

# Program Handbook and Abstracts

IMBC 2013



10<sup>TH</sup>

INTERNATIONAL  
**MARINE**  
BIOTECHNOLOGY  
CONFERENCE

BRISBANE  
• AUSTRALIA 2013 •

*Genome to phenome:  
understanding to  
sustainable use*

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11-15 November 2013

Brisbane Convention & Exhibition Centre

*INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE*

*10<sup>TH</sup> TRIENNIAL CONFERENCE*

*IMBC 2013*

*PROGRAM AND ABSTRACT  
HANDBOOK*



11 - 15 November 2013

Brisbane Convention and Exhibition Centre  
Queensland, Australia

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### **Conference Secretariat**

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# HANDBOOK CONTENTS

Please Note: **Daily Timetables** - Tuesday - Friday are printed on coloured paper for ease of finding.  
**Posters, sorted by Presenter Last Name**, giving a quick way to find the poster number. are on pp 37-39.  
 All posters are listed with full title and authors on pages 29-36.  
 A quick reference to Presenters (alpha by last name) with details of the day, time, session, room and title of each presentation are printed on A3 sheets and displayed on the Notice Board near the Registration Office.

<b>Venue Location map</b> .....	<b>4</b>
<b>Brisbane Convention and Exhibition Centre Floor Plan</b> .....	<b>5</b>
<b>President's Welcome Message (Prof Tadashi Matsunaga)</b> .....	<b>6</b>
<b>Marine Drugs - Proceedings of the 10th International Marine Biotechnology Conference</b> .....	<b>6</b>
<b>Welcome from Chairs of Organising Committee (Prof. Bernie Degnan and Prof. Joe Baker)</b> .....	<b>7</b>
<b>International Marine Biotechnology Committees (National Organising Committee, International Program Committee and International Marine Biotechnology Board members)</b> .....	<b>8</b>
<b>Venue and Conference Structure</b> .....	<b>9</b>
<b>Conference Social Functions</b> .....	<b>10</b>
<b>Student Prizes</b> .....	<b>10</b>
<b>Plenary Speakers</b> .....	<b>11-13</b>
<i>Professor Asao Fujiyama</i>	
<i>Professor Bill Gerwick (University of California, San Diego)</i>	
<i>Professor Ben Hankamer (University of Queensland)</i>	
<i>Professor Ute Hentschel (University of Wuerzburg)</i>	
<i>Professor Ron Quinn (Griffith University)</i>	
<i>Professor Amir Sagi (The Negev Ben Gurion University)</i>	
<i>Professor Anchalee Tassanakajon (Chulalongkorn University)</i>	
<b>Keynote Speakers</b> .....	<b>13-14</b>
<b>Thank You to our Sponsors</b> .....	<b>15</b>
<i>Sunshine Coast University</i>	<b>16</b>
<i>The University of Queensland</i>	<b>17</b>
<i>CSIRO</i>	<b>17</b>
<i>BGI Tech Solutions</i>	<b>18</b>
<i>Queensland University of Technology</i>	<b>18</b>
<b>Timetable and Program Description</b> .....	<b>19</b>
<b>Timetable - Tuesday Morning</b> .....	<b>20</b>
<b>Timetable - Tuesday Afternoon</b> .....	<b>21</b>
<b>Timetable - Wednesday (Auditorium and Boulevard 1)</b> .....	<b>22</b>
<b>Timetable - Wednesday (Boulevard 2 and 3)</b> .....	<b>23</b>
<b>Timetable - Thursday Morning</b> .....	<b>24</b>
<b>Timetable - Thursday Afternoon</b> .....	<b>25</b>
<b>Timetable - Friday</b> .....	<b>26</b>
<b>ANZ-CHINA Collaboration Forum on Marine Biotechnology</b> .....	<b>27-28</b>
<b>POSTERS (Display Order by Poster Number &amp; Topic)</b> .....	<b>29-36</b>
<b>POSTER numberS (Presenter Alpha Order)</b> .....	<b>37-38</b>
<b>IMBC 2013 ABSTRACTS</b> .....	<b>39-170</b>
<b>Delegate List</b> .....	<b>171-184</b>



## PRESIDENT'S WELCOME MESSAGE

Dear Friends and Colleagues,

It is my great pleasure to welcome you to the 10<sup>th</sup> International Marine Biotechnology Conference at Brisbane.

Since the first IMBC in 1989 in Tokyo, we had successive conferences in various countries – the United States in 1991, Norway in 1994, Italy in 1997, Australia in 2000, Japan in 2003, Canada in 2005, Israel in 2007 and China in 2010. All these conferences were remarkable boosters to Marine Biotechnology research, and this IMBC2013 will also serve as another significant milestone in the history of this research area.



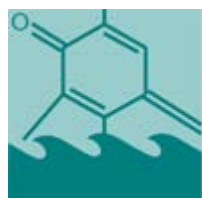
Year by year, the Marine Biotechnology research has been more focused on, and expected to contribute to solving the global issues such as climate changes and energy problems, and to realize the sustainable society as soon as possible. The ocean is a mother of all living creatures, and a treasure box of resources and potentiality. We can see many miracles in the Ocean, and we need to use it appropriately for the earth and humanity, saving the beautiful environment, and co-existing each other.

Of course, it requires huge efforts from broad range of perspectives and multidisciplinary approaches beyond countries and any kind of frameworks, so this international conference is a great opportunities to exchange cutting-edge information, new research findings and innovations. Moreover, many distinguished lectures that the top scientists provide us and many workshops and sessions organized by world-leading researchers will encourage young promising scientists. I wish you all the participants can have a fruitful time, and get something to accelerate your research further.

Finally, I would like to appreciate all the effort of Symposium Chairs and the staffs, the members of the International Program Committee and National Organizing Committee, and many supporting organizations that make this conference successful.

Thank you again for joining us.

*Tadashi Matsunaga*



*marine drugs*  
an open access journal  
by MDPI

## PROCEEDINGS OF THE 10TH INTERNATIONAL MARINE BIOTECHNOLOGY CONFERENCE

Proceedings of IMBC2013 will be published in a Special Issue of *Marine Drugs* (impact factor 3.85), ***Advances and New Perspectives in Marine Biotechnology***.

We cordially invite all presenters at IMBC2013 to submit a comprehensive/mini review, an original research article or a short communication to this Special Issue.

Please go to the special IMBC2013 web page for instructions on how to prepare and submit your manuscript ([http://www.mdpi.com/journal/marinedrugs/special\\_issues/marine-biotechnology](http://www.mdpi.com/journal/marinedrugs/special_issues/marine-biotechnology)).

*Marine Drugs* will defray publication costs for a number of the submitted manuscripts.

**DEADLINE FOR MANUSCRIPT SUBMISSIONS: 31 JANUARY 2014**

### Special Issue Editors

**Prof. Bernie Degan**

School of Biological Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia

**Mr Pabulo Henrique Rampelotto**

Interdisciplinary Center for Biotechnology Research, Federal University of Pampa, Antônio Trilha Avenue, P.O.Box 1847, 97300-000, São Gabriel – RS, Brazil

**Dr Paul Long**

Institute of Pharmaceutical Science & Department of Chemistry, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom

## WELCOME FROM CHAIRS OF ORGANISING COMMITTEE



Welcome to Brisbane and the 10th International Marine Biotechnology Conference. This is the latest in a series of highly successful meetings that date back to 1989, when the first IMBC was held in Tokyo, Japan. Since then IMBCs have been held in seven countries on three continents. This meeting marks its return to Australia; the 5th IMBC was in Townsville, Queensland.

The International Marine Biotechnology Conferences are widely recognised as the world's premier conferences in marine biotechnology. This year, as in the past, there is extensive and wide-sweeping participation and support from both the private and public sectors. The 10th IMBC has nearly 300 delegates from over 20 countries participating.

As the "Century of Biology" begins to bear fruit, through the translation of predictive biological understanding into applications that enhance the human condition and maintain biodiversity, the almost infinite potential of marine biological resources will be unlocked.

The theme of the 10th IMBC is *Genome to phenome: understanding to sustainable use*. This summarises the world's opportunity to profit from and protect our oceans. Already marine biotechnology has delivered products for medicine, food, biofuels, nanomaterials, and bioremediation.

The 10th IMBC will include a wide range of Plenary and Keynote Speakers. These will allow delegates to experience cutting-edge developments from around the world – our invited speakers come from all continents on Earth. As in the past, this year's IMBC features the most recent development in the field of marine biotechnology, including recent advances in algal biofuels and bioenergy, marine genomics and metagenomics, aquaculture reproductive technologies, microbial-based technologies and small molecule applications.

Of course 10th IMBC would struggle to happen at all without the generous support from a wide range of Australian and international agencies and institutes. Our sponsors are listed in the program. Please take time to find out more about them. We are fully appreciative of all they have done to make the 10th IMBC a highly successful meeting – thank you.

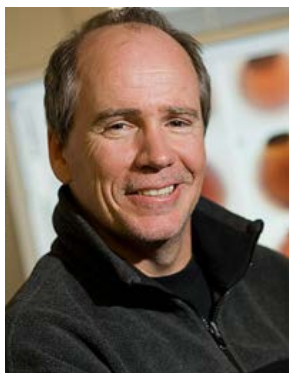
Finally, before, during and after the meeting take some time to explore Brisbane, Queensland and Australia. Brisbane remains one of Australia's most desirable cities. Surrounding it is an amazing range of natural settings, including world famous beaches, ancient rainforests, coral reefs and the outback.

On behalf of the National Organising Committee, we thank you for attending the 10th International Marine Biotechnology Conference. We hope that you find the meeting as exciting and stimulating as we hope it will be.

Best wishes

A handwritten signature in blue ink, appearing to read 'Bernie Degnan'.

Bernie Degnan, Director of 10th International Marine Biotechnology Conference and co-Chair of the National Organising Committee



A handwritten signature in dark ink, appearing to read 'J. J. Baker'.

Professor Joe Baker  
AO, OBE, FTSE, FRACI,  
C.Chem.

Joe Baker, co-Chair of  
the National Organising  
Committee



# INTERNATIONAL MARINE BIOTECHNOLOGY COMMITTEES

## NATIONAL ORGANISING COMMITTEE

**Co-Chairs**     **Bernie Degnan** (co-Chair) (The University of Queensland): marine genomics  
**Joe Baker** (co-Chair) (Consultant)

### Members

Andy Barnes (The University of Queensland): aquaculture – immunology, vaccine development  
Chris Battershill (The University of Waikato, New Zealand): marine chemistry and microbes  
Kirsten Benkendorff (Southern Cross University): natural product chemistry and biology  
Michael Borowitzka (Murdoch University): algal biotechnologies  
Rocky de Nys (James Cook University): natural products, biofouling, biofuels  
Abigail Elizur (University of the Sunshine Coast): biomineralization; Aquaculture – reproductive molecular biology  
Peer Schenk (University of Queensland): microalgal biotechnology and bioenergy  
Melony Sellars (CSIRO Marine & Atmospheric Research) aquaculture – transgenics  
Torsten Thomas (The University of New South Wales): marine microbes and metagenomics  
Robyn Williams (Australian Broadcasting Corporation) science journalist and communicator  
Wei Zhang (Flinders University): bioprocessing and marine bioproducts



## INTERNATIONAL PROGRAM COMMITTEE

**Nobuhiro Fusetani** (Japan): marine pharmacology (Chair)

### Members

Peter Alestrom (Norway): genomics and model organisms  
Takashi Aoki (Japan): disease, omics, aquaculture  
Joe Baker (Australia): bioactives, policy, etc.  
Chris Battershill (New Zealand): sponge, aquaculture  
Roberto Berlinck (Brazil): bioactives, bioproducts  
Xiguang Chen (China): biomaterials  
Mike Hall (Australia): aquaculture  
Russell Hill (United States): biodiversity conservation and drug discovery  
San-Jin Kim (Korea): microbiology, extremophiles, biocatalysts  
Song Qin (China): algal biotechnology  
Haruko Takeyama (Japan): microbiology, metagenomics  
John van der Meer (Canada): algal biotechnology, aquaculture  
Shugo Watabe (Japan): genomics, molecular biology, biomineralization  
Rene Wijffels (Netherlands): biofuels, microalgae, etc.  
Yonathan Zohar (USA): aquaculture

## IMBA BOARD MEMBERS

Prof. Tadashi Matsunaga, President, Japan  
Prof. Werner Müller, Vice-President, Germany  
Prof. Russell Hill, Secretary-Treasurer, USA  
Prof. Jan Olafsen, Immed Past President, Norway  
Prof. Bernie Degnan, Conference Director, Australia  
Prof. Joseph Baker, Chair Nominating Committee, Australia  
Prof. Christopher Battershill, New Zealand  
Dr. Pamela Chavez-Crooker, Chile  
Prof. Vernon Coyne, South Africa  
Prof. Nobuhiro Fusetani, Japan  
Prof. Se-Kwon Kim, Korea  
Dr. S. Raghu Kumar, India  
Dr. Hanzhi Lin, China  
Prof. Song Qin, China  
Dr John van der Meer, Canada  
Dr Joy Watts, United Kingdom  
Prof. Yonathan Zohar, USA



# VENUE AND CONFERENCE STRUCTURE

## **REGISTRATION**

The Registration Office will be located on the Boulevard Level and will be staffed from 1500 - 1730 on Monday 11th and - 0815 - 1700 Tuesday - Thursday and 0815 -1330 Friday.

## **NAME BADGES**

Delegates are requested to wear their name badge at all times during the conference. This badge is also your ticket to included functions.

## **PRESENTATION UPLOADS – ARBOUR LEVEL SPEAKERS PREPARATION ROOM**

All presentations are to be loaded onto the Convention Centre laptop computers in Speakers Prep in advance - you cannot use your own laptop. Please ensure that you take your CD / USB to the Speakers Prep area to be loaded well before your session (preferably the day before). While you can check that the presentation works after uploading, there is no computer availability for major changes to be done. Please do not leave your upload until the last moment.

<b>NOTE Opening Hours</b>	<b>Monday 11th</b>	<b>1500 - 1730</b>
	<b>Tuesday 12th</b>	<b>0800 - 1600</b>
	<b>Wednesday 13th</b>	<b>0800 - 1600</b>
	<b>Thursday 14th</b>	<b>0800 - 1600</b>
	<b>Friday 15th</b>	<b>0800 - 1100</b>

## **CONFERENCE STRUCTURE**

### **For session details, refer to the Timetable pages (printed in colour)**

Each morning in the Boulevard Auditorium is a plenary session which includes the plenary speakers for that day. After morning tea, concurrent sessions will commence in the breakout rooms and continue throughout the day.

Most talks in the concurrent sessions in the breakout rooms are 20 minutes (15 minute presentations with 5 minutes for questions). Every effort will be made by the chairpersons to keep to the allotted times, allowing delegates to move between rooms and presentations. If you are a presenter, please assist the program by keeping your talk within the allotted timeframe.

The scientific program finishes at 1315 on Friday, with the last 15 minutes in the Boulevard Auditorium with conference closing statements.

## **POSTERS**

Posters will be on display for the entire conference in the Boulevard Foyer, where lunch and morning/afternoon teas will be served. The Poster Cocktail Session will be held on Tuesday evening from 1730 - 2000. Poster presenters will be standing with their posters during this session to answer any questions. Student posters will be judged during this Poster Session. A selection of canapés and drinks will be served.

## **EXHIBITION BOOTH DISPLAYS**

Exhibition booth displays from our sponsors and exhibitors will be in the Boulevard Foyer for the duration of the conference and can be accessed throughout the conference, Tuesday to Friday. All refreshments will be served in this area during the conference to enable maximum time for delegates to meet Exhibition Stand holders and study the Posters. Exhibitors have put in enormous cost and effort to exhibit to the marine biotechnology audience. Please make them feel welcome.

## **CONFERENCE DRESS CODE**

Dress for the conference is business-casual comfortable clothing. Ties and jackets are not necessary. Dress for the Conference Dinner on Thursday 11 July is smart casual.

## **MESSAGES**

Please check the notice board by the Conference Secretariat regularly for messages.  
During conference hours: Secretariat Telephone is: 0400 358 302

## **PUBLIC TRANSPORT, TAXIS, ATM AND BANKING**

Please check with the Convention Centre Reception on the Ground Level.

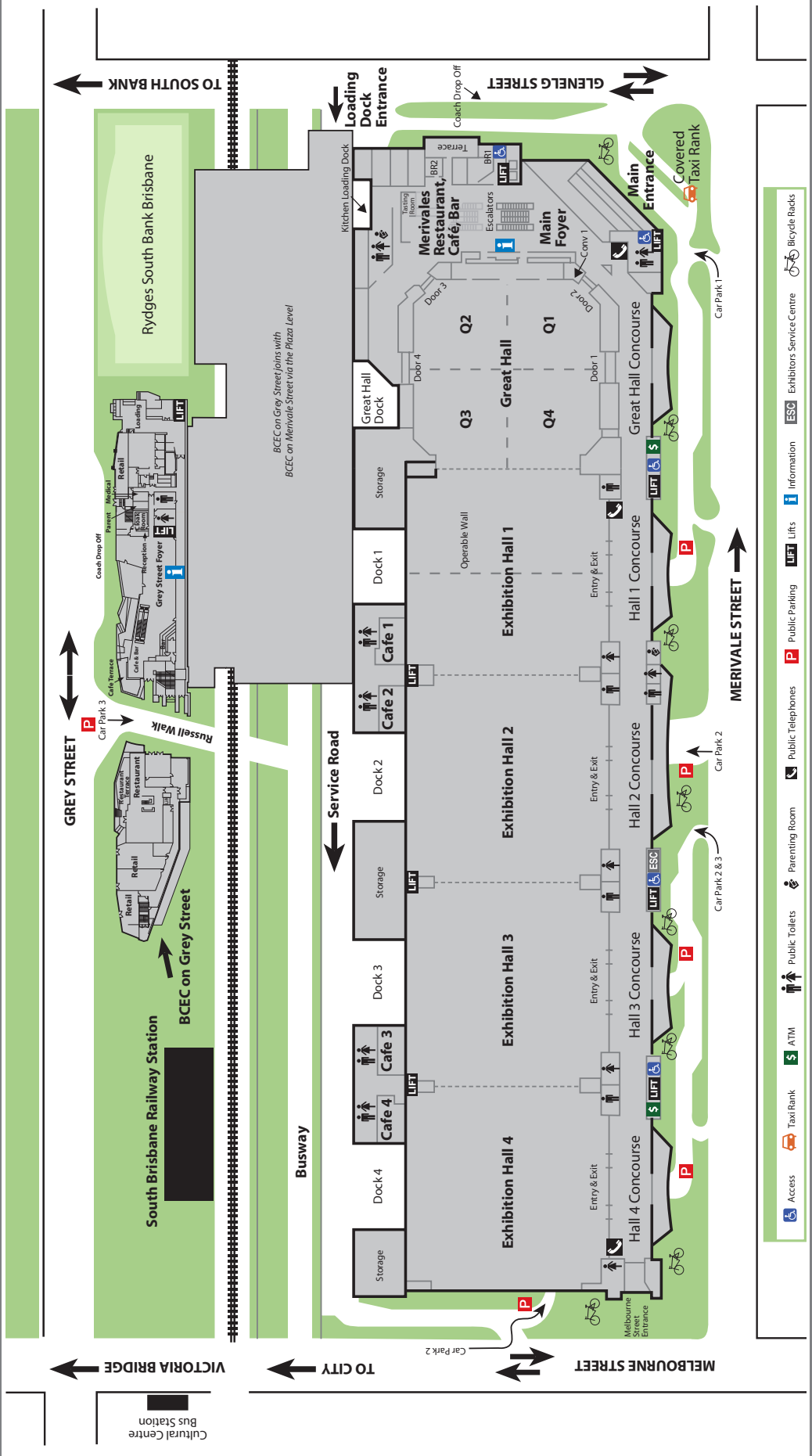


# VENUE LOCATION MAP

Note Grey Street (for Boulevard Level Entrance) on the left of map

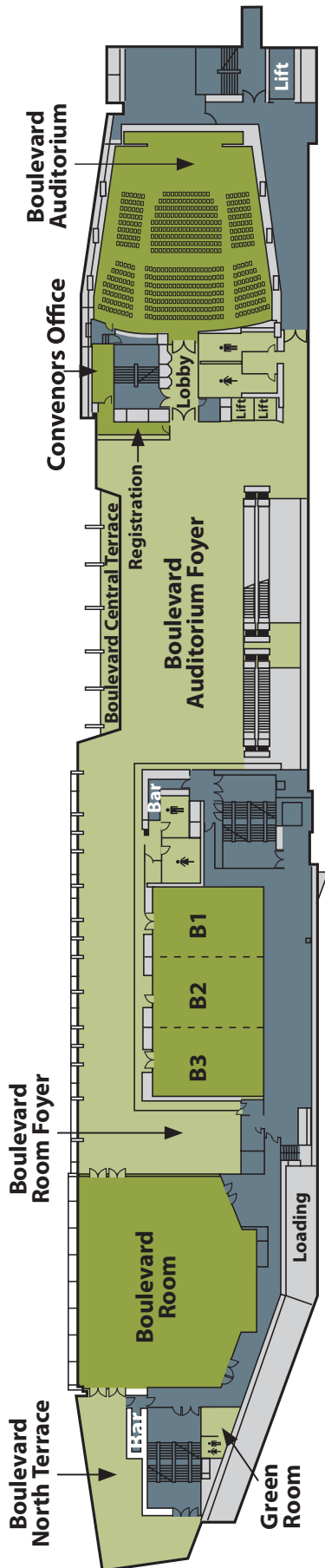
**BRISBANE**  
convention  
& exhibition  
**CENTRE**

Location & Access Map  
BCEC on Merivale Street



- Access
- Taxi Rank
- ATM
- Public Toilets
- Parenting Room
- Public Telephones
- Public Parking
- LIFT Lifts
- Information
- ESC Exhibitors Service Centre
- Bicycle Racks

# BRISBANE CONVENTION AND EXHIBITION CENTRE



The IMBC 2013 conference, held at the Brisbane Convention and Exhibition Centre, Grey Street, South Brisbane, is walking distance from the Brisbane CBD and Southbank Parklands. The main entry for the Boulevard Level is on Grey Street.

The conference will be concentrated on the Boulevard Level, with the main plenary in the Boulevard Auditorium and breakout rooms in B1, B2 and B3 along the foyer towards the Boulevard Room. The Conference Dinner will be held in the Boulevard Room

## EXHIBITION STAND DISPLAYS

Exhibition booth displays from our sponsors and exhibitors will be in the Boulevard Foyer for the duration of the conference and can be accessed at any time, from Monday evening through to Friday.

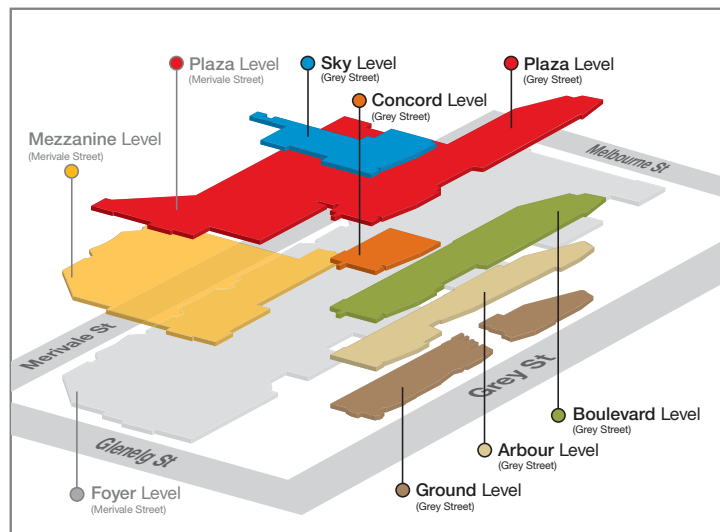
All refreshments will be served on this level during the conference so delegates will have ample opportunity to meet Exhibitors.

Sometime during the conference please take time to have a look at these and talk to the representatives occupying the displays. Please make them feel welcome.

## POSTERS

Posters will be on display for the entire conference in the Boulevard Foyer, in the Exhibition area and where lunch and morning/afternoon tea will be served. Posters are to be judged during the Tuesday Poster Session.

Poster presenters are requested to stand with their posters during the Poster Session to answer any questions. It may also be helpful for delegates if poster presenters have morning and afternoon tea and lunch near their poster. The prizes for the winning student posters will be presented at the Conference Dinner on Thursday evening.



# CONFERENCE SOCIAL FUNCTIONS

**BOULEVARD LEVEL, BRISBANE CONVENTION AND EXHIBITION CENTRE**

## **REGISTRATION - GALLERY AREA FROM 3:00PM - 5:30PM**

The Welcome, Poster and Dinner Functions are Included with all full registrations.

Extra tickets are available for purchase for guests and with single day registrations from the Conference Secretariat.

## **FUNCTIONS**

### **MONDAY - WELCOME FUNCTION - 1800-2000**

A **Welcome Reception** will be held on Monday 11th November from 1800 - 2000 hrs. The Welcome Function is sponsored by CSIRO. Formalities will include a welcome from the Chair of the Conference Organising Committee, and a brief overview of the CSIRO.

### **TUESDAY - POSTER COCKTAIL SESSION - 1730-2000**

This Poster Session, during which canapés and drinks will be served, is designed to give poster presenters the opportunity to discuss their work with conference participants. Authors stand with their posters for discussions. Student posters are judged at this time.

### **WEDNESDAY - FREE EVENING**

### **THURSDAY - CONFERENCE DINNER, BOULEVARD ROOM 1900 - 2330**

The **Conference Dinner**, with a three-course table-service meal, will be held in the Boulevard Room on Thursday 14 November. The function commences with drinks served from 1900 and will conclude at 2330.

Professor Werner E.G. Müller, Incoming IMBA President, is the Speaker at the Conference Dinner.

At the Conference Dinner he will summarise highlights of his research and its relevance, and share his vision for the IMBA and for marine biotechnology.

## **STUDENT PRIZES**

**All student prizes will be judged by a panel of judges during the conference and the prizes will be awarded during the conference dinner on Thursday evening.**

### **PAMBA BEST ORAL PRESENTATION**

Pan American Marine Biotechnology Association has donated a cash prize for the best oral presentation.

Three second prizes will be awarded to runner-ups of the best oral presentation.



### **IMBA BEST POSTER PRESENTATION**

The Board of the International Marine Biotechnology Association has donated a cash prize for the Best Poster Presentation.



### **FACULTY OF 1000 PRIZES FOR RUNNER-UP BEST POSTERS**

F1000 will provide three awards for excellence in the "Best Posters" Competition at the 10th IMBC.



In addition to the prizes, these posters will be uploaded onto the established open access poster repository, F1000 posters (<http://f1000.com/posters>) with a brief evaluation. These posters will be assessed by Faculty of 1000 (<http://f1000.com>) and could be selected as the F1000 submission of the week, which gets 1000+ hits and gets heavily promoted via their social media avenues.

## PLENARY SPEAKERS

The committee is pleased to present plenary speakers at *IMBC 2013 Genome to phenome: understanding to sustainable use*, a wonderful mix of the finest researchers in marine biotechnology.

### **PROFESSOR ASAO FUJIYAMA**

Comparative Genomics Laboratory, National Institute of Genetics, Yata 1111, Mishima, Shizuoka 411-8540, JAPAN

#### **Toward understanding marine lifestyles using new-generation sequencing and genomic technologies: Red alga *Pyropia yezoensis* and other case studies**



I have been involved in genome research since the beginning of International Human Genome Project dated back to the year 1990. Thirteen years later, and after the so called completion of the human genome, I shifted my research interest to comparative genomics; to begin with, we sequenced particular chromosomes of chimpanzee and found that the difference among human and chimp is about 1.8%.

Genomics is not only a key technology for any field of life sciences but provides us the insight of organisms with which we are working. It is needless to say that aquatic life is quite complex and full of secrets, for example, we recently published coelacanth genome and found that two coelacanth species, Tanzanian and Indonesian, are quite similar in terms of genomic differences. We are currently analyzing genome of red agar which has complex symbiosis dependent life.

### **PROFESSOR WILLIAM (BILL) GERWICK**

Distinguished Professor of Oceanography and Pharmaceutical Sciences, Scripps Institution of Oceanography and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA 92093

#### **Integrating genomics and biosynthesis to discover new classes of bioactive secondary metabolites from marine cyanobacteria**



Dr Gerwick received a B.S. in Biochemistry from the University of California Davis in 1976, and a Ph.D. in Oceanography from the Scripps Institution of Oceanography, University of California San Diego, in 1981 where he worked with Bill Fenical. He held a postdoctoral position in the area of natural product biosynthesis with Steven Gould in the School of Pharmacy at the University of Connecticut, was Assistant Professor of Chemistry at the University of Puerto Rico, Rio Piedras (1983-84) and then advanced through the ranks to become Full Professor in 1992 at the College of Pharmacy, Oregon State University. In 2005 he returned to La Jolla to hold a joint professorship at Scripps Institution of Oceanography and the Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego.

His work has focused on the characterization of the unique natural products of marine algae and cyanobacteria, their biological activities of use to biomedicine, and the pathways of their biosynthesis. This latter area has expanded into genomic characterization of cyanobacteria, the molecular evolution of natural product pathways, heterologous expression of cyanobacterial pathways in other prokaryotes, and studies of the mechanistic chemistry of natural products biosynthesis.

### **PROFESSOR BEN HANKAMER**

University of Queensland, Institute for Molecular Bioscience

#### **Towards High-Efficiency Microalgae Biofuel Systems**



In 2002, Ben moved from Imperial College London to take up his position as a Principle Investigator at The University of Queensland's Institute for Molecular Bioscience. Ben has focused on the development of environmentally friendly high-efficiency microalgae biofuel production systems. In 2006, he established and directs the Solar Biofuels Consortium which now includes 8 international teams, ~100 researchers and ~10 industry partners. In 2009, Ben was awarded the prestigious Eisenhower Fellowship, awarded to individuals identified as international leaders in areas of energy technology and supply. In 2013, Ben was also awarded the Discovery of Outstanding Researcher Award from the Australian Research Council.

Over the past 10 years, Ben Hankamer has focused on the development of environmentally friendly high-efficiency biofuel production systems. This area represents a rapidly expanding biotechnology. His specialisation is in the structural biology of the photosynthetic machinery, which drives the conversion of solar energy into chemical energy (fuels) and has published extensively on the water splitting Photosystem II complex, its light harvesting antenna system and V-type ATPase (Nature, Nature Structural Biology, TIBS, PNAS). Using this knowledge of the photosynthetic machinery, he embarked on the targeted engineering of the green alga *Chlamydomonas reinhardtii* for high-efficiency biofuel production. To facilitate the development of high efficiency biofuel systems, he founded the Solar Biofuels Consortium which he now directs. The consortium includes eight international teams and conducts economic analysis, bio-discovery, marine biology, structural biology, molecular biology, microbiology, genomics, metabolomics, culture optimisation and bioreactor scale up within a coordinated research program of parallel research streams.

## PLENARY SPEAKERS

### PROFESSOR UTE HENTSCHEL

Dept of Botany II, Julius-von-Sachs-Institute for Biological Sciences, University of Wuerzburg, 97082 Wuerzburg, Germany

#### Microbial diversity, function and biotechnological potential of marine sponges



Professor Ute Hentschel obtained her Ph.D. degree in marine biology at Scripps Institution of Oceanography, La Jolla, USA and performed her postdoctoral research in infection biology. Since 2008, she is full professor at the Julius-von-Sachs Institute for Biological Sciences at the University of Würzburg, Germany. Dr Hentschel has published over 109 publications and 3 patents. Her research interests include host-microbe interactions, with special focus on diversity, function and biotechnological potential of marine sponges and their microbial consortia. In 2008, she was awarded the Recognition Award of Marine Biotechnology (Springer).

Current projects include:

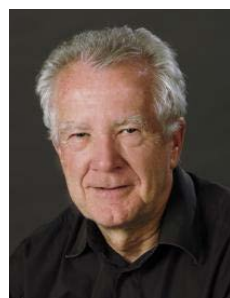
•-Omics (metagenomics, single cell genomics, metatranscriptomics) of the sponge microbiome

- Unravelling patterns of global microbial biodiversity in sponges
- Investigations on sponge diseases
- Novel anti-infectives discovery from marine-sponge associated actinomycetes

### PROFESSOR RON QUINN

Eskitis Institute for Cell and Molecular Therapies, Griffith University, Nathan Qld 4111

#### The Future for, and the challenges of, commercializing Marine Bioactives



Professor Quinn obtained his PhD from the University of New South Wales (Australia) in 1970. Following post-doctoral research at Arizona State University, University of Hawaii, and ANU, he joined the Roche Research Institute of Marine Pharmacology in Sydney (1974 – 1981). He joined Griffith University in 1982 and is the Foundation Director of the Eskitis Institute for Cell and Molecular Therapies at Griffith University. He is the author of over 170 publications and patents. His research concentrates on the use of molecules as tools to understand interactions in biological systems and to build concepts around molecular recognition.

His research has concentrated on:

1. Biodiscovery involving high throughput screening against molecular and cellular targets, isolation and structure elucidation of bioactive natural products
2. Design and synthesis of receptor ligands in the adenosine area, enzyme inhibitors of protein phosphatase 1 and 2A and Factor XIa
3. Understanding of natural product recognition for biosynthetic enzymes and correlation with therapeutic targets as a rational approach to drug discovery, and
4. Developing concepts of biological structure space embedded in natural product scaffolds.

The current direction of his research is to transform approaches to the discovery of new bioactive molecules from nature. He has developed a front-loading of physicochemical properties of constituents of extracts and is using an understanding of biological structure space to discover new biologically relevant molecules. Through funding from the Gates Grand Challenge Exploratory grant, he is developing a novel approach to study latent stages of malaria using natural product fragments.

### PROFESSOR AMIR SAGI

Department of Life Sciences and the National Institute for Biotechnology, The Negev Ben Gurion University, P.O. Box 653, Beer Sheva 84105, Israel

#### Insulin-like androgenic hormones and monosex culture of prawns: The first implementation of RNA-interference in aquaculture



Research interests of Professor Sagi include: Genes and gene products in comparative and applied endocrinology: Regulation of sexual differentiation, reproduction, growth and calcium mobilization in marine and freshwater invertebrates; Crustacean models are employed in his laboratory for the study of genes and gene products related to processes of sexual differentiation and skeletal bio-mineralization. In particular, study of the endocrine regulation by steroids and insulin-like androgenic gland factors of sexual differentiation, gonad maturation, growth, molt and the related processes of calcium mobilization and bio-mineralization. Control of the above events have been enabling the development of biotechnological tools for crop improvement via crustacean monosex culturing, soft shell-based products as well as for human food additives and drugs.

Professor Sagi is Past President, International Society for Invertebrate Reproduction and Development (ISIRD) and holds the Lily and Sidney Oelbaum Chair in Applied Biochemistry. He is also a Member of the National Institute for Biotechnology in the Negev (NIBN), co- Founder of Amorphical Ltd and Enzootic Ltd, and Former dean of the Faculty of Natural Sciences.

## PLENARY & KEYNOTE SPEAKERS

### PROFESSOR ANCHALEE TASSANAKAJON



Shrimp Molecular Biology and Genomics Laboratory, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330 Thailand

#### **How does the immune system of shrimps fight against pathogens**

Prof. Anchalee Tassanakajon is a pioneer in shrimp genome research. Her work focuses on applying genome information to study the immune system of shrimp. She and her research team have successfully identified several immune-related genes in the commercially important black tiger shrimp, *Penaeus monodon*, by principally Expressed Sequence Tag (EST) analysis and also other related approaches including differential and suppression subtraction hybridization analysis. The functions of some of these genes have been further characterized to unveil the important immune mechanisms in shrimp.

### PROFESSOR WERNER E.G. MÜLLER

#### **INCOMING IMBA PRESIDENT - CONFERENCE DINNER ADDRESS**



University Medical Center of the Johannes Gutenberg University Mainz

- . distinguished Professor at the University Medical Center of Mainz, Germany,
- . awardee of a prestigious ERC Advance Grant,
- . coordinator of the EU Integrated Project “BlueGenics”,
- . recipient of the prestigious Friendship Award of China,
- . senator of the “Akademie gemeinnütziger Wissenschaften zu Erfurt (Germany)”,
- . member of the “Croatian Academy of Science and Arts”,
- . more than 20 patents and 1100 publications with a scientific research impact h-index of 61

## KEYNOTE SPEAKERS AND SESSIONS



**Borowitzka, Michael (Murdoch University)**

***Marine Algal Oleomics: New Development of Biofuel Production Research and Technology***

**Monday, Auditorium**

**Physiology and metabolism of lipid production by marine and halotolerant microalgae**



**Chavez-Crooker, Pamela (Aquamarina SA)**

**Microbial Bioresources**

**Tuesday Boulevard 1**

***Bioleaching of conventional and non conventional materials: new approaches***



**Abigail Elizur (University of the Sunshine Coast)**

***Reproductive Technologies in Aquaculture***

**Wednesday, Boulevard 2**

***Reproductive technologies in aquaculture***



**Fang, Xiaodong (BGI Tech Solutions Co. Limited, Guangdong)**

**Genomics**

**Friday Auditorium**

***View from oyster genome: The opportunity and challenge for aquatic organism genome study***

## KEYNOTE SPEAKERS AND SESSIONS



**Do-Hung Kang**

**Algal Fuels & Bioenergy**

**Wednesday, Auditorium**

*A mini review on algae biofuel in Korea*



**Kim, Se Kwon (Pukyong National University, Marine Bio-Process Reseach Center, Busan)**

**Marine Bioresources**

**Tuesday, Boulevard 1**

*Investigation and development of bioactive substances from marine organisms*



**Müller, Werner (University Medical Center of the Johannes Gutenberg University Minz)**

**Genomics**

**Thursday, Boulevard 1**

*Sustainable Oceans – our Treasure in the Past and in the Future: The power of marine genomics*



**Nichols, Peter (CSIRO Marine and Atmospheric Research)**

**Nutraceuticals & Functional Foods**

**Friday, Boulevard 2**

*A Journey from Marine Genes to New Sustainable Land Plant Sources of Long-chain Omega-3 Oils*



**Prinsep, Michele (University of Waikato, New Zealand)**

**Marine Natural Products**

**Thursday, Auditorium**

*Trends in Marine Natural Products Research-A New Zealand Perspective*



**Qin, Song (Yantai Institute of Coastal Zone Research, CAS)**

**Marine Natural Products**

**Thursday, Boulevard 2**

*Algal Biotechnology: reshaping the coasts*



**Schenk, Peer (University of Queensland)**

**Algal Production and Biotechnology**

**Thursday, Boulevard 2**

*Development of low-cost high-efficiency algae energy farms*

# THANK YOU TO OUR SPONSORS

Sponsor support is essential to support marine biotechnology conferences, and the sponsorship of the below organisations is very much appreciated. The Organising Committee would like to thank each of these organisations for their generous contribution to our IMBC2013 conference.

The International Marine Biotechnology Conferences provide the opportunity for interaction between scientists, technologists, industry and policy-makers, and heighten national and international awareness of the role of marine biotechnologies in the sustainable development of marine bioindustries and the protection of the marine environment.

## IMBC 2013 Sponsors

**University of the Sunshine Coast** has supported as a Bronze Sponsor. Their Exhibition Stand is hosted by staff from the Genecology laboratory.

**The University of Queensland** has supported as a Bronze Sponsor. Their Exhibition Stand is hosted by staff from the Centre for Marine Studies.

**CSIRO** sponsored the Welcome Function on Monday evening. Dr Sue Blackburn will speak on behalf of CSIRO during the evening.

**Office of Naval Resources Global** contributed by way of a Grant.

**Maryland Sea Grant Program** supported travel for graduate students to attend from the United States.

**BGI Tech** and **QUT ife** have Exhibition Stands.

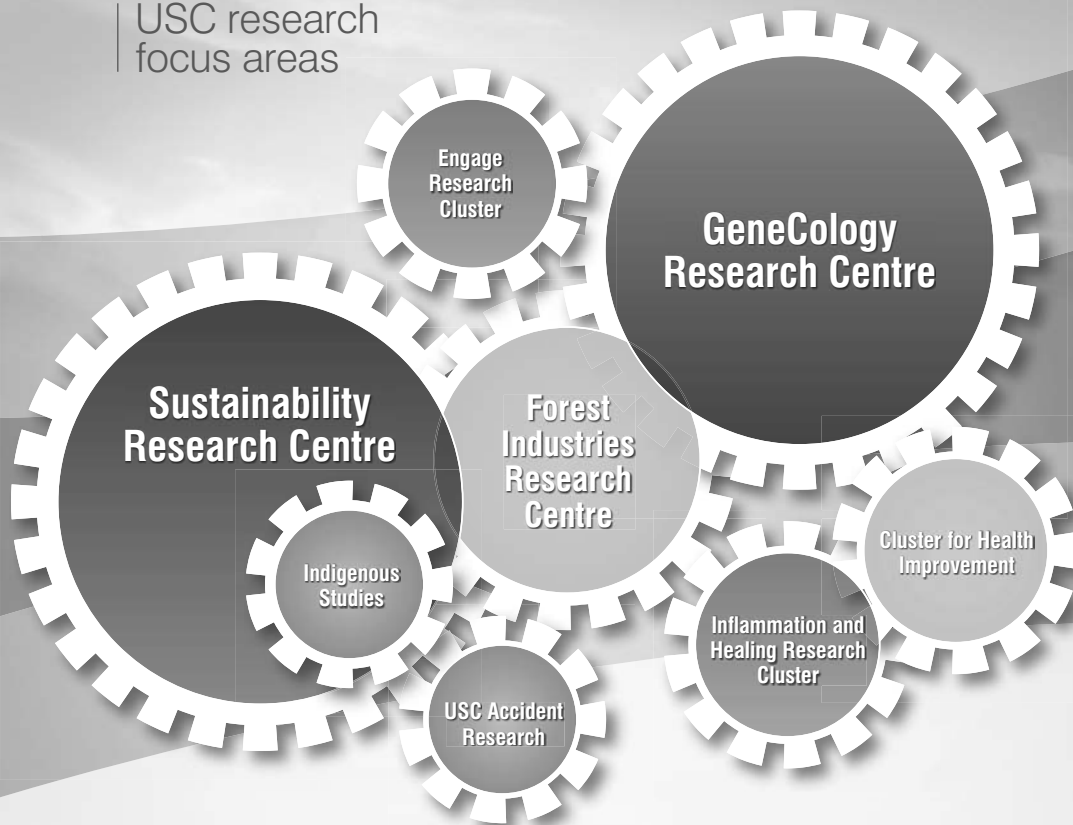
**Japanese Science and Technology Agency** supported keynotes for the Oleomics symposium.

**On behalf of the International Marine Biotechnology Association and the organisers of IMBC 2013, we thank all sponsors for their support.**





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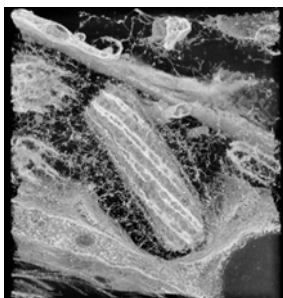
# THE UNIVERSITY OF QUEENSLAND

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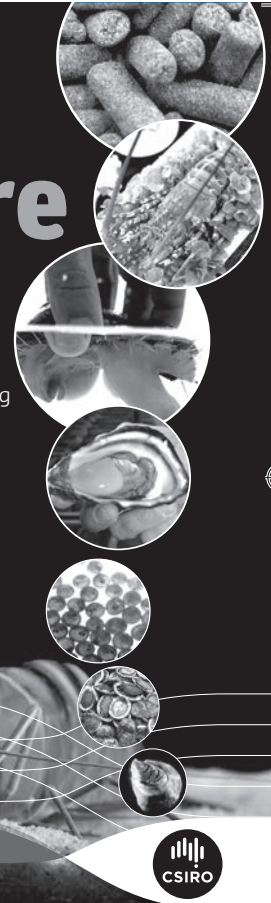
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- Genotyping

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- RNA sequencing (Quantification)
- Digital Gene Expression (DGE)
- Small RNA sequencing
- Degradome sequencing

## Epigenomics research

- Bisulfite sequencing
- ChIP sequencing
- MeDIP sequencing

# ***TIMETABLE AND PROGRAM***

## ***OVERALL CONFERENCE TIMETABLE AND PROGRAM EXPLANATION***

The Daily Timetables, giving details of rooms, sessions and speakers, from Tuesday 12th to Friday 15th can be found on the following coloured pages.

The mornings commence in the Boulevard Auditorium and continue until Morning Tea. before breaking into concurrent sessions. As well as the Boulevard Auditorium, we have three other breakout rooms - Boulevard 1, Boulevard 2 and Boulevard 3.

All abstracts follow after the Timetables, in alphabetical order by the last name of the first author, with the presenting author marked with an asterisk.

Poster abstracts are marked with [Poster] at the end of the title in the abstracts, with a list in **author last name** order with **poster number**. Posters are displayed in topic order, and a list with authors, titles and the assigned topic category follow the author name list to assist with finding the poster number for a specific author.

Posters will be displayed in numerical order. Authors will be beside their posters during the Poster Cocktail Session on Tuesday evening to discuss their work and answer questions.

Student posters will be judged during this session.

### ***MONDAY 11TH NOVEMBER 2013***

Boulevard Foyer, Brisbane Convention and  
Exhibition Centre

From 1500 Registration (Boulevard Foyer)  
Hanging of Posters

1800 - 2000 Welcome Function  
(sponsored by CSIRO)

### **INFORMATION DISCLAIMER**

The speakers, topics and times are correct at the time of publishing. In the event of unforeseen circumstances, the organisers reserve the right to alter or delete items from the Conference Program.

## TIMETABLE - TUESDAY MORNING

Time	<b>Tuesday, 12th November 2013</b>		
	<b>Morning Timetable</b>		
8:00	<b>Registration (Boulevard Foyer)</b>		
	<b>Boulevard Auditorium - Opening Ceremony &amp; Plenary Address</b>		
9:00	<b>Welcome to Country : Songwoman Maroochy</b>		
	<i>Chair: Bernie Degnan</i>		
9:15	<b>Official Opening: Robyn Williams AM FAA ABC International Science Broadcaster and Communicator</b>		
9:40	<b>Announcements</b>		
9:45	<b>Plenary Address: William Gerwick (Scripps Institute of Oceanography, USA)</b> <i>Integrating genomics and biosynthesis to discover new classes of bioactive secondary metabolites from marine cyanobacteria</i>		
10:30	<b>Morning Tea</b>		
Room	<b>Boulevard Auditorium</b>	<b>Boulevard 1</b>	<b>Boulevard 2</b>
	<b>Marine Algal Oleomics: New Development of Biofuel Production Research and Technology</b>	<b>Marine Bioresources</b>	<b>Aquaculture Disease and Immunology</b>
	<i>Chairs: Yoshihiro Shiraiwa &amp; Navid Moheimani</i>	<i>Chair: Pamela Chavez-Crooker</i>	<i>Chair: Bernie Degnan</i>
10:50	<b>Keynote: Matsunaga, Tadashi</b> - Japanese project on algal biofuel production	<b>Keynote: Kim, Se Kwon</b> - Investigation and development of bioactive substances from marine organisms	<b>Keynote: Coyne, Vernon</b> - The abalone haemocyte proteome: an indicator of animal health
11:20	<b>Shiraiwa, Yoshihiro</b> - Metabolic pathway of alkenones and alkenes by marine haptophytes and its application to biofuel production	<b>Nazareth, Sarita</b> - Production of compatible solutes by halophilic fungi	<b>Kim, Ji Hyung</b> - Evaluation of protective efficacy of a novel Aeromonas phage PAS-1 against A. salmonicida subsp. salmonicida infections in rainbow trout ( <i>Oncorhynchus mykiss</i> ) model
11:40	<b>Tanaka, Tsuyoshi</b> - Metabolic engineering of marine oleaginous diatom towards biofuel production	<b>Kang, Kyong-Hwa</b> - High-pressure extract of <i>Phaeodactylum tricornutum</i> inhibits HGF-induced proliferation in human gastric cancer SNU-1 and AGS cells	<b>Rungrassamee, Wanilada</b> - Feasibility study of bacterial lipopolysaccharide to increase black tiger shrimp survival under <i>Vibrio harveyi</i> challenge
12:00	<b>Sode, Koji</b> - A synthetic biology approach to develop a novel marine cyanobacterial bioprocesses -The CyanofactoryTM	<b>Inuoe, Akira</b> - Efficient degradation of alginate using alginate lyases from <i>Flavobacterium</i> sp.	<b>Thomas, Ancy</b> - Recombinant viral protein 24 (rVP24) of white spot syndrome virus-a new vaccine candidate in aqua vaccinology against WSSV
12:20	<b>Fukuzawa, Hideya</b> - Genetic engineering of microalgae for photosynthetic biofuel production.	<b>Himaya, S.W.A.</b> - EGFR Tyrosine Kinase Inhibitory Peptide Isolated from Marine Chlamydomonas Sp. Attenuates Helicobacter Pylori-Mediated Carcinogenic Responses	<b>Somboonwiwat, Kunlaya</b> - Study of Protein Interaction between <i>Penaeus monodon</i> Anti-lipopolysaccharide Factor Isoform 3 and White Spot Syndrome Virus
12:40	<b>Lunch</b>		

## TIMETABLE - TUESDAY AFTERNOON

Time	<b>Tuesday, 12th November 2013</b>		
	Afternoon Timetable		
Room	Boulevard Auditorium	Boulevard 1	Boulevard 2
<b>12:40</b>	<b>Lunch</b>		
	<b>Marine Algal Oleomics</b>	<b>Microbial Bioresources</b>	<b>Aquaculture Disease and Immunology</b>
	<i>Chair: Yoshihiro Shiraiwa</i>	<i>Chair: Se-Kwon Kim</i>	<i>Chair: Vernon Coyne</i>
<b>13:30</b>	<i>Keynote: Read, Betsy - The use of <i>Emiliana huxleyi</i> in biofuel applications: Opportunities and challenges</i>	<i>Keynote: Chavez-Crooker, Pamela - Bioleaching of conventional and non conventional materials: new approaches</i>	<i>Keynote: TBA</i>
<b>14:00</b>	<b>Moheimani, Navid</b> - Sustainable conversion of light to chemical and electrical energy	<b>Yamagishi, Ayana</b> - Morphological regulation of cubo-octahedral magnetite crystal by the coordinated action of Mms proteins in magnetotactic bacteria	<b>Saetang, Sureerat</b> - Identification of candidate genes controlling the resistance to Taura syndrome virus in Pacific white shrimp ( <i>Litopenaeus vannamei</i> )
<b>14:20</b>	<b>Olaizola, Miguel</b> - Economies of scale and markets for microalgal products on the way to biofuels	<b>Lu, Jenn-kan</b> - Marine Microbial Bioactive Biosurfactant for Cosmeceutical Industry	<b>Wang, Lingling</b> - The Nervous-Endocrine System Mediates Immune Regulation in Scallops
<b>14:40</b>	<b>Ohi, Nobuaki</b> - Shotgun lipidomic profiling in marine alga <i>Emiliana huxleyi</i> : Identification of intermediates for lipid and very-long-chain alkene biosynthesis	<b>Li, Fuchao</b> - Genome Screening and Biosynthesis of Manumycins-type Compounds from Marine Streptomyces	<b>Li, WeiWei</b> - Three novel C-type lectins from <i>Eriocheir sinensis</i> functions as pattern recognition receptor (PRR)
<b>15:00</b>	<b>Lim, David</b> - Identifying the bottlenecks of microalgal lipid production: a new transcriptional profiling approach	<b>Kwon, Young-Kyung</b> - Gene characterization, cloning and over-expression of the acetyl xylan esterase from <i>Ochrovirga pacifica</i>	<b>Hong, Jiann</b> - RNA Interference (RNAi) Technology Applied on the Blocking of Betanodavirus Replication and Host Cell Death
<b>15:20</b>	<b>Afternoon Tea</b>		
	<b>Marine Algal Oleomics</b>		<b>Aquaculture Biotechnology</b>
	<i>Chair: Navid Moheimani</i>		<i>Chair: Tomer Ventura</i>
<b>15:40</b>	<b>Tsuji, Yoshinori</b> - Photosynthetic Carbon Partitioning into Lipids and Polysaccharides in the coccolithophore <i>E. huxleyi</i>		<b>Kabeya, Naoki</b> - Modification of EPA/DHA biosynthetic pathway by transgenesis in a marine teleost, nibe croaker
<b>16:00</b>	<b>Tanaka, Masayoshi</b> - Functional analysis of a key enzyme in PUFA synthesis, Delta-9 desaturase, identified from the oleaginous diatom <i>Fistulifera</i>		<b>Katayama, Naoto</b> - Germ cell-specific excision of the loxP-flanked transgene in rainbow trout
<b>16:20</b>	<b>Araie, Hiroya</b> - Transcriptome analysis of genes associated with cold-inducible lipid biosynthesis in <i>Emiliana huxleyi</i>		<b>Rane, Gargi</b> - Isolation, culturing of a 'wild strain' of marine microalga and effect of temperature on its growth and lipid content
<b>16:40</b>	<b>Duong, Van Thang</b> - Effects of environmental Conditions on Lipid Accumulation and Diversity of Microalgae at the South East Coast of Queensland – Australia		<b>Thomas-Hall, Skye</b> - Fine-tuning maximum productivity from microalgae in large-scale
<b>17:00</b>	<b>CLOSE OF TUESDAY SESSIONS</b>		
<b>17:30-20:00</b>	<b>POSTER COCKTAIL FUNCTION SESSION (BOULEVARD ROOM FOYER)</b>		

# TIMETABLE - WEDNESDAY (AUDITORIUM AND BOULEVARD 1)

Time	<b>Wednesday, 13th November 2013</b>	
8:00	<b>Auditorium and Boulevard 1 - full day timetable</b>	
	<b>Registration (Boulevard Foyer)</b>	
	<b>Boulevard Auditorium - Plenary Addresses</b>	
8:55	<i>Chair: Russell Hill</i>	
9:00	<b>Introduction and announcements</b>	
9:45	<b>Plenary Address: Ute Hentschel Humeida (University of Wuerzburg, Germany)</b> <i>Microbial diversity, function and biotechnological potential of marine sponges</i>	
10:30	<b>Plenary Address: Amir Sagi (The Negev Ben Gurion University, Israel)</b> <i>Insulin-like androgenic hormones and monosex culture of prawns: The first implementation of RNA-interference in aquaculture</i>	
	<b>Morning Tea</b>	
	<b>Boulevard Auditorium</b>	<b>Boulevard 1</b>
	<b>Algal Fuels &amp; Bioenergy</b>	<b>Microbial Symbionts</b>
	<i>Chair: Susan Blackburn</i>	<i>Chair: Ute Hentschel Humeida</i>
10:50	<b>Keynote: de Nys, Rocky</b> - Renewable fuels from macroalgae: revising the paradigm for algal fuels	<b>Keynote: Zhang, Wei</b> - Novel approach to decipher interactions between marine sponges and their microbial symbionts/pathogens
11:20	<b>Paul, Nicholas</b> - Selection of robust and high productivity marine macroalgae for renewable fuels	<b>Zhukova, Natalia</b> - Exceptional Lipids in Nudibranch Mollusks: Evolution, Diets, Symbionts and Biosynthesis
11:40	<b>Roberts, David</b> - Biochar from marine macroalgae and their waste streams: yields, characteristics and uses	<b>Vincente, Jan</b> - Diversity and functionality of microbial symbionts associated with a two sponge symbioses in the Caribbean
12:00	<b>Powell, Ryan</b> - Merging Metabolism and Power: Development of a Novel Photobioelectric Device Driven by Photosynthesis and Respiration	<b>Zhang, Fan</b> - Characterizing the role of diazotrophs in the symbiotic microbial community associated with two marine sponges
12:20	<b>Packer, Michael</b> - Growing algae and cyanobacteria: photobioreactor technology for product optimisation at the Cawthron Institute	<b>Keren, Ray</b> - Arsenic tolerant sponge-associated bacteria of the Red Sea <i>Theonella swinhoei</i> and their implication for water remediation
12:40	<b>Lunch</b>	
	<b>Algal Fuels &amp; Bioenergy</b>	<b>Microbial Bioresources</b>
	<i>Chair: Rocky de Nys</i>	<i>Chair: Chris Battershill</i>
13:30	<b>Keynote: Kang, Do-Hung</b> - A mini review on algae biofuel in Korea	<b>Keynote: Zhang, Jiquan</b> - An efficient <i>E. coli</i> secretory expression system to produce recombinant chitin-degrading related enzymes
14:00	<b>Blackburn, Susan</b> - Developing Australian Native Microalgae for Algal Biofuels and Bioproducts	<b>Li, Fuli</b> - Untapped Bacterial community Enriched from Coastal Marine Sediment under Anaerobic and Thermophilic Conditions
14:20	<b>Puri, Munish</b> - Exploring Australian marine biodiversity for producing next generation of biofuels	<b>Rajasabapathy, Raju</b> - Bacterial diversity, a comparison between the hydro-thermal vent and the non-vent region of Espalamaca
14:40	<b>Wang, Guangyi</b> - Production biofuels and bioproducts using marine microalgae isolated from the coastal waters of China	<b>Eythorsdottir, Arnheidur</b> - Isolation of antimicrobial marine bacteria from sub-arctic hydrothermal sites
15:00	<b>Jeon, Seon-Mi</b> - Biomass Evaluation of a Novel Green Microalga <i>Chlamydomonas</i> sp. KIOST-1 for Biofuel Production Isolated from Korea	<b>Lee, Youngdeuk</b> - Molecular cloning, overexpression and purification of a novel laminarinase from <i>Mesoflavibacter zeaxanthinifaciens</i> S86
15:20	<b>Afternoon Tea</b>	
	<b>Algal Biotechnology</b>	<b>Microbiotechniques</b>
	<i>Chair: Nick Paul</i>	<i>Chair: Russell Hill</i>
15:40	<b>Suhartono, Maggy Thenawidjaja</b> - Study of Proteins that Catalyze Silica formation and Polyunsaturated Fatty Acid synthesis in Marine Diatom ( <i>Chaetoceros gracilis</i> )	<b>Sonkar, Shailendra Pratap</b> - Metabolic engineering of Polyunsaturated fatty acid biosynthetic pathway in Yeast
16:00	<b>Ye, Bo-Ram</b> - Seasonal variations on organic and inorganic components of <i>Ulva pertusa</i> with environmental factors in Jeju, Korea	<b>Jain, Deepti</b> - Production, purification and characterization of halothermotolerant solvent stable lipase and its application in ester synthesis
16:20	<b>Reddy, Amelia</b> - Characterisation of the effect stress on nitrogen metabolism in the commercially important agarophyte <i>Gracilaria gracilis</i>	<b>Hu, Xiaoke</b> - Bioremediation Of Crude Oil Using Indigenous Marine Bacteria
16:40	<b>Rengasamy, Ragupathirajakannan</b> - Phenolic derivatives from South African kelps: pharmacological screening and in silico approaches towards new functional foods	<b>Delpin, Marina</b> - Seagrass restoration using hessian: silane coating of hessian reduces <i>Escherichia coli</i> attachment and fouling by marine bacteria
17:00	<b>Gupta, Vishal</b> - Development and characterization of interspecific somatic hybrids through protoplast fusion between <i>Ulva fasciata</i> Delie (x) <i>U. reticulata</i> Forskal	
17:20	<b>CLOSE OF SESSIONS</b>	

## TIMETABLE - WEDNESDAY (BOULEVARD 2 AND 3)

Time	<b>Wednesday, 13th November 2013</b>	
8:00	<b>Boulevard 2 and Boulevard 3 - full day timetable</b>	
	<b>Registration (Boulevard Foyer)</b>	
	<b>Boulevard Auditorium - Plenary Addresses</b>	
	<i>Chair: Russell Hill</i>	
8:55	<b>Introduction and announcements</b>	
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9:45	<b>Plenary Address: Amir Sagi (The Negev Ben Gurion University, Israel)</b> <i>Insulin-like androgenic hormones and monosex culture of prawns: The first implementation of RNA-interference in aquaculture</i>	
10:30	<b>Morning Tea</b>	
	<b>Boulevard 2</b>	<b>Boulevard 3</b>
	<b>Reproductive Technologies in Aquaculture</b>	<b>Dinoflagellate &amp; Algal Genomics</b>
	<i>Chair: Shugo Watabe</i>	<i>Chairs: Rose Jagus &amp; Tsetso Bavaroff</i>
10:50	<b>Keynote: Elizur, Abigail</b> - Reproductive technologies in aquaculture	<b>Keynote: Bachvaroff, Tsvetan/Jagus, Rosemary</b> - The amazing dinoflagellates
11:20	<b>Zhang, Quanqi</b> - Female specific markers and attempts of all-female production in half-smooth tongue sole ( <i>Cynoglossus semilaevis</i> )	<b>Rosic, Nedeljka</b> - Dinoflagellates in symbiosis with reef building corals
11:40	<b>Cummins, Scott</b> - Brain stimulants and sex smells: Decoding peptide communication systems in marine molluscs	<b>Bachvaroff, Tsvetan</b> - Dissecting Dinoflagellate Evolution
12:00	<b>Botwright, Natasha</b> - Identification of key signalling peptides involved in abalone sexual maturation	<b>Jagus, Rosemary</b> - Involvement of multiple eIF4Es in mRNA recruitment in dinoflagellates
12:20	<b>Jung, Hyungtaek</b> - Transcriptomic identification of genes affecting growth and reproduction, and SNP association studies with individual growth performance in giant freshwater prawn ( <i>Macrobrachium rosenbergii</i> )	<b>Reith, Michael</b> - Draft genomes of four <i>Chlorella</i> strains
12:40	<b>Lunch</b>	
	<b>Reproductive Technologies in Crustacean Aquaculture</b>	<b>ANZ-China Collaboration Forum</b> (note: times vary from standard schedule)
	<i>Chair: Abigail Elizur</i>	<i>Chair: Wei Zhang</i>
13:30	<b>Keynote: Chung, J. Sook</b> - A female sex hormone is required for developing adult female features of blue crabs	<b>Opening Address: Murphy, Barry</b> -Goals of the ANZ-China Collaboration Forum
14:00	<b>Ventura, Tomer</b> - First steps towards an environmentally friendly monosex population culture of spiny lobsters	<b>13:50 Keynote: Battershill, Chris</b> - New Zealand Marine Bioresources: a new approach to R&D needed fast
14:20	<b>Rotlant, Guiomar</b> - Development of nutrigenomic tools to assess reproductive performance in shrimp	<b>14:10 Keynote: Xiang, Jianhai</b> - Marine Biotechnology enables development of the Blue Bioeconomy
14:40	<b>Leelatanawit, Rungnapa</b> - Study of effects of nutrients on testicular maturation in the black tiger shrimp by sperm performance assessment and cDNA microarray analysis	<b>14:30 Keynote: Quinn, Ron</b> - Australia's Nagoya Approach and Opportunities from Nature Bank (Eskitis Institute)
15:00	<b>Lezer, Yaara</b> - Masculine sex differentiation pathways in the fresh water prawn <i>Macrobrachium rosenbergii</i> , a hinge around the major component, insulin- like androgenic gland hormone (Mr-IAG)	<b>14:55 Evans-Illidge, Elizabeth</b> : Access to Australian mega-diverse marine bioresources in a modern (post-Nagoya Protocol) world
15:20	<b>Afternoon Tea</b>	<b>15:10 Qin, Qiwei</b> - Coastal biotechnology: Transforming changing bioresources to sustainable green industries
	<b>Aquaculture Biotechnology</b>	<b>Afternoon Tea</b>
	<i>Chair: Scott Cummins</i>	<i>Chair: Joe Baker</i>
15:40	<b>Apitanyasai, Kantamas</b> - Role of hemocyte homeostasis associated protein (HHAP) in regulation of hemocyte apoptosis from black tiger shrimp <i>Penaeus monodon</i>	<b>15:50 Olsen, Danette</b> - Marine Extracts: New opportunities for high value export
16:00	<b>Chan, Siu Ming</b> - Potential role of MeNP in shrimp ovary maturation: RNAi silencing resulted in inhibition of vitellogenesis	<b>16:05 Qin, Song</b> - An overview of research and development of marine biotechnology for medicine, aquaculture and industrial materials in China
16:20	<b>Sukumaran, Vrinda</b> - Regulation of glycaemia with the application of recombinant CHH 1 and its polyclonal antiserum in <i>Penaeus monodon</i>	<b>16:20 Slim, George</b> - Biotechnology at the last bus stop: a New Zealand industry perspective
16:40	<b>CLOSE OF SESSION</b>	<b>16:35 Zhang, Wei</b> - Marine Biotechnology Industry Development in Australia: An Ocean of Opportunities for Australian and International Partners
		<b>16:50 Chair: Barry Murphy</b> , Chair Panel-led Discussion
		<b>17:30 Lewis, Rob</b> - Forum summary
		<b>17:40 Zhang, Wei</b> -Closing remarks
		<b>18:00 CLOSE OF SESSION</b>



## TIMETABLE - THURSDAY MORNING

Time	<b>Thursday, 14th November 2013</b>		
	<b>Morning Timetable</b>		
<b>8:00</b>	<b>Registration (Boulevard Foyer)</b>		
	<b>Boulevard Auditorium - Plenary Addresses</b>		
	<i>Chair: Nobuhiro Fusetani</i>		
<b>8:55</b>	Introduction and announcements <b>(including for the 10th Asia-Pacific Marine Biotechnology Conference (2014 APMBC))</b>		
<b>9:00</b>	<b>Plenary Address: Ben Hankamer (The University of Queensland, Australia)</b> <i>Towards High-Efficiency Microalgae Biofuel Systems</i>		
<b>9:45</b>	<b>Plenary Address: Asao Fujiyama (National Institute of Genetics, Japan)</b> <i>Understanding marine lifestyles using new-generation sequencing and genomic technologies: Red alga Pyropia yezoensis and other cases</i>		
<b>10:30</b>	<b>Morning Tea</b>		
	Boulevard Auditorium	Boulevard 1	Boulevard 2
	<b>Marine Natural Products</b>	<b>Genomics</b>	<b>Algal Production &amp; Biotechnology</b>
	<i>Chair: Kirsten Benkendorff</i>	<i>Chair: Ka Hou Chu</i>	<i>Chair: John van der Meer</i>
<b>10:50</b>	<b>Keynote: Prinsep, Michele</b> - Trends in Marine Natural Products Research- A New Zealand Perspective	<b>Keynote: Müller, Werner</b> - Sustainable Oceans – our Treasure in the Past and in the Future: The power of marine genomics	<b>Keynote: Schenk, Peer</b> - Development of low-cost high-efficiency algae energy farms
<b>11:20</b>	<b>Grkovic, Tanja</b> - NMR-guided approaches to natural product-based drug discovery	<b>Adamski, Marcin</b> - Genomes of calcareous sponges: simple body plans and surprisingly complex developmental toolkits	<b>Reith, Michael</b> - The NRC Algal Carbon Conversion Flagship Program – a Canadian approach to sustainable algal biorefineries
<b>11:40</b>	<b>Akanbi, Taiwo</b> - Profiling the phospholipid residues of krill oil by 31P-NMR and regioisomeric distribution of polyunsaturated fatty acids in its triacylglycerol	<b>Degnan, Bernie</b> - Gene regulation in the demosponge Amphimedon queenslandica and insights into the construction of animal body plans	<b>Ward, Andrew</b> - Methane production from saline derived microalgae biomass
<b>12:00</b>	<b>Baharum, Syarul Nataqain</b> - Metabolite Extraction Strategies from Whole Tissue Samples of Tropical Fish Using Gas Chromatography Mass Spectrometry Metabolomics	<b>Bannister, Stephanie</b> - Efficient precision genome engineering in the marine polychaete Platynereis dumerilii using transcriptional activator like effector nucleases (TALENs)	<b>Oh, Chulhong</b> - Bioethanol production from cyanobacteria biomass
<b>12:20</b>	<b>Bahrami, Yadollah</b> - Isolation and structure elucidation of novel saponins from the sea cucumber <i>Stichopus hermanni</i> viscera using HPCPC and mass spectrometry	<b>Li, Yingrui</b> - View from oyster genome: The opportunity and challenge for aquatic organism genome study	<b>Kim, Taeho</b> - The Feasibility of pilot production of <i>Spirulina (Arthrospira)</i> maxima cultivated newly constructed raceway pond in Republic of Korea
<b>12:40</b>	<b>Lunch</b>		

## TIMETABLE - THURSDAY AFTERNOON

Time	<b>Thursday, 14th November 2013</b>		
	Afternoon Timetable		
<b>12:40</b>	Lunch		
	Boulevard Auditorium	Boulevard 1	Boulevard 2
	Marine Bioresources	Genomics	Algal Production & Biotechnology
	Chair: Michele Prinsep	Chair: Bernie Degnan	Chair: Peer Schenk
<b>13:30</b>	<b>Keynote: Benkendorff</b> , Kirsten - <i>Dicathais orbita</i> as a model for marine natural product screening and nutraceutical development	<b>Keynote: Chu</b> , Ka Hou - Genome Sequencing of the Shrimp <i>Neocaridina denticulata</i> : Insights into Crustacean Aquaculture and Arthropod Evolution	<b>Keynote: Qin</b> , Song: Algal Biotechnology: reshaping the coasts
<b>14:00</b>	<b>Rudd</b> , David - Unravelling muricid secondary metabolite biosynthesis, <i>in situ</i> , using surface assisted mass spectrometry imaging	<b>Mao</b> , Yunxiang - Global transcriptome profiling of <i>Pyropia yezoensis</i> in response to temperature stresses	<b>Long</b> , Lijuan - Simple lipid extraction method without heating from wet microalga <i>Picochlorum sp.</i>
<b>14:20</b>	<b>Prentis</b> , Peter - Identifying the digestive enzyme repertoire of a herbivorous intertidal snail	<b>Aguilera</b> , Felipe - The evolutionary origin of molluscan shell matrix genes: comparative analysis of ten molluscan mantle transcriptomes	<b>Powell</b> , Ryan - Rapid Harvest of Microalgae using a Novel Bacterial Isolate
<b>14:40</b>	<b>Davis</b> , Jeanette - Characterization of the bacterial community of Hawaiian sea slug <i>Elysia rufescens</i> .	<b>Wang</b> , Wei - De novo Transcriptome Sequencing of the Heat-stressed Snail <i>Echinolittorina malaccana</i>	<b>Li</b> , Yan - Microalgae nutrient efficacy as aquafeed additives: a booster of aquaculture sustainable development
<b>15:00</b>	<b>Sairi</b> , Fareed - Rhogocyte cell isolation and characterization from mollusc's tissue: The answer to hemocyanin biosynthesis bottleneck	<b>Hu</b> , Tsung-Han - The analyses of de novo milkfish transcriptome assembly in response to salinity and temperature changes	<b>Xiang</b> , Wenzhou - Optimization of medium using response surface methodology for the lipid production by <i>Scenedesmus sp.</i>
<b>15:20</b>	Afternoon Tea		
	Marine Bioresources		Algal Production & Biotechnology
	Chair: Carmel McDougall		Chair: Song Qin
<b>15:40</b>	<b>Annuar</b> , Nurul Izzati - Anti-atherosclerotic activity of marine sponge <i>Xestospongia sp.</i>		<b>Nalder</b> , Tim - Investigation of lipids and lipases from the microalgae <i>I. galbana</i> and <i>P. lutheri</i>
<b>16:00</b>	<b>Watson</b> , Jabin - Analysis of the biomass composition of the demosponge <i>Amphimedon queenslandica</i> reveals marked variation within and between individuals		<b>Ahmed</b> , Faruq - Microalgae as a source of phyosterols
<b>16:20</b>	<b>Mori</b> , Tetsushi - Defining the producers of marine natural compounds in marine sponges using the single-cell analytical approach		<b>Heo</b> , Soo-Jin - Anti-inflammatory and Anti-tumor activity of a carotenoid isolated from brown algae through MAPKs regulation
<b>16:40</b>	<b>Ismail</b> , Noraznawati - Potential Compounds from Marine <i>Xestospongia sp.</i> , <i>Chicoreus sp.</i> and <i>Acanthaster planci</i> as Peroxisome Proliferator Activated Receptor Gamma (PPAR ?) Ligand for Anti-Atherosclerotic Activity		<b>Adame-Vega</b> , Catalina - Photosynthetic micro-algae: a sustainable source of omega-3 fatty acids from for nutraceuticals and aquaculture feed
<b>17:00</b>	CLOSE OF SESSION		
<b>18:30 - 11:59</b>	CONFERENCE DINNER (BOULEVARD ROOM)		

## TIMETABLE - FRIDAY

Time	<b>Friday 15th November 2013</b>		
<b>8:00</b>	<b>Registration (Boulevard Foyer)</b>		
	<b>Boulevard Auditorium - Plenary Addresses</b>		
	<i>Chair: Joe Baker</i>		
<b>8:55</b>	<b>Introduction and announcements</b>		
<b>9:00</b>	<b>Plenary Address: Ron Quinn (Griffith University, Australia)</b> <i>The Future for, and the challenges of, commercializing Marine Bioactives</i>		
<b>9:45</b>	<b>Plenary Address: Anchalee Tassanakajon (Chulalongkorn University, Thailand)</b> <i>How does the immune system of shrimps fight against invading pathogens</i>		
<b>10:30</b>	<b>Morning Tea</b>		
	<b>Boulevard Auditorium</b>	<b>Boulevard 1</b>	<b>Boulevard 2</b>
	<b>Genomics</b>	<b>Bioactive Marine Resources</b>	<b>Nutraceuticals &amp; Functional Foods</b>
	<i>Chair: Werner Müller</i>	<i>Co-Chairs:</i> <b>Libby Evans-Illidge and Raymond Tham</b>	<i>Co-Chairs: Madhavi Indap and Joe Baker</i>
<b>10:50</b>	<b>McDougall</b> , Carmel - It pays to be tough: the prevalence of proteins with repetitive, low complexity domains in marine biomaterials	<b>Keynote: Evans-Illidge</b> , Elizabeth - The Nagoya Protocol – a new legally binding international regime for access to biodiversity and benefit sharing. Can it solve the uncertainty?	<b>Keynote: Nichols</b> , Peter - A Journey from Marine Genes to New Sustainable Land Plant Sources of Long-chain Omega-3 Oils
<b>11:20</b>	<b>Ali</b> , Muhammad Yousef - Transcriptome analysis of freshwater crayfish ( <i>Cherax quadricarinatus</i> ) and characterisation of gill-expressed carbonic anhydrase genes	<b>Tham</b> , Raymond - Mapping and matching hotspots of biodiversity, biochemical and bioactivity diversity for advanced Marine Park policy in South Australia	<b>Barrow</b> , Colin (presenting Dobson, Polly) - Controlled formation of mono- and dihydroxy-resolvins and protectin analogues from omega-3 DHA and EPA using soybean 15-lipoxygenase
<b>11:40</b>	<b>Xiang</b> , Jianhai -RNA-Seq reveals the dynamic features of transcriptome during early development in pacific white shrimp <i>Litopenaeus vannamei</i>	<b>Rosic</b> , Nedeljka - Coral algae as a source of UV-absorbing compounds	<b>Barrow</b> , Colin - Omega-3 biotechnology: Sources, concentration methods, microencapsulation and bioactive derivatives
<b>12:00</b>	<b>Othman</b> , Roohaida - Identification of <i>Eucheuma denticulatum</i> and <i>Kappaphycus alvarezii</i> genes	<b>Wang</b> , Xiaohong - The deep-sea natural products, biogenic polyphosphate (Bio-PolyP) and biogenic silica (Bio-Silica), as biomimetic scaffolds for bone tissue engineering: fabrication of a morphogenetically-active polymer	<b>Ohara</b> , Katsuyoshi - Fucoxanthin Production from Microalgae
<b>12:20</b>	<b>Qin</b> (presenting Huang <i>et al</i> ), Qiwei - The transcriptome analysis of grouper, <i>Epinephelus coioides</i> in response to Singapore grouper iridovirus (SGIV) infection	<b>Yamashita</b> , Michiaki - Se-containing antioxidant "selenoneine" in tuna blood and its roles in selenium redox metabolism and methylmercury detoxification	<b>Hori</b> , Kanji - The lectins from the genus <i>Codium</i>
<b>12:40</b>	<b>Pavasovic</b> , Ana - <i>Anadara trapezia</i> functional genomics	<b>Ageenko</b> , Natalya - Pigment Cell Differentiation in Blastula-derived primary Cell Cultures of Sea Urchins	
<b>13:00</b>	<b>Boulevard Auditorium - Close of Conference - IMBC 2013 Chairs</b>		
<b>13:15</b>	<b>CLOSE OF CONFERENCE</b>		

# Australia, New Zealand and China

## 2013 Collaboration Forum on Marine Biotechnology

An Australia - New Zealand Marine Biotechnology Network event

Wednesday 13<sup>th</sup> November **Room B3** Boulevard Level, Brisbane Convention and Exhibition Centre

### Forum OPENING

1.30

#### Mr Barry Murphy



Barry Murphy is currently Chairman of the Board of Advisers for the Centre for Bioproducts Development at Flinders University. He is a chemical engineer, having spent 30 years with Shell and Caltex in the downstream refining and marketing parts of the oil industry in Australia, America and South-East Asia. In 1991, this culminated in his appointment as Chairman and Chief Executive of the publicly-listed Caltex Australia Limited plus other Caltex Group companies in Australia.

During his career, he has filled a number of other senior roles in the public and private business sectors in Australia, including as Chairman of the Australian Quality Council, President of the American Chamber of Commerce in Australia, Chairman of the Customs Advisory Board, a Trustee of the Royal Botanic Gardens and Domain Sydney, a Trustee of the Art Gallery of NSW Foundation, and Chairman of the Australian Rail Track Corporation Limited.

Mr Murphy is a Fellow of the Institution of Chemical Engineers, a Chartered Scientist of the UK Science Council, and a Foundation Fellow of the Australian Institute of Company Directors. He is an ex-President and Honorary Life Member of the University of Queensland Union.

1.40

**Prof. Wei Zhang**, Director Centre for Marine Bioproducts, Development, Flinders University, Aust.

**Forum background, structure and objectives**

### KEYNOTE PRESENTATIONS Chair: Wei Zhang

1.50

**Prof. Chris Battershill** Chair, Coastal Science, Environmental Research Institute, University of Waikato, NZ.

***New Zealand Marine Biotechnology: a successful past should now inform a successful future***

2.10

**Prof. Jianhai Xiang** Past Director, Qingdao Institute of Oceanology, Chinese Academy of Sciences.

***Marine Biotechnology enables development of the Blue Bioeconomy***

2.30

**Prof. Ron Quinn** Director, Eskitis Institute, Griffith University, Aust.

***Australia's Nagoya Approach and Opportunities from Nature Bank (Eskitis Institute)***

### INVITED PRESENTATIONS Chair: Joe Baker

2.55

**Ms Libby Evans-Illidge** Manager, AIMS Bioresources Library, Australian Institute of Marine Science.

***Access to Australian diverse marine bioresources in a modern (post-Nagoya Protocol) world***

3.10

**Prof. Song Qin** Vice-Director, Yantai Coastal Zone Research Institute, Chinese Academy of Sciences.

***Coastal biotechnology: Transforming changing bioresources to sustainable green industries***

3.25

#### TEA BREAK

3.50

**Ms Danette Olsen** General Manager Science – Seafood, The New Zealand Institute for Plant & Food Research Limited.

***Marine Extracts – New opportunities for high value export products from NZ***

4.05

**Prof. Qiwei Qin** Director of CAS Key Laboratory of Marine Bioresource Sustainable Utilisation, South China Sea Institute of Oceanology, Chinese Academy of Sciences.

***An overview of research and development of marine biotechnology for medicine, aquaculture and industrial materials in China***

4.20

**Dr George Slim** CEO, NZBIO, Wellington, NZ.

***Biotechnology at the last bus stop: a New Zealand industry perspective***

4.35

**Prof. Wei Zhang** Director, Centre for Marine Bioproducts, Development, Flinders University, Aust.

***Marine Biotechnology Industry Development in Australia: An Ocean of Opportunities for Australian and International Partners***

# ANZ-CHINA COLLABORATION FORUM ON MARINE BIOTECHNOLOGY

## PANEL-LED DISCUSSION, SUMMING UP and CLOSE Chair: Barry Murphy

4.50	<b>Invited panellists</b> (listed below) <b>Panel Chair: Barry Murphy</b>	<b>Panel Discussion</b> Questions and suggestions from the floor, towards forming a 'White paper' report, and suggestions for a collaborative platform including working groups to take this forward.
5.30	<b>Prof. Rob Lewis</b>	<b>Summing up</b> and what happens next. Formation of working group.
5.50	<b>Prof. Wei Zhang</b>	<b>Thanks and close</b>

### Chairs

**Prof. Rob Lewis** Director Science Without Bounds Pty Ltd. Adelaide, Aust.

**Prof. Joe Baker** Co-chair IMBC conference. Member, Board of Advisors, Flinders University Centre for Marine Bioproducts Development, Adelaide, Aust.

**Mr Barry Murphy** Chair, Board of Advisors, Flinders University Centre for Marine Bioproducts Development, Adelaide, Aust.

### Panellists

**Prof. Ben Hankamer** Institute for Molecular Bioscience, Uni Queensland, Aust.

**Dr John Hooper** Head, Biodiversity and Geosciences Programs, Queensland Museum, Aust.

**Prof. Bin Liu** Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China

**Dr Doug Mountfort** Cawthron Institute, NZ.

**Prof. Rocky de Nys** School of Marine and Tropical Biology, James Cook Uni, Aust.

**Dr Michele Prinsep** Senior lecturer, Organic Chemistry, Waikato University, NZ.

**Prof. Qun Wang** School of Life Sciences, East China Normal University, Shanghai, China

## PURPOSE

The purpose of this Forum is to examine the marine biological resources, R&D capacity, industry development and government blue economy policy in Australia, New Zealand and China, in order to identify and develop collaboration and partnership opportunities among the three countries.

The expected outcomes of this Forum are:

- (1) A short 'white paper' report for the governments of China, Australia and New Zealand on the drivers for marine biotechnology and recommendations for government policy support and a partnership model;
- (2) A collaboration and communication platform between the A-NZ Marine Biotechnology Network and Chinese Marine Biotechnology Society and communities;
- (3) Collaborations between individual researchers, institutions and industries.

### This Forum has been organised by

Prof. Wei Zhang, Mr Raymond Tham and Ms Shirley Sorokin, CMBD, Flinders University, Adelaide.

Prof. Song Qin, Vice-Director, Yantai Coastal Zone Research Institute, Chinese Academy of Sciences.

Prof. Chris Battershill, Chair, Coastal Science, Environmental Research Institute, University of Waikato, NZ.

In collaboration with Prof. Joe Baker and Prof. Bernie Degnan, co-chairs of IMBC.

With thanks to the IMBA Board.

Organising committee contact: Shirley Sorokin [marine.bioproducts@flinders.edu.au](mailto:marine.bioproducts@flinders.edu.au)

[Australia – New Zealand Marine Biotechnology Network](#)

## POSTERS (DISPLAY ORDER BY POSTER NUMBER & TOPIC)

Presenter LN	Presenter FN	All Authors	Title of Presentation	Poster Number & Topic
<b>Chang</b>	Jo-Shu	Chen, Chun-Yen <sup>1</sup> , Po-Jen Lee <sup>2</sup> , and Jo-Shu Chang <sup>1,3,4*</sup>	Engineering strategies for improving protein production from microalgal <i>Chlorella vulgaris</i> FSP-E using novel photobioreactor illuminated with cold cathode fluorescent lamps	<b>1</b> (Algal Biotech)
<b>Chen</b>	Chun-Yen	Chen, Chun-Yen <sup>1*</sup> , Hsin-Yueh Chang <sup>1</sup> , Chun-Hua Huang <sup>1</sup> and Jo-Shu Chang <sup>1,2,3</sup>	Characterization of photosynthetic carbon dioxide fixation ability of indigenous <i>Chlorella pyrenoidosa</i> CY10 isolate	<b>2</b> (Algal Biotech)
<b>Chen</b>	Chun-Yen	Chen, Chun-Yen <sup>1*</sup> , Pei-Chun Kao <sup>2</sup> and Jo-Shu Chang <sup>1,2,3</sup>	Technology development of CO <sub>2</sub> fixation, C-phycocyanin production and purification with <i>Spirulina platensis</i>	<b>3</b> (Algal Biotech)
<b>Chen</b>	Chun-Yen	Chen, Chun-Yen <sup>1*</sup> , Yu-Chun Chen <sup>2</sup> , Chen-Chun Liu <sup>2</sup> , Hsiao-Chen Huang <sup>2</sup> and Jo-Shu Chang <sup>1,2,3</sup>	Enhancing the production of EPA from <i>Nannochloropsis oceanica</i> CY2 by using LED photobioreactor	<b>4</b> (Algal Biotech)
<b>Chen</b>	Chun-Yen	Chen, Chun-Yen <sup>a*</sup> , Yu-Mei Chen <sup>a</sup> , and Jo-Shu Chang <sup>a,b,c</sup>	Enhancing EPA production from <i>Nannochloropsis oceanica</i> using deep-sea water supplemented cultivation medium	<b>5</b> (Algal Biotech)
<b>Chen</b>	Chun-Yen	Chen, Chun-Yen <sup>a*</sup> , Yu-Mei Shen <sup>a</sup> , Hsin-Yueh Chang <sup>a</sup> , and Jo-Shu Chang <sup>a,b,c</sup>	Characterization of photosynthetic carbon dioxide fixation ability of indigenous <i>Chlorella pyrenoidosa</i> NNK-A isolate	<b>6</b> (Algal Biotech)
<b>Chen</b>	Chun-Yen	Chen, Chun-Yen <sup>a</sup> , Po-Cheng Lin <sup>a</sup> , and Jo-Shu Chang <sup>a,b,c*</sup>	UV mutagenesis of an isolated green microalga <i>Chlamydomonas orbicularis</i> for enhanced lipid production	<b>7</b> (Algal Biotech)
<b>Cheon</b>	Jihyeon	Bo-kyung, Kim, Jihyeon Cheon*, Myeongjeong Jeon, SeoYeon Kim, Mira Park and Mihyang Kim	The Effects of <i>Ishige okamurae</i> on Collagen Synthesis of Osteoblastic MC3T3-E1	<b>8</b> (Algal Biotech)
<b>Chun</b>	Byung-Soo	Meillisa, Aviannie <sup>1</sup> , Yin Shipeng <sup>1</sup> , Hee-Chul Woo <sup>2</sup> and Byung-Soo Chun <sup>*1</sup>	Characterization of Bio-prospecting Compounds of Brown Seaweed <i>Sargassum horneri</i> by Liquefied Pressurized System	<b>9</b> (Algal Biotech)
<b>Chun</b>	Byung-Soo	Siahaan, Evi Amelia <sup>1</sup> , Yin Shipeng <sup>1</sup> , Hee-Chul Woo <sup>2</sup> , Byung-Soo Chun <sup>*1</sup>	Measurements of antioxidant activities in edible brown seaweeds extracts Obtained from supercritical CO <sub>2</sub> and solvent extraction	<b>10</b> (Algal Biotech)
<b>Gao</b>	Zhengquang	Gao, Zhengquan <sup>1,2*</sup> , David KY Lim <sup>1</sup> , Peer M Schenk <sup>1</sup>	Laser-induced mutation and selection leads to improved <i>Tetraselmis</i> sp. microalgae as a hopeful candidate for biodiesel production	<b>11</b> (Algal Biotech)
<b>Heo</b>	Soo-Jin	Heo, Soo-Jin <sup>*1</sup> , Bo-Ram Ye <sup>1</sup> , Jiyi Jang <sup>1</sup> , Min-Sun Kim <sup>1</sup> , Junseong Kim <sup>2</sup> , Won-Kyo Jung <sup>3</sup> , Chulhong Oh <sup>1</sup> , Do-Hyung Kang <sup>1</sup>	Osteoclastogenic effect of marine algae in human osteoblast-like MG-63 cells	<b>12</b> (Algal Biotech)
<b>Heo</b>	Soo-Jin	Heo, Soo-Jin <sup>*1</sup> , Bo-Ram Ye <sup>1</sup> , Jiyi Jang <sup>1</sup> , Min-Sun Kim <sup>1</sup> , Junseong Kim <sup>2</sup> , Won-Kyo Jung <sup>3</sup> , Chulhong Oh <sup>1</sup> , Do-Hyung Kang <sup>1</sup>	Evaluation of anti-inflammatory activity of marine algae in LPS-stimulated RAW 264.7 cells	<b>13</b> (Algal Biotech)
<b>Jeon</b>	Myeongjeong	Bo-kyung, Kim, Myeongjeong Jeon*, Seoyeon Kim, Jihyeon Cheon and Mihyang Kim	Effects of <i>Ishige okamurae</i> Extract on Serum Lipid Content of Ovariectomized Rats	<b>14</b> (Algal Biotech)
<b>Jeon</b>	Myeongjeong	Jeon, Myeongjeong <sup>*1</sup> , Seo-yeon Kim <sup>1</sup> , Ji Hyeon Cheon <sup>1</sup> , Seong-Hwan Park <sup>2</sup> , Sang-Hyeon Lee <sup>2</sup> and Mihyang Kim <sup>1</sup>	The Effects of Seaweed Gongjindan on Estrogen Like Activities, Platelet Aggregation and Serum Lipid Levels in Ovariectomized Rats	<b>15</b> (Algal Biotech)

## POSTERS (DISPLAY ORDER BY POSTER NUMBER & TOPIC)

<b>Jeon</b>	Seon-Mi	Jeon, Seon-Mi*, Ji Hyung Kim, Areumi Park, Taeho Kim, Su-Jin Lee, Chulhong Oh, Soo-Jin Heo and Do-Hyung Kang	A novel coccoid-shaped cyanobacterium, <i>Myxosarcina</i> sp. KIOST-1 isolated from Mangrove Forest in Chuuk State, Federated States of Micronesia	<b>16</b> (Algal Biotech)
<b>Kanda</b>	Hideki	Kanda, Hideki <sup>1,2</sup> , Yuichi Kamo <sup>1</sup> , Shuhei Shintani <sup>1</sup> , Siti Machmudah <sup>1,3</sup> , Wahyudiono <sup>1</sup> and Motonobu Goto <sup>1</sup>	Extraction of carotenoids from raw macroalgae excluding drying and cell wall disruption by liquefied dimethyl ether	<b>17</b> (Algal Biotech)
<b>Kawano</b>	Yumi	Kawano, Yumi <sup>1</sup> , Tomoyuki Suenaga <sup>1</sup> , Yousuke Taoka <sup>1</sup> , Motonari Sibakami <sup>2</sup> and Masahiro Hayashi <sup>1</sup>	Mixotrophic cultivation of <i>Euglena gracilis</i> using waste from food industry	<b>18</b> (Algal Biotech)
<b>Kim</b>	Ji Hyung	Kim, Ji Hyung*, Seon-Mi Jeon, Taeho Kim, Su-Jin Lee, Areumi Park, Soo-Jin Heo, Chulhong Oh, Do-Hyung Kang	Comparative analysis of biochemical components in <i>Spirulina maxima</i> Cy-23 and the newly isolated <i>Leptolyngbya</i> sp. KIOST-1	<b>19</b> (Algal Biotech)
<b>Kim</b>	Seoyeon	Kim, Seoyeon*, Myeong-Jeong Jeon, Jihyeon Cheon, Mira Park, Changsuk Kong and Mihyang Kim	The Effect of <i>Scytosiphon lomentaria</i> on Differentiation of Osteoblastic MC3T3-E1 Cells	<b>20</b> (Algal Biotech)
<b>Kim</b>	Seoyeon	Kim, Seoyeon*, Myeong-Jeong Jeon, Jihyeon Cheon, Mira Park, Changsuk Kong and Mihyang Kim	Effects of <i>Eisenia bicyclis</i> Fractions on Osteoblast Differentiation and Osteoclast Formation	<b>21</b> (Algal Biotech)
<b>Kong</b>	Chang-Suk	Kim, Jung-Ae <sup>1</sup> and Chang-Suk Kong <sup>*2</sup>	Preventive Effect of Marine algae on Bone Loss in C2C12 Myoblasts	<b>22</b> (Algal Biotech)
<b>Kong</b>	Chang-Suk	Kim, Jung-Ae <sup>1</sup> and Chang-Suk Kong <sup>*2</sup>	Osteogenic and anti-adipogenic activities of <i>Salicornia herbacea</i>	<b>23</b> (Algal Biotech)
<b>Kong</b>	Chang-Suk	Kim, Jung-Ae <sup>1</sup> , Mihyang Kim <sup>2</sup> , Sang-Hyeon Lee <sup>3</sup> and Chang-Suk Kong <sup>*2</sup>	Protective Effects of <i>Ecklonia cava</i> on Osteoporosis and Adipogenesis in Mesenchymal Cells	<b>24</b> (Algal Biotech)
<b>Kong</b>	Chang-Suk	Kim, Jung-Ae <sup>1</sup> , Mihyang Kim <sup>2</sup> , Sang-Hyeon Lee <sup>3</sup> and Chang-Suk Kong <sup>*2</sup>	Inhibitory Effects of <i>Sargassum thinbergii</i> on Adipogenic Differentiation in Mouse Mesenchymal Cells	<b>25</b> (Algal Biotech)
<b>Lee</b>	Sang Man	Lee, Sang-Man <sup>*1</sup> , Soon-Sun Bak <sup>2</sup> , Ratih Pangestuti <sup>2</sup> , Byul-Nim Ahn <sup>2</sup> , Sun-Ju Park <sup>1</sup> , Jin Eun Lee <sup>3</sup> , Jung Chul Kim <sup>3</sup> , Moon Kyu Kim <sup>3</sup> , Young Kwan Sung <sup>3</sup> and Se-Kwon Kim <sup>1,2</sup>	The hair growth promoting effects of <i>Eucheuma cottonii</i>	<b>26</b> (Algal Biotech)
<b>Matsuda</b>	Ryuya	Matsuda, Ryuya <sup>*1</sup> , Katsuaki Takechi <sup>1</sup> , Hiroyoshi Takano <sup>1</sup> and Susumu Takio <sup>1,2</sup>	Sporophyte-specific expression of bromoperoxidase gene in a red alga, <i>Pyropia yezoensis</i>	<b>27</b> (Algal Biotech)
<b>Meng</b>	Chunxiao	Meng, Chunxiao <sup>1,2*</sup> , Zhengquan Gao <sup>1,2</sup> , Ahmed Faruq <sup>1</sup> , Yan Li <sup>3</sup> , Peer M Schenk <sup>1</sup>	The role of astaxanthin biosynthesis genes in <i>Haematococcus pluvialis</i> during carotenoid induction by salinity and nutrient starvation stress	<b>28</b> (Algal Biotech)
<b>Okado</b>	Yu	Okado, Yu <sup>1*</sup> , Yousuke Taoka <sup>1</sup> , Mayumi Ueda <sup>2</sup> , Daiske Honda <sup>3</sup> and Masahiro Hayashi <sup>1</sup>	Catalase production and H <sub>2</sub> O <sub>2</sub> tolerance of <i>Aurantiochytrium limacinum</i> strain mh0186	<b>29</b> (Algal Biotech)
<b>Osada</b>	Kyoko	Osada, Kyoko <sup>*1</sup> , Masahito Hosokawa <sup>1</sup> , Tomoko Yoshino <sup>1</sup> and Tsuyoshi Tanaka <sup>1,2</sup>	Time-lapse analysis of oleaginous diatom <i>Fistulifera</i> sp. strain JPCC DA0580 during the triglyceride accumulation using single-cell patterning	<b>30</b> (Algal Biotech)
<b>Qin</b>	Song	Cui, Hongli <sup>1</sup> , Xiaona Yu <sup>2</sup> , Yan Wang <sup>1</sup> , Yulin Cui <sup>1</sup> and Song Qin <sup>*1</sup>	Molecular cloning, characterization and expression analysis of a glucokinase gene from the mixotrophic green alga <i>Chlorella kessleri</i>	<b>31</b> (Algal Biotech)

## POSTERS (DISPLAY ORDER BY POSTER NUMBER & TOPIC)

<b>Suenaga</b>	Tomoyuki	Suenaga, Tomoyuki* <sup>1</sup> , Yumi Kawano <sup>1</sup> , Yousuke Taoka <sup>1</sup> , Motonari Sibakami <sup>2</sup> and Masahiro Havashi <sup>1</sup>	Paramylon production by fed-batch cultivation of <i>Euglena gracilis</i> using waste in food industry	<b>32</b> (Algal Biotech)
<b>Takahashi</b>	Chisato	Takahashi, Chisato* <sup>1</sup> , Masaki Muto <sup>1,2</sup> , Masayoshi Tanaka <sup>1,2</sup> , Mitsufumi Matsumoto <sup>2,3</sup> , Tomoko Yoshino <sup>1</sup> and Tsuyoshi Tanaka <sup>1,2</sup>	Modification of fatty acid composition by gene silencing of $\Delta 9$ desaturase in oleaginous diatom <i>Fistulifera</i> sp. strain JPCC DA0580	<b>33</b> (Algal Biotech)
<b>Takahashi</b>	Mami	Takahashi, Mami <sup>1,2</sup> , Tetsushi Mori <sup>1,2</sup> , Naoko Midorikawa <sup>1,2</sup> , Toshiyuki Shibata <sup>2,3</sup> , Kouichi Kuroda <sup>2,4</sup> , Seinen Chow <sup>5</sup> , Mitsuyoshi Ueda <sup>2,4</sup> , Haruko Takayama <sup>1,2</sup>	Screening for exolytic alginate lyase genes of bacteria isolated from marine environmental samples	<b>34</b> (Algal Biotech)
<b>Tanbirul haque</b>	A S M	Tanbirul haque, ASM and Byung-Soo Chun*	Micronization of fucoxanthin from <i>Laminaria japonica</i> with biodegradable polymer-associated particles from gas saturated solution process	<b>35</b> (Algal Biotech)
<b>Tonon</b>	Angela	Tonon, P Angela, Helena Vilella, Pio Colepicolo	Strategy to improve the cellular synthesis of lipids in two <u>microalgas</u>	<b>36</b> (Algal Biotech)
<b>Xu</b>	Nianjun	Sun, Xue, Weiwei Wang, Nianjun Xu*	The transcriptome sequencing and carbonic anhydrase analyses of marine microalga <i>Chlorella pyrenoidosa</i> (Chlorophyta)	<b>37</b> (Algal Biotech)
<b>Xu</b>	Nianjun	Xu, Nianjun*, Xue Sun, Xili Cai	Physiological response of marine red algae <i>Gracilaria lemaneiformis</i> to different salinities stress	<b>38</b> (Algal Biotech)
<b>Ye</b>	Bo-Ram	Ye, Bo-Ram <sup>1,2,*</sup> , Jiyi Jang <sup>1</sup> , Young-Kyung Kwon <sup>1,2</sup> , Taeho Kim <sup>1</sup> , Seon-Mi Jeon <sup>1</sup> , Do-Hyung Kang <sup>1</sup> , Chulhong Oh <sup>1</sup> , Soo-Jin Heo <sup>1</sup>	Potential antioxidant capacities of ethanol and enzymatic extracts of <i>Pylaiella littoralis</i> collected from Federated States of Micronesia	<b>39</b> (Algal Biotech)
<b>Ye</b>	Bo-Ram	Ye, Bo-Ram <sup>1,2,*</sup> , Jiyi Jang <sup>1</sup> , Young-Kyung Kwon <sup>1,2</sup> , Ji Hyung Kim <sup>1</sup> , Youngdeuk Lee <sup>1</sup> , Su-Jin Lee <sup>1</sup> , Do-Hyung Kang <sup>1</sup> , Chulhong Oh <sup>1</sup> , Soo-Jin Heo <sup>1</sup>	Anti-proliferative effect of <i>Pylaiella littoralis</i> extract on HT29 cells	<b>40</b> (Algal Biotech)
<b>Apitanyasai</b>	Kantamas	Apitanyasai, Kantamas* <sup>1</sup> , Walaiporn Charoensapsri <sup>1</sup> , Piti Amparyup <sup>1,2</sup> and Anchalee Tassanakajon <sup>1</sup>	Role of hemocyte homeostasis associated protein (HHAP) in regulation of hemocyte apoptosis from black tiger shrimp <i>Penaeus monodon</i>	<b>41</b> (Aqua Biotech)
<b>Asaduzzaman</b>	A K M	Asaduzzaman, A K M and Byung-Soo Chun*	Antihypertensive effect and antioxidant activities in mackerel muscle hydrolyzate recovered by subcritical water	<b>42</b> (Aqua Biotech)
<b>Chen</b>	Wei-Jung	Chen, Wei-Jung*, Wei-Chen Tsai and Tsun-Yung Kuo	Evaluation of therapeutic efficacy of antimicrobial peptides against marine pathogens using in vitro and in vivo infection models	<b>43</b> (Aqua Biotech)
<b>Chen</b>	Tzong-Yueh	Hsu, Hao-Hsuan <sup>1,2,3</sup> and Tzong-Yueh Chen* <sup>1,2,3,4</sup>	Marker-assistant Selection and Breeding through Phylogenetic Relationships in Taiwan Giant Grouper ( <i>Epinephelus lanceolatus</i> ) by Using Microsatellite and Mitochondria Markers	<b>44</b> (Aqua Biotech)
<b>Chiang</b>	Cheng-yi	Chiang, Cheng-yi*, Yi-lin Chen and Huai-jen Tsai	Different visible colors were obtained from the mutated purple chromoprotein isolated from sea anemone	<b>45</b> (Aqua Biotech)



## POSTERS (DISPLAY ORDER BY POSTER NUMBER & TOPIC)

<b>Chou</b>	Hsin-Yiu	Chou, Hsin-Yiu <sup>*1,2</sup> , Ming-Horng Wu <sup>1</sup> and Jiann-Horng Leu <sup>1,2</sup>	Transcriptomic study and different gene expression profiling of two kinds of grouper iridoviruses infection in orange-spotted grouper ( <i>Epinephelus coioides</i> )	<b>46</b> (Aqua Biotech)
<b>Chung</b>	Chia-Ling	Chung Chia-Ling <sup>*1</sup> , Fang-Huar Ngou <sup>1</sup> , Sook-Ping Tan <sup>1</sup> , Hsiang-Ping Kuo <sup>1</sup> , Zwe-Ling Kong <sup>1</sup> , Hong-Ting Lin <sup>1</sup> , Yu-Shen Lai <sup>2</sup> , Ming-Wei Wu <sup>1</sup>	The Overexpression and Bioactivity Assay of Fish Type I Interferons on Grouper Cell Line	<b>47</b> (Aqua Biotech)
<b>Chung</b>	J. Sook	Chung, J. Sook <sup>*1</sup> , Sirinart Techa <sup>1</sup> , Karrie Bulski <sup>2</sup> , Anna N. Walker <sup>3</sup> and Richard, F Lee <sup>2</sup>	Effects of dispersed and emulsified oil on molting, ecdysone and EcR/RXR complex in the grass shrimp and the blue crab	<b>48</b> (Aqua Biotech)
<b>Chunhua</b>	Zhu	Li, Yu liao, Luan Luan Chen, Zhu Chunhua*	Effects of Tributyltin on the activities of immunologic enzyme in blood serum of the <i>Macrobrachium rosenbergill</i>	<b>49</b> (Aqua Biotech)
<b>Gong</b>	Hong-Yi	Gong, Hong-Yi <sup>*1</sup> , Tse-Yu Tai <sup>1</sup> , Fcng-You Lin <sup>2</sup> , Hsin-Yiu Chou <sup>1</sup> , Chang-Wen Huang <sup>1</sup> , Shinn-Lih Yeh <sup>2</sup> and Jen-Leih Wu <sup>1,3</sup>	Development of Type I microsatellite markers from transcriptome of giant grouper for marker-assisted selection	<b>50</b> (Aqua Biotech)
<b>Hidaka</b>	Kazuaki	Hidaka, Kazuaki*, Yousuke Taoka and Masahiro Hayashi	Applicability of aquaculture effluents to production of docosahexaenoic acid by oleaginous microbe, <i>Aurantiochytrium limacinum</i> strain mh0186	<b>51</b> (Aqua Biotech)
<b>Hollander</b>	Lian	Hollander-Cohen, Lian*, Joseph Aizen, Levavi-Sivan Berta	Characterization of recombinant gonadotropins activity and their receptors in the Common Carp	<b>52</b> (Aqua Biotech)
<b>Hu</b>	Yau-Chung	Hu, Yau-Chung*, Yan-Shuo Liu and Tsung-Han Lee	Multi-chloride channels from two clades of the CIC members involve in chloride absorption of tilapia gills	<b>53</b> (Aqua Biotech)
<b>Huang</b>	Shin-Jie	Huang, Shin-Jie <sup>*1</sup> , Mary-nia M. Santos <sup>2</sup> , Chih-Lun Cheng <sup>3</sup> , Jen-Leih Wu <sup>1,2,3</sup>	Specific transcriptional response and cold tolerance ability in PUFA zebrafish	<b>54</b> (Aqua Biotech)
<b>Jagus</b>	Rosemary	Gillespie, Kate, Erica Dasi, Rosemary Jagus*	The roles of eIF4E family members in zebrafish ( <i>Danio rerio</i> )	<b>55</b> (Aqua Biotech)
<b>Jagus</b>	Rosemary	Liu, Chieh Lun <sup>1</sup> , Erica Dasi <sup>1</sup> , Shau-Chi Chi <sup>2</sup> , Yu-Hsuan Kai <sup>2</sup> , Allen R. Place <sup>1</sup> , & Rosemary Jagus <sup>1*</sup>	eIF2 expression and phosphorylation in response to nutritional status and stressors in fish	<b>56</b> (Aqua Biotech)
<b>Ken</b>	Chui-an-Fu	Ken, Chui-an-Fu* and Chih-Chui-an Pan	Overexpress glutathione reductase to prevent thioacetamide induce oxidative stress in zebrafish	<b>57</b> (Aqua Biotech)
<b>Leethochavalit</b>	Supanee	Leethochavalit, Supanee*, Janjarus Watanachote and Nareerat Rittirut	<b><i>Perkinsus atlanticus</i> Infestation in undulated surf clam, <i>Paphia undulata</i>, along the east coast of Thailand</b>	<b>58</b> (Aqua Biotech)
<b>Liu</b>	Lei	Liu, Lei <sup>1</sup> , Zhencheng Wei <sup>1</sup> , Xueming Liu <sup>1</sup> and Lixin Huang <sup>*2</sup>	Characterisation of water-soluble collagen from tilapia skin	<b>59</b> (Aqua Biotech)
<b>Liu</b>	Wangta	Liu, Wangta <sup>*1</sup> , Yen-Chun Chen <sup>1</sup> , Chi-Hsueh Lin <sup>1</sup> , Shin-Jie Huang <sup>1</sup> , Hong-Yi Gong <sup>2</sup> and Jen-Leih Wu <sup>1</sup> .	The role of foxm1 in the initiation mechanism of intrahepatic cholangiocarcinogenesis in zebrafish	<b>60</b> (Aqua Biotech)
<b>Lu</b>	Ming-Wei	Lu Ming-Wei <sup>*1</sup> , Jen-Leih Wu <sup>2</sup> , Yu-Chen Lin <sup>1</sup> , Chia-Ling Chung <sup>1</sup> , Ya-Ting Juhn <sup>1</sup>	Application of RNA vaccine in grouper nervous necrosis virus	<b>61</b> (Aqua Biotech)
<b>Mo</b>	Zhaolan	Mo, Zhaolan <sup>*1</sup> , Guiyang Li <sup>1</sup> , Lin Huang <sup>2,4</sup> , Jie Li <sup>1</sup> , Bin Hao <sup>3</sup>	Japanese flounder ( <i>Paralichthys olivaceus</i> ) spleen transcriptome and expression profile involved in immunity during <i>Vibrio anguillarum</i> infection	<b>62</b> (Aqua Biotech)

## POSTERS (DISPLAY ORDER BY POSTER NUMBER & TOPIC)

<b>Powell</b>	Daniel	Powell, Daniel* <sup>1</sup> , Abigail Elizur <sup>1</sup> Trevor Anderson <sup>1</sup> , Courtney Remilton <sup>2</sup> and Wayne Knibb <sup>1,3</sup>	Transcriptome characterisation and gene discovery in the marine shrimp <i>Fenneropenaeus</i> <i>merguiensis</i>	<b>63</b> (Aqua Biotech)
<b>Shen</b>	Kang-Ning	Shen, Kang-Ning* <sup>1,2</sup> , Chih-Wei Chang <sup>3,4</sup> , Jean-Dominique Durand <sup>5</sup>	Species identification and reproductive characteristic of the three <i>Mugil cephalus</i> cryptic species in Taiwan	<b>64</b> (Aqua Biotech)
<b>Watana- chote</b>	Janjarus	Watanachote, Janjarus*, Supanee Leethochavalit and Nareerat Rittirut	Comparison of immune parameters in cultured oyster ( <i>Saccostrea</i> sp.) along the eastern coast of Thailand	<b>65</b> (Aqua Biotech)
<b>Wei</b>	Jingguang	Wei, Jingguang <sup>1</sup> , Minglan Guo <sup>1</sup> , Huasong Ji <sup>2</sup> , Qiwei Qin* <sup>1</sup>	Molecular cloning, characterization of one key molecule of teleost innate immunity from orange-spotted grouper ( <i>Epinephelus coioides</i> ): serum amyloid A	<b>66</b> (Aqua Biotech)
<b>Yang</b>	Shi Ping	Yang, Shi-Ping* <sup>1,2</sup> , Hui-Ling Liu <sup>1</sup> , Cheng-Gui Wang <sup>3</sup> , Ping Yang <sup>1</sup> , Cheng-Bo Sun <sup>1</sup> , Siu-Ming Chan <sup>1</sup>	Effect of oxidized fish oil on growth performance and oxidative stress of <i>Litopenaeus vannamei</i>	<b>67</b> (Aqua Biotech)
<b>Zhang</b>	Zhuhuai	Zhang, Zhuhuai* <sup>1</sup>	Artificial breeding technology of <i>Bohadschia argus</i> made great progress in China	<b>68</b> (Aqua Biotech)
<b>Agrawal</b>	Sweta	Agrawal, Sweta*, Madhavi Indap	Anti-angiogenic potential of a marine gastropod <i>Euchelus asper</i>	<b>69</b> (Biores & Biotech)
<b>Ahn</b>	Byul-Nim	Ahn, Byul-Nim* <sup>1</sup> , Yong-Xin Li <sup>1</sup> and Se-Kwon Kim <sup>1,2</sup>	Neoechinulin A isolated from marine-derived <i>Microsporium</i> sp. suppresses sebum accumulation in insulin-like growth factor (IGF)- 1 differentiated human sebocytes	<b>70</b> (Biores & Biotech)
<b>Bose</b>	Utpal	Bose, Utpal* <sup>1</sup> , Mark P. Hodson <sup>2</sup> , P. Nicholas Shaw <sup>1</sup> , John A. Fuerst <sup>3</sup> and Amitha K. Hewavitharana <sup>1</sup>	Intraspecific variability in secondary metabolites of the Great Barrier Reef sponge- associated marine bacteria "Salinispora pacifica"	<b>71</b> (Biores & Biotech)
<b>Chaugule</b>	Sachin	Balakrishnan, Babita <sup>1</sup> , Sachin Chaugule* <sup>1</sup> , Madhavi Indap <sup>1</sup> and Shubhada Chiplunkar <sup>2</sup>	Effect of Bioactive Compounds from Marine Sponge <i>Tethya</i> spp. on Bone resorption	<b>72</b> (Biores & Biotech)
<b>Chen</b>	Jyh-Yih	Chen, Jyh-Yih* <sup>1</sup> , Tsui-Chin Huang <sup>1</sup>	Proteomic Analysis Reveals That Pardaxin Triggers Apoptotic Signaling Pathways in Human Cervical Carcinoma HeLa Cells: Crosstalk among the UPR, c-Jun, and ROS	<b>73</b> (Biores & Biotech)
<b>Chieh-yu</b>	Pan	Chieh-Yu, Pan <sup>1*</sup> , Jian-Chyi Chen <sup>2</sup> , Jenn-Feng Sheen <sup>3</sup> , Tai-Lang Lin <sup>4</sup> , Jyh-Yih Chen <sup>1</sup>	Epinecidin-1 has immunomodulatory effects, facilitating its therapeutic use in a mouse model of <i>Pseudomonas</i> <i>aeruginosa</i> sepsis	<b>74</b> (Biores & Biotech)
<b>Chiou</b>	Pinwen	Chuang, Hsiang-Chieh, Fang-Yao Lee, Nai-Yu Chen and Pinwen P. Chiou*	Structural characteristic and immuno-regulatory function of class-A CpG oligodeoxynucleotide in grouper	<b>75</b> (Biores & Biotech)
<b>Choi</b>	Yoo Seong	Han, Yohan, Hyerin Kim and Yoo Seong Choi*	Calcium Carbonate Crystallization using marine-derived recombinant glycine-rich Proteins	<b>76</b> (Biores & Biotech)
<b>Dehasakul- watana</b>	Chutiwan	Dechasakulwatana, Chutiwan* and Sumaitt Putchakarn	Antioxidant activity of extracts from sponge-associated bacteria collected from Tao Island, Gulf of Thailand	<b>77</b> (Biores & Biotech)

## POSTERS (DISPLAY ORDER BY POSTER NUMBER & TOPIC)

<b>Eom</b>	So Hyeon	Xiao, Bin <sup>1,2</sup> , Min Hi Park <sup>1</sup> , Mingzhi Su <sup>1</sup> , So Hyeon Eom <sup>1*</sup> , Jongki Hong <sup>3</sup> , Hae Young Chung <sup>1</sup> , Jun Yin <sup>2</sup> , Jee H. Jung <sup>1</sup>	Design of Phthalimide Derivatives Based on Paecilocin A as PPAR- $\gamma$ Activators	<b>78</b> (Biores & Biotech)
<b>Evans-Illidge</b>	Elizabeth	Evans-Illidge, Elizabeth A <sup>1,*</sup> , Murray Logan <sup>1</sup> , Jason Doyle <sup>1</sup> , Jane Fromont <sup>2</sup> , Christopher N Battershill <sup>1,3</sup> , Gavin Ericson <sup>1</sup> , Carsten W Wolff <sup>1,4</sup> , Andrew Muirhead <sup>1</sup> , Philip Kearns <sup>1</sup> , David Abdo <sup>1,5</sup> , Stuart Kininmonth <sup>1,6</sup> , and Lyndon Llewellyn <sup>1</sup>	Phylogeny drives large scale patterns in Australian marine bioactivity - a chemical ecology rationale for future biodiscovery	<b>79</b> (Biores & Biotech)
<b>Fukaya</b>	Kazuto	Fukaya, Kazuto <sup>*1</sup> , Mayumi Ueda <sup>2</sup> , Naoki Nagano <sup>1</sup> , Daiske Honda <sup>3</sup> , Masahiro Hayashi <sup>1</sup> , and Yousuke Taoka <sup>1</sup>	Evaluation of the ligninolytic activity by the marine eukaryotes, Thraustochytrids which using the Remazol Brilliant Blue R as indicator	<b>80</b> (Biores & Biotech)
<b>Han</b>	Bingnan	Han, Bingnan <sup>*1</sup> , Xiuwen Tang <sup>2</sup> , Zachary Kemmerer <sup>3</sup> , Aimee L. Egger <sup>3</sup> , and Hou-Wen Lin <sup>1</sup>	Discovery of marine natural products targeting Keap1-Nrf2-ARE signalling pathway and mechanism study	<b>81</b> (Biores & Biotech)
<b>Indap</b>	Madhavi	Pathak, Rupa, Madhavi Indap <sup>*</sup> , Sweta Agrawal, Gargi Rane	Insecticidal activity of marine organism <i>Scomber</i> spp. against <i>Tribolium castaneum</i>	<b>82</b> (Biores & Biotech)
<b>Jung</b>	Jee Hyung	Li, Huayue <sup>1</sup> , Mingzhi Su <sup>1</sup> , Eun La Kim <sup>1</sup> , John J. Bowling <sup>2</sup> , Jongki Hong <sup>3</sup> , Mark T. Hamann <sup>2</sup> , Jee Hyung Jung <sup>1*</sup>	New cystine knot peptides of a marine sponge, and its potential as a scaffold for oral peptide drug delivery	<b>83</b> (Biores & Biotech)
<b>Kim</b>	Eun La	Kim, Eun La <sup>1*</sup> , Haibo Wang <sup>1</sup> , Jongki Hong <sup>2</sup> , Jee Hyung Jung <sup>1</sup>	Bioactive Metabolites from a Jellyfish-derived Fungus <i>Phoma</i> sp.	<b>84</b> (Biores & Biotech)
<b>Kim</b>	Young-Sang	Kim, Young-Sang <sup>1*</sup> , Yong-Xin Li <sup>2</sup> , Kyong-Hwa Kang <sup>2</sup> , Sun-Ju Park <sup>1,2</sup> , Se-Kwon Kim <sup>1,2</sup>	Bis(methylthio)gliotoxin isolated from marine fungus <i>Aspergillus fumigatus</i> inhibits HGF-induced cell proliferation in human gastric epithelial AGS cells	<b>85</b> (Biores & Biotech)
<b>McCauley</b>	Janice	McCauley, Janice <sup>*1</sup> , Pia Winberg <sup>2</sup> and Danielle Skropeta <sup>1,3</sup>	Anti-inflammatory and anticancer potential of Australian marine macroalgae; role in gut health as dietary therapeutics	<b>86</b> (Biores & Biotech)
<b>Sakaguchi</b>	Toshifumi	Sakaguchi, Toshifumi <sup>*1</sup> , Yusuke Nochida <sup>1</sup> , Nobuyasu Tanabe <sup>1</sup> , Katsuya Doi <sup>1</sup> , Kaoru Nakasone <sup>2</sup> , Chiaki Kato <sup>3</sup>	Elemental selenium and tellurium formation by <i>Shewanella</i> microbes newly isolated from deep sea sediments in Japan Trench	<b>87</b> (Biores & Biotech)
<b>Schwartz</b>	Inbar	Schwartz, Inbar <sup>1*</sup> , Shmuel Carmeli <sup>2</sup> and Micha Ilan <sup>1</sup>	Antimicrobial activity of the coral reef sponge <i>Crella cyathophora</i>	<b>88</b> (Biores & Biotech)
<b>Siranonthana</b>	Nisa	Siranonthana, Nisa <sup>*</sup> , Rawiwan Watanadilok and Somrat Taweedet	Antimicrobial activity of total lipids extracted from Thai marine sponges	<b>89</b> (Biores & Biotech)
<b>Takagi</b>	Ryosuke	Takagi, Ryosuke <sup>*</sup> , Akiko Takami and Tomoyuki Miyashita	The <i>Pinctada fucata</i> BMP-2 induced the osteogenic differentiation of C3H10T1/2 murine mesenchymal stem cells	<b>90</b> (Biores & Biotech)
<b>Takahashi</b>	Koji	Takahashi, Koji <sup>*</sup> , Keiichi Kushibe, Kazumi Sato, Yasushi Hasegawa	Isolation and identification of glycoproteins inhibiting adipocyte differentiation from scallop shells	<b>91</b> (Biores & Biotech)
<b>Wang</b>	Tianfang	Wang, Tianfang <sup>*1,2</sup> , Stewart Michael <sup>1</sup> , Hammond Michael <sup>1,2</sup> , Bernie Degnan <sup>2</sup> and Cummins Scott <sup>1</sup>	Composition and antimicrobial activity of partial peptidome of the Great Barrier Reef sponge <i>Amphimedon queenslandica</i>	<b>92</b> (Biores & Biotech)

## POSTERS (DISPLAY ORDER BY POSTER NUMBER & TOPIC)

<b>Muñoz-Márquez</b>	Maria Enriqueta	Muñoz-Márquez, María Enriqueta* <sup>1</sup> , Elizabeth Ponce-Rivas <sup>2</sup> and Ashraf A. Khan <sup>3</sup>	Class I integrons in multiresistant <i>Escherichia coli</i> isolates from poultry litter	<b>93</b> (Food Safety)
<b>Rotllant</b>	Guiomar	Rotllant, Guiomar* <sup>1</sup> , Enric Gisbert <sup>2</sup> , Guillermo Guerao <sup>2</sup> , Marc Uya <sup>3</sup> and Luis Cardona <sup>3</sup>	Stable isotopes as a tool for identifying <i>Maja</i> commercial species origin to guarantee its market traceability	<b>94</b> (Food Safety)
<b>Uchida</b>	Junya	Uchida, Junya*, Sho Sakita, Daisuke Ichikawa, Shihori Takanashi, Tomoyo Narita, Koko Abe, Shiro Itoi and Haruo Sugita	Isolation of lactic acid bacteria from the intestinal tract of bivalves	<b>95</b> (Food Safety)
<b>Chiang</b>	Keng-Yu	Chiang, Keng-Yu* <sup>1,2</sup> , Yen-Hsing Li <sup>1</sup> , Ya-Wen Li <sup>1</sup> , Jen-Lieh Wu <sup>1</sup>	Progranulin is required for liver regeneration in the partial hepatectomized zebrafish	<b>96</b> (General)
<b>Hipolito</b>	Sheryll	Hipolito, Sheryll*, Hidehiro Kondo and Ikuo Hirono	Role of <i>Marsupenaeus japonicus</i> crustin-like peptide against <i>Vibrio penaeicida</i> and white spot syndrome virus infection	<b>97</b> (General)
<b>Itoi</b>	Shiro	Itoi, Shiro*, Junya Uchida, Kento Ishizuka, Narumi Takimoto, Ryoko Mitsuoka, Shihori Takanashi, Shunsuke Noguchi, Keita Ishikawa, Takamasa Okamura, Keitaro Komori, Motoki Tamai, Susumu Domeki, and Haruo Sugita	A possible process of tetrodotoxin accumulation in marine pufferfish of the genus <i>Takifugu</i>	<b>98</b> (General)
<b>Li</b>	Ya-Wen	Li, Ya-Wen* <sup>1,2</sup> , Yen-Hsing Li <sup>2</sup> , Wangta Liu <sup>2</sup> , Keng-Yu Chiang <sup>2</sup> , Jen-Leih Wu <sup>2</sup>	Study of the microRNA 145 mediated regulatory mechanism for liver development in zebrafish	<b>99</b> (General)
<b>Wu</b>	Sung-Yu	Wu, Sung-Yu*, Wangta Liu, Jen-Leih Wu	Transdifferentiation of duct-like cells from hepatocyte through progenitor cells in zebrafish model of Intrahepatic cholangiocarcinoma	<b>100</b> (General)
<b>Amin</b>	Shorash	Amin, Shorash* <sup>1</sup> , Peter J. Prentis <sup>2</sup> , Edward K. Gilding <sup>3</sup> , Christopher Collet <sup>1</sup> and Ana Pavasovic <sup>1</sup>	Identifying genes involved in physiological adaptation of <i>Merita melanotragus</i> to temperature stress using comparative transcriptome sequencing	<b>101</b> (Genomics)
<b>Antunes</b>	Agostinho	Antunes, Agostinho <sup>1,2*</sup>	Next-generation genomics for bioproducts discovery	<b>102</b> (Genomics)
<b>Benkendorff</b>	Kirsten	Baten, Abdul <sup>1</sup> , Ajit Ngangbam <sup>2</sup> , Kirsten Benkendorff* <sup>2</sup>	Preliminary genome sequencing, denovo assembly and annotation of the Neogastropoda <i>Dicathais orbita</i>	<b>103</b> (Genomics)
<b>Kim</b>	Sang-Jin	Patra, Ajit Kumar <sup>1,2</sup> , Yoshihiro Fujiwara <sup>3</sup> and Sang-Jin Kim* <sup>1,2</sup>	Energy metabolic relationship of <i>Lamellibrachia satsuma</i> with its endosymbiont revealed by metagenomic analysis	<b>104</b> (Genomics)
<b>Koyama</b>	Hiroki	Koyama, Hiroki* <sup>1</sup> , Sanit Piyapattanakorn <sup>2</sup> and Shugo Watabe <sup>1,3</sup>	Expression and tissue distribution of skeletal myosin heavy chain genes from adult and larvae of shrimps	<b>105</b> (Genomics)
<b>Ochiai</b>	Yoshihiro	Ochiai, Yoshihiro* <sup>1</sup> , Hideo Ozawa <sup>2</sup>	Strategies of acclimation to deep sea: Structure simulation of myoglobin molecules from aquatic animals under high pressure	<b>106</b> (Genomics)
<b>Tsubouchi</b>	Taishi	Tsubouchi, Taishi*, Shinro Nishi, Keiko Usui, Yasuhiro Shimane, Tadashi Maruyama and Yuji Hatada	Draft genome sequence of the dimorphic prosthecate bacterium <i>Brevundimonas abyssalis</i> , isolated from deep-subsea floor sediment	<b>107</b> (Genomics)

## **POSTERS (DISPLAY ORDER BY POSTER NUMBER & TOPIC)**

<b>Dewapriya</b>	Pradeep	Dewapriya, Pradeep <sup>1,*</sup> and Se-Kwon Kim <sup>1,2</sup>	Isolation and Characterization of Marine-Derived <i>Mucor</i> sp. for Fermentative Production of Tyrosol	<b>108</b> (Microbiotech)
<b>Gupta</b>	Adarsha	Gupta, Adarsha*, Colin J. Barrow, Munish Puri	Screening of marine microorganisms: Thraustochytrids from Victorian environment for advancing omega-3 biotechnology	<b>109</b> (Microbiotech)
<b>Handayani</b>	Midia	Handayani, Midia* <sup>1</sup> , Hiroyuki Sasaki <sup>1</sup> , Ryuya Matsuda <sup>1</sup> , Katsuaki Takechi <sup>1</sup> , Hiroyoshi Takano <sup>1</sup> , Susumu Takio <sup>1,2</sup>	Characterization of an epiphytic bacterium <i>Neptunomonas</i> sp. BPy-1 on a red alga <i>Pyropia yezoensis</i>	<b>110</b> (Microbiotech)
<b>Indap</b>	Madhavi	Phatak, Rupa, Shashank More, Madhavi Indap*	Anti-microbial potential of crude extracts of marine sponge- <i>Tethya</i> spp. and edible fish- <i>Scomber</i> spp.	<b>111</b> (Microbiotech)
<b>Kim</b>	Mihyang	Lee, Dong-Geun <sup>1</sup> , Seong-Hwan Park <sup>1</sup> , Myong Je Jeon <sup>1</sup> , Mihyang Kim <sup>2*</sup> , Chang-Suk Kong <sup>2</sup> and Sang-Hyeon Lee <sup>1</sup>	Recombinant production and characterization of a thermophilic arylsulfatase from the marine bacterium <i>Thermotoga maritima</i>	<b>112</b> (Microbiotech)
<b>Kuwahara</b>	Hitomi	Kuwahara, Hitomi* <sup>1</sup> , Junko Ninomiya <sup>1</sup> , Yosuke Tabel <sup>2</sup> , Hiroshi Morita <sup>2</sup>	Luminescence behaviour of marine luminous bacteria under nutrient-saved conditions	<b>113</b> (Microbiotech)
<b>Lavy</b>	Adi	Lavy, Adi*, Keren Ray, Haber Markus, Schwartz Inbar, Ilan Micha	Anaerobic conditions and poor nutrient media reveal antibacterial properties of sponge-associated bacteria	<b>114</b> (Microbiotech)
<b>Li</b>	Zhiyong	Su, Jing, Fengli Zhang, Wei Sun, Zhiyong Li*, Qun Jiang*	A novel alkaline lipase obtained from the metagenome of marine sponge <i>Ircinia</i> sp.	<b>115</b> (Microbiotech)
<b>Lipton</b>	Anuj Nishanth	Lipton, Anuj Nishanth*	Purification, characterization and optimisation of metalloprotease from <i>Pseudomonas poae</i> PGPR2	<b>116</b> (Microbiotech)
<b>Ngnangbam</b>	Ajit	Ngangbam, Ajit*, Steve Whalan, Kirsten Benkendorff	Bacterial communities associated with biosynthetic organs of the marine mollusc <i>Dicathais orbita</i>	<b>117</b> (Microbiotech)
<b>Park</b>	Seong-Hwan	Park, Seong-Hwan <sup>1</sup> , Dong-Geun Lee <sup>1</sup> , Mihyang Kim <sup>2*</sup> , Chang -Suk Kong <sup>2</sup> , and Sang-Hyeon Lee <sup>1</sup>	Effects on cancer cell growth of saponin and fucoidan treated with thermophilic xylose isomerase from the marine bacterium <i>Thermotoga maritima</i>	<b>118</b> (Microbiotech)
<b>Park</b>	Si Jae	Park, Si Jae* <sup>1</sup> , Seung Hwan Lee <sup>2</sup> , Young Hoon Oh <sup>2</sup> and Jeong Geol Na <sup>3</sup>	Biosynthesis of polyhydroxyalkanoate in recombinant microorganisms using carbon source derived from terrestrial and marine biomass	<b>119</b> (Microbiotech)
<b>Srivibool</b>	Rattanaporn	Srivibool, Rattanaporn* <sup>1</sup> , Rawiwan Watanadilok <sup>1</sup> and Subuntith Nimrat <sup>2</sup>	Anti-MRSA and antioxidant activities of actinomycetes isolated from marine sponges	<b>120</b> (Microbiotech)
<b>Tareq</b>	Fakir Shahidullah	Tareq, Fakir Shahidullah <sup>1,*</sup> , Ji-Hyi <sup>2</sup> , Min-Ah <sup>2</sup> , Hyi-Seung <sup>2</sup> , Jong-Seok <sup>2</sup> , Yeon-Ju <sup>2</sup> , Hee-Jae Shin <sup>1,2</sup>	Novel Lipopeptides, Kiostostatins A-E from a Marine-Derived Bacterium <i>Bacillus subtilis</i>	<b>121</b> (Microbiotech)

The following pages list the posters in two columns by Presenter order, giving part of the title for ease of identification, with the Poster Number and topic.

## POSTER NUMBERS (PRESENTER ALPHA ORDER)

Presenter LN	[ref] Title of Presentation	Poster Number & Topic
<b>Agrawal</b>	Anti-angiogenic potential of a marine	<b>69</b> (Biores & Biotech)
<b>Ahn</b>	Neoechinulin A isolated from marine-	<b>70</b> (Biores & Biotech)
<b>Amin</b>	Identifying genes involved in	<b>101</b> (Genomics)
<b>Antunes</b>	Next-generation genomics for	<b>102</b> (Genomics)
<b>Apitanyasai</b>	Role of hemocyte homeostasis	<b>41</b> (Aqua Biotech)
<b>Asaduzza-man</b>	Antihypertensive effect and antioxidant	<b>42</b> (Aqua Biotech)
<b>Benkendorff</b>	Preliminary genome sequencing, denovo	<b>103</b> (Genomics)
<b>Bose</b>	Intraspecific variability in	<b>71</b> (Biores & Biotech)
<b>Chang</b>	Engineering strategies for	<b>1</b> (Algal Biotech)
<b>Chaugule</b>	Effect of Bioactive Compounds from	<b>72</b> (Biores & Biotech)
<b>Chen</b>	Characterization of photosynthetic	<b>2</b> (Algal Biotech)
<b>Chen</b>	Technology development of CO2	<b>3</b> (Algal Biotech)
<b>Chen</b>	Enhancing the production of EPA	<b>4</b> (Algal Biotech)
<b>Chen</b>	Enhancing EPA production from	<b>5</b> (Algal Biotech)
<b>Chen</b>	Characterization of photosynthetic	<b>6</b> (Algal Biotech)
<b>Chen</b>	UV mutagenesis of an isolated green	<b>7</b> (Algal Biotech)
<b>Chen</b>	Evaluation of therapeutic efficacy	<b>43</b> (Aqua Biotech)
<b>Chen</b>	Marker-assistant Selection and	<b>44</b> (Aqua Biotech)
<b>Chen</b>	Proteomic Analysis Reveals That	<b>73</b> (Biores & Biotech)
<b>Cheon</b>	The Effects of Ishige okamurae on	<b>8</b> (Algal Biotech)
<b>Chiang</b>	Different visible colors were obtained	<b>45</b> (Aqua Biotech)
<b>Chiang</b>	Progranulin is required for liver	<b>96</b> (General)
<b>Chieh-yu</b>	Epinecidin-1 has immunomodulatory	<b>74</b> (Biores & Biotech)
<b>Chiou</b>	Structural characteristic and	<b>75</b> (Biores & Biotech)
<b>Choi</b>	Calcium Carbonate Crystallization using	<b>76</b> (Biores & Biotech)
<b>Chou</b>	Transcriptomic study and different gene	<b>46</b> (Aqua Biotech)
<b>Chun</b>	Characterization of Bio-prospecting	<b>9</b> (Algal Biotech)
<b>Chun</b>	Measurements of antioxidant activities	<b>10</b> (Algal Biotech)
<b>Chung</b>	The Overexpression and Bioactivity Assay	<b>47</b> (Aqua Biotech)
<b>Chung</b>	Effects of dispersed and emulsified oil on	<b>48</b> (Aqua Biotech)

<b>Chunhua</b>	Effects of Tributyltin on the activities of	<b>49</b> (Aqua Biotech)
<b>Dehsakul-watana</b>	Antioxidant activity of extracts from sponge-	<b>77</b> (Biores & Biotech)
<b>Dewapriya</b>	Isolation and Characterization of	<b>108</b> (Microbiotech)
<b>Eom</b>	Design of Phthalimide Derivatives Based on	<b>78</b> (Biores & Biotech)
<b>Evans-Illidge</b>	Phylogeny drives large scale patterns	<b>79</b> (Biores & Biotech)
<b>Fukaya</b>	Evaluation of the lignolytic activity by	<b>80</b> (Biores & Biotech)
<b>Gao</b>	Laser-induced mutation and	<b>11</b> (Algal Biotech)
<b>Gong</b>	Development of Type I microsatellite	<b>50</b> (Aqua Biotech)
<b>Gupta</b>	Screening of marine microorganisms:	<b>109</b> (Microbiotech)
<b>Han</b>	Discovery of marine natural products	<b>81</b> (Biores & Biotech)
<b>Handayani</b>	Characterization of an epiphytic bacterium	<b>110</b> (Microbiotech)
<b>Heo</b>	Osteoclastogenic effect of marine algae	<b>12</b> (Algal Biotech)
<b>Heo</b>	Evaluation of anti-inflammatory activity	<b>13</b> (Algal Biotech)
<b>Hidaka</b>	Applicability of aquaculture effluents	<b>51</b> (Aqua Biotech)
<b>Hipolito</b>	Role of Marsupenaeus japonicus crustin-like	<b>97</b> (General)
<b>Hollander</b>	Characterization of recombinant	<b>52</b> (Aqua Biotech)
<b>Hu</b>	Multi-chloride channels from two	<b>53</b> (Aqua Biotech)
<b>Huang</b>	Specific transcriptional	<b>54</b> (Aqua Biotech)
<b>Indap</b>	Insecticidal activity of marine organism	<b>82</b> (Biores & Biotech)
<b>Indap</b>	Anti-microbial potential of crude	<b>111</b> (Microbiotech)
<b>Itoi</b>	A possible process of tetrodotoxin	<b>98</b> (General)
<b>Jagus</b>	The roles of eIF4E family members in	<b>55</b> (Aqua Biotech)
<b>Jagus</b>	eIF2 expression and phosphorylation in	<b>56</b> (Aqua Biotech)
<b>Jeon</b>	Effects of Ishige okamurae Extract on	<b>14</b> (Algal Biotech)
<b>Jeon</b>	The Effects of Seaweed Gongjindan	<b>15</b> (Algal Biotech)
<b>Jeon</b>	A novel coccoid-shaped	<b>16</b> (Algal Biotech)
<b>Jung</b>	New cystine knot peptides of a marine	<b>83</b> (Biores & Biotech)
<b>Kanda</b>	Extraction of carotenoids from raw	<b>17</b> (Algal Biotech)
<b>Kawano</b>	Mixotrophic cultivation of Euglena	<b>18</b> (Algal Biotech)
<b>Ken</b>	Overexpress glutathione reductase	<b>57</b> (Aqua Biotech)
<b>Kim</b>	Comparative analysis of biochemical	<b>19</b> (Algal Biotech)

## POSTER NUMBERS (PRESENTER ALPHA ORDER)

<b>Kim</b>	The Effect of Scytosiphon	<b>20</b> (Algal Biotech)	<b>Park</b>	Biosynthesis of polyhydroxyalkanoate	<b>119</b> (Microbiotech)
<b>Kim</b>	Effects of Eisenia bicyclis Fractions on	<b>21</b> (Algal Biotech)	<b>Powell</b>	Transcriptome characterisation and	<b>63</b> (Aqua Biotech)
<b>Kim</b>	Bioactive Metabolites from a Jellyfish-	<b>84</b> (Biores & Biotech)	<b>Qin</b>	Molecular cloning, characterization and	<b>31</b> (Algal Biotech)
<b>Kim</b>	Bis(methylthio)gliotoxin isolated from	<b>85</b> (Biores & Biotech)	<b>Rotllant</b>	Stable isotopes as a tool for identifying	<b>94</b> (Food Safety)
<b>Kim</b>	Energy metabolic relationship of	<b>104</b> (Genomics)	<b>Sakaguchi</b>	Elemental selenium and tellurium	<b>87</b> (Biores & Biotech)
<b>Kim</b>	Recombinant production and	<b>112</b> (Microbiotech)	<b>Schwartz</b>	Antimicrobial activity of the coral reef	<b>88</b> (Biores & Biotech)
<b>Kong</b>	Preventive Effect of Marine algae on Bone	<b>22</b> (Algal Biotech)	<b>Shen</b>	Species identification and reproductive	<b>64</b> (Aqua Biotech)
<b>Kong</b>	Osteogenic and anti-adipogenic activities	<b>23</b> (Algal Biotech)	<b>Siranonthana</b>	Antimicrobial activity of total lipids	<b>89</b> (Biores & Biotech)
<b>Kong</b>	Protective Effects of Ecklonia cava on	<b>24</b> (Algal Biotech)	<b>Srivibool</b>	Anti-MRSA and antioxidant activities	<b>120</b> (Microbiotech)
<b>Kong</b>	Inhibitory Effects of Sargassum thinbergii	<b>25</b> (Algal Biotech)	<b>Suenaga</b>	Paramylon production by fed-batch	<b>32</b> (Algal Biotech)
<b>Koyama</b>	Expression and tissue distribution of	<b>105</b> (Genomics)	<b>Takagi</b>	The Pinctada fucata BMP-2 induced the	<b>90</b> (Biores & Biotech)
<b>Kuwahara</b>	Luminescence behaviour of marine	<b>113</b> (Microbiotech)	<b>Takahashi</b>	Modification of fatty acid composition by	<b>33</b> (Algal Biotech)
<b>Lavy</b>	Anaerobic conditions and poor nutrient	<b>114</b> (Microbiotech)	<b>Takahashi</b>	Screening for exolytic alginate lyase genes	<b>34</b> (Algal Biotech)
<b>Lee</b>	The hair growth promoting effects of	<b>26</b> (Algal Biotech)	<b>Takahashi</b>	Isolation and identification of	<b>91</b> (Biores & Biotech)
<b>Leethochavalit</b>	<i>Perkinsus atlanticus</i>	<b>58</b> (Aqua Biotech)	<b>Tanbirulhaque</b>	Micronization of fucoxanthin from	<b>35</b> (Algal Biotech)
<b>Li</b>	Study of the microRNA 145	<b>99</b> (General)	<b>Tareq</b>	Novel Lipopeptides, Kiostostatins A-E	<b>121</b> (Microbiotech)
<b>Li</b>	A novel alkaline lipase obtained from	<b>115</b> (Microbiotech)	<b>Tanon</b>	Strategy to improve the cellular synthesis	<b>36</b> (Algal Biotech)
<b>Lipton</b>	Purification, characterization and	<b>116</b> (Microbiotech)	<b>Tsubouchi</b>	Draft genome sequence of the	<b>107</b> (Genomics)
<b>Liu</b>	Characterisation of water-soluble	<b>59</b> (Aqua Biotech)	<b>Uchida</b>	Isolation of lactic acid bacteria from the	<b>95</b> (Food Safety)
<b>Liu</b>	The role of foxm1 in the initiation	<b>60</b> (Aqua Biotech)	<b>Wang</b>	Composition and antimicrobial activity	<b>92</b> (Biores & Biotech)
<b>Lu</b>	Application of RNA vaccine in grouper	<b>61</b> (Aqua Biotech)	<b>Watanachote</b>	Comparison of immune parameters	<b>65</b> (Aqua Biotech)
<b>Matsuda</b>	Sporophyte-specific expression of	<b>27</b> (Algal Biotech)	<b>Wei</b>	Molecular cloning, characterization of	<b>66</b> (Aqua Biotech)
<b>McCauley</b>	Anti-inflammatory and anticancer	<b>86</b> (Biores & Biotech)	<b>Wu</b>	Transdifferentiation of duct-like cells from	<b>100</b> (General)
<b>Meng</b>	The role of astaxanthin	<b>28</b> (Algal Biotech)	<b>Xu</b>	The transcriptome sequencing and	<b>37</b> (Algal Biotech)
<b>Mo</b>	Japanese flounder (Paralichthys	<b>62</b> (Aqua Biotech)	<b>Xu</b>	Physiological response of marine	<b>38</b> (Algal Biotech)
<b>Muñoz-Márquez</b>	Class I integrons in multiresistant	<b>93</b> (Food Safety)	<b>Yang</b>	Effect of oxidized fish oil on growth	<b>67</b> (Aqua Biotech)
<b>Ngnangbam</b>	Bacterial communities associated with	<b>117</b> (Microbiotech)	<b>Ye</b>	Potential antioxidant capacities of ethanol	<b>39</b> (Algal Biotech)
<b>Ochiai</b>	Strategies of acclimation to deep	<b>106</b> (Genomics)	<b>Ye</b>	Anti-proliferative effect of Pylaiella	<b>40</b> (Algal Biotech)
<b>Okado</b>	Catalase production and H2O2 tolerance	<b>29</b> (Algal Biotech)	<b>Zhang</b>	Artificial breeding technology of	<b>68</b> (Aqua Biotech)
<b>Osada</b>	Time-lapse analysis of oleaginous diatom	<b>30</b> (Algal Biotech)			
<b>Park</b>	Effects on cancer cell growth of saponin	<b>118</b> (Microbiotech)			

# ***IMBC 2013 ABSTRACTS***

## ***TABLE OF CONTENTS***

Abstracts are in alphabetical order of first author, with the presenting author marked with an asterisk.





A synthetic biology approach to develop a novel marine cyanobacterial bioprocesses -The Cyanofactory™-	58
<b>Abe, Koichi, Kotone Miyake, Mayumi Nakamura, Amr M.A.K.I. Badary, Wataru Yoshida, Stefano Ferri, Kazunori Ikebukuro, Koji Sode*</b>	
Genomes of calcaronean sponges: simple body plans and surprisingly complex developmental toolkits	58
<b>Adamski, Marcin*, Sven Leininger, Sofia Fortunato, Hans Torre Rapp and Maja Adamska</b>	
Photosynthetic microalgae: a sustainable source of omega-3 fatty acids from for nutraceuticals and aquaculture feed	59
<b>Adarme-Vega, T. Catalina*, Skye R. Thomas-Hall, Catherine Lovelock and Peer M. Schenk</b>	
Pigment cell differentiation in blastula-derived primary cell cultures of sea urchins	59
<b>Ageenko, Natalya*, Konstantin Kiselev, Pavel Dmitrenok and Nelly Odintsova</b>	
Anti-angiogenic potential of a marine gastropod <i>Euchelus asper</i> [Poster]	59
<b>Agrawal, Sweta*, Madhavi Indap</b>	
The evolutionary origin of molluscan shell matrix genes: comparative analysis of ten molluscan mantle transcriptomes	60
<b>Aguilera, Felipe*, Carmel McDougall and Bernard Degnan</b>	
Microalgae as a source of phytosterols	60
<b>Ahmed, Faruq*, Wenxu Zhou, Peer M Schenk</b>	
Neoechinulin A isolated from marine-derived <i>Microsporium</i> sp. suppresses sebium accumulation in insulin-like growth factor (IGF)-1 differentiated human sebocytes [Poster]	60
<b>Ahn, Byul-Nim*, Yong-Xin Li and Se-Kwon Kim</b>	
Profiling the phospholipid residues of krill oil by <sup>31</sup> P-NMR and regioisomeric distribution of polyunsaturated fatty acids in its triacylglycerol	61
<b>Akanbi, Taiwo* and Colin Barrow</b>	
Transcriptome analysis of freshwater crayfish ( <i>Cherax quadricarinatus</i> ) and characterisation of gill-expressed carbonic anhydrase genes	61
<b>Ali, Muhammad Yousuf*, Ana Pavasovic, Peter Mather and Peter Prentis</b>	
Identifying genes involved in physiological adaptation of <i>Nerita melanotragus</i> to temperature stress using comparative transcriptome sequencing [Poster]	62
<b>Amin, Shorash*, Peter J. Prentis, Edward K. Gilding, Christopher Collet and Ana Pavasovic</b>	
Next-generation genomics for bioproducts discovery [Poster]	62
<b>Antunes, Agostinho*</b>	
Role of hemocyte homeostasis associated protein (HHAP) in regulation of hemocyte apoptosis from black tiger shrimp <i>Penaeus monodon</i> [Poster]	63
<b>Apitanyasai, Kantamas*, Walaiporn Charoensapsri, Piti Amparyup and Anchalee Tassanakajon</b>	
Transcriptome analysis of genes associated with cold-inducible lipid biosynthesis in <i>Emiliana huxleyi</i>	63
<b>Araie, Hiroya*, Masato Baba, Iwane Suzuki and Yoshihiro Shiraiwa</b>	
Antihypertensive effect and antioxidant activities in mackerel muscle hydrolyzate recovered by subcritical water [Poster]	64
<b>Asaduzzaman, A K M and Byung-Soo Chun*</b>	
Dissecting dinoflagellate evolution	64
<b>Bachvaroff, Tsvetan*</b>	
Isolation and structure elucidation of novel saponins from the sea cucumber <i>Stichopus hermanni</i> viscera using HPCPC and mass spectrometry	64
<b>Bahrami, Yadollah*, Wei Zhang Christopher M Franco</b>	

Effect of Bioactive Compounds from Marine Sponge <i>Tethya spp.</i> on Bone resorption [Poster]	65
<b>Balakrishnan, Babita, Sachin Chaugule*, Madhavi Indap and Shubhada Chiplunkar</b>	
Efficient precision genome engineering in the marine polychaete <i>Platynereis dumerilii</i> using Transcriptional activator like effector nucleases (TALENs).	65
<b>Bannister, Stephanie*, Antonova, Olga, Polo, Alessandra, Hallay, Natalia, Valinciute, Agne, Raible, Florian, Tessmar-Raible, Kristin.</b>	
Omega-3 biotechnology: Sources, concentration methods, microencapsulation and bioactive derivatives.	66
<b>Barrow, Colin*, Taiwo Akanbi, Tim Nalder, Adarsha Gupta, Dilip Singh, Polly Dobson, Jacqui Adcock and Munish Puri</b>	
Preliminary genome sequencing, denovo assembly and annotation of the Neogastropoda <i>Dicathais orbita</i> [Poster]	66
<b>Baten, Abdul, Ajit Ngangbam, Kirsten Benkendorff*</b>	
New Zealand Marine Biotechnology: a successful past should now inform a successful future.	67
<b>Battershill, Chris*; Prinsep, Michele, Murray, Munro</b>	
<i>Dicathais orbita</i> as a model for marine natural product screening and nutraceutical development	67
<b>Benkendorff, Kirsten*, Vicki Edwards, Fiona Young, Babak Esmaelian and Catherine Abbott</b>	
Growing algae and cyanobacteria: photobioreactor technology for product optimisation at the Cawthron Institute	68
<b>Beuzenberg, Veronica, Gemma Gimenez Papiol, Susanna A Wood, Michael A Packer*</b>	
Developing Australian Native Microalgae for Algal Biofuels and Bioproducts	68
<b>Blackburn, Susan*, Albinsson, Maria Elisabeth, Clementson, Lesley, Dunstan, Graeme, Frampton, Dion, Jameson, Ian, Jovanovic, Tom, Lee Chang, Kim Jye</b>	
The Effects of <i>Ishige okamurae</i> on Collagen Synthesis of Osteoblastic MC3T3-E1 [Poster]	69
<b>Bo-kyung, Kim, Jihyeon Cheon*, Myeongjeong Jeon, SeoYeon Kim, Mira Park and Mihyang Kim</b>	
Effects of <i>Ishige okamurae</i> Extract on Serum Lipid Content of Ovariectomized Rats [Poster]	69
<b>Bo-kyung, Kim, Myeongjeong Jeon*, Seoyeon Kim, Jihyeon Cheon and Mihyang Kim</b>	
Physiology and metabolism of lipid production by marine and halotolerant microalgae	69
<b>Borowitzka, Michael A.</b>	
Intraspecific variability in secondary metabolites of the Great Barrier Reef sponge-associated marine bacteria " <i>Salinispora pacifica</i> " [Poster]	70
<b>Bose, Utpal*, Mark P. Hodson, P. Nicholas Shaw, John A. Fuerst and Amitha K. Hewavitharana</b>	
Identification of key signalling peptides involved in abalone sexual maturation	70
<b>Botwright, Natasha A.*, Michelle L. Colgrave, Scott F. Cummins, Mathew T. Cook, Harry King, Nick G. Elliott</b>	
Potential role of MeNP in shrimp ovary maturation: RNAi silencing resulted in inhibition of vitellogenesis	71
<b>Chan, Siu Ming*, Cheng Bo Sun</b>	
Bioleaching of conventional and non conventional materials: new approaches	71
<b>Chavez-Crooker, Pamela</b>	
Technology development of CO2 fixation, C-phycocyanin production and purification with <i>Spirulina platensis</i> [Poster]	71
<b>Chen, Chun-Yen*, Pei-Chun Kao and Jo-Shu Chang</b>	
Engineering strategies for improving protein production from microalgal <i>Chlorella vulgaris</i> FSP-E using novel photobioreactor illuminated with cold cathode fluorescent lamps [Poster]	72
<b>Chen, Chun-Yen, Po-Jen Lee, and Jo-Shu Chang*</b>	

Enhancing the production of EPA from <i>Nannochloropsis oceanica</i> CY2 by using LED photobioreactor [Poster]	72
<b>Chen, Chun-Yen*, Yu-Chun Chen, Chen-Chun Liu, Hsiao-Chen Huang and Jo-Shu Chang</b>	
Enhancing EPA production from <i>Nannochloropsis oceanica</i> using deep-sea water supplemented cultivation medium [Poster]	73
<b>Chen, Chun-Yen<sup>a*</sup>, Yu-Mei Chen<sup>a</sup>, and Jo-Shu Chang<sup>abc</sup></b>	
Characterization of photosynthetic carbon dioxide fixation ability of indigenous <i>Chlorella pyrenoidosa</i> NNK-A isolate [Poster]	73
<b>Chen, Chun-Yen<sup>a*</sup>, Yu-Mei Shen<sup>a</sup>, Hsin-Yueh Chang<sup>a</sup>, and Jo-Shu Chang<sup>abc</sup></b>	
Characterization of photosynthetic carbon dioxide fixation ability of indigenous <i>Chlorella pyrenoidosa</i> CY10 isolate [Poster]	73
<b>Chen, Chun-Yen*, Hsin-Yueh Chang, Chun-Hua Huang and Jo-Shu Chang</b>	
UV mutagenesis of an isolated green microalga <i>Chlamydomonas orbicularis</i> for enhanced lipid production [Poster]	74
<b>Chen, Chun-Yen<sup>a</sup>, Po-Cheng Lin<sup>a</sup>, and Jo-Shu Chang<sup>abc*</sup></b>	
Proteomic Analysis Reveals That Pardaxin Triggers Apoptotic Signaling Pathways in Human Cervical Carcinoma HeLa Cells: Crosstalk among the UPR, c-Jun, and ROS [Poster]	74
<b>Chen, Jyh-Yih*, Tsui-Chin Huang</b>	
Evaluation of therapeutic efficacy of antimicrobial peptides against marine pathogens using in vitro and in vivo infection models [Poster]	75
<b>Chen, Wei-Jung*, Wei-Chen Tsai and Tsun-Yung Kuo</b>	
Different visible colors were obtained from the mutated purple chromoprotein isolated from sea anemone [Poster]	75
<b>Chiang, Cheng-yi*, Yi-lin Chen and Huai-jen Tsai</b>	
Progranulin is required for liver regeneration in the partial hepatectomized zebrafish [Poster]	75
<b>Chiang, Keng-Yu*, Yen-Hsing Li, Ya-Wen Li, Jen-Lieh Wu</b>	
Epinecidin-1 has immunomodulatory effects, facilitating its therapeutic use in a mouse model of <i>Pseudomonas aeruginosa</i> sepsis [Poster]	76
<b>Chieh-Yu, Pan*, Jian-Chyi Chen, Jenn-Feng Sheen, Tai-Lang Lin, Jyh-Yih Chen</b>	
Transcriptomic study and different gene expression profiling of two kinds of grouper iridoviruses infection in orange-spotted grouper ( <i>Epinephelus coioides</i> ) [Poster]	76
<b>Chou, Hsin-Yiu *, Ming-Horng Wu and Jiann-Horng Leu</b>	
Genome Sequencing of the Shrimp <i>Neocaridina denticulata</i> : Insights into Crustacean Aquaculture and Arthropod Evolution	77
<b>Chu, Ka Hou*, Yung Wa Sin, Nathan Kenny, Kevin Z. Qu, Wei Wang, Fiona K.M. Cheung, Ka Wo Chan, Zora C.K. Chan, Ricky W.T. Leung, Nicola W.Y. Wong, Sam P.S. Cheong, Katie W.S. Chan, Ting Fung Chan, Stephen S. Tobe and Jerome H.L. Hui</b>	
Structural characteristic and immuno-regulatory function of class-A CpG oligodeoxynucleotide in grouper [Poster]	77
<b>Chuang, Hsiang-Chieh, Fang-Yao Lee, Nai-Yu Chen and Pinwen P. Chiou*</b>	
The Overexpression and Bioactivity Assay of Fish Type I Interferons on Grouper Cell Line [Poster]	78
<b>Chung Chia-Ling*, Fang-Huar Ngou, Sook-Ping Tan, Hsiang-Ping Kuo, Zwe-Ling Kong, Hong-Ting Lin, Yu-Shen Lai, Ming-Wei Lu</b>	
A female sex hormone is required for developing adult female features of blue crabs	78
<b>Chung, J. Sook*, In Sook, Ahn and Nilli Zmora</b>	
Effects of dispersed and emulsified oil on molting, ecdysone and EcR/RXR complex in the grass shrimp and the blue crab [Poster]	78
<b>Chung, J. Sook*, Sirinart Techa, Karrie Bulski, Anna N. Walker and Richard, F Lee</b>	
The abalone haemocyte proteome: an indicator of animal health.	79
<b>Coyne, Vernon E*, Caroline GG Beltran, Bridget Calder, Valera L Dias</b>	

Molecular cloning, characterization and expression analysis of a glucokinase gene from the mixotrophic green alga <i>Chlorella kessleri</i> [Poster]	79
<b>Cui, Hongli, Xiaona Yu, Yan Wang, Yulin Cui and Song Qin*</b>	
Brain stimulants and sex smells: Decoding peptide communication systems in marine molluscs	80
<b>Cummins, Scott*, Abigail Elizur, Bernie Degnan<sup>2</sup>, Gregg Nagle<sup>3</sup></b>	
Characterization of the bacterial community of Hawaiian sea slug <i>Elysia rufescens</i> .	80
<b>Davis, Jeanette*, W. Florian Fricke, Mark T. Hamann and Russell T. Hill</b>	
Renewable fuels from macroalgae: revising the paradigm for algal fuels	80
<b>de Nys, Rocky</b>	
Antioxidant activity of extracts from sponge-associated bacteria collected from Tao Island, Gulf of Thailand [Poster]	81
<b>Dechasakulwatana, Chutiwan* and Sumaitt Putchakarn</b>	
Gene regulation in the demosponge <i>Amphimedon queenslandica</i> and insights into the construction of animal body plans	81
<b>Degnan, Bernie</b>	
Seagrass restoration using hessian: silane coating of hessian reduces <i>Escherichia coli</i> attachment and fouling by marine bacteria	81
<b>Delpin, Marina W.*, Natasha Friend, Samuel Ogden, Kirsten Benkendorff, Sue Murray-Jones and Jamie Quinton</b>	
Isolation and Characterization of Marine-Derived <i>Mucor</i> sp. for Fermentative Production of Tyrosol [Poster]	82
<b>Dewapriya, Pradeep,*and Se-Kwon Kim</b>	
Controlled formation of mono- and dihydroxy-resolvins and protectin analogues from omega-3 DHA and EPA using soybean 15-lipoxygenase	82
<b>Dobson, Polly, Colin J. Barrow*, Jaroslav A. Kralovec and Jacqui L. Adcock</b>	
Effects of environmental Conditions on Lipid Accumulation and Diversity of Microalgae at the South East Coast of Queensland – Australia	82
<b>Duong, Van Thang*, Ekaterina Nowak, David KY Lim, Lilia C Carvalhais, Peer M Schenk</b>	
Technologies to assist reproductive performance in finfish aquaculture	83
<b>Elizur, Abigail</b>	
Access to Australian marine bioresources in a modern (post Nagoya Protocol) world	83
<b>Evans-Illidge, Elizabeth</b>	
The Nagoya Protocol – a new legally binding international regime for access to biodiversity and benefit sharing. Can it solve the uncertainty?	83
<b>Evans-Illidge, Elizabeth A*</b>	
Phylogeny drives large scale patterns in Australian marine bioactivity - a chemical ecology rationale for future biodiscovery [Poster]	84
<b>Evans-Illidge, Elizabeth A*, Murray Logan, Jason Doyle, Jane Fromont, Christopher N Battershill, Gavin Ericson, Carsten W Wolff, Andrew Muirhead, Philip Kearns, David Abdo, Stuart Kininmonth, and Lyndon Llewellyn</b>	
Isolation of antimicrobial marine bacteria from sub-arctic hydrothermal sites	84
<b>Eythorsdottir Arnheidur*, Sesselja Omarsdottir, Hjorleifur Einarsson</b>	
View from oyster genome: The opportunity and challenge for aquatic organism genome study	85
<b>Fang, Xiaodong</b>	
A fatal reovirus of blue crab, <i>Callinectes sapidus</i> , has potential to impact the host throughout its entire geographic range	85
<b>Flowers, Emily M., Kahil A. Simmonds, Robert Aguilar, Holly A. Bowers, Oded Zmora, Eric J. Schott*</b>	

Toward understanding marine lifestyles using new-generation sequencing and genomic technologies: Red alga <i>Pyropia yezoensis</i> and other case studies	86
<b>Fujiyama, Asao*, Hideki Noguchi, Shoji Tatsumoto, Hideki Hirakawa, Hong-Seog Park, Koji Mikami, Naotsune Saga, Atsushi Toyoda and Satoshi Tabata</b>	
Evaluation of the ligninolytic activity by the marine eukaryotes, Thraustochytrids which using the Remazol Brilliant Blue R as indicator [Poster]	86
<b>Fukaya, Kazuto*, Mayumi Ueda, Naoki Nagano, Daiske Honda, Masahiro Hayashi and Yousuke Taoka</b>	
Genetic engineering of microalgae for photosynthetic biofuel production	87
<b>Fukuzawa, Hideya*, Takashi Yamano, Masataka Kajikawa, Emi Sato, Yuri Sawaragi</b>	
Laser-induced mutation and selection leads to improved <i>Tetraselmis</i> sp. microalgae as a hopeful candidate for biodiesel production [Poster]	87
<b>Gao, Zhengquan*, David KY Lim, Peer M Schenk</b>	
Integrating genomics and biosynthesis to discover new classes of bioactive secondary metabolites from marine cyanobacteria	88
<b>Gerwick, William H.*</b>	
The roles of eIF4E family members in zebrafish ( <i>Danio rerio</i> ) [Poster]	88
<b>Gillespie, Kate, Erica Dasi, Rosemary Jagus*</b>	
Development of Type I microsatellite markers from transcriptome of giant grouper for marker-assisted selection [Poster]	88
<b>Gong, Hong-Yi*, Tse-Yu Tai, Fcng-You Lin, Hsin-Yiu Chou, Chang-Wen Huang, Shinn-Lih Yeh and Jen-Leih Wu</b>	
NMR-guided approaches to natural product-based drug discovery	89
<b>Grkovic, Tanja*, Ronald J Quinn</b>	
Screening of marine microorganisms: Thraustochytrids from Victorian environment for advancing omega-3 biotechnology [Poster]	89
<b>Gupta, Adarsha*, Colin J. Barrow, Munish Puri</b>	
Development and characterization of interspecific somatic hybrids through protoplast fusion between <i>Ulva fasciata</i> Delile (×) <i>U. reticulata</i> Forsskål	89
<b>Gupta, Vishal*, CRK Reddy, B Jha</b>	
Calcium Carbonate Crystallization using marine-derived recombinant glycine-rich Proteins [Poster]	90
<b>Han, Yohan, Hyerin Kim and Yoo Seong Choi*</b>	
Discovery of marine natural products targeting Keap1-Nrf2-ARE signalling pathway and mechanism study [Poster]	90
<b>Han, Bingnan*, Xiuwen Tang<sup>2</sup>, Zachary Kemmerer<sup>3</sup>, Aimee L. Egger<sup>3</sup>, and Hou-Wen Lin</b>	
Characterization of an epiphytic bacterium <i>Neptunomonas</i> sp. BPy-1 on a red alga <i>Pyropia yezoensis</i> [Poster]	90
<b>Handayani, Midia*, Hiroyuki Sasaki, Ryuya Matsuda, Katsuaki Takechi, Hiroyoshi Takano, Susumu Takio, 2</b>	
Towards High-Efficiency Microalgae Biofuel Systems	91
<b>Hankamer, Ben</b>	
The effect of Z-Nisin and Sodium citrate on increasing of shelf-life of Kutum filets ( <i>Rutilus frisii kutum</i> ) stored at 4°C	91
<b>Hedayatifard, M*, M. Alinejhad, R. Safari</b>	
Microbial diversity, function and biotechnological potential of marine sponges	91
<b>Hentschel, Ute*</b>	

Osteoclastogenic effect of marine algae in human osteoblast-like MG-63 cells [Poster]	92
<b>Heo, Soo-Jin*, Bo-Ram Ye, Jiyi Jang, Min-Sun Kim, Junseong Kim, Won-Kyo Jung, Chulhong Oh, Do-Hyung Kang</b>	
Evaluation of anti-inflammatory activity of marine algae in LPS-stimulated RAW 264.7 cells [Poster]	92
<b>Heo, Soo-Jin*, Bo-Ram Ye, Jiyi Jang, Min-Sun Kim, Junseong Kim, Won-Kyo Jung, Chulhong Oh, Do-Hyung Kang</b>	
Anti-inflammatory and Anti-tumor activity of a carotenoid isolated from brown algae through MAPKs regulation	92
<b>Heo, Soo-Jin*, Bo-Ram Ye, Ji Hyung Kim, Youngdeuk Lee, Su-Jin Lee, Chulhong Oh, Do-Hyung Kang</b>	
Applicability of aquaculture effluents to production of docosahexaenoic acid by oleaginous microbe, <i>Aurantiochytrium limacinum</i> strain mh0186 [Poster]	93
<b>Hidaka, Kazuaki*, Yousuke Taoka and Masahiro Hayashi</b>	
EGFR Tyrosine Kinase Inhibitory Peptide Isolated from Marine <i>Chlamydomonas</i> Sp. Attenuates <i>Helicobacter Pylori</i> -Mediated Carcinogenic Responses	93
<b>Himaya, S.W.A.* and Se-Kwon Kim,2,†</b>	
Role of <i>Marsupenaeus japonicus</i> crustin-like peptide against <i>Vibrio penaeicida</i> and white spot syndrome virus infection [Poster]	94
<b>Hipolito, Sheryll*, Hidehiro Kondo and Ikuo Hirono</b>	
Characterization of recombinant gonadotropins activity and their receptors in the Common Carp [Poster]	94
<b>Hollander-Cohen, Lian*, Joseph Aizen, Levavi-Sivan Berta</b>	
The lectins from the genus <i>Codium</i>	94
<b>Hori, Kanji* and Makoto Hirayama</b>	
Marker-assistant Selection and Breeding through Phylogenetic Relationships in Taiwan Giant Grouper ( <i>Epinephelus lanceolatus</i> ) by Using Microsatellite and Mitochondria Markers [Poster]	95
<b>Hsu, Hao-Hsuan and Tzong-Yueh Chen*</b>	
Bioremediation Of Crude Oil Using Indigenous Marine Bacteria	95
<b>Hu, Xiaoke*, Hui Wang, Meng Lin</b>	
The analyses of de novo milkfish transcriptome assembly in response to salinity and temperature changes	95
<b>Hu, Yau-Chung*, Tsung-Han Lee</b>	
Multi-chloride channels from two clades of the CIC members involve in chloride absorption of tilapia gills [Poster]	96
<b>Hu, Yau-Chung*, Yan-Shuo Liu and Tsung-Han Lee</b>	
Specific transcriptional response and cold tolerance ability in PUFA zebrafish [Poster]	96
<b>Huang, Shin-Jie*, Mary-nia M. Santos, Chih-Lun Cheng, Jen-Leih Wu</b>	
The transcriptome analysis of grouper, <i>Epinephelus coioides</i> in response to Singapore grouper iridovirus (SGIV) infection	96
<b>Huang, Youhua, Xiaohong Huang, Yang Yan, Qiwei Qin*</b>	
Efficient degradation of alginate using alginate lyases from <i>Flavobacterium</i> sp.	97
<b>Inoue, Akira*, Ryuji Nishiyama, Kohei Takadono and Takao Ojima</b>	
Potential compounds from marine <i>Xestospongia</i> sp., <i>Chicoreus</i> sp. and <i>Acanthaster planci</i> as peroxisome proliferator activated receptor gamma (PPAR $\gamma$ ) ligand for anti-atherosclerotic activity	97
<b>Ismail, N.*, Nurul Izzati, M.A., Nur Maisarah, S., Faridah, M., Nurul Hazirah M.L., Asari A., Mariam T., Aziz A., Habsah M., Tengku Muhammad T.S.</b>	
Anti-atherosclerotic activity of marine sponge <i>Xestospongia</i> sp.	98
<b>Ismail, N., Mohd Annuar, N.I *, Faridah, M., Asari A., Mariam T., Aziz A., Tengku Muhammad T.S., Habsah M.</b>	

A possible process of tetrodotoxin accumulation in marine pufferfish of the genus <i>Takifugu</i> [Poster]	98
<b>Itoi, Shiro*, Junya Uchida, Kento Ishizuka, Narumi Takimoto, Ryoko Mitsuoka, Shihori Takanashi, Shunsuke Noguchi, Keita Ishikawa, Takamasa Okamura, Keitaro Komori, Motoki Tamai, Susumu Domeki, and Haruo Sugita</b>	
Production, purification and characterization of halothermotolerant solvent stable lipase and its application in ester synthesis	99
<b>Jain, Deepti*, Mishra Sandhya</b>	
The Effects of <i>Seaweed Gongjindan</i> on Estrogen Like Activities, Platelet Aggregation and Serum Lipid Levels in Ovariectomized Rats [Poster]	99
<b>Jeon, Myeongjeong*, Seo-yeon Kim, Ji Hyeon Cheon, Seong-Hwan Park, Sang-Hyeon Lee and Mihyang Kim</b>	
Biomass Evaluation of a Novel Green Microalga <i>Chlamydomonas</i> sp. KIOST-1 for Biofuel Production Isolated from Korea	100
<b>Jeon, Seon-Mi*, Ji Hyung Kim, Areumi Park, Taeho Kim, Su-Jin Lee, Chulhong Oh, Soo-Jin Heo and Do-Hyung Kang</b>	
A novel coccoid-shaped cyanobacterium, <i>Myxosarcina</i> sp. KIOST-1 isolated from Mangrove Forest in Chuuk State, Federated States of Micronesia [Poster]	100
<b>Jeon, Seon-Mi*, Ji Hyung Kim, Areumi Park, Taeho Kim, Su-Jin Lee, Chulhong Oh, Soo-Jin Heo and Do-Hyung Kang</b>	
Untapped Bacterial community Enriched from Coastal Marine Sediment under Anaerobic and Thermophilic Conditions	101
<b>Ji, Shiqi, Yang Tan, Shi'an Wang, Fuli Li*</b>	
Involvement of multiple eIF4Es in mRNA recruitment in dinoflagellates	101
<b>Jones, Grant D, Tsvetan R. Bachvaroff, Earnest Williams, Allen R. Place and Rosemary Jagus*</b>	
Transcriptomic identification of genes affecting growth and reproduction, and SNP association studies with individual growth performance in giant freshwater prawn ( <i>Macrobrachium rosenbergii</i> ).	102
<b>Jung, Hyungtaek*, Russell E. Lyons, David A. Hurwood and Peter B. Mather</b>	
Modification of EPA/DHA biosynthetic pathway by transgenesis in a marine teleost, nibe croaker	102
<b>Kabeya, Naoki*, Yutaka Takeuchi, Yoji Yamamoto, Ryosuke Yazawa, Yutaka Haga, Shuichi Satoh, Goro Yoshizaki</b>	
Extraction of carotenoids from raw macroalgae excluding drying and cell wall disruption by liquefied dimethyl ether [Poster]	103
<b>Kanda, Hideki*, Yuichi Kamo, Shuhei Shintani, Siti Machmudah, Wahyudiono and Motonobu Goto</b>	
A mini review on algae biofuel in Korea	103
<b>Kang, Do-Hyung*</b>	
High-pressure extract of <i>Phaeodactylum tricornutum</i> inhibits HGF-induced proliferation in human gastric cancer SNU-1 and AGS cells	103
<b>Kang, Kyong-Hwa* and Se-Kwon Kim</b>	
Germ cell-specific excision of the loxP-flanked transgene in rainbow trout	104
<b>Katayama, Naoto*, Sachi Kume, Sakiko Sadaie, Shoko Ihara and Goro Yoshizaki</b>	
Mixotrophic cultivation of <i>Euglena gracilis</i> using waste from food industry [Poster]	104
<b>Kawano, Yumi*, Tomoyuki Suenaga, Yousuke Taoka, Motonari Sibakami and Masahiro Hayashi</b>	
Overexpress glutathione reductase to prevent thioacetamide induce oxidative stress in zebrafish [Poster]	104
<b>Ken, Chuian-Fu* and Chih-Chiuan Pan</b>	

Arsenic tolerant sponge-associated bacteria of the Red Sea <i>Theonella swinhoei</i> and their implication for water remediation	105
<b>Keren Ray*, Lavy Adi, Mayzel Boaz, Ilan Micha</b>	
Bioactive Metabolites from a Jellyfish-derived Fungus <i>Phoma</i> sp. [Poster]	105
<b>Kim, Eun La*, Haibo Wang, Jongki Hong, Jee Hyung Jung</b>	
Comparative analysis of biochemical components in <i>Spirulina maxima</i> Cy-23 and the newly isolated <i>Leptolyngbya</i> sp. KIOST-1 [Poster]	106
<b>Kim, Ji Hyung*, Seon-Mi Jeon, Taeho Kim, Su-Jin Lee, Areumi Park, Soo-Jin Heo, Chulhong Oh, Do-Hyung Kang</b>	
Evaluation of protective efficacy of a novel <i>Aeromonas</i> phage PAS-1 against <i>A. salmonicida</i> subsp. <i>salmonicida</i> infections in rainbow trout ( <i>Oncorhynchus mykiss</i> ) model	106
<b>Kim, Ji Hyung*, Se Chang Park, Do-Hyung Kang, Soo-Jin Heo, Chulhong Oh</b>	
Preventive Effect of Marine algae on Bone Loss in C2C12 Myoblasts [Poster]	107
<b>Kim, Jung-Ae and Chang-Suk Kong*</b>	
Osteogenic and anti-adipogenic activities of <i>Salicornia herbacea</i> [Poster]	107
<b>Kim, Jung-Ae and Chang-Suk Kong*</b>	
Protective Effects of <i>Ecklonia cava</i> on Osteoporosis and Adipogenesis in Mesenchymal Cells [Poster]	107
<b>Kim, Jung-Ae, Mihyang Kim, Sang-Hyeon Lee and Chang-Suk Kong*</b>	
Inhibitory Effects of <i>Sargassum thinbergii</i> on Adipogenic Differentiation in Mouse Mesenchymal Cells [Poster]	108
<b>Kim, Jung-Ae, Mihyang Kim, Sang-Hyeon Lee and Chang-Suk Kong*</b>	
Investigation and development of bioactive substances from marine organisms	108
<b>Kim, Se-Kwon</b>	
The Effect of <i>Scytosiphon lomentaria</i> on Differentiation of Osteoblastic MC3T3-E1 Cells [Poster]	109
<b>Kim, Seoyeon*, Myeong-Jeong Jeon, Jihyeon Cheon, Mira Park, Changsuk Kong and Mihyang Kim</b>	
Effects of <i>Eisenia bicyclis</i> Fractions on Osteoblast Differentiation and Osteoclast Formation [Poster]	109
<b>Kim, Seoyeon*, Myeong-Jeong Jeon, Jihyeon Cheon, Mira Park, Changsuk Kong and Mihyang Kim</b>	
The Feasibility of pilot production of <i>Spirulina (Arthrospira) maxima</i> cultivated newly constructed raceway pond in Republic of Korea.	110
<b>Kim, Taeho*, Seon-Mi Jeon, Ji Hyung Kim, Areumi Park, Ji Hyun Lee, Chulhong Oh, Soo-Jin Heo, Kwang-Sik Choi and Do-Hyung Kang</b>	
Bis(methylthio)gliotoxin isolated from marine fungus <i>Aspergillus fumigatus</i> inhibits HGF-induced cell proliferation in human gastric epithelial AGS cells [Poster]	110
<b>Kim, Young-Sang*, Yong-Xin Li, Kyong-Hwa Kang, Sun-Ju Park, Se-Kwon Kim</b>	
Expression and tissue distribution of skeletal myosin heavy chain genes from adult and larvae of shrimps [Poster]	110
<b>Koyama, Hiroki*, Sanit Piyapattanakorn and Shugo Watabe</b>	
Bioactive potential of some intertidal molluscs collected from Mumbai coast (West coast of India)	111
<b>Kulkarni, Balasaheb*, Nair Madhu, Argekar Anant</b>	
Luminescence behaviour of marine luminous bacteria under nutrient-saved conditions [Poster]	111
<b>Kuwahara, Hitomi*, Junko Ninomiya, Yosuke Tabei, Hiroshi Morita</b>	
Gene characterization, cloning and over-expression of the acetyl xylan esterase from <i>Ochrovirga pacifica</i>	111
<b>Kwon, Young-Kyung*, Chulhong Oh, Jung-Ho Hyun, Youngdeuk Lee, Su-Jin Lee, Ji Hyung Kim, Taeho Kim, Do-Hyung Kang</b>	



Anaerobic conditions and poor nutrient media reveal antibacterial properties of sponge-associated bacteria [Poster]	112
<b>Lavy, Adi*, Keren Ray, Haber Markus, Schwartz Inbar, Ilan Micha</b>	
Recombinant production and characterization of a thermophilic arylsulfatase from the marine bacterium <i>Thermotoga maritima</i> [Poster]	112
<b>Lee, Dong-Geun, Seong-Hwan Park, Myong Je Jeon, Mihyang Kim*, Chang-Suk Kong and Sang-Hyeon Lee</b>	
The hair growth promoting effects of <i>Eucheuma cottonii</i> [Poster]	112
<b>Lee, Sang-Man*, Soon-Sun Bak, Ratih Pangestuti, Byul-Nim Ahn, Sun-Ju Park, Jin Eun Lee, Jung Chul Kim, Moon Kyu Kim, Young Kwan Sung and Se-Kwon Kim</b>	
Cloning, expression and characterization of L-Asparaginase from <i>Mesoflavibacter zeaxanthinifaciens</i> S86.	113
<b>Lee, Su-Jin, Youngdeuk Lee, Ji Hyung Kim, Young-Kyung Kwon,2, Seon-Mi Jeon, Soo-Jin Heo, Chulhong Oh, Do-Hyung Kang*</b>	
Molecular cloning, overexpression and purification of a novel laminarinase from <i>Mesoflavibacter zeaxanthinifaciens</i> S86	113
<b>Lee, Youngdeuk*, Su-Jin Lee, Ji Hyung Kim, Young-Kyung Kwon, Soo-Jin Heo, Do-Hyung Kang and Chulhong Oh</b>	
Study of effects of nutrients on testicular maturation in the black tiger shrimp by sperm performance assessment and cDNA microarray analysis	114
<b>Leelatanawit, Rungnapa*, Umaporn Uawisetwathana, Amornpan Klanchui, Juthatip Kudej, Suwanchai Phomklad, Somjai Wongtripop, Pikul Jiravanichpaisal, Nitsara Karoonuthaisiri</b>	
<i>Perkinsus atlanticus</i> Infestation in undulated surf clam, <i>Paphia undulata</i> , along the east coast of Thailand [Poster]	114
<b>Leethochavalit, Supanee*, Janjarus Watanachote and Nareerat Rittirut</b>	
Masculine sex differentiation pathways in the fresh water prawn <i>Macrobrachium rosenbergii</i> , a hinge around the major component, insulin- like androgenic gland hormone (Mr-IAG).	115
<b>Lezer, Yaara*, Omri Sharabi, Rivka Manor, Eliahu D. Aflalo, Amir Sagi</b>	
Genome Screening and Biosynthesis of Manumycins-type Compounds from Marine <i>Streptomyces</i>	115
<b>Li, Fuchao*, Zhongyao Huang, Peng Jiang and Song Qin</b>	
New cystine knot peptides of a marine sponge, and its potential as a scaffold for oral peptide drug delivery [Poster]	116
<b>Li, Huayue, Mingzhi Su, Eun La Kim, John J. Bowling, Jongki Hong, Mark T. Hamann, Jee Hyung Jung*</b>	
Three novel C-type lectins from <i>Eriocheir sinensis</i> functions as pattern recognition receptor (PRR)	116
<b>Li, Wei-Wei, Xing-Kun Jin, Xiao-Nv Guo, Shuang Li, Ai-Qing Yu, Min-Hao Wu, Shang-Jian Tan, You-Ting Zhu, Qun Wang*</b>	
Microalgae nutrient efficacy as aquafeed additives: a booster of aquaculture sustainable development	117
<b>Li, Yan*, Guoqian Xiao, 2, Arnold Mangott, 3, Megan Kent, Igor Pirozzi,</b>	
Study of the microRNA 145 mediated regulatory mechanism for liver development in zebrafish [Poster]	117
<b>Li, Ya-Wen*, Yen-Hsing Li, Wangta Liu, Keng-Yu Chiang, Jen-Leih Wu</b>	
Effects of Tributyltin on the activities of immunologic enzyme in blood serum of the <i>Macrobrachium rosenbergii</i> [Poster]	117
<b>Li, Yu liao, Luan Luan Chen, Zhu Chunhua*</b>	
Identifying the bottlenecks of microalgal lipid production: a new transcriptional profiling approach	118
<b>Lim, David KY*, Holger Schuhmann, Skye R Thomas-Hall, Kenneth Chan, David Edwards, Peer M Schenk</b>	
Purification, characterization and optimisation of metalloprotease from <i>Pseudomonas poae</i> PGPR2 [Poster]	118
<b>Lipton, Anuj Nishanth*</b>	

eIF2 expression and phosphorylation in response to nutritional status and stressors in fish [Poster]	118
<b>Liu, Chieh Lun, Erica Dasi, Shau-Chi Chi, Yu-Hsuan Kai, Allen R. Place, &amp; Rosemary Jagus*</b>	
Characterisation of water-soluble collagen from tilapia skin[Poster]	119
<b>Liu, Lei, Zhencheng Wei, Xueming Liu and Lixin Huang*</b>	
The role of foxm1 in the initiation mechanism of intrahepatic cholangiocarcinogenesis in zebrafish [Poster]	119
<b>Liu, Wangta*, Yen-Chun Chen, Chi-Hsueh Lin, Shin-Jie Huang, Hong-Yi Gong and Jen-Leih Wu.</b>	
Application of RNA vaccine in grouper nervous necrosis virus[Poster]	119
<b>Lu Ming-Wei*, Jen-Leih Wu, Yu-Chen Lin, Chia-Ling Chung, Ya-Ting Juhn</b>	
Marine Microbial Bioactive Biosurfactant for Cosmeceutical Industry	120
<b>Lu, Jenn-Kan*, Wei-Haw, Xu</b>	
Global transcriptome profiling of <i>Pyropia yezoensis</i> in response to temperature stresses	120
<b>Mao, Yunxiang*, Peipei Sun, Fanna Kong, Guiyang Li, Zhaolan Mo, Guiqi Bi, Min Cao</b>	
Sporophyte-specific expression of bromoperoxidase gene in a red alga, <i>Pyropia yezoensis</i> [Poster]	121
<b>Matsuda, Ryuya*, Katsuaki Takechi, Hiroyoshi Takano and Susumu Takio</b>	
Anti-inflammatory and anticancer potential of Australian marine macroalgae; role in gut health as dietary therapeutics [Poster]	121
<b>McCauley, Janice*, Pia Winberg and Danielle Skropeta</b>	
It pays to be tough: the prevalence of proteins with repetitive, low complexity domains in marine biomaterials	122
<b>McDougall, Carmel*, Ben Woodcroft, Felipe Aguilera and Bernard M. Degnan</b>	
Characterization of Bio-prospecting Compounds of Brown Seaweed <i>Sargassum horneri</i> by Liquefied Pressurized System [Poster]	122
<b>Meillisa, Aviannie, Yin Shipeng, Hee-Chul Woo and Byung-Soo Chun*</b>	
The role of astaxanthin biosynthesis genes in <i>Haematococcus pluvialis</i> during carotenoid induction by salinity and nutrient starvation stress [Poster]	122
<b>Meng, Chunxiao*, Zhengquan Gao, Ahmed Faruq, Yan Li, Peer M Schenk</b>	
Japanese flounder ( <i>Paralichthys olivaceus</i> ) spleen transcriptome and expression profile involved in immunity during <i>Vibrio anguillarum</i> infection [Poster]	123
<b>Mo, Zhaolan*, Guiyang Li, Lin Huang, Jie Li, Bin Hao</b>	
Marine pigmented bacterium <i>Serratia rubidaea</i> (NIO/PPB/01) and its potential towards antifouling properties	123
<b>Mohandass, Chellandi*, Mohan A. Dhale, Chinnarajan Ravindran and Mangalaa K. Rajasekaran</b>	
Sustainable conversion of light to chemical and electrical energy	124
<b>Moheimani, Navid Reza and David Parlevliet</b>	
Defining the producers of marine natural compounds in marine sponges using the single-cell analytical approach	124
<b>Mori, Tetsushi, Micheal C Wilson, Rimi Miyaoka, Masahiro Ando, Hiro-o Hamaguchi, Shigeki Matsunaga, Joern Piel, Haruko Takeyama</b>	
Our shared challenge and our shared objective: Developing and applying marine biotechnologies to achieve sustainable use of marine resources for human benefit and ecosystem protection	124
<b>Müller, Werner E.G.</b>	
Sustainable Oceans – our Treasure in the Past and in the Future: The power of marine genomics	125
<b>Müller, Werner E.G.* and Wang, Xiaohong</b>	
Class I integrons in multiresistant <i>Escherichia coli</i> isolates from poultry litter [Poster]	125
<b>Muñoz-Márquez, María Enriqueta*, Elizabeth Ponce-Rivas and Ashraf A. Khan</b>	

Investigation of lipids and lipases from the microalgae <i>I. galbana</i> and <i>P. lutheri</i>	126
<b>Nalder, Tim D*,2, Susan N Marshall2, Colin J Barrow</b>	
Production of compatible solutes by halophilic fungi	126
<b>Nazareth, Sarita* and Valerie Gonsalves</b>	
Bacterial communities associated with biosynthetic organs of the marine mollusc <i>Dicathais orbita</i> [Poster]	126
<b>Ngangbam, Ajit*, Steve Whalan, Kirsten Benkendorff</b>	
A Journey from Marine Genes to New Sustainable Land Plant Sources of Long-chain Omega-3 Oils	127
<b>Nichols, Peter D.*, Petrie, James and Singh, Surinder</b>	
Metabolite Extraction Strategies from Whole Tissue Samples of Tropical Fish Using Gas Chromatography Mass Spectrometry Metabolomics	127
<b>Nurdalila, A'wani Aziz and Syarul Nataqain Baharum*</b>	
Strategies of acclimation to deep sea: Structure simulation of myoglobin molecules from aquatic animals under high pressure [Poster]	128
<b>Ochiai, Yoshihiro*, Hideo Ozawa</b>	
Bioethanol production from cyanobacteria biomass	128
<b>Oh, Chulhong*, Youngdeuk Lee, Su-Jin Lee, Ji Hyung Kim, Young-Kyung Kwon, Seon-Mi Jeon, Taeho Kim, Soo-Jin Heo, Do-Hyung Kang</b>	
Fucoxanthin Production from Microalgae	128
<b>Ohara, Katsuyoshi*, Kurokawa Hiroshi, Maeno Katsuhiko and Matsumoto Mitsufumi</b>	
Shotgun lipidomic profiling in marine alga <i>Emiliania huxleyi</i> : Identification of intermediates for lipid and very-long-chain alkene biosynthesis	129
<b>Ohi, Nobuaki*, Iwane Suzuki, Yoshihiro Shiraiwa</b>	
Metabolic pathway of alkenones and alkenes by marine haptophytes and its application to biofuel production	129
<b>Ohi, Nobuaki, Hiroya Araie, Hideto Nakamura, Ken Sawada, Tomonori Kotajima, Yoshinori Tsuji, Manami Satoh, Iwane Suzuki and Yoshihiro Shiraiwa*</b>	
Catalase production and H <sub>2</sub> O <sub>2</sub> tolerance of <i>Aurantiochytrium limacinum</i> strain mh0186 [Poster]	130
<b>Okado, Yu*, Yousuke Taoka, Mayumi Ueda, Daiske Honda and Masahiro Hayashi</b>	
Economies of scale and markets for microalgal products on the way to biofuels.	130
<b>Olaizola, Miguel</b>	
Marine Extracts: New opportunities for high value exports	130
<b>Olsen, Danette</b>	
Time-lapse analysis of oleaginous diatom <i>Fistulifera</i> sp. strain JPCC DA0580 during the triglyceride accumulation using single-cell patterning [Poster]	131
<b>Osada, Kyoko*, Masahito Hosokawa, Tomoko Yoshino and Tsuyoshi Tanaka</b>	
Identification of <i>Eucheuma denticulatum</i> and <i>Kappaphycus alvarezii</i> genes	131
<b>Othman, Roohaida*, Diana Mohd Nor, Mohammad Akhmal Ilias and Zeti-Azura Mohammed Hussein</b>	
Effects on cancer cell growth of saponin and fucoidan treated with thermophilic xylose isomerase from the marine bacterium <i>Thermotoga maritima</i> [Poster]	132
<b>Park, Seong-Hwan, Dong-Geun Lee, Mihyang Kim*, Chang -Suk Kong, and Sang-Hyeon Lee</b>	
Biosynthesis of polyhydroxyalkanoate in recombinant microorganisms using carbon source derived from terrestrial and marine biomass [Poster]	132
<b>Park, Si Jae*, Seung Hwan Lee, Young Hoon Oh and Jeong Geol Na</b>	
Insecticidal activity of marine organism <i>Scomber</i> spp. against <i>Tribolium castaneum</i> [Poster]	132
<b>Pathak, Rupa, Madhavi Indap*, Sweta Agrawal, Gargi Rane</b>	

Energy metabolic relationship of <i>Lamellibrachia satsuma</i> with its endosymbiont revealed by metagenomic analysis [Poster]	133
<b>Patra, Ajit Kumar, Yoshihiro Fujiwara and Sang-Jin Kim*</b>	
Selection of robust and high productivity marine macroalgae for renewable fuels.	133
<b>Paul, Nicholas*</b>	
<i>Anadara trapezia</i> functional genomics	134
<b>Pavasovic, Ana and Peter J. Prentis</b>	
Identifying the digestive enzyme repertoire of a herbivorous intertidal snail	134
<b>Pavasovic, Ana, Shorash Amin and Peter J. Prentis*</b>	
Anti-microbial potential of crude extracts of marine sponge- <i>Tethya spp.</i> and edible fish- <i>Scomber spp.</i> [Poster]	134
<b>Phatak, Rupa, Shashank More, Madhavi Indap*</b>	
Transcriptome characterisation and gene discovery in the marine shrimp <i>Fenneropenaeus merguensis</i> [Poster]	135
<b>Powell, Daniel*, Abigail Elizur Trevor Anderson, Courtney Remilton and Wayne Knibb</b>	
Rapid Harvest of Microalgae using a Novel Bacterial Isolate	135
<b>Powell, Ryan J.* and Russell T. Hill</b>	
Merging Metabolism and Power: Development of a Novel Photobioelectric Device Driven by Photosynthesis and Respiration	136
<b>Powell, Ryan J.*, Ryan White2 and Russell T. Hill</b>	
Trends in marine natural products research-a New Zealand perspective.	136
<b>Prinsep, Michèle*, Jonathan Puddick, Susie Wood, Nikki Webb, Chris Lockley, Alice Wang, Richard Hales, Chris Battershill, Ryan Martinus, John Blunt, Murray Munro, Robert Keyzers and Brent Copp</b>	
Exploring Australian marine biodiversity for producing next generation of biofuels	137
<b>Puri, Munish*, Adarsha Gupta, TamilSelvi Thyagrajan, Avinesh ByByreddy, Dilip Singh, Colin J Barrow</b>	
Algal Biotechnology: reshaping the coast and reforming algal industry	137
<b>Qin Song*, Zhengyi Liu, Wenjun Li, Jun Chen</b>	
Coastal Algal Biotechnology: Transforming changing bioresources to sustainable green industries	137
<b>Qin, Song*, Zhengyi Liu, Yinchu Wang, Yulin Cui and Hongli Cui</b>	
The Future for, and the challenges of, commercializing Marine Bioactives	138
<b>Quinn, Ronald J</b>	
Australia's Nagoya Approach and Opportunities from Nature Bank (Eskitis Institute)	138
<b>Quinn, Ronald J</b>	
Bacterial diversity, a comparison between the hydro-thermal vent and the non-vent region of Espalamaca	138
<b>Rajasabapathy, Raju*, Chellandi Mohandass, Ram Murti Meena</b>	
Isolation, culturing of a 'wild strain' of marine microalga and effect of temperature on its growth and lipid content	139
<b>Rane, Gargi*, Madhavi Indap</b>	
Lipid metabolism in <i>Emiliania huxleyi</i> : Recent advances in gene identification and biochemical analysis	139
<b>Read, Betsy</b>	
Characterisation of the effect stress on nitrogen metabolism in the commercially important agarophyte <i>Gracilaria gracilis</i> .	139
<b>Reddy, Amelia F.* and Coyne, Vernon E.</b>	

Draft genomes of four <i>Chlorella</i> strains.	140
<b>Reith, Michael*</b>	
The NRC Algal Carbon Conversion Flagship Program – a Canadian approach to sustainable algal biorefineries.	140
<b>Reith, Michael*, Stephen J.B. O’Leary and Aleks Patrzykat</b>	
Phenolic derivatives from South African kelps: pharmacological screening and <i>in silico</i> approaches towards new functional foods	140
<b>Rengasamy RR Kannan*, Mutalib A Aderogba, Wendy A Stirk and Johannes Van Staden</b>	
Biochar from marine macroalgae and their waste streams: yields, characteristics and uses.	141
<b>Roberts, David*</b>	
Coral algae as a source of UV-absorbing compounds	141
<b>Rosic, Nedeljka* and Sophie Dove</b>	
Dinoflagellates in symbiosis with reef building corals	141
<b>Rosic, Nedeljka*, E. Ling, C.-K. K. Chan, Paulina Kaniewska, D. Edwards, Sophie Dove and O. Hoegh-Guldberg</b>	
Stable isotopes as a tool for identifying <i>Maja</i> commercial species origin to guarantee its market traceability [Poster]	142
<b>Rotllant, Guiomar*, Enric Gisbert, Guillermo Guerao, Marc Uyà and Luis Cardona</b>	
Development of nutrigenomic tools to assess reproductive performance in shrimp	142
<b>Rotllant, Guiomar*, Nicholas M. Wade, Stuart J. Arnold, Melony J. Sellars, Gregory J. Coman, Nigel P. Preston and Brett D. Glencross</b>	
Unravelling muricid secondary metabolite biosynthesis, in situ, using surface assisted mass spectrometry imaging	143
<b>Rudd, David*<sup>1</sup>, Maurizio Ronci<sup>2</sup>, Taryn Guinan<sup>2</sup>, Nicolas Voelcker<sup>2</sup> and Kirsten Benkendorff<sup>3</sup></b>	
Feasibility study of bacterial lipopolysaccharide to increase black tiger shrimp survival under <i>Vibrio harveyi</i> challenge	143
<b>Runggrassamee, Wanilada*, Sawarot Maibunkaew, Nitsara Karoonuthaisiri and Pikul Jiravanichpaisal</b>	
Insulin-like androgenic hormones and monosex culture of prawns: The first implementation of RNA-interference in aquaculture	144
<b>Sagi, Amir</b>	
Rhogocyte cell isolation and characterization from mollusc’s tissue: The answer to hemocyanin biosynthesis bottleneck	144
<b>Sairi, Fareed *, Peter Valtchev, Vincent Gomes, Fariba Dehghani</b>	
Elemental selenium and tellurium formation by <i>Shewanella</i> microbes newly isolated from deep sea sediments in Japan Trench [Poster]	145
<b>Sakaguchi, Toshifumi*, Yusuke Nochida, Nobuyasu Tanabe, Katsuya Doi, Kaoru Nakasone, Chiaki Kato</b>	
Development of low-cost high-efficiency algae energy farms	145
<b>Schenk, Peer*, Skye Thomas-Hall, Ekaterina Nowak, David Lim, Tania Catalina Adarme-Vega, Kalpesh Sharma, Sourabh Garg, Rakesh Narala, Faruq Ahmed, Duong Van Thang, Ali Malekizadeh, Forough Ghasemi, Alexander Britten, Edilberto Medina-Cabrera, Nadia Abd Halim, Meng Chunxiao, Zhengquang Gao, Steven Pratt, Simon Tannock</b>	
Antimicrobial activity of the coral reef sponge <i>Crella cyathophora</i> [Poster]	146
<b>Schwartz, Inbar*, Shmuel Carmeli and Micha Ilan</b>	
Species identification and reproductive characteristic of the three <i>Mugil cephalus</i> cryptic species in Taiwan [Poster]	146
<b>Shen, Kang-Ning*, Chih-Wei Chang, Jean-Dominique Durand</b>	

Measurements of antioxidant activities in edible brown seaweeds extracts Obtained from supercritical CO <sub>2</sub> and solvent extraction [Poster]	147
<b>Siahaan, Evi Amelia, Yin Shipeng, Hee-Chul Woo<sup>2</sup>, Byung-Soo Chun*</b>	
Antimicrobial activity of total lipids extracted from Thai marine sponges [Poster]	147
<b>Siranonthana, Nisa*, Rawiwan Watanadilok and Somrat Taweedet</b>	
Biotechnology at the last bus stop: a New Zealand industry perspective	147
<b>Slim, George</b>	
Bioassay experiments reveal that the cyanobacteria <i>Anabaena variabilis</i> is associated with Loose Shell Disease (LSD) in shrimps [Poster]	148
<b>Somaraj, K Abhilash*, Babu T.D and Alavandi S.V (Vrinda Sukumaran presenting)</b>	
Immune Defence Mechanisms of Cultured Marine Invertebrates	148
<b>Song, Linsheng*</b>	
Metabolic engineering of Polyunsaturated fatty acid biosynthetic pathway in Yeast	148
<b>Sonkar, Shailendra P.*, Munish Puri and Colin J Barrow</b>	
Anti-MRSA and antioxidant activities of actinomycetes isolated from marine sponges [Poster]	149
<b>Srivibool, Rattanaporn*, Rawiwan Watanadilok and Subuntith Nimrat</b>	
A novel alkaline lipase obtained from the metagenome of marine sponge <i>Ircinia</i> sp. [Poster]	149
<b>Su, Jing, Fengli Zhang, Wei Sun, Zhiyong Li*, Qun Jiang*</b>	
RNA interference (RNAi) technology applied on the blocking of betanodavirus replication and host cell death	149
<b>Su, Yi C, Horng C Wu and Jiann R Hong*</b>	
Paramylon production by fed-batch cultivation of <i>Euglena gracilis</i> using waste in food industry [Poster]	150
<b>Suenaga, Tomoyuki*, Yumi Kawano, Yousuke Taoka, Motonari Sibakami and Masahiro Hayashi</b>	
Study of Proteins that Catalyze Silica formation and Polyunsaturated Fatty Acid synthesis in Marine Diatom <i>Chaetoceros gracilis</i>	150
<b>Suhartono, Maggy Thenawidjaja*, Alberta Rika Pratiwi, Dahrul Syah, Linawati Hardjito</b>	
Regulation of glycaemia with the application of recombinant CHH1 and its polyclonal antiserum in <i>Penaeus monodon</i> .	150
<b>Sukumaran Vrinda*, Jasmine C, Rosamma Philip and Bright Singh I.S.</b>	
The transcriptome sequencing and carbonic anhydrase analyses of marine microalga <i>Chlorella pyrenoidosa</i> (Chlorophyta) [Poster]	151
<b>Sun, Xue, Weiwei Wang, Nianjun Xu*</b>	
Study of Protein Interaction between <i>Penaeus monodon</i> Anti-lipopolysaccharide Factor Isoform 3 and White Spot Syndrome Virus	151
<b>Suraprasit, Sivalee, Thanachai Methatham, Phattarunda Jaree, Pakkakul Sangsuriya, Saengchan Senapin, Ikuo Hirono, Anchalee Tassanakajon and Kunlaya Somboonwiwat*</b>	
The <i>Pinctada fucata</i> BMP-2 induced the osteogenic differentiation of C3H10T1/2 murine mesenchymal stem cells [Poster]	151
<b>Takagi, Ryosuke*, Akiko Takami and Tomoyuki Miyashita</b>	
Modification of fatty acid composition by gene silencing of $\Delta 9$ desaturase in oleaginous diatom <i>Fistulifera</i> sp. strain JPCC DA0580 [Poster]	152
<b>Takahashi, Chisato*, Masaki Muto, Masayoshi Tanaka, Mitsufumi Matsumoto, Tomoko Yoshino and Tsuyoshi Tanaka</b>	
Isolation and identification of glycoproteins inhibiting adipocyte differentiation from scallop shells [Poster]	152
<b>Takahashi, Koji*, Keiichi Kushibe, Kazumi Sato, Yasushi Hasegawa</b>	

Screening for exolytic alginate lyase genes of bacteria isolated from marine environmental samples [Poster]	153
<b>Takahashi, Mami, Tetsushi Mori, Naoko Midorikawa, Toshiyuki Shibata, Kouichi Kuroda, Seinen Chow, Mitsuyoshi Ueda, Haruko Takeyama</b>	
Functional analysis of a key enzyme in PUFA synthesis, $\Delta 9$ desaturase, identified from the oleaginous diatom <i>Fistulifera</i>	153
<b>Tanaka, Masayoshi*, Masaki Muto, Chihiro Kubota, Akira Satoh, Mitsufumi Matsumoto, Tomoko Yoshino and Tsuyoshi Tanaka</b>	
Metabolic engineering of marine oleaginous diatom towards biofuel production	154
<b>Tanaka, Tsuyoshi*</b>	
Micronization of fucoxanthin from <i>Laminaria japonica</i> with biodegradable polymer-associated particles from gas saturated solution process [Poster]	154
<b>Tanbirul haque, ASM and Byung-Soo Chun*</b>	
<b>Identification of candidate genes</b> controlling the resistance to Taura syndrome virus in Pacific white shrimp ( <i>Litopenaeus vannamei</i> )	155
<b>Tang, Sureerat*, Siriporn Pongsomboon, Suchonma Sookruksawong and Anchalee Tassanakajon</b>	
Novel Lipopeptides, Kiostostatins A-E from a Marine-Derived Bacterium <i>Bacillus subtilis</i> [Poster]	155
<b>Tareq, Fakir Shahidullah*, Ji-Hyi, Min-Ah, Hyi-Seung, Jong-Seok, Yeon-Ju2, Hee-Jae Shin</b>	
How does the immune system of shrimps fight against pathogens	156
<b>Tassanakajon, Anchalee</b>	
Recombinant viral protein 24 (rVP24) of white spot syndrome virus-a new vaccine candidate in aqua vaccinology against WSSV	156
<b>Thomas, Ancy*, Issac Bright Singh, Viswanath Kiron, Rangarajan Badri Narayanan</b>	
Strategy to improve the cellular synthesis of lipids in two microalgas [Poster]	156
<b>Tonon, P Angela, Helena Vilella, Pio Colepicolo</b>	
Draft genome sequence of the dimorphic prosthocatace bacterium <i>Brevundimonas abyssalis</i> , isolated from deep-subsea floor sediment [Poster]	157
<b>Tsubouchi, Taishi*, Shinro Nishi, Keiko Usui, Yasuhiro Shimane, Tadashi Maruyama and Yuji Hatada</b>	
Photosynthetic Carbon Partitioning into Lipids and Polysaccharides in the coccolithophore <i>E. huxleyi</i>	157
<b>Tsuji, Yoshinori*, Masatoshi Yamazaki, Iwane Suzuki, Yoshihiro Shiraiwa</b>	
Isolation of lactic acid bacteria from the intestinal tract of bivalves [Poster]	158
<b>Uchida, Junya*, Sho Sakita, Daisuke Ichikawa, Shihori Takanashi, Tomoyo Narita, Koko Abe, Shiro Itoi and Haruo Sugita</b>	
First steps towards an environmentally friendly monosex population culture of spiny lobsters	158
<b>Ventura, Tomer*, Abigail Elizur, Amir Sagi, Quinn Fitzgibbon and Stephen Battaglione</b>	
Diversity and functionality of microbial symbionts associated with a two sponge symbioses in the Caribbean	158
<b>Vicente, Jan*, Russell T. Hill</b>	
Production biofuels and bioproducts using marine microalgae isolated from the coastal waters of China	159
<b>Wang, Guangyi*, Hui Guo, Liu Ying, Xuwei Yang, Guanyi Chen and Xianhua Liu</b>	
The Nervous-Endocrine System Mediates Immune Regulation in Scallops	159
<b>Wang, Lingling*, Linsheng Song</b>	
Composition and antimicrobial activity of partial peptidome of the Great Barrier Reef sponge <i>Amphimedon queenslandica</i> [Poster]	160
<b>Wang, Tianfang*, Stewart Michael, Hammond Michael, Bernie Degnan and Cummins Scott</b>	

De novo transcriptome sequencing of the heat-stressed snail <i>Echinolittorina malaccana</i>	160
<b>Wang, Wei*, Jerome H.L. Hui, Ka Hou Chu</b>	
The deep-sea natural products, biogenic polyphosphate (Bio-PolyP) and biogenic silica (Bio-Silica), as biomimetic scaffolds for bone tissue engineering: fabrication of a morphogenetically-active polymer	161
<b>Wang, Xiaohong* and Werner E.G. Müller</b>	
Methane production from saline derived microalgae biomass	161
<b>Ward, Andrew*, Andrew Ball, David Lewis</b>	
Comparison of immune parameters in cultured oyster ( <i>Saccostrea</i> sp.) along the eastern coast of Thailand [Poster]	161
<b>Watanachote, Janjarus*, Supanee Leethochavalit and Nareerat Rittirut</b>	
Analysis of the biomass composition of the demosponge <i>Amphimedon queenslandica</i> reveals marked variation within and between individuals	162
<b>Watson, Jabin, Bernard Degnan, Sandie Degnan and Jens Krömer</b>	
Molecular cloning, characterization of one key molecule of teleost innate immunity from orange-spotted grouper ( <i>Epinephelus coioides</i> ): serum amyloid A [Poster]	162
<b>Wei, Jingguang, Minglan Guo, Huasong Ji, Qiwei Qin*</b>	
Transdifferentiation of duct-like cells from hepatocyte through progenitor cells in zebrafish model of <i>Intrahepatic cholangiocarcinoma</i> [Poster]	163
<b>Wu, Sung-Yu*, Wangta Liu, Jen-Leih Wu</b>	
The Marine Biotechnology Enable Development of the Blue Bioeconomy in China	163
<b>Xiang, Jianhai*</b>	
RNA-Seq reveals the dynamic features of transcriptome during early development in pacific white shrimp <i>Litopenaeus vannamei</i>	163
<b>Xiang, Jianhai*, Jiankai Wei, Xiaojun Zhang and Fuhua Li</b>	
Design of Phthalimide Derivatives Based on Paecilocolin A as PPAR- $\gamma$ Activators [Poster]	164
<b>Xiao, Bin, Min Hi Park, Mingzhi Su, So Hyeon Eom*, Jongki Hong, Hae Young Chung, Jun Yin, Jee H. Jung</b>	
Physiological response of marine red algae <i>Gracilaria lemaneiformis</i> to different salinities stress [Poster]	164
<b>Xu, Nianjun*, Xue Sun, Xili Cai</b>	
Morphological regulation of cubo-octahedral magnetite crystal by the coordinated action of Mms proteins in magnetotactic bacteria	164
<b>Yamagishi, Ayana*, Atsushi Arakaki, Ayumi Fukuyo, Masayoshi Tanaka, and Tadashi Matsunaga</b>	
Se-containing antioxidant "selenoneine" in tuna blood and its roles in selenium redox metabolism and methylmercury detoxification	165
<b>Yamashita, Michiaki*, Shintaro Imamura, Takeshi Yabu, Ken Touhata, Kenji Ishihara, Yumiko Yamashita</b>	
Optimization of medium using response surface methodology for the lipid production by <i>Scenedesmus</i> sp.	165
<b>Yang, Fangfang, Lijuan Long, Junde Dong, Xiumei Sun, Tao Li, Hualian Wu, Wenzhou Xiang*, Yunming Lu*</b>	
Simple lipid extraction method without heating from wet microalga <i>Picochlorum</i> sp.	165
<b>Yang, Fangfang, Wenzhou Xiang, Xiumei Sun, Tao Li, Hualian Wu, Lijuan Long*</b>	
Effect of oxidized fish oil on growth performance and oxidative stress of <i>Litopenaeus vannamei</i> [Poster]	166
<b>Yang, Shi-Ping*, Hui-Ling Liu, Cheng-Gui Wang, Ping Yang, Cheng-Bo Sun, Siu-Ming Chan</b>	
Seasonal variations on organic and inorganic components of <i>Ulva pertusa</i> with environmental factors in Jeju, Korea	166
<b>Ye, Bo-Ram*, Jiye Jang, Young-Kyung Kwon, Do-Hyung Kang, Chulhong Oh, Soo-Jin Heo</b>	



Anti-proliferative effect of <i>Pylaiella littoralis</i> extract on HT29 cells [Poster]	167
<b>Ye, Bo-Ram*, Jiyi Jang, Young-Kyung Kwon,, Ji Hyung Kim, Youngdeuk Lee, Su-Jin Lee, Do-Hyung Kang, Chulhong Oh, Soo-Jin Heo</b>	
Potential antioxidant capacities of ethanol and enzymatic extracts of <i>Pylaiella littoralis</i> collected from Federated States of Micronesia [Poster]	167
<b>Ye, Bo-Ram*, Jiyi Jang, Young-Kyung Kwon, Taeho Kim, Seon-Mi Jeon, Do-Hyung Kang, Chulhong Oh, Soo-Jin Heo</b>	
Characterizing the role of diazotrophs in the symbiotic microbial community associated with two marine sponges	168
<b>Zhang, Fan* and Russell T. Hill</b>	
An efficient <i>E. coli</i> secretory expression system to produce recombinant chitin-degrading related enzymes	168
<b>Zhang, Jiquan*, Sun Yuying, Wang Jing, Gui Tianshu, Xiang Jianhai</b>	
Female specific markers and attempts of all-female production in half-smooth tongue sole ( <i>Cynoglossus semilaevis</i> )	168
<b>Zhang, Quanqi*, Xubo Wang, Haiyang Yu, Zhigang Wang and Xinglian Wang</b>	
Marine Biotechnology Industry Development in Australia: An Ocean of Opportunities for Australian and International Partners	169
<b>Zhang, Wei*, Tham, Raymond</b>	
Novel approach to decipher interactions between marine sponges and their microbial symbionts/pathogens	169
<b>Zhang, Wei</b>	
Mapping and matching hotspots of biodiversity, biochemical and bioactivity diversity for advanced Marine Park policy in South Australia	170
<b>Zhang, Wei, Jan Bekker, JingJing Wang, Shuang Peng, Shirley Sorokin, Jason E Tanner, Raymond Tham*</b>	
Artificial breeding technology of <i>Bohadschia argus</i> made great progress in China [Poster]	170
<b>Zhang, Zhuhuai*</b>	
Exceptional lipids in nudibranch mollusks: evolution, diets, symbionts and biosynthesis	170
<b>Zhukova, Natalia V.</b>	

# ***IMBC 2013 ABSTRACTS***

***ALPHABETICAL ORDER BY AUTHOR LAST NAME***



## A synthetic biology approach to develop a novel marine cyanobacterial bioprocesses -The Cyanofactory™-

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Our research group is engaged in the development of a novel bioprocess based on the synthetic biology concept, for bioenergy production, designated as the "Cyanofactory™". The Cyanofactory™ is composed of **1)** synthetic marine cyanobacterial host strains, **2)** synthetic operons for the production of biofuel-related compounds, and **3)** the employment of ionic liquids for downstream processing. The combination of downstream processes employing the "Green solvents", ionic liquids realizes the sustainable production of biofuel-related compounds based on synthetic cyanobacterial processes with minimal energy and waste. "Synthetic marine cyanobacterial host strains" are the engineered cyanobacteria whose functions are highly controlled by the artificial signal transduction system, which is composed of the light sensors / histidine kinases, the response regulator and the riboregulator / riboswitch systems. This system is regulated by the light stimulation frequency and aiming the control of cell growth, biofuel-related compound production, self-aggregation and auto-lysis by regulation. In order to realize the light regulated artificial signal transduction system, we have developed the expression vector for green light induction system, based on engineered two component regulation system. Using this expression vector, green light regulated cell lysis of cyanobacteria was realized. The current progress in the development of riboregulators functioning in cyanobacteria will also be reported in this paper.

## Genomes of calcaronean sponges: simple body plans and surprisingly complex developmental toolkits

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Sponges are an important subject of biological research as evolutionarily ancient animals, producers of highly bioactive compounds and exquisite silica or calcium based skeletal elements. We are using calcareous sponge *Sycon ciliatum* as a model for comparative developmental biology studies. We have used Illumina HiSeq technology, including paired end and mate pair libraries, to sequence its genome and annotate it with an extensive transcriptome dataset. We have first focused our analysis on key components of the developmental toolkit: signaling pathways and transcription factors. Many of these gene families are significantly larger in *Sycon* than in the previously sequenced *Amphimedon queenslandica*, a representative of demosponges. In several cases, gene numbers within *Sycon* subfamilies exceed these in eumetazoans. Phylogenetic analyses indicate that gene family expansions leading to complexity in *Sycon* are independent of these leading to complexity in the eumetazoans. To gain insight into evolutionary history of this complexity, we have next sequenced genome and transcriptome of *Leucosolenia complicata*, a distantly related calcaronean with a different body plan. With some minor differences, the two genomes appear to contain equally complex developmental toolkits, suggesting the expansions of the families predates or coincides with the emergence of calcaroneans. Further investigation led us to sequencing genomes of two *Clathrina* species representing Calcineans, a sister group to the Calcaroneans: *C. coriacea* from North Atlantic and *C. laminoclathrata* from Indian Ocean (West Australia), and we are currently analysing their genomes. The generated resources will provide background for evolutionary and developmental studies and beyond.

## Photosynthetic microalgae: a sustainable source of omega-3 fatty acids from for nutraceuticals and aquaculture feed

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Omega-3 fatty acids ( $\omega$ -3) eicosapentaenoic acid (EPA) and docohexaenoic acid (DHA), provide significant health benefits for brain function/development and cardiovascular conditions. Due to these valuable properties commercial demand for  $\omega$ -3 has significantly increased over the past decades. Most EPA and DHA for human consumption are sourced from small fatty fish caught in coastal waters and, with depleting global fish stocks, recent research has been directed towards more sustainable sources. These include krill, marine microalgae and genetically-modified plants. Photosynthetic microalgae are the primary producers of  $\omega$ -3 in the marine food chain and, to meet the increasing demand for EPA and DHA, developments are underway towards land-based sources, in particular microalgae.  $\Omega$ -3 content is strongly correlated with the conditions at which microalgae are cultivated. Our studies on abiotic stress factors during microalgal growth led to an induction of fatty acid accumulation, for storage of chemical energy and as a protection mechanism against oxidative stress. Results varied greatly for different species, but generally optimum temperature, high irradiance and low salinity often induced higher proportions of EPA. The integrated interaction of these parameters constitutes a further optimisation step for large-scale  $\omega$ -3 production from microalgae.

## Pigment cell differentiation in blastula-derived primary cell cultures of sea urchins

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Quinone pigments from sea urchins constitute a group of polyketide compounds with highly effective antioxidant, antibacterial, antifungal, and antitumor activities and may play a vital role in immune defense. One of them, echinochrome, is synthesized in sea urchin pigment cells in larvae and in adults. We have developed in vitro technology for inducing pigment differentiation in the blastula-derived cell culture. Two-fold differences in the pigment cell number were detected between the cells cultivated in coelomic fluids of normal and injured sea urchins. The origin of this phenomenon seems to be due to the protein composition changes in the coelomic fluid after injury, as shown by MALDI MS. In addition, the pks gene expression was found to increase in the cells cultivated in the coelomic fluid of injured sea urchins at the presence of shikimic acid, a precursor of naphthoquinone pigments. Intensification of pigment differentiation was accompanied by a simultaneous decrease in the number of dividing cells. Thus, our results could contribute to the development of new techniques in marine biotechnology. This study was supported by the RFBR (12-04-00363a), FEB RAS (12-I-P6-07, 12-III-A-06-084), and the Program at the FEFU (11 G34.31.0010).

## Anti-angiogenic potential of a marine gastropod *Euchelus asper* [Poster]

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It is known that cancer growth and metastasis are angiogenesis dependent and inhibition of tumour angiogenesis can be a successful tool in cancer therapeutics. Now-a-days, emphasis is being given on natural products as a source of therapeutic drugs as they are more promising than the synthetic drugs. The marine environment is a rich source of bioactive natural products. Many of the marine bioactive compounds have been derived successfully from molluscs. The shell and flesh of gastropods is used in traditional ethno-medicine for weakness, stomach disorders, asthma, TB, eye related problems, etc in India. *Euchelus asper* is one of the marine molluscs which are commonly found in the intertidal rocky regions of the Mumbai coast. Methanolic extract of *Euchelus asper* has shown immunomodulatory and anti-osteoporetive activities. The present study focused on evaluating anti-angiogenic activity of methanolic extract of *Euchelus asper*. Anti-angiogenic activity was assessed by chick chorio-allantoic membrane (CAM) model by using different concentrations of the extract. Analysis of CAM images revealed that the extract is effective in reducing the branching points of the 1<sup>st</sup> order blood vessels (capillaries) of CAM. Samples treated with lower concentration (50 $\mu$ g/ml, 100 $\mu$ g/ml) showed slight decrease in their capillary density while those treated with higher concentration (200 $\mu$ g/ml, 400 $\mu$ g/ml, 800 $\mu$ g/ml) had a marked decrease in the capillary density as compared to control. Histological images of capillary plexus in the CAM were also analysed and results were found to be confirming the image analysis data. Also, cytotoxic effect of the extract was evaluated by using SRB assay.

## The evolutionary origin of molluscan shell matrix genes: comparative analysis of ten molluscan mantle transcriptomes

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The Mollusca is one of the most successful metazoan phyla and its evolutionary success can be in part attributed to the evolvability of an external mineralised skeleton – the shell. Here, we analyse genes expressed in the shell-forming tissue, the mantle, of ten gastropod and bivalve species to study the evolutionary origin and conservation of molluscan shell matrix gene families. We identify 792 gene families expressed in these mantles that encode secreted proteins. Of these, over 19% of the families appear to be mollusc-specific innovations and are not found in any other animal or eukaryotic genomes. A further 48%-73% appear to be even more recent innovations and are restricted to specific gastropod or bivalve taxa. This high proportion of taxon-restricted shell matrix genes suggests that these genes contribute to phenotypic differences observed between the shells of different, sometimes closely-related, molluscs. For example, there are 46 new gene families inferred to have evolved in the pearl oyster lineage (*Pinctada* spp.) whose most recent common ancestor lived only ~13 million years ago. Most of these gene families encode proteins containing repetitive, low-complexity domains (RLCDs). The intrinsic instability of repetitive sequences encoding the RLCDs appears to increase gain, loss and shuffling of these domains and motifs. This mode of molecular evolution is likely to contribute to structural characteristics and evolvability of gastropod and bivalve shells during the course of molluscan evolution.

## Microalgae as a source of phytosterols

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Phytosterols are now well established as health compounds and find applications in the pharmaceutical (therapeutic steroid production), nutritional (additives in functional foods to lower cholesterol and anticancer properties), and cosmetics (creams, lipstick) industries. With the expansion of the market (currently \$300M), research is focussing on identifying new natural sources of phytosterols. Microalgae have recently been demonstrated to produce large amounts of these functional compounds. Biomass from eleven microalgae strains from south-east Queensland were analysed for phytosterol composition by GC-MS. *Pavlova lutheri* had the highest total sterol content (25.9 mg/g dry weight) followed by *Tetraselmis striata* (4.3 mg/g), *Nannochloropsis* spp. (4.04 mg/g) and *T. chui* (3.6 mg/g). The number of sterol compounds in each strain varied from two (*T. suecica*, *T. striata*, *T. chui* and *Isochrysis galbana*) to ten (*P. lutheri*). Among the different sterol compounds identified, 24-methylcholest-5-enol was found in almost all strains and was the dominant sterol (>90%) in all three *Tetraselmis* species. The second most abundant sterol was cholesterol and was the main compound in *Nannochloropsis* spp. and *Chaetoceros muelleri* (80%). Further induction studies (salinity and nutrient variation) are underway for the top two phytosterol producers (*P. lutheri* and *T. striata*) to identify their commercial suitability for sterol production.

## Neoechinulin A isolated from marine-derived *Microsporum* sp. suppresses sebum accumulation in insulin-like growth factor (IGF)-1 differentiated human sebocytes [Poster]

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Acne vulgaris is one of the most common skin diseases and the pathogenesis of this disorder involved the increased production of sebum. Insulin like growth factor-1 (IGF)-1 stimulated lipid production in sebaceous gland. In this study, we evaluated effects of neoechinulin A on sebum accumulation of IGF-1 differentiated sebocytes by measuring lipid accumulation and transcription factors capable of regulating lipid production. Treatment with neoechinulin A reduced the triglyceride production in Oil-Red O staining and down-regulated lipid biosynthesis related factors SREBP-1, PPAR $\alpha$  and C/EBP $\beta$ . Forkhead box O1 (FoxO1) transcription factors regulate the activity of important target genes involved in the pathogenesis of acne. The potent functions of FoxO1 protein are tightly controlled by phosphoinositide-3kinase (PI3K)/Akt signaling pathways under physiological conditions. The results indicated that neoechinulin A inhibited the expression phosphorylation of FoxO1 was decreased via blocking the PI3K/Akt signaling pathway. These findings may contribute to a understanding of the molecular mechanisms of neoechinulin A for acne treatment and prevention.

## Profiling the phospholipid residues of krill oil by $^{31}\text{P}$ -NMR and regioisomeric distribution of polyunsaturated fatty acids in its triacylglycerol

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Phospholipid (PL) and triacylglycerol (TAG) from krill oil were isolated and purified using thin layer chromatography (TLC) via a series of carefully selected and controlled solvent systems. The separation and purification of both PL and TAG was monitored using capillary chromatography with flame ionisation detector (Iatroscan) while their fatty acid compositions were determined using gas chromatography (GC).  $^{13}\text{C}$  nuclear magnetic resonance (NMR) was used to determine the regioisomeric distribution of polyunsaturated fatty acids in its TAG while Phosphorus ( $^{31}\text{P}$ ) NMR was used to identify the PL classes. GC data shows higher amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the PL as compared to the TAG while  $^{13}\text{C}$  NMR results showed that DHA is more in positions 1 and 3 (*sn*-1,3) and EPA is enriched in position 2 (*sn*-2) of the TAG.  $^{31}\text{P}$ -NMR analysis showed that the major PL classes are phosphatidylcholine (PC), alkylacylphosphatidylcholine (AAPC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE) and N-acyl-ethanolamine phospholipids (NAPE) and their relative amounts were determined by integration of the peak areas of each residue. The structures and functions of these PLs are also presented and discussed briefly.

## Transcriptome analysis of freshwater crayfish (*Cherax quadricarinatus*) and characterisation of gill-expressed carbonic anhydrase genes

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Carbonic anhydrase (CA) is a family of Zn-metalloenzymes that catalyze the inter-conversion of carbon dioxide and water to  $\text{HCO}_3^-$  and  $\text{H}^+$  playing an important role in systemic acid-base balance. While Redclaw crayfish (*Cherax quadricarinatus*) occur naturally in streams that vary in pH range, to date no studies have focused on the identification and characterization of CA genes in this species, which is essential to address pH stress-physiology. Transcriptome analysis of Redclaw gills was undertaken and CA genes expressed in the gill tissues exposed to low pH (approximately 5) were identified and characterized. We obtained a total of 72,382,710 Illumina paired end reads, which were assembled into a total of 36,128 contigs with an average length of 800bp. The assembled sequences were used as BLASTx queries and also assigned gene ontology functions. Approximately 37% of the contigs received significant BLAST hits and 22 % were able to be assigned gene ontology terms. We identified three complete CA isoforms; cytoplasmic CA (CAC), glycosyl-phosphatidylinositol-linked CA (CAG), and  $\beta$ -CA; and two partial CA cds. CAC consists of 271 amino acids (total length 1527 bp), CAG 269 aa (total 3265 bp) and  $\beta$ -CA 255 aa (total 1021 bp). The two partial cds have a length of 128 aa (total 390 bp) and 165 aa (total 495 bp). Phylogenetic analysis of the CA genes showed that both the partial CA genes appear to be a recently diverged, duplicated copy of the CAG gene. Protein alignments with other marine decapods showed that most amino acids in active and metal binding sites are conserved in each of the three different CA genes. These data will provide valuable information on CA genes and their functions in crayfish that can assist further in-depth study of this gene family. Additionally, the large number of transcripts recovered here will also provide a good resource for identification of novel genes in freshwater crayfish.

## Identifying genes involved in physiological adaptation of *Nerita melanotragus* to temperature stress using comparative transcriptome sequencing [Poster]

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As climate change continues to impact marine environments, shifts in biotic and abiotic factors within marine ecosystems are evident. How species respond and adapt to these climate driven changes is critical in determining which marine species will be most affected. The understanding of the physiology of many marine species, in particular molluscs, is limited and making it difficult to predict the future effects of climate change. This research aims to increase our understanding of physiologically important genes in intertidal snails by: (1) developing large transcriptomic datasets for 2 closely related intertidal gastropod species *Nerita melanotragus* and *N. albicilla*, (2) selecting candidate genes involved in temperature, salinity and oxygen responses, (3) calculating the synonymous and non-synonymous substitution rates for candidate genes and (4) comparing key candidate gene sequences with both close and distantly related species. *Nerita albicilla* was collected from the species southern most distribution, while the *N. melanotragus* specimen was collected from its northern most distribution. Illumina sequencing of *Nerita melanotragus* and *N. albicilla* produced 68,678,334 and 61,367,256 reads respectively, from whole organism tissue. From these reads, *de novo* assembly produced 77098 and 78577 contigs respectively. A variety of genes involved in response to temperature, salinity and oxygen have been identified from the dataset, including a suite of extensively duplicated heat shock proteins, carbonic anhydrases and globin genes. The large number of contigs that have been annotated and functionally characterized in this study provide a first step towards a systems biology approach to understanding the response of gastropod species to environmental stress associated with climate change.

## Next-generation genomics for bioproducts discovery [Poster]

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The completion of the human genome sequencing in 2003 opened a new perspective into the importance of whole genome sequencing projects, and currently multiple species are having their genomes completed sequenced, from simple organisms, such as bacteria, to more complex taxa, such as mammals. This voluminous sequencing data generated across multiple organisms provides also the framework to better understand the genetic makeup of such species and related ones, allowing the assessment of its evolutionary histories and patterns of genetic diversity that can be highly valuable for bioproducts discovery and the understanding of the molecular diversity associated with such biomolecules. The utility of genomics in the biodiversity assessment will be discussed, with emphasis on case studies comprehending the identification of bioproducts in marine and terrestrial species, and their relevance for biomedical applications.

## Role of hemocyte homeostasis associated protein (HHAP) in regulation of hemocyte apoptosis from black tiger shrimp *Penaeus monodon* [Poster]

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Hemocyte plays a key role in the defense against pathogen infection in invertebrates. The production of hemocyte as well as the controlling of hemocyte level is important for health and immune function in shrimp. Hemocyte homeostasis-associated protein (*PmHHAP*) has been shown to be essential for shrimp survival and plays an important role in controlling the hemocyte homeostasis in the black tiger shrimp *Penaeus monodon*. However, the role of *PmHHAP* in maintaining shrimp hemocyte homeostasis remains unknown. In the present study, the potential function of *PmHHAP* was further characterized. Gene silencing of *PmHHAP* by RNA interference resulted in the characteristic of apoptotic DNA ladder. Moreover, double-stranded RNA (dsRNA)-mediated gene silencing of *PmHHAP* resulted in increased the number of annexin V-positive apoptotic cells compared to the dsGFP RNA injected control. This result suggests that *PmHHAP* might play a role in mediating shrimp hemocyte apoptosis. To further identify the *PmHHAP*-target genes, a suppression subtractive hybridization (SSH) cDNA library was constructed. Interestingly, several up-regulated genes identified as likely to be associated with homeostasis including transcription factor and apoptotic-related proteins were obtained. Some apoptotic genes were confirmed by real-time RT-PCR. Correspondingly, the results revealed that suppression of *PmHHAP* gene affects the transcript levels of apoptotic genes. These results suggest that *PmHHAP* might play a role in hemocyte homeostasis through the regulation of the apoptotic genes.

## Transcriptome analysis of genes associated with cold-inducible lipid biosynthesis in *Emiliana huxleyi*

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Haptophyte algae are known to produce very long (C<sub>35</sub>-C<sub>40</sub>) straight-chain ketones, namely alkenones. A molecule of alkenone has 2 to 4 trans-type unsaturation bonds and such feature is different from most fatty acids possessing cis-type unsaturation bonds. Alkenones are known to be detected from only Isochrysidales in Haptophyta. The number of unsaturation bond and the amount of alkenones produced by cells is known to increase when cells were grown at low temperature and therefore the unsaturation index of alkenones has been used as a paleothermometer for reconstructing paleotemperature in marine paleoenvironments. However, the function and synthetic pathway of alkenone are not known yet. In this study, we performed transcriptome analysis to obtain some hints on alkenone biosynthetic pathway under conditions where the unsaturation degree of alkenones increased without change in its amount by transferring cells to low temperature. Almost all genes related to fatty acid biosynthesis and very long chain fatty acid biosynthesis are expected to be up-regulated under low temperature condition. By factor analysis, we found four such genes predicted as fatty acid desaturase, acetylcholine esterase, choline transporter-like protein and phospholipid scramblase in *E. huxleyi* database. These evidences suggest that the biosynthesis of fatty acids and very long chain fatty acids may be contributed to alkenone biosynthesis. Finally, the fatty acid desaturase identified in this study may specifically function as alkenone desaturase in *E. huxleyi*.



## Antihypertensive effect and antioxidant activities in mackerel muscle hydrolyzate recovered by subcritical water [Poster]

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Food obtained bioactive peptides show a wide range of physiological functions including antihypertensive, antioxidative, antimicrobial etc. Fish proteins have massive potentiality as novel sources of bioactive peptides. The aim of this study was to produce peptide containing hydrolyzate from mackerel muscle protein which has antihypertensive and antioxidant effect. The subcritical water hydrolysis was carried out in 200 ml of a batch reactor made of 276 Hastelloy with temperature control. Supercritical carbon dioxide (SC-CO<sub>2</sub>) extracted mackerel muscle residues and water with different catalysts such as sodium chloride, sodium bicarbonate, sodium hydroxide, acetic acid, formic acid, carbon dioxide gas and nitrogen gas were put in the reactor and closed. Material to water ratio of this experiment was 1:16 (w/v). High pressure pump was applied to flow air from tank to reactor for getting initial pressure 2 bar. The reactor was heated by an electric heater which was previously heated to the desired temperature (220-260) °C and pressure (40-100) bar. The sample was stirred by magnetic stirrer at 150 rpm. The reaction time for each sample was taken 3 min. After cooling to room temperature, the hydrolyzed sample from the reactor was collected and filtered using a filter paper. Hydrolysis yield was increased after increasing the temperature and pressure. Bioactive peptides in the hydrolyzate will be analyzed by SDS-PAGE electrophoresis and antihypertensive effect of the hydrolyzate will be analyzed by o-phthalaldehyde (OPA) method. Beside this, antioxidant activities of the hydrolyzate also will be analyzed by DPPH, ABTS and reducing power assay.

## Dissecting dinoflagellate evolution

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Dinoflagellates are ubiquitous members of the marine phytoplankton and are important primary producers. The focus of this study is on the ability of dinoflagellates to acquire and incorporate genes from other lineages. For example, the peridinin plastid found in dinoflagellates has lost most of its genes to the nuclear genome, as has the mitochondrial genome. Aside from organellar genes, specific genes, such as a form of rubisco otherwise only found in anoxygenic bacteria were likely acquired via horizontal transfer. The genes from transcriptomes of several dinoflagellates were sorted into three categories, host, plastid and likely bacterial gene transfers. Generally carbon- processing enzymes appear to be derived from the bacterial category. These genes also appear to be subject to duplication and fusion, although gene duplication in dinoflagellates includes bone fide host genes as well. Opportunities for gene transfer include symbioses and flexible trophic modes including mixotrophy. The unique dinoflagellate genome, with tandem gene repeats, less dependence on transcriptional regulation, and trans-splicing might also facilitate gene transfer. Overall, gene transfer in dinoflagellates provides strong support for a new Lamarkian synthesis of eukaryotic evolution.

## Isolation and structure elucidation of novel saponins from the sea cucumber *Stichopus hermanni* viscera using HPCPC and mass spectrometry

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Sea cucumbers, sometimes referred to as marine ginseng, produce numerous compounds with diverse functions, and are potential sources of active ingredients for agricultural, nutraceutical, pharmaceutical and cosmeceutical products. This project aimed to identify and characterise novel bioactive compounds from the viscera of an Australian sea cucumber *Stichopus hermanni* Semper, 1868, with an emphasis on the triterpene glycosides, saponins. The viscera were extracted with 70% ethanol and this extract was purified by a liquid-liquid partition process and solid column chromatography using Amberlite XAD-4 resin, followed by iso-butanol extraction. The iso-butanol saponins-enriched mixture was further purified by high performance centrifugal partition chromatography (HPCPC). The resultant purified polar samples were analysed using high performance liquid chromatography (HPLC) and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-ToF/ MS) and ESI-MS, and MS/MS to identify saponins and characterise their molecular structures. As a result, at least 35 saponins were tentatively identified in the viscera of *S. hermanni* with a high structural diversity, including 20 new sulphated and non-sulphated triterpene glycosides, containing different aglycone and sugar moieties. The TLC profiles of the purified saponins mixture and MALDI analyses revealed that this species possesses a unique saponins pattern, which could be used for taxonomic classification of this species. The high structural diversity of saponins from *S. hermanni* with potential functional activities presents a great opportunity to exploit their applications for industrial, agricultural and pharmaceutical use.

## Effect of Bioactive Compounds from Marine Sponge *Tethya spp.* on Bone resorption [Poster]

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Estrogen deficiency associated osteoporosis following menopause, is by far the most common cause of age-related bone loss. Although estrogen is established to have direct effects on bone cells, animal studies have identified additional regulatory effects of estrogen centered at the level of the adaptive immune response. Since many years marine sponges have been considered as a gold mine with respect to the discovery of bioactive compounds with high potential pharmaceutical applications. The present study investigates the anti-osteoporotic activity of petroleum ether extract of marine sponge "*Tethya spp.*" collected from intertidal zone along rocky shore of Khardanda, Mumbai in the *in vivo* ovariectomized (OVX) mice model. An effect of extract on osteogenic differentiations was assessed by Histopathological, Biochemical methods, Immunophenotypic analysis and Raman spectrometry. Oral administration of SPPE (Sponge petroleum ether extract) successfully inhibited the detrimental effect brought about by estrogen deficiency in bone architecture and restored broken trabeculation as well as bone morphology. Extract significantly promoted osteogenesis by elevation in alkaline phosphatase activity and serum calcium level to normal level in a dose-dependent manner indicating a decrease in osteoclastogenesis. The immunophenotyping data also shows that the SPPE was able to regulate bone resorption through T cell regulation by reducing the elevation; activation and stimulation of T cells in estrogen deficient mice. Raman spectra analysis also revealed an increase in mineralization of the bones treated with SPPE. These data suggests that SPPE has a role to play in bone reversal in OVX mice model.

## Efficient precision genome engineering in the marine polychaete *Platynereis dumerilii* using Transcriptional activator like effector nucleases (TALENs).

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TALENs are a class of recombinant nucleases that have been rapidly adopted as the tool of choice for precision genome engineering in conventional model organisms. TALENs can be custom designed to induce DNA double-strand breaks (DSBs) at specific genomic loci, which promotes the introduction of loss-of-function, indel mutations during repair of DSBs by non-homologous end joining (NHEJ). This, together with the fact that TALENs can be designed to target any gene of interest, makes TALENs powerful tools for *in vivo* genome modification in any species of interest. *Platynereis dumerilii* (lophotrochozoa, annelida) is a slowly-evolving marine polychaete worm that features an ancestral body plan. In addition to its use as a model for understanding the evolution of developmental gene regulatory networks, *P. dumerilii* is a key model organism for studying the genetic control of lunar reproductive timing. This is because spawning in *P. dumerilii* is synchronised to the lunar cycle: a phenomenon observed for many marine invertebrates. With the particular aim of allowing in-depth functional analyses of genes implicated in the control of reproductive timing, we optimised a platform for generating transgenic loss-of-function mutations in *P. dumerilii* using TALENs. Using this platform, we have engineered loss-of-function mutations in 3 endogenous genes. TALEN-mediated mutations are heritable, making this an efficient approach for generating homozygous knockout lines. Our established TALEN platform allows us to pursue functional genetics studies for the first time in *P. dumerilii*, and more widely demonstrates the utility of TALENs as tools for precision genome engineering in marine species.

## **Omega-3 biotechnology: Sources, concentration methods, microencapsulation and bioactive derivatives.**

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The health benefits of long-chain omega-3 fatty acids are well established, especially for eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) from fish and microbial sources. In fact, a billion dollar market exists for these compounds as nutritional supplements, functional foods and pharmaceuticals. This presentation will describe some aspects of our omega-3 biotechnology research being carried out at Deakin University. These include the use of lipases for the selective concentration of omega-3 fats, through immobilization of these lipases on nanoparticles, and the microencapsulation and stabilization of omega-3 oils for functional foods. Work on the enzymatic production of resolvins using lipoxygenases will also be described. Finally, the collection and utilization of marine micro-organisms for the production of omega-3 oils and biofuels will be discussed.

## **Preliminary genome sequencing, denovo assembly and annotation of the Neogastropoda *Dicathais orbita* [Poster]**

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Currently the complete genome has only been published for one molluscan species (*Crassostrea gigas*, Bivalvia) and whilst a few gastropods have been sequenced, their assembly and annotation is still in progress. In this study we report the genome sequencing, denovo genome assembly and annotation of the Australian marine gastropod *Dicathais orbita* (Muricidae Neogastropoda), which is well known for the production of anticancer precursors of the pigment Tyrian purple. With the absence of a reference genome it is a challenging task to assemble its large and complex ~2.2 billion base pair genome in a denovo fashion. DNA was extracted from the foot tissue and sequenced on an Illumina GAII with 150nt reads from 400bp and 700bp insert size libraries. After quality control of the data we had ~15x coverage of the genome. Initial CLC denovo assembly produced ~1.2 million contigs totalling ~800mb with an N50 size of ~1kb. The highly fragmented assembly output indicates the complex and repetitive nature of the *Dicathais* genome. Initial repeat analysis reveals ~35% of the genome is repetitive. Maker gene annotation pipeline consisting of SNAP and Augustus gene finding modules and trained with oyster genes predicted ~104,000 gene models. A large number of the multi exon genes are predicted as separate genes due to fragmented assembly, however, search against UNIPROT database, oyster genes and *Lottia gigantea* (Gastropoda) genes reveal good similarity. Our preliminary results indicate the need for generating further data and analysis to improve the assembly and annotation of this complex gastropod genome.

## New Zealand Marine Biotechnology: a successful past should now inform a successful future.

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Marine biotechnology *per se* in New Zealand currently, is to many an emerging developmental sector with public and politicians alike frequently assuming the field is centred on aquaculture and development of value added products from aquaculture waste. New Zealand has however had a long and rich history in marine biotechnology, particularly in drug discovery from the sea. The short corporate memory is sadly due to a downturn in national public good funded scientific effort in recent times. New Zealand has been acknowledged for its pioneering work in the field of development of drugs from the sea. Very early on, collaborations between chemists, notably the University of Canterbury marine natural products research group led by Professors Munro and Blunt together with biologists like the Late Dame Professor Pat Bergquist and her students, together with the work done at the Cawthron Institute and University of Auckland, created novel approaches to exploration of bioactive medicinal leads from the marine estate. For a relatively limited investment in effort to date in New Zealand, the success of marine leads as therapeutics in particular, promotes enhanced focus on marine biodiversity as a source of useful medicinal agents. Notable are the leads from the Halichondrins, Mycalamides and Pateamine. New Zealand has arguably one of the highest success rates for discovery of leads that advance to pre-clinical trial of any country based on unit research effort. This is due in part to high endemism in biodiversity and an approach based on chemotaxonomy, and chemical ecology. With this successful backdrop, it is surprising then that the country is not advancing with more unified strength in developing marine natural products for medicine and indeed other applications. With the Treaty of Waitangi and Wai262 legislation, New Zealand should also benefit from a clear understanding of the provenance of its' biodiversity in terms that satisfy the Access and Benefit Sharing components of the Convention of Biodiversity. The country is arguably poised for a new generation of research and development initiatives that will benefit from advances in marine natural products screening and chemistry but also from the significant recent progress in synthesis and supply. We will provide a review of marine biotechnology with a focus on the biomedical sector, in as much as New Zealand has been involved, and explore a pathway for re-asserting this field down under.

## *Dicathais orbita* as a model for marine natural product screening and nutraceutical development

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The Australian marine mollusc *Dicathais orbita* has been identified as a useful model species for brominated indole biosynthesis, and is currently under development for potential nutraceutical production. Useful biological traits for the selection of model research species include ready availability and life history features that make them easy to manipulate and maintain in the laboratory, as well as genetic knowledge and potential economic benefit. *D. orbita* is a relatively large, long-lived and highly fecund gastropod that is common on rocky reefs in temperate Australian and can be easily maintained in laboratory aquaria and on aquaculture farms. Its taxonomy is well resolved and preliminary genome sequencing is underway. To be suitable as a model system for innovative natural products chemistry research, extensive familiarity with the secondary metabolism system is also required. Muricidae molluscs are well known for the production of brominated indoles with associated anticancer activity. The organic extracts and purified compounds from *D. orbita* have been screened in a wide range of *in vitro* assays and subjected to preliminary *in vivo* efficacy and safety testing. These well characterized extracts have been used to optimize the efficiency of the preclinical screening process by maximizing the data obtained from a single experiment. Examples include the simultaneous assessment of progesterone production from cell proliferation assays using female reproductive cancers and the collection of blood toxicity data from an *in vivo* rodent model for colon cancer prevention. These innovations will be highlighted using *Dicathais orbita* as a model system for marine natural product development.

## Growing algae and cyanobacteria: photobioreactor technology for product optimisation at the Cawthron Institute

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The Cawthron Institute operates a shellfish hatchery where several species of marine microalgae are grown in medium-scale continuous culture. The technology has been commercialised through partnerships with biotechnology and aquaculture companies. Current research includes developing automated photobioreactor systems for rapid optimisation of growth and target product generation. Central composite design is used to provide a robust experimental design framework that enables multiple variables to be adjusted and optimised simultaneously. This builds on experience with systems to monitor and control research photobioreactors, used for investigation of high value products and toxin production by marine and freshwater microalgae and cyanobacteria. Growth systems for benthic algae and cyanobacteria are also being investigated. A multitude of different photobioreactor designs are available for planktonic species but there has been little research on photobioreactors for benthic species. Some benthic cyanobacteria become positively buoyant as they approach stationary phase, detaching from the growth surface to form floating mats. This 'self-harvesting' capability could be advantageous in commercial processes by reducing dewatering costs. Benthic species are also useful in bioelectrical systems, where the redox state of the algae is influenced by the delivery of electrons which in turn affects the range and yield of potential products that can be produced by a particular species. Our investigations are concentrating on high value products including evaluating these for pharmaceutical, nutraceutical and cosmaceutical applications as well as ingredients for healthy and functional foods.

## Developing Australian Native Microalgae for Algal Biofuels and Bioproducts

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There is a great interest in microalgae as a renewable source of biofuels and other bioproducts. Australia has intrinsically attractive features for a developing an algal industry including favourable climatic zones, and available, non-arable land including coastal land. There is a vast native microalgal biodiversity, a sub-set of which is held in the Australian National Algae Culture Collection (ANACC). Of more than 1000 strains held in ANACC, several hundred that have potential for biofuels, isolated from habitats ranging from tropical to temperate Australia, have been screened for their biofuel potential, with a CSIRO in-house database developed. These data go part way to addressing for Australia the issue highlighted by the U.S. Department of Energy: "Currently, no database(s) exists that would provide global information on the characteristics of currently available algal strains" (USDoe 2010). The CSIRO database allows ready identification of high performing strains for biofuels and other bioproducts, including high value oils and pigments, and provides a benchmark against which biodiscovery of new strains can be compared. CSIRO has identified 'types' of microalgae according to temperature and salinity tolerances and matched these types to different climatic conditions, water and land types across Australia in a multi-criteria analysis to inform selection of microalgae for development in different regions. Utilisation and development of Australian native microalgae protects against unwanted imported 'weed' strains and avoids rigorous biosecurity legislative scrutiny. Collection and isolation of native microalgae for commercial purposes must consider access and benefit sharing arrangements which vary between the States and the Commonwealth.

## The Effects of *Ishige okamurae* on Collagen Synthesis of Osteoblastic MC3T3-E1 [Poster]

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*Ishige okamurae* (IO) is a brown alga that occurs mainly in the northwest Pacific Ocean on rocks near the coasts of China, Japan, and Taiwan. Plants like the shape of the dark brown wire grow by making a kind of two-pronged. The IO ethanol extract showed 75% activity by the DPPH assay. The SOD-like activity of the IO ethanol extract was 55%. The total polyphenol content of the IO ethanol extract was 2.2mg/g. The IO ethanol extract heavily inhibited ACE activity. Proliferation of osteoblastic MC3T3-E1 cells treated with IO ethanol extract increased in a dose-dependent manner. In particular, IO ethanol extract at a concentration of 50 µg/mL showed a higher (growth rate) than that observed in the control group. ALP activity was significantly higher in the IO ethanol extract group than that in the control group, and the highest activity was observed with the IO ethanol extract concentration of 50 µg/mL. The collagen synthesis ability was concentration-dependent increase when IO ethanol extract at 10 ~100 µg / mL was added. These results indicate that the IO extracts may have an anabolic effect on bones through the promotion of osteoblastic differentiation.

## Effects of *Ishige okamurae* Extract on Serum Lipid Content of Ovariectomized Rats [Poster]

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*Ishige okamurae* (IE) is an edible brown alga that is abundantly found along the coast of Jeju Island, Korea. Brown algae harbor a variety of biological compounds, including fucoïdan, and polyphenol compounds. This study examined the effects of an IE ethanol extract on the serum lipid concentration and platelet aggregation in ovariectomized rats. Twenty-eight 9-week old female Sprague-Dawley rats were randomly assigned to four groups: sham-operated rats (SHAM), ovariectomized rats (OVX-CON), 17-beta-estradiol rats (OVX-ES) and ovariectomized rats that were treated with IE extracts (OVX-IE50; 50 mg/kg and OVX-IE200; 200 mg/kg). The rats were placed on prescribed diets for 7 weeks following ovariectomy. The total cholesterol and triglyceride contents on serum were lower in the SHAM group than in the OVX-CON group. As significant decrease of triglycerides, a decrease of total cholesterol level, as well as a significantly elevated level of HDL-cholesterol on serum observed in the ovariectomized rats when fed the diet containing IE extracts. The IE extract-supplemented groups were shorter than the times in the untreated group (OVX-CON). HDL-cholesterol levels on serum were higher in the IE extract treated group than in the OVX-ES group. Platelet aggregation in the OVX-IE50 and OVX-IE200 groups was lesser than that in the OVX-CON group. These results suggest that the IE extract may be used to improve the quality of life of menopausal women.

## Physiology and metabolism of lipid production by marine and halotolerant microalgae

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The sustainable production of lipid-rich microalgae for biofuels requires marine or halophilic algae in order to minimise the use of freshwater. Furthermore, high lipid productivity (g lipid m<sup>-2</sup> day<sup>-1</sup> or g lipid L<sup>-1</sup> day<sup>-1</sup>) is required rather than just a high lipid content. Most microalgae accumulate high levels of lipids when they are nutrient limited, especially nitrogen limited. In diatoms silicon limitation also greatly stimulates lipid formation. Under these conditions growth is greatly reduced or has stopped. However, there are a few algae such as some *Tetraselmis* spp. which have the highest cellular lipid content (up to ~40% of afdw) during the exponential phase of growth rather than in stationary phase. The lipid content is also highest when the cells are not carbon limited. The synthesis of fatty acids, the major building blocks of lipids, occurs mainly in the chloroplast and is strongly dependent on the rate of photosynthesis. Thus it is not surprising that light and the availability of an inorganic carbon source are the principal factors affecting the rate of lipid formation. Nutrient limitation (i.e. N and Si) modulates the metabolic pathways enhancing the flow of organic C to lipids in most species. Environmental factors such as temperature and salinity mainly affect the types of lipids and their degree of unsaturation. This paper will consider how an understanding of the physiology and metabolism of lipid production can be used to optimise growth conditions to enhance lipid productivity and lipid quality. Differences between different microalgae taxa also will be highlighted.

## **Intraspecific variability in secondary metabolites of the Great Barrier Reef sponge-associated marine bacteria "*Salinispora pacifica*" [Poster]**

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To better understand patterns of chemical ecology and biodiversity in the environment it is necessary to increase our knowledge of chemical diversity both within and between species. As an obligate marine Actinobacterium, the *Salinispora* species have proven to be a robust source of secondary metabolites; these represent the largest class of functional traits differentiating the species. Those bacterial species that are members of the genus *Salinispora* have been shown to produce their secondary metabolites in a species specific pattern. However, the extent of metabolite diversity observed within *Salinispora* species remains unexplored. Here, we compare the metabolite profiles of 25 strains of "*Salinispora pacifica*" obtained from sponges sampled from a ~2500km stretch of Australian Great Barrier Reef. We used Principal Component Analysis, Orthogonal Partial Least Squares Discriminant Analysis and Hierarchical Clustering Analysis to examine the patterns of variation in chemical diversity within this species. This screening revealed a high level of intraspecific diversity in the "*Salinispora pacifica*" secondary metabolome. The tentative identification of several non-ubiquitous candidate compounds greatly exceeds the small number of secondary metabolites previously known to have been derived from this species. These results indicate that "*Salinispora pacifica*" may be a promising source of bioactive natural products.

## **Identification of key signalling peptides involved in abalone sexual maturation**

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Sexual maturation impacts on the commercial culture of Australian temperate abalone. However, little is known about it and the associated metabolic processes. Closed-life-cycle breeding of elite animals, where sexual maturation, conditioning and spawning are synchronised, is essential for enhancing commercial culture. Studies in other marine molluscs have shown there to be a link between maturation and spawning processes with impaired immune status and increased pathogen susceptibility. For example, stressors including elevated water temperatures increase susceptibility to mortality. Also, abalone growth may be impaired by metabolic requirement for gonad development over the peak growing season. Currently the genes and proteins involved in maturation in temperate abalone are poorly understood. The present study aims to develop knowledge of the molecular basis of abalone maturation using genomic and transcriptomic sequencing in combination with novel peptide analyses. For this, brain and gonad tissues samples were collected over a 12 month period from a range of maturational stages (immature juvenile to mature adult). We used a liquid-liquid peptide extraction method with MarkerView software analysis (traditionally used for metabolite studies) to identify a number of peptide profiles linked to key maturation events. Known and/or predicted peptides were identified by automated database searching. For novel peptides mass, charge and retention time data will facilitate their identification as more genomic data becomes available through other DNA sequencing projects. We anticipate results gained from this work will eventually be used to assist in improving management of commercial abalone production.

## Potential role of MeNP in shrimp ovary maturation: RNAi silencing resulted in inhibition of vitellogenesis

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The full-length MeNP cDNA of the shrimp encodes a protein homologous to the insect neuroparsin and vertebrate insulin like growth factor binding protein (IGFBP) was cloned. MeNP cDNA is 1385 bp in length with the longest open reading frame of 306 bp. By SignalP analysis, MeNP has an estimated molecular weight of 7.83 kDa and pI=5.0. It shows high amino acid sequence similarities (42% - 68%) to the neuroparsin of the insects and insulin like growth factor binding of vertebrates. The cysteine residues responsible for the formation of the disulfide bonds in MeNP are conserved as in other neuroparsin like protein. MeNP shows no difference in expression in both sexes with the highest expression level in the hepatopancreas and a corresponding decrease in vitellogenin protein level in the hemolymph. In summary, the result of this study has provided evidence that MeNP may be involved in the initial stage of ovary maturation in shrimp. (Funded by Guangdong Ocean University)

## Bioleaching of conventional and non conventional materials: new approaches

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Even when Chilean mining industry is focused on copper extraction, it's been diversification to other values as gold and lanthanides, although not all the values come directly from the ore, there are big amounts of recoverable values on dumps or tailings as occurs with flotation tails usually stocked, smelter slag or fines dust produced during the ore crushing, normally avoided by leaching stages. Supporting the diversification implies studies and research efforts focused on solving problems more than absolutely understanding the microbiological phenomena behind it. Working this way several advances have been reached on the improves of bioaugmentation plants using CO<sub>2</sub>, bacterial on line monitoring into solutions and attached to the ore in order to improve extraction from traditional ores as chalcocite, digenite, etc. trough modifying simple operational conditions as irrigation rate, also allowing cyanide degradation, electrochemical treatment of chalcopyrite and lanthanides extraction. The efforts should be focused on the process optimization and scale up, due the fact that binding microbiological phenomena and electrochemical principles (among others) is not utopic.

## Technology development of CO<sub>2</sub> fixation, C-phycocyanin production and purification with *Spirulina platensis* [Poster]

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*Spirulina platensis* mitigates CO<sub>2</sub> emissions and simultaneously produces nutraceutical product C-phycocyanin (C-PC) during its growth. This work was firstly undertaken to optimize the microalgae cultivation processes to obtain higher biomass productivity, CO<sub>2</sub> fixation rate and C-phycocyanin productivity. The *Spirulina platensis* was cultivated with an innovated pH control system, in which the pH value was controlled via the feeding of 2.5% CO<sub>2</sub>, instead of acid/alkaline titration. The results show that controlling pH at 9.0 and 9.5 was suitable for cell growth, but further increased in pH slightly inhibited the cell growth. In addition, the CO<sub>2</sub> removal efficiency sharply increased from 13.6% to 62.3% by applying the CO<sub>2</sub>-mediated pH control strategy. Moreover, the C-PC content and productivity were also increased to 16.8% and 0.17 g/L/d, respectively, with the CO<sub>2</sub>-mediated pH control. Therefore, the proposed pH control system could avoid the problem of excessive addition of acid or alkaline, resulting in better CO<sub>2</sub> fixation efficiency and higher C-phycocyanin productivity. The purification process of C-phycocyanin was also developed in this study. Using fractional precipitation, the purity and recovery of C-PC could be achieved as 1.92 and 91%, respectively. Using the anion exchange chromatography was able to enhance the purity to 3.70 with 40% recovery. Combination of the foregoing two purification methods further increased the purity of C-PC to 4.33 purity with a slight decrease in recovery (33%)



## Engineering strategies for improving protein production from microalgal *Chlorella vulgaris* FSP-E using novel photobioreactor illuminated with cold cathode fluorescent lamps [Poster]

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Fish meal is widely used as a protein source in commercial aquacultural feed. The yearly production of fish meal heavily relies on fish harvest. According to recent report from Food and Agriculture Organization (FAO), the amount of fish production from wild capture has reached saturation stage in the past decades. Thus, finding an alternative protein source is an urgent demand. Increasing attention has been paid to protein-rich microalgae which show a great potential as a candidate to replace fish meal in commercial aquaculture feed. In this study, protein-rich indigenous microalgal isolate, identified as *Chlorella vulgaris* FSP-E, was enhanced by using innovative light source to obtain higher microalgal protein productivity. The results show that the microalgae biomass and protein productivity reached 411 mg/L/d and 183 mg/L/d, respectively. Next, the nitrogen concentration was adjusted to further enhance the protein production performance. The results show that using a nitrogen concentration of 24.8 mM could effectively enhance the protein production, leading to a biomass and protein productivity of 613 mg/L/d and 301 mg/L/d, respectively. For medium improvement, the PBR was conducted under different concentrations of ferric ion concentrations. The result show that using a ferric ion concentration of 90 mM, the biomass productivity and protein productivity were further enhanced to 699 mg/L/d and 365 mg/L/d, respectively. Analysis of protein content and amino acid profile of the microalga shows that over 60% of protein in the microalga was indispensable amino acids. This amino acid quality is suitable for the use as aquacultural feed.

## Enhancing the production of EPA from *Nannochloropsis oceanica* CY2 by using LED photobioreactor [Poster]

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The omega-3 fatty acids, especially EPA and DHA, are important elements for human health. Nowadays, marine cold water fish are the major source of omega-3 fatty acids. However, the global fish harvest has been nearly saturated, an urgent need in developing an alternative source of omega-3 fatty acids. Among the potential alternatives, microalgae have emerged as a new resource of omega-3 fatty acids since microalgae can synthesize and accumulate large amounts of omega-3 fatty acids in their cells. Besides, producing omega-3 fatty acids from microalgae could avoid the problems of biomagnification and odd smell. From our previous results, the specific light wavelength can improve the cell growth and EPA production of *Nannochloropsis oceanica* CY2 as compared with conventional light sources (i.e., fluorescent lamp). In this study, four different colors of LEDs (i.e., white, blue, yellow and red) were selected to verify whether the multi-wavelength could enhance the microalgal cell growth and EPA production of *N. oceanica* CY2. Compared with single-wavelength (i.e., white-LED), using blue-red LED resulted in a higher EPA productivity, achieving a maximal biomass productivity and EPA productivity of 639 mg/l/d and 12.9 mg/l/d, respectively. Next, the light shading effect of microalgal culture is another critical problem of mass production of microalgae. In this study, an immersed multi-wavelength LED light source was designed and use as the light source to improve microalgal biomass and EPA productivity. Compared with control experiment, the immersed light source could further increase biomass productivity and EPA productivity to 752 mg/l/d and 15.9 mg/l/d, respectively.

## Enhancing EPA production from *Nannochloropsis oceanica* using deep-sea water supplemented cultivation medium [Poster]

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The aim of this study is to use deep-sea water (DSW) to promote the autotrophic growth and EPA accumulation of an indigenous microalga *Nannochloropsis oceanica* with the aid of innovative engineering strategies. First, different quantity of deep-sea water was supplemented to culture broth to investigate its stimulating effects on algae growth and EPA production. The results show that cultivating the microalga on 100% DSW resulted in marked enhancement in the EPA production, giving a EPA content and algae biomass concentration of 3.07% and 3.2 g/l, respectively. Next, the effect of CO<sub>2</sub> aeration rate was also investigated using 100% deep-sea water as the culture medium. It was observed that the EPA content was further enhanced to 3.46% by using an optimal CO<sub>2</sub> aeration rate of 0.15 vvm. In contrast, varying the concentration of NaNO<sub>3</sub> concentration from 0.5 g/L to 2 g/L did not significantly affect the oil/lipid content in the microalga, as the EPA content and algae biomass concentration maintained at a level of 3.46% and 3.2 g/l, respectively, for all the nitrogen source concentration examined. Finally, a plastic bag-type microalgae cultivation system with a working volume of 5.0 liter was used to grow the *N. oceanica* strain for EPA production. The results show that with an inoculum size of 0.8 g/l, the EPA content and biomass concentration were 4.12% and 2.84 g/l, respectively. The results mentioned above suggest that combining deep-sea water and plastic bag-type microalgae cultivation system demonstrates the potential of enhancing microalgae growth and EPA productivity.

## Characterization of photosynthetic carbon dioxide fixation ability of indigenous *Chlorella pyrenoidosa* NNK-A isolate [Poster]

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In this study, autotrophic growth of indigenous microalgal isolate, identified as *Chlorella pyrenoidosa* NNK-A, was selected on CO<sub>2</sub> fixation ability. First, different carbon dioxide concentrations were used to confirm the effect of higher CO<sub>2</sub> concentration on CO<sub>2</sub> fixation rate and microalgae growth rate. The results show that increasing the carbon dioxide concentration to 15.0% did not significantly inhibit the growth of *C. pyrenoidosa* NNK-A, while the CO<sub>2</sub> consumption rate and algae biomass concentration reached 424 mg/l/d and 242 mg/l/d, respectively. Next, different operation temperature was also investigated to simulate outdoor cultivation conditions. The results show that CO<sub>2</sub> consumption rate and algae biomass concentration still maintained at the level of 416.8 mg/l/d and 229 mg/l/d, respectively, at the temperature of 35°C. Finally, when the working volume of the microalgae cultivation system was increased to 50 L, the CO<sub>2</sub> consumption rate with *C. pyrenoidosa* NNK-A became lower but can still reach the level of 219 mg/l/d.

## Characterization of photosynthetic carbon dioxide fixation ability of indigenous *Chlorella pyrenoidosa* CY10 isolate [Poster]

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In this study, autotrophic growth of indigenous microalgal isolate, identified as *Chlorella pyrenoidosa* CY10, was selected on CO<sub>2</sub> fixation ability. First, different carbon dioxide concentrations were used to confirm the effect of higher CO<sub>2</sub> concentration on CO<sub>2</sub> fixation rate and microalgae growth rate. The results show that increasing the carbon dioxide concentration to 15.0% did not significantly inhibit the growth of *C. pyrenoidosa* CY10, while the CO<sub>2</sub> consumption rate and algae biomass concentration reached 429 mg/l/d and 236 mg/l/d, respectively. Next, different operation temperature was also investigated to simulate outdoor cultivation conditions. The results show that CO<sub>2</sub> consumption rate and algae biomass concentration still maintained at the level of 403.5 mg/l/d and 222 mg/l/d, respectively, at the temperature of 35°C. Finally, when the working volume of the microalgae cultivation system was increased to 50 L, the CO<sub>2</sub> consumption rate with *C. pyrenoidosa* CY10 became lower but can still reach the level of 219 mg/l/d.

## **UV mutagenesis of an isolated green microalga *Chlamydomonas orbicularis* for enhanced lipid production [Poster]**

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Microalgae are photosynthetic organisms that convert carbon dioxide into various energy-rich compounds. Microalgae can grow on non-arable land with a much faster rate than that of most terrestrial plants and without serious seasonal restrictions. Some microalgal strains are known to accumulate a considerable amount of lipid. Therefore, microalgae have been considered as promising sustainable feedstocks for biofuels production. The quantity and rate of lipid accumulation are the determining factors for production of cost-effective algal biofuels. In this study, UV mutagenesis was used to enhance the oil production efficiency of *Chlamydomonas orbicularis*, which is an indigenous microalgal strain isolated from seawater in southern Taiwan. High-throughput Nile red method was developed to identify the high lipid content mutants. After screening 320 mutants, 16 mutants with higher Nile red fluorescence intensity were obtained, and the strains were further cultivated in 250 ml photobioreactor to characterize the lipid production. Among them, 5 mutants with higher lipid content were discovered. The lipid content could exceed 40% after 7 days of cultivation. Among those strains, one mutant (No. 215) with the highest lipid production efficiency was selected for scale-up cultivation in 1 L photobioreactor. The growth rate and lipid content of No. 215 were significantly higher than that of the wild type. After 9 days of cultivation, the biomass concentration of No. 215 was 2.36 g/l, indicating an 8.3% increase over the wild type. The lipid content of the mutant No. 215 is also 20.8% higher than the wild-type strain (increased from 38.5% to 46.5%). Furthermore, the highest lipid productivity (121.8 mg/l/day) was obtained at 9 days of cultivation. This lipid productivity is 30.8% higher than that of the wild type. Therefore, this study shows that UV mutagenesis seems to be a feasible approach for strain improvement to achieve better microalgal lipids production.

## **Proteomic Analysis Reveals That Pardaxin Triggers Apoptotic Signaling Pathways in Human Cervical Carcinoma HeLa Cells: Crosstalk among the UPR, c-Jun, and ROS [Poster]**

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Pardaxin, an antimicrobial peptide, secreted by the Red Sea flatfish *Pardachirus marmoratus*, was shown to exhibit antiproliferative activity against and induce apoptosis of human cancer cell lines. However, the underlying molecular mechanisms are at present only partially understood. In this study, we applied proteomics approaches and network reconstruction to clarify the mechanism of pardaxin-induced apoptosis in human cervical carcinoma HeLa cells. We identified that pardaxin-regulated proteins predominantly functioned in the unfolded protein response, oxidative stress, and cytoskeletal distribution. Molecular examination of signal transduction and cellular localization demonstrated that the activator protein-1 transcription factor was activated, and eventually caused apoptosis via both caspase- and apoptosis-inducing factor-dependent pathways. Restoration of reactive oxygen species (ROS) alleviated c-Jun activation and small interfering RNA knockdown of c-Jun abrogated pardaxin-induced caspase activation and cell death, which demonstrated the involvement of ROS and c-Jun in pardaxin-induced apoptosis signaling. In summary, the present study provides the first protein-interacting network maps and novel insights into the biological responses and potential toxicity of pardaxin.

## Evaluation of therapeutic efficacy of antimicrobial peptides against marine pathogens using in vitro and in vivo infection models [Poster]

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*Photobacterium damsela* and *Vibrio parahaemolyticus* are Gram-negative, halophilic bacteria which occur naturally in estuarine environments world-wide. They are universal marine pathogens causing severe fish and shellfish diseases, and have caused great economic loss to the fish farming industries in many countries. Antibiotics were applied to prevent infections by these marine pathogens, however, the extensive use of antibiotics has led to the growing emergence of many resistant strains of pathogenic microorganisms. Therefore, the development of novel therapeutic agents that could overcome the resistance problem has become critical. Evidence suggested that antimicrobial peptides (AMPs) are of greatest potential to replace classical antibiotics. In the current study, MIC values of natural and synthetic AMPs against these marine pathogens have been determined. AMPs Q6, H1 and pleurocidin were found to be the most potent ones. We have also identified AMP-responsive protein profiles via 2-DE-based proteomic approaches. Furthermore, we demonstrate the efficacy of AMPs as therapeutic agents against these marine pathogens using in vitro (grouper kidney cell lines) and in vivo (cobia) infection models, respectively. Cobia of 20-25 g were intraperitoneally injected with 100  $\mu$ l ( $10^8$  CFU) of bacterial suspension. AMPs were applied to the infected fish by immersion, and Q6 of 0.5  $\mu$ g/mL was found to increase the relative survival rate of infected cobia by 20%. These findings would provide support for future application of these novel cationic AMPs as potential therapeutic agents for treatment of these marine pathogens.

## Different visible colors were obtained from the mutated purple chromoprotein isolated from sea anemone [Poster]

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Many proteins in the GFP-like protein family have been studied with the aim of developing fluorescent proteins. Since the property of color variation has received little attention, we isolated a novel GFP-like chromoprotein from the tentacles of the carpet anemone *Stichodactyla haddoni*, termed shCP. The maximum absorption wavelength peak ( $\lambda_{max}$ ) of shCP is located at 574 nm, resulting in a purple color. The amino acid sequence of shCP consists of 227 amino acids, sharing 96% sequence identity with the GFP-like nonfluorescent chromoprotein of *Heteractis crispa*. To examine whether coloration could be altered, we mutated amino acid(s) E63-Y64-G65 located at the shCP chromophore and its surrounding microenvironment. When E63 was replaced by serine (E63S), the  $\lambda_{max}$  of mutated protein shCP-E63S was shifted to 560 nm and exhibited a pink color. Meanwhile, Q39 in the chromophore's microenvironment was replaced by serine (Q39S), and the  $\lambda_{max}$  of this mutated protein, shCP-Q39S, was shifted to 518 nm and exhibited a red color. To confirm whether shCP and its mutated derivatives could be expressed *in vivo*, we found that the transformed bacteria colonies harboring shCP and shCP-E63S cDNAs turned purple and pink, respectively. Additionally, we constructed expression vectors driven by muscle-specific  $\alpha$ -actin promoter and microinjected them into one-celled zebrafish (*Danio rerio*) embryos. Similar to the bacterial colonies, the muscle of transgenic zebrafish embryos harboring shCP and shCP-E63S cDNAs was observed to express purple and pink color, respectively, suggesting that the cDNAs of shCP and its mutated varieties are faithfully and stably expressed *in vivo*.

## Progranulin is required for liver regeneration in the partial hepatectomized zebrafish [Poster]

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Physiologic liver growth not only occurs in embryonic development, but also in the liver regeneration process that was activated after liver injury. According to our previous research, it indicated that progranulin could modulate MET, the receptor of hepatocyte growth factor, signaling to regulate liver growth. Progranulin is a multifunctional secreted glycoprotein. It has been known that plays a critical role in cell growth, wound healing, tumorigenesis, inflammation response, neurodevelopment, and neurodegeneration. This research would study the regulatory role of progranulin that involve in liver regeneration. We established a liver regeneration model after partial hepatectomy in liver-specific fluorescent adult zebrafish. The result of quantitative-PCR showed that mRNA expression of progranulin, cell cycle and proliferation related genes were induced after partial hepatectomy. To study the functional progranulin A in liver regeneration. We use the loss of function and gain of function assay. In our *grnA* morphant showed a weak recovery with delayed cell cycle related gene and suppressed cell proliferation related gene that specifically at 36 hours after partial hepatectomy. On the other side, in our liver specific *grnA* over expression transgenic zebrafish showed a faster recovery than wild-type zebrafish after partial hepatectomy. In conclusion, *grnA* mediate met signaling is required for liver regeneration.

## **Epinecidin-1 has immunomodulatory effects, facilitating its therapeutic use in a mouse model of *Pseudomonas aeruginosa* sepsis [Poster]**

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The emergence of multidrug-resistant strains of species such as *Pseudomonas aeruginosa* has necessitated the search for novel antimicrobial agents. Antimicrobial peptides (AMPs) are garnering attention as possible alternatives to antibiotics. We recently reported that a fish AMP, named epinecidin-1, effectively eradicated Gram-positive and Gram-negative bacteria, fungi, and viruses in vitro. Here, we describe the antimicrobial properties of epinecidin-1 against multi-drug resistant clinical isolates of *P. aeruginosa* (*P. aeruginosa* (R)) and *P. aeruginosa* from ATCC (*P. aeruginosa* (19660)) both in vitro and in vivo. The minimum inhibitory concentrations (MICs) of the peptide against *P. aeruginosa* (R) and *P. aeruginosa* (19660) were determined in comparison with imipenem. We report that *P. aeruginosa* (R) was more susceptible than *P. aeruginosa* (19660) to epinecidin-1 in vitro, and the MICs for 90% of *P. aeruginosa* (R) and *P. aeruginosa* (19660) were 3.12 and 50 µg/ml, respectively. Moreover, epinecidin-1 was highly effective at combating peritonitis infection caused by *P. aeruginosa* (R) and *P. aeruginosa* (19660) in mouse models. No significant adverse effects of epinecidin-1 on the liver or kidney were observed, and it did not affect behavior. In addition, epinecidin-1 had bacteriostatic effects against *P. aeruginosa* in mice. Microarray analysis demonstrated that epinecidin-1 modulated several *P. aeruginosa*-responsive genes in mice. Taken together, our results indicate that epinecidin-1 enhanced the survival rate of mice against the bacterial pathogen *P. aeruginosa* through both antimicrobial and immunomodulatory functions. These properties make the epinecidin-1 peptide a good candidate for the development of novel antimicrobial drugs for use against nosocomial bacterial infections caused by multidrug-resistant Gram-negative bacteria, especially *P. aeruginosa*. If there is a list of items in your abstract, please use bullet indents.

## **Transcriptomic study and different gene expression profiling of two kinds of grouper iridoviruses infection in orange-spotted grouper (*Epinephelus coioides*) [Poster]**

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Iridovirus infections have led to serious economic losses in grouper aquaculture in Taiwan since 1995. Two iridoviruses were obtained and were designated as grouper iridovirus of Taiwan (TGIV) of genus *Megalocyttivirus* and grouper iridovirus (GIV) of genus *Ranavirus* within the family *Iridoviridae*. These two iridoviruses were found to be highly pathogenic and resulted in high mortality in a variety of farming marine fishes. First, we analyze the gene expression profiling in spleen of orange-spotted grouper infected with TGIV and GIV respectively at the 1, 3, 5 days after virus infection. Subsequently, RNA-seq technology combines transcriptome sequencing and bioinformatics analysis method of digital gene expression (DGE) was used to detect genes with changes in expression levels of at least 2-fold. And the up-regulated and down-regulated genes (transcripts) in the spleen of TGIV-infected and GIV-infected groupers are 204 (up), 713 (down) and 614 (up), 829 (down), respectively. Moreover, hemoglobin subunit beta-2 (Hb) ∖ C-C motif chemokine 19 precursor (CCL19) ∖ Mx 10-3 and Toll-like receptor 9 isoform A ∖ B (TLR9-A ∖ B) were selected as target genes. The abundance of these target genes in spleen and kidney of infected fish were calculated using real time quantitative PCR (qPCR). Furthermore, we compare the expression patterns of these five target genes among vaccinated and non-vaccinated groups separately against TGIV and GIV. Different expression profiles suggest that Hb-regulated gene might be related to the onset of hypoxia, splenomegaly symptoms; and other genes might be involve in pathogenesis of these two iridoviruses.

## Genome Sequencing of the Shrimp *Neocaridina denticulata*: Insights into Crustacean Aquaculture and Arthropod Evolution

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The crustaceans form a large subphylum of arthropods after the subphylum Insecta, and include ecologically and environmentally important classes such as Branchiopoda, Cephalocarida, Maxillopoda, Ostracoda, Remipedia, and Malacostraca (including decapods, isopods, amphipods, and stomatopods). To date, the only publically available sequenced crustacean genome is the water flea *Daphnia pulex* from the Branchiopoda. Whereas this animal is a well-established ecotoxicology model, its genome has undergone many lineage-specific gene duplications. To better understand arthropod evolution and improve crustacean aquaculture (which mainly comprises the decapods such as shrimps, lobsters, and crabs), we have sequenced the genome of a new shrimp model - *Neocaridina denticulata*. A library of 180bp fragment size was constructed from DNA of a starved single adult and sequenced using the Illumina HiSeq2000 platform, providing 3,776,650,691bp. Developmental genes, hormonal pathway genes, and other regulatory elements such as microRNAs were then compared to other metazoans. The *N. denticulata* genomic resources presented here will provide a better understanding of how genomes in the Decapoda, the Crustacea, and the Arthropoda evolve, and will be useful for a wide range of further developmental and genetic research to improve crustacean aquaculture.

## Structural characteristic and immuno-regulatory function of class-A CpG oligodeoxynucleotide in grouper [Poster]

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Synthetic unmethylated CpG oligodeoxynucleotides (ODNs) have been widely used in mammals as Toll-like receptor 9 (TLR9) agonists to trigger innate immune response or to serve as vaccine adjuvant. Based on the structural and functional characteristics, CpG ODN is classified into four classes: A, B, C and P. In teleost, most of the CpG ODNs being tested fall into the category of class B. In this study, we analyzed the relationship between structural property and immuno-stimulatory effect of class-A CpG ODN in orange-spotted grouper (*Epinephelus coioides*), an important aquaculture species in many Asian countries. The structural characteristic of a class-A CpG ODN includes: a central palindromic sequence containing unmethylated CpG motif, and poly-G tails at the 3' and/or 5' ends. Our data showed that the palindromic sequence is critical to the immuno-regulatory function as the ability of inducing IL-1 $\beta$  expression was abolished when the palindromic sequence was compromised. The 5' poly-G tail is also crucial to the induction of IL-1 $\beta$ , whereas the 3' poly-G tail is dispensable. In mammals, the CpG motif is crucial to the immuno-regulatory function of CpG ODN as a reversion in the CG sequence will diminish the activity. Our data indicated that this specific sequence requirement is less stringent in fish as ODNs containing GpC motif are also effective in grouper cells. The result of this study provides valid information for the design of optimal CpG ODN as immuno-simulant in fish.

## The Overexpression and Bioactivity Assay of Fish Type I Interferons on Grouper Cell Line [Poster]

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Type I interferons (IFNs) a family of cytokines which play essential roles in host defence, in response of bacterial and viral infections. In this work, we overexpressed the grouper type I interferon (gIFN), salmon interferon (sIFN), European seabass interferon (sbIFN), and tilapia interferon in *Escherichia coli* BL21(DE3). The overexpressed IFNs have a protein size of 18 – 18.5 kDa for gIFN, sIFN, sbIFN and tpIFN. IFNs showed the ability to activate the interferon downstream gene, Mx, which has function of inducing host into an antiviral state *in vitro*, after 12 to 36 hours treated with a concentration of 10 µg/ml in grouper kidney cell (GK). The real-time PCR results showed that there is a decrease trend in induction of Mx with the decreasing concentrations of IFNs of 10, 1 and 0.1 µg/ml treated *in vitro*. The IFNs treated cells has a delayed of cytopathic effect (CPE), and inhibition of viruses replications for nervous necrosis virus and iridovirus infections; with the increase of the possibility of survival of GK cells. The pre-treatment of IFNs prior virus infections showed a better protection effect as compared to the co-treatment and post-treatment of IFNs.

## A female sex hormone is required for developing adult female features of blue crabs

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The current paradigm in sex differentiation of malacostracan crustaceans is based solely on the androgenic gland, a male specific endocrine organ, and its hormonal product, the androgenic gland hormone (AGH). Accordingly, female differentiation and secondary traits essential for reproduction are determined by default, in the absence of the masculinizing action of AGH. Adult female crustaceans display a wide range of reproductive strategies but the endocrine systems supporting them have not yet been found. We identified a hitherto unknown crustacean female sex hormone (CFSH), predominantly expressed in distinct neurons in the eyestalk ganglia of female blue crab *Callinectes sapidus*. The full-length cDNA encodes a precursor of a novel protein consisting of a signal peptide, a precursor-related peptide and a mature protein of 167 amino acids. *CFSH* knock-down by double-stranded RNA interference reduced the levels of *CFSH* expression and *CFSH* protein in the eyestalks of pubertal females, resulting in abnormal development of structures essential for successful mating and brooding, such as a pair of gonopores and a brooding egg attachment system comprised of enlarged semi-circular abdomen and ovigerous setae. The ovigerous setae in *CFSH* knocked-down females displayed smaller and less complex structure and the gonopores were small, misplaced or absent. These data provide the first evidence for the presence of a female specific hormone in decapod crustaceans and its functional role in the development of adult female-secondary morphology. *CFSH* is probably needed to support the specific reproductive strategy of crustacean species with internal fertilization and brooding behavior.

## Effects of dispersed and emulsified oil on molting, ecdysone and EcR/RXR complex in the grass shrimp and the blue crab [Poster]

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It has been known that the exposure to crude oil inhibits molting in crustaceans. We hypothesized that such inhibition in molting process is possibly due to the change in the levels of ecdysone and its receptor (Ecdysone receptor= EcR /Retinoid-X receptor= RXR) complex. Grass shrimp or blue crabs were exposed to sediment or food containing water-oil emulsions of oil, followed by assays for ecdysone and EcR/RXR complex in the Y-organs and hepatopancreas, as well as morphological studies. Blue crabs fed food containing emulsified oil had distended hemocytes with eosinophilic material composed of glycoproteins. Grass shrimp embryos were exposed to dispersed oil (dispersants added to crude oil to produce dispersed oil droplets), followed by assays for ecdysone and the EcR/RXR complex. After exposure to dispersed oil the late embryo stages showed abnormalities with respect to molting and hatching from the egg sacs. There was evidence of the effects of both emulsified and dispersed oil on ecdysone and the EcR/RXR complex.

## The abalone haemocyte proteome: an indicator of animal health.

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Despite the many benefits derived from marine organisms, the health of the marine environment is being damaged enormously due to rapidly elevating levels of anthropogenic pollution, over-fishing and the effects of climate change. In order for us to enjoy the benefits that the marine environment offers humanity through marine biotechnology and mitigate the damage done to the marine environment, it is necessary for us to utilise modern technologies to better understand the functioning of the organisms that inhabit the marine environment in terms of their ecology and biology. Proteomics provides a unique opportunity to characterize the phenotype of marine organisms which dynamically responds to changing environmental conditions and associated stresses. Wild stocks of the South African abalone *Haliotis midae*, already depleted as a consequence of over-exploitation and rampant poaching, will decrease further as environmental stress due to climate change increasingly impacts the immune system of the animal. Although studies of the abalone immune system have been conducted at the physiological level, very little is known regarding the signalling pathways that induce the immune system to respond to environmental stress, the metabolic pathways that comprise the abalone immune system or their regulation. We have employed a proteomics approach to characterise the stress response of the abalone immune system at the molecular level. An increased understanding of the stress response of this commercially important shellfish will lead to a more accurate assessment of its ability to withstand environmental stress and thus, more appropriate management decisions.

## Molecular cloning, characterization and expression analysis of a glucokinase gene from the mixotrophic green alga *Chlorella kessleri* [Poster]

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*Chlorella kessleri* is a particular green alga that can grow well photoautotrophically as well as heterotrophically. To understand the responds to the presence and uptake of glucose at the molecular level, a cDNAs encoding glucokinase was isolated from the mixotrophic green alga *Chlorella kessleri* CGMCC 4917. The full-length cDNA of glucokinase, designated *CkeGCK* (Genbank Accession No. KF011248), comprised 1,421-bp with an open reading frame (ORF) of 1,134-bp encoding a 377 amino acid protein. It was flanked by a 21-bp of 5-untranslated region (UTR) and a 266-bp of 3-UTR including the poly-A tail. The deduced *CkeGCK* protein had a calculated molecular weight of 40.61 kDa and a predicted isoelectric point (*pI*) of 6.01. Multiple alignment analysis revealed that the deduced amino acid sequence of *CkeGCK* shared high identity of 54-66% with corresponding GCKs from other green algae. The catalytic motifs of glucokinase, including ATP- and glucose-binding sites were detected in the amino acid sequence of *CkeGCK*. The origin of glucokinase from algae is uncertain. The biomass accumulation, glucose concentration, and transcriptional expression patterns of *CkeGCK* were observed under different glucose-based cultures (light or dark). The decrease of glucose concentration in dark condition was found to be associated with the up-regulation of *CkeGCK* gene. However, it was absent under light condition. Our results implied that there may be another enzyme responsible for uptake of glucose under light condition. These results provide us valuable information on further investigating the molecular mechanism of presence and uptake of glucose for *Chlorella kessleri* under mixotrophic culture.



## Brain stimulants and sex smells: Decoding peptide communication systems in marine molluscs

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Molluscs represent the second largest animal phylum, inhabiting both land and aquatic environments. Peptides are used by marine molluscs for cell-cell communication, both within an animal as neuropeptides and between animals as pheromones. Neuropeptides are a diverse class of chemical messengers, instrumental in orchestrating complex physiological events from metabolism and growth to reproduction. By a combination of *in silico* data mining analysis and mass spectral analysis, we have identified neuropeptides within the Akoya pearl oyster, Pacific oyster and abalone that encode precursors for over 200 predicted bioactive peptide products. The findings include neuropeptides: allototropin, conopressin, FMRFamide, egg laying hormone and gonadotropin-releasing hormone. This work greatly expands our understanding of molluscan neuropeptides and further stimulates advances in molluscan aquaculture. Researchers have discovered thousands of pheromones, however, only in recent years has there been progress in our understanding of mollusc pheromones. Our studies have aimed at decoding their pheromone system, as a means toward understanding molluscan behaviour. This multidisciplinary research has demonstrated that: 1. *Aplysia* (sea slug) mate attraction and resultant breeding aggregations is the result of a cocktail of water-borne peptide pheromones. 2. Male Longfin squid become extremely aggressive within spawning grounds upon contact with a peptide pheromone embedded within egg capsules. 3. Male oysters have a peptide/protein on the surface of the sperm membranes that signal to others that it is time to spawn. These investigations have demonstrated the importance of peptide pheromones in mollusc communication.

## Characterization of the bacterial community of Hawaiian sea slug *Elysia rufescens*.

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Sacoglossans are characterized by the ability to sequester both functional chloroplast and bioactive compounds from their algal diet through a process called kleptoplasty and kleptochemistry, respectively. These sacoglossans are then able to photosynthesize and secrete bioactive compounds in their mucus for chemical defense. However, the bacterial diversity associated with the sacoglossan is not well understood. We coupled traditional cultivation-based methods with 454 pyrosequencing to examine the bacterial communities of the chemically defended Hawaiian sacoglossan *Elysia rufescens* and its secreted mucus. There was a diverse bacterial assemblage associated with *E. rufescens* and its mucus with secreted mucus harboring higher bacterial diversity and specific operational taxonomic units (OTUs) that were detected only in mucus samples. Furthermore the most abundant bacterial groups belong to *Mycoplasma* spp. and *Vibrio* spp. for the communities associated with *E. rufescens* and its mucus, respectively. Our analyses revealed that the *Vibrio* spp. that were highly represented in the cultivable assemblage were also abundant in the culture-independent community. This work forms the basis for describing new bacterial species for these sacoglossan-associated isolates and making them accessible for bioactive screening.

## Renewable fuels from macroalgae: revising the paradigm for algal fuels

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Macroalgae are a scalable and sustainable feedstock for the delivery of renewable fuels. The transformation of macroalgal biomass to biocrude through thermochemical conversion using hydrothermal liquefaction, and subsequent refining to advanced 'drop in' fuels, has radically revised the paradigm for algal fuels. We describe the process of hydrothermal liquefaction (HTL) for macroalgal biomass highlighting the key drivers for optimising the quantity and quality of biocrude. We also demonstrate how these key drivers can be manipulated under culture, and through post-harvest processing, and subsequently translated through the HTL process to improve both the quantity and quality of algal biocrude. Tailored feedstock, coupled with refined hydrothermal liquefaction techniques, is delivering an innovative platform for the delivery of sustainable renewable fuels, and the potential for multiple bioproduct streams from macroalgal biomass.

## Antioxidant activity of extracts from sponge-associated bacteria collected from Tao Island, Gulf of Thailand [Poster]

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Marine derived microbial natural products have been largest and many unique microorganisms, which produce biological active compounds to adapt to particular environmental conditions. The aim of this study was to screen antioxidant activity of extracts from sponge-associated bacteria. A total of 109 isolates from 15 sponges collected from northern to western coast of Tao Island, Suratthani Province, Gulf of Thailand were pre-screened for antibacterial activity using disc diffusion agar assay. Then 21 promising-bacteria exhibited antagonistic activity against the test bacteria. Then cultured supernatant and cell pellets were extracted with ethyl acetate and mix solvents of methanol and chloroform (ratio 2:1) respectively, and were evaporated by Rotary vacuum evaporator. All extracts were investigated antioxidant activity by using TLC spray with DPPH. The results showed that 15 of supernatant extracts and 10 cells extracts indicated the potential of antioxidant activity. Among them, extracts from strain T55A 4-1, T55A 5-13, T55 H 1-6 and T55J 2-6 showed high potential of both activities. These isolates were identified as *Pseudoalteromonas* spp., *Pseudomonas* sp., *Alteromonas* sp., *Flavobacterium* sp. The results obtained in this study suggest that sponge-associated bacteria may be an interesting source for discovery of bioactive agents.

## Gene regulation in the demosponge *Amphimedon queenslandica* and insights into the construction of animal body plans

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Comparison of the *Amphimedon queenslandica* (demosponge) genome with eumetazoan (vertebrates, insects, cnidarians and their allies) genomes has identified deeply conserved genomic features that have been maintained over some 700-800 million years of independent evolution. These constitute the 'zootypic' genomic ground plan and are likely to be responsible for a range of metazoan-specific traits, including cell cycle control and growth, development, somatic- and germ-cell specification, cell adhesion, innate immunity and allorecognition. From this ground plan arose an array of distinct animal body plans (phyla), each with their own genomic innovations. As a first step towards identifying the genomic features underlying phyletic body plans, I will use *A. queenslandica* transcriptome data as a proxy to understand the regulatory networks that control gene expression. This information is largely manifested through the regulated expression of transcription factors, which interact with non-coding *cis*-regulatory elements proximal to specific genes of the genome. Comparative analysis of sponge and eumetazoan transcriptomes can reveal developmental and morphological differences between these animal groups. For example, *A. queenslandica* possesses a near-complete set of genes to specify and build nerve cells, yet lack this cell type. This sponge does not co-regulate neuronal genes to the same extent as neural eumetazoans, suggesting that the evolution of a regulatory circuitry that underlies the co-expression of neuronal proteins was critical for the formation of this cell type.

## Seagrass restoration using hessian: silane coating of hessian reduces *Escherichia coli* attachment and fouling by marine bacteria

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Hessian sandbags are being deployed in denuded South Australian seagrass meadows to facilitate seedling recruitment in metropolitan areas impacted by nutrient rich waste water. As the hessian is degrading prematurely, we are trialling coatings to limit surface bacterial adhesion, using compounds that do not have environmental toxicity: an antibacterial, polyethylene glycol; and two silanes, tetraethoxysilane and propyltrimethoxysilane. The aim of this study was to compare the ability of *E. coli*, a model human pathogenic bacterium found in waste water, to attach and persist on coated versus native hessian. *E. coli* was inoculated into separate flasks containing each hessian type in (i) artificial seawater (pure culture) or (ii) unfiltered seawater (competition with marine species). Enumeration of *E. coli* in solution and on the hessian revealed higher survival rates in artificial seawater, but comparison of the coated and native hessian revealed similar trends. The initial attachment of *E. coli* to the coated hessian was similar to, or higher than native hessian, but the persistence on the coated hessian was significantly lower from day 7 post-inoculation. Overall tetraethoxysilane performed marginally better, with significantly less attached *E. coli* compared to any other coated or native hessian during testing in pure culture over the entire 42 day experiment. Environmental scanning electron microscopy confirmed that there was more bacterial attachment on native compared to coated hessian in both artificial seawater and seawater cultures. Overall, it appears that antifouling coatings, such as tetraethoxysilane, could be used to reduce bacterial loads on hessian bags used for seagrass restoration in metropolitan coastal waters.

## Isolation and Characterization of Marine-Derived *Mucor* sp. for Fermentative Production of Tyrosol [Poster]

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Zygomycota is one of the less characterized phyla of marine microorganisms. An investigation of marine algae surface-associated fungal community led to the isolation of previously un-described *Mucor* strain. All the characteristic features of the genus *Mucor*; sporangiophores, sporangium, sporangiospores and columellae, were apparent in Scan Electron Microscope (SEM) images of the isolated strain. The sequence of the internal transcribed spacer (ITS) rDNA and cellular fatty acid analysis revealed that the strain exhibits 97% of homology to the genus *Mucor*. Tyrosol, (2-(4-hydroxyphenyl)ethanol), was isolated as a major secondary metabolites from ethyl acetate extract of the culture broth and structure of the compound was established through spectroscopic data (1H NMR, 13C NMR and MS). Productivity of tyrosol was considerably higher than that of reported tyrosol producing microorganisms and optimum conditions, culture media, incubation period, temperature and pH, were identified for the production of tyrosol. In conclusion, the finding clearly demonstrates that the novel strain have potential to be developed as a natural source for producing tyrosol for industrial purposes.

## Controlled formation of mono- and dihydroxy-resolvins and protectin analogues from omega-3 DHA and EPA using soybean 15-lipoxygenase

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This study describes the enzymatic synthesis and characterisation of a range of anti-inflammatory and pro-resolution compounds derived from omega-3 polyunsaturated fatty acids (PUFAs) DHA and EPA, which are typically found in high concentrations in marine oils. In this work we have developed a simple and effective method for controlling the progression of resolvins and protectin analogue synthesis using an isolated 15-lipoxygenase enzyme from soybean (*Glycine max*). Soybean 15-lipoxygenase-1 (15-sLOX-1) utilises the *cis,cis*-1,4-pentadiene moiety of PUFAs to catalyse sequential dioxygenation reactions in a regio- and stereospecific manner, generating a range of mono- and di-hydroxy isomers with potentially potent anti-inflammatory properties. We have investigated the effect of experimental conditions on the catalytic activity of 15-sLOX, with pH and enzyme concentration found to have a significant effect on product formation. As a result two methods have been developed for the controlled synthesis of mono- and dihydroxy compounds from five biologically significant PUFAs including arachidonic acid, EPA, DHA, DPA (n-3) and DPA (n-6). Furthermore these compounds have been characterised by NP-HPLC, GC-MS, TOF-MS, NMR spectroscopy and UV-visible spectroscopy to elucidate the complete structure, including stereochemistry. The described methods can be further utilised in the synthesis of such mediators from hydrolysed fish and krill oils which are high in DHA, EPA and DPA concentration using crude soybean sources, thus offering an economical and 'green' method for large scale production of anti-inflammatory mediators from natural products.

## Effects of environmental Conditions on Lipid Accumulation and Diversity of Microalgae at the South East Coast of Queensland – Australia

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Environmental conditions affect the distribution and lipid accumulation capability of marine microalgae. Water samples were collected over time for three representative environmental coastal habitats in South East Queensland, Australia, including a tidal river (Brisbane River), a mangrove forest (Wellington Point) and a rock pool (Mooloolaba). Each habitat presented at least one main stress factor on microalgal survival, such as water flow, nutrient limitation, high salinity and/or light exposure. Results from flow cytometry (FACS) analyses showed that rock pools had only 35% of the cell density than mangrove forests or the Brisbane River. 18S rRNA amplicon pyrosequencing analysis revealed hundreds of eukaryotic species. The diversity of microalgae communities was highest in the Brisbane River which also harboured a large population of zooplankton but diversity and composition of microalgae changed significantly over time. Overall cellular lipid accumulation capability was highest when coinciding with adverse environmental conditions, in particular in rock pools. The relative lipid fluorescence intensity per cell was 1924 in rock pools compared to 409 and 332 in mangrove forest and river water, respectively. Isolation of approx. 200 pure microalgae strains and screening in a standard cultivation assay resulted in 6 strains with outstanding lipid productivity. Out of these, *Nannochloropsis* sp. BR2, *Chlorella* sp. BR2 and *Tetraselmis* sp. M8 were found to be best suited for the development of large-scale outdoor cultivation systems and appropriate harvesting and lipid extraction protocols.

## **Technologies to assist reproductive performance in finfish aquaculture**

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Closing of the life cycle is an important aspect of domesticating aquaculture species, and includes securing reliable reproductive performance. Among the challenges facing various finfish industries are late maturing fish (such as the southern Bluefin tuna, which reaches sexual maturity at 12 years of age), dysfunctional spawning in captivity requiring hormonal manipulations to obtain seed production and large size of some broodstock, which can prevent handling. This talk will describe some of the strategies and technologies developed for hormonal manipulations using peptides to advance pubertal development and induce spawning in finfish as well as approaches for the development of an alternative broodstock system using germ cell transplantation and surrogate technology in fish.

## **Access to Australian marine bioresources in a modern (post Nagoya Protocol) world**

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Australia is well placed to support and participate in the next renaissance of marine natural products research. Collectively, Australian universities and research organisations provide a wide range of infrastructure and capability for biodiscovery at all stages of the pipeline, and Cost-efficient and legally certain access to Australia's marine biodiversity is facilitated through extensive, well curated and value-added ex-situ collections such as the AIMS Bioresources Library and Naturebank. Such access is fundamental to fuel natural product discovery campaigns, and once leads have been identified, ongoing sustainable access to larger quantities of target bioresources is often necessary, to support lead development. The current opportunity to develop the chondropsins, a therapeutic lead in the treatment of cancer and osteo-disease, is a good example of an exciting opportunity which continues to rely on sustainable supply of natural marine material from the AIMS Bioresources Library.

The Australian government understands the marine biodiscovery opportunity and has taken some steps to remove impediments and encourage research activity. Its draft implementation model for the Nagoya Protocol protects Australia's interest in its biodiversity while being careful to avoid the creation of any new legal obstacles or impediments to the use of Australian biodiversity in biodiscovery research. For example, this implementation model includes measures to recognise trusted ex-situ collections and streamline legal compliance procedures when samples are accessed through them. Australia has identified a suite of strategic research priorities for future investment, which are based on key societal challenges, and biotechnology innovation has the potential to be relevant to them all.

## **The Nagoya Protocol – a new legally binding international regime for access to biodiversity and benefit sharing. Can it solve the uncertainty?**

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The world's mega marine biodiversity, and its extraordinary arsenal of metabolic machinery, remains a relatively untapped source of raw materials for biodiscovery. However, the process discovery and product development is fraught with many obstacles and challenges both in the research and innovation required, and the policy, legal and regulatory regimes that govern the use of biodiversity and translation of the outcomes. By the time the Convention on Biological Diversity CBD was opened for signature in 1992, the term 'biopiracy' had been coined and despite its intention to do the opposite, the CBD spawned an era of global uncertainty over legal and jurisdictional issues around access to biodiversity, and the equitable sharing of the benefits arising from the use of inherent genetic resources. This has been blamed, at least in part, for a downturn in global confidence in biodiscovery and subsequent investment to stimulate and grow activity in this field. After over 10 years of robust international negotiations, the parties to the CBD reached consensus and in 2010 opened a new legally binding protocol to the CBD for access and benefit sharing (ABS), which is likely to come into force in 2013 or 2014. The Nagoya Protocol presents an opportunity for a clear and coherent global standard for ABS, which supports and facilitates biodiscovery across international borders by creating certainty and transparency. This presentation will outline the Nagoya Protocol's key points of clarification, some practicalities of its implementation including potential pitfalls, and the outlook for the future.

## Phylogeny drives large scale patterns in Australian marine bioactivity - a chemical ecology rationale for future biodiscovery [Poster]

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Twenty-five years of Australian marine bioresources collecting and research by the Australian Institute of Marine Science (AIMS) has explored the breadth of latitudinally and longitudinally diverse marine habitats that comprise Australia's ocean territory. The resulting AIMS Bioresources Library and associated relational database integrate biodiversity with bioactivity data, and these resources were mined to retrospectively assess biogeographic, taxonomic and phylogenetic patterns in cytotoxic, antimicrobial, and central nervous system (CNS)-protective bioactivity. While the bioassays used were originally chosen to be indicative of pharmaceutically relevant bioactivity, the results have qualified ecological relevance regarding secondary metabolism. In general, metazoan phyla along the deuterostome phylogenetic pathway (eg to Chordata) and their ancestors (eg Porifera and Cnidaria) had higher percentages of bioactive samples in the assays examined. While taxonomy at the phylum level and higher-order phylogeny groupings helped account for observed trends, taxonomy to genus did not resolve the trends any further. In addition, the results did not identify any biogeographic bioactivity hotspots that correlated with biodiversity hotspots. We conclude with a hypothesis that high-level phylogeny, and therefore the metabolic machinery available to an organism, is a major determinant of bioactivity, while habitat diversity and ecological circumstance are possible drivers in the activation of this machinery and bioactive secondary metabolism. This study supports the strategy of targeting phyla from the deuterostome lineage (including ancestral phyla) from biodiverse marine habitats and ecological niches, in future biodiscovery, at least that which is focused on vertebrate (including human) health.

## Isolation of antimicrobial marine bacteria from sub-arctic hydrothermal sites

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Marine sponges, other sessile invertebrates and algae were sampled from two different geothermal sites in North - Icelandic waters; at a hydrothermal vent site in Eyjafjordur and at Kolbeinsey Island, north of Grimsey Island. Bacteria were isolated from the organisms using selective media for Actinomycetes and screened for antimicrobial activity. From Eyjafjordur, 14% of the total bacterial isolates yielded antimicrobial activity, whereas from Kolbeinsey 5% of screened isolates have shown such activity so far. The active isolates were identified by PCR and 16SrRNA sequencing. Both lots are dominated by class Actinobacteria, but the genera distribution are different between sampling sites, possibly reflecting difference in sampled organisms. A difference in growth potential was also observed. Surprisingly, sponges did not host higher percentage of active microorganisms than other macroorganisms and a sample consisting of sea anemone larvae and a nudibranch, retrieved from the vent site, showed the highest rate of active isolates. Growth inhibition was observed in various patterns e.g. against Gram positive and/or Gram negative test strains - indicating different substances causing the antimicrobial activity. Ethyl acetate extracts from growing cultures of active bacterial isolates are being produced and will be screened further as well as using chromatographical methods in order to isolate and identify active compounds. Preliminary results indicate that presence of target bacterium in a culture might stimulate production of compounds which will be further investigated.

## View from oyster genome: The opportunity and challenge for aquatic organism genome study

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The fast developing of sequencing and other high throughput technologies make it possible to construct reference genome and functional genomics study with acceptable cost. Aquatic organisms represent high proportion of biodiversity in the earth but only a few genomes have been decoded. Now it is the high time for aquatic organism research using Omics technology, but many aquatic organisms are with high level of heterozygosity and abundant of repetitive sequences which are challenged for de novo assembly. Here I will give a summary of the advance of aquatic genome study discuss the technical opportunity and challenge for aquatic genome de novo assembly and taking oyster genome as an example to show the genomic study using sequencing technology in whole genome scale which reveals stress adaptation and complexity of shell formation in the Pacific oyster.

## A fatal reovirus of blue crab, *Callinectes sapidus*, has potential to impact the host throughout its entire geographic range

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Diseases have the potential to significantly affect wild populations of marine species. However, because of a lack tools to identify and track disease agents, there are few studies on the impact of diseases throughout the life history of species in the wild. We have identified a reovirus that is associated with mortality in the blue crab, *Callinectes sapidus*, which supports one of the most valuable fishery species along the Atlantic coasts of North and South America. The virus, termed RLV, was identified using a simple yet powerful physicochemical method to enrich virus genomic RNA from host tissue. The method is applicable to any species and has been used to identify multiple additional viruses from blue crab, as well as from marine zooplankton. Using a quantitative PCR method to detect RLV, we have documented it throughout the North American range of the blue crab. Prevalence of RLV varies widely, but is generally over 10% and localized outbreaks of 40 to 50% are not uncommon. RLV is associated with the vast majority of blue crab mortality in soft shell aquaculture, and we are investigating the possibility that fishing and aquaculture activities may contribute to the prevalence of RLV in the wild. Biotechnological tools such as those employed in this study have the potential to revolutionize our understanding of how disease affects fluctuating natural mortality of marine species.

## **Toward understanding marine lifestyles using new-generation sequencing and genomic technologies: Red alga *Pyropia yezoensis* and other case studies**

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*Pyropia yezoensis*, *Susabi-nori* in Japanese, and several other *Pyropia* (aka *Porphyra*) species are edible sea weeds and cultivated widely in the coastal areas among Japan, Korea and China. It is also known that these species go through an yearly dimorphic life cycle with alternating haploid and diploid stages linked with spores of different types. Because of the economical and biological interests, many studies have been accumulated high-lighting characteristics of these species, for example, growth requirement of bacterially produced vitamins as well as symbiotic dependence of proper development were suggested. To understand genetic backbone of these phenomena, we have been conducting genomic analyses of *Pyropia yezoensis* using a battery of new-generation sequencers and high-performance computers. Since extremely high G/C % of the genomic DNA, which is close to 70%, high content of repetitive sequences and co-existing bacterial cells, although some of them might be symbiotic, production of sequence data and subsequent assembly was very complicated. We constructed fosmid library from conchospore DNA and sequenced pools of clones to construct initial framework of the genome, then carefully incorporating other data such as sequences from other tissues or RNA-seq results. The entire process is still on-going but we have to be careful on the processes because simple minded assembly results in erroneous genomic structure. The genomes of recently sequenced rare deep-sea fish will also be discussed.

## **Evaluation of the ligninolytic activity by the marine eukaryotes, Thraustochytrids which using the Remazol Brilliant Blue R as indicator [Poster]**

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It is urgently necessary to develop the microbial biofuel production technique because of the depletion of fossil fuel resources. It is very important to use woody and plant biomass as for microbial cultivation based on the concept "carbon neutral". Thraustochytrids are marine eukaryotic microorganisms and considered as potential agents for single cell oil production. We have focused on the cultivation of thraustochytrids using unutilized plant biomass. However, it is necessary to degrade refractory components contained in plants, such as lignin. In this study, we investigated the distribution of lignolytic activities in thraustochytrids. Remazol Brilliant Blue R (RBBR) was used as a yield substrate to evaluate lignolytic activities. *Aurantiochytrium limacinum* strain SR21 was cultivated in a GY31 medium (3% glucose, 1% yeast extract, 0.01% vitamins mixed solution, 50% ASW) with 0.03% RBBR for 72 hrs at 28°C with shaking at 110 rpm. The biomass was determined as dry cell weight (g/L). The concentration of glucose was measured with glucose CII-test wako (Wako Pure Chemical Industries, Ltd., Japan). Decolorization of RBBR was assayed as the decrease of absorbance at 595 nm. The cell morphology and the colour were observed with a light-microscopy. The concentration of glucose in the medium decreased and exhausted at 72 hours. The absorbance of the culture at 595 nm decreased from 1.6 to 0.8 (0 hrs and 72 hrs, respectively). The cells cultured in a medium with RBBR was coloured in blue. These results indicated that strain SR21 absorbed the RBBR dye in the cells with growing.

## Genetic engineering of microalgae for photosynthetic biofuel production

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Many microalgae accumulate triacylglycerols (TAGs) under nutrients (N and S) starvation conditions. To date, genetic modification has not yet achieved in microalga to enhance biofuel production. We have focused on the carbon-concentrating mechanism (CCM) and regulatory mechanism of the TAG accumulation in *Chlamydomonas reinhardtii*. CCM is essential to support photosynthetic carbon assimilation under CO<sub>2</sub>-limiting conditions, and constituted by inorganic carbon transporters, carbonic anhydrases, and a subcellular compartment, pyrenoid, which maintains high levels of CO<sub>2</sub> around ribulose-1, 5-bisphosphate carboxylase/oxygenase. To enhance the photosynthetic carbon assimilation, low-CO<sub>2</sub> (LC)-inducible genes during the induction of the CCM were identified by DNA array or RNA-seq. we focused on several LC-inducible genes encoding putative transporters on the chloroplast envelope and plasma membrane. By using inducible promoter, we have succeeded to over express two genes in a cell, resulting that the photosynthetic affinity against dissolved inorganic carbon were enhanced. By using FACS system, we have isolated a mutant with increased TAG content under the normal growth condition before N-starvation. The fact that the TAG content was 2.5-fold higher in the mutant than wild-type cells, suggests that a gene encoding negative regulator of lipid accumulation might be disrupted in the mutant. We have also isolated a mutant with reduced level of TAG content after N- and S-deprivation. Regulatory mechanisms of the TAG accumulation might be defective in these mutants. The tag-inserted genomic region and the causal genes of these mutants will be discussed.

## Laser-induced mutation and selection leads to improved *Tetraselmis* sp. microalgae as a hopeful candidate for biodiesel production [Poster]

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Energy crisis, global warming and pollution due to the use of fossil resources have resulted in detrimental effects for our planet. Microalgal biodiesel appears promising because of its unparalleled advantages including high photosynthetic efficiency, fast growth, without occupying farmlands. The isolation and selection of elite microalgae strains with high lipid production and fast growth is crucial. Lasers have been widely applied for breeding of industrial microbes, field and horticultural crops. In the present study, *Tetraselmis* sp. M8 was induced by He-Ne and YAG lasers. By comparing growth rates, four fast growing strains, S2 and S3, were selected from He-Ne laser treatments, while strains B and I were selected from YAG laser treatments. These strains were usually smaller in cell size and reached significantly higher cell densities than the wild-type strain M8 with increases of 67.7%, 18.0%, 39.2% and 34.4%, respectively. In addition, five high lipid productivity candidates were selected from the two laser treatments. S10 and S11 were selected from He-Ne laser and A, H, I from YAG laser treatments. They possessed 64.6%, 104.7%, 52.0%, 15.6% and 56.9% higher lipid productivity than M8. Moreover, eight high carotenoid production candidates were selected from laser treatments. S8, S9, S10, S14 had 418.9%, 325.3%, 213.3% and 206.9% higher carotenoid productivity (generated by He-Ne laser treatment) and E, G, H, I had 63.2%, 81.8%, 116.0% and 143.6% higher carotenoid productivity than M8 (YAG laser treatment). These results indicate that laser treatment is a simple but effective breeding technology for improving microalgae strains for biodiesel and carotenoid production.



## **Integrating genomics and biosynthesis to discover new classes of bioactive secondary metabolites from marine cyanobacteria**

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Marine microorganisms, including marine cyanobacteria, have emerged as major sources for new and biologically active secondary metabolites isolated from the marine environment. Phylogenetic analysis of various collections of marine cyanobacteria from around the world is revealing that there is much more genetic diversity among these organisms than previously realized. Further, genomic information from filamentous marine cyanobacteria has revealed that these organisms have the capacity to produce many more metabolites than have currently been isolated. In combination, these findings encourage the continued exploration of marine cyanobacteria as a resource of high chemical diversity to be evaluated for useful biomedical properties. Our recent work which integrates phylogenetics with natural product isolation and characterization is continuing to be highly productive with many new classes of secondary metabolites being discovered. Moreover, use of MS/MS data with molecular networks is giving fresh insights into the depth of expressed metabolites in cultured cyanobacteria, and MALDI MS of intact cyanobacterial filaments is providing keen knowledge of in vivo biosynthetic processes. Coupling the increase in genomic information with post-genomic successes in heterologous expression of complex natural product pathways, this is an exceptional time during which to study these fascinating marine photosynthetic prokaryotes for their bioactive natural products.

## **The roles of eIF4E family members in zebrafish (*Danio rerio*) [Poster]**

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eIF4E functions to recruit mRNAs to the ribosome through its interaction with the 5'-cap of mRNA. Most eukaryotes express a family of related proteins that play significant roles in growth and development. eIF4Es from multicellular eukaryotes have been grouped into Class I eIF4Es that function as translation factors, and Classes II and III that regulate mRNA recruitment. *Danio rerio* has six eIF4Es; eIF4E-1A, -1B, -1C, eIF4E-2A and -2B, and eIF4E-3 as compared to four mammalian eIF4Es. eIF4E-1C has similar characteristics to eIF4E-1A as demonstrated by its binding to cap analogue (m<sup>7</sup>GTP), eIF4G, and 4EBP. eIF4E-1C rescues growth of a yeast eIF4E knockout (eIF4E-KO) strain, indicative of a functional translation factor. Transcript levels of eIF4E-1A are higher than those of eIF4E-1C in all adult tissues tested, but lower than eIF4E-1C during early embryogenesis. The regulatory eIF4E-1B binds m<sup>7</sup>GTP weakly, is non-interactive with eIF4G/4E-BPs and does not rescue the yeast eIF4E-KO strain. eIF4E-1B transcripts are highest in heart, ovary, testis, kidney, and during early embryogenesis. eIF4E-2A and -2B transcripts in both adult tissues and during embryogenesis are lower than those of the other eIF4Es. Neither protein interacts with eIF4G. Surprisingly, eIF4E-2B is able to rescue the yeast eIF4E-KO strain. Further study is needed to determine how this occurs. Although eIF4E-3 binds to m<sup>7</sup>GTP and eIF4G, it is unable to rescue the yeast eIF4E-KO, suggestive of a mRNA recruitment regulator. eIF4E-3 represents the predominant eIF4E transcript in brain, testis, gill, muscle, and during early embryogenesis and eIF4E-3 protein levels are high in ovary, testis, and zfl cells.

## **Development of Type I microsatellite markers from transcriptome of giant grouper for marker-assisted selection [Poster]**

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The 2728 Type I microsatellite/SSR DNA markers with designed PCR primers in >20,000 non-redundant functional Unigenes were identified from giant grouper (*Epinephelus lanceolatus*) transcriptome and were classified by enriched GeneOntology. From analysis of giant grouper sibling (5-inch 79 fish) by multiple microsatellites genotyping, we had identified 30 polymorphic microsatellite markers from 55 microsatellite markers in skeletal myogenesis control related Unigenes. The different allele frequencies of 9 microsatellite DNA markers and one deletion DNA marker were found from 30 polymorphic microsatellites to be associated with the body weight at a significant level ( $P < 0.05$ ). In another fast growing giant grouper sibling (5-inch 96 fish) selected by traditional breeding, 7 DNA markers from previous identified 10 DNA markers were coincident with fast growth genotypes. In addition, we are screening polymorphic microsatellite DNA markers associated with disease-resistance of giant grouper from 164 SSRs in 126 functional genes related with immune cell activation including B cell, T cell, NK cell and Mast cell activation for MAS of giant grouper with both disease-resistance and fast growth by multiple DNA markers of two economic traits.

## NMR-guided approaches to natural product-based drug discovery

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The isolation and identification of new and novel natural products is a challenging task; one that requires the separation of a single component from hundreds of others like it. The development of a suitable dereplication and metabolic fingerprinting technique early in the isolation workflow is therefore crucial for successful natural product-based drug discovery programmes. Herein, we present an approach based on proton nuclear magnetic resonance (<sup>1</sup>H NMR) analysis of a library of lead-like enhanced fractions. Examples of new natural products sourced from marine sponges and microbes, as well as bioactive compounds identified from high throughput screens using this methodology, will be presented.

## Screening of marine microorganisms: Thraustochytrids from Victorian environment for advancing omega-3 biotechnology [Poster]

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Marine world has a lot to offer in terms of magnified biodiversity, especially microorganisms for the production of lipids (saturated and unsaturated fatty acids). In addition, marine microbes are the potential producers of carotenoids, enzymes and therapeutic metabolites. Thraustochytrids are marine heterotrophic protists sized between 10-80 µm commonly isolated from the mangrove areas and estuarine environments. They have been known for accumulation of large amounts of nutritionally important omega-3 fatty acids and carotenoids. At Deakin, University, we have isolated thirteen strains of thraustochytrids from the Victorian marine environment near the Queenscliff region. Pollen baiting technique was found to be simple and effective in isolation of the thraustochytrids. The fatty acid methyl esters (FAMES) profile of the isolates obtained, exhibited a varied range of docosahexaenoic acid (DHA) accumulation from 11- 41% of total fatty acid content (TFA) while high amounts of saturated fatty acids was also observed in selected isolates showing their potential use towards biodiesel production. Selected isolates were identified on the basis of 18S rRNA sequencing technique as *Thraustochytrium* species, *Schizochytrium* species and *Ulkenia* species. One of the isolates was found to utilise a variety of carbon sources for fatty acid production.

## Development and characterization of interspecific somatic hybrids through protoplast fusion between *Ulva fasciata* Delile (x) *U. reticulata* Forsskål

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Marine macroalgae (Seaweeds) are nowadays gaining prominence as a potential renewable feedstock for bioenergy and chemicals besides their conventional utilization as food, feed, fertilizer and phycocolloids. The growing industrial demands for biomass necessitated the cultivation and subsequent strain selection studies. In order to overcome the barriers with conventional breeding and regulatory hurdles involved in cultivation of genetically modified seaweeds in the open sea, somatic hybridization through protoplast fusion was chosen to generate elite strains. In this study, a total of three interspecific hybrids were generated from protoplast fusion between *Ulva fasciata* and *U. reticulata*. The generated hybrids showed allopolyploidy based on chromosome count. Further the biochemical (isozyme profile of lipoxxygenase) and molecular characterization (RAPD, ISSR and AFLP) along with organelle specific markers confirmed the introgression of fusion partner's genome into hybrids. Functional trait analysis of hybrids showed mid-parent heterosis for growth and heterosis for thermal tolerance correlating with the contents of gibberellins (GA<sub>4</sub>) and ABA. The lower content of primary metabolites over secondary metabolites in hybrids also attributes for hybrid vigour. Additionally, hybrids showed an increased contents of essential fatty acids i.e. C16:0, C18:0, C22:0, C18:4 (n-3) and C22:6 (n-3) over parental partners. Further, the epigenetic regulatory mechanism for heterosis as analyzed from DNA methylation polymorphism showed reduction in methylation attributing to higher genetic expression resulting in heterosis in hybrids. This study not only provided the molecular evidences of interspecific hybrids with improved functional traits but also showed possible mechanisms underpinning the heterosis.

## Calcium Carbonate Crystallization using marine-derived recombinant glycine-rich Proteins [Poster]

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The layer of molluscan shells consists of structure of highly organized calcium carbonate crystals, which provides remarkable strength and toughness compared to pure mineral calcium carbonate. Here, we successfully produced some marine-derived recombinant glycine-rich proteins in *E. coli* based on genetic redesign and codon-optimization, which are expected to be involved in calcium carbonate biomineralization. Calcium carbonate binding properties of the recombinants were investigated, and it was tried to regulate the morphology in calcium carbonate crystallization. From this study, we expect that the recombinant proteins can regulate *in vitro* biomineralization for the production of calcium carbonate with superior mechanical properties. This study can give potential to understand biomineralization mechanism and to fabricate marine-inspired notable materials.

## Discovery of marine natural products targeting Keap1-Nrf2-ARE signalling pathway and mechanism study [Poster]

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The Keap1-Nrf2-ARE regulator pathway plays a central role in the cellular antioxidant response mechanisms. Modern medicine has proved Keap1-Nrf2-ARE signaling pathway activated by drugs, can enhance the body's antioxidant capacity, resulting in anti-inflammatory, anti-cancer and neuroprotective effects. Applicant using the antioxidant response element assay (ARE-Luciferase activity) as a screening model, combined with glutathione Michael reaction probe technology, has obtained a pigment, scytonemin from the extract of cyanobacteria *Scytonema* sp. Scytonemin has demonstrated moderate ARE activity in HepG2 and MCF7 cancer cell lines, compared with control chemicals, t-BHQ and sulforaphane. In addition, we have found scytonemin can induce phase II enzymes over-expression via Keap1-Nrf2-ARE signaling pathway. ARE activation was also evaluated for both scytonemin and nostodione in a normal, non-cancer-derived keratinocyte cell line, HaCaT, using the ARE reporter pGL4.37 from Promega. The current project will be extended to develop a DAD-HPLC-MS based analytical method, in combination with bioactivity and chemical NMR profiling to quickly isolate the compounds with characteristic functional group, from the extracts of the sponge *Dysidea avara* and *Halichondria* sp., collected from the Xisha Islands, China.

## Characterization of an epiphytic bacterium *Neptunomonas* sp. BPy-1 on a red alga *Pyropia yezoensis* [Poster]

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The gametophyte of red alga *Pyropia yezoensis* (TU-1) has been maintained properly in our laboratory. However, an overgrowth of epiphytic bacteria sometimes occurred due to an ethanol contaminated from the room air system. Recently, we isolated an ethanol-eating bacterium *Neptunomonas* sp. BPy-1 from the gametophytes of *P. yezoensis*. BPy-1 showed 100% identity of 16S rRNA sequences with *Neptunomonas* sp. 0536, which was identified as the probiotic bacterium of green shell mussels in New Zealand. Physiological tests showed that 23 characters were identical between BPy-1 and 0536 strains but 4 characters (gelatin liquefaction, glucose acidification, citrate assimilation and maltose assimilation) differed. To further characterize BPy-1, other physiological properties of BPy-1 and the abundance of BPy-1 in the epiphytic bacterial population were analysed. *Neptunomonas* sp. BPy-1 was able to use not only ethanol (or butanol) but also agar for the sole carbon source. Antibiotic resistance tests revealed that BPy-1 has a higher resistant to aztreonam compared to *Neptunomonas* sp. 0536. Total DNA of epiphytic bacteria was isolated from the gametophytes grown under normal conditions, and 16S rRNA sequences were amplified using two primer sets (27F/1492, 75F/1492). Of 51 clones, 42 clones showed an identical sequence, which was most related to Flavobacteria. Other 9 clones including BPy-1 were divided into 6 different groups. DGGE analysis also revealed the similar composition of epiphytic bacteria. These findings suggest that *Neptunomonas* sp. BPy-1 is attached to gametophytes with restricted growth as a minor component of epiphytic bacteria under normal growth conditions.

## Towards High-Efficiency Microalgae Biofuel Systems

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The development of renewable fuels is an urgent challenge facing our society, due to the importance of reducing CO<sub>2</sub> emissions, increase fuel security and providing a sustainable basis for economic development. Global energy demand is ~0.5ZJ yr<sup>-1</sup> (~83% used as fuels and 17% as electricity) and is rising due to the population growth and the demand for continued economic growth. Global documented 1P resources (fossil fuels with a 90% probability of recovery using current technologies and prices) and Ultimately Recoverable Resources (5% probability of recovery using current technologies and prices) of oil, gas, coal and uranium are reported to be 36.5ZJ and 82.7ZJ respectively (BP statistical review). In contrast to finite fossil fuel supplies, global solar energy incident upon the Earth's surface vastly exceeds global energy demand (~3020 ZJ yr<sup>-1</sup>). Of this 1300 ZJ yr<sup>-1</sup> is photosynthetically active radiation that can be used for the production of biofuels. This presentation will provide an overview of the physical constraints of photo-biological biofuel production in terms of land and ocean based systems and advances made by the Solar Biofuels Consortium ([www.solarbiofuels.org](http://www.solarbiofuels.org)) in terms of developing such microalgal systems not only for fuel but also food and high value product production. Microalgae as the can be produced in marine systems and on non-arable land offer an opportunity to contribute to the 70% increase in food and 50% increase in fuel demand predicted by 2050.

## The effect of Z-Nisin and Sodium citrate on increasing of shelf-life of Kutum fillets (*Rutilus frisii kutum*) stored at 4°C

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The effects of nisin Z (0.15 g) and sodium citrate (2%) in packed Kutum fillets during of refrigerated storage was investigated. Changes in some quality indexes of fish including chemical parameters (TBA and TVB-N contents) and microbial parameters (total viable counts: psychrotrophic, lactic acid bacteria and Mesophilic) during 12 days (0, 3, 6, 9 and 12) were determined. The results showed that TVB-N and TBA contents in the Nisin-Z and sodium citrate treatments increased more slowly than the control samples and didn't increase more than the fish acceptability limit (TVB-N and TBA contents of 30-35 mg N/100 g and 5 mg Malonaldehyde/kg is generally regarded as the fish acceptability limit, respectively) during storage time. Up to 6 days TVB-N and TBA contents was higher than limiting level in the control samples. In addition bacteria total counts was higher than limiting level after 6 and 12 days in the control samples and preservatives treatments, respectively. The results of current study revealed that nisin Z in combination with sodium citrate can therefore be used as the effective preservatives to maintain the quality of fillets during refrigerated storage.

## Microbial diversity, function and biotechnological potential of marine sponges

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Many species of sponges (phylum Porifera) harbor enormously dense and diverse communities of symbiotic microorganisms in their tissues, which can comprise up to 35% of the total sponge biomass. As many as 29 bacterial phyla, among them 12 candidate phyla and two archaeal lineages were identified in sponges thus far. Recent amplicon pyrosequencing studies have indicated that as many as several thousand lineages of symbionts exist, making sponges one of the most diverse host-microbe associations in the marine environment. Collectively, the animals and their microbial consortia boast an impressive metabolic and chemical repertoire that not only contributes to their nutritional ecology but has also elicited the interest of the pharmaceutical industry due to their production of bioactive compounds. This presentation will cover global patterns of microbial biodiversity, it will discuss symbiont function in the context of the sponge holobiont, and it will identify products of biotechnological relevance (i.e., anti-infective secondary metabolites) from sponge microbiomes. Overall, these efforts are directed at providing a deeper understanding of the high-complexity microbial ecosystems within sponges, and at providing research strategies to a sustainable use of this natural marine resource.

## **Osteoclastogenic effect of marine algae in human osteoblast-like MG-63 cells [Poster]**

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Useful secondary metabolites are obtained from marine algae, various species of which are found in Jeju Island, Korea. We aimed to screen marine algae products for their ability to suppress osteoclastogenic factors. Sargachromanol G (SG) which isolated from marine algae has anti-osteoclastogenic activity, but its mechanism of action and its active components remain largely unknown. In the present study, we investigated the anti-osteoclastogenic effects of SG on the expression of interleukin-1 $\beta$  (IL-1 $\beta$ )-induced osteoclastogenic factors (PGE2, COX-2, IL-6, OPG, and RANKL) in the human osteoblast cell line MG-63. We also examined the role of the nuclear factor- $\kappa$ B (NF- $\kappa$ B) and the mitogen-activated protein kinase (MAPK) signaling pathways in IL-1 $\beta$ -stimulated MG-63 cells. SG dose-dependently inhibited the production of osteoclastogenic factors in MG-63 cells. SG also inhibited phosphorylation of MAPK (ERK1/2, p38, and JNK) and NF- $\kappa$ B (p65, p50, and I $\kappa$ B- $\alpha$ ). These results suggest that the anti-osteoporotic effect of SG may be because of the modulation of osteoclastogenic factors via suppression of MAPK and NF- $\kappa$ B activation.

## **Evaluation of anti-inflammatory activity of marine algae in LPS-stimulated RAW 264.7 cells [Poster]**

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Inflammation is complex process involving a variety of immune cells that defend the body from harmful stimuli. However, pro-inflammatory cytokines and inflammatory mediators can also exacerbate diseases such as cancer. The aim of this study was to identify a natural effective remedy for inflammation. We isolated a functional algal compound from marine algae and identified as a kinds of chromene, sargachromanol G (SG). In this study, the anti-inflammatory effect and the action mechanism of SG have been investigated in murine macrophage cell line RAW 264.7. SG dose-dependently inhibited the production of inflammatory markers [nitric oxide (NO), inducible nitric oxide synthase (iNOS), prostaglandin E2 (PGE2), and cyclooxygenase-2 (COX-2)] and pro-inflammatory cytokines [tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , and IL-6] induced by LPS treatment. To further elucidate the mechanism of this inhibitory effect of SG, we studied LPS induced nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation and mitogen-activated protein kinases (MAPKs) phosphorylation. SG inhibited the phosphorylation I $\kappa$ B- $\alpha$  and NF- $\kappa$ B (p65 and p50) and MAPK (ERK1/2, JNK, and p38) in a dose dependent manner. These results suggest that the anti-inflammatory activity of SG results from its modulation of pro-inflammatory cytokines and mediators via the suppression of NF- $\kappa$ B activation and MAPK phosphorylation.

## **Anti-inflammatory and Anti-tumor activity of a carotenoid isolated from brown algae through MAPKs regulation**

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Fucoxanthin (FX), a major carotenoid of edible brown algae, has reported to have potent anti-inflammatory and anti-tumor activity both in vitro and in vivo. However, the mechanism underlying FX-induced actions remains unclear. In the present study, we designed to evaluate the molecular mechanism of FX isolated from a marine algae in RAW 264.7 cells for anti-inflammation and against HL-60 and B16F10 cell lines for anti-tumor effects. FX induced dose-dependent reductions in the levels of iNOS and COX-2 proteins and concomitant reductions in the production of NO and PGE2. Additionally, FX was shown to suppress the production of inflammatory cytokines and shown to induce a dose-dependent inhibition of the phosphorylation of mitogenactivated protein kinases (MAPKs; JNK, ERK and p38). In case of anti-tumor effect, we found that ROS are generated during FX-induced apoptosis in HL-60 cells, and NAC which is a scavenger of ROS, suppressed FX-induced cytotoxicity and apoptosis. Furthermore, the treatment with NAC dramatically inhibited FX-induced phosphorylation of JNK and p38 kinase in HL-60 cells. Moreover, FX reduced the viability of B16F10 cells in a dose-dependent manner accompanied by the induction of cell cycle arrest during the G0/G1 phase and apoptosis. These findings reveal, in part, the molecular basis underlying the anti-inflammatory and anti-tumor properties of FX.

## Applicability of aquaculture effluents to production of docosahexaenoic acid by oleaginous microbe, *Aurantiochytrium limacinum* strain mh0186 [Poster]

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Aquaculture has been a fast-growing industry because of increases in demand for seafood throughout the world. On the other hand, discharge of the aquaculture wastewater becomes the problem. In this study, we investigated the reusing of aquaculture effluents to the production of docosahexaenoic acid (DHA) by oleaginous microbe, *Aurantiochytrium limacinum* strain mh0186. *A. limacinum* mh0186 was cultivated in 1/5 concentration GY broth (1/5GY broth: glucose 6 g, yeast extract 2 g and vitamin mix solution 1 ml in artificial sea water 1 L, pH 6.8) as a control group and red sea bream rearing water broth (RW broth) as a test group. The concentration of carbon, nitrogen and phosphorus in the RW broth was adjusted by addition of glucose, yeast extract and potassium dihydrogenphosphate to those of 1/5GY broth respectively. The biomass yield was determined as dry cell weight (DCW) to evaluate the cell growth. The total lipid in the cells was extracted by the Floch method, and the compositions of fatty acid were analysed by gaschromatography. The DCW increased with the decrease of glucose, and the glucose was exhausted after 18 hours in both groups. The DCW in a 1/5GY group and a RW group at 18 hours was 2.07 g/L and 1.90 g/L respectively, and the yield of DHA in the culture medium was 259.23 mg/L and 217.09 mg/L respectively. No significant differences were observed in respective analysis items. These results indicated that potentials of aquaculture effluents for its utilization as the medium for strain mh0186.

## EGFR Tyrosine Kinase Inhibitory Peptide Isolated from Marine *Chlamydomonas* Sp. Attenuates *Helicobacter Pylori*-Mediated Carcinogenic Responses

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The protective effects of an isolated active peptide H-P-6 (Pro-Gln-Pro-Lys-Val-Leu-Asp-Ser) from microbial hydrolysates of marine *Chlamydomonas* sp. against *H. pylori*-induced carcinogenesis was examined in this study. *H. pylori* infection activates the EGFR tyrosine kinase signaling and nuclear translocation of the  $\beta$ -catenin which has the potential to induce carcinogenesis. It was found that EGFR activation led to the up-regulation of PI3K/Akt signaling pathway and the nuclear translocation levels of  $\beta$ -catenin were significantly increased as a result of Akt mediated down-regulation of GSK3/ $\beta$  protein levels in the cytoplasm. Interestingly, the isolated peptide potently inhibited *H. pylori*-mediated EGFR activation and thereby down-regulated the subsequent P13K/Akt signaling leading to  $\beta$ -catenin nuclear translocation. The peptide activity was confirmed with the use of EGFR tyrosine kinase inhibitor AG1487 and molecular docking studies. Collectively this study identifies a potent peptide which binds to EGFR tyrosine kinase and thereby regulates the *H. pylori*-induced hyper-proliferation and migration of AGS cells at molecular level.

## Role of *Marsupenaeus japonicus* crustin-like peptide against *Vibrio penaeicida* and white spot syndrome virus infection [Poster]

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An essential component of the shrimp's innate immune system is the release of antimicrobial peptides (AMPs), which are critical factors for defense against pathogen invasion. One important AMP that has been identified in *Marsupenaeus japonicus* is crustin-like peptide; it contains the highly conserved 12 cysteine-rich region and the characteristic WAP domain. In *M. japonicus*, its antimicrobial activity has not been determined. In the present study, the role of *M. japonicus* crustin-like peptide was elucidated using RNA interference (RNAi). Shrimps were first injected with dsRNA specific to crustin-like peptide (dsCRS), non-specific green fluorescent protein (dsGFP) and phosphate buffer saline (PBS) and subsequently infected by either *Vibrio penaeicida* or white spot virus. Tissue expression, changes in gene expression and total hemocyte counts (THCs) were also determined. dsCRS treated group resulted to a significant mortality compared to PBS and dsGFP treated groups at day-1 post-infection with *V. penaeicida*. A significant mortality rate was also observed in dsCRS treated group at day-5 post-infection with white spot virus. Results showed that crustin-like peptide is highly expressed in hemocytes. Transcript levels of crustin-like peptide were downregulated at day-1 and returned to a level similar to day-0 at day-3 and day-5 post-infection with *V. penaeicida*. On the other hand, a significant upregulation was observed at 12 hours post-infection with white spot virus. dsRNA treatment alone decreased THCs and subsequent *V. penaeicida* or white spot virus infection further decreased THCs significantly. The results demonstrated the importance of crustin-like peptide against *V. penaeicida* and white spot virus.

## Characterization of recombinant gonadotropins activity and their receptors in the Common Carp [Poster]

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The Common Carp (*Cyprinus carpio*) is the largest group of cultured aquatic organisms. In Carp aquaculture, artificial spawning is often used to induce synchronized ovulation. In order to optimize carp production an effective synthetic substitute for the current spawning agents needs to be developed. Previous studies have shown that the gonadotropins (GtHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and their receptors play critical roles in vertebrate reproduction. Using the methylotrophic yeast *Pichia Pastoris*, Carp's recombinant LH and FSH (cLH and cFSH) were produced as a single-chain polypeptide, their biological activity was primarily tested on fragments of carp's ovaries in two different developmental stages and in both cases the GtHs elicited estradiol secretion as expected. The receptors of each gonadotropin (FSHR and LHR) were cloned and expressed in COS-7 cells, their activity was tested using cLH and cFSH, Carp pituitary extract (CPE), tilapia recombinant GtHs (tLH and tFSH) and human GtHs (HCG and hFSH). The most efficient response was to CPE which activated both receptors. Each of the Carp's recombinant GtHs activated its own cognate receptor; however some cross-activity between the receptors was detected. Ligand selectivity and binding specificity has important implications from an evolutionary aspect, the current dogma argues that in Cypriniformes FSHRs show a preference for FSH but also respond to LH, whereas LHRs respond specifically to LH. In the case of the Carp, which is part of the Cypriniforms order, we suspect that both ligands may activate both receptors with different efficiency.

## The lectins from the genus *Codium*

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About 50 species of the genus *Codium* inhabit worldwide and some of them can be utilized as edibles and sources for chemicals. We isolated and characterized the lectins from several species of the genus *Codium* and found that the *Codium* lectins are classified in three groups based on their carbohydrate-binding properties and primary structures. A major group includes GalNAc-specific lectins from *C. fragile* (CFA), *C. pugniforme* (CPA), and the other four species. These lectins mostly consisted of a hexamer ( $\alpha_6$ ) or a tetramer ( $\alpha_2\beta_2$ ). The  $\alpha$  (9.5 kDa) and  $\beta$  (8.5 kDa) subunits share almost the same N-terminal sequence. Interestingly, the complete sequence of the CFA subunit contained a H-type lectin domain that is commonly found in invertebrates. CFA was specific for  $\alpha$ -GalNAc whereas CPA for  $\beta$ -GalNAc. The second group includes the lectin from *C. latum* (CLA), which had no affinity for mono- and oligosaccharides examined. CLA was a monomeric protein consisting of 129 amino acids including three intrachain disulfide bonds, and belongs to the "fascin" superfamily having a beta-trefoil topology with the internal three repeats. The recombinant CLA exhibited hemagglutination activity, suggesting that CLA is the first lectin possessing the fascin domain. The third group includes the lectin from *C. barbatum* (CBA). CBA had no affinity for mono- and oligosaccharides examined, however its hemagglutination activity was inhibited by porcine thyroglobulin. CBA consisted of a S5-linked homodimer of a 9257 Da-polypeptide (84 amino acids) and was a novel protein. Thus, the properties of lectins were diverse within the genus *Codium*.

## Marker-assistant Selection and Breeding through Phylogenetic Relationships in Taiwan Giant Grouper (*Epinephelus lanceolatus*) by Using Microsatellite and Mitochondria Markers [Poster]

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Giant grouper is an economically important fish species in Taiwan's aquaculture industry. Thus, we are attempting to develop a technique that can investigate the parent-offspring/sibling relationship of giant grouper. This technique will improve the inbreeding management of fish farms. In this study, we would like to apply two systems which are microsatellite and mitochondrial genetic markers as tools to analyze genetic distance of giant grouper population. The high variable mitochondrial D-loop sequence is selected to study the siblings' relationship of giant groupers. Analysis of data collected from 3 fish farms, with total samples of 118 giant groupers, we found and categorized 42 haplotypes as well as calculate their genetic distance. Genetically close relative giant groupers would be separated to different farms. On the other hand, we have applied microsatellites system as parent-offspring investigation tool. Based on previous findings, 6 microsatellite markers from other grouper species will be used on giant grouper. By amplifying selected microsatellites via polymerase chain reaction, we can understand their genetic variety and diversity. Combining both techniques would enhance the efficacy for marker-assistant selection and breeding management in fish farm.

## Bioremediation Of Crude Oil Using Indigenous Marine Bacteria

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Bioremediation, mainly by indigenous bacteria, has been regarded as an effective way to clean up oil pollution after oil spill. In order to obtain a systematic understanding of succession of bacterial communities associated with oil bioremediation, sediments collected from Penglai 19-3 oil platform were co-incubated with crude oil. Oil biodegradation was assessed on the basis of changes in oil composition monitored by GC-MS. Changes in the bacterial community structure were detected by two 16S rRNA gene based culture-independent methods, denaturing gradient gel electrophoresis and clone library. Using enrichment culture technique, two isolates that brought a significant degradation and dispersion of crude oil were obtained from the contaminated sediments. Cotton fibers were used as a biocarrier for bacteria immobilization. Our results suggested that crude oil was rapidly degraded during 30 days' bioremediation period. Bacteria affiliated into the genus *Pseudomonas* dominated all the three clone libraries. But dramatic changes were also detected during the process of biodegradation of crude oil. 16S rRNA gene sequencing and phylogenetic analysis indicated that the two bacterial strains affiliated into the genera *Vibrio* and *Acinetobacter*. Among the two isolates, marine bacteria *Vibrio* sp. HC8-3S showed a strong binding to the cotton fibers. Both free and immobilized bacteria showed relatively high biodegradation (>60%) of saturated hydrocarbons fraction of crude oil, in the pH range of 5.6 to 8.6. This study will be useful to develop *in situ* strategies for the bioremediation of Penglai 19-3 oil spills.

## The analyses of de novo milkfish transcriptome assembly in response to salinity and temperature changes

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Milkfish (*Chanos chanos*) is a popular aquaculture marine species in southern Taiwan with great euryhaline ability but low tolerance to temperature disturbance. Milkfish is quite unique because it is the only member in its family. It has been reported that this species sustains in the winter by some strategies. Our previous studies revealed that seawater-acclimated milkfish tolerated lower temperature rather than the fresh water group. Although the tolerance to low-temperature between fresh water- and seawater-acclimated milkfish was different, no related molecular evidence was reported. To investigate the molecular and biological processes involved in the non-model species upon environmental changes, transcriptomic analyses by high through-put next generation sequencing (NGS) was used to verify the massive functional genes of the milkfish. Total RNA extracted from the brain, gill, liver and kidney were mixed and the quality was checked. The RQI values of each group were over 8 which is a recommended value by Illumina. A total 238121 unigenes were identified in an average length of approximate 600 base pairs. GO analysis was performed after gene annotation. Huge amounts of genes in molecular regulation were retrieved by transcriptome comparison with KEGG analysis. The ongoing process and future work will follow the results to deeper levels such as particular pathways involved in low-temperature tolerance or new mechanisms of milkfish acclimated to environments with different salinities or temperature changes.



## Multi-chloride channels from two clades of the CIC members involve in chloride absorption of tilapia gills [Poster]

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Fish gill is a well-defined organ delicately responsible for respiration and ionic homeostasis. Many studies have addressed on the balance of ions manipulated by different ionocytes. However, it is still complicated when focusing on one particular ion. In fresh water, the scarcity of ions promotes the needs of chloride. Chloride channels (CICs) are thought to be localized at basolateral membrane to transport chloride ion. CIC-2 and CIC-3 belong to different subfamily of CIC members. CIC-2 was reported mainly expressed in the gill rather than other CICs in the zebrafish, while CIC-3 was localized in the endosome for acidification in mammals and regulated in the gill of tilapia, pufferfish and European sea bass. In this study, we have cloned partial sequences of tilapia CIC-2s of tilapia defined as isoform -1, -2 and -3. They were widely distributed in tilapia organs like reported in the mammals. Relative mRNA levels of tilapia CIC-2s which were abundant in the brain, gill, and kidney were up-regulated in low-ionic milieus, like CIC-3. However, these chloride channels in the gill might have additional features. In our data, CIC-2 isoform 3 was present in the more acid environment. We also found evidence of intracellular CIC-3 by cell compartment isolations. Thus, the differences or coordination of these two chloride channels in contribution to chloride absorption should be further examined in tilapia gills.

## Specific transcriptional response and cold tolerance ability in PUFA zebrafish [Poster]

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In recent years, temperature has been demonstrated to influence fatty acid N-3 polyunsaturated fatty acids (PUFA) synthesis and desaturases enzyme activity. Enhancement of cold-tolerance can improve survival rate of fish under cold stress conditions. A number of research has been done on the physiological, biochemical and molecular responses of various fish species to low temperature stress. However, there is still no appropriate model to explore the mechanisms between PUFA and cold tolerance. The most significant response to cold stress is the increased level of unsaturated fatty acids. The present study aims to investigate the interaction between desaturases and elongase, key enzymes involved in PUFA biosynthesis pathway, in response to cold stress in zebrafish (*Danio rerio*) model. Transgenic zebrafish was developed using L-FABP, a strong hepatocyte specific promoter to constitute express high levels of Omega-3 biosynthesis genes in liver, each carrying  $\Delta 4$ 、 $\Delta 5$ 、 $\Delta 6$  desaturase and elongase gene. The response of transgenic zebrafish to cold stress will be assessed by swimming performance, balance and orientation. Using these new cold tolerant tet-off models, we will look at the changes in gene expression profile using microarray and next generation sequencing (NGS). We hope to find a PUFA specific marker for cold stress response. These new transgenic fish model is ideal for studies of PUFA and low-temperature environments. This study could also contribute to aquaculture industry and fishing science.

## The transcriptome analysis of grouper, *Epinephelus coioides* in response to Singapore grouper iridovirus (SGIV) infection

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Grouper, *Epinephelus coioides* is an important fish species being maricultured in China and Southeast Asian countries. With rapid development of aquaculture industry, the outbreaks of newly emerging viral pathogens such as iridovirus and nodavirus have severely affected grouper aquaculture and resulted in heavy economic losses. However, limited available information on grouper genomics restricted the understanding of mechanisms of viral pathogenesis and development of antiviral strategies. In this study, we investigated the global transcriptome of *E. coioides* in response to a novel iridovirus, Singapore grouper iridovirus (SGIV), using 454 pyrosequencing method. The abundant high-quality ESTs were obtained from two cDNA libraries constructed from mock- and SGIV-infected grouper spleen. A total of 42501 and 37759 non-redundant ESTs were produced in control and infected libraries, respectively. Of these ESTs, 22.6% (9616) and 27.6% (10426) were matched against known genes in NCBI. Assembled sequences were annotated with gene ontology and clusters of orthologous group terms. Many infection and immune related genes were existed in the infected library, and several important genes were cloned and characterized. KEGG analysis suggested that several immune signalling pathways involved in SGIV infection, including MAPK, chemokines, toll-like receptors and RIG-I-like receptor signals. Taken together, our data will not only contribute to the identification of novel genes from marine fish, but also help to the development of virus-controlling biotechnologies.

## Efficient degradation of alginate using alginate lyases from *Flavobacterium* sp.

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Alginate is a heteropolysaccharide biosynthesized by brown algae and some kinds of bacteria. It consists of  $\beta$ -D-mannuronic acid and  $\alpha$ -L-guluronic acid, and is utilized for food, medical and various industries. Alginate degradation is important for not only decreasing in viscosity of alginate solution but also producing of oligosaccharides, which have physiological activities such as an improvement of plant growth and an antihypertensive action. Recently, alginate is also paid attention as one of bioenergy source. Although many researches on alginate lyases have been published, there is little information regarding an effective production of an  $\alpha$ -keto acid, namely 4-deoxy-L-erythro-5-hexoseulose uronic acid (DEH). DEH is a final product by alginate lyases in bacteria having an alginate metabolism system, and is converted in cytoplasm to pyruvate and D-glyceraldehyde-3-phosphate. In this study, efficient alginate degradation by enzymes was investigated to establish DEH production. We isolated *Flavobacterium* sp. UMI-01 from decaying brown algae *Coccolophora langsdorffi* at the shore of Otaru, Hokkaido, Japan. Cell lysate showed an alginate degradation activity, and DEH was detected. Two alginate lyases, FIAlyA and FIAlex, were purified and properties were characterized. FIAlyA showed an endolytic activity and minimal degradation products were disaccharides. FIAlex yielded DEH, but its activity was poor against alginate polymer compared with the activities against oligosaccharides. Moreover, cDNAs for both enzymes were cloned and recombinant proteins, rFIAly and rFIAlex, were functionally expressed in *Escherichia coli*. Enzymatical properties of recombinant enzymes were matched with those of native enzymes, respectively. DEH was efficiently produced by a combination of these recombinant enzymes.

## Potential compounds from marine *Xestospongia* sp., *Chicoreus* sp. and *Acanthaster planci* as peroxisome proliferator activated receptor gamma (PPAR $\gamma$ ) ligand for anti-atherosclerotic activity

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The current drugs used for atherosclerosis has been reported with side effects in patients such as weight gain, fluid retention and risk of heart failure. In order to treat atherosclerosis, there is much interest in targeting specific receptor in the body that plays an important role in the formation of the disease, in this case the human Scavenger Receptor Class B Type-1 (SR-B1). We report the potential of *Xestospongia* sp. (marine sponge), *Chicoreus* sp. (marine mollusk) and *Acanthaster planci* (marine echinoderm) as producer of Peroxisome Proliferator Activated Receptor Gamma (PPAR $\gamma$ ) ligand that activate the SR-B1 for anti-atherosclerotic activity. The compounds obtained were sterol (*Xestospongia* sp.), hexadecanoic acid (*Chicoreus* sp.) and methyl benzoate (*Acanthaster planci*). These compounds with various concentrations (0.78 to 25.00  $\mu$ g/ml) were treated onto HepG2 which was stably transfected with SR-B1 promoter. Liver Receptor Homolog-1 (LRH-1) was used as positive control in the experiment. All compounds showed positive results towards luciferase activities by increasing the transcriptional regulations of SR-B1 promoter at specific effective concentrations which are 6.25, 12.5 and 3.12  $\mu$ g/ml respectively. The compounds increased the transcriptional regulations of SR-B1 promoter activity and subsequently increased the Luciferase activity of the assay system which also reflects the ability as PPAR $\gamma$  ligand. The efficiency of these compounds was comparable with the LRH-1 suggesting that the *Xestospongia* sp., *Chicoreus* sp. and *Acanthaster planci* have the potential as candidates for anti-atherosclerotic agent proving that marine organism possess compounds that beneficial towards human.

## Anti-atherosclerotic activity of marine sponge *Xestospongia* sp.

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Atherosclerosis or hardening of the arteries which occur due to gradual deposition of lipid, fibrin and calcium in arteries is one of the diseases with a high demand of new remedy. Marine sponges have been proven to contain bioactive compounds that possess many biological activities that are beneficial to humans. This study reports the potential of bioactive compound from *Xestospongia* sp. as an anti-atherosclerotic agent through its ability as the Peroxisome Proliferator Activated Receptor Gamma (PPAR $\gamma$ ) ligand. The methanolic extract (MCE), Diethyl ether and butanol fractions (DEF and BUF) as well as sterol compound of *Xestospongia* sp. were treated onto HepG2 cells transfected with pGL3-PPRE in various concentrations (0.78 to 50.00  $\mu\text{g/ml}$ ) and Rosiglitazone was used as positive control in the experiment. The MCE, DEF, BUF and sterol compound showed positive results to luciferase activities by increasing the transcriptional regulation of pGL3-PPRE at effective concentrations of 6.25, 12.5, 50.0 and 6.25  $\mu\text{g/ml}$  respectively. The result shows that the MCE, DEF and BUF fractions of *Xestospongia* sp. exhibits a potential activity in increasing the transcriptional regulations of pGL3-PPRE promoter activity and subsequently increase the Luciferase activity of the assay system which also reflects the ability of the samples to acts as PPAR $\gamma$  ligand. The efficiency of the extract, fractions and sterol of *Xestospongia* sp. which was comparable with the Rosiglitazone used shows that *Xestospongia* sp. has the potential to be used as candidates for anti-atherosclerotic agent and finally lowering the risk of atherosclerosis in the body.

## A possible process of tetrodotoxin accumulation in marine pufferfish of the genus *Takifugu* [Poster]

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Pufferfish possess a potent neurotoxin, tetrodotoxin (TTX), which is specific blocker against voltage-gated sodium channels on excitable membrane of muscle and nerve tissues. Since TTX have been also detected from various organisms including food animals of pufferfish and TTX-producing bacteria have been isolated from these animals, TTX in marine pufferfish would be accumulated in pufferfish via food web consisting of several steps starting with marine bacteria. On the other hand, there is also another possibility that TTX in the pufferfish body could not be accumulated via the food web in the quantities to explain intoxication of pufferfish, because marine bacteria were found to produce TTX in only limited quantities. Thus, the intoxication process in pufferfish still remain ambiguous. In this study, a lot of eggs were found in the intestinal contents of the pufferfish *Takifugu niphobles*, and direct sequencing analysis for mitochondrial DNA identified the eggs to be those of the another pufferfish *Takifugu pardalis*. The eggs in the total intestinal contents of *T. niphobles* in March 2013 were detected in 14 and 15 specimens of 15 females and 21 males, respectively, and the content was 0.7–67.9% and 2.5–67.9%, respectively. The peak corresponding to TTX was detected in the egg samples by LC-MSMS analysis. The concentrations of TTX in these egg samples were 0.1–24.2  $\mu\text{g/g}$  (0.46–121 MU/g). These results suggested that *T. niphobles* fed the eggs of *T. pardalis* to intoxicate themselves effectively.

## Production, purification and characterization of halothermotolerant solvent stable lipase and its application in ester synthesis

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Enzymes as replacement of chemical catalysts in various synthesis reactions are nowadays gaining global attention. The enzyme catalyzed reactions circumvents the need of hazardous chemicals and their re-usability can make the process economically feasible. This study describes isolation and characterization of an extracellular lipase having catalytic potential for various esterification reactions. Lipase was found to be an extracellular secretion of a poly-extremotolerant bacterium *Bacillus* sp. (MTCC no. 5549). Enzyme showed optimal activity at pH 7.0 and temperatures 10 °C to 30 °C. The enzyme stabilized its activity at broad ranges of pH (5.0-9.0), temperature (10°C-70°C) and salinity (2 M). Further, the enzyme showed stability in various organic solvents for 7 days at a concentration as high as 50% (v/v). Purification of lipase by dialysis, ion exchange and gel permeation chromatography resulted in enzyme recovery of 34.6 % with purity fold of 23. The enzyme was found to have single polypeptide of mol wt. of about 113 kDa. The Km and Vmax for the enzyme was 0.338 mM and 3.07 mM mL<sup>-1</sup> min<sup>-1</sup> respectively. The industrial utility of lipase was demonstrated by catalyzing transesterification reactions for various oils, and esterification for the synthesis of ethyl lactate and butyl acetate. The enzyme was immobilized over magnetic iron oxide particles for easier recovery. The immobilized enzyme was found active for 3 recovery cycles. Also, the enzyme production was optimized on agro-waste e.g. wheat bran. The efficient catalytic properties of lipase in synthetic chemistry together with easier/efficient recovery after immobilization substantiate its industrial utility.

## The Effects of Seaweed *Gongjindan* on Estrogen Like Activities, Platelet Aggregation and Serum Lipid Levels in Ovariectomized Rats [Poster]

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Postmenopausal women are at an increased risk of developing coronary artery disease. The risk increase is due primarily to dyslipidemia accompanying the loss of estrogen secretion. Estrogen-like activities were evaluated using ethanol and hot water extracts of *Gongjindan* and 3 species of blown algae by an *in vitro* detection system. *L. japonica* and *U. pinnatifida* represented statistically significant estrogen-like activities, whereas *Gongjindan* and *E. stolonifera* did not. Furthermore, activities of hot water extracts of *L. japonica* and three mixes were stronger than 10<sup>-7</sup> M 17 β -estradiol. These results suggest that *L. japonica* contains estrogen-like compounds. These results allowed us to perform further investigation utilizing *Gongjindan*. Prepared seaweed *Gongjindan* (GJD) was used for animal experiments to detect its effects on the inhibition of platelet aggregation and serum lipid levels in ovariectomized rats. Old female Sprague-Dawley rats were randomly assigned to 3 groups: sham-operated rats (SHAM), ovariectomized rats (OVX-CON) and ovariectomized rats treated with GJD. Following the ovariectomy procedure, the rats were placed on different diets for 5 weeks. Total cholesterol and triglyceride contents on serum were lower in the SHAM group than those in the OVX-CON group. The GJD diet fed to the ovariectomized rats resulted in a significant decrease of triglyceride levels, a decrease in total cholesterol level, as well as a significant increase in the level of HDL-cholesterol on serum. Less platelet aggregation was observed in the group of ovariectomized rats treated with GJD than that in the OVX-CON group. These results suggest that GJD may be used to prevent or treat the metabolic syndrome of menopausal women.

## **Biomass Evaluation of a Novel Green Microalga *Chlamydomonas* sp. KIOST-1 for Biofuel Production Isolated from Korea**

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From the early 20<sup>th</sup> century, *Chlamydomonas* (Chlorophyceae, Chlorophyta) has been thoroughly investigated as an important model for microalgal physiology and genetics. Recently, those researches also have been expanded on bioremediation and biofuel production. In this study, we evaluated the biofuel production potential of a newly isolated unicellular green microalga from freshwater in Korea. The isolated microalga was preliminarily analyzed by microscopic investigations and its taxonomical position was confirmed by two phylogenetic analysis using 18S rRNA and ITS1-5.8S-ITS2 sequences, respectively. Based on these results, the isolated microalga belongs to the genus *Chlamydomonas*, and finally designated as *Chlamydomonas* sp. KIOST-1. In order to evaluate its potential for biofuel production, cellular components (protein, lipid, carbohydrate, ash and moisture) of the isolate were analyzed. According to the biochemical analysis of *Chlamydomonas* sp. KIOST-1, its protein, lipid, carbohydrate, ash and moisture contents were estimated to  $53.5 \pm 0.8\%$ ,  $20.6 \pm 0.8\%$ ,  $16.9 \pm 1.1\%$ ,  $5.2 \pm 1.2\%$  and  $3.9 \pm 0.1\%$ , respectively. Furthermore, protein and carbohydrate of the microalga were mainly comprised of glutamic-acid (11.6%) and D-glucose (49.7%), respectively. Interestingly, oleic acid ( $33.12 \pm 0.1\%$ ) known as important sources for biodiesel production, were dominantly found from *Chlamydomonas* sp. KIOST-1. Based on these results, the newly isolated *Chlamydomonas* sp. KIOST-1 will be a potential biomass not only for biofuel production but also for protein production in future microalgal biotechnology.

## **A novel coccoid-shaped cyanobacterium, *Myxosarcina* sp. KIOST-1 isolated from Mangrove Forest in Chuuk State, Federated States of Micronesia [Poster]**

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A cyanobacterium was isolated from root sediment of mangrove forest in Chuuk State, Federated States of Micronesia (FSM), and then the isolate was cultured in f/2 medium. From the culture medium, an entangled, dark-brown, coccoid cyanobacterium was observed and identified by its morphological and molecular characteristics. Microscopic analysis revealed that size of yellow-brown or grayish periphytic cell ranged 3–5  $\mu\text{m}$  in diameter, and the colonies were densely packed 4–64 non-polarized blastoparenchymatous cells, arranged more or less regularly. Ultrastructural analysis showed progress of cell division in the inner sheath, while some motile nanocytes of 0.5–0.8  $\mu\text{m}$  in diameter was observed during its reproduction under light microscopic analysis. To determine the taxonomical position of the cyanobacterium, 16S rRNA gene was sequenced and phylogenetic analysis was constructed. Accordingly, the result indicated that the isolated cyanobacterium was most similar to the genus *Myxosarcina*, however, it not clearly separated from the genera *Chroococidiopsis* and *Dermocarpella*. Therefore, the reproduction type and motility of progeny cells were considered as key characteristics in the final identification, and the isolated cyanobacterium was finally classified in the genus *Myxosarcina* and designated *Myxosarcina* sp. KIOST-1. These findings will help to advance our understanding of the cyanobacterial biodiversity in the mangrove forest in the FSM.

## Untapped Bacterial community Enriched from Coastal Marine Sediment under Anaerobic and Thermophilic Conditions

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Normally, marine thermophiles are thought to distribute widely in some anaerobic and thermophilic environments, such as deep sea vent and subsurface petroleum reservoir. Our recent studies demonstrate that hypothermal marine environments, such as coastal sediments, also contain diverse populations of thermophiles which could become active at thermal conditions (>55°C). Studies showed that most of the thermophilic bacteria were belonging to Firmicutes species, and shared extremely low 16S rRNA gene similarities. These bacteria showed various polysaccharide-degrading enzymes including cellulases, alginases. The untapped microbial resource offers a great potential in the search for enzymes with novel catalytic properties, and strains with interesting metabolic pathways. For example, bacterial cellulose-degradation in marine environments is much more unusual than terrestrial environments because of its high salinity, and therefore is likely to evolve different cellulose-degrading systems. The cellulose degradation by anaerobic and thermophilic bacteria under marine environment still remains unexplored. One thermophilic bacterial community with strong cellulose-degrading ability was enriched from coastal marine sediment. By constructing a 16S rRNA gene library, we found that most of the bacteria shared 16S rRNA similarities lower than 90%, and all of them shared 16S rRNA similarities below 94%, which meant that they were novel at least on species level. Sixty percent of the clones were most related to the type strain of *Clostridium thermocellum* with 16S rRNA gene identity around 87-89%. The cellulase activity of the community was comparable to that of *C. thermocellum* LQR1.

## Involvement of multiple eIF4Es in mRNA recruitment in dinoflagellates

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A wide range of studies has implicated mRNA recruitment as a major site of the regulation of gene expression in dinoflagellates. In eukaryotes, eIF4E functions to recruit mRNAs to the ribosome through its interaction with the 5'-cap of mRNA. Similar to plants and metazoans, dinoflagellates express a family of eIF4Es that are expected to function in mRNA recruitment or its regulation. The 5'-caps of dinoflagellates mRNAs derive from spliced leader RNAs and represent a unique cap-4 structure(s). Phylogenetic analysis of RNAseq data on multiple dinoflagellate species demonstrated three separate clades of eIF4E. In *Amphidinium carterae* and *Karlodinium veneficum*, Clade 1 eIF4Es, which show up to seven closely related subtypes, displayed the highest expression. eIF4Es from Clades 2 and 3 showed lower expression. Using binding to m<sup>7</sup>GTP binding as a measure of affinity to the 5'-mRNA cap, differential binding of the eIF4Es from the three clades were observed; Clade 1 and 3 eIF4Es bind to m<sup>7</sup>GTP, Clade 2 eIF4Es do not. The high level of expression and m<sup>7</sup>GTP binding ability of Clade 1 eIF4Es is consistent with their role as functional initiation factors. Clade 1 eIF4Es contain extended amino acid stretches between the structural units of the eIF4E core that show marked heterogeneity between the subtypes. This may reflect differing functions between the subtypes, such as selectivity for different mRNAs. The low cap binding ability of Clade 2 and the low level of Clade 3 eIF4Es suggest they fulfill regulatory functions.

## **Transcriptomic identification of genes affecting growth and reproduction, and SNP association studies with individual growth performance in giant freshwater prawn (*Macrobrachium rosenbergii*).**

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Giant freshwater prawn (*Macrobrachium rosenbergii* or GFP), supports the largest culture industry of any freshwater crustacean species in tropical and subtropical areas around the world, yet little is known currently about this species genome. Here we used 454 GS-FLX pyrosequencing and Ion-Torrent PGM to characterise the GFP transcriptome and to identify genes that influence growth and reproductive traits. A collection of 13,733,210 sequence reads (1719.86 Mb) obtained from muscle, ovary and testis tissues taken from 18 adult prawns was assembled using three different *de novo* assemblers applying multiple *k*-mer approaches. Results of the CLC Genomic Workbench assembly provided the best assembled contig dataset based on number of contigs (44,407), average contig length (437 bp), N50 length (438 bp) and maximum contig length (9,495 bp). 44,407 contigs generated in CLC possessed high similarity with published sequences in the GenBank non-redundant database, with the highest proportion of matches (75%) evident with crustacean and insect sequences. Downstream analyses of GO, KEGG and IPRS identified putative members of several biological proteins, genes and pathways potentially important for growth and reproduction. Association studies of individual growth phenotype with SNPs in target genes identified three exonic SNPs and six intronic SNPs that showed significant associations with estimated breeding values (EBVs) in the experimental animals. Individually, they explained 2.6–4.8% of the genetic variance ( $R^2 = 0.026-0.048$ ). This is the first large set of SNP markers reported for *M. rosenbergii* and will be useful for confirmation of associations in other samples or culture lines for marker-assisted selection.

## **Modification of EPA/DHA biosynthetic pathway by transgenesis in a marine teleost, nibe croaker**

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Marine fishes are generally unable to produce eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) as they are deficient in the key fatty acid-metabolizing enzymes in the EPA/DHA biosynthetic pathway. It is therefore necessary to supplement with fish oil to diets for cultured marine fish species, which is a dietary source of EPA and DHA. However, since fresh water fishes are capable of synthesizing both EPA and DHA, and they presumably express all of the enzymes required for this biosynthetic pathway, we hypothesized that transgenic marine species carrying the aforementioned fatty acid-metabolizing enzymes could be reared without addition of fish oil to their food. As the first step towards this goal, we produced a transgenic marine fish, the nibe croaker (*Nibea mitsukurii*), carrying an elongation of very long chain fatty acids protein 2 (*elovl2*) gene (*OmElo2*) isolated from masu salmon (*Oncorhynchus masou*), which has been predicted to catalyze the elongation step required for producing C22 and C24 fatty acids from C20 and C22 fatty acids, respectively. Fatty acid analysis revealed that the liver EPA (20:5n-3) content in the *OmElo2* transgenic fish decreased (3.3% vs. 7.7%). However, docosapentaenoic acid (DPA; 22:5n-3) content in the transgenic fish was 2.28-fold (4.1% vs. 1.8%) higher than those of non-transgenic fish. Further, tetracosapentaenoic acid (TPA; 24:5n-3), which was not detected in non-transgenic fish was specifically detected in the transgenic fish. We therefore concluded that transgenesis of fatty acid-metabolic enzymes could be a powerful tool for manipulating fatty acid metabolic pathways in fish.

## Extraction of carotenoids from raw macroalgae excluding drying and cell wall disruption by liquefied dimethyl ether [Poster]

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Co-production of value-added materials is important toward inexpensive algae fuel. In this study, extraction of fucoxanthin from raw macroalgae *Undaria pinnatifida* was conducted with simple method using liquefied dimethyl ether (DME) as solvent. The operation absolute pressure was 0.59 MPa, and the temperature in the extractor and distillation tower was around 25°C. Liquefied DME is mixed with wet algae in the extractor, and lipid and carotenoid are extracted. The mixture of lipid and DME is separated from the algae residue and ejected from the extractor. Next, DME in the mixture is evaporated in the heat exchanger at 40°C, and the lipid is separated from DME in the distillation tower. The DME vapor is then condensed in the heat exchanger at 10°C. This method saves energy, because the waste heat around the operation temperature can be used for the circulation of DME. Despite DME extraction omits cell wall disruption, drying and heating, 0.390 mg of fucoxanthin from 1 g of *U. pinnatifida* was extracted. The extracted fucoxanthin amount was 7.8 times bigger than that by typical ethanol Soxhlet extraction. The current study provides a safe, eco-friendly method that combines the high yield extraction of the active ingredient and drying of macroalgae in a single step.

## A mini review on algae biofuel in Korea

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Recently, very productive photosynthetic algae are highlighted in the presence of renewable natural biomass. The carbon-captured algae can be processed into both biofuels and valuable co-products. In Korea, algae biofuel research was initiated in the early 1990s. The research activities were unfortunately stopped due to limited governmental budget and low petroleum prices. Interest on algal biofuels in Korea has been growing recently after 2000 due to an increased oil prices, energy security, greenhouse gas emissions, and the potential for other biofuel feedstock to compete for limited agricultural resources. Despite various efforts and techniques have been reported to produce biofuels from various feedstock, numerous technical bottlenecks have still remained to be economically feasible. Only few reports seemed to meet the economic feasibility although numerous results have been reported to date. We developed a high-pressure liquefying conversion process under semi-supercritical conditions, to directly convert the cellulose to glucose of macroalgae without uses of acid and/or alkali treatments and expensive cellulases. Therefore, the extracts through this process yielded 15–20% (v/v) of alcohol concentrations by the yeast, compared to 8–10% (v/v) of alcohol using conventional treatments. For biodiesel production, some microalgae have been cultivated in different culture systems to obtain biomass with high lipid concentrations. We found that light intensity and growth medium greatly affected the lipid concentration within the cells in some strains. Therefore our systems demonstrate the possibility of economically producing biofuels from algae, while some barriers of microalgal biofuels need to be overcome for industrialization.

## High-pressure extract of *Phaeodactylum tricornutum* inhibits HGF-induced proliferation in human gastric cancer SNU-1 and AGS cells

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Hepatocyte Growth Factor (HGF) is known to enhance proliferation and reproduction in several human cell types including gastric system cells. Recent studies demonstrated that HGF can induce the progression of various gastrointestinal diseases. Certain natural remedies have been used to treat above mentioned gastric disease. Among all, microalgae is a well studied source for its ability to possess various bioactive materials which are being utilized in several fields such as pharmacology and cosmetic. In this study, we examined the anti-proliferative effect of high-pressure extract of *Phaeodactylum tricornutum* and its possible bioactive fraction on HGF-induced human cancer gastric cell lines. ERK pathway protein levels and the translocation of E-cadherin were measured by western blotting and immunofluorescent assay, respectively. All results from above assays confirmed in accordance an effective protection against HGF-induced proliferation on both SNU-1 and AGS gastric cell lines. Therefore, *P. tricornutum* is a promising natural source for bioactive materials to be utilized in order to treat common gastric diseases enhanced by HGF.



## Germ cell-specific excision of the loxP-flanked transgene in rainbow trout

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Targeted cell ablation using a toxin gene is a powerful tool for understanding cell function. In fish, however, transient and non-specific expression of an episomally located toxic gene injected into one-cell-stage egg cytoplasm can result in unexpected cell death shortly after the mid-blastula transition. In the mouse, cell type-specific Cre/loxP-mediated gene activation and inactivation through excision of the loxP-flanked gene has become an invaluable tool and has been widely used in targeted cell ablation. As a first step toward targeted cell ablation in rainbow trout, this study examined the feasibility of germ cell-specific Cre/loxP-mediated gene excision and activation of the transgene. We established a stable transgenic line Tg (*vasa-cre*) that strongly expressed *cre* mRNA in both male and female gonads under the control of a germ cell-specific promoter. We also established a stable reporter transgenic line, Tg (*heat shock cognate71:loxP-DsRed-loxP-EGFP [hsc:LRLG]*), which carries two fluorescence protein genes, *DsRed* and *EGFP*, driven by a ubiquitous promoter. Crossing of the male Tg (*vasa-cre*) with the female Tg (*hsc:LRLG*) induced excision of the loxP-flanked *DsRed* gene in the *hsc:LRLG* genome and expression of the *EGFP* gene specifically in the germ cells of the double transgenic line. Our data showed that Cre activity was responsible for excising the loxP-flanked *DsRed* gene from the genome of the germ cells. We concluded that a cell type-specific Cre/loxP-mediated gene activation and inactivation system can be used for targeted cell ablation using a toxin gene in rainbow trout.

## Mixotrophic cultivation of *Euglena gracilis* using waste from food industry [Poster]

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*Euglena gracilis* is a unique protist that can grow either autotrophically, heterotrophically, or mixotrophically. However, autotrophic cultivation of *Euglena gracilis* gave a low yield of the biomass same as the cultivation of other microalgae. For an efficient CO<sub>2</sub> fixation and a practical production of valuables from CO<sub>2</sub> by *Euglena*, its high density culture is required under illumination. In the present study, the waste from food industry, such as waste beer, was utilized as organic carbon source for the mixotrophic cultivation of *Euglena*. The mixotrophic cultivation of *Euglena* in the medium that yeast extract and vitamins B1 and B12 were added into waste beer gave a rapid growth and quite high density of *Euglena* compare to its autotrophic cultivation. Moreover, semi-continuous autotrophic cultivation of *Euglena*, following the high density mixotrophic cultivation using waste beer, resulted in the efficient autotrophic cultivation in high density. The *Euglena* cells that obtained by the high density autotrophic cultivation were rich in protein and seemed to be suitable for fishery feed. As a result, the high density mixotrophic cultivation of *Euglena* using waste from food industry made an efficient CO<sub>2</sub> fixation and a practical production of valuables, such as fishery feed, from CO<sub>2</sub> possible.

## Overexpress glutathione reductase to prevent thioacetamide induce oxidative stress in zebrafish [Poster]

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Thioacetamide (TAA) is one of the hepatotoxin that can cause liver fibrosis, cirrhosis and hepatocarcinoma in mice and zebrafish, but its toxicity to embryonic development is still unclear. In this study, we found TAA can induce edema formation in pericardial sac (eps), heart failure-like syndrome, un-hatched out and embryo death. The level of hydrogen peroxide, nitric oxide and lipid hydroperoxide are increased significantly. The liver-specific overexpress glutathione reductase (GR) transgenic fish are generated by using Tol2 transposon system. The level of GR mRNA is increase about two-fold, GR protein is increase about 1.5-fold, and GR enzyme activity is increase about 2.5-5 fold. The GR transgenic fish larve were used to test the inhibition of TAA induced oxidative stress. The hydrogen peroxide and oxidative stress markers of GR TG-fish were significantly lower than control after TAA treatment. The TAA induced cell death is also reduced in GR TG-fish larva by acridine orange staining. When exposed to 4mM H<sub>2</sub>O<sub>2</sub> for 72-96hr, the survival rate was increased and the deformation rate of embryos were decreased in GR TG-fish. Our results showed TAA can induced H<sub>2</sub>O<sub>2</sub> that damage to embryos, and overexpress GR can prevent TAA induced oxidative damages.

## Arsenic tolerant sponge-associated bacteria of the Red Sea *Theonella swinhoei* and their implication for water remediation

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*Theonella swinhoei*, a common Indo-Pacific sponge, contains high amounts of arsenic and barium with a bioaccumulation factor of over 10<sup>6</sup>. Sponge fractionation to bacterial and sponge-enriched fractions analyzed by ICP-AES showed that 60-80% of both elements were found in the bacterial fraction. Further analysis of arsenic species by HPLC-ICP-MS revealed that the dominant soluble form is arsenate, the majority of which is localized in *Entotheonella* sp., and that a large part of the arsenic pool is insoluble. Culture media were designed to select for a variety of arsenic-modifying bacteria, with 5 mM of arsenate or arsenite as the selective agent. Two approaches were taken to isolate arsenic-tolerant sponge-associated bacteria: direct inoculation on solid media or acclimatization in a system mimicking the 3D environment of the mesohyl, achieved with a dried sponge skeleton in liquid medium. While the classic culturing approach yielded isolates mainly on two media, bacterial growth was observed on all seven media after acclimatization. Arsenic tolerance assays with arsenate-grown bacteria, revealed 13 isolates tolerating up to 100 mM arsenate. Several isolates were observed to precipitate arsenic salts on top of the colonies, as revealed by SEM-EDX, while others accumulated arsenic or barium. These arsenic-tolerant sponge-associated bacteria have the potential to be utilized in bioremediation of polluted water sources. The World Health Organization declared arsenic contamination in Bangladesh the worst mass poisoning in history, while the Agency for Toxic Substances and Disease Registry placed arsenic first in the hazardous substance priority list. These statements reinforce the importance of bioremediation.

## Bioactive Metabolites from a Jellyfish-derived Fungus *Phoma* sp. [Poster]

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Endobiotic environment is the habitat of many groups of microorganisms within the tissues of plants and animals and also is a complex milieu in association with specific microbial biota. These ecological interactions exist not only between the host and their endobiotic microorganisms, but also between the endobiotic bacteria and fungi, which share a common substrate. Their host provides organic nutrition or habitat and endobiotic microorganisms act as chemical guards. Unlike free-living marine microorganisms, they biosynthesize unique secondary metabolites in special ecological niche, which have interesting pharmacological properties. To search for bioactive secondary metabolites and understand the ecological function of these compounds, 12 bacterial and 12 fungal strains were isolated from the inner tissue of the marine jellyfish *Nemopilema nomurai*. Investigation of endozoic microorganisms associated with the jellyfish *N. nomurai* led to the isolation of fungal strain *Phoma* sp. (J08NF-7) that exhibited high toxicity against brine shrimp. Bioactivity-guided fractionation of the culture broth of *Phoma* sp. yielded five new steroids including new C<sub>25</sub> steroid, 23,25-dihydroxyergosta-4,6,8(14),20-tetraen-3-one, and three cholic acid derivatives, eleven cytochalasin derivatives, and one tentoxin derivative in addition to known congeners. Their structures were elucidated on the basis of FABMS and 1D and 2D NMR (principally COSY, HMBC and HSQC). The compounds were evaluated for cytotoxicity against a small panel of human solid tumor cell lines (A549, SK-OV-3, SK-MEL-2, XF 498, and HCT15), and cytochalasins exhibited considerable cytotoxicity.

## Comparative analysis of biochemical components in *Spirulina maxima* Cy-23 and the newly isolated *Leptolyngbya* sp. KIOST-1 [Poster]

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Numerous approaches have been attempted to produce microalgal biomass for commercial exploitations. In recent years, *Leptolyngbya* sp. KIOST-1 was isolated from the *Spirulina* culture raceway in Korea. In order to evaluate its potential for industrial applications, the compositions of cellular components including amino acids, mono-saccharide and fatty acids (FAs) in the isolate were investigated and compared to those in *Spirulina maxima* Cy-23 strain which was cultured at the raceway. From the *Leptolyngbya* strain, ash, carbohydrate, lipid and moisture contents were estimated to be 10.2±0.1%, 24.5±0.7%, 11.4±0.5% and 1.3±0.1%, respectively. And its protein content was estimated to be 52.6±0.1%, thus proving that it is major component in the isolate. Similarly, protein (50.7±0.3%) was dominant cellular component in *Spirulina* strain, and its ash, carbohydrate, lipid and moisture contents were estimated to be 20.3±0.3%, 17.8±0.3%, 9.1±0.5% and 2.2±0.1%, respectively. Moreover, glutamic-acid (14.2% and 14.7%) and aspartic-acid (11.0% and 10.2%) were identified as dominant amino acids from the *Leptolyngbya* and *Spirulina* strains, and D-glucose (75.9% and 80.3%) was dominant mono-saccharide in those two cyanobacteria. In the FAs analysis, unsaturated FAs such as 16:1 w9c (18.5%), 18:1 w9c (21.4%) and 18:2 w6,9c/18:0 ANTE (16.9%), and 16:0 saturated FAs (17.3%) were dominantly found from the isolate. However, the *Spirulina* strain dominantly contained 16:0 saturated FAs (39.3%), and the 18:0 iso FAs (16.2%) and the 18:2 w6,9c/18:0 ANTE (16.3%) unsaturated FAs were also detected from it. Based on these results, *Leptolyngbya* sp. KIOST-1 will might have potential for the industrial production of microalgal biomass alike *Spirulina*.

## Evaluation of protective efficacy of a novel *Aeromonas* phage PAS-1 against *A. salmonicida* subsp. *salmonicida* infections in rainbow trout (*Oncorhynchus mykiss*) model

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To search for candidate control agents against antibiotic-resistant *A. salmonicida* subsp. *salmonicida* infections in salmonid culture, one lytic bacteriophage (phage), designated as PAS-1, was isolated from the environmental water, and its several biological properties were investigated. The phage showed broad host ranges to other subspecies of *A. salmonicida* as well as *A. salmonicida* subsp. *salmonicida* including antibiotic-resistant strains. The PAS-1 was morphologically classified as *Myoviridae* and possessed approximately 48 kb of double-strand genomic DNA. Moreover, partial genomic and structural proteomic analysis of PAS-1 revealed that the phage was closely related to other *Myoviridae* phages infecting Enterobacteriaceae or Aeromonadaceae. For the evaluation of potential protective efficacy of PAS-1, the phage was preferentially co-cultured with one virulent *A. salmonicida* subsp. *salmonicida* strain that possesses the *ascV* gene, and strong bacteriolytic activity was observed against the bacteria. The administration of PAS-1 in rainbow trout (*Oncorhynchus mykiss*) demonstrated that it was cleared within 200 h post-administration from the fish kidneys, and temporal neutralizing activity against the phage was detected in the phage-administrated fish serums. The protective effects of the phage were verified in experimental rainbow trout-furunculosis model therapy, showing increased survival rates and mean time to death. Based on these results, phage PAS-1 could be considered as potential therapeutic or prophylactic candidate against antibiotic-resistant *A. salmonicida* subsp. *salmonicida* infections in salmonid culture.

## Preventive Effect of Marine algae on Bone Loss in C2C12 Myoblasts [Poster]

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Bone loss may be due to the increased bone resorption and widely recognized as a major public health problem. Recent studies have shown that marine algae and their extracts can help to prevent bone loss. However, these effects have not yet been clarified in vitro and vivo. Thus, the effect of various algae on bone calcification was investigated in C2C12 myoblasts by measuring alkaline phosphatase activity and bone mineralization. Brown algae, *Sargassum horneri*, *Eisenia bicyclis*, *Ecklonia cava*, *Sargassum thinbergii* and *Sargassum hemiphyllum*, were extracted three times with MeOH and their effects on osteogenic differentiation was investigated in C2C12 cells. Bone alkaline phosphatase activity, which is an enzyme for calcification, was significantly enhanced by treatment with algae extracts. Additionally, intracellular calcium contents also elevated in the presence of algae extract. Moreover, pretreatment of C2C12 cells with various algae extracts dramatically induced the expression of the osteoblast markers such as alkaline phosphatase (ALP), bone morphogenic protein-2 (BMP-2), collagen and osteocalcin (OC). Comparative analysis demonstrates that *S. horneri* extract showed highest effect on the bone calcification in C2C12 cells in a dose-dependent manner. Therefore, present study demonstrate that the intake of algae extract has preventive effect of bone loss in C2C12 cells.

## Osteogenic and anti-adipogenic activities of *Salicornia herbacea* [Poster]

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Mesenchymal stem cells have been reported to contribute to both self-renewal and multi-lineage differentiation into mesoderm-type of cells such as osteoblasts and adipocytes. In this study, we investigated the effects of *Salicornia herbacea* on the adipogenic-differentiation in 3T3-L1 adipocytes and D1 mouse mesenchymal cells using MeOH extracts from *S. herbacea*. Osteogenic activity was examined by measuring ALP activity and the expression of osteogenesis-related genes. The level of lipid accumulation was determined by measuring Oil-Red O staining at the end of differentiation. Lipid accumulation along with the expression of several genes associated with adipogenesis was examined at the end of differentiation. The expression levels of adipose genes were examined using reverse-transcription polymerase chain reaction (RT-PCR) analysis. *S. herbacea* extract significantly reduced lipid accumulation in 3T3-L1 adipocytes. Treatment with *S. herbacea* extract down-regulated peroxisome proliferator-activated receptor- $\gamma$  (PPAR  $\gamma$ ), CCAAT/enhancer-binding proteins  $\alpha$  (C/EBP $\alpha$ ) and differentiation-dependent factor 1/sterol regulatory element-binding protein 1 (SREBP1) in 3T3-L1 adipocytes and D1 mouse mesenchymal cells. In addition *S. herbacea* enhanced the osteoblast differentiation by the activation of ALP, BMP-2, osteocalcin and collagen. Therefore, these results suggest that *S. herbacea* has promising potential to act as a functional ingredient effective in improving osteoporosis and obesity.

## Protective Effects of *Ecklonia cava* on Osteoporosis and Adipogenesis in Mesenchymal Cells [Poster]

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*Ecklonia cava* (Laminariaceae) has emerged as the most abundant edible marine algae and consumed as a kind of seasoned vegetable in the coastal area of Asian countries including Korea and Japan. It has been treated as a source of natural marine product due to its biological activity in a broad range. In this study, we investigated the effects of *E. cava* on the adipogenic differentiation and osteoblast differentiation. Mesenchymal stem cells have been reported to contribute to both self-renewal and multi-lineage differentiation into mesoderm-type of cells such as osteoblasts and adipocytes. The anti-adipogenic activity was investigated by measuring Oil-red O staining, morphological changes, and the expression of adipocyte differentiation biomarkers. The effects on osteoblast differentiation was investigated by measuring ALP activity and the expression of ALP, BMP-2, osteocalcin and collagen mRNA. *E. cava* extract significantly reduced lipid accumulation in adipocytes and decreased the expression of peroxisome proliferator-activated receptor- $\gamma$  (PPAR  $\gamma$ ), CCAAT/enhancer-binding proteins  $\alpha$  (C/EBP $\alpha$ ) and differentiation-dependent factor 1/sterol regulatory element-binding protein 1 (SREBP1) mRNA in 3T3-L1 adipocytes and D1 mouse mesenchymal cells. *E. cava* extract enhanced the osteoblast differentiation by activation of ALP and upregulation of ALP BMP-2, osteocalcin and collagen. Therefore, these results suggest that *E. cava* could be developed as a functional ingredient effective in improving both osteoporosis and obesity.

## **Inhibitory Effects of *Sargassum thunbergii* on Adipogenic Differentiation in Mouse Mesenchymal Cells [Poster]**

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Brown algae *Sargassum* sp. are found throughout tropical and subtropical areas of the world and are valuable source to produce various bioactive secondary metabolites. In this study, the effect of *Sargassum thunbergii* on the adipogenic differentiation in D1 mouse mesenchymal cells using EtOH extracts from *S. thunbergii*. The anti-adipogenic activity was investigated by measuring Oil-red O staining, morphological changes, and the expression of adipocyte differentiation biomarkers. *S. thunbergii* significantly reduced lipid accumulation in adipocytes and decreased the expression levels of peroxisome proliferator-activated receptor- $\gamma$  (PPAR  $\gamma$ ), CCAAT/enhancer-binding proteins  $\alpha$  (C/EBP $\alpha$ ) and differentiation-dependent factor 1/sterol regulatory element-binding protein 1 (SREBP1) mRNA in 3T3-L1 adipocytes and D1 mouse mesenchymal cells. In western blot analysis, the expression level of adipogenic target protein such as RXR $\alpha$ , RXR $\beta$ , LXR $\alpha$  and LXR $\beta$  also down-regulated by *S. thunbergii*. It was known that mesenchymal stem cells contribute to both self-renewal and multi-lineage differentiation into mesoderm-type of cells such as osteoblasts and adipocytes. Therefore, these results suggest that *S. thunbergii* could be developed as a functional ingredient effective in improving both osteoporosis and obesity.

## **Investigation and development of bioactive substances from marine organisms**

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While more than 80% of living organisms are found in marine ecosystems, only less than 5% of the marine resources have been utilized as human food materials. Nutritional properties of fish, shellfish, algae and marine microorganisms are generally well known. However, their functional characteristics have not been fully revealed. It is believed that they contain biologically active compounds including potential nutraceuticals. For example, marine macroorganisms produce a vast array of secondary metabolites including terpenes, steroids, polyketides, peptides, alkaloids, porphyrins and polysaccharides. These secondary metabolites serve many biopharmaceutical purposes (antitumor, anti-inflammation, anti-allergy, antioxidant, antifungal, anti-HIV, and antihypertensive). However, development of a new drug requires sufficient amounts of pure compounds that exceed by large quantities, but it is extremely difficult to collect them in higher amounts from a marine environment. If the compound of interest was originally isolated from a bacterium, fungus, or microalga, the organisms could be cultured at a large scale by fermentation. With limits for the recovery of natural bioactive compounds from different resources, molecular biological and genetic approaches should be integrated as standard husbandry practices that play an increasingly important role in the enhancement of production efficiency of bioactive substances through biotechnological improvement of the transformed microorganism species. Many bioactive substances, such as antitumor, anti-inflammation, anti-allergy, antioxidant, antifungal, anti-HIV, antihypertensive and skin whitening agent, have been identified and investigated from marine organisms using various marine biotechnologies. Moreover, with the respect to investigation and development of marine bioactive substances for industry applications, many studies have been conducted to develop marine biotechnologies, such as membrane bioreactor, bioconversion and continuous mass producing process technology. Industrially developed marine medicinal substances have been widely popular because of their biological activities. The biotransformation technology consisting of membrane bioreactor-assisted bioconversion and continuous massproduction made significant contributions to the commercial development of marine nutraceutical and biomedical substance. A membrane bioreactor equipped with ultrafiltration for the production of bioactive compounds has recently been considered as a potential method to bioprocess marine organisms and byproducts efficiently.

## The Effect of *Scytosiphon lomentaria* on Differentiation of Osteoblastic MC3T3-E1 Cells [Poster]

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*Scytosiphon lomentaria* is an irregularly lobed, brown seaweed found in temperate, littoral waters worldwide. The process where mature bone tissue is removed from the skeleton and new bone tissue is formed is called bone remodeling. This lifelong process remodels approximately 10% of an adult's skeleton annually. The osteoblast activities are important for bone remodeling. In this study, the effect of the *Scytosiphon lomentaria* extracts on proliferation, alkaline phosphatase (ALP) activity, and nodule formation of cells were investigated by using osteoblasts. When osteoblasts were processed with *Scytosiphon lomentaria* hot water extract or *Scytosiphon lomentaria* ethanol extract, the proliferative rate significantly increased at each concentration level. The highest proliferation rate of 115% was obtained when the *Scytosiphon lomentaria* hot water concentration level was 10 µg/mL. Further, the ALP activity was 101% higher when *Scytosiphon lomentaria* hot water extract was processed at a concentration of 50 µg/mL. The effect of nodule formation increased in the supplemented *Scytosiphon lomentaria* hot water extracts more than that in the *Scytosiphon lomentaria* ethanol extracts. In addition, ALP, BMP2, macrophage-colony stimulating factor (M-CSF), and RUNX2 were expressed in osteoblastic MC3T3-E1 cells. The results indicate that *Scytosiphon lomentaria* extracts promote differentiation inducement and proliferation of osteoblasts and, therefore may provide beneficial effects on the bones.

## Effects of *Eisenia bicyclis* Fractions on Osteoblast Differentiation and Osteoclast Formation [Poster]

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Limited to the temperate Pacific Ocean, *Eisenia bicyclis* is found mostly around Japan. Because this species is high in calcium, iodine, iron, magnesium and vitamin A as well as many other minerals, it has been cultured in other locations, including South Korea. The effects of *Eisenia bicyclis* extracts on osteoblast differentiation and osteoclast formation were investigated. First, the proliferation of the MC3T3-E1 osteoblastic cells was tested by MTT assay. Treatment with *Eisenia bicyclis* hot water extract increased cell proliferation by approximately 180% at a concentration of 50 µg/mL. The ALP activities in the MC3T3-E1 cells treated with *Eisenia bicyclis* ethanol extract increased by 170% on 50 µg/mL reased. Second, the proliferation of the RAW 264.7 osteoclastic cells was tested by MTT assay. Proliferation decreased significantly in response to treatment with the *Eisenia bicyclis* extracts. Moreover, the proliferation of the RAW 264.7 osteoclastic cells treated with *Eisenia bicyclis* hot water extract decreased by nearly 92%. In addition, the *Eisenia bicyclis* extracts reduced the number of tartrate-resistant acid phosphatase-positive (TRAP+) multinucleated cells from osteoclastic RAW 264.7 cells. These results indicate that *Eisenia bicyclis* extracts have an anabolic effect on bone through the promotion of osteoclast differentiation and suggest that the extracts could be used for the treatment of common metabolic bone diseases.

## The Feasibility of pilot production of *Spirulina (Arthrospira) maxima* cultivated newly constructed raceway pond in Republic of Korea.

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This study reports a novel microalgae raceway with 40 ton culture medium, which is modified from traditional paddlewheel-driven raceways. To control cold and heat in the raceway during winter season, the pond is excavated to 1 m-deep substrate with passive solar green house constructed by building information modeling (BIM). The flow and mixing rate (15-30 rpm) of seawater is controlled by new developed paddlewheel and controller. For optimizing the design factors of the mixing ground, flow visualization, velocity measurement and cell density ultrasonic detector were employed to investigate the flow mixing features. An optimized pilot plant design for microalgae mass culture in temperate area and details of mixing are presented. The capability of the pilot production of *Spirulina maxima* was tested in Ansan, South Korea (37.287°N, 126.833°E). In a pre-industrial trial using raceway ponds, the strain displayed satisfactory growth under batch condition. In 2-years trial in the ponds, average production was recorded 0.99±0.16 g/L which showed stable productivity in a year. Maximum production was estimated 1.418±0.09 g/L in August 2011 while minimum production was estimated 0.597±0.05 g/L in October 2011. In conclusion, intensive pilot production of *S. maxima* is feasible in Korean climates, a region previously thought to be outside its geographic limits. The study presents an inventive design for microalgae mass culture system, which is significant model to the microalgae and biofuel industry in temperate area.

## Bis(methylthio)gliotoxin isolated from marine fungus *Aspergillus fumigatus* inhibits HGF-induced cell proliferation in human gastric epithelial AGS cells [Poster]

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Hepatocyte Growth Factor (HGF) is known to enhance proliferation and reproduction in several human cell types including gastric system cells. Recent studies demonstrated that HGF can induce the progression of various gastrointestinal diseases. In this study, we examined the inhibition of cell proliferation of Bis(methylthio)gliotoxin on HGF-induced human gastric epithelial AGS cells. For check cell viability, AGS cells were treated with recombinant human HGF and Bis(methylthio)gliotoxin spontaneously and observed by MTT assay. Also, AGS cells were treated with recombinant human HGF and mRNA and protein levels were determined by RT-PCR and Western blot analysis, respectively. All results from above assays confirmed in accordance an effective protection against HGF-induced proliferation on gastric cancer cell lines. Therefore, Bis(methylthio)gliotoxin is a promising natural compound for bioactive materials to be utilized in order to treat common gastric diseases.

## Expression and tissue distribution of skeletal myosin heavy chain genes from adult and larvae of shrimps [Poster]

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The present study focused on myosin from kuruma *Marsupenaeus japonicus*, black tiger *Penaeus monodon* and Pacific white *Penaeus vannamei* shrimps. Myosin occupies the most part of skeletal muscle and has a variety of isoforms with different properties. It consists of two heavy chain subunits (MYH) and four light chain subunits with biologically important abilities such as ATP and actin binding. Full-length MYH genes (MYHs) were cloned from abdominal fast muscle in adults of the above-mentioned three shrimps and defined as MYH1 and MYH2. On *in situ* hybridization the transcripts of MYH1 were detected in the flexor muscle, whereas the transcripts of MYH2 in the extensor and flexor muscle. Pleopod muscle did not express either MYH1 or MYH2. Northern blot analysis using different tissues from abdominal and pleopod muscle supported these results. Two MYHs, named MYH3 and MYH4, were determined from pleopod muscle of kuruma shrimp, and two MYHs, named MYH4 and MYH5, were from Pacific white shrimp pleopod. In contrast, only MYH3 were cloned from black tiger shrimp pleopod. Larval type MYHs were also cloned from zoea, mysis and postlarvae of black tiger and Pacific white shrimps. The phylogenetic tree revealed that most MYHs from pleopod muscle formed a clade with MYH1, whereas larval and postlarval MYHs formed a clade with MYH2.

## Bioactive potential of some intertidal molluscs collected from Mumbai coast (West coast of India)

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Mumbai located on west coast India is endowed with long coast line. In spite of pollution of Intertidal areas in and around Mumbai, moderate diversity of macrobenthos like molluscs has been recorded at selected sites of it. During present investigation bioactive potential of gastropods *Trochus radiatus* and *Echaleus asper* was assayed using their methanolic extract for antimicrobial and antitumor activities. Anti-microbial activity of crude extracts in various solvents was tested against five fish and eleven human pathogens using standard agar disc diffusion method. Acetonitrile extract of *E. asper* showed inhibitory activity against fish pathogens *Vibrio harveyi* and *Aeromonas hydrophila* and human pathogen *Pseudomonas aeruginosa*. However, there was no inhibition by the acetonitrile extract of *T. radiatus* on any of the pathogens studied. Methanolic extract of *E. asper* has shown inhibitory activity on human pathogen *Staphylococcus aureus*, whereas the methanolic extract of *T. radiatus* did not show any inhibitory activity. Ethyl acetate extract of *E. asper* as well as *T. radiatus* gave inhibitory zone against *E. coli* and *S. aureus*. Hexane and petroleum ether extract of both gastropods did not show any inhibitory activity on any of the pathogens studied. Anti-tumour activity was assayed by using human breast cancer cell line Mc-F<sub>7</sub> and Human lung cancer cell line A<sub>549</sub>. The studies on antitumor activity of *E. asper* and *T. radiatus* of methanol extracts revealed that methanol extract of *E. asper* and *T. radiatus* have potential to inhibit growth of tumour cells. Growth inhibition of both cell lines was measured by estimating protein at the end of drug treatment as well as net loss of 50% cells following the treatment.

## Luminescence behaviour of marine luminous bacteria under nutrient-saved conditions [Poster]

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Despite the knowledge is accumulated about Quorum Sensing, it is not known about luminescence behaviour under nutrient-starved conditions. In this study, we investigated about luminescence behaviour using various marine luminescent bacteria under artificial seawater medium (ASW composition, NaCl, KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> · 6H<sub>2</sub>O, NaHCO<sub>3</sub>, MgSO<sub>4</sub> · 7H<sub>2</sub>O). In nutrient-starved conditions, *Photobacterium liognathi* ATCC 33469 and *Vibrio harveyi* ATCC 14126 did not induce the luminescence. When these strains cultured nutrient enrichment conditions, the luminescence increased. Therefore these strains regulate the luminescence dependent on Quorum Sensing. But, *Vibrio fischeri* ATCC 49387 and *Allivibrio fischeri* ATCC7744, although the very low cell densities conditions, they induce the luminescence. Since the cell density of these strains increase for 6 h, these results suggested these strains regulation of luminescence was cell density-independent. This regulation of luminescence intensity is not dependent on Quorum Sensing, because the cell densities in this medium were not reached a critical threshold concentration. When *V. fischeri* was cultured in the ASW medium without MgSO<sub>4</sub>-starved conditions, luminescence intensity was not increase. The luminescence intensities in the *V. fischeri* are controlled by exogenous sulfur source under the nutrient-starved condition. On the other hand, the luminescence intensities in the *A. fischeri* are controlled by exogenous sulfur and potassium. These results suggested that *V. fischeri* need sulfur to induce the luminescence under nutrient-starved conditions. Sulfur and potassium were required for *A. fischeri* to induce the luminescence under these conditions. These induction of luminescence system were different from Quorum Sensing.

## Gene characterization, cloning and over-expression of the acetyl xylan esterase from *Ochrovirga pacifica*

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We isolated novel genus strain from seaweed included seawater from coastal area of Chuuk state in Micronesia, and named *Ochrovirga pacifica* in family *Flavobacteriaceae*. Genome was analyzed from the strain by genome sequencer-FLX. Acetyl xylan esterase gene (Axe) was detected from the genome. Acetyl xylan esterase hydrolyzes ester linkages of acetic acid in xylan polysaccharide. It is known to help xylanase activity. The Axe was 864 bp which is encoding 287 amino acids. The deduced amino acid sequence of the Axe showed 35.1% similarity with both endo-1,4-β-xylanase B from *Robiginitalea bififormata* HTCC2501. It has a theoretical molecular mass and an isoelectric point (pI) of 32 kDa and 5.9, respectively. The mature protein displays the catalytic residues classically found in the enzymes belonging to GH16 family. We cloned Axe into pET11a vector and expressed in *E. coli* BL21(DE3). Purified his-tagged acetyl xylan esterase checked optimum conditions and specific activity.



## Anaerobic conditions and poor nutrient media reveal antibacterial properties of sponge-associated bacteria [Poster]

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Many of the sponge-derived bioactive compounds are considered products of their associated microbiota based on their structure resemblance to typical microbial metabolites, or through the use of localization studies. If such compound-producing microbe can be cultured, the big caveat of the supply problem of marine natural products that hinders pharmaceutical exploitation of many such compounds can be solved. The Indo-Pacific common *Theonella swinhoei* is a high microbial abundance (HMA) coral reef sponge, with up to 40% of its body volume made of bacteria. Numerous secondary metabolites were extracted from *T. swinhoei*, some are novel antitumor active polyketides, while others have antifungal or antibacterial activity. Considering that only a small fraction of all *T. swinhoei* symbionts has ever been cultured, improving bacteria cultivation methods from this sponge could provide many new bioactive compounds, of ecological and pharmaceutical interests. In order to increase the culturable bacterial diversity we used diverse culture media compositions and a variety of growth conditions, determined according to ecological data. Overall 59 OTUs, based on 97% similarity of 16s rRNA gene, were retrieved. These OTUs represent 44 genera of 6 phyla. Twenty two OTUs are yet uncharacterized species, genera and families. Eleven of these novel OTUs were strict anaerobic, and 21 were specific to one type of culture media. Antibacterial tests showed that 28 OTUs have antibacterial properties against environmental and laboratory bacterial strains. Our study encourages the use of diverse culturing media and conditions planned according to the organisms' natural environment to overcome the "unculturable" bacteria barrier.

## Recombinant production and characterization of a thermophilic arylsulfatase from the marine bacterium *Thermotoga maritima* [Poster]

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Production of low sulfated agar or agarose from agar or agaropeptins by enzymatic hydrolysis has advantages but a high melting temperature is needed. The arylsulfatase gene from thermophilic *Thermotoga maritima* was cloned and expressed in *Escherichia coli* W3110 with pCol-MICT as the vector. The gene was comprised of 1,782 bp and encoded a protein of 593 amino acids with a molecular weight of 65 kDa. The recombinant arylsulfatase was partially purified by heat treatment (70°C, 30 min) and characterized. The enzyme was prepared with a total protein content of 2.4 mg and a specific activity of 20.63 U/mg. Optimal temperature and pH of the enzyme were 70–80°C and 7.0, respectively, for hydrolysis of *p*-nitrophenyl sulfate and sulfate content of agar was diminished to 40% after a 12 h treatment at that condition. Enhanced electrophoretic movement of DNA was observed in enzyme-treated agar gel compared to that in a non-treated agar gel. These results suggest that thermophilic arylsulfatase expressed in *E. coli* could be useful for producing a low sulfated agar and electrophoretic grade agarose.

## The hair growth promoting effects of *Eucheuma cottonii* [Poster]

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*Eucheuma cottonii*, an edible species of pacific red algae, has a high amount of polyunsaturated fatty acids and phenol compounds. Previous studies have reported that intake of *E. cottonii* has antioxidant activity in hypercholesterolemic rat and wound healing and hair growth promoting activities in wounded SD rat. In this study, we evaluated the effects of *E. cottonii* on elongation of the human hair shaft *in vitro* and promotion of hair growth in C57BL/6 mice. Treatment with *E. cottonii* extract significantly increased the proliferation of human follicle dermal papilla cell (DPCs) and outer root sheath (ORS) cells. It also stimulated hair shaft elongation in cultured human hair follicles. In addition, treatment with *E. cottonii* extract induced expression of keratinocyte growth factor (KGF), insulin like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF) in DPCs. Moreover, *E. cottonii* promoted induction of anagen phase from telogen phase of the hair shaft on the dorsal skin of C57BL/6 mice.

## Cloning, expression and characterization of L-Asparaginase from *Mesoflavibacter zeaxanthinifaciens* S86.

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L-Asparaginase(EC 3.5.1.1) catalyzes the hydrolysis of L-asparagine to L-aspartic acid and ammonia. L-asparaginase has been used as therapeutic agents in the treatment of acute childhood lymphoblastic leukemia. We carried out genome sequencing for *Mesoflavibacter zeaxanthinifaciens* S86, which isolated from seawater of Chuuk State in Micronesia. L-Asparaginase was detected from the genome and analyzed the sequence. The coding region is 1053 bp, encoding 350 amino acids. The predicted molecular mass was 39 kDa. The amino acid sequence of L-asparaginase from *M. zeaxanthinifaciens* S86 showed highest identity (69%) with type I L-asparaginase of *Cellulophaga lytica* DSM 7489. A gene encoding a L-asparaginase from *Mesoflavibacter zeaxanthinifaciens* S86 was cloned and expressed in *E. coli* BL21(DE3). The purified his-tag fusion L-asparaginase was tested activity with L-asparagine. Furthermore, we are going to study of the enzyme activity in other tumor cells.

## Molecular cloning, overexpression and purification of a novel laminarinase from *Mesoflavibacter zeaxanthinifaciens* S86

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Laminarin is a linear polymer of  $\beta$ -1,3-D-glucose with some  $\beta$ -1,6-D-glucose branching. It is a major structural and storage polysaccharide of various brown seaweeds and microalgae. Laminarinase (endo-1,3(4)-D-glucanase) catalyse the hydrolysis of  $\beta$ -1,3-D-glucosidic linkages in glucan. Laminarinase coding genes have not been demonstrated in *Mesoflavibacter zeaxanthinifaciens* S86. Here, we first report a novel laminarinase gene from the *Mesoflavibacter zeaxanthinifaciens* S86. *Mesoflavibacter zeaxanthinifaciens* S86 was isolated from seawater of Chuuk State in Micronesia. The genome of *M. zeaxanthinifaciens* S86 was sequenced by Genome Sequencer-FLX (GS-FLX), a next generation sequencing (NGS) technology. A unique nucleotide sequence that showed homology to known laminarinase was identified by the BLAST algorithm. It was cloned and designated as MzLam. MzLam was 2106 bp which is encoding 702 amino acid residues and includes a glycosyl hydrolase family 16 (GH16) laminarinase module. The deduced amino acid sequence showed highest identity (34.9%) and similarity (45.1%) with laminarinase form *Leeuwenhoekiella blandensi*. The predicted molecular mass of mature protein was 74 kDa. His-tagged MzLam was overexpressed in *Escherichia coli* and purified as a fusion protein. Optimal temperature for rMzLam was 50°C, while optimal pH was 7.0. The rMzLam activity was enhanced by 20% with 25 mM of MnSO<sub>4</sub>. The substrate specific activities of MzLam towards laminarin,  $\beta$ -glucan, lichenan, curdlan were 261, 128, 115 and 92 Unit/mg, respectively. In contrast, activity of against carboxymethyl-cellulose (CMC) was not observed. These characteristics indicate that rMzLam is a useful candidate for bioethanol production using brown seaweeds and microalgae.

## **Study of effects of nutrients on testicular maturation in the black tiger shrimp by sperm performance assessment and cDNA microarray analysis**

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Poor reproductive maturation of the black tiger shrimp in captivity is one of serious threats to sustainability of the farming industry. Feeds are believed to be crucial for testicular maturation of the shrimp. In this study, sperm performance assessment, gene expression analysis by cDNA microarray and morphological study by scanning electron microscope were conducted to compare the effects between reproduction-boosting polychaetes and commercial pellets on testicular maturation. Measurements were made before a four-week feeding trial (Week 0), and 3 and 4 weeks after feeding. For the sperm performance, total sperm counts of polychaetes-fed shrimp were significantly higher than those of pellet-fed shrimp at Weeks 3 and 4. While % abnormal sperms increased at Weeks 3-4 from Week 0 in both groups, the % from polychaetes group was significantly lower than the pellet group at Week 4. Acrosome reaction rate in the polychaetes-fed shrimp was significantly higher than that in the pellet-fed shrimp at Week 4. A cDNA microarray analysis revealed differentially expressed transcripts between the two groups which were further investigated using bioinformatic tools such as gene ontology, pathway mapping and gene enrichment analysis. Quantitative realtime PCR of selected transcripts, such as *ADP ribosylation factor 4*, *Gins complex subunit 4*, and *MCM2*, confirmed significantly differential expressed patterns in testis between the two shrimp groups. These results demonstrate the importance of nutrients on the shrimp testicular maturation at the molecular levels which serves as an initial step to rationally improve feed formulation and farming practice for this important industry.

## ***Perkinsus atlanticus* Infestation in undulated surf clam, *Paphia undulata*, along the east coast of Thailand [Poster]**

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The Apicomplexa parasites of the genus *Perkinsus* are responsible for a perkinsosis disease in many marine bivalves and gastropods. Animals infected with *Perkinsus* often exhibit retarded growth, impaired reproduction and pathologic symptoms around the infected cells and tissues that ultimately lead to death of the hosts. In this study, we investigated the prevalence of *Perkinsus atlanticus* in undulated surf clam from along the east coast of Thailand. We performed a monthly survey in Chonburi and Trat province during October 2010 to September 2011 and during January to December 2012. The highest infection intensity from Chonburi and Trat province were  $207,536 \pm 28,969$  and  $528,150 \pm 98,749$  cell/individual. The results manifested significant differences ( $p < 0.05$ ) in *Perkinsus atlanticus* infection intensities between sample collected from Chonburi and Trat province.

## Masculine sex differentiation pathways in the fresh water prawn *Macrobrachium rosenbergii*, a hinge around the major component, insulin- like androgenic gland hormone (Mr-IAG).

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Insulin- like androgenic gland hormone (IAG) is a major key regulator of sex differentiation in crustaceans. In the fresh water prawn *Macrobrachium rosenbergii*, Mr-IAG was silenced through repeated injections of Mr-IAG dsRNA into juvenile males. The silencing caused full and functional sex reversal. However, the sex determinations switch, which turns on or off the IAG hormone in males and females, respectably is not known yet. Moreover, the downstream cascade to IAG is also not discovered yet. By using *M. rosenbergii* Next Generation Sequencing transcriptomic library, we have been mining candidates that may play a role in the sex determination and differentiation mechanisms. In the transcriptomic library, we found some homologues transcripts of conserved genes which are known to have a role in the sexual determination/ differentiation cascade. In order to generate a full sequence we assembled *in silico* few shorter contigs together. Silencing these transcripts using dsRNA injections seems to affect sexual development. Changes in testis formation and AG hypertrophy were observed in the silenced individuals. The relation between the silenced transcripts and Mr-IAG is studied. Here we present some novel transcripts that may play an important role in the sexual differentiation cascade. Nowadays, there is a growing interest in crustacean monosex cultured which requires sexual manipulations, therefore, finding sex differentiation related mechanism is of importance.

## Genome Screening and Biosynthesis of Manumycins-type Compounds from Marine *Streptomyces*

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Marine streptomycetes are robust sources of new bioactive compounds. However, the discovery ratio of novel compounds from *Streptomyces* has decreased in recent years. With the development of sequencing technology, the genome sequences of streptomycetes provide the abundant secondary metabolite genes to elucidate the probable biosynthesis pathways. Manumycins-type compounds have C7N and C5N structures with significant anti-tumor and anti-inflammatory bioactivity. In this study, we focused on the discovery of manumycins-type compounds based on marine streptomycetes genome and established the genetic screening and biosynthesis methods to find manumycins-type compounds. We presented the genome sequence of marine *Streptomyces griseoaurantiacus* M045 that produced manumycin A and chinikomycins antibiotics. Genome analysis revealed a number of genes related to biosynthesis of secondary metabolites. The gene cluster for manumycin A contains 32 genes, including 3-amino-4-hydroxybenzoic acid synthase, 3,4-AHBA carrier protein, 3-oxoacyl-(acyl carrier protein) synthase, and 5-aminolevulinic acid synthase. Chinikomycins contain a pABA core component, but share partial gene cluster with manumycin A. Based on the genome of *S. griseoaurantiacus* M045, we established the PCR and hybridization methods to screen the manumycins-type compounds from marine streptomycetes. And new manumycins-type compounds were biosynthesized by genetic engineering. This work provides insight into discovering cryptic metabolic potential, directing traditional natural product isolation and biosynthesis of bioactive compounds produced by marine streptomycetes based on the genome sequences.

## **New cystine knot peptides of a marine sponge, and its potential as a scaffold for oral peptide drug delivery [Poster]**

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New cystine knot peptides were isolated from a marine sponge *Asteropus* sp., collected off the coast of Geoje Island, Korea. The primary structure of asteropsin A (ASPA) was determined by Edman degradation and corroborated by sequence specific resonance assignment based on NMR (DQF-COSY, TOCSY, and NOESY) data. The crystal structure of asteropsin A was determined by X-ray at a high resolution of 0.87 Å. For comparison of asteropsin A in crystal and solution states, the solution structure was independently completed using NMR techniques. The 37-residue peptide is folded by three cystine bridges indicative of the knot peptide class. The unique properties of ASPA with *N*-terminal blocking, absence of basic residues, *cis*-prolines and henceforth different bioactivities distinguish it from other reported knottin families such as conotoxins and spider toxins. Additional analogous peptides (asteropsins B-E) were isolated, and they were found to share unique properties derived from an *N*-terminal pyroglutamate modification, two conserved *cis* prolines, and a highly acidic nature, which distinguish them from other knottin family peptides. The tertiary structures of ASPB and ASPC were determined by solution NMR spectroscopy and that of ASPD by homology modeling. Asteropsins were found to share a highly conserved structural framework and remarkable stability against the enzymes in gastrointestinal tract (chymotrypsin, elastase, pepsin, and trypsin) and human plasma, which enable their use as orally available peptide scaffolds. This new subclass of knottin peptides was structurally described and characterized regarding to their potential for oral peptide drug development, and their recombinant synthesis was pursued.

## **Three novel C-type lectins from *Eriocheir sinensis* functions as pattern recognition receptor (PRR)**

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The basic mechanism of host fighting against pathogens is pattern recognition receptors recognized pathogen-associated molecular patterns. However, the specificity of recognition within the innate immune molecular of invertebrates remains largely unknown. For that reason, the immune function of three pattern recognition receptors, C-type lectin EsLecA, EsLecG and EsLecD were investigated in this study. The EsLecA cDNA contained a 480-bp open reading frame that encoded a putative 159 aa protein, EsLecG cDNA contained a 465-bp open reading frame that encoded a putative 154 aa protein, and EsLecD contained a 468-bp open reading frame that encodes a putative protein of 155 aa residues. EsLecA and EsLecG mRNA expression in *E. sinensis* were (a) both detected in all tissues, including the hepatopancreas, gills, hemocytes, testis, accessory gland, ovary, muscle, stomach, intestine, heart, thoracic ganglia and brain, and (b) responsive in hepatopancreas, gill, hemocytes post-LPS stimulation all appeared dramatically variation. The EsLecD transcript was mainly detected in the hepatopancreas but rarely in other tissues, and it was significantly up regulated in the hepatopancreas after LPS stimulation. The recombinant EsLecD protein (rEsLecD) exhibited the ability to bind to all tested microorganisms, including bacteria and yeast. Meanwhile, calcium significantly increased the binding affinity of rEsLecD toward microorganisms. The binding of rEsLecD induced the aggregation of microbial pathogens. Moreover, rEsLecD was capable of inhibiting the growth of microorganisms and even directly killing bacteria. Interestingly, rEsLecD could stimulate cellular encapsulation *in vitro*. Collectively data demonstrated the successful isolation of three novel C-type lectins from Chinese mitten crab, and their role in the innate immune system of an invertebrate.

## Microalgae nutrient efficacy as aquafeed additives: a booster of aquaculture sustainable development

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Intensive aquaculture operations are heavily regulated in Australia due to the high levels of nutrient present in discharge water and this is a major limiting factor on the growth of the domestic industry. Over the past decade, aquaculture has grown and reached a global landmark in 2009, supplying greater than half of the fish destined for human consumption. Fish are an important resource to supply protein and nutrients to humans and accounts for one-third of total animal protein intake in the human diet. However, with the decline of global fisheries and an increase of human population, pressure from aquaculture operations has increased competition with forage fisheries for highly contested fishmeal derived from bait fish species that provide essential animal protein in fin fish diets. Microalgae systems provide an elegant clean technology solution that can bioremediate the excess nutrients present in an aquaculture farm while rapidly producing plant biomass. This forms the basis of the aquatic food chain and hence their high nutritional value and protein content hold the promise of unlocking a more sustainable source of aquaculture feed. The overall approach of this study is to evaluate indigenous microalgae strains for aquaculture nutrient removal and to also develop competitive and functional microalgae aquafeed additive.

## Study of the microRNA 145 mediated regulatory mechanism for liver development in zebrafish [Poster]

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The liver is the essential metabolic organ in the body. The regulated mechanism of embryonic liver organogenesis remains elusive. Zebrafish are a suitable model for studying organogenesis. Our preliminary results have identified a known tumorigenic growth factor, Progulin (Pgrn), which regulates MET signaling in the zebrafish liver developmental morphogenesis. Additionally, the microRNA is a crucial regulatory mechanism to fine-tune the gene expression that could modulate the developmental process and tumorigenesis. The miR-145 has been shown that differentially expressed in HCC patients and involved in liver tumorigenesis. However, little is known about the miR-145 regulatory mechanism in the liver development. In our study, we modulate the miR-145 expression pattern in zebrafish by miR-145 mimic and miR-145 hairpin inhibitor treatment. We further confirm the role of miR-145 in liver development by using whole-mount in situ hybridization with series developmental marker. The results show that overexpression of miR-145 could inhibit hepatic outgrowth. Hence, we further search potential target genes of miR-145 related to liver development in zebrafish by bioinformatics software and microarray analysis. We examine that Pgrn might be one of potential target genes of miR-145 by luciferase reporter assay. Further we will examine that miR-145 mediated Pgrn signaling in liver development by gain/loss of function experiments. We will also evaluate the more potential direct targets of miR-145 in the liver cells. Therefore, our findings indicate the regulatory mechanism between miR-145 and potential target genes - Pgrn signaling in liver development. We believe the results could contribute to the further therapeutic studies of liver development and cancer formation.

## Effects of Tributyltin on the activities of immunologic enzyme in blood serum of the *Macrobrachium rosenbergii* [Poster]

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The activities of different immunological enzymes in the serum of *Macrobrachium rosenbergii* exposed to different doses of tributyltin (TBT) (0.1mg/L, 0.2mg/L, 0.4mg/L) was investigated. The results showed that the activity of SOD in serum were significantly increased ( $P<0.05$ ) at the high dose group within 12h. However, the activity was then decreased after 48h. The activity of ACP in the serum of all tested doses were significantly inhibited ( $P<0.05$ ) within 12h, but the activities of ACP in serum were significantly increased ( $P<0.05$ ) at the high dose group after 48h. The activities of AKP in the serum were significantly decreased ( $P<0.05$ ) in the early stage at medium and high dose groups, but the effect on the activity of AKP in serum was no-significantly at the low dose group. The activities of AKP in serum were inhibited initially but then increased at medium and high dose groups. In summary, different immunological enzymes showed different pattern of responses when the shrimp were exposed at different concentrations.

## Identifying the bottlenecks of microalgal lipid production: a new transcriptional profiling approach

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Lipid induction in microalgae is considered essential to achieve high triacylglyceride contents for commercial biodiesel production. Nitrogen deprivation is an effective method of lipid induction in microalgae. Aside from lipid accumulation, nitrogen deficiency also has major consequences for other microalgal cell functions such as carbon metabolism and photosynthesis. Using next generation RNA sequencing and metabolic profiling, this study revealed molecular changes of *Tetraselmis* sp. under N deprivation, particularly in the first 24 hours of N deprivation. Physiological observations revealed that early lipid accumulation was predominately due to a reduced fatty acid degradation rate rather than lipid biosynthesis. This included the downregulation of acyl-CoA synthetase-encoding genes, the first step in fatty acid degradation. This process was accompanied by reduced chlorophyll content and growth rates. This first report on molecular mechanisms of lipid accumulation in *Tetraselmis* sp. provides a new approach for metabolic engineering to maximise lipid accumulation in microalgae.

## Purification, characterization and optimisation of metalloprotease from *Pseudomonas poae* PGPR2 [Poster]

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The novel isolate of *Pseudomonas poae* PGPR2 produced a maximum extracellular metalloprotease with a proteolytic activity of 195U/ml at 28h. Among the different substrates, carbon and nitrogen sources, the highest protease production could be recorded in casein, fructose and ammonium carbonate. Purification was done in three steps by 0-80% ammonium sulphate precipitation followed by anion exchange chromatography on DEAE- cellulose resin and Gel filtration chromatography using Sephadex G-100 column. The highest activity of protease was detected in 60 to 80% ammonium sulphate precipitation step. The enzyme had a low molecular weight of approximately 35kDa. Zymogram activity staining also revealed clear zone of proteolytic activity against the blue background for the purified sample at the corresponding positions in SDS-PAGE. The purified metalloprotease exhibited maximum activity at pH 6.0. This protease exhibited 75 to 85% of activity at a pH range of 5.0 to 9.0. Proteolytic activity attained a maximum level at 60°C. However, the protease exhibited only 65 and 85% of the maximum activity at the temperature range of 40<sup>o</sup> to 70<sup>o</sup>C. The metalloprotease was stable at 40°C for 60 min. However, the stability of this protease decreased drastically between 60 and 70°C with half-life of 60 and 20 minutes respectively. Among the metals tested, Cu<sup>2+</sup> had a strong inhibitory effect, whereas Zn<sup>2+</sup> and Cs<sup>2+</sup> have exerted mild effects on protease activity. In contrast, Mg<sup>2+</sup> strongly induced the protease activity. MALDI analysis showed the masses values are matched with the amino acid sequence of protease of *Pseudomonas aeruginosa* strain PA7. Using 'protident' tool, it was confirmed as a metalloprotease.

## eIF2 expression and phosphorylation in response to nutritional status and stressors in fish [Poster]

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The phosphorylation of the translational initiation factor, eIF2, on its  $\alpha$ -subunit is an adaptive response to a variety of stressors in eukaryotes from protists to vertebrates. There are four eIF2 $\alpha$ -specific kinases in vertebrates, GCN2, PERK, PKR and HRI, each of which can be activated by different stressors. With the known relationship between eIF2 $\alpha$  phosphorylation in nutritional status and food choices, it was of interest to determine whether eIF2 $\alpha$  phosphorylation can be used as an early marker to evaluate diets in fish. Studies were initiated in zebrafish, *Danio rerio*, to lay the groundwork for investigating aquaculture species. All the eIF2 $\alpha$ -kinases are present in the zebrafish genome and are expressed in the zebrafish embryonic liver cell line, zfl. Two forms of eIF2 $\alpha$  are expressed, eIF2 $\alpha$ -1 and eIF2 $\alpha$ -2, with eIF2 $\alpha$ -1 transcripts ~5-fold higher than those of eIF2 $\alpha$ -2 in zfl cells. The two gene products are 96 % identical. Phosphorylation of eIF2 $\alpha$  in zfl cells is increased by a variety of conditions; starvation, leucinol, ER stress, PIC and *N*-methylprotoporphyrin, suggesting activation of GCN2, PERK, PKR and HRI, respectively. Two of these eIF2 $\alpha$ -kinases, GCN2 and PERK, respond to changes in nutritional status. The sequence of eIF2 $\alpha$  cDNA from cobia, *Rachycentron canadum*, shows close identity to the zebrafish eIF2 $\alpha$ s. Cobia has two eIF2 $\alpha$  genes although the coding sequence of each is identical. Investigation of a new cobia muscle cell line has shown that these cells are responsive to activators of GCN2 and PERK. Furthermore, preliminary studies have shown that in cobia larvae, both water temperature and probiotics affect the phosphorylation state of eIF2 $\alpha$ .

## Characterisation of water-soluble collagen from tilapia skin[Poster]

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In the present study, we investigated physicochemical characterisation of water-soluble collagen (WSC) from tilapia skin. The SDS-PAGE showed that the molecular weight of WSC was mainly focused on up 50000 Da, accounting for 95.4%. The percentage of main amino acid composition of WSC was Gly(20.4%), Glu(11.4%), Pro(10.6%), Arg(10.3%), Hyp(9.3%), respectively. In addition, the effects of concentration, ethanol, pH on the viscosity of WSC were investigated. The results indicated that the viscosity of WSC increased with the increase of concentration of WSC or ethanol. The viscosity of WSC was the highest at pH 12.0 and retained the lowest level at pH5.0-8.0, while it was the second highest at pH 3.0. Moreover, the effects of freeze and spray drying on the micro-morphology of WSC were observed by scanning electron microscope. It was found that WSC was flake by freeze drying, while it was elliptic granule particle by spray drying. Our result can give guidance for application of WSC on food and medicinal adhesive.

## The role of foxm1 in the initiation mechanism of intrahepatic cholangiocarcinogenesis in zebrafish [Poster]

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Intrahepatic Cholangiocarcinoma (ICC) is the second common liver cancer worldwide. Our previous results show that livers of one-month-old HBx+HCP transgenic zebrafish exhibit several predominant features of hepatobiliary disorder and ICC formation at three months of age. To investigate the initiation mechanism of ICC formation, we histologically and genetically analyzed the liver of one-month-old zebrafish by IHC and digital gene expression (DGE), respectively. The results show that the different gene expression profiles in the liver include genes involved in cell cycle initiation, development and cytoskeletal remodeling among the top 10 GeneGo pathways at one-month-old zebrafish. The different expression profiles of genes are related to cell cycle processes and DNA damage among the top 10 GeneGo process networks. Furthermore, the expression profiles were consistent with genes involved in genomic instability and in regulating several types of neoplasms among the top 10 GeneGo disease biomarker networks. Our data also showed that foxm1 was activated in HBx- and HCP-induced ICC. ICC was markedly reduced by knockdown of foxm1 by vivo morpholinos injections. These results reveal that foxm1 plays an important role in HBx- and HCP-induced ICC tumorigenesis. If there is a list of items in your abstract, please use bullet indents.

## Application of RNA vaccine in grouper nervous necrosis virus[Poster]

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Grouper industry in Taiwan has leading position worldwide; however, viral diseases have caused great damage on it long time ago. Development of anti-viral vaccine could be an efficient management to protect fish from viral diseases. Nervous Necrosis Virus (NNV) is one of the most devastating viral diseases in grouper culture. In present, the anti-NNV vaccines such as inactivated vaccines, subunit vaccines and recombinant vaccines which rely on adaptive immunity can only be applied to 30-days-post-hatching grouper larvae. RNA interference (RNAi) systems are fully established in zebrafish model system, and we believe that this can provide a new, appropriate strategy on NNV prevention in grouper. We found out that EPC had the best delivery efficiency among different encapsulating materials *in vivo*. Additionally, four siRNA plasmids which targeted NNV RNA2 were constructed at nucleotide positions of 93, 585, 730 and 1024. The plasmid contained NNV RNA2 and EGFP reporter gene was co-transfected with siRNA plasmid into cells to examine the efficiency of siRNA. Simultaneously, the survival rate was increased from 2% to 76% *in vivo*. To evaluate the safety of RNA vaccine, we administrated RNAi vaccines and PBS as control by i.p injection to grouper larvae of 1.5-2 inches in length. No abnormal behavior was observed and survival rate of all injected fish was found to be 100% after two weeks injection. Based on the results, we can confirm RNAi vaccine is effective and safe.



## Marine Microbial Bioactive Biosurfactant for Cosmeceutical Industry

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Among the various marine bioactive compounds, biosurfactants (BS) are attracting major interest and attention due to their structural and functional diversity. The features that make biosurfactant commercially promising alternatives to chemically synthesized surfactants are their lower toxicity, higher biodegradability and hence, greater environmental compatibility. The objectives of present study are 1) to study the chemical properties and biological function of this surfactin product, 2) to optimize the conditions of surfactin production. 3) to employ surfactin as an additive in cosmetics and skin care products. The results of susceptibility test of surfactin indicated that surfactin showing inhibitory activity in gram-positive bacteria, gram-negative bacteria, fungi and virus. Using surfactin as a transdermal permeation enhancer. We have demonstrated that the lipophilic surfactin are capable of increasing skin penetration of drugs (i.e. dexamethasone) and cosmetic moisturizers such as hyaluronic Acid and  $\gamma$ -polyglutamic acid. We are currently testing that using surfactin as transdermal carrier can enhance Au-NPs penetration through skin SC of mouse skin. After permeation, Au-NP transformed fibroblast with multiplications of mRNA in KGF and EGF. Due to application of nano gold, it effected the indication of balance for SOD, metallothionein, and EGF indirectly. The high yield of surfactin production can be accomplished by an innovative semi-solid state fermentation (SSSF) method and the yield was approximately 6.7g/kg. As a conclusion, surfactin has antibacterial and antiviral activity, emulsifier and is able to enhance skin permeation ability. Surfactin serve as bactericidal, preservatives, emulsifier and transdermal penetration enhancer, and it might be applied in animal culture, cosmetics and pharmaceutical industry.

## Global transcriptome profiling of *Pyropia yezoensis* in response to temperature stresses

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*Pyropia yezoensis* is an economically important marine crop and a model organism of the intertidal zone. To define its critical mechanisms responding to the temperature stresses, we profiled gene expression pattern under four different temperature treatments: normal temperature (NT, 8°C), high temperature (HT, 24°C), chilling stress (CS, 0°C) and freezing stress (FS, -8°C), using the Illumina/Solexa technology. Two biological replicates for each treatment were set up. After filtering out low quality data, about 10.7~12.1 million clean reads remained for each sample. Differential expression analysis was performed using the DESeq R package. Between HT and NT, a total of 2202 differentially expressed genes (DEGs) were detected. Comparing the gene expression profile of NT with that of CS, as well as with that of FS, 1334 and 592 DEGs were found respectively. Among the DEGs responding to heat stress, 116 GO terms were significantly enriched. After heat stress, 10 heat shock protein genes and 59 transcription factors genes differentially expressed. After chilling stress, GO enrichment analysis revealed there were 1 and 7 significantly enriched GO terms in the up-regulated and down-regulated genes respectively. Nine genes encoding for fatty acid desaturases were up-regulated after chilling stress, indicating that the fluidity of membrane could be a key factor for chilling stress acclimation. The similar DEGs pattern between treatment of freezing and that of chilling could be observed. The comprehensive high-resolution analysis of gene expression changes associated with temperature stresses provided key resources for understanding the biology of extreme temperatures tolerance in intertidal red seaweed.

## Sporophyte-specific expression of bromoperoxidase gene in a red alga, *Pyropia yezoensis* [Poster]

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Bromoperoxidases (BPOs) catalyze the halogenation of organic compounds and are distributed among marine macroalgae and cyanobacteria. Although the red alga *Pyropia yezoensis* is widely used for laver farming and laboratory experiments, its BPO activity has not been reported. Recently, we found that the BPO gene of *P. yezoensis* (*PyBPO1*) is highly expressed in sporophytes but strictly repressed in gametophytes and that BPO activity and bromoform production were detected only in sporophytes. Since appropriate growth conditions differ between sporophytes and gametophytes, there are at least two possible mechanisms to explain the sporophyte-specific expression of *PyBPO1*; sporophyte-generation dependent manner and growth-conditions dependent one. In this study, to address this question, changes in the *PyBPO1* mRNA level and *PyBPO1* activity in sporophytes cultured under gametophyte conditions were investigated. Sporophytes maintained under sporophyte conditions (23°C on constant irradiation without aeration) were transferred to gametophyte conditions (15°C on photoperiod of 10L:14D with aeration). *PyBPO1* mRNA level in sporophytes was maintained after transfer to gametophyte conditions. *PyBPO1* activity was also maintained. Recently, sodium pump genes of *P. yezoensis*, *PyKPA1* and *PyKPA2*, were identified as sporophyte-generation specific and gametophyte-generation specific genes, respectively. We also observed that *PyKPA1* mRNA level in sporophytes was not changed by transfer to gametophyte conditions. On the other hand, *PyKPA2* mRNA was not detected in sporophytes. These results suggested that *PyBPO1* expression is regulated by generation-dependent manner.

## Anti-inflammatory and anticancer potential of Australian marine macroalgae; role in gut health as dietary therapeutics [Poster]

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Currently there is a push for the development of bio-functional molecules in the area of gut health. Marine algae are an abundant and readily available source of both primary and secondary bioactive metabolites. These metabolites can offer essential components for human and animal nutrition as functional foods, but also offer a source of novel bioactives for therapeutics, particularly for diet and lifestyle related diseases such as inflammatory bowel disease (IBD). IBD is characterised by chronic inflammation of the bowel, often triggered by genetics and/or diet, correlating with complex alterations of the microbiome. An emerging field in the correction of the microbiome is the development of prebiotics, non-digestible carbohydrates that promote the growth of beneficial bacteria from the unique sulphated polysaccharides isolated from seaweeds, as well as the development of other high-value ingredients from secondary metabolite bioactives. In a separate study we have identified a number of Australian macroalgae (*Ecklonia*, *Phyllospora*, *Hormosira*, *Myriogloea Gracilaria* and *Ulva*) with potential gut health benefits related to their polysaccharide content. Currently, we are profiling the biological activity of both polar and non-polar extracts obtained from these algae. Herein, an *in vitro* MTS assay was used to evaluate cytotoxic activity of the algal extracts in human (MIA-Pa-Ca-2) pancreatic carcinoma cells, as well as investigating anti-inflammatory effects (via inhibition of NO production) of the extracts on lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. The results of the current screening efforts along with work towards the isolation and identification of purified bioactive components will be presented.

## **It pays to be tough: the prevalence of proteins with repetitive, low complexity domains in marine biomaterials**

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Spider silks are comprised of highly repetitive and modular proteins that are well known for their exceptional strength and elasticity. Interestingly, proteins with similar repetitive, low complexity domains (RLCDs) are common in animals found in marine environments, for example, in scallop hinge ligaments, mussel byssal threads, fish fertilization envelopes, molluscan shells and echinoderm spicules. All of these biomaterials play important structural, elastic or strength roles within the organism, therefore it is likely that the common motifs found in their constituent proteins are key for the physical properties of these extracellular structures. Given these sequence similarities, we have developed a bioinformatics-based method to identify previously unknown silk-like proteins from large scale sequence databases. We find that the genes encoding these proteins are largely expressed in tissues that are responsible for producing tough extracellular structures. As a case study, we investigate two families (the KRMPs and Shematrins) of such proteins from pearl oyster shells. We find that the genes for these proteins are highly expressed, rapidly evolving, and likely arose within the pearl oyster lineage. Given the diversity of structures with which these sequences are involved, the genetic distance of the organisms they are found in, and their rapid evolutionary rates, we propose that RLCD containing proteins evolved multiple times independently in different metazoan lineages.

## **Characterization of Bio-prospecting Compounds of Brown Seaweed *Sargassum horneri* by Liquefied Pressurized System [Poster]**

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Seaweeds or marine macro algae are the potential renewable resource in marine environment. It has unexplored bioactive compound, which could be potentially as functional food for human health. The procurement of bioactive potential of the brown seaweed would enhance their utility values. Our work is intended to use eco-friendly technology to obtain bioactive properties of edible brown seaweed. The purpose of this study is to determine biological properties of edible brown seaweed *Sargassum horneri* that will be produced by subcritical water hydrolysis. The liquefied pressurized system will be done on brown seaweed by subcritical water hydrolysis treatment with batch system. The experiment condition of this work will be set up at 180 to 260 °C for the reaction temperatures and 1.3 to 6.5 MPa for the pressures. The ratio of material to water is 1:25 (w/v) and the reaction time of each condition will be maintained for 5 min. Analysis of physical properties will be done by colorimeter and molecular weight determination. Whilst bio-active properties of hydrolysate water will be determined by antioxidant properties (TFC, TPC, DPPH and ABTS), total sugar and reducing sugar.

## **The role of astaxanthin biosynthesis genes in *Haematococcus pluvialis* during carotenoid induction by salinity and nutrient starvation stress [Poster]**

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The green microalga *Haematococcus pluvialis* accumulates large amounts of astaxanthin under a variety of environmental stresses which result in a visible colour change. In this study, the transcriptional pattern of eight carotenoid genes involved in astaxanthin biosynthesis in *H. pluvialis* culture exposed salinity stress or nutrient starvation, was revealed using qRT-PCR. Both, salinity and nutrient deprivation led to an increase in astaxanthin production, but the combination of both stresses showed the most promising approach. Results from qRT-PCR showed that differential expression of carotenoid genes reflected the observed increase in astaxanthin production in *H. pluvialis*. Further experiments are underway to compare four different *Haematococcus* strains and to optimise astaxanthin biosynthesis by applying additional treatments of *H. pluvialis* cultures, including the addition of ferrous sulfate and/or plant hormones. These findings will be evaluated for future large-scale production of astaxanthin from *H. pluvialis*.

## Japanese flounder (*Paralichthys olivaceus*) spleen transcriptome and expression profile involved in immunity during *Vibrio anguillarum* infection [Poster]

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Japanese flounder (*Paralichthys olivaceus*) is an economically important marine fish in China suffering from severe outbreaks of infectious disease caused by bacterial pathogens, such as *Vibrio anguillarum*, resulting in great economic losses. However, the mechanisms involved in the immune response of this fish to bacterial infection are not fully understood. To understand the molecular mechanisms underlying the immune response to such pathogenic bacteria, we used high-throughput deep sequencing technology to investigate the transcriptome and comparative expression profiles of the Japanese flounder infected with *V. anguillarum*. A total of 12,196,968 reads were generated and assembled into 314,377 contigs from the spleen of the flounder infected with *V. anguillarum*. Via annotation to the NCBI database, 21,392 unigenes were identified. For function classification and pathway assignment, 19,548 genes were classified into Clusters of Orthologous (COG) classification, 12,504 into Gene Ontology (GO), and 10,650 into 20 Kyoto Encyclopedia of Genes and Genome (KEGG) pathways. Among the identified KEGG categories, 1536 unigenes were characterized to be involved in immune-response pathway, including 110 genes in Toll-like receptor signaling, 164 in T cell receptor signaling, 197 in chemokine signaling. By using illumina's DESeq, 189 differential expressed genes (P value < 0.05) were detected in comparative analysis of the expression file between *V. anguillarum*-infected fish and control fish, including 38 remarkably upregulated genes and 151 remarkably downregulated genes. This study produced the most comprehensive genomic resources of flounder, and will provide insights into the molecular mechanisms underlying flounder immune response to bacterial pathogen infection.

## Marine pigmented bacterium *Serratia rubidaea* (NIO/PPB/01) and its potential towards antifouling properties

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A red pigmented bacterium, isolated from Mandovi estuary, Goa, India identified as *Serratia rubidaea* (NIO/PPB/01) by 16S rDNA sequencing tested their bacterial biomass and its derivative towards antifouling properties. The bacterial compounds could yield a clear zone of 28 mm with 850 µg per disc concentration against strain FB 06. The RFLP pattern showed that the variation among the 6 different strains tested for antifouling belongs to four different groups. The bacterial product released by *S. rubidaea* analyzed with multiple criteria like nuclear magnetic resonance spectroscopy (NMR), electron impact mass spectroscopy (EtMR), infra red spectrum (IR) and UV visible absorbance showed similar to Dioctyl Phthalates (DOP). *S. rubidaea* and its DOP were discussed in this paper for its potential towards future use.

## Sustainable conversion of light to chemical and electrical energy

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The Earth receives around 1000W.m<sup>-2</sup> of power from the Sun and only a fraction of this light energy is able to be converted to biomass (chemical energy) via the process of photosynthesis. Out of all photosynthetic organisms, microalgae, due to their fast growth rates, have been identified as potential source of raw material for chemical energy production. Electrical energy can also be produced from this same solar resource via the use of photovoltaic modules. In this work we propose a novel method of combining both of these energy production processes to make full utilisation of the solar spectrum and increase the productivity of light-limited microalgae systems. These two methods of energy production would appear to compete for use of the same energy resource (sunlight) to produce either chemical or electrical energy. However, some groups of microalgae (i.e. Chlorophyta) only require the blue and red portions of the spectrum whereas photovoltaic devices can absorb strongly over the full range of visible light. This suggests that a combination of the two energy production systems would allow for a full utilization of the solar spectrum allowing both the production of chemical and electrical energy from the one facility making efficient use of available land and solar energy. In this work we propose to introduce a filter above the algae culture to modify the spectrum of light received by the algae and redirect parts of the spectrum to generate electricity. The electrical energy generated by this approach can then be directed to running ancillary systems or producing extra illumination for the growth of microalgae. We have modelled an approach whereby the productivity of light-limited microalgae systems can be improved by using an LED array to increase the total amount of illumination on the microalgae culture.

## Defining the producers of marine natural compounds in marine sponges using the single-cell analytical approach

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Marine sponges are known to produce a vast range of pharmaceutically and industrially important natural compounds. Recent speculations however, suggest that such natural compounds may be highly associated to the bacterial symbionts living within them. In recent years, studies on the metagenomes of the microbial flora within sponges have further supported this by the identification of polyketide synthase biosynthetic gene clusters. Nevertheless, the producers of such natural compounds are still to be determined. Thus, our goal in this study was to identify the producers of PKSs in marine sponges by implementing the single-cell approach. The yellow chemotype of the marine sponge, *Theonella swinhoei*, were collected from Hachijo island and were processed to attain bacterial fractions. The bacterial fractions were analysed by flow cytometry, single-cells were sorted and subjected to genomic DNA amplification and nested-PCR for identification of the target PKSs and the producers. To further support our discovery, other single-cell analytical tools such as Raman microspectroscopy etc. were also used. In this work, we targeted the onnamide A compound, found highly abundant within *T. swinhoei*. Thus far, we were successful in the identification of the biosynthesis gene cluster fragments of the PKS associated to onnamide A within genomic DNA samples of wells positive for the bacteria from the *Entotheonella* genus. We show here that the implementation of the single-cell approach was useful in allowing us to verify the producers of marine natural compounds.

## Our shared challenge and our shared objective: Developing and applying marine biotechnologies to achieve sustainable use of marine resources for human benefit and ecosystem protection

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Incoming IMBA President, Professor Werner Mueller will comment on the major challenges facing the world in the sustainable development of its marine bioresources, and propose ways in which members of the IMBA will be able to share knowledge and experience in marine biotechnologies, to assist their own Nations, and the world. He will refer to his own extensive research on marine sponges and to their potential, both to provide products of economic benefit, and to better understand the complex interactions and interdependencies within and between marine biota.

## Sustainable Oceans – our Treasure in the Past and in the Future: The power of marine genomics

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During the last decade, the principles of biomineralization have increasingly attracted multidisciplinary scientific attention, not only because they touch the interface between the organic/inorganic worlds but also because they offer fascinating bioinspired solutions to notorious problems in the fields of biotechnology and medicine. However, only one group of animals has the necessary genetic/enzymatic toolkit to control biomineralization: siliceous sponges (Porifera). Using a unique blend of cutting-edge techniques in molecular/structural biology, biochemistry, bioengineering, and material sciences, we approach for the first time a comprehensive analysis of natural biomineralization, from gene to biomineral to hierarchically ordered structures of increasing complexity [Müller WEG. *Chemistry Eur. J.* 19: 5790; Wang XH. *Soft Matter* 8:9501; Wang XH. *FEBS J* 279: 1721.]. The groundbreaking discoveries expected are of tremendous potential benefit for humans from the applied point of view. They can contribute to the development of innovative nanobiotechnological and -medical approaches that aim to elicit novel (biogenous) optical waveguide fibers and self-repairing inorganic-organic bone substitution materials. By application of the quantitative real-time RT-PCR analysis technique, it is shown that silica-impregnated scaffold induces SaOS-2 cells to express osteoprotegerin [OPG] and bone morphogenetic protein 2 [BMP-2]. It is proposed that biosilica might function also as a morphogenetic material *in vivo*. Animal experiments are in progress. In conclusion, the data compiled and integrated show that the elucidation of the mechanism of biosilica formation resulted in a paradigm shift in bioinorganic chemistry, which surely will add new technologies in chemical and biomedical fields in an exploitable and sustainable way.

## Class I integrons in multiresistant *Escherichia coli* isolates from poultry litter [Poster]

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Resistance to fluoroquinolones (FQR) has been increasingly reported among human and veterinary isolates. Apparently FQR can contaminate poultry by products and be acquired by human beings via the food supply. A substantial proportion of resistant determinants in Gram-negative bacteria reside in class 1 integrons that are capable of capturing and expressing genes contained in cassette-like structures. In this work we screened 19 fluoroquinolone-resistant *Escherichia coli* strains isolated from poultry house litter samples from 2000 to 2003. Isolates were assayed for susceptibility to 14 antibiotics and the presence of *qnr* genes and class 1 integron resistance gene was determined by PCR. Genetic characterization of isolates was performed by pulsed-field gel electrophoresis (PFGE) and plasmid analysis of the isolates. PCR data suggests that *qnrS* and *qnrA* were the predominant genes and were detected in association with class 1 integrons. Nucleotide sequence analysis of class 1 integrons of multiresistant *E. coli* strains showed that streptomycin and trimethoprim were the most common cassettes. One of the isolate presented an uncommon array of genes encoding tobramycin-chloramphenicol and trimethoprim resistance. An additional putative cassette was observed in this array that had a unique coding sequence. Antibiotic resistance, PFGE and plasmid analysis provided a good discrimination of the isolates indicating that resistance has emerged among multiple avian pathogenic *E. coli* chromosomal backgrounds.

## Investigation of lipids and lipases from the microalgae *I. galbana* and *P. lutheri*

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The consumption of omega-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been shown to provide numerous health benefits. Examples include roles in cardiovascular health, neurological disorders, visual function and inflammatory diseases to name a few. As a result commercial interest has risen significantly in omega-3 concentrates for use in supplement, drug and food products, creating a multi-billion dollar industry. Traditional chemical and physical oil processing methods for concentrating omega-3 PUFA can be harsh and may initiate degradative oil oxidation, particularly with EPA and DHA. Occurrence of degradation is detrimental to the associated bio-active properties and thus directly affects the commercial value of these compounds. New lipid processing methods using lipases offer an alternative way to concentrate omega-3 fatty acids. The use of lipases would enable milder oil processing conditions than those used in conventional methods, producing superior quality and value-added products. Lipases are also potentially more selective as they themselves vary in specificity for different fatty acids. Microalgae provide an untapped source of novel lipases that may possess PUFA-specific activities due to the fact they produce large quantities of omega-3. The work presented here first investigates the growth and lipid production of several microalgal species and then focuses on the identification, isolation and characterisation of lipases from the organisms.

## Production of compatible solutes by halophilic fungi

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It was believed that fungi are merely tolerant of unfavourable extreme conditions, or extremotolerant, and are able to grow/survive such untoward situations, but are not necessarily extremophilic in nature. However, it has now been shown that some fungi are indeed extremophilic, well demonstrated in the finding of halophilic fungi. Halophilic microorganisms are exposed to the high osmotic pressure of their hypertonic environment caused by salt. Consequently, they develop strategies to maintain an isoosmotic balance with their surroundings, in order to survive the otherwise harsh conditions of hypersalinity and the consequently low water activity of their surroundings. This occurs by two mechanisms: a salt-in-cytoplasm, and/or an accumulation of compatible solutes, so termed because these do not interfere with the physiology of the organism. While in the archaea, osmoadaptation occurs mainly by the former mechanism, the fungi survive by the build-up of compatible solutes or osmolytes, which may occur either by synthesis or by direct uptake from the surroundings. The filamentous halophilic fungi studied were obligate halophiles in some instances, and some were facultative halophiles. In both obligate as well as facultative halophiles, erythritol was the major sugar alcohol detected, followed closely by arabitol and inositol, sucrose, trehalose and galactose; in some instances, glucose was also identified. Osmolytes find wide applications, particularly as mobility controllers, thickeners and humectants in cosmetics and food industry, and drug carriers in medicines. The genes involved can also be used for transfer to crops to prevent desiccation in saline soils and/or excessively dry climates.

## Bacterial communities associated with biosynthetic organs of the marine mollusc *Dicathais orbita* [Poster]

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*Dicathais orbita*, an Australian mollusc of the Muricidae family, is well known for the production of the dye Tyrian Purple. The brominated indole precursors of this dye have anticancer and antimicrobial properties. The hypobranchial gland, reproductive organs and egg capsules of Muricidae molluscs are the only known source of Tyrian Purple, a brominated derivative of indigo, which is also produced by a range of bacteria and certain terrestrial plants. The generation of indigo in Muricidae molluscs may therefore present an interesting case of convergent evolution, although the alternative hypothesis of production through symbiotic bacteria cannot yet be ruled out. The aim of this project is to isolate and identify the symbiotic bacteria from *D. orbita* using culture techniques and molecular approaches targeting the bacterial 16S rRNA. The biochemical profiles of microbial communities were investigated using the API20 E biochemical kit. All together 28 distinct bacterial colonies were isolated from the targeted tissues of the foot, hypobranchial gland, reproductive organs and egg capsules of *D. orbita*. Although bacterial communities from foot tissue were similar to seawater, the hypobranchial gland, reproductive organs and egg capsules showed distinct differences. No reliable bacterial colonies were identified from hypobranchial gland homogenates. Egg capsules had high bacterial diversity and were similar to the female capsule gland. Since bacterial communities associated with the hypobranchial gland in both the sexes appear to be unculturable, genetic approaches are required to establish whether symbiotic bacteria contribute to Tyrian Purple production in Muricidae molluscs.

## A Journey from Marine Genes to New Sustainable Land Plant Sources of Long-chain Omega-3 Oils

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Marine derived long-chain polyunsaturated fatty acids (LC-PUFA) contain 20 or more carbon atoms and multiple double-bonds. LC-PUFA are grouped into omega-3 or omega-6 based on the position of the first double-bond from the methyl (omega) end of the fatty acid. The two main omega-3 LC-PUFA fatty acids are - EPA (eicosapentaenoic acid, 20:5 $\omega$ 3) and DHA (docosahexaenoic acid, 22:6 $\omega$ 3). These two omega-3 LC-PUFA (also termed LC omega-3 oils) have critical roles in human health and development. Many studies indicate that their deficiency increases the risk or severity of cardiovascular disease, inflammatory diseases and rheumatoid arthritis, hypertension and neuropsychiatric disorders such as depression or dementia. These two fatty acids are predominantly sourced from fish and algal oils. However, wild-harvest marine fish stocks are recognised to be threatened. When taken together with the expanding global population, and to be able to meet increasing demand for these oils in feeds, foods, nutraceuticals and pharmaceuticals, there is a need for new, alternative and sustainable sources. We will outline the production of EPA and DHA in land plant oils through use of metabolic engineering. The CSIRO Omega-3 team capabilities included: the unique CSIRO Australian National Algae Culture Collection; oils chemistry and marine lipids; molecular genetics, plant genetics and breeding; food technology and chemistry; livestock and human nutrition and aquaculture. This combined capability and skill base has enabled: the first demonstration of DHA in an oilseed; highly efficient and selective genes discovered and used in the LC Omega-3 pathway in model and oilseed plants; recent land plant oil profiles mimicking fish oils. An R & D partnership has commenced involving - Nuseed, CSIRO and GRDC - and aims to produce and commercialize Canola DHA.

## Metabolite Extraction Strategies from Whole Tissue Samples of Tropical Fish Using Gas Chromatography Mass Spectrometry Metabolomics

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Metabolomic analysis of tissue samples can be applied across multiple fields including medicine, toxicology, and environmental sciences. A thorough evaluation of several metabolite extraction procedures from tissues is therefore highly needed. This has been achieved at the research laboratories using whole tissues from local tropical fish, the Tiger grouper (*Epinephelus fuscoguttatus*). Multiple replicates of homogenous tissues were extracted using the following solvent systems of varying polarities of acetonitrile: water, methanol: water, and methanol: chloroform: water and accompanied with derivatizing reagent of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA). Extraction of metabolites from homogenized tissue was used in this study. It is essential to compare which solvent extractions produce what metabolites and which in the end extracted more metabolites. After extraction, the tissues were subjected to gas chromatography mass spectrometry (GC-MS, Perkin Elmer) and the spectra evaluated using multivariate analysis, the principal components analysis (PCA). Based on the results, we observed that the yields of low molecular weight metabolites were extracted from using methanol: water solvent while acetonitrile: water and methanol: chloroform: water extracted lipids and macromolecules with highest reproducibility. Overall, single organic solvent extractions are quick and easy and produce reasonable results. However, considering both yield and reproducibility of the tropical fish metabolites, we conclude that the acetonitrile: water and methanol: chloroform: water extraction is the preferred method.



## Strategies of acclimation to deep sea: Structure simulation of myoglobin molecules from aquatic animals under high pressure [Poster]

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Deep sea organisms and diving animals are capable to resist against high hydraulic pressure. Especially, oxygen incorporation is one of the most important requirements they should achieve. Myoglobin plays a very important role in storing oxygen in muscle tissues to facilitate sustainable contraction. Sequence alignment is one of the ways to detect the strategies for molecular evolution. However, molecular dynamics simulation could provide a lot more detailed information about the behaviour of proteins under a given atmosphere. Thus in the present study, structure simulation of myoglobins from whale, tuna and sea hare was performed to understand what happens to the proteins under high hydraulic pressure and to know the molecular mechanisms involved in acclimation to deep sea. Amber 11 was used for the simulation throughout. As the templates, the atomic structures of myoglobins from slug sea hare *Aplysia limacina* Mb (PDB ID: 1MBA), blackfin tuna *Thunnus atlanticus* (2NRT), and sperm whale *Physeter macrocephalus* (1U7R) were used. The temperature range was set to from 273 K. All the production runs were performed for 100 ns under the pressure of 1, 10 and 100 atm. Root mean square deviation (RMSD) and root mean square fluctuation (RMSF) were used to compare the changes in structure. The results obtained revealed that whale myoglobin stayed much more flexible than other myoglobins even at 100 atm. Sea hare myoglobin, which would never experience high pressure, seemed to become very rigid under the same condition.

## Bioethanol production from cyanobacteria biomass

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Glycogen is a multibranched polysaccharide with glucose polymer that is main energy storage source in many cyanobacteria. This source is useful for production of bioethanol. We isolated two strains of cyanobacteria as *Spirulina maxima* and *Leptolyngbya* sp. including glycogen. The *Spirulina maxima* was cultured by 10 ton open culture system for 1 year. The biomass was collected with each different time and analysed carbohydrate contents and other environmental conditions. Highest carbohydrate was showed as 56% in total dry biomass. We carried out pre-treatment with different organic solutions at 121 °C, 1.5 atm for 30 min and saccharification with spirizyme (Novozymes, Inc.). Reducing suger was analysed by DNS method. Bioethanol was highly produced with the optimum saccharified supernatant. Genome sequence was analysed from *Leptolyngbya* sp.. Glycogen hydrolysis relative genes were detected from the genome as glycogen debranching enzyme, glycogen phosphorylase and alpha-amylase. The genes were cloned into pET11a and pET16b. Now we are going to study about expression in *E. coli*. Also, we analysed carbohydrate and monosaccharide contents from the *Leptolyngbya* sp.. This biomass also may useful for bioethanol production.

## Fucoxanthin Production from Microalgae

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Fucoxanthin (FX) contained in the brown algae and diatoms is reported to have anti-cancer, anti-obesity and anti-inflammatory effects. FX is commercially produced from brown algae. But the production method for FX from brown algae is complicated due to a high content of polysaccharides and sturdy cell walls. On the other hand, diatoms have a higher FX content but have a lower polysaccharides content compared with brown algae. Therefore, in this study, the efficient FX production from marine diatom, *Fistulifera* sp. strain JPCC DA0580, was investigated. *Fistulifera* sp. strain JPCC DA0580 was centrifuged and then dried. FX was extracted from dried algae by ethanol and purified by column chromatography using synthetic adsorbents with 80%vol ethanol as the eluent. The main fraction containing 30% FX was obtained by this process, in which monogalactosyldiacylglycerol (MGDG) and fatty acids (FA) were also contained. Since usable solvents and adsorbents are restricted for use in food, it is difficult to enhance FX content by only column chromatography. Next, separation of FX from MGDG and FA using the differences in the solubility of each of them in ethanol/water was examined before column chromatography. After adding water to the algae extract in ethanol followed by cooling, it was found that MGDG and FA were precipitated and FX dissolved in the ethanol/water mixture. As a result of chromatographic separation of supernatant, the purity of FX in the main fraction was improved by 50%.

## Shotgun lipidomic profiling in marine alga *Emiliana huxleyi*: Identification of intermediates for lipid and very-long-chain alkene biosynthesis

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We developed sensitive and high throughput approach method for profiling membrane lipid molecular species in marine alga haptophyceae *Emiliana huxleyi* CCMP 2090 using electrospray ionization tandem mass spectrometry (ESI-MS). Then we succeeded to identify molecular species of fatty acyl-CoAs, glycerolipids (DAG, TAG, MGDG, DGDG, SQDG) and glycerophospholipids (PC, PE, PI, PG, PS) based on the simultaneous automated acquisition and processing of 84 precursor's ion spectra, specific acyl anions of common fatty acids moieties and several lipid class-specific fragment ions. Here we report the profiling of more than 166 apparent molecular species of polar glycerolipids, alkenones, and very-long-chain fatty acyl-CoAs in *E. huxleyi*. Especially, the profiles of C24:0, C28:0 and C30:0 of fatty acyl-CoAs were the first report in *E. huxleyi*. In addition, very-long-chain alkenes of C27 and C29 in *E. huxleyi* were also identified as products by *E. huxleyi* although their biosynthetic pathway is unclear yet. According to the present results, alkene biosynthetic pathway is considered to involve the elongation process of C3- to C30-fatty acyl-CoA. Further detailed analysis of alkene and fatty acid biosynthesis is needed to elucidate whole processes.

## Metabolic pathway of alkenones and alkenes by marine haptophytes and its application to biofuel production

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Marine phytoplankton plays an important role in producing half of the global photosynthetic primary production and has changed the global environment by fixing carbon dioxide and producing oxygen. Especially the marine unicellular, calcifying coccolithophores in the Haptophytes, are well-known as producers of limestone and petroleum during the Cretaceous era. The white cliffs of Dover and the petroleum in the Middle Eastern countries were produced by coccolithophores. Coccolithophores are still producing huge blooms even in the ocean, especially at high latitudes, such as the North Atlantic Ocean and the Bering Sea. This talk will focus on "the potential of haptophytes" for the reduction of atmospheric CO<sub>2</sub> and the production of lipids as a renewable energy source. Haptophyte algae hardly produce triglycerides but rather produce oil droplets of long-chain ketones, namely alkenones and alkenes. These metabolites occupy 20-30% of the cell dry mass and are candidates for liquid and gaseous hydrocarbons as biofuels. Very similar components to native crude oils were produced when *Emiliana huxleyi* was co-cultured as consortia comprised of alga, bacteria, and archaea, and degraded by pyrolysis. Based on our studies on alkenone producing haptophytes, we will present new findings on the mechanisms of their growth regulation, metabolic pathway of alkene and alkenone biosynthesis and efficient production of other useful metabolites and the development of lipid droplets. This study was supported in part by the fund of CREST, JST, Japan, to YS (FY2010-2015).

## Catalase production and H<sub>2</sub>O<sub>2</sub> tolerance of *Aurantiochytrium limacinum* strain mh0186 [Poster]

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In aerobic organisms, oxygen is important to generate energy during respiration. The reactive oxygen in the process of respiration, reactive oxygen species (ROS) was produced and causes the serious cellular damages. Environmental agents such as high temperature, UV radiation, and various compounds that generate intracellular ROS cause the oxidative stress. Polyunsaturated fatty acids (PUFA) in the cell membranes are directly attacked by ROS. Aerobic organisms have defense mechanism for the oxidative stress called the antioxidant. Marine eukaryotes, thraustochytrids are aerobic microorganisms and accumulate large amount of PUFA in the cells. The antioxidant activity of 22 thraustochytrids strains representing 8 genera was determined. The test strains grown on the B1 plate medium was subjected to the catalase test using 3% H<sub>2</sub>O<sub>2</sub> solution. To evaluate the H<sub>2</sub>O<sub>2</sub> tolerance, *Aurantiochytrium limacinum* strain mh0186 was cultured in a GY medium with absence and presence of H<sub>2</sub>O<sub>2</sub> (5 mM, 10 mM). The cell biomass and the catalase activity were determined. The definition of one catalase unit is the amount of enzyme decomposing 1.0 μM of H<sub>2</sub>O<sub>2</sub> per mg-protein per minute. In catalase test, 21 out of 22 strains showed positive results. In H<sub>2</sub>O<sub>2</sub> tolerance test, the beginning of the logarithmic growth phase was delayed in the 5 mM H<sub>2</sub>O<sub>2</sub> as compared with that in the 0 mM group and grew well after 48 hrs. In case of the 10 mM group, the growth was remarkably inhibited during cultivation. However, the catalase activity in 10 mM group significantly increased to around 70, 000 unit.

## Economies of scale and markets for microalgal products on the way to biofuels.

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Microalgal technology offers a lot of promise: new and old products with varied applications from very high value compounds with medical applications to still high value nutraceuticals to relatively low value food and feed applications and even lesser valued biofuels. Here we will describe some of the opportunities for economic activity that are based on microalgal technology. We will also discuss the economic impacts of such activities, including two specific examples from the Southwest United States. Furthermore, we will make the case that the economics of microalgal technology are very much dependent on the type of product and the scale at which it is being produced. We will make the argument that these economic activities have positive impacts but not without a few caveats. For example, it is our belief that some anticipated microalgal products will have difficulty finding markets without large increases in productivity (and concomitant decreases in costs). Improvements in strains (over wild type; both genetically modified and classically improved) and processes will be critical to increase the positive economic impacts of microalgal technology. Finally, as with any economic activity, those based on microalgal technology may also have significant environmental impacts. Some of these are expected to be positive (e.g., CO<sub>2</sub> capture, waste water remediation): the magnitude of these impacts is also very much dependent on the scale at which the specific activity is carried out. By considering the different products and scales of microalgal technology we can forge a path towards microalgal based biofuels.

## Marine Extracts: New opportunities for high value exports

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Plant and Food Research is a New Zealand Crown Research Institute that undertakes scientific discovery and innovation to grow New Zealand's food, plants and seafood resources. The Seafood Technologies research group is interested in growing the value of the seafood industry through improved production technologies, and the development of seafood and marine-based products. An area of interest is high-value marine products from the non-fillet portion of harvested seafood. A particular focus of the team is the development of products based on marine peptides, collagens, enzymes and lipids. This presentation will discuss our research programmes and our efforts to develop new marine products for New Zealand companies.

## Time-lapse analysis of oleaginous diatom *Fistulifera* sp. strain JPCC DA0580 during the triglyceride accumulation using single-cell patterning [Poster]

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Our research group screened the oleaginous diatom, *Fistulifera* sp. strain JPCC DA0580 as the highest triglyceride producer from the marine microalgae culture collection. The lipid amount significantly increases under nutrition deprivation condition. However, the triglyceride accumulation mechanism associated with chloroplast structure was poorly understood. In this study, we analysed the morphological behaviour of chloroplast during triglyceride accumulation at the single-cell level. For tracking single-cellular organelles, we used a simple and rapid single-cell patterning technique using the microcavity array. The microcavity array contains 10,000 cavities, which were designed for highly efficient entrapment of single cells by applying negative pressure. Here, the captured cells on the microcavities were immersed and transferred to an agarose gel. Single-cell patterning was demonstrated on an agarose gel in a highly ordered fashion. Furthermore, using confocal microscope for time-lapse analysis, the chloroplast volume in the diatom was observed. In a two-phase cultivation process (a nutrient sufficient phase followed by a nutrient deprived phase to boost the lipid synthesis), the chloroplast volume was drastically decreased from 55  $\mu\text{m}^3$  to 15  $\mu\text{m}^3$  per single cell cooperating with the increase of lipid volume from 10  $\mu\text{m}^3$  to 40  $\mu\text{m}^3$  in 96 hours. From these results, it was suggested that the lipid compounds in chloroplast might be transferred to triglyceride during the accumulation. The developed novel technique for continuous long-term observation of single-cells has a great potential especially for the understanding of organelle functions with morphological changes in a single cell.

## Identification of *Eucheuma denticulatum* and *Kappaphycus alvarezii* genes

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*Eucheuma denticulatum* and *Kappaphycus alvarezii* are economically important red algae as important source of iota- and kappa-carrageenans, respectively, which have high demands in the food, pharmaceutical and manufacturing industries. Expressed sequence tags (EST) approach was undertaken to investigate gene expression in these red algae. For *E. denticulatum*, 10,031 ESTs were obtained and automatically processed using ESTFrontier, an in-house automated EST analysis pipeline. StackPack EST assembly pipeline was used to assemble raw EST data resulting in 2,275 unique transcripts (UTs) that comprised 1,320 consensus sequences and 955 singletons. Sequence similarity search against NCBI nr-database showed that 961 *E. denticulatum* UTs have significant matched homologues, 145 UTs were categorised as predicted proteins whilst 138 UTs were grouped into hypothetical and unknown proteins. Meanwhile, for *K. alvarezii*, 1405 ESTs were obtained which gave 1392 UTs clustered into 13 contigs and 1379 singletons. The UTs consisted of 294 hits with known proteins, 12 predicted proteins and 774 hypothetical and unknown proteins. BLASTX analysis showed that both sets of ESTs have significant match with sequences in non-redundant protein database mostly with *Chondrus crispus* and *Ectocarpus siliculosus* gene sequences. However, when the ESTs were compared with each other, there was no significant match between the sequences. Further sequencing of the *K. alvarezii* is being carried out to gain more information about this carrageenophyte.

## Effects on cancer cell growth of saponin and fucoidan treated with thermophilic xylose isomerase from the marine bacterium *Thermotoga maritima* [Poster]

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The gene encoding for xylose isomerase from the thermophilic marine bacterium *Thermotoga maritima* was cloned and recombinantly expressed in *E. coli* cells. Optimal activity was shown at 90° and pH 8.0. Treatment of saponin by recombinant xylose isomerase increased the growth inhibitory effect against human gastric cancer (AGS) cells and human colon cancer (HT-29) cells. On the other hand, treatment of fucoidan by the enzyme could not change the growth inhibitory effect against the same cancer cells. One µg/ml of enzyme-treated saponin exhibited the same or higher growth inhibitory effect against both cancer cells compared with 100 µg/ml of enzyme-untreated saponin. These results would be useful in the development of functional food or drug.

## Biosynthesis of polyhydroxyalkanoate in recombinant microorganisms using carbon source derived from terrestrial and marine biomass [Poster]

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Polyhydroxyalkanoates (PHAs) is one of the most promising alternatives of chemically synthesized polymers because their mechanical properties are similar to those of petroleum-based plastics and they can be easily modified by altering the composition of monomers. Biological synthesis of PHA is composed of two distinguished steps. Firstly, hydroxyacyl-CoAs which are the monomers of PHA are produced by natural and engineered microbial strains. Secondly, the PHA synthase, which is the key enzyme in PHA synthesis, accepts its specific monomers and then synthesizes PHAs. Development of fermentative production of PHAs that can be used for substituting petroleum-based polymers have extensively been examined to solve the energy and environmental problems caused by depletion of fossil resources and CO<sub>2</sub> accumulation in the atmosphere. Since the cost of carbon sources has significant effect on the production cost of PHAs and the costs of specific carbon sources such as glucose, sucrose, xylose, and glycerol are different depending on the location and market environment, it is necessary to develop host strains that can use various carbon sources for the efficient production of target products. In this study, we compared different carbon sources derived from terrestrial and marine biomass for their applicability for the production of PHAs using recombinant microorganisms. Poly(3-hydroxybutyrate) [P(3HB)] was examined as a model polymer. Detailed results will be presented in this presentation.

## Insecticidal activity of marine organism *Scomber* spp. against *Tribolium castaneum* [Poster]

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*Tribolium castaneum* is considered as major pest of stored grains in tropical regions. Stored grain insect pests have been damaging our economy by infesting agricultural stored products. The uncontrolled use of synthetic insecticides causes great hazard for environment and consumers due to residual property. In addition the increasing public concern over pesticide safety has resulted in increasing attention being given to natural products to control these stored grain pests. In comparison to search for new pharmaceutical compounds from plants little effort has been devoted to exploration of agro-chemical compounds from marine organisms. In the light of this background, we chose a marine organism *Scomber* spp. (Mackerel) as a potential insecticidal substance. Crude methanol extract of *Scomber* spp. (MHA) along with its fractions were used for studying insecticidal activity. Insect repellent property of extract was studied by spraying the extract on paper and observing repellence of *T. castaneum* introduced. MHA at concentration of 2.5 mg/cm<sup>2</sup> showed 88% repellence. Emergence of F1 progeny of *T. castaneum* was studied by introducing adults in wheat flour treated with extract. Progeny production was totally suppressed with MHA extract (3.57% at 0.144 mg/g) and fractions also showed significant effect on it. Effect of MHA on larval hatching and weight was also studied. It was found that both the parameters showed decrease with increasing concentration of MHA. Present work reveals that there is significant effect of MHA on *T. castaneum* at level of larval emergence, larval growth and F1 progeny production.

## Energy metabolic relationship of *Lamellibrachia satsuma* with its endosymbiont revealed by metagenomic analysis [Poster]

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*Lamellibrachia satsuma* is a vestimentiferan tube worm; lack mouth, gut and anus. They are nourished by chemoautotrophic bacterial endosymbionts growing in a specialized tissue called the trophosome. The *L. satsuma* was found at depth 82-110 m in Kagosima Bay, the shallowest depth record for a vestimentiferan. In this study, a sample of trophosome contents from the tubeworm was sequenced by Illumina HiSeq 2000. Data were assembled using SOAP de novo. Assembled data were submitted to the IMG/MER pipeline. Metagenomic analysis suggests that they harbor mostly  $\gamma$ -proteobacterial endosymbiont in the trophosome very closely related to the endosymbiont of the vent tubeworm *Riftia pachyptila*. The genes involved in carbon and sulfur metabolism indicate a sulfide-oxidizing chemoautotrophic endosymbiont. The symbionts contain all genes required for sulfur-oxidizing metabolism including those needed for the oxidation of reduced sulfur compounds mediated through the cytoplasmic enzymes adenylylsulfate reductase (AprA/AprB), periplasmic sulfite oxidase enzyme complex (Sox). The membrane bound respiratory nitrate reductase (NarI), a cytochrome cd1/ nitrite reductase (Nir), nitric oxide reductase (Nor) and nitric-oxide reductase (Nos) genes required for nitrate assimilation are also found in these symbionts. Surprisingly, the endosymbiont harbors genes for two different carbon fixation pathways, the Calvin-Benson-Bassham (CBB) cycle as well as the reductive tricarboxylic acid (rTCA) cycle, as has been reported in the endosymbiont of the various tubeworms like *Riftia pachyptila*, *Tevnia jerichonana*, *Escarpia laminata* and *Lamellibrachia spp.* This study suggests that, regardless of different geographical location, most of the vestimentiferan tubeworms might possess endosymbiont of common energy metabolism capability.

## Selection of robust and high productivity marine macroalgae for renewable fuels.

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Macroalgae offer outstanding potential as a renewable biomass energy feedstock. This is because of their high productivities and the ability to deliver a diversity of fuels. Furthermore, macroalgae deliver renewable biomass on non-arable land avoiding competition with food crops. Production can be linked directly to wastewater streams providing low-cost high-productivity outputs. We describe the selection process of two species with high biomass productivity for renewable fuels, *Derbesia tenuissima* and *Ulva ohnoi*. These species had the highest growth rates in across a range of macroalgae in intensive outdoor cultures, and had biochemical compositions suited to the thermochemical conversion of biomass to biological crude oil (biocrude). We highlight the unique properties of both of these macroalgae, including the high lipid content of *Derbesia*, and demonstrate that the biochemical profile of these feedstocks can be manipulated, both during culture and with post-harvest techniques. This enables us to tailor the feedstock specifically for biocrude production, providing the technological platform to develop renewable fuels and alternative bioproducts from marine macroalgae.

## **Anadara trapezia functional genomics**

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Understanding how species respond to temporal and spatial changes in their abiotic environments is a central goal in evolutionary genomics. *Anadara trapezia* is a widespread intertidal mollusc, distributed across a number of temporally and spatially fluctuating environmental gradients, including clines in water temperature and salinity. Understanding the molecular genetic basis of physiological traits that are associated with environmental variation has been limited by a lack of genomic resources for this species. In this study we undertook large scale sequencing of the *A. trapezia* transcriptome to generate genomic resources for functional genomic analyses. Over 2.4 Gb of sequence data were assembled into 75, 024 contigs using the Trinity de novo assembler and paired end information. This de novo assembly resulted in contigs with an average length 505 bp and an N50 of 597 bp. Overall 29, 013 (38.7%) contigs received significant BLASTx hits and gene ontology (GO) terms were assigned to 13718 of these sequences. Over 194 212 high confidence SNPs and small indels were identified in this transcriptome dataset. A number of candidate genes involved in adaptation to changes in temperature, pH and salinity including heat shock protein 70, carbonic anhydrase and sodium potassium ATPase were also identified. In this study we have provided the first transcriptome sequence and identified a large set of polymorphic markers for *A. trapezia*. This large transcriptome resource will provide the genomic markers to facilitate physiological genomic studies to test the gene expression response of *A. trapezia* to various environmental stresses.

## **Identifying the digestive enzyme repertoire of a herbivorous intertidal snail**

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A large number of intertidal gastropods are algal grazers and have been reported to digest complex carbohydrates from algae. Until recently the enzymes required to break down complex carbohydrates were thought to be of bacterial origin, but recent studies have identified endogenous genes for cellulose degradation in some mollusc species including gastropods. Only few studies, however, have tried to document the entire digestive enzyme repertoire that gastropods possess to degrade complex polysaccharides of plant origin. In this study we identified and functionally annotated digestive enzymes from two intertidal snails, *Nerita melanotragus* and *N. albicilla*, in particular those involved in the digestion of complex polysaccharides. We identified a number of genes involved in the breakdown of complex carbohydrates including endocellulases, exocellulases, cellobiases, as well as mannanases and pectinases in both snail species. Comparative analysis of these digestive enzymes with other mollusc species showed many of these proteins are highly conserved, and that *Nerita* species possess multiple isoforms of cellulase genes which may have slightly different functions. Overall this study has demonstrated that intertidal snails possess all the digestive enzymes needed to digest complex polysaccharides from algae.

## **Anti-microbial potential of crude extracts of marine sponge-*Tethya* spp. and edible fish-*Scomber* spp. [Poster]**

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Infectious diseases are one of the major causes of death in tropical countries. The striking rise in the prevalence of bacterial resistance currently poses a serious threat to public health worldwide. Of particular concern are infections caused by methicillin resistant *Staphylococcus aureus* (MRSA), penicillin resistant *Streptococcus pneumoniae*, vancomycin resistant *Enterococcus* (VRE) and *Mycobacterium tuberculosis*. Many of these organisms have developed resistance to several classes of established antibiotics. Therefore drugs from natural sources particularly from marine source are proving to be an alternative to synthetic antibiotics. Marine organism produces biologically active secondary metabolites which possess pharmacological and toxicological properties. Many marine invertebrates as well as vertebrates have been successfully used as a source of anti-oxidant, anti-cancer, anti-inflammatory as well as anti-bacterial drugs. Screening of organic extracts from marine sponges and common edible fishes may provide some new bioactive substances having anti-microbial activity. Our present work focuses on marine sponge *Tethya* spp and common edible fish *Scomber microlepidotus* (Mackerel) as potential anti-bacterial agents. *Tethya* spp-petroleum ether extract was labelled as SPPE and hexane-acetone extract of mackerel was labelled as MHA. Anti-microbial activity of the extract and their fractions was assessed by the disc diffusion assay against *S.typhi*, *S.aureus*, *B.subtilis*, *S.epidermidis*, *S.flexneri*, *C.diphtheria* and *E.coli*. SPPE showed good activity against *S.typhi*, *S.epidermidis* and *C.diphtheria*. MHA at all concentration showed good activity against *S.flexneri* and weak activity against *S.epidermidis* at concentration of 100µg/ml, 200µg/ml and against *E.coli* at concentration of 50 µg/ml and 100 µg/ml.

## Transcriptome characterisation and gene discovery in the marine shrimp *Fenneropenaeus merguensis* [Poster]

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Marine shrimp form part of one of the most important species groups for aquaculture worldwide with production reaching over 3 million tonnes in 2010. *Fenneropenaeus merguensis* is one of the most commercially significant shrimp species in South East Asia, India and Australia with over 86,000 tonnes being produced in 2007. However, only minimal genetic information is available for this species. Improvement in our basic knowledge of genomics and genetics of these shrimp could greatly benefit the development of this industry. The development of next generation sequencing technologies has greatly expanded the capacity for gene discovery in non-model species. Here we carried out high-throughput RNA sequencing of farmed *F. merguensis*. Messenger RNA was isolated from eight tissue types including eye stalk, nervous system, hepatopancreas, stomach, muscle, gonads and androgenic gland. Individual cDNA libraries were produced from the mRNA from each tissue and sequenced at the AGRF using 454 FLX Titanium chemistry generating a combined set of 822,076 high quality reads. These reads were assembled *de novo* into 50,257 unique transcripts representing approximately 32Mb of transcriptome sequence. Average length was 650 and N50 was 698. Putative genes involved in growth and reproduction were predicted using BLAST sequence homology searches against publicly available databases. Transcriptomic data generated in this study provides a resource for further gene discovery and can be exploited for the development of functional markers. This information can lead to a better understanding of shrimp biology and contribute to improvements in aquaculture of this species.

## Rapid Harvest of Microalgae using a Novel Bacterial Isolate

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Algal derived biofuels are one of the best alternatives for economically replacing liquid fossil fuels with a fungible renewable energy source. One hurdle that must be overcome before algal biofuels can be used at scale is finding a way to efficiently harvest and concentrate the algae. We are investigating the roles of bacteria associated with microalgae that are potential biofuel producers, including *Nannochloropsis* sp. IMET1. We isolated a novel, fast growing bacterium that when added to *Nannochloropsis* sp. IMET1 rapidly aggregates the algae into large flocs which settle and can easily be separated from the culture media. The bacterium was identified by 16S rRNA gene sequencing as a *Bacillus* sp., designated as strain RP1137. The flocculation process occurs in as little as 15 seconds and requires one bacterial cell for every five algal cells aggregated. This bacterium aggregates several diverse freshwater and marine photosynthetic organisms including *Tetraselmis* species and a marine diatom within the *Chaetoceros* genus suggesting the strain may be useful for harvesting many different types of algae. The mechanism of aggregation is pH dependent and reversible. It does not require live cells, suggesting the cells surface is the source of the aggregation phenotype and that the cells may be reused. Aggregation is dependent on divalent cations, specifically calcium and magnesium. The aggregation process has proved scalable up to 20 L, with a 1500 L test planned. Together the characteristics of this bacterium may be valuable in developing an efficient, low energy method of harvesting microalgae from large culture volumes.



## Merging Metabolism and Power: Development of a Novel Photobioelectric Device Driven by Photosynthesis and Respiration

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In many biological systems energy is stored in proton gradients. While organisms generate internal proton gradients to power their metabolism, the by-products of their metabolisms also affect the concentration of protons in their surrounding environment through the production or consumption of carbon dioxide. Bacterial respiration can decrease pH of the surrounding media to 3 while algal photosynthesis can increase pH to 11.5. By separating these two forms of metabolism a proton gradient on the order of  $10^8$  can be generated. By harnessing this gradient a biologically driven solar cell can be created that works in concert with the metabolisms of any organism that consumes or produces  $\text{CO}_2$  in an aqueous environment. Here we show a proof of principle that photosynthetic and respiratory metabolism can be harnessed to drive energy producing reactions in a high power bio-electric solar cell. With field trials we demonstrate the system is robust enough to work with an undefined natural microbial community. Power generated is light and photosynthesis dependent. The cell achieved a peak power output of 33 watts/m<sup>2</sup> electrode, more than an order of magnitude higher than previous photo-bioelectric cells. The design is simple, low cost and works with the biological processes driving the system by removing waste products that impede growth. Our results demonstrate a proof of principle of tapping into the core carbon pumping capacity common to all life to generate electrical power.

## Trends in marine natural products research-a New Zealand perspective.

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Although the scope of research on marine natural products encompasses many diverse areas, a number of trends are becoming evident. Some of these include:

- research on problems associated with human impact/environmental issues
- miniaturisation of technology
- marine and terrestrial commonalities
- research into diseases other than cancer.

This talk will present examples of research from the author's own group and from other research groups in New Zealand that typify these (and other) trends in the area.

## Exploring Australian marine biodiversity for producing next generation of biofuels

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Marine microbes have the potential in accumulating large amounts of lipids and are thus considered as feedstock for next generation biofuels. Few isolates from Australian marine environment were obtained after rigorous screening based on fatty acid (FA) composition, omega-3 and omega-6 polyunsaturated fatty acids. The other prominent fatty acids recorded in all isolates were palmitic acid (24.1-49.7 %), stearic acid (4.5-25.1 %), eicosapentaenoic acid (EPA: 5.6-12.9 %) and docosapentaenoic acid (DPA: 6.8-17.9 %). Overall, these isolates exhibited a comprehensive fatty acid profile accommodating saturated and polyunsaturated fatty acids. The molecular identification based on 18S rDNA sequencing and FT-IR spectroscopy confirmed their uniqueness. In addition, the isolate produced fair amount of carotenoids (such as  $\beta$ -carotene) which needs further medium optimisation studies. Further comparative analyses will be presented which will help to elucidate suitability of the isolate as a potential biomass for producing biodiesel.

## Algal Biotechnology: reshaping the coast and reforming algal industry

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Algal biotechnology is referred to biotechnology employed in algal research and applications. Regarding applications, algal biotechnology works in determination, production and utilization of algal biomass. Recently, global warming, ocean acidification and human activities offer great changes in distribution of coastal algal resources, thus making algal supply unstable and eventually algal industry unsustainable. Algal biotechnology aiming at constructing a stable supply as well as intensive/extensive utilizing algal resources becomes a realistic challenge in China. In China more than 50% of coastal line has become artificial constructs and seaweed resources declined. Seaweed diversity protection centers were set up by us in recent years, and the so called "ecological artificial coastal line" by cultivating seaweed/seagrass along artificial coastal line was demonstrated by our colleagues. Integrated biorefinery technology was developed by Chinese scientists to optimize utilizing seaweed resources to provide more value-added products. In the past decade, cultivated microalgal biomass increased significantly in China by using integrated low-cost technology combining with using CO<sub>2</sub> as a carbon source. Phycocyanins, carotenoids and other high value products from microalgae were produced and new circular technology was employed in microalgal manufacturing and processing plants in China. The presentation will showcase technology progress in both microalgal and seaweed industries. It is believed that algal biotechnology should be playing significant role in reshaping coastal flora and meanwhile upgrading algal industry towards a sustainable one in China.

## Coastal Algal Biotechnology: Transforming changing bioresources to sustainable green industries

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Coastal algae play important roles in global biogeochemistry and have been used in China as daily food and traditional medicine for thousands of years. Since 1949 China has successfully established the largest scale of seaweed cultivation in the world with about 3 million tons (fresh weight) a year, which is one of so called three waves in blue revolution in China. Global warming and human behaviors offer coastal environment great challenges. The *Enteromorpha* formed green tide which annually happened in Huanghai Sea since 2008 is the world's largest green tide, which causes high costs of cleaning up the seaweeds of up to 3 million tons of biomass (fresh weight). In Shandong Province, more than 52% natural coast line has been replaced by artificial ones, which causes dramatic decline of seaweeds biodiversity. While coastal biodiversity is losing, bio-resources turn to be unstable and unsustainable. The aim of seaweeds biotechnology is to rebuild a sustainable resource utilization system. For traditional biomass such as artificially cultivated seaweeds, iodine, alginate and mannitol are 3 common products in bio-refinery technology. With the technology development, 2 more products: fucoidan and seaweeds fertilization are added and have been industrialized. Combined with bio-finery, genetic engineering has developed to be a reliable method of producing upgraded products such as recombinant HBV antigen, rt-PA and other products with bioactivity. While to the new emerging biomass such as green tide seaweeds, the value added products from intensive and extensive utilizations can be very promising. At present the production of crude oil from *Enteromorpha* has completed at pilot scale.

## The Future for, and the challenges of, commercializing Marine Bioactives

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The value of marine natural products arises because of their novel chemotypes and because of the inherent difficulty associated with producing synthetic libraries that contain molecules that interact with biology space. Halichondrin B was first isolated from the sponge *Halichondria okadai* in 1986. The supply of halichondrin B hampered the early development and it was not until it was demonstrated that its biological activity resided in the smaller macrolactone moiety that development of a simplified fully synthetic molecule was possible. Eribulin is a tubulin microtubule dynamics inhibitor, shows low- to sub-nM potency against cancer cells in vitro, leading to apoptosis after 10-12 hours of irreversible mitotic block. Eribulin (as Halaven®) was initially approved in November 2010. *Ecteinascidia turbinata* is a colonial ascidian species found in the Caribbean and the Mediterranean. Aqueous ethanol extracts of *Ecteinascidia turbinata* were shown to have antitumor effects in 1969, but it was not until 1990 that isolation and structural elucidation was achieved. ET-743 was the most abundant member of the family (0.0001% yield. Early clinical development used material obtained by aquaculture. The supply problem was finally solved by development of a semisynthetic production of the drug. Trabectedin (ET-743) (as Yondelis®) was first approved in September 2007 by the European Commission. These compounds highlight the supply question around marine bioactives and the success stories are informative to the future challenges. The lecture will reflect on the role of scaffolds and non-flat marine natural products in this context.

## Australia's Nagoya Approach and Opportunities from Nature Bank (Eskitis Institute)

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The revival of natural products arises because of their novel chemotypes and because of the inherent difficulty associated with producing synthetic libraries that contain molecules that interact with biology space. Our lead discovery program is based on the drug-like natural product metabolome. We have previously reported a strategy (requiring no knowledge of structure) for the generation of lead-like enhanced (LLE) extracts and fractions with a protocol that allowed the retention of lead- and drug-like constituents by selecting favourable physicochemical properties such as  $\log P < 5$ . Natural products and their analogues have had high impact as drugs because of the embedded biosynthetic molecular recognition that transfers to therapeutic targets as described by protein fold topology (PFT). Nature Bank consists of around 45,000 biota samples collected from a range of mega-biodiverse countries. I will discuss our collaboration model and the Australian response to the Nagoya protocol.

The lecture will present two approaches to collaboration around the Nature Bank resource.

1. **Screening of 200,000 lead-like enhanced fractions:** The model involves supply of the library in assay ready format to collaborators with validated biological targets.
2. **Fragment-based screening of a naturally occurring fragment library:** We have observed ligand-protein complexes using FT-ICR-MS. The model involves supply of protein from collaborators for bioaffinity screening at Eskitis.

We will report outcomes from the two strategies.

## Bacterial diversity, a comparison between the hydro-thermal vent and the non-vent region of Espalamaca

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A new shallow water hydrothermal vent field was discovered during 2010 at a depth of 30 m in Espalamaca (38°33'N; 28°39'W) Azores, North Atlantic Ocean. To understand the community variation between the vent and non-vent, the culturable bacterial diversity were analyzed through molecular approach. Nutrient agar media amended with lead, iron, manganese and thiosulfate were used to isolate a variety of metal and element resistant bacteria. 16S rRNA gene sequence results 74 phylotypes exclusively from the vent, 18 from the non-vent and 21 reported to be common in both the places. *Proteobacteria* ( $\alpha$ -,  $\beta$ - and  $\gamma$ -) and *Firmicutes* were observed to be common whereas *Bacteroidetes* and *Actinobacteria* could be retrieved only from the vent area indicated the existence of bacterial groups with highly variable physiological and metabolic properties. Rarefaction curve for species richness, Shannon-Weaver index ( $H'$ ) and Chao-I richness clearly indicated that bacterial diversity is rich in vent than non-vent area. Interestingly, a total of 26 phylotypes from the vent and 11 phylotypes from the non-vent area found to be novel. Results from the current study assure many metal resistant bacteria with high detoxifying potential against various heavy metals.

## Isolation, culturing of a 'wild strain' of marine microalga and effect of temperature on its growth and lipid content

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Microalgal species are known to produce oil at per hectare yields 20 times greater than traditional agri-crops do today. Therefore biodiesel production from microalgae lipids is increasingly regarded as a more sustainable and feasible alternative to conventional biodiesel feed-stocks derived from terrestrial bioenergy crops. A successful and economically viable algae-based biofuel industry mainly depends on the selection of appropriate algal strains. The aim of present work was to study the effect of temperature on lipid content of a wild hypersaline strain of green microalgae; in view of its possible utilization as novel raw material for biodiesel production. The microalga was isolated from a salt-pan and cultured in laboratory. Isolation was done by standard algal plating method and the culture was maintained at 150 ppt, 10:14 D:N cycle with 4000LUX light intensity. A batch experiment was set for 12 days. A 3°C temperature rise was given to the batch culture with 48 hrs exposure at each range of temperature elevation. The temperature rise was given from 30°C to 45°C. Parameters like cell-count and transmittance were analysed daily and biomass and lipid content was analysed at each temperature rise. The cell-count and biomass showed an increase till 42°C with the highest count at 39°C. The extracted lipids were qualitatively and quantitatively analysed by gas chromatography. Above results will be discussed.

## Lipid metabolism in *Emiliana huxleyi*: Recent advances in gene identification and biochemical analysis

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The lipid profile of *E. huxleyi* is particularly noteworthy and consistent with enzyme systems that have been characterized by genetic and biochemical analysis. Many of these systems enable the coccolithophorid to alter the composition of its cellular membranes and lipid stores in response to fluctuating oceanic conditions. *E. huxleyi* stores energy, for example, in the form of the long chain (C<sub>37-39</sub>) polyunsaturated alkenones, alkenoates and alkenes (PULCA). The number of *trans* double bonds in these unusual neutral lipids changes in response to growth temperature, and to a lesser extent in response to nutrient and light limitation (Prah et al., 2003). *E. huxleyi* also has the ability to alter the composition of its cellular membranes when subjected to severe oligotrophic conditions. To maintain growth when phosphorus is limiting, *E. huxleyi* reduces phospholipid biosynthesis by substituting non-phosphorus betaine lipids for phosphatidylcholine (Mooy et al., 2009). The sphingolipid complexes in *E. huxleyi*, are also interesting as they are inextricably linked to the *E. huxleyi* virus, and perhaps apoptosis (Monier et al, 2009; Vardi et al., 2009). Moreover, some are of the methylated type, that occur only in fungi. Finally, *E. huxleyi* has the ability to synthesize the unusual long chain fatty acids (PUFAs), eicosapentaenoic (EPA), and docosahexaenoic acid (DHA). The genome of *E. huxleyi* has enabled the metabolic pathways for the synthesis and degradation of these different lipids to be reconstructed and new insights into the basic physiology of lipid metabolism in *E. huxleyi* to be gained. These insights and the potential to exploit *E. huxleyi* for commercial applications will be discussed, particularly in relation to very long chain PUFAs and biofuels.

## Characterisation of the effect stress on nitrogen metabolism in the commercially important agarophyte *Gracilaria gracilis*.

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Modelling algal physiology and growth in response to abiotic stresses such as nutrient limitation requires an understanding of the underlying metabolic processes. The present study aimed to address this by investigating nitrogen metabolism and the mechanisms regulating nitrogen metabolism in the red macroalga *Gracilaria gracilis*. This was achieved by profiling changes in gene and protein expression, and activity of two major nitrogen metabolic enzymes, nitrate reductase and glutamine synthetase. Long term culture of *G. gracilis* in nitrogen replete and free media indicated that nutrient limitation induces physiological changes as well as changes in nitrate reductase and glutamine synthetase mRNA, protein and activity. In addition, gene expression profiles suggested that *G. gracilis* may possess multiple nitrate reductase and glutamine synthetase isoforms that are differentially regulated via transcriptional, post-transcriptional, translational and post-translational mechanisms. Immunocytochemical investigations confirmed the presence of multiple nitrate reductase and glutamine synthetase isoforms. A novel finding was the immuno-localisation of glutamine synthetase to intracellular starch granules. Overall, findings in the current study have suggested multiple roles for these metabolic enzymes that include nitrogen assimilation/transport, cell wall biosynthesis and senescence.

## Draft genomes of four *Chlorella* strains.

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A number of species of algae are being explored for potential utilization in large scale biorefinery systems to produce biofuels and other products from the lipid extracted biomass. Genomic information from these strains is useful to understand the biochemical potential of the strain, for gene expression analyses and to identify target genes and regulatory regions for genetic manipulation. Draft genomes of four *Chlorella* strains, *C. vulgaris* UTEX26 and three strains isolated from Alberta, Canada (AB02, AB04 & AB06), were constructed from a single lane of 100 bp paired-end Illumina HiSeq reads using Ray 2.2 and the scaffolding and gapcloser modules from Soapdenovo. The draft genomes range considerably in size and show varying degrees of completeness: the UTEX26 draft genome is 37.71 Mb in 945 contigs (>1000 bp), AB02 is 86.44 Mb in 8045 contigs, AB04 is 56.68 Mb in 2411 contigs and AB06 is 63.25 Mb in 4028 contigs. Chloroplast and mitochondrial sequences were identified in the genome assemblies and complete genomes constructed by PCR between contigs. There is considerable variation in the sizes of both organellar genomes among these four strains: UTEX26 has the largest genomes (ct – 161612 bp, mt – 93803 bp) while AB04 has the smallest (ct – 101122 bp, mt – 51583 bp). Comparisons among these genomes and that of *C. variabilis* NC64A indicate regions of conserved gene order, but also variations in the size of intergenic regions, as well as regions novel to a particular strain. The taxonomic and biochemical implications of these differences will be discussed.

## The NRC Algal Carbon Conversion Flagship Program – a Canadian approach to sustainable algal biorefineries.

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The concept of culturing algae on an industrial scale for the production of biofuels and other products has gained substantial momentum in the last few years. Most efforts to establish such biorefineries have utilized open ponds in warm, sunny climates since the cost of energy for light and heating are presumed to make large scale photobioreactors economically infeasible. In northern climates, the winter temperatures and limited daylength prevent this approach from being viable. The National Research Council of Canada’s Algal Carbon Conversion Flagship Program aims to test the technological and economic feasibility of co-locating large photobioreactors in an industrial environment where CO<sub>2</sub>, excess heat, waste water and co-generated electricity can be used for the production of algal biomass. NRC, along with partners Canadian Natural Resources Ltd. (CNRL) and Pond Biofuels, will construct a demonstration facility housing a 100,000 L photobioreactor at the CNRL Primrose oil sands site near Bonnyville, Alberta. The demonstration facility will be outfitted with ancillary equipment for the harvesting and dewatering of the algal biomass as well as for the extraction of oils. A research and development team will support the demo facility by developing alternative technologies in four key areas: (1) algal strains and growth conditions; (2) photobioreactor technologies; (3) processing technologies; and, (4) biomass utilization. This research aims to develop suites of algal strains, technologies and products that will enhance the further scale-up and commercialization of similar biorefineries for deployment at industrial locations.

## Phenolic derivatives from South African kelps: pharmacological screening and *in silico* approaches towards new functional foods

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Marine algae are extensively used mainly for the industrial production of alginate, as an animal feed and fertilizers. Despite extensive research on the biological potential of marine algae throughout the world, screening of pharmacological properties and identification of active molecules from South African marine algae is not yet fully explored. The aim of this study was to investigate the phenolic constituents of kelp seaweeds. Five phenolic derivatives namely 1,3,5-trihydroxybenzene (phloroglucinol) (1), dibenzo [1,4] dioxine-2,4,7,9-tetraol (2), hexahydroxyphenoxydibenzo [1,4] dioxine (eckol) (3), 4-(2-hydroxyethyl)phenol (tyrosol) (4) and 4-(1,2, dihydroxyethyl)phenol (5) were isolated from *Ecklonia maxima* (1-3) and *Macrocystis angustifolia* (4-5). The spectral data of compound 2 is reported for the first time, while compounds 4 and 5 were identified for the first time from a marine alga. The isolated compounds were evaluated for various biological activities. *In silico* prediction of biological activity of all identified compounds showed remarkable potential with P>7. This indicates that the chance to find the observed activity by experimentation is high. Various molecular properties, toxicity profiles and other physico-chemical properties of these compounds were also determined. The bioactive potential and promising drug-likeness profile of these compounds make them valuable, leads requiring further experimentation. The isolated compounds also suggest that they could be used either as drugs or functional food ingredients with promising roles in formulation of medicines and nutritional supplements.

## Biochar from marine macroalgae and their waste streams: yields, characteristics and uses.

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Algae are a promising feed stock for bioenergy production through slow pyrolysis, producing carbon-rich biochar as a by-product. Biochar has potential uses in a wide range of applications including long-term carbon sequestration, agricultural soil amendments and in the bioremediation of industrial waste waters. In addition, the extraction of phycocolloids such as carrageenan and agar from marine macroalgae produces a solid waste stream that is currently underutilised and undervalued. I have quantified biochar yield from intensively cultivated macroalgae and, where relevant, the remnant solid waste streams. Macroalgal biochar has relatively low carbon content (10-30%), but high concentrations of extractable inorganic nutrients and pH. As a result it has great potential as a soil amendment, particularly in acidic soils. I will demonstrate two potential uses of algal-derived biochar; as an agricultural soil amendment to stimulate plant growth and, as a biosorbent to remove contaminants from complex industrial waste water.

## Coral algae as a source of UV-absorbing compounds

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Reef-building corals form mutualistic symbioses with unicellular photosynthetic dinoflagellate of the genus *Symbiodinium*. Exposure to ultraviolet radiation especially when combined with thermal stress, has been recognized as an important abiotic factor leading to oxidative stress and break down of the coral-algal endosymbiosis. In nature, many marine organisms use Mycosporine-like Amino Acids (MAAs) as biological sunscreens in UV protection and the prevention of oxidative stress. Corals acquire MAAs from their symbiotic algae and diet. Higher diversity of MAAs discovered within the coral host compared to their algal endosymbionts may be due to a regulation of MAA synthesis by host factors and/or due to host heterotrophy. The biosynthesis of MAAs has been proposed to occur via either the shikimate and/or pentose phosphate pathways. Until now the complete enzymatic pathway of MAA synthesis is not known nor is the extent of their regulation by environmental conditions. Here, using a transcriptome mining approach, we distinguish the gene homologs from the shikimate and pentose phosphate pathway involved in MAA biosynthesis within the sequences of coral dinoflagellates. We also describe the highly similar sequences of genes from the proposed MAA biosynthetic pathway involved in the metabolism of 4-deoxygadusol (direct MAA precursor) in different *Symbiodinium* strains confirming their algal origin and evolutionary conserved nature. Finally, we unveil the separate identity of 4 genes from the proposed MAA (shinorine) biosynthetic gene cluster in symbiotic dinoflagellates. This work provides a novel phylogenetic/biochemical outline of the genes involved in MAA biosynthesis in coral dinoflagellates.

## Dinoflagellates in symbiosis with reef building corals

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Dinoflagellates from the genus *Symbiodinium* form a mutualistic symbiotic relationship with reef-building corals. The susceptibility of coral to stress and bleaching may vary depending on algal endosymbionts. Here, we applied Illumina sequencing to produce transcriptomic data for four *Symbiodinium* clades (clades A, B, C and D) that are commonly associated with corals. We applied novel genomic tools to assess genetic similarity and diversity among four phylogenetically diverse dinoflagellate clades and obtained more than 30,000 predicted transcripts per each *Symbiodinium* clade. To understand better molecular mechanisms of symbiosis between corals and their dinoflagellate protists here we have focused on the uniqueness of symbiotic dinoflagellates genes. Our results represent the commonly preserved *Symbiodinium* transcripts that provide important segment of the molecular recognition between the coral and their algal endosymbionts. Within the Kyoto Encyclopedia of Genes and Genomes (KEGG) database that contains well described proteins and ubiquitous biochemical pathways we have discovered six pathways common in all four *Symbiodinium* clades: Phosphatidylinositol signalling system, Inositol phosphate metabolism, Spliceosome, Ribosome, Endocytosis and sucrose metabolism pathways. A list of common *Symbiodinium* transcripts included conserved genes like Heat shock proteins (HSP70 and HSP90), a number of ribosomal, photosynthetic and cytochrome genes. Mutual antioxidant genes important in stress response have been discovered and also a number of calcium-dependent and Calcium/calmodulin-dependent protein kinases that may play role in establishment of symbiosis. Our findings disclose new knowledge about the foundation of coral-algal symbiosis and genetic uniqueness of symbiotic dinoflagellates.

## Stable isotopes as a tool for identifying *Maja* commercial species origin to guarantee its market traceability [Poster]

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In the Spanish market, during the whole year, but particularly during the spider crab closed season (May to November approximately, annual variations depending on total fishing quotas), fisheries market is supplied by *Maja brachydactyla* specimens caught in Ireland, France, United Kingdom and/or Morocco, although crabs from Galicia (Spain) fetch higher prices. The geographic distribution of the spider crabs *M. brachydactyla* and *M. squinado* from several areas (three in the Atlantic and two Mediterranean) were studied through the analysis of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) stable isotope ratios in the exoskeleton of post-pubertal specimens. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of *M. brachydactyla* and *M. squinado* differed between populations (e.g. higher  $\delta^{15}\text{N}$  values were observed in Atlantic populations when compared with Mediterranean ones). The reported  $\delta^{15}\text{N}$  values were low in all tested specimens; the nitrogen stable isotope profile did not differ between males and females. However, the enrichment in  $\delta^{13}\text{C}$  of females across populations and species suggested that females tended to use shallower habitats than males before the terminal molt. The results reported here are useful for commercial *Maja* species traceability for allowing the identification of their geographical origin of capture and do not involve the sacrifice of the animal for this purpose since only one piece of a leg is enough for the analysis. Thus, this methodology could be used for fisheries management in order to identify the origin of the product, prevent fraud and guarantee its traceability along the commercial chain.

## Development of nutrigenomic tools to assess reproductive performance in shrimp

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Future development of crustacean aquaculture worldwide currently faces a significant bottleneck, because many culture industries still rely on the collection of wild ovigerous female broodstock to assure a regular seed supply. The development of genetic markers that correlate reproductive performance with broodstock nutrition will be a significant step toward solving this problem. In a separate experiment, the reproductive performance (egg quantity, hatching rate) of wild and domesticated *Penaeus monodon* (Black Tiger Shrimp) females was quantified following a series of reciprocal matings. Using samples of hepatopancreas and gonads from that experiment, this study analyzed gene expression levels of key regulators of a range of metabolic or biosynthetic pathways, as well as lipid and fatty acid profiles from females that had been sampled before the first spawning or after spawning. Specific quantitative RT-PCR assays were developed in *P. monodon* for ten genes that are orthologous to genes involved in vitellogenesis, eicosanoid, steroid and fatty acid biosynthesis pathways. Relative fold-change of target genes was examined for each group against the wild pre-spawning group and correlated with tissue lipid levels. A strong positive correlation was observed between the expression of *Elovl4* (elongation of very long chain fatty acids – 4) and two independent measures of reproductive performance, namely relative fecundity and hatching rate. This result suggests that sufficient capacity to produce long chain poly-unsaturated fatty acids from shorter-chain essential fatty acids exists in this species, and that high expression levels of this gene may be a predictive marker of reproductive success.

## Unravelling muricid secondary metabolite biosynthesis, in situ, using surface assisted mass spectrometry imaging

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In the last two decades there has been an increasing trend in the discovery of mollusc derived marine natural products, which generally come in the form of bioactive secondary metabolites. Unfortunately only a fraction of these discoveries develop further than structural elucidation. One underlying feature that aided in the development and supply of successful marine natural products was knowledge of the in situ natural synthesis of the active compound, or the functional ecology of the secondary metabolite. Here we present a new approach to understanding the in situ biosynthesis and functional ecology of muricid secondary metabolites using surface assisted mass spectrometry imaging. Desorption / ionization on porous silicon (DIOS) and nano-assisted laser desorption / ionization (NALDI) provide alternative functionalized scaffolds for mass spectrometry imaging of low molecular weight compounds without suppression of interfering matrix signals. These novel methodologies were employed to spatially analyse two classes of secondary metabolites, and their precursors, from the hypobranchial gland of the Australian muricid, *Dicathais orbita*. DIOS and NALDI proved to effectively map highly insoluble brominated indoles and highly soluble choline ester compounds, in situ, from fresh frozen tissue sections. DIOS is also being applied to describe the biodistribution of secondary metabolites during the reproductive cycle of both male and female *D. orbita* and maternally derived secondary metabolites in intracapsular fluids during larval development. By locating the precise site of biosynthesis and deployment of secondary metabolites we hope to contribute to our understanding of their ecological relevance and further their development as medicinal compounds.

## Feasibility study of bacterial lipopolysaccharide to increase black tiger shrimp survival under *Vibrio harveyi* challenge

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The effect of bacterial lipopolysaccharide (LPS) as a feed supplement to improve immunity of the black tiger shrimp (*Penaeus monodon*) was investigated. The commercial feed pellets were coated with LPS and given to 2-month-old juveniles once or twice a day for 10 days (4 ug of LPS/g of shrimp body weight). The growth rates, percent weight gains, total hemocyte and granulocyte counts, and survival rates were compared between the LPS-coated pellet fed groups and a non-LPS commercial pellet fed control group. After 10 days of the feeding trials, growth rates were not significantly different in all groups, suggesting no toxicity from LPS supplement. To determine beneficial effect of LPS in the diets, each group was subsequently exposed to *Vibrio harveyi* by an immersion method and the survival rates were recorded for seven days after the immersion. Regardless of the dosages of LPS, the shrimp groups fed with LPS-coated pellets showed significant higher survival rates than the control group. There was no significant difference in survival rates between the two LPS dosages groups. Gene expression analysis in the *P. monodon* intestines revealed that *antilipopolysaccharide factor isoform 3*, *C-type lectin*, and *mucine-like peritrophin* were expressed significantly higher in the groups fed with LPS supplemental diet once or twice a day than in the control group. The up-regulation of the immune gene levels in intestines and higher resistance to *V. harveyi* of the shrimp fed with LPS provide the evidence for potential application of LPS as an immunostimulant in the *P. monodon* farming.



## Insulin-like androgenic hormones and monosex culture of prawns: The first implementation of RNA-interference in aquaculture

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Monosex culture is a desirable practice in animal husbandry. Differences between males and females of the same species, in growth rate, alimentary needs and behavioral patterns, dictate the need to establish management procedures specifically adjusted to one sex or the other. In many crustacean species a bimodal growth pattern is exhibited where females grow larger than males or vice versa. In one of the economically important cultured freshwater species, the prawn *Macrobrachium rosenbergii*, males grow faster and reach larger size at harvest than females. Thus, to produce large specimens an all-male population culture is economically beneficial. The androgenic gland (AG) is the key regulator of masculine sexual differentiation in crustaceans. Recent discoveries, in our laboratory, of AG-specific insulin-like peptides (IAGs) have deepened our understanding of the gland's mode of action. Temporal IAG knockdown, using RNA interference, in the prawn *M. rosenbergii* has recently enabled the alteration of the phenotypic sex of genetic males into functional 'neo-females' capable of producing an all-male progeny. The intervention through RNAi does not require the use of hormones or other exogenous chemicals. It is temporal, applied at the youngest broodstock stage, and does not pose genetic modifications (non-GMO). This is the first instance of an aquaculture commercial use of a monosex population derived from an RNAi-based induced sex reversal.

Over the past six decades the role of the androgenic gland (AG) was established as the key regulator of masculine sexual differentiation in crustaceans. In *M. rosenbergii*, AG removal from immature males results in sex reversal. Sex-reversed males (neo-females) are capable of mating and spawning. Crossing neo-females with normal males results in all-male progenies. This was tested and found to be feasible and a two-step scheme for commercial all-male production was established and paved the way to more focused and specific interventions in the sexual differentiation processes in crustaceans.

Recent discoveries of AG-specific insulin-like peptides (IAGs) have deepened our understanding of the gland's mode of action. Temporal IAG knockdown, using RNA interference, in *M. rosenbergii* has recently enabled us to alter the phenotypic sex of genetic males into functional 'neo-females' capable of producing an all-male progeny. The intervention does not use hormones or other exogenous chemicals. It is temporal, applied at the youngest broodstock stage, and does not pose genetic modifications (non-GMO). The all-male progeny population is similar to that of males in normal mixed populations. This is the first instance of an aquaculture commercial use of a monosex population derived from a single gene silencing-induced sex reversal.

## Rhogocyte cell isolation and characterization from mollusc's tissue: The answer to hemocyanin biosynthesis bottleneck

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Rhogocyte cells are known as the hemocyanin biosynthesis site, scattered within mollusc's connective tissue. The protein immunogenic properties are extensively studied and used in biomedical application. In contrast to its application, knowledge for its biosynthesis is still obscure due to challenges in isolating and culturing the rhogocyte cells. In this study, we aim to solve the challenges of rhogocyte cells isolation and its characterization as a single cell. To achieve this, cells were isolated using Fluorescence-activated cell sorting (FACS) based on simultaneous staining of hemocyanin antibody and its mRNA probes (IF-FISH). Further observation with confocal microscopy was performed to characterize the cells before and after sorting. The results of this study demonstrated the isolation of two distinctive cells populations with overlapping signals. Both populations had varied cell morphology, sizes and IF-FISH signal distribution. The population with high antibody signal had irregular and elongated cell morphology with punctate mRNA probes signal. The second population with lower antibody had ovoid morphology and wide distribution of mRNA probes signals. Hemocyanin localization in the membrane was detected for both populations when observed by confocal microscopy followed by mRNA probes signal in the cytoplasm. Thus, we confirmed the isolation of rhogocyte cells from mollusk using a combination of IF-FISH and FACS. The result of this study can be a turning point to further understand the mechanism of hemocyanin biosynthesis in vitro.

## Elemental selenium and tellurium formation by *Shewanella* microbes newly isolated from deep sea sediments in Japan Trench [Poster]

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Existence of many useful elements such as gold, vanadium, tungsten, manganese, nickel, cobalt, selenium and tellurium has been confirmed in seawater and various marine environments. Much amounts of various metallic and metalloids elemental resources distribute in marine sediment. Therefore, it is predicted and shown the presence of various microorganisms capable of growing with metal and/or metalloid compound by their redox-reactions. In this study, we have attempted to isolate abyssal microorganism capable reducing selenium or tellurium oxyanions from deep sea sediments in Japan Trench of Sanriku offing. Furthermore, we have also performed the biochemical characterization, the phylogenetic analysis based on the 16SrDNA, and TEM observation and EDX analysis of biogenic nano-depositions. Samples of deep-sea sediments in depth from 6,500m to 7,000m were collected via the ocean survey research by the ROV, KAIKO 7000 II submersible in 2007 November. Many isolates had the ability to respire selenium (or tellurium) oxyanion to elemental selenium (or tellurium) by using acetate or lactate as a sole carbon source under anaerobic conditions. TEM observation and EDX analysis of cells in selenite-reducing strains and biogenic nano-particles indicated that pure selenium globules with diameters from 100 to 300nm were produced by their microbial reduction of selenite. On the other hands, tellurate-reducing isolates exhibited an intracellular formation of pure tellurium nano-sized crystalline. Furthermore, phylogenetic analyses based their 16SrDNA sequences showed that their isolates were bacteria belonged in genus *Shewanella*. These results showed biological candidate of the selenium and tellurium conversion and reduction was considered to be such species microbes.

## Development of low-cost high-efficiency algae energy farms

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Microalgae are highly efficient producers of biomass for bioenergy that can be farmed in large-scale without competing for arable land or biodiverse landscapes. They are able to use polluted water, brackish or seawater. However, current costs of algal biofuel production are too high, mainly because of expensive harvesting and extraction procedures. To overcome this hurdle, students, scientists and engineers in our team have developed low-cost cultivation, harvesting and product extraction technology to sustainably produce biodiesel, protein-rich animal feed and nutraceuticals. Using next generation sequencing and chemical engineering, we have improved local microalgae strains, and established innovative low-cost algal cultivation and harvesting systems by applying our lipid induction and settling (LIS) technology. A 250,000 Liter Algae Energy Farm has been constructed adjacent to the tidal Brisbane River in Pinjarra Hills, Australia, to provide a cost- & energy-effective biodiesel and animal feed production module. This module fully utilises the potential of microalgae as a zero-waste biorefinery concept, producing not only bioenergy, but also cattle feed supplement as well as omega-3 fatty acids, carotenoids and phytosterols. Research is underway to combine biodiesel from microalgae with biogas production that will allow recycling of fertiliser to provide fully sustainable energy farms. A new project will be introduced that focuses on carbon capture from cars coupled to stationary production of microalgae.

## **Antimicrobial activity of the coral reef sponge *Crella cyathophora* [Poster]**

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Sponges are an important source of natural bioactive compounds from marine environment. *Crella cyathophora* is a common sponge in the Red Sea and Indian Ocean, however, little is known about its physicochemical properties. In the present study natural compounds extracted from *C. cyathophora* were screened in anti-bacterial and fungal assays, of ecological and pharmacological relevance. The target microorganisms were: bacteria isolated from the sponge environment, laboratory bacterial strains, environmental marine fungi and human pathogenic fungi. The extract was fractionated using different chromatography methods and was found as non-active against bacteria and environmental fungi, yet highly active against *Candida glabrata* and *C. tropicalis*, with one non polar fraction showing a over 90% of growth inhibition. These *Candida* species are known human pathogens that cause nosocomial fungal bloodstream infections and account for many non-superficial *Candida* infections. The shortcomings of the present treatments include unfavourably interactions with other medications, resistance problems, low spectrum of activity, limited formulation, fungistatic compounds (as opposed to fungicidal) and are often toxic. In effort to find novel antifungal drugs, we are now focusing on chemical elucidation of the bioactive compound/s.

## **Species identification and reproductive characteristic of the three *Mugil cephalus* cryptic species in Taiwan [Poster]**

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A Flathead mullet *Mugil cephalus* is a commercially important fish species for both capture fisheries and aquaculture. Three cryptic species of *M. cephalus* (NWP1, NWP2, NWP3) were recently identified by genetic analysis and were found to exist sympatrically in the waters around Taiwan. Different *M. cephalus* species may have its own reproductive characteristics which are important for aquaculture applications. In this study, we used rapid COI haplotype-specific PCR screening to identify the species of *M. cephalus* in a tropical estuary in southern Taiwan. Then the spawning periods of different *M. cephalus* cryptic species were examined using the temporal changes in their gonadosomatic index (GSI) from August 2008 to March 2009. Meanwhile, the species compositions of the larval *M. cephalus* in the nearby Baoli Stream from December 2011 to March 2012 were also examined. The results indicated that the temperate species NWP1 is not common in the tropical Kaoping Estuary, but its higher GSI value during December coincided with the spawning timing of its spawning stock mainly in the south Taiwan Strait. The GSI of NWP2 increased starting in September and probably went for spawning in the offshore waters during October to early December. The appearance of the newly recruited NWP2 larvae from December to February also proved the similar spawning period for NWP2. However, the GSI of the tropical NWP3 indicated that it has a longer spawning period that commenced in October and will last until March of the following year. These reproductive characteristics are important for the species selection for aquaculture.

## Measurements of antioxidant activities in edible brown seaweeds extracts Obtained from supercritical CO<sub>2</sub> and solvent extraction [Poster]

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Recently, much attention has been paid on the anti-tumor activity, anti-cholesterolemic activity and antioxidant activity of seaweed constituents. Consequently, antioxidant activity is intensively focused due to the currently growing demand from the pharmaceutical industry where there is interest in anti-aging and anti-carcinogenic natural bioactive compounds, which possess health benefits. Supercritical CO<sub>2</sub> and solvent extraction will be applied to samples of the two edible brown seaweeds, *Ecklonia cava* and *Sargassum horneri* to compare their phenolic contents and antioxidant activities. SCO<sub>2</sub> conditions will be tested at 30 °C and 25 MPa, giving solvent densities of 699-923 kg m<sup>-3</sup> applied at a flow rate of 38.5 x 10<sup>-5</sup> kg s<sup>-1</sup>, and ethanol will be used as co-solvent with flow rate of 1 mL/min for 120 min. Fatty acid contents of the recovered oil will be determined by gas chromatographic quantification of their methyl esters (FAMES). Three different solvents (ethanol, methanol and acetone) will be used to extract the phenolic content of the edible brown seaweeds. Solvent extraction will be run in a 200 mL flask with magnetic stirring overnight under the dark at 25 °C and the ratio of material to solvent is 1:10 (w/v). Antioxidant activity properties of two marine edible brown seaweeds will be evaluated and compared on the extracts obtained.

## Antimicrobial activity of total lipids extracted from Thai marine sponges [Poster]

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Fifteen of marine sponges from the gulf of Thailand were investigated antimicrobial activity and the fatty acid compositions. The total lipids were extracted by the Floch method and were classified by solid phase extraction technique (SPE). Fatty acid compositions were analysed by GC/FID. The result showed that the lipid extract of *Dysidea* sp. "White" showed antibacterial activity against *Vibrio vulnificus*. Whereas the fatty acids were saturated fatty acids (SFAs; 18.55 %), mono-unsaturated fatty acids (MUFAs; 4.53 %) and polyunsaturated fatty acids (PUFAs; 13.47%), which included linoleic acid (18:2 n-6, 0.50 %), α-linolenic acid (18:3 n-3, 0.83 %), γ-linolenic acid (20:3 n-6, 2.66 %) and arachidonic acid (20:4 n-6, 2.79%), eicosapentaenoic acid (EPA; 20:5 n-3, 2.57% and docosahexaenoic acid (DHA; 22:6 n-3, 3.54%).

## Biotechnology at the last bus stop: a New Zealand industry perspective

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Like every industry in New Zealand Biotechnology faces the twin challenges of lack of scale, given the country's small population and resource base, and distance from markets. None the less the industry is showing strong signs of success based partly on New Zealand's historical expertise in the primary sector and also on some outstanding science groups turning their ideas into commercial reality. How can we build on this success? It seems obvious that with China recently displacing Australia as New Zealand's largest trading partner, part of the answer must lie in New Zealand's relationship with these two countries. In this presentation I will look at some of the obstacles and opportunities that have driven New Zealand's Biotechnology industry and consider how strengthening links with Australia and China can benefit Biotechnology in all three countries.

## **Bioassay experiments reveal that the cyanobacteria *Anabaena variabilis* is associated with Loose Shell Disease (LSD) in shrimps [Poster]**

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Loose Shell Disease (LSD) is increasingly being observed around the shrimp aquaculture facilities in India. This disease is characterized by muscular atrophy of the body and usually develops by 30-35 days of culture practices. A filterable agent is believed responsible for the disease but there exist huge lacunae regarding the proper etiology and an experimental validation of the pathogen. In this study, we have isolated dominant microalgae from shrimp aquaculture ponds infested with loose shell disease. In bioassay experiment with these microalgae, the cyanobacterium, *Anabaena variabilis* was observed causing loose shell disease like symptoms in otherwise naïve shrimp post larvae. The observation was further confirmed by histopathological studies also. The shrimps post larvae fed with *Anabaena* caused similar lesions to loose shell disease like hypertrophied and clumped cells of hepatopancreas. Atrophied nucleated cells, large number of B-cells were consistently observed along with a constricted lumen area while the control groups remained normal. Based on these results it is proposed that the loose shell disease in shrimps is caused by *Anabaena variabilis*, but the nature of interaction remains unexplored.

## **Immune Defence Mechanisms of Cultured Marine Invertebrates**

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A number of important genes involved in the host immune defence were cloned from marine invertebrate, and the preliminary immune network was constructed. Substantial genes from high-quality cDNA libraries of scallop and Chinese mitten crab were obtained; part of these genes were proved to be related to the host immune defence, from pathogens recognition, signals transduction, effector synthesis to pathogens clearance. More than 40 pathogen recognition receptors (PRRs) were reported to play important roles in pathogen recognition, including C-type lectins (CTLs), Toll-like receptors (TLRs), thioester-containing proteins (TEPs) and scavenger receptors. In addition, the consequent immune responses to pathogen invasion were determined according to their binding properties to pathogen-associated molecular patterns (PAMPs). Moreover, the existence of Myd88-dependent TLR-mediated signal pathway was first revealed in molluscs. The G-type lysosomal enzyme, complement analogues and the complement activation pathway were also elucidated to exist in invertebrates. Immune priming phenomenon was demonstrated in scallop, which was of great importance to understand immune memory mechanism in invertebrates. Furthermore, over 30 recombinant proteins were obtained with biological activities, which had the great potential to activate immune response against pathogens. A novel method based on immune activation by CpG ODN dietary treatment was established to prevent white spot syndrome virus (WSSV) infection. Moreover, genetic maps of scallops were constructed and over 10 single nucleotide polymorphisms (SNPs) were found to be involved in pathogen-resistance, the relationship between pathogen resistance and genetic diversity was illustrated, which was of great value to the scallop genetic breeding.

## **Metabolic engineering of Polyunsaturated fatty acid biosynthetic pathway in Yeast**

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Omega-3 and omega-6 fatty acids have been shown to play significant roles in human health. However, limited fish resources and increasing global demand for polyunsaturated fatty acid (PUFA) as functional food and nutritional ingredients have stimulated the development of renewable PUFA sources, including microbial cell factory systems as an alternate PUFA sources. This study investigates the potential of *Saccharomyces cerevisiae* to produce PUFA by exploiting synthetic biology and metabolic engineering tools. The genes involved in the fatty acid synthesis pathway, such as Diacylglycerol acyl transfers (*DGA1*), Acyl CoA synthase (*FAA3*), Delta 9 desaturase (*D9D*), Delta 12 desaturase (*D12D*), Delta 6 desaturase (*D6D*), Delta 6 elongase (*D6E*), Delta 5 desaturase (*D5D*) and Delta 17 desaturase (*D17D*), were selected from closely related eukaryotes. Selected pathway genes were synthesized after codon optimization. Synthetic genes were cloned under Gal1 and 10 promoters in Yeast Epitope Tagging Vectors. Engineered *S. cerevisiae* were grown on various carbon sources in a nitrogen limited synthetic medium at 30 °C for fatty acid analysis. Results revealed an increase in major fatty acid followed by lipid accumulation after expression of *DGA1* and *FAA3* genes. Significant conversion of stearic acid [18:0, SA] to Oleic acid [18:1, OA] was observed. Furthermore, expression of *D12D* converted OA into Linoleic acid [C18:2n6, LA] and  $\alpha$ -Linoleic acid [C18:2n6, ALA] was optimised. In conclusion, engineered *S. cerevisiae* showed increased ability to synthesize Linoleic acid and alpha linoleic acid without supplying exogenous fatty acid in the medium. Further studies are in progress to expend pathway to synthesise omega-3 fatty acids including the fish derived long-chain PUFA eicosapentaenoic acid (EPA).

## Anti-MRSA and antioxidant activities of actinomycetes isolated from marine sponges [Poster]

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Sixteen samples of marine sponges were collected at Wangnok island, Nakhon Sithamarat Province, to isolate for actinomycetes. Isolation media were ISP2 (International *Streptomyces* Project 2), actinomycete isolation agar and starch casein agar. Each sample of sponge was ground with normal saline before the fine suspension was inoculated and spread onto the medium plates, triplicately. The plates were incubated at 30°C for 4 weeks observation. Two isolates of actinomycetes, *Micromonospora* POR 02(MP02) and *Micromonospora* POR 06 (MP06) were found from *Chalinidia* sp. and *Dysidea* sp., respectively. Both isolates were found to be antimicrobial producing strains against methicillin resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis*. Crude extract products were prepared from both cells and cultured medium of MP02 and MP 06. Interestingly, by disc diffusion assay, all of 23 hospital strains of tested MRSA were inhibited by methanol crude extract from cells of MP 06 which showing a small difference of inhibition distance zone to that of vancomycin (45 µg/disc). The MIC<sub>50</sub> of (medium) MP02 and (cell) MP06 crude extracts to MRSA SP83 showed the same range, 64-128 µg/ml, while MIC<sub>50</sub> of (cell) MP02 crude extract ranging from 128-256 µg/ml. Antioxidant activity was assessed on the basis of scavenging effect on stable 1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) free radicals. We found that the crude ethyl acetate extract from growth medium of MP06, but not MP02, showed good antioxidant activity compared to that of ascorbic acid. Although not many actinomycetes were found from sponges, the recovered isolates were rather promising to study further for some other bioactive metabolites.

## A novel alkaline lipase obtained from the metagenome of marine sponge *Ircinia* sp. [Poster]

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Marine sponge-associated microorganisms are considered as potential resources for marine enzymes. In this study, for marine sponge *Ircinia* sp. from the South China Sea, a metagenomic library including 6,568 clones with inserts of 2-10 kb was constructed to screen lipase. A clone (35F4) with lipolytic activity was obtained by plating on a tributyrin medium. Sequence analysis revealed that the insert DNA of 35F4 contained an open reading frame lipA of 921 bp, which was responsible for encoding a 32.98 kDa lipase with 61% amino acid similarity to the lipase of *Aeromonas media* WS. The amino acid sequence analysis also revealed the presence of the conserved domain GX SXG which was essential to lipase activity. After removing the signal peptide, the lipase gene lipA was successfully expressed in *E. coli* BL21 (DE3) using pET28a (+) expression system and further purified by Ni-nitrilotriacetic acid affinity chromatography. Spectrophotometric assays demonstrated the purified recombinant enzyme LipA preferentially hydrolyzed p-nitrophenyl esters with long length acyl chain, especially showing highest activity toward p-nitrophenylmyristate (C<sup>14</sup>). Moreover, enzymatic assays showed the LipA displayed a high degree of activity at 40°C and pH 9.0. Thermal stability analysis showed that the LipA was stable at 4°C, but the activity had a significant decline at 60°C for 120 min. The pH stability analysis showed that the LipA was stable at pH 9.0-11.0. This study led to the discovery of a novel alkaline lipase from the metagenome of marine sponge *Ircinia* sp..

## RNA interference (RNAi) technology applied on the blocking of betanodavirus replication and host cell death

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The RNA nervous necrosis virus (NNV) triggers secondary necrotic cell death in fish cells, but its molecular death mechanism is still unsolved. In this present, we demonstrated that betanodavirus-induced necrotic cell death is initiated at the genomic level of RNA replication and required viral death inducer-dependent pathway at late replication stage. To identify the viral genomic replication is whether required for RGNNV-induced cell death, by using loss-of-function approach for testing this hypothesis. In the results, the effectively knockdown of RNA dependent RNA polymerase gene (RdRp, 110 kDa) can either completely block viral genomic replication or blocked viral death inducers protein  $\alpha$  and protein B2 expression, which correlate to inhibit the mitochondria-mediated death signaling for increasing cellular viability. Taken together results indicate that RGNNV replication is correlated to induce host cell death, which provided new insight into RNA viral pathogenesis.

## **Paramylon production by fed-batch cultivation of *Euglena gracilis* using waste in food industry [Poster]**

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*Euglena gracilis* accumulates remarkable amount of beta-1,3-glucan, called paramylon, in the cells. It has been recently shown that the introduction of acyl chains into paramylon molecules gave thermoplasticity to paramylon. However, for the utilization of paramylon as a raw material of thermoplastics, development of an efficient mass production method of paramylon at a low cost is required. In the present study, the waste in food industry, such as waste beer, was utilized as cheap organic carbon source for the heterotrophic cultivation of *Euglena*. The heterotrophic cultivation of *Euglena* in the medium that yeast extract and vitamins B1 and B12 were added into waste beer gave a rapid growth and high density of *Euglena* cells same as that in the medium containing glucose as a sole carbon source. Moreover, fed-batch cultivation of *Euglena* using waste beer resulted in the efficient heterotrophic cultivation in quite high density. The *Euglena* cells that obtained by the fed-batch cultivation in high density were rich in paramylon and seemed to be suitable for raw material of thermoplastics. As a result, the fed-batch cultivation of *Euglena* using waste in food industry made an efficient paramylon production in high density and a practical production of valuables, such as bio-plastics, possible.

## **Study of Proteins that Catalyze Silica formation and Polyunsaturated Fatty Acid synthesis in Marine Diatom *Chaetoceros gracilis***

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Silica polymers and polyunsaturated fatty acid (PUFA) are valuable substances due to their benefits for food and health industry. They are abundantly produced by diatom. The mechanism of their synthesis is controlled by several proteins. The aim of this research was to study the proteins involved in silica and PUFAs formation in *Chaetoceros gracilis*. Research methods include analysis of growth, cellular content of protein and lipid as well as fatty acid composition during growth. Protein characteristics were conducted by two-dimensional electrophoresis to estimate the molecular weight and isoelectric point. Proteins were identified by bioinformatic study. The cellular content of proteins, lipids and fatty acids were influenced by the growth phase. There were five silica proteins detected from siliceous cell wall of this diatom. Silicic acid transport proteins which are active in the transport of silicic acid for silica synthesis was also identified during exponential to death growth phase. Eleven proteins involved in synthesis of PUFAs were identified, these included three microsomal desaturase proteins ( $\Delta 9$  DES,  $\Delta 6$  DES, ELO  $\Delta 6$ , prekursor  $\omega$ -6 DES, prekursor  $\omega$ -3 DES, spingolipid  $\Delta 8$  DES,  $\Delta 12$  DES,  $\Delta 4$  DES, microsomal  $\Delta 4$  DES, microsomal  $\omega$ -6 DES and microsomal  $\Delta 6$  DES).

## **Regulation of glycaemia with the application of recombinant CHH1 and its polyclonal antiserum in *Penaeus monodon*.**

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Crustacean hyperglycaemic hormone (CHH) family neuropeptides have been in the research limelight for the past two decades due to their importance in the regulation of glycaemia, moulting and gonad development in crustaceans. Under natural conditions, the low levels of the CHH neuropeptide and the structural similarity of the three CHH family neuropeptides limit their purification directly from the animal. In this study, we carried out sequence analysis and homology modelling of CHH1 hormone gene, isolated the mature region of the CHH1 gene, constructed the recombinant translation expression vector (pET32a<sup>+</sup>- PmCHH1) and produced the recombinant protein in *E.coli* (BL21 (DE3) pLysS). The translation expression vector construct (pET32a<sup>+</sup>- PmCHH1) was successfully built for the production of recombinant CHH1 protein (rCHH1-29.47 kDa). rCHH1 produced a hyperglycaemic effect when experimentally injected into adult *Penaeus monodon* similar to that of the eyestalk extract. Polyclonal antibody (anti-rCHH1) was developed in mice for the purified recombinant CHH1 protein. A hypoglycaemic effect was induced by the polyclonal antiserum when injected into adult *P.monodon*, observed by 50% and 94.76 % reduction in glucose and CHH1 hormone level. Therefore, rCHH1 and its antibody could be useful tools to better understand the endocrine mechanisms regulating hyperglycaemia and reproduction in *P. monodon*.

## The transcriptome sequencing and carbonic anhydrase analyses of marine microalga *Chlorella pyrenoidosa* (Chlorophyta) [Poster]

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High-throughput RNA-Seq technique has rapidly developed in recent years. In this study, de novo transcriptome assembly and annotation were conducted in a genome information deficient marine green alga *Chlorella pyrenoidosa* 820. And the carbonic anhydrase (CA) family, an important component of the CO<sub>2</sub>-concentrating mechanism (CCM), were analyzed in this alga. In the sequenced 4.93G clean nucleotides, a total of 57,090 contigs with 435nt mean length and 36,826 unigenes with 1089nt mean length were assembled. The GC percentage of *C. pyrenoidosa* was 53.69%. In total, 23,015 unigenes were annotated in the NR, NT, SwissProt, KEGG, COG and GO databases with e-value lower than 1e<sup>-5</sup>. In the annotated sequences, 21 unigenes were identified as CA genes. The CAs were further matched to  $\alpha$ -,  $\beta$ -,  $\gamma$ -CA by aligning with other green algae *Chlamydomonas reinhardtii* and *C. variabilis*. This work will enrich the CA information and be helpful to understand the CCM in the genus *Chlorella*.

## Study of Protein Interaction between *Penaeus monodon* Anti-lipoplysaccharide Factor Isoform 3 and White Spot Syndrome Virus

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A broad spectrum antimicrobial peptide from *Penaeus monodon*, namely anti-lipoplysaccharide factor isoform3 (ALFPm3) exhibited the activity against bacteria, fungi and a shrimp pathogenic virus, white spot syndrome virus (WSSV). To study in depth on ALFPm3 function in shrimp viral responses, we screened the WSSV library for ALFPm3 interacting protein using yeast two hybrid screening (Y2H) technique. Five true positive clones were identified. The plasmids were extracted from these positive clones and then analyzed for their sequences. WSSV proteins including WSSV186, WSSV189, WSSV395, WSSV458 and WSSV471 were found to be the ALFPm3-interaction proteins. Temporal transcriptional analysis in WSSV-infected *P. monodon* revealed that all WSSV genes were expressed in the late phase of infection (24 h and 48 h post infection). Then WSSV189, an unknown protein, was selected for further analysis. The recombinant proteins of WSSV189 was expressed in *Escherichia coli* and purified by affinity chromatography. The *in vitro* pull-down assay using rWSSV189 as bait confirmed the true interaction between ALFPm3 with WSSV189 protein. Moreover, pre-incubation of rWSSV189 protein to rALFPm3 could impede the neutralization effect of rALFPm3 on WSSV as shown by the increase in the mortality of shrimp injected with mixture of rWSSV189, rALFPm3 and WSSV when compared to those infected with rALFPm3 and WSSV. To our knowledge, the specific binding of ALFPm3 to WSSV189 might involve in anti-WSSV activity of ALFPm3.

## The *Pinctada fucata* BMP-2 induced the osteogenic differentiation of C3H10T1/2 murine mesenchymal stem cells [Poster]

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BMP-2 plays an important role in morphogenesis in both vertebrates and invertebrates and is one of the most powerful bioactive substances known to induce the osteogenic differentiation of mesenchymal cells. In this study, the structural conservation of the *Pinctada fucata* BMP-2 (*Pf*BMP-2) was examined, and the capability of the *Pf*BMP-2 that induces differentiation from mouse mesenchymal stem cells C3H10T1/2 to osteoblast cells was also investigated. To examine the homology between *Pf*BMP-2, Drosophilae family members, and vertebrate homolog, we performed a Dot matrix analysis of the conserved amino acid sequences. *Pinctada fucata* BMP-2 has a high overall amino acid sequence homology with that of human BMP-2 (hBMP-2) and *Xenopus* BMP-2, as well as that of the Drosophilae homolog Dpp and *Chordata Amphioxus* BMP2/4. The synthetic polypeptide PGSVPKPCCVPTLSSLSLL of the *Pf*BMP-2 functional domain carried out differentiation induction of the C3H10T1/2 murine mesenchymal stem cell to osteoblast cells. Osteogenic induction was confirmed by an increase in ALP activity, and the accumulation of calcium. Further, it was also confirmed by performing the RTPCR analysis of the expression of bone cell-specific marker genes and bone cell-specific transcription factor genes in C3H10T1/2 cells. It was shown that the BMP-2 gene is highly conserved structurally and functionally in animals from invertebrates to vertebrate.



## **Modification of fatty acid composition by gene silencing of $\Delta^9$ desaturase in oleaginous diatom *Fistulifera* sp. strain JPCC DA0580 [Poster]**

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An oleaginous microalga, *Fistulifera* sp. strain JPCC DA0580, was selected as a promising resource toward biodiesel fuel (BDF) production. The microalgal triglyceride used as a BDF precursor is consisted with various fatty acids including polyunsaturated fatty acids (PUFAs). As the degree of unsaturation of fatty acids affects on the oxidative stability of BDF, the PUFA content is one of the limitations for commercial use. Toward addressing this issue, the knock-down of *desaturase* gene is an effective approach for the modification of fatty acid profile in microalgae. In this study,  $\Delta^9$  *desaturase* gene, which is the first enzyme to introduce a double bond into saturated fatty acids in the PUFA synthesis pathway, was suppressed by RNA interference to reduce the PUFA content. *Fistulifera* sp. was transformed by a vector containing the anti-sense sequence of  $\Delta^9$  *desaturase* gene (250-bp). Five candidate clones were selected through 2-step methods based on the evaluation of cell growth and the confirmation of RNAi cassette integrated in the genome. The transformants showed different fatty acid profiles of eicosapentaenoic acid (C20:5) and linolenic acid (C18:3) at 1.42 and 0.40-fold changes compared with wild-type, while the growth and lipid content were similar level. The results showed that the strategy of *desaturase* gene knock-down is effective for the modification of fatty acid profile in oleaginous microalga. Further regulations of other *desaturase* genes would provide the oleaginous microalga transformant with decreased PUFA contents, highly suitable for BDF.

## **Isolation and identification of glycoproteins inhibiting adipocyte differentiation from scallop shells [Poster]**

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Many scallop shells are generated as industrial wastes in Hokkaido, Japan. Scallop shells consist of 98–99% calcium carbonate and 1–2% organic compounds. For the novel effective utilization, we have searched the bioactive components in organic components isolated from scallop shells (scallop shell extract). Previously, we found that rats fed with scallop shell extract showed a reduction in white adipose tissue weight compared to rats fed a control diet, and the scallop shell extract inhibited adipocyte differentiation in vitro. In this study, we isolated differentiation-inhibiting substances and determined its partial structure. The scallop shell extract was applied to Lens culinaris (LCA) and Concanavalin A (ConA) lectin affinity columns sequentially, and each lectin-binding glycoproteins were collected. 3T3-L1 preadipocytes were cultured in DMEM including 10 % FBS and differentiated by treatment with Insulin, 3-isobutyl-1-methylxanthine and dexamethasone in the absence or presence of LCA- or ConA-binding glycoproteins. LCA- and ConA-binding glycoproteins inhibited the differentiation. Isolated LCA-binding glycoprotein had a molecular weight of 16,000 and MALDI-TOF/TOF analysis identified 4 sequences AGEE(L/I)NSFD(Q/K)(Q/K)NK, FFDN(L/I)CPE(L/I)K, (L/I)MSADGR and S(Q/K)DCVR. Structural analysis of sugar chains of a LCA-binding glycoprotein showed the existence of N-linked oligosaccharide with core fucose. Now we attempt to determine the structures of LCA- and ConA-binding glycoproteins.

## Screening for exolytic alginate lyase genes of bacteria isolated from marine environmental samples [Poster]

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Brown algae are known as an important resource for bioethanol production. Nevertheless, degradation of brown algae is still considered as a challenging process due to the presence of its main component alginate. One approach to degrade alginate is by the use of bacterial alginate lyases. Furthermore, exotype enzymes, those that degrade alginate to monosaccharide are rare and are of high demand. Thus, our goal in this research is to search for exolytic alginate lyases from marine bacteria isolated from various marine environmental samples including gut and feces of invertebrates, various types of fermented algae, sea sand and sediment. Briefly, alginate lyase producing bacteria were isolated using marine broth spiked with 1% alginate and their species were identified via 16S rRNA sequence analysis. Subsequently, screening for exolytic alginate lyase producing bacteria was conducted and the presence for exolytic alginate lyase from these bacteria was evaluated using thin layer chromatography (TLC). From the 16S rRNA sequence analysis, 19 strains of bacteria from 9 genera types were identified, among which 3 strains, *Rhodobacter*, *Vibrio* and *Alteromonas*-like species showing sequence homology < 97% were considered as novel. Based on the TLC analysis of degraded alginate using these identified bacteria, a total of 11 bacterial strains showed the ability to degrade alginate to monosaccharides suggesting the presence of exolytic alginate lyases. Our future plans include the extraction of the exolytic alginate lyase genes from these bacteria and to subsequently apply them in the degradation of alginate using yeast.

## Functional analysis of a key enzyme in PUFA synthesis, $\Delta^9$ desaturase, identified from the oleaginous diatom *Fistulifera*

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*Fistulifera* sp. strain JPCC DA0580 accumulates high amounts of neutral lipid (60% (wt/wt)) mainly composed of palmitic acid (C16:0) and palmitoleic acid (C16:1), suitable for biodiesel fuel. Now a challenge task is the decrease of the long-chain polyunsaturated fatty acids (PUFAs) content affecting on the BDF oxidative stability by using gene manipulation techniques. However, only the limited knowledge has been available concerning the fatty acid and PUFA synthesis pathways in microalgae. Especially, the function of  $\Delta^9$  desaturase, which is a key enzyme in PUFA synthesis pathway, has not been determined in diatom. In this study, 4  $\Delta^9$  desaturase genes (*fD9desA*, *fD9desB*, *fD9desC* and *fD9desD*) from the oleaginous diatom *Fistulifera* sp. were newly isolated and functionally characterized. The putative  $\Delta^9$  acyl-CoA desaturases in the endoplasmic reticulum (ER) showed 3 histidine clusters that are well-conserved motifs in the typical  $\Delta^9$  desaturase. Furthermore, the function of these  $\Delta^9$  desaturases was confirmed in the *Saccharomyces cerevisiae ole1* gene deletion mutant ( $\Delta ole1$ ). All the putative  $\Delta^9$  acyl-CoA desaturases showed  $\Delta^9$  desaturation activity for C16:0 fatty acids; *fD9desA* and *fD9desB* also showed desaturation activity for C18:0 fatty acids. This study represents the first functional analysis of  $\Delta^9$  desaturases from oleaginous microalgae and from diatoms as the first enzyme to introduce a double bond in saturated fatty acids during PUFA synthesis. The findings will provide beneficial insights into applying metabolic engineering processes to suppressing PUFA synthesis in this oleaginous microalgal strain.

## Metabolic engineering of marine oleaginous diatom towards biofuel production

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Oleaginous microalga is one of the promising resource of nonedible biodiesel fuel (BDF) feedstock alternatives. A metabolic engineering technique has a great impact to improve the BDF productivity using microalgae. However, due to the lack of genomic information and genetic transformation techniques, enhancing the metabolic pathway for triacylglyceride (TAG) production has been limited in oleaginous microalgae. We have discovered a marine pennate diatom, *Fistulifera* sp. strain JPCC DA0580, as a high TAG producer. This strain accumulates high amounts of TAG up to 60% w/w. In this study, the whole genome was sequenced by using Genome sequencer FLX System. Identified 20,455 ORFs were then annotated based on BLAST searches and assigned to metabolic pathways including 2 *glycerol kinase* (GK) genes. As a next step, to improve the BDF productivity, the enhancement of glycerol assimilation in this strain was attempted because the glycerol is major byproduct during BDF production from TAG. *Fistulifera* sp. was transformed with a vector for the over-expression of endogenous GK gene, which is a key enzyme in glycerol metabolism. The transformant clone (GK2\_16) showed 12% and 41% increases in lipid productivity and in consumption of glycerol compared with wild-type, respectively. The results obtained in this study will significantly contribute to the development of the technology capable of not only improving the BDF productivity through the recycling of glycerol but also avoiding the limitation of unstable light supply for photosynthesis.

## Micronization of fucoxanthin from *Laminaria japonica* with biodegradable polymer-associated particles from gas saturated solution process [Poster]

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Seaweeds have been used as human foods, cosmetics, fertilizers and source of chemicals for medicine and industries. Fucoxanthin is xanthophylls which found in brown seaweed. This yellowish brown pigment has recently attracted much attention as a free radical scavenger and as an anticarcinogenic, anti-inflammatory and antiobesity agent. In this study, fucoxanthin will be purified from *Laminaria japonica*. After purification of fucoxanthin PGSS process will be used to micronize by supercritical carbon dioxide (SC-CO<sub>2</sub>). The particle formation of functional material with biodegradable polymer will be performed by SC-CO<sub>2</sub> in thermo stated stirred vessel. PGSS process will be carried out at different temperature ranging from 30 °C to 60 °C and different pressure ranging from 20 MPa to 40 MPa to measure the optimum condition for the formation of fucoxanthin particle. One nozzle (300 μm) and different pH ranging from 6 to 8 will be used during PGSS process for 1 hr. Different agitation speed also will be used. The produced particles will be characterized by scanning electron microscope (SEM) and particle size analyzer (PSA) to determine their shape and distribution size.

## Identification of candidate genes controlling the resistance to Taura syndrome virus in Pacific white shrimp (*Litopenaeus vannamei*)

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Shrimp cultivation is often affected by outbreaks of deadly infectious diseases caused mainly by viruses. To gain more knowledge on shrimp defense against viral infection, *Litopenaeus vannamei* 44 K oligo array based on 60-mers was employed to analyze levels of transcript abundance in hemocytes between taura syndrome virus (TSV) resistant and susceptible *L. vannamei*. The average survival rate after TSV infection of resistant and susceptible shrimps was 80% and 20%, respectively. For analysis, the ratio of hybridization of fluorescent cRNA probes prepared from hemocyte RNA of resistant shrimp was compared with those of the susceptible shrimp. The comparisons were performed between normal resistant and susceptible shrimps and between day 2 TSV injected resistant and susceptible shrimps. Of the 21,864 unique oligo probes on the array, a total of 1,049 and 2,920 genes were differentially expressed with at least 2-fold change under normal and TSV injected conditions, respectively. Most of the differentially expressed genes were genes with unknown function or hypothetical proteins. Among the genes of known function, the abundant genes with higher transcript abundance in the resistant shrimp were homologous genes classified into signaling and communication, while those in the susceptible shrimp were genes related to chaperone, detoxification, proteasomes and ubiquitin system, energy and electron transport and genes encoding for tubulin. These finding implied a number of genes that were implicated in the shrimp defense against TSV and also that may need to be suppressed for survival of shrimp.

## Novel Lipopeptides, Kiostostatins A-E from a Marine-Derived Bacterium *Bacillus subtilis* [Poster]

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As part of our continuing interest to discover secondary metabolites from marine sponge and sediment derived bacteria, a bacterial strain 109GGC020 showing good antimicrobial activity was isolated from a marine sediment sample collected from Gageocho, Republic of Korea. The bacterium was identified by 16s rRNA sequence analysis as *Bacillus subtilis*. After 7 days fermentation of the strain in optimum growth condition, five linear lipopeptides, kiostostatins A-E (1-5), were isolated using chromatographic procedures. These lipopeptides were characterized by extensive 1D, 2D and high resolution ESIMS data analysis and their stereoconfigurations were assigned by chemical derivatization studies. The minimum inhibitory concentrations (MICs) of kiostostatins 1-5 were evaluated against bacteria (*B. subtilis*, *S. aureus*, *S. typhi* and *P. aeruginosa*) and fungi (*R. solani*, *B. cinerea* and *C. acutatum*) by broth dilution assay. Kiostostatins exhibited antibacterial and antifungal activity with MICs values of 0.003-0.022  $\mu$ M. These results demonstrated 1-5 as potent antibiotics and fungicides. Furthermore, kiostostatins 1-5 displayed cytotoxicity against six human cancer cell lines with GI<sub>50</sub> values of 0.01-0.04  $\mu$ M.

## How does the immune system of shrimps fight against pathogens

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Shrimp, like other invertebrates, lack a true adaptive immune system and rely mainly on innate immune responses. The innate immune system, although considered less sophisticated, is efficiently recognize and destroy foreign materials enable shrimp to live in an environment enriched in microbes. Pattern recognition receptors (PRRs) recognize and bind to microbial cell wall components and subsequently activate a variety of immune responses. Several PRRs have been reported in shrimp including lectins, lipopolysaccharide- and beta glucan binding proteins (LGBPs), toll-like receptors and Down Syndrome Cell Adhesion Molecule (DSCAM) receptors. Shrimp LGBP recognizes and binds to LPS and  $\beta$ -1,3-glucan and subsequently activate the melanization cascade which is an important immune response of shrimp against bacterial infection. Recently, we demonstrated that the shrimp viral pathogen, white spot syndrome virus (WSSV) is capable of suppression of melanization in shrimp by inhibition of the proteinase enzyme in the host proPO cascade. Clotting system prevents intrusion of invading microbes by entrapment into the clots while alpha-2-macroglobulin (A2M) plays a critical role in preventing fibrinolysis of blood clots from bacterial proteases, and so restraining bacterial escape into the circulation. Antimicrobial peptides are important components of the shrimp defense system. They exhibit broad spectrum of antimicrobial activity against various shrimp pathogens and their production is possibly regulated by the Toll and IMD pathways like those found in insects. These major immune pathways as well as the function of effector components which defend shrimp from invading microbes will be described.

## Recombinant viral protein 24 (rVP24) of white spot syndrome virus-a new vaccine candidate in aqua vaccinology against WSSV

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Vaccinology has become a recognized science that combines disciplines of immunology, protein chemistry, and molecular biology which accelerates third generation subunit vaccines using recombinant technology offering many advantages over traditional whole cell vaccines. Invertebrates, like shrimps are believed to be incapable of acquiring immunity and there are no vaccines available for shrimps against white spot syndrome virus, one of the most devastating viral diseases in aqua farms. But strategies are reported to induce an immune boost in shrimps; we investigated the potential of a new subunit vaccine candidate consisting of a WSSV envelope protein. The protection conferred by oral vaccination with recombinant viral protein 24 (rVP24) from Indian isolate of white spot syndrome virus (WSSV) was examined in black tiger shrimp *Penaeus monodon* juveniles infected with WSSV. Bacterially expressed rVP24 was purified by immobilized metal affinity chromatography (IMAC) and coated on a commercial feed to be employed as an oral vaccine. *P. monodon* juveniles were fed this vaccine for 10 days and orally challenged with WSSV. The survival rate of the vaccinated shrimp juveniles was significantly higher until day 10 post challenge. Further the expression levels of three WSSV genes immediate early gene 1 (IE1), DNA polymerase (DNApol) and latency 1 (LAT1) at different duration of the vaccine regime was assessed and their down regulation or absence of expression in vaccinated animals substantiated the protection offered by the vaccine. Our findings indicate the adequacy of rVP24 as a new oral vaccine candidate to protect *P. monodon* juveniles against WSSV infection.

## Strategy to improve the cellular synthesis of lipids in two microalgas [Poster]

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Microalgae mutant are being used to generate high lipid content to channel toward biofuel production. To further increase lipid yield, we are investigating the overexpression of enzymes from different biochemistry pathways. DGAT (Diacylglycerol acyltransferase) represents a limiting step on the lipid pathway, producing TAGs. The *Chlamydomonas reinhardtii* wall-less strain, CC424 and *Dunaliella tertiolecta* Butcher, was used in the experiments of the transformations. Both genes were solely overexpressed in grown photoheterotrophically conditions. The effects of mutations on oil content from clones were monitored under normal and nitrogen starvation conditions through Nile Red phenotypic expression assays. Statistical analysis of initial results indicates that some of the *C. reinhardtii* transformants increased lipid production under normal and nitrogen starvation conditions. However more analysis have to be done and this results will be discussed in more detail.

## Draft genome sequence of the dimorphic prosthecate bacterium *Brevundimonas abyssalis*, isolated from deep-subsea floor sediment [Poster]

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Bacterial adhesion is the first station nodes of formation of microbial colonization or biofilm composed of cells and the extracellular polysaccharide (EPS) secreted by itself. The mechanism for mediating attachment to surfaces depends on physiological features of microbes, however macromolecules such as EPS or filamentous cell appendages function to form a bridge between microbial cell and surface, therefore they are essential factors for bacterial adhesion. In an aquatic bacterium *Caulobacter crescentus*, which is famous for its strong adherence property, three extracellular appendages, flagellum, pili and holdfast are required and whose biosynthesis is regulated in the level of cell cycle. The polysaccharide component of the holdfast is comprised in part of oligomers of *N*-acetylglucosamine. *Brevundimonas abyssalis* TAR-001<sup>T</sup> is a novel *Brevundimonas* species previously isolated from deep-subsea floor sediment in Japan, is a dimorphic prosthecate bacterium and possesses extracellular apparatus called holdfast like that of *Caulobacter crescentus* and shows adhesiveness to one another or to the surface of abiotic substance. Phylogenetically, this strain is affiliated with the family of *Caulobacteraceae* and is located in the boundary of the genus *Brevundimonas* and *Caulobacter*. Furthermore, strain TAR-001<sup>T</sup> combines the chemotaxonomic features of the two genus. As the result of the draft genome sequencing of *B. abyssalis* TAR-001<sup>T</sup> performed, TAR-001<sup>T</sup> consists of 2,979,700 bp in 128 contigs, with a G + C content of 68.2%, 2,946 potential coding sequences (CDS), 3 rRNAs and 41 tRNAs. In this symposium, we show the differences in the adhesion mechanism of the two genus.

## Photosynthetic Carbon Partitioning into Lipids and Polysaccharides in the coccolithophore *E. huxleyi*

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There are diverse organisms in eukaryotic algae including the primary and secondary endosymbiotic organisms. According to wide phylogenetic variation, such algae are promising candidates for producers of industrially applicable metabolites. Despite the extensive studies on the photosynthetic carbon metabolisms in the primary endosymbiotic algae such as green algae, studies on ecologically important marine phytoplanktons which are mostly the secondary endosymbiotic algae are quite limited. Among them, coccolithophorids are unicellular calcifying algae and major primary producers in the ocean. Those cells are covered with calcareous scales (coccoliths) consisting of CaCO<sub>3</sub> and acidic polysaccharide (AP). *Emiliana huxleyi*, the representative coccolithophorid, are characterized by unique products such as AP, β-glucan and long-chain unsaturated ketones called alkenones. In this study, we intended to elucidate functions of β-glucan and alkenones experimentally using <sup>14</sup>C-radiotracer experiments. To reveal the allocation of photosynthetically fixed carbons into various cellular components, photosynthetic <sup>14</sup>C-partitioning into various compounds were investigated by using NaH<sup>14</sup>CO<sub>3</sub> as a substrate. Then we found that 17% of fixed <sup>14</sup>C was allocated into alkenones while <sup>14</sup>C-incorporation into β-glucan was below 1%, irrespective of growth stages of cells. The results implicate that alkenones function as carbon storage in *E. huxleyi*, but not β-glucan. As alkenones are expected to possess a potential as renewable energy source, further studies are essentially required to elucidate biosynthetic pathway and related regulatory mechanisms.

## Isolation of lactic acid bacteria from the intestinal tract of bivalves [Poster]

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Lactic acid bacteria (LAB) have been used for industry to manufacture fermented foods and silages, and have been isolated from various environments to isolate the strains which have novel functions. LAB have been readily isolated from plants and related products, suggesting that herbivores and other plant-associated organisms may be excellent sources for LAB in aquatic environments. In this study, therefore, we attempted to isolate LAB from the intestinal tracts of marine and freshwater bivalves, which feeds on phytoplankton (filter feeder). Intestinal contents from wild specimens of *Meretrix lamarckii*, *Atrina pectinata* (marine bivalves) and *Anodonta japonica* (freshwater bivalve) were pre-cultured in MRS broth, and then a loopful of culture was incubated on MRS agar plates. Presumptive LAB isolates were chosen based on some properties including solubilization of calcium carbonate, oxidase test and Gram staining. Then, DNA sequences of their 16S rRNA gene were analyzed using a ABI 3130xl autosequencer. As the results, several species of LAB were recognized in each bivalve: the LAB from *M. lamarckii* were classified into the genera *Lactococcus*, *Lactobacillus* and *Pediococcus*, whereas those from *A. pectinata* were classified into the genera *Leuconostoc*, *Lactobacillus* and *Enterococcus*. In addition, the LAB from *A. japonica* were classified into the genus *Lactococcus*. These results suggests that the filter feeder-bivalves are a high-potency source of LAB.

## First steps towards an environmentally friendly monosex population culture of spiny lobsters

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Monosex population culture is important for both commercial and environmental reasons. It is abundantly practiced in poultry and cattle, enabling better yields and more efficient broodstock management and is a desirable outcome for aquaculture. In several commercially important fish species, either all-male or all-female populations is gained through hormonal treatments that can be environmentally hazardous. In crustaceans on the other hand, recent studies have demonstrated the commercial viability of using RNA interference to silence the masculinising androgenic gland hormone. Due to the species-specific impact of such intervention and the fact that the silencing agent is a naturally occurring highly degradable compound, this technology mitigates the environmental risk of hazardous chemicals. In an attempt to harness this novel biotechnology to the lobster industry, we have characterised, for the first time in spiny lobsters, the androgenic gland in the eastern rock lobster *Sagmariasus verreauxii* and commenced with characterisation of the androgenic gland hormone, termed *Sagmariasus verreauxii* insulin-like androgenic gland factor (Sv-IAG).

## Diversity and functionality of microbial symbionts associated with a two sponge symbioses in the Caribbean

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Two new *Plakortis* species have been found living in a specialized association with *Xestospongia deweerdtiae* and *Xestospongia* sp. in cryptic habitats of the Caribbean. A free-living form of the two *Plakortis* sp. has yet to be observed, suggesting a possible mutualism between both sponge pairs. Although structural benefits provided by both *Plakortis* species to *Xestospongia deweerdtiae* have been previously studied, the diversity and functional aspects of their associated microbial symbionts remains unresolved. We study the role of the microbial community in shaping this unique symbiosis. *Plakortis* species are known to be a "high microbial abundance" sponge while *Xestospongia* species belong to the order *Haplosclerida* which have many species that are "low microbial abundance" sponges. We are investigating the possibility that in addition to structural support the *Plakortis* serves as a source for key symbionts with important metabolic pathways that *Xestospongia* spp. would not be able to access otherwise. Careful dissections were performed on the *Plakortis/Xestospongia* sponge pairs followed by subsequent 16S rRNA 454 deep sequencing analysis on genomic DNA and cDNA extracted from sponge tissues. In addition a *nifH* clone library of both *Plakortis* spp. and its associated *Xestospongia* sp. were analysed. The *nifH* clone library revealed that both *Xestospongia* and *Plakortis* sponges shared *Alphaproteobacteria*, *Cyanobacteria* and *Chlorobi*. However, *Deltaproteobacteria* and *Archaea* were exclusively found in *Xestospongia deweerdtiae*. Our work is revealing new interactions between host sponges and their microbial associates.

## Production biofuels and bioproducts using marine microalgae isolated from the coastal waters of China

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Major challenges of the modern world, including energy security, oil price, resources depletion and climate change, have prompted significant advances in research and development of biomass-derived fuels and by-products. Microalgal biomass has been considered as one of the most promising feed stocks for bioenergy production and for alleviation of climate change for the years to come. Major advantages of microalgae are potentially high yield and no competition with food crops for arable land and fresh water resource. This talk discusses our efforts to isolate microalgal strains from the coastal waters of China and details their diversity analysis and selection processes of those strains with capabilities to production high-yield of carbohydrates and/or fatty acids. Particularly, we focus on the optimization processes of cultivation conditions for high-yield production of biomass and saccharification of microalgal biomass for fermentation production of bioethanol and high-value marine bioproducts (e.g., DHA). We also discuss the production of biodiesel via in situ transesterification of microalgal biomass derived from those strains with high-yield of lipids.

## The Nervous-Endocrine System Mediates Immune Regulation in Scallops

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Several key genes included in catecholaminergic pathway, acetylcholinergic pathway and nitric oxide (NO) metabolic system were cloned from scallop *Chlamys farreri*, and it was suspected that there existed primary nervous-endocrine immune system in bivalves. A comparative complete catecholamine metabolism pathway was revealed in *C. farreri*, and a number of genes believed to be involved in this pathway were obtained, including *phenylalanine hydroxylase (PAH)*, *dopa decarboxylase (DDC)*, *dopamine beta hydroxylase (DBH)* and *monoamine oxidase (MAO)*. In addition, it was proved that liposaccharide from Gram-negative bacteria treatment activated the catecholamine metabolism pathway, leading to the increased expression level of these genes. The acetylcholinergic pathway was disclosed in *C. farreri*, genes of acetylcholinesterase (CfAChE) and two acetylcholine receptors (CfnAChR-L and CfnAChR-W) were identified, which were reported to play pivotal roles in acetylcholinergic pathway. Furthermore, the tissue expression pattern of CfAChE was determined, and the expression patterns in scallop hemocytes were characterized under the stimulation of TNF- $\alpha$  and PAMPs respectively. In addition, the lysosomal enzyme genes were found up-regulated by inhibition of CfAChE enzyme activity. Nitric oxide (NO) metabolic system in scallops was found significantly different from that of higher vertebrates, with one NO synthase (NOS) in scallops and three isoforms in vertebrates. Catecholaminergic system regulated NO system activity by NE- $\alpha$  /  $\beta$ -AR-cAMP/Ca<sup>2+</sup> pathway under the pathogens stimulation conditions. Moreover, transcript factors, such as NF- $\kappa$ B, STAT, were also involved in the regulation of NOS transcription and expression. Further studies showed that NOS located at the intracellular membrane with the assistance of PSD-95, while NOS translocated from intracellular membrane to cytoplasm, altered the synthesis efficiency of NO, and finally modulated host innate immune response under the stimulation.



## Composition and antimicrobial activity of partial peptidome of the Great Barrier Reef sponge *Amphimedon queenslandica* [Poster]

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Australia has enormous marine biological resource capacity, and is becoming a 'hotspot' for the discovery of novel natural products. Sponges are one family of widely-distributed marine organisms, from which thousands of novel products have been found, and the relevant research on bioactive peptides and proteins is highly demanding. We made use of the first ever sponge genome of *Amphimedon queenslandica* of the Great Barrier Reef, to help identify and screen for bioactive peptides/proteins. Sponge peptide crude extraction with molecular weight less than 3,000 Da was purified using high performance liquid chromatography (HPLC), and different fractions were then subjected to antimicrobial activity screening (both Gram-positive and Gram-negative pathogens were tested, including *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenes* and *V.harveyi*) with one fraction showing the most promising activity. The overall antibacterial activity of these crudes in general was determined using Kirby-Bauer disk-diffusion assays. The inhibitory effects of this fraction on *P. aeruginosa* and *V.harveyi* were 50 times more effective in scale to that reported for antibiotics. To elucidate the peptide profile of this fraction, a nano-HPLC-ESI-QToF study involving CID spectra was carried out to clarify the composition of the peptidome. This approach allowed determination of 110 individual peptides, which corresponds to 87 proteins by analysing the protein database built from the genome. Besides several novel genome-predicted only proteins, the identified proteins include fanconi anemia, histone, tRNA methyltransferase homolog, microtubule-actin cross-linking factor, mbt repeat family protein etc. Most peptides were reported in the peptidome of *A. queenslandica* for the first time.

## De novo transcriptome sequencing of the heat-stressed snail *Echinolittorina malaccana*

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The periwinkle *Echinolittorina malaccana* is commonly distributed along the coast of South China Sea. How these snails can tolerate different thermal stresses is currently unknown. To reveal the underlying coping mechanism, two cDNA libraries were constructed from the normal and heat-stressed snails obtained in Hong Kong. Illumina paired-end sequencing was carried out on these two libraries. By Trinity program, a total of 115,211 unique transcripts (unigenes) could be assembled from ~106 million filtered reads. The mean length, N50 size, and maximum length of the two combined transcriptomes were of 453, 492, and 15,478 bp, respectively. Searching with Blastx against the major databases, 38,821 unigenes corresponding to 23,098 non-redundant genes were annotated. Cutting off at a threshold of  $|\log_2 \text{fold changes}| \geq 1$  between the two transcriptomes, we found 1,267 up-regulated and 6,667 down-regulated genes in the heat-stressed animals. When assigning these genes by Gene Ontology terms, it was found that genes involved in the biological regulation, metabolic and cellular processes, and response to stimulus were significantly enriched. Further KEGG mapping narrowed these transcripts to 257 pathways, including the important ubiquitin mediated proteolysis and MAPK signalling pathways. Our *de novo* transcriptome sequencing data presented here provides a valuable reservoir of candidate genes for further study on snail thermal adaptation.

## The deep-sea natural products, biogenic polyphosphate (Bio-PolyP) and biogenic silica (Bio-Silica), as biomimetic scaffolds for bone tissue engineering: fabrication of a morphogenetically-active polymer

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Bone defects in human, caused by fractures/nonunions or trauma, gain increasing impact and have become a medical challenge in the present-day aging population. Frequently, those fractures require surgical intervention which ideally relies on autografts or suboptimally on allografts. Therefore, it is pressing and likewise challenging to develop bone substitution materials to heal bone defects. During the differentiation of osteoblasts from their mesenchymal progenitor/stem cells and of osteoclasts from their hemopoietic precursor cells, a lineage-specific release of growth factors and a trans-lineage homeostatic cross-talk via signaling molecules take place. Hence, the major hurdle is to fabricate a template that is functioning in a way mimicking the morphogenetic, inductive role(s) of the native extracellular matrix. In the last few years, two naturally occurring polymers that are produced by deep-sea sponges, the biogenic polyphosphate (bio-polyP) and biogenic silica (bio-silica) have also been identified as promoting morphogenetic on both osteoblasts and osteoclasts. These polymers elicit cytokines that affect bone mineralization (hydroxyapatite formation). In this manner, bio-silica and bio-polyP cause an increased release of BMP-2, the key mediator activating the anabolic arm of the hydroxyapatite forming cells, and of RANKL. In addition, bio-polyP inhibits the progression of the pre-osteoclasts to functionally active osteoclasts. Based on these findings, new bioinspired strategies for the fabrication of bone biomimetic templates have been developed applying 3D-printing techniques. Finally, a strategy is outlined by which these two morphogenetically active polymers might be used to develop a novel functionally active polymer.

## Methane production from saline derived microalgae biomass

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Anaerobic digestion of high salinity microalgae is being considered as a key unit process that could be integrated into the production of microalgae biofuels. This integration is due to the potential for anaerobic digestion to improve the economic viability and reduction in the carbon footprint associated with the production of biofuels from saline microalgae feed stocks. However the anaerobic digestion of saline microalgae biomass is potentially problematic due to the varying salinity of the biomass. An increase in salinity has been shown to have a direct effect on Methanogen *Archaea* bacterial species, hence limiting gas production. To overcome the issue associated with increased salinities, high salinity tolerant Methanogen *Archaea* bacteria were utilised to anaerobically digest saline microalgae biomass. This experimental work presents methane bio-gas production data from saline anaerobically digested *Tetraselmis spp.* microalgae biomass. Results presented here indicate that bio-gas production from anaerobic digestion of saline derived biomass is achievable.

## Comparison of immune parameters in cultured oyster (*Saccostrea sp.*) along the eastern coast of Thailand [Poster]

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Marine bivalves are found to possess a nonspecific immune system by cellular and biochemical substances in haemolymph. Due to an industrial development in the eastern coast along the Gulf of Thailand, shellfish may be contaminated by microorganisms. In this study, oyster haemolymph was collected from three different farms during October 2010 to December 2012. Some immunological parameters in haemolymph of oyster were determined and compared among Chonburi, Rayong and Chanthaburi Provinces. The results show that there was no significant difference ( $p > 0.05$ ) in the total haemocyte count (THC), agglutination titer and antibacterial activity against *Vibrio cholerae*, *V. harveyi* and *V. parahaemolyticus*. THC was found in the range of  $2.2 \times 10^5$ – $5.9 \times 10^5$  cells/ml. The cell-free haemolymph showed the average haemagglutinating activity at 256 titer and the highest average antibacterial activity at  $39.89 \pm 5\%$  against *V. parahaemolyticus*. Lytic activity to *Micrococcus luteus* of the protein in oyster haemolymph was measured by inhibition zone using hen egg white lysozyme as standard. The highest lysozyme produced was  $0.10 \pm 0.05$  mg/ml in cultured oysters from Chanthaburi Province which no significantly differed from that in Chonburi and Rayong ( $p > 0.05$ ). This information can help indicating suitable areas for oyster farms to reduce infectious diseases.

## **Analysis of the biomass composition of the demosponge *Amphimedon queenslandica* reveals marked variation within and between individuals**

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Knowing the biochemical composition of an organism is an important first step in understanding, at a system level, the complex metabolic processes responsible for growth and the production of secondary metabolites. Although the secondary metabolites of marine sponges have been extensively studied, the central and secondary pathways leading to their production remain largely unknown. Indeed there is little understanding of the overall biochemical composition of sponges. The demosponge *Amphimedon queenslandica* is currently the only sponge that a genome scale reconstruction of metabolic networks and subsequent flux balance analysis (FBA) can be undertaken. To allow for this analysis, here we quantify the biomass and characterise the biochemical composition of *A. queenslandica*. Using a volume displacement method, we have developed a reliable method to translate dry weight to live biomass composition. We found that there was marked variation in the biochemical composition across an individual and that significant variations occurred between individuals within a given population. The most abundant macromolecule in *A. queenslandica* were lipids and carbohydrates were the most variable. We quantified the composition of protein and lipids, the two most abundant macromolecules, using an amino acid analysis and a fatty acid methyl ester analysis. Together, the biochemical data from this study, and the *A. queenslandica* genome, lay the foundation for the rational investigation of metabolic pathways of a sponge at the level of the whole organism.

## **Molecular cloning, characterization of one key molecule of teleost innate immunity from orange-spotted grouper (*Epinephelus coioides*): serum amyloid A [Poster]**

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The orange-spotted grouper (*Epinephelus coioides*), a favorite marine food fish, is widely cultured in China and Southeast Asian countries. However, little is known about its acute phase response (APR) caused by viral diseases. Serum amyloid A (SAA) is a major acute phase protein (APP). In this study, a new SAA homologous (EcSAA) gene was cloned from grouper, *E. coioides*, by rapid amplification of cDNA ends (RACE) PCR. The full-length cDNA sequence of SAA was 508 bp and contained a 363 bp open reading frame (ORF) coding for a protein of 121 aa. Similar to other fish known SAA genes, the EcSAA gene contained four exons and three introns. Quantitative real-time PCR analysis revealed that EcSAA mRNA is predominately expressed in liver and skin of grouper. Furthermore, the expression of EcSAA was differentially up-regulated in liver after infection with *Staphylococcus aureus*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Saccharomyces cerevisiae* and Singapore grouper iridovirus (SGIV). Recombinant EcSAA (rEcSAA) was expressed in *Escherichia* BL21 (DE3) and purified for mouse anti-EcSAA serum preparation. The rEcSAA fusion protein was demonstrated to bind to all tested bacteria and yeast. Overexpression of EcSAA in grouper spleen (GS) cells could inhibit the replication of SGIV. These results suggest that EcSAA may be an important molecule in the innate immunity of grouper.

## Transdifferentiation of duct-like cells from hepatocyte through progenitor cells in zebrafish model of *Intrahepatic cholangiocarcinoma* [Poster]

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Intrahepatic cholangiocarcinoma (ICC), a member of primary liver cancers, has the highest incidence in South-Eastern Asia. The ICC worldwide incidence increased over the past three decades and its 5-year survival rate is below 5%. The cellular basis mechanisms in ICC formation are related unclear. Previously, we have developed transgenic ICC models in zebrafish. We hypothesize that the dedifferentiation process of hepatocyte is responsible for hepatocyte acquired ductal phenotype in ICC formation. Primary culture of hepatocyte was used to observation the process of hepatocyte to cholangiocyte transdifferentiation. Here we demonstrate that zebrafish hepatocytes in culture have the capacity to convert into duct-like cells, expressing duct markers. In order to demonstrate that hepatocyte is the origin of duct-like cell. The liver specific promoter of fatty acid binding protein 10 was used to generate LFABP-GFP transgenic fish. Primary culture of GFP expressing hepatocyte confirmed that the cellular origin of duct-like cells is hepatocyte. Furthermore, the expression of some hepatic progenitor cells markers is upregulated in the process of hepatocyte to cholangiocyte transdifferentiation. This indicated that dedifferentiation of hepatocyte is required for hepatocyte to cholangiocyte transdifferentiation.

## The Marine Biotechnology Enable Development of the Blue Bioeconomy in China

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Marine biotechnology in China has been developed since the last decades of the 20th century. The blue economy is emerging in the critical junctures of the world economic transformation during the global recession. This presentation focuses the concept and possible connotation of the blue bioeconomy. The priority and key areas of research and development of the marine biotechnology in China are summarizes. The market potential of the global blue bioeconomy is enormous. Obviously, R & D of marine biotechnology is vital enabling sector of the blue bioeconomy. The author emphasizes the sustainable development of the Chinese blue bioeconomy and the importance of international cooperation.

## RNA-Seq reveals the dynamic features of transcriptome during early development in pacific white shrimp *Litopenaeus vannamei*

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The penaeid shrimp, with high commercial value, has a distinctive early development process. Morphology and physiology of this developmental process has been investigated for decades, yet a comprehensive understanding of its transcriptome is still lacking. In this study, we used RNA sequencing (RNA-seq) to explore the transcriptome of *Litopenaeus vannamei* at 20 distinct developmental stages. We obtained an average of 9 million clean reads for each stage and 92% were successfully mapped to 66815 unigenes assembled by us before. By calculating RPKM (reads per kilo bases per million reads) value, we acquired expression patterns of all unigenes. In addition, hierarchical clustering analysis and principal component analysis all clustered the 20 stages into 3 groups. Furthermore, the differentially expressed genes (DEGs) between each adjacent stages were identified and gene expression patterns were clustered. In total, 13073 DEGs were determined and clustered into 8 groups. The maximum DEGs were identified between gastrula and limb bud embryo early stage, then followed by that between nauplius VI and protozoa I stage, indicating that most dramatic changes occurred in these periods. We also made the GO and KEGG pathway enrichment analysis. In conclusion, these results would provide much support to better understand the physiological changes during embryonic and larval development of *L. vannamei*.

## Design of Phthalimide Derivatives Based on Paecilocin A as PPAR- $\gamma$ Activators [Poster]

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Paecilocin A is a natural product that was isolated from a jellyfish-derived fungus *Paecilomyces variotii*. And it shows PPAR-  $\gamma$  agonistic activity. Peroxisome proliferator-activated receptors (PPARs) are a ligand-activated transcription factors including the nuclear receptor superfamily. PPARs comprise three isoforms : PPAR- $\alpha$ , PPAR-  $\beta/\delta$ , and PPAR- $\gamma$ . PPAR- $\gamma$  is mostly expressed in adipose tissue, macrophages, monocytes, intestinal cells, skeletal muscle, and endothelium, and plays an important role in the regulation of insulin sensitivity, lipid metabolism, adipogenesis, and glucose homeostasis. PPAR-  $\gamma$  agonist are therefore used to treat type 2 diabetes. In the present study, a series of *N*-substituted phthalimide derivatives were synthesized based on pharmacophore study of Paecilocin A. and they were evaluated for PPAR-  $\gamma$  agonistic activity on rat liver Ac2F cells by luciferase assay. A 3-hydroxy-*N*-phenethyl phthalimide derivative (**9**) showed significant activity. Further optimization of 3-hydroxy-*N*-phenethyl phthalimide derivative (**9**) generated the potency-enhanced derivative **15**. A free hydroxyl group on the phthalimide head and a phenyl hydrophobic tail were favorable for binding to PPAR- $\gamma$ . Further biological evaluation on (**15**) is in progress.

## Physiological response of marine red algae *Gracilaria lemaneiformis* to different salinities stress [Poster]

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The growth, cell ultrastructure, membrane permeability, antioxidative enzymes, lipid peroxidation, photosynthetic pigments, optimal quantum yield, osmo-regulation substances and phytohormones were studied to reveal the physiological responses of marine algae *Gracilaria lemaneiformis* to low and high salinity stress of 10‰ and 35‰. Results showed that adverse salinity stress could inhibit the growth rate of *G. lemaneiformis*, the Pit connection, intracellular floridean starches and chromatoplast were changed under salinity stress. The Na<sup>+</sup> content increased and K<sup>+</sup>, Ca<sup>2+</sup> content decreased under high salinity stress. Under two kinds of stresses, the SOD and POD activity increased significantly in the later phase to clear the ROS of stresses. The content of phycoerythrin, phycocyanin and chlorophyll *a* decreased in the initial phase and then rebounded in the later phase under low salinity stress. Two kinds of stresses contributed to the decline in optimal quantum yield (Fv/Fm). Three kinds of photosynthetic pigments declined to average 71.80% under high salinity stress. Under high salinity stress, the proline and mannitol content increased by 75.66% and 29.40% respectively. The IAA content decreased slightly, ABA and JA showed significant increase under high salinity stress; while IAA significantly decreased, ABA, JA, SA, RA showed significant increase under low salinity stress. In conclusion, the adverse salinity stress inhibited *G. lemaneiformis*, and low salinity showed vigour damage.

## Morphological regulation of cubo-octahedral magnetite crystal by the coordinated action of Mms proteins in magnetotactic bacteria

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Magnetotactic bacteria are microorganisms that produce magnetite crystals surrounded by a lipid bilayer membrane containing proteins. The uniform size and morphology of these crystals suggest that they are morphologically regulated within the bacteria. Since some strains of these bacteria produce crystals that cannot be chemically synthesized, the mechanism of crystal formation is an area of interest. We have previously isolated the Mms5, Mms6, Mms7, and Mms13 proteins that are specifically localized on the cubo-octahedral magnetite crystals in *Magnetospirillum magneticum* strain AMB-1. By analyzing an *mms6*-deletion mutant strain ( $\Delta mms6$ ), Mms6 was shown to regulate magnetite crystal morphology during crystal growth. In this study, in order to analyze the function of the other Mms proteins, we constructed the relevant gene deletion mutants by homologous recombination and characterized the phenotype of the magnetite crystals. Transmission electron micrograph analyses revealed that the  $\Delta mms7$  strain produced elongated crystals that have similar morphologies to those in the  $\Delta mms6$  strain. In contrast, the magnetite crystals synthesized in the  $\Delta mms5$  and the  $\Delta mms13$  strains were small cubo-octahedral crystals, as compared with the wild type strain. These results indicated that the protein functions can be categorized into two groups: the first involves Mms6 and Mms7, which are responsible for anisotropic crystal growth, and the second involves Mms5 and Mms13, which are responsible for isotropic crystal growth. Thus, the specified and co-operative functions of these proteins in magnetite biomineralization enable the formation of cubo-octahedral shaped crystals in strain AMB-1.

## Se-containing antioxidant "selenoneine" in tuna blood and its roles in selenium redox metabolism and methylmercury detoxification

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The novel Se-containing strong antioxidant selenoneine, 2-selenyl- $N_{\alpha}$ ,  $N_{\alpha}$ ,  $N_{\alpha}$ -trimethyl-L-histidine, has recently been discovered to be the predominant form of organic Se in tuna blood (Yamashita & Yamashita, JBC, 285, 18134, 2010). A substantial proportion of the total amount of selenium is present as selenoneine in the muscles of ocean fish. This compound is thought to play a key role in the Se redox antioxidant mechanism in animal cells. Cell growth of human cultured cells were enhanced in the presence of selenoneine at 5-100 nM, and GPx1 gene expression was induced in dose-dependent manner. The uptake of selenoneine was mediated by organic cations/carnitine transporter-1 (OCTN1). Selenoneine in culture medium was incorporated into human cultured cells and zebrafish embryo by OCTN1. Selenoneine accelerates the excretion and demethylation of methylmercury (MeHg), mediated by OCTN1. When such OCTN1-expressing cells and embryos were exposed to MeHg-cysteine (MeHgCys), MeHg accumulation was decreased and the excretion and demethylation of MeHg were enhanced by selenoneine. In addition, exosomal secretion vesicles were detected in the culture water of embryos that had been microinjected with MeHgCys. In contrast, OCTN1-deficient embryos accumulated MeHg, and MeHg excretion and demethylation were decreased. Furthermore, Hg accumulation was decreased in OCTN1-overexpressing HEK293 cells, but not in mock vector-transfected cells, indicating that selenoneine and OCTN1 can regulate MeHg detoxification in human cells. Thus, the selenoneine-mediated OCTN1 system regulates secretory lysosomal vesicle formation and MeHg demethylation. Thus, the dietary intake of selenoneine, by consuming fish, may decrease the formation of ROS, oxidized damages and MeHg accumulation.

## Optimization of medium using response surface methodology for the lipid production by *Scenedesmus* sp.

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The effects of medium composition on the lipid production by *Scenedesmus* sp. were investigated using response surface methodology. The result revealed that three factors,  $\text{NaHCO}_3$ , which reacted mutually with the other two,  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  and  $\text{NaNO}_3$ , influenced significantly the lipid production. The Box-Behnken design was employed to optimize further the levels of three variables. The optimal medium was found to be  $3.07 \text{ mg} \cdot \text{L}^{-1} \text{NaHCO}_3$ ,  $15.49 \text{ mg} \cdot \text{L}^{-1} \text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  and  $803.21 \text{ mg} \cdot \text{L}^{-1} \text{NaNO}_3$ . Apopting the optimal condition, the lipid production ( $304.02 \text{ mg} \cdot \text{L}^{-1}$ ) increased 54.64% than that using the initial medium, which agreed well with predicted value  $309.50 \text{ mg} \cdot \text{L}^{-1}$ . Additionally, lipid analysis found the palmitic acid (C16:0) and oleic acid (C18:1) dominantly constituted the algal fatty acids (about 60%) and a much higher content of neutral lipid accounted for 82.32 % of total lipids, which can strongly prove that *Scenedesmus* sp. is a very promising feedstock for biodiesel production.

## Simple lipid extraction method without heating from wet microalga *Picochlorum* sp.

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A simple and low-cost technology was proposed for extracting directly lipids from wet microalga biomass of *Picochlorum* sp., which was performed by using ethanol as extractant, requiring neither drying nor heating. In this study, the Central Composite Designs (CCD) was employed to optimize the conditions of lipids extraction. The results revealed that the solvent-biomass ratio had significant effect on the lipid yield, followed by extraction time and temperature. The lipid yield with 33.04 % was provided under the following optimum extraction conditions: 4.85 ml solvents per gram of wet biomass at room temperature for 40 min with gentle stirring, which was similar to that obtained by the conventional Bligh-Dyer method. Furthermore, no significant differences in the distribution of lipid classes and fatty acid composition were observed according different extraction methods. In conclusion, these results indicated that ethanol can extract efficiently lipids from wet biomass and has promising potential in lipid extraction at large scale.

## Effect of oxidized fish oil on growth performance and oxidative stress of *Litopenaeus vannamei* [Poster]

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A four-week feeding trial was conducted to determine the effects of oxidized fish oil (OFO, POV: 234.84 meq·kg<sup>-1</sup>) on growth performance and oxidative stress of *Litopenaeus vannamei*. Five diets containing various OFO levels (0, 25, 50, 75, and 100 g·kg<sup>-1</sup>) with the same dietary lipid levels were fed to *L. vannamei*. There is a significant decrease ( $p < 0.05$ ) in body weight gain and specific growth rate but a significant increase ( $p < 0.05$ ) in hepatosomatic index in shrimp fed with 50, 75, and 100 g·kg<sup>-1</sup> of OFO diets. The malondialdehyde concentrations in the serum and muscle of the shrimp fed with 50, 75, and 100 g·kg<sup>-1</sup> of OFO diets were significantly higher than that of the shrimp fed with fresh fish oil ( $p < 0.05$ ). The total antioxidant competence decreased significantly compared with the control group. In conclusion, dietary OFO affects the growth performance and increases the oxidative stress of shrimp.

## Seasonal variations on organic and inorganic components of *Ulva pertusa* with environmental factors in Jeju, Korea

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*Ulva pertusa* is known to cause eutrophication in worldwide. This study, we confirmed Seasonal variations on organic and inorganic components of *U. pertusa* with environmental factors in two years. The environmental parameters have shown a clear seasonal variation and the greatest fluctuating seasonal period have been observed during from Jun to October. The nitrogen, carbon, amino acid and protein, lipid contents showed higher values in spring and lower values in summer. These values confirmed by the positive correlation found between these contents and negative correlation with temperature and positive correlation with salinity. Indeed, even though protein contents decreased, essential amino acids increased in summer periods ( $p < 0.05$ ). Carbohydrate chlorophyll and carotenoid contents did not showed correlation of temperature, salinity and nitrogen ( $p > 0.05$ ), But these indicated positive correlation with carbon content ( $p < 0.05$ ). On the contrary, the maximum ash contents occurred during summer and decreased during autumn-winter. It also related to environmental effect by negative correlation with temperature ( $p < 0.01$ ) and positive correlation with salinity ( $p < 0.001$ ). Especially, Ca and Fe contents of major element and Cu and Zn contents of trace element observed high amount compare with other elements in summer season. These patterns were increased sharply towards summer and the decreased during in autumn-winter months. This study determined biochemical compositions values of *U. pertusa* by identifying their organic and inorganic components on the seasonal variations. We suggest that the aim of the present study was to determine ecological, physiological significance and knowledge of *U. pertusa*.

## Anti-proliferative effect of *Pylaiella littoralis* extract on HT29 cells [Poster]

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Most antitumor agents induce apoptosis. Apoptosis plays an important role in physiological process as to regulate the homeostasis through cell proliferation, differentiation, survival and death in healthy tissues. This study confirmed that five tumorigenic cells, AGS, DU145, SK-HEP, NCI-H1299 and HT-29 cells were treated with *Pylaiella littoralis* extract (PLE) to determine anti-proliferative activity. PLE showed anti-proliferative activities in the tested tumorigenic cells ranged from 20.2% to 67.9%. The highest inhibitory activity showed in HT-29 cells and exhibited no cytotoxic effect with increasing concentration for normal cells and inhibited cell growth of HT-29 cells depending on concentration and time. Also, we identified that growth inhibition rate on HT-29 cells of PLE was associated by as nuclear condensation, apoptotic body formation, DNA fragmentation and sub-G1 DNA accumulation in HT-29 cells. Thus, we next focused on identifying the cellular mechanisms whereby PLE induced apoptosis in HT-29 cells. PLE induced increase of mitochondrial membrane permeabilization compare with untreated PLE and exhibited decreasing Bcl-2 protein, increasing Bax protein, activating Caspase-3 and PARP expressions via caspases pathway. Indeed, PLE increased expression of phosphorylation of JNK, P38 and ERK and attenuated specific inhibitors of JNK, P38 and ERK via MAPKs pathway. In conclusion, this study demonstrated that PLE could inhibit the proliferation of HT-29 cells by caspases and MAPKs pathways involving the induction of apoptosis. Our data suggest that the *P. littoralis* might be a potential antitumor agent through the inhibition of human colorectal cancer cell line.

## Potential antioxidant capacities of ethanol and enzymatic extracts of *Pylaiella littoralis* collected from Federated States of Micronesia [Poster]

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*Pylaiella littoralis* was collected in the Chuuk lagoon of the Federated States of Micronesia (FSM). FSM has a variety of coral reef ecosystems and provides abundant marine algae because of its high biodiversity value and productivity capacity. In this study, we evaluated antioxidant activities of ethanol and enzymatic extracts of *P. littoralis* collected from FSM by measuring the scavenging activities on DPPH free radical, Alkyl radical, hydroxyl radical and cell viability. The enzymatic extracts were hydrolyzed to prepare water soluble extracts by using five carbohydrate degrading enzymes (AMG, Celluclast, Termamyl, Ultraflo, Viscozyme) and five proteases (Alcalase, Flavourzyme, Kojizyme, Neutrase, Protamex). As a result, the enzymatic extracts of Flavourzyme, Ultraflo, Kojizyme exhibited higher antioxidant activity in DPPH free radical and alkyl radical scavenging activity and had greater antioxidant effect than commercial antioxidants. Ethanolic extract exhibited higher antioxidant activity in Hydroxyl radical. *P. littoralis* also showed high phenolic content around 14mg g<sup>-1</sup> and higher cell viability around 90%. Therefore, this study suggests the *P. littoralis* is a good source for natural antioxidants.



## Characterizing the role of diazotrophs in the symbiotic microbial community associated with two marine sponges

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The transformation of N<sub>2</sub> to biologically available nitrogen by biological nitrogen fixation is mediated only by prokaryotes. Sponges that harbor microalgal or cyanobacterial symbionts may benefit from photosynthetically derived carbohydrates, which are rich in carbon but devoid of nitrogen, and may therefore encounter nitrogen limitation. Two Caribbean sponges, *Ircinia strobilina* and *Mycale laxissima* were studied in a time series during which three individuals of each sponge were collected in four time points (noon, 5:00pm, 10:00pm and 5:00am) and samples were immediately immersed in RNAlater for subsequent DNA/RNA extraction and analysis. *nifH* genes were successfully amplified from the corresponding genomic DNA and cDNA pools and sequenced by high throughput 454 amplicon sequencing, providing a deep insight into the diversity of nitrogen fixing bacteria in the sponge hosts. In both sponges, ca. half the OTUs were from cyanobacteria and the remainder from heterotrophic bacteria. Various groups of bacteria actively express the *nifH* gene during the entire day, an indication that the nitrogen fixation potential was fully exploited by different nitrogen fixing bacteria groups in the bacterial communities associated with their hosts. Anaerobic culture and cell separation techniques were used in this study to culture the diverse diazotrophs from the sponge-associated communities. One filamentous cyanobacterial culture belonging to the genus *Leptolyngbya* was shown to carry a *nifH* gene sharing 92% identity in DNA sequence with the previously characterized *nifH* from a sponge-derived clone library. Further immuno-fluorescent assay with nitrogenase antibody indicated the presence of nitrogenase protein in both *Vibrio* and cyanobacterial cultures.

## An efficient *E. coli* secretory expression system to produce recombinant chitin-degrading related enzymes

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Chitooligosaccharides and N-acetylchitooligosaccharides have various applications in physiological, pharmaceutical, agricultural and food fields. Generally, chitooligosaccharides come from the degradation of chitosan and N-acetylchitooligosaccharides come from the degradation of chitin. At present, most of chitosan is produced by the thermo-alkaline deacetylation of chitin. The process shares a multi-step chemical procedure which mainly is being environmentally unsafe, tedious to control and resulting into heterogeneous range of products. Alternatively, chitosan can also be produced through chitin deacetylase (CDA, EC 3.5.1.41) under mild conditions which overcome most of disadvantages in the alkali treatment method. Chitosanase and chitinase play important role in bioconversion of chitosan into chitooligosaccharides and chitin into N-acetylchitooligosaccharides. In our laboratory, we obtained many genes encoding chitin-degrading related enzymes including chitin deacetylase, chitosanase, and chitinase from shrimp and bacteria. In order to exploit the potential use of these genes encoding chitin-degrading related enzymes, we constructed an expression plasmid pCT7-CHISP6H which contained several elements including T7 promoter, signal peptide sequence of mschito, 6×His-tag sequence, and *PmaC*I restriction enzyme clone site, etc. Based on the information of plasmid pCT7-CHISP6H and target genes, we constructed several expression plasmids and succeeded in obtaining recombinant chitin deacetylase, chitosanase, and chitinase. The recombinant enzyme could be secreted into culture broth, which facilitates downstream processing and enables production of soluble and biologically active proteins at a reduced process cost. The enzymatic characterization of the purified recombinant enzymes was also studied. These results make it possible to explore the chitin source in nature.

## Female specific markers and attempts of all-female production in half-smooth tongue sole (*Cynoglossus semilaevis*)

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Half-smooth tongue sole (*Cynoglossus semilaevis*) is an important aquaculture flatfish showing significant fast growth in females than in males. Its sex determination system is female heterogametic (ZZ/ZW). Production of all-female progeny has attracted great attention. Several methods were tried in obtaining WW super females for all-female production.

- Meiotic and mitotic gynogenesis was induced by pressure shocks. Hundreds of gynogens from both treatments were obtained. But all the survivors were ZZ males.

- W chromosome-specific library was constructed after chromosome micro-dissection, and W-specific probes were developed. Large number of pseudo-males was identified from commercially fish using these probes. Crosses between pseudo-males and normal female (ZW × ZW) yielded thousands of individuals. Molecular assessment showed that 63.6% individuals were genetic females, but all these individuals showed ZW chromosome constitution. The WW super female was not identified. Growth performance of this high-female population was not as good as control population.
- Triploids were induced by suppression of the second polar body. The triploidy rate in juvenile progeny was 73%. PCR assessment with W-specific markers showed that 46% triploids were females. Chromosomes were observed for 36 females and all of them showed ZWW chromosome constitution. ZZW chromosome constitution was not observed. Again, growth performance of this triploid population was not as good as control diploid population.

Our results showed that production of all-female population in half-smooth tongue sole through gynogenesis and sex reversed ZW pseudo-male is not possible at present stage, because the WW super female is inviable.

## **Marine Biotechnology Industry Development in Australia: An Ocean of Opportunities for Australian and International Partners**

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Australia enjoys a reputation as the world's fifth most 'megadiverse' country, with a huge proportion of endemic biota, especially in marine waters. Australia has over 16.1 million km<sup>2</sup> of oceanic jurisdiction, 70 thousand kilometres of continental coastline and 8.6 million km<sup>2</sup> of continental marine territory. This unique marine biological resource presents an ocean of opportunities for Australian and international partners for the development of novel marine bioproducts and new marine biotechnology industry. This presentation will focus on a high level overview of the marine biotechnology industry development in Australia; identification of issues and opportunities for future industry developments; and discussion on the opportunities for national and international collaborations to benefit not only Australian, but the global economy and society. Case studies will be used to demonstrate the model of partnership and collaborations among government, academia, industry and investors.

## **Novel approach to decipher interactions between marine sponges and their microbial symbionts/pathogens**

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Sponges (Porifera) filter organic matter and bacteria from the water column, and are host to microorganisms up to 40% of the total living tissue and 60% of the mesohyl volume (Vacelet, 1975). These microbial communities are known as sponge-associated bacteria (SAB), or symbionts. It is an important, but unresolved debate as to whether the rich diversity of sponge metabolites originates from SAB or sponge cells. Our hypothesis is that 'high diversity of secondary metabolites and biological activities in sponges is regulated by the symbiotic and challenging interactions between sponges and their associated bacteria and/or foreign bacteria of non-pathogenic and pathogenic origin'. This presentation will report on the development of novel approaches to understand the interactions between bacteria and their host sponge and the roles of such interactions in biosynthesis of bioactive metabolites, toward design and development of sustainable production technology of sponge-derived bioactives. The novel approach is based on the establishment of controlled sponge explant and sponge cell culture systems, therefore defined challenge and interactive experiments can be designed and carried out. The dynamic changes of sponge-associated bacteria community and metabolome, as well as bioactivities of metabolites can be investigated using a range of molecular and modern analytical tools with the aid of bio- and chemo-informatics tools.

## Mapping and matching hotspots of biodiversity, biochemical and bioactivity diversity for advanced Marine Park policy in South Australia

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A project was conducted to develop and demonstrate methodology to map and devise conservation policy and protocols for establishing biodiscovery zones in South Australian Marine Parks. The aims were to (1) establish a knowledge base of biochemical and bioactivity diversity of key representative species such as marine sponges from South Australian waters; and (2) determine the correlation (pilot scale), if any, between species diversity, biochemical diversity and bioactivity to facilitate the identification of biodiscovery hotspots. Key project outcomes included methodology for mapping and matching biodiversity, biochemical and bioactivity diversity; biochemical fingerprinting and bioactivity assays on marine sponges collected from five sites in South Australian waters; demonstration of the biochemical and bioactivity diversity of sponges from South Australian waters; indication that marine sponges in South Australian waters possess many unique compounds, within which there is a high degree of pharmaceutical activity; and indication that high biochemical and bioactivity diversity from a small number of species demonstrates the need to highlight this discovery potential and how this may affect marine park management policy.

## Artificial breeding technology of *Bohadschia argus* made great progress in China [Poster]

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*Bohadschia argus*, the leopard sea cucumber, is a species of marine invertebrate belonging to Echinodermata, Holothuroidea, Aspidochirotid, Holothuriidae, *Bohadschia* Jaeger, 1833. It lives on coral reefs and exposed, sandy areas of the seabed at depths of between 10 feet (3.0 m) and 120 feet (37 m). Its geographic distribution is from the Seychelles Islands and Sri Lanka to the Ryukyu Islands, east to Tahiti, south to northern Australia, including south of Taiwan, Hainan Island and Hsisha Archipelago. In Singapore, Malaysia and the south of China it has traditionally been regarded as seafood treasures with high economic value, which can only be obtained by diving harvest. At present, there is no report on artificial breeding of *B. argus*. After more than three years of harvesting and culturing, my group obtained 270 kg parental individuals of *B. argus* Jaeger. Through continuous establishing and optimizing the conditions of feeding, ripening and reproduction, larvae and juveniles were finally successfully bred in 2012. The artificial breeding technology of *B. argus* was nearly mature, which will lay a ground for it to be a new high-value economic species and also promote the development of the matchable mode of sowing it in tropical shallow seabed.

## Exceptional lipids in nudibranch mollusks: evolution, diets, symbionts and biosynthesis

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The molecular diversity of chemical compounds found in marine animals descends from evolution of organisms with unique physiological and biochemical adaptations and offers good prospect for finding novel bioactive compounds with a variety of unique structural features and diverse biological activities. Nudibranch mollusks, not protected by a shell and producing chemicals for various ecological uses attract great interest to their lipid composition. Lipid analysis of 17 Nudibranchia species revealed 1-O-alkyldiacylglycerol ethers along with phospholipids and sterols. Among polar lipids ceramide-aminoethylphosphonates, were found. Plasmalogens predominated in the mollusks. A noticeable feature was abundance of novel *iso*-17:0 and *anteiso*-17:0 fatty aldehydes with predominant 16:0. The fatty acid compositions of the nudibranchs exhibited a wide diversity and differed greatly from that of other marine gastropods. They displayed large amounts of very long chain fatty acids known as demospongiac acids, thus suggesting predation on sponges. A series of non-methylene-interrupted fatty acids including novel 21:2Δ7,13 was identified. Another unique feature was an abundance of various odd and branched fatty acids typical of bacteria. Ultrastructural observations revealed heterotrophic symbiotic bacteria in the *Dendrodoris nigra* epithelium. Their density, appearance and localization indicated their functional relationships and active reproduction. We suggested that symbiotic bacteria participate in production of some compounds, which serve for chemical defense of the nudibranch mollusk. Lipid and fatty acid composition of the nudibranchs is determined by taxonomic relationships, food supply, internal biosynthetic activities and intracellular symbiotic microorganisms. [This work was supported by RFBR grant 11-04-98507-p\_vostok\_a, the Government of the Russian Federation grant 11.G34.31.0010.]

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# 10th International Marine Biotechnology Conference

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## 10th International Marine Biotechnology Conference

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## 10th International Marine Biotechnology Conference

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# 10th International Marine Biotechnology Conference

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Brisbane 11-15 November 2013



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Brisbane 11-15 November 2013



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**WEEK AT A GLANCE - Refer to Daily Timetables for Details**

**Monday 11 November**

3:00 - 5:30 pm Registration and Poster hanging

Welcome Reception 18:00 - 20:00

**November 2013**

<b>Rooms</b>	<b>Tuesday 12</b>	<b>Wednesday 13</b>	<b>Thursday 14</b>	<b>Friday 15</b>
<b>Boulevard Auditorium - Plenary</b>	Welcome to Country <b>Songwoman Maroochy</b>	<b>Invited Plenary: Ute Hentschel Humeid</b>	<b>Invited Plenary: Ben Hankamer</b>	<b>Invited Plenary: Ron Quinn</b>
	Official Opening: <b>Robyn Williams AM FAA</b> ABC International Science Broadcaster and Communicator			
	<b>Invited Plenary: William Gerwick</b>	<b>Invited Plenary: Amir Sagi</b>	<b>Invited Plenary: Asao Fujiyama</b>	<b>Invited Plenary: Anchalee Tassanakajon</b>
<b>Morning Tea</b>	10:30 - 10:50	10:30 - 10:50	10:30 - 10:50	10:30 - 10:50
<b>Boulevard Auditorium</b>	Marine Algal Oleomics: New Development of Biofuel Production Research and Technology	Algal Biofuels and Energy	Marine Natural products	Genomics
<b>Boulevard 1</b>	Marine Bioresources	Microbial symbionts	Genomics	Bioactive Marine Resources
<b>Boulevard 2</b>	Aquaculture Disease and Immunology	Reproductive Tech-nologies in Aquaculture	Algal Production and Biotechnology	Nutraceuticals and functional food
<b>Boulevard 3</b>		Dianoflagellate & algal genomics		
<b>Lunch</b>	12:40 - 13:30	12:40 - 13:30	12:40 - 13:30	1:00:00 PM Conference Close
<b>Boulevard Auditorium</b>	Marine Algal Oleomics: New Development of Biofuel Production Research and Technology	Algal Biofuels and Energy	Marine Bioresources	
<b>Boulevard 1</b>	Microbial Bioresources	Microbial Bioresources	Genomics	
<b>Boulevard 2</b>	Aquaculture Disease and Immunology	Reproductive Tech-nologies in Crustacean Aquaculture	Algal Production and Biotechnology	
<b>Boulevard 3</b>		ANZ-China Collaboration Forum		
<b>Afternoon Tea</b>	15:20 - 15:40	15:20 - 15:40	15:20 - 15:40	
<b>Boulevard Auditorium</b>	Marine Algal Oleomics: New Development of Biofuel Production Research and Technology	Algal Biotechnology	Marine Bioresources	
<b>Boulevard 1</b>		Microbiotechniques		
<b>Boulevard 2</b>	Aquaculture Biotechnology	Aquaculture Biotechnology	Algal Production and Biotechnology	
<b>Boulevard 3</b>		ANZ-China Collaboration Forum		
	Poster Cocktail Function, Boulevard Foyer		Conference Dinner, Boulevard Room	

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