



Genomic approaches to unlock the biotechnological potential of marine organisms

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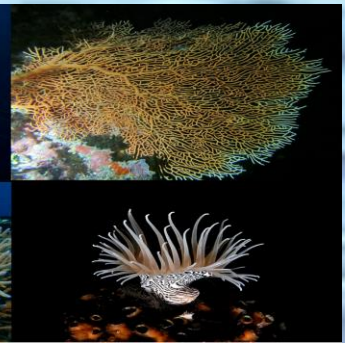
“From ocean to lab: Marine genetic resources and application to new bioactive molecules production”. VIGO Spain, November 9th, 2018.

Structure of the Talk

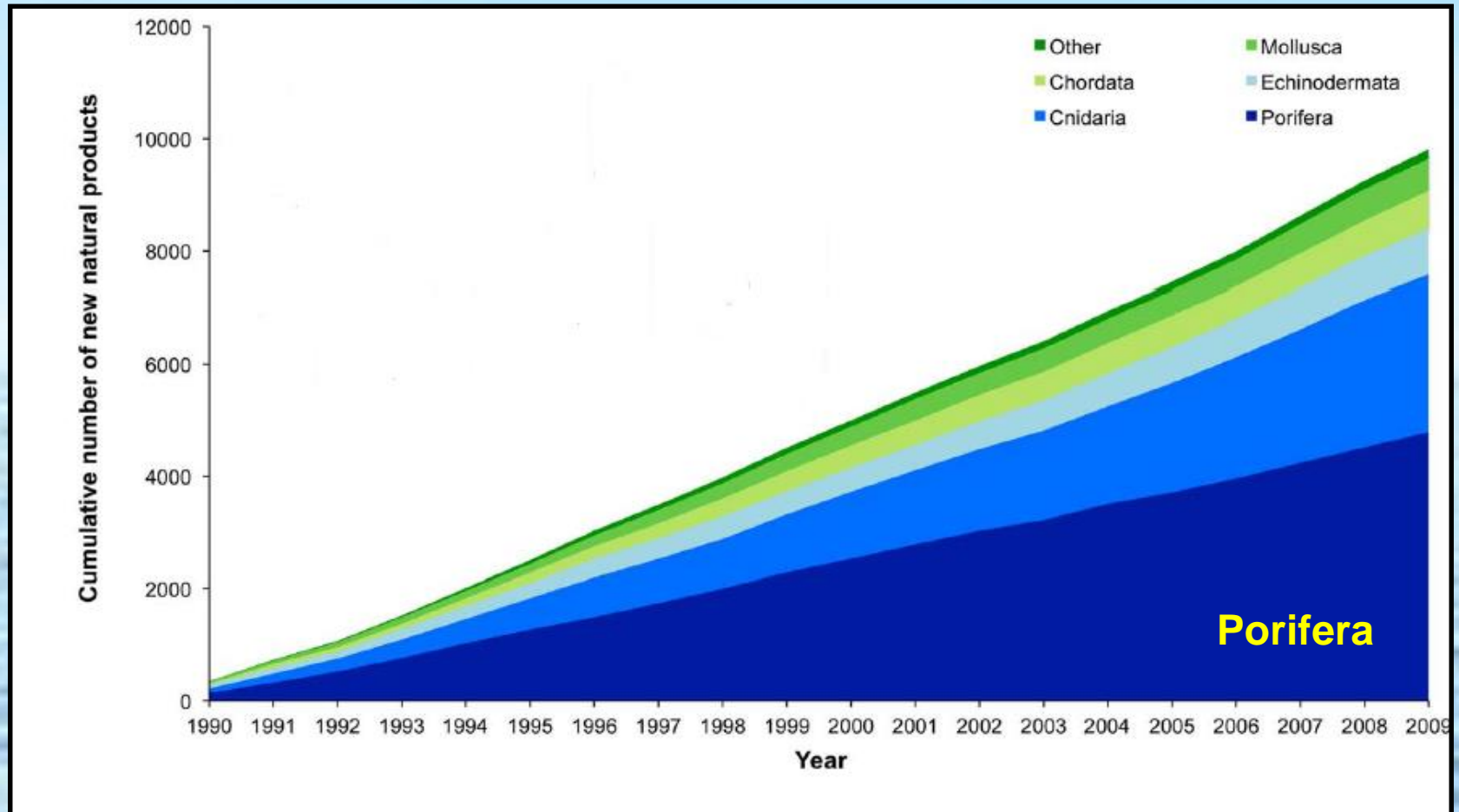
- * Marine ecosystems as a source of novel bioactive compounds.
- * Genome mining of marine sponge derived bacteria for genes encoding novel bioactivity bioactive molecules in marine *Streptomyces* and *Pseudovibrio* strains.
- * Metagenomic approaches to identify novel enzymes with industrial applications.

Marine Natural Products

- Over the past 25 years, two thirds of drugs which have been developed for the treatment of human diseases have been derived from natural products (NPs) or their derivatives.
- Bioprospecting for new marine natural products (MNPs) has increased significantly over the past decades, leading to an unprecedented discovery of new molecules.
- Most compounds isolated from marine invertebrates
 - **sponges** are biggest single source
 - followed by **bacteria** and **cnidarians**.



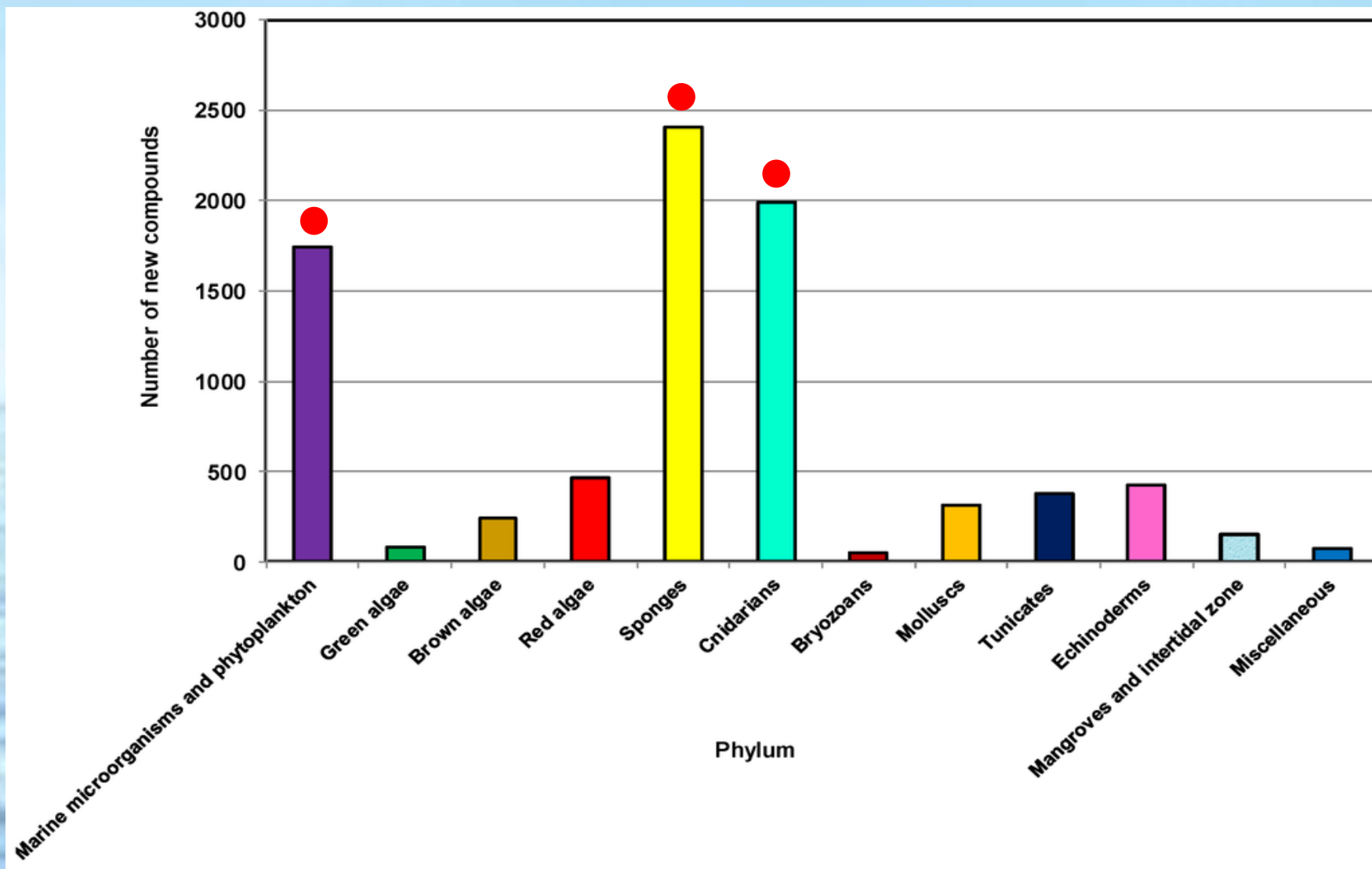
Marine natural product discovery from marine phyla from 1990-2009.



Other Phyla include *Annelida*, *Arthropoda*, *Brachiopoda*, *Hemichordata*, *Platyhelminthes* and *Bryozoa*

Adapted from Leal *et al.*, Plos ONE 2012

Total number of new compounds isolated from different types of marine sources, 2001–2010.



Current Pipeline of Marine Drugs

Clinical status	Compound name	Marine organism	Chemical class	Disease area
Approved	Cytarabine, ara-C	Sponge	Nucleoside	Cancer, leukemia
	Brentuximab vedotin (SGN-35)	Mollusk/cyanobacterium	ADC (MMAE)	Cancer, lymphoma
	Vidarabine, ara-A	Sponge	Nucleoside	Anti-viral
	Omega-3-acid ethyl esters	Fish	Omega-3 fatty acid	Hypertriglyceridemia
	Ziconotide	Cone snail	Peptide	Pain
	Eribulin mesylate (E7389)	Sponge	Macrolide	Breast cancer
Phase III	Trabectedin (ET-743)	Tunicate	Alkaloid	Cancer
	Plitidepsin	Tunicate	Depsipeptide	Cancer
	Tetrodotoxin	Pufferfish	Guanidinium alkaloid	Chronic pain
Phase II	Soblidotin (TZT 1027)	Bacterium	Peptide	Cancer
	DMXBA (GTS-21)	Worm	Alkaloid	Cognition, Alzheimers disease, schizophrenia
Phase I	Plinabulin (NPI-2358)	Fungus	Diketopiperazine	Cancer
	Glembatumumab vedotin	Mollusk/cyanobacterium	ADC (MMAE)	Breast cancer, melanoma
	Elisidepsin	Mollusc	Depsipeptide	Cancer
	PM1004	Nudibranch	Alkaloid	Cancer
	Tasidotin, synthadotin (ILX-651)	Bacterium	Peptide	Cancer
	Pseudopterosins	Soft coral	Diterpene glycoside	Wound healing
	Bryostatin 1	Bryozoa	Polyketide	Cancer
	Pinatuzumab vedotin (DCDT-2980S) and (DCDS-4501A)	Mollusk/cyanobacterium	ADC (MMAE)	Non-Hodgkin lymphoma, chronic lymphocytic leukemia
	Hemiasterlin (E7974)	Sponge	Tripeptide	Cancer
	HuMax [®] -TF-ADC	Mollusk/cyanobacterium	ADC (MMAE)	Cancer for ovary, endometrium, cervix, prostate
Preclinical	Marizomib (salinosporamide A)	Bacterium	Beta-lactone-gamma lactam	Cancer
	Chrysopaentin A	Alga <i>Halobacillus salinus</i>	Shikimate	Bacterial infections
	Phenethylamine	Bacterium <i>lyngbyoic acid</i>	Shikimate	Bacterial infections
	Geodisterol sulfates	Sponge	Peptide	Fungal infections
	<i>Pseudoalteromonas</i> sp. metabolites	Bacteria	Polyketide	Bacterial infections
	<i>Peziza vesiculosa</i> β -carboline	Bryozoa	Alkaloid	Fungal infections
	Bromophycolides	Alga	Terpene	Malaria
	Plakortin	Sponge	Polyketide	Malaria
	Homogentisic acid	Sponge	Shikimate	Malaria
	<i>Cladonia cervicornis</i> diterpene	Alga	Terpene	Protozoal infections
	Hymenidin	Sponge	Alkaloid	Tuberculosis
	Ggyrosanols	Soft coral	Terpene	Viral infections
	Dysidine	Sponge	Terpene	Diabetes
	Arenamides A and B	Bacteria	Peptide	Inflammation
	Capnellene	Soft coral	Terpene	Inflammation
	Floridosides	Alga	Glycolipid	Inflammation
	Grassystatins A-C	Bacteria	Peptide	Immunity
	Callyspongidiol	Sponge	Polyketide	Immunity
	Calyculin A	Sponge	PKS/NRPS	Nervous system
Pulicatin A	Bacteria	Alkaloid	Nervous system	
Dysideamine	Sponge	Terpene	Nervous system	



Malve (2016) J. Pharmacy + Bioallied Sciences 8, 83-91.

ADC: Antibody drug conjugate, MMAE: Monomethylauristatin E, PKS: Polyketide synthases, NRPS: Nonribosomal peptide synthases, DMXBA: 3-(2,4 dimethoxy) benzylidene-anabaseine

CYTABARINE

Chemotherapy medication used to treat acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, and non-Hodgkin's lymphoma.



Nucleoside called
Arabinosyl cytosine (ara-C).

Discovery in Florida (1945).

Produced by the sponge
Cryptotethia crypta.



Cryptotethia crypta

VIDARABINE

An antiviral drug which is active against herpes simplex and varicella zoster viruses.



Copyright Museum of Health Care

Nucleoside called 9- β -D-arabinofuranosyladenine (ara-A).

Discovery in Caribbean sponge *Tethya crypta* in early 1960s.



Tethya crypta

ZICONOTIDE

Powerful analgesic drug which blocks calcium channels, interrupting pain signalling at the level of the spinal cord.



Peptide with a 25 amino acid sequence.

Discovery in Cone Snail
Conus magus

Patented by Neurex Corp., a U.S. company purchased in 1998 by Elan Corporation.



Conus magus

ERIBULIN

Eribulin is a drug used to treat metastatic breast cancer.



Synthetic analogue of the marine natural product Halichondrin B.

Approved by USFDA 2010.
Also being tested as a treatment for lung and prostate cancer.

Discovered in sponge *Halicondria okadai*



Halicondria okadai

TRABECTEDIN

Used as a drug to treat advanced soft-tissue sarcomas, liposarcoma, and leiomyosarcoma.

Complicated heterocyclic ring structure. Semisynthetic process using the antibiotic SAFRACIN B obtained by fermentation of the bacterium *Pseudomonas fluorescens*.

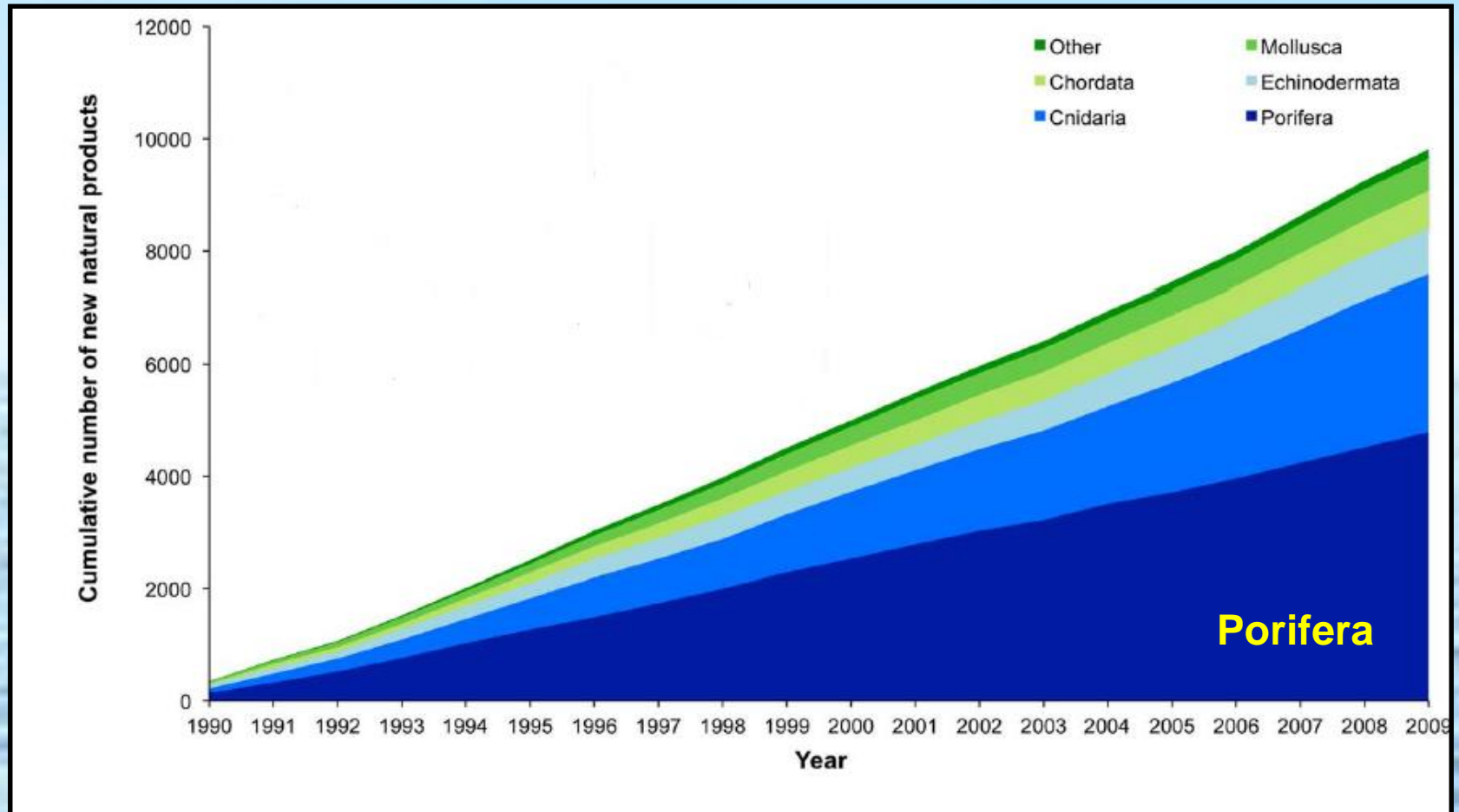
Approved by US FDA 2015

Discovery in tunicate *Ecteinascidia turbinate*.



Ecteinascidia turbinate

Marine natural product discovery from marine phyla from 1990-2009.



Other Phyla include *Annelida*, *Arthropoda*, *Brachiopoda*, *Hemichordata*, *Platyhelminthes* and *Bryozoa*

Adapted from Leal *et al.*, Plos ONE 2012

Marine Sponges *Porifera*

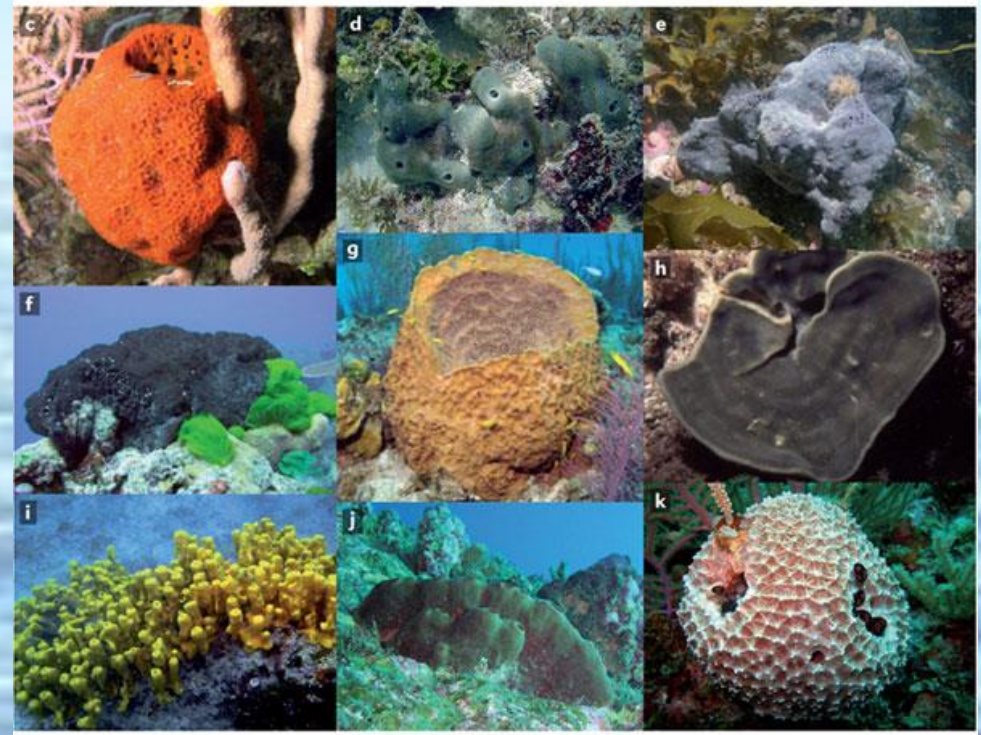
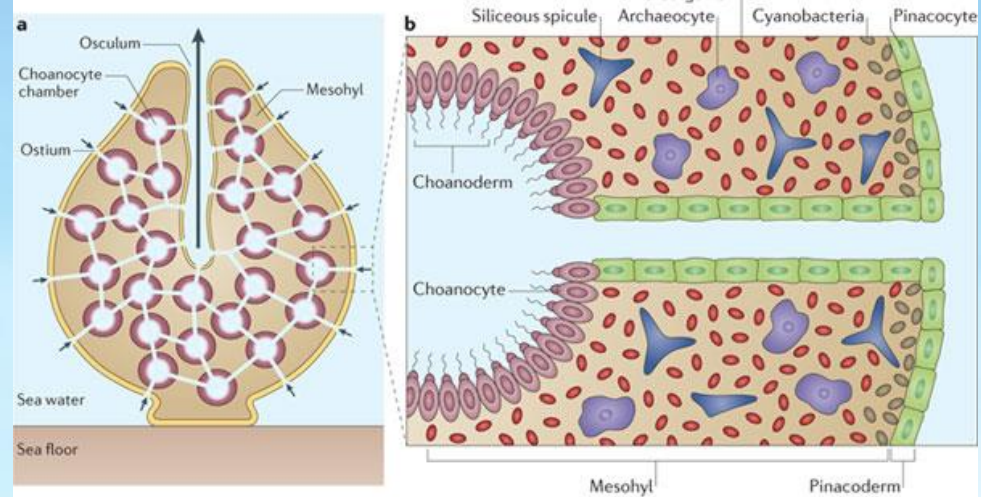
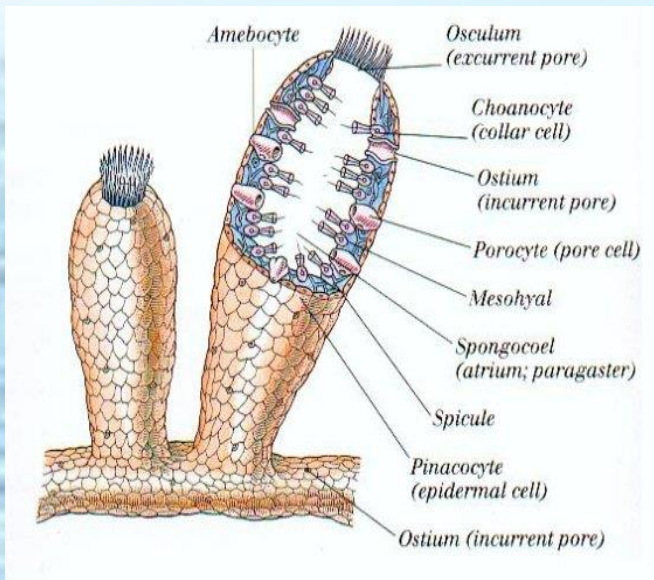
Simple animals

No nervous system

No internal organs

Sessile filter feeders

< 24,000 L kg⁻¹ day⁻¹



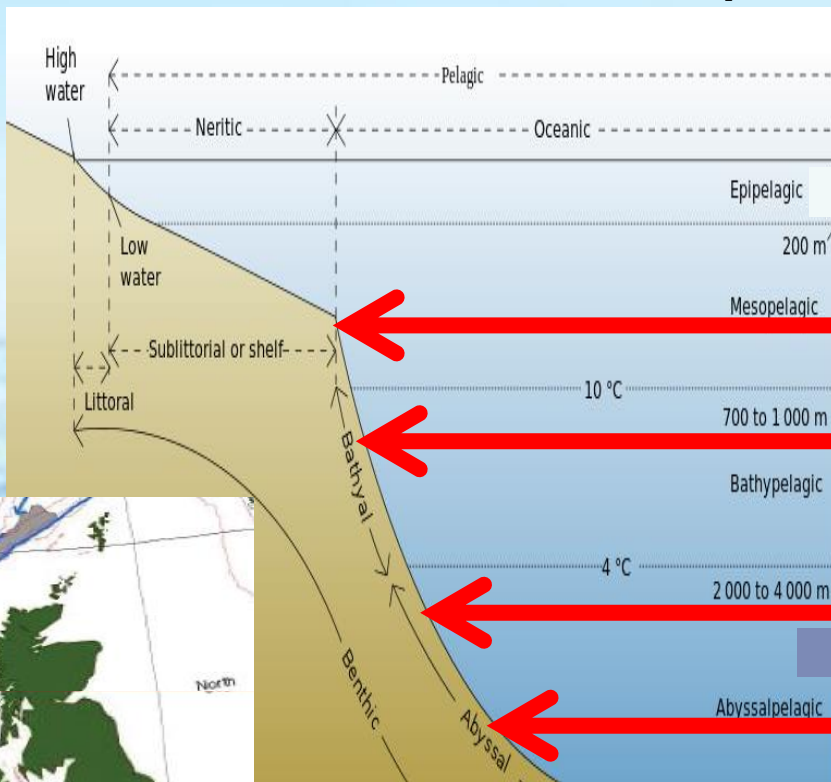
Globally distributed

- marine: tropical, polar, temperate oceans;
- intertidal zones to bathypelagic zones
- freshwater

Hentschel *et al.*, 2012. *Nature Reviews Microbiology* 10, 641-654.

<https://www.youtube.com/watch?v=q7mKS4bCfuo>

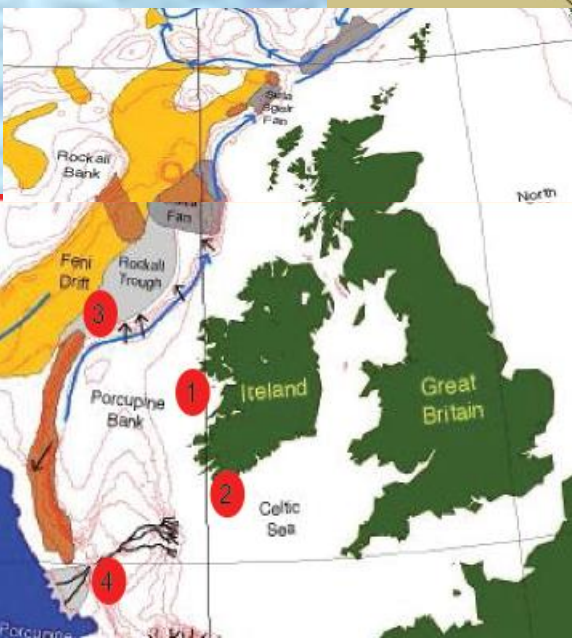
Genomic based studies in deep-sea sponges



RV Celtic Explorer



ROV Holland I



Inflatella pellicula
(750 m)



Lissodendoryx diversichela
(1300 m)



Stelletta normani
(1300 m)



Poecillastra compressa
(2100 m)



Inflatella pellicula
(2900 m)



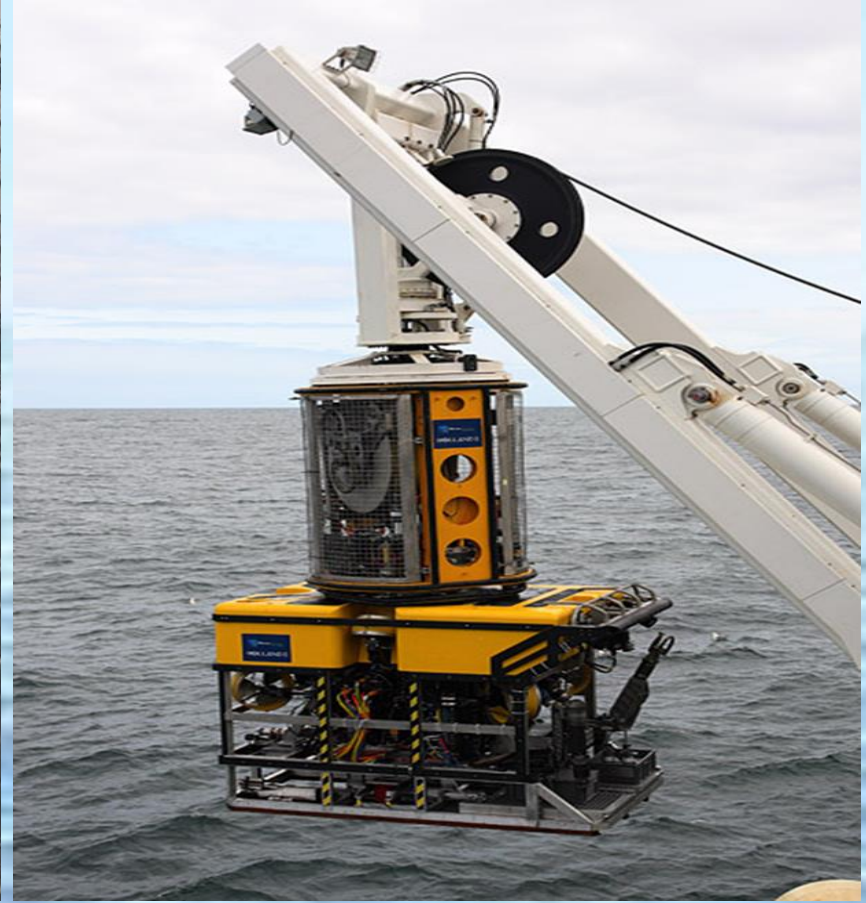


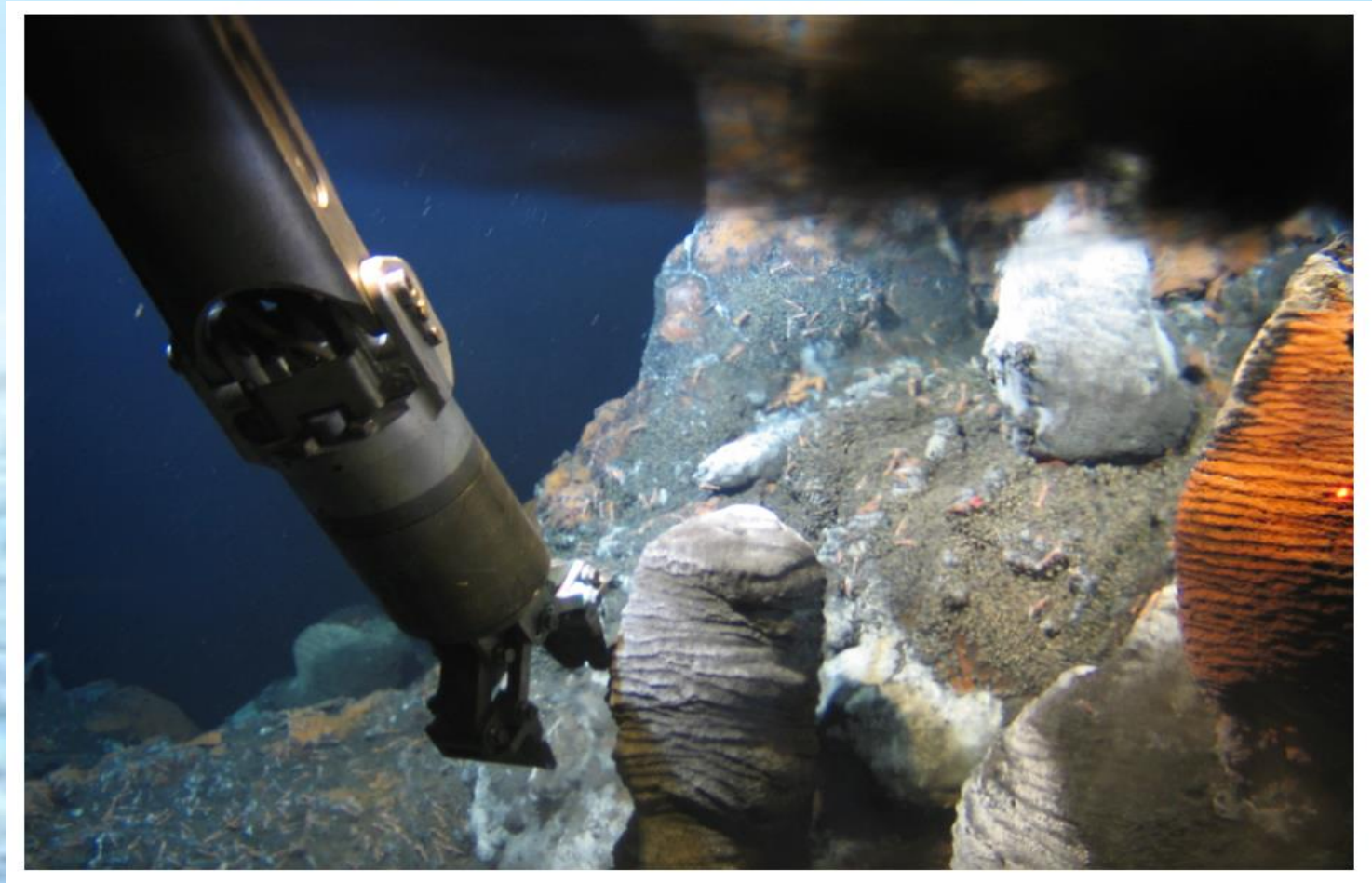
CELTIC EXPLORER



CELTIC VOYAGER

ROV HOLLAND 1

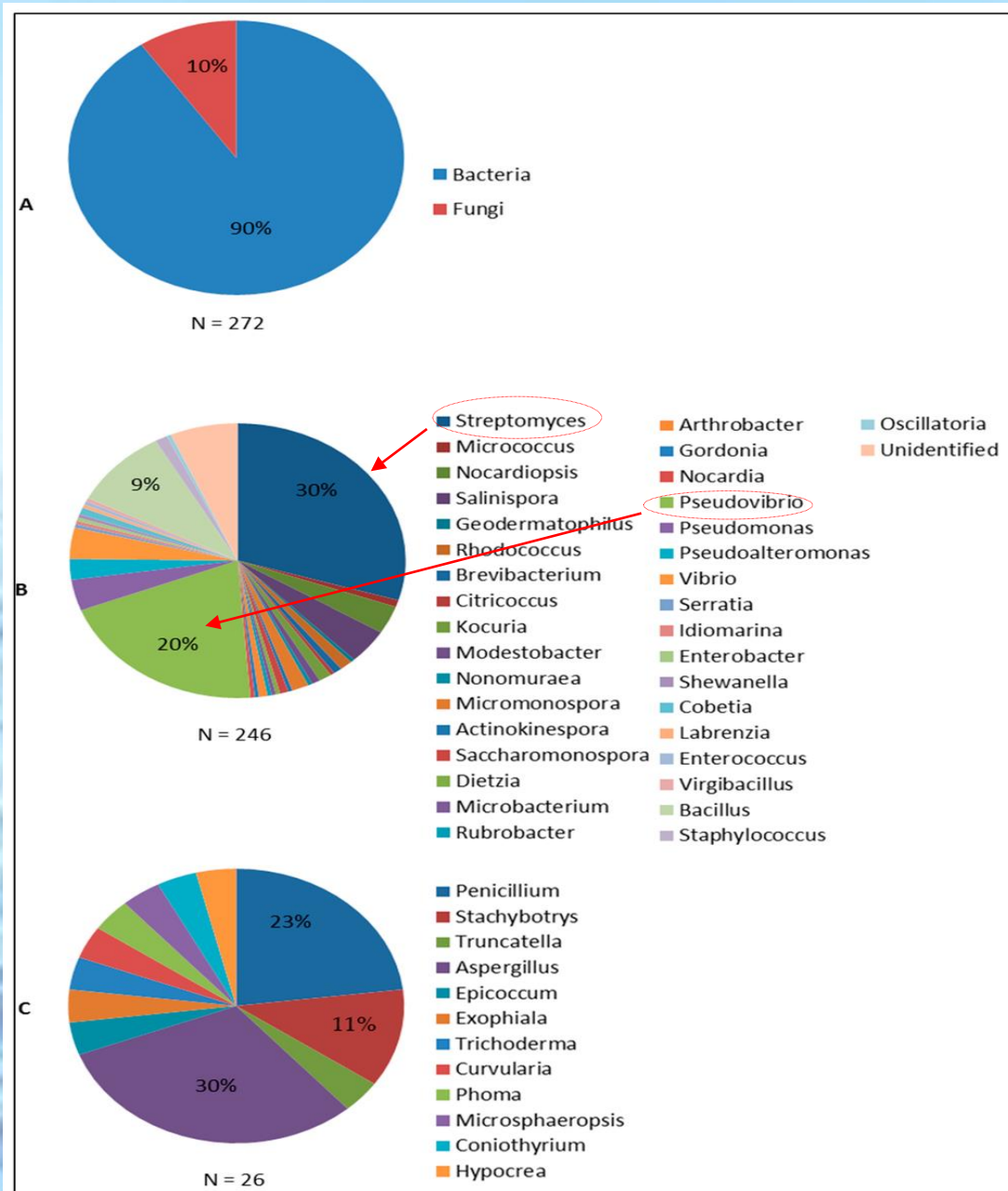




Microbes in Marine Ecosystems

- **Bacteria account for most oceanic biomass and metabolism.**
- **Total number of bacteria in marine environments including the sub-surface is estimated at $\sim 3.67 \times 10^{30}$.**
- **Up to 10^6 bacteria are present in 1 ml of seawater.**

Distribution of Sponge-Associated Microorganisms producing antimicrobial compounds



BIOMINING BIOACTIVE MARINE *STREPTOMYCES* STRAINS

Over 65% of the natural antibiotics produced in the pharmaceutical industry are from Streptomycetes.



Streptomyces venezuela



Streptomyces kanamyceticus



Streptomyces aureofaciens



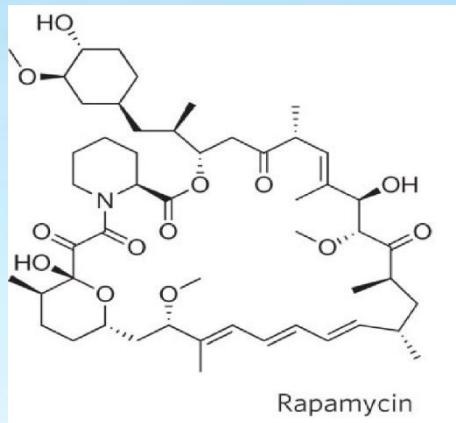
Streptomyces fradiae



Streptomyces roseosporus



Streptomyces lincolnensis



Streptomyces hydroscopicus

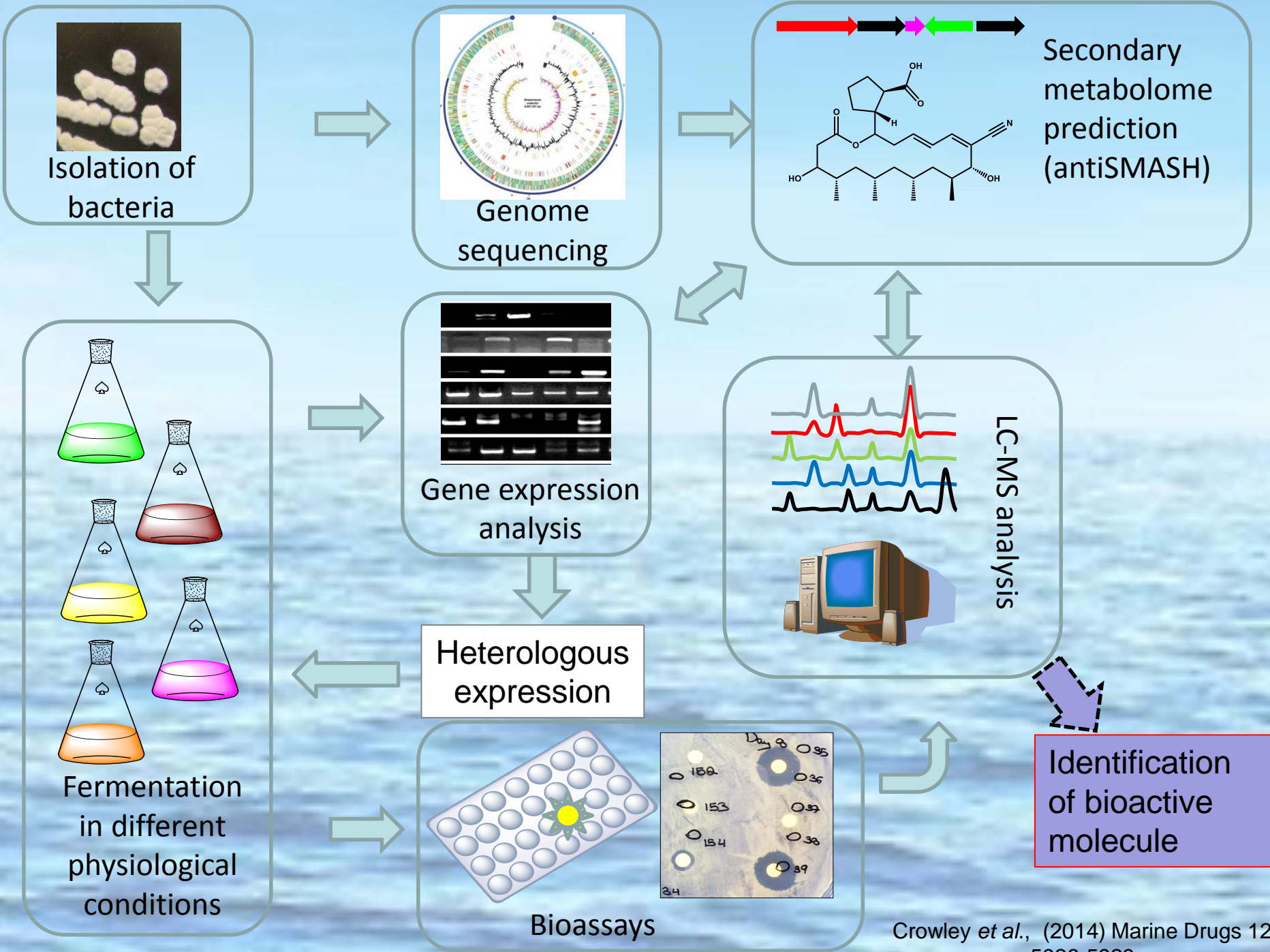
Used to coat coronary stents, to prevent organ transplant rejection.



Streptomyces peucetius



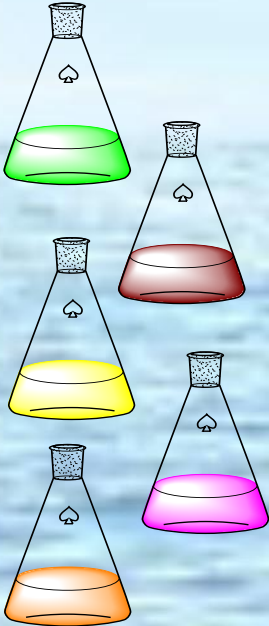
Used to treat acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), and Kaposi's sarcoma.



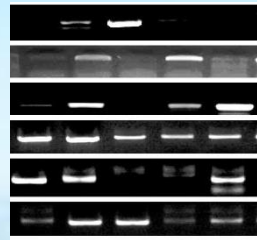
Isolation of bacteria

Genome sequencing

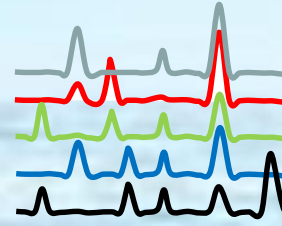
Secondary metabolome prediction (antiSMASH)



Fermentation in different physiological conditions

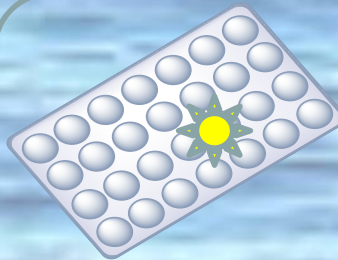


Gene expression analysis

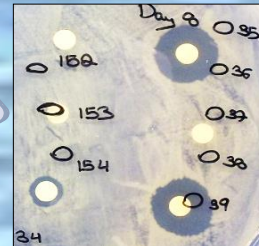


LC-MS analysis

Heterologous expression



Bioassays



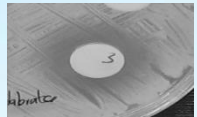
Identification of bioactive molecule

540 actinomycetes, isolated from shallow water and deep-sea sponges, were screened for growth inhibition of a number of clinically relevant bacterial and fungal/yeast species.

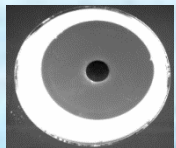
Thirteen of these strains which displayed the most interesting range of bioactive antimicrobial activities, including growth inhibition of problematic anti-microbial resistant (AMR) human pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-intermediate *Staphylococcus aureus* (ViSA), were identified.



MRSA



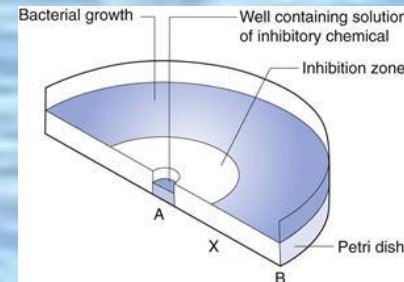
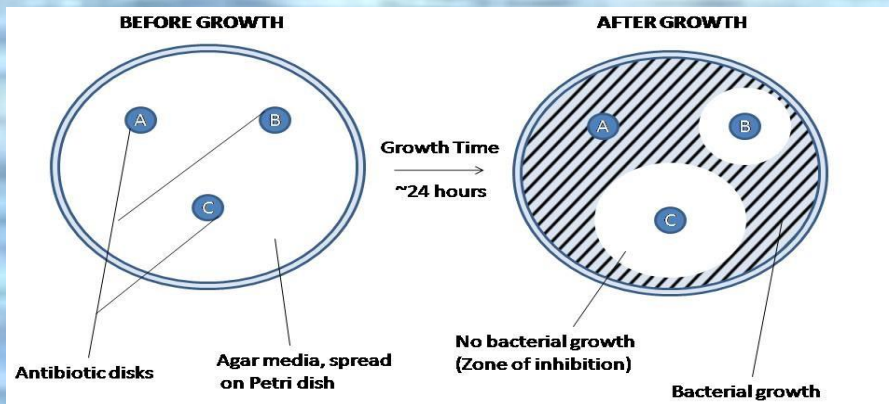
C. glabrata



C. difficile



B. subtilis



Antimicrobial activities of 13 cultured sponge *Streptomyces* strains

Test strain	Gram negative bacteria			Gram positive bacteria		Yeasts	
	<i>E. coli</i> NCIMB 12210	<i>P. aeruginosa</i> PAO1	<i>Bacillus</i> spp.	<i>Staphylococcus</i> spp.	<i>L. monocyete genes</i> F2365	<i>Candida</i> spp.	<i>A. fumigatus</i> ATCC 46645
	SM1*	+	-	+ ^{1,2}	+ ^a	+	+
SM5*	+	-	+ ^{1,2}	+ ^a	-	-	n.d.
SM9*	+	-	-	-	-	-	n.d.
SM10*	+	n.d.	-	-	-	-	n.d.
SM11*	-	-	+ ²	+ ^{a,b}	-	-	n.d.
SM12*	-	+	-	+ ^a	-	-	n.d.
SM14*	-	-	+ ^{1,2}	+ ^a	-	-	n.d.
SM16*	-	+	+ ²	+ ^c	-	-	n.d.
SM17*	+	-	-	+ ^b	-	+	n.d.
SM18*	-	-	+ ²	+ ^b	-	-	n.d.
FMC008*	-	+	+ ²	+ ^d	-	-	n.d.
B226SN101	-	-	- ³	-	n.d.	+	+
B188M101	-	-	- ³	-	n.d.	+	+

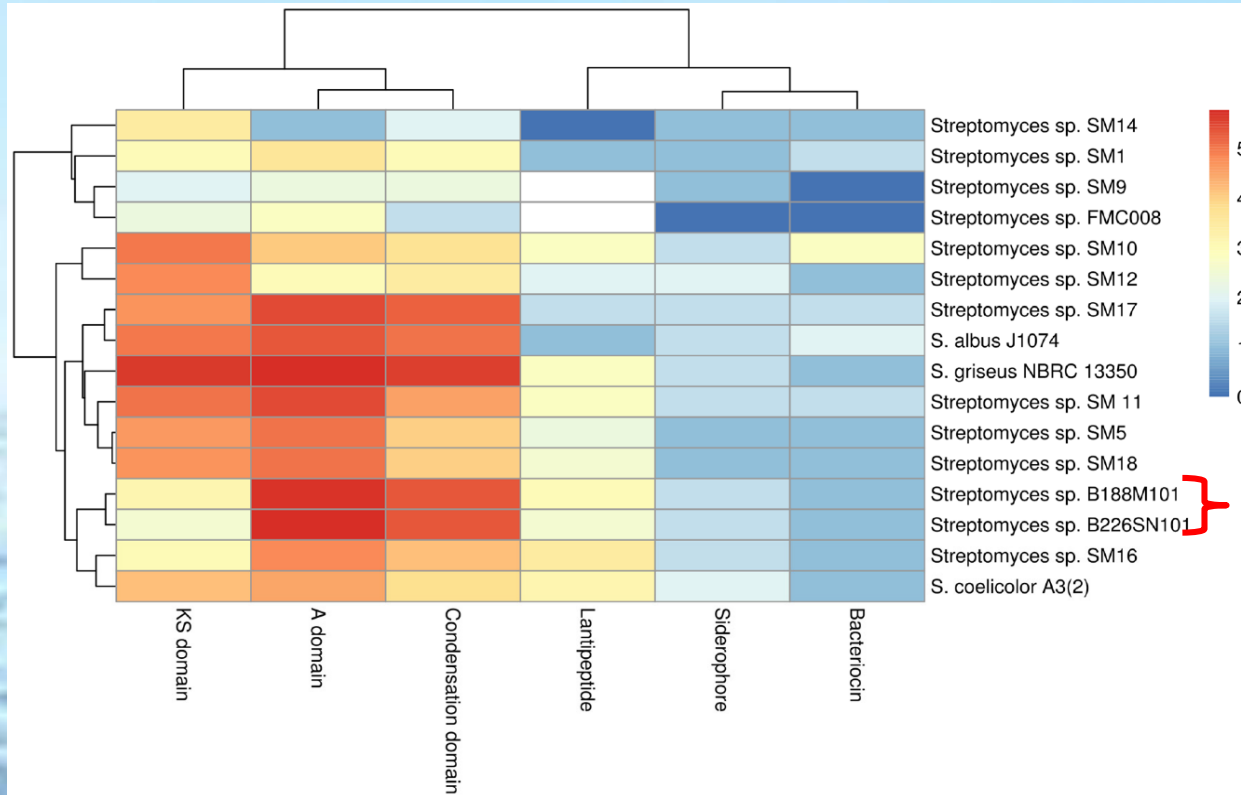
Deep
Sea

Antimicrobial activities of cultured sponge bacteria using deferred antagonism assays. ¹: *Bacillus cereus* FPL1; ²: *Bacillus subtilis* 1A40; ³: *B. subtilis* 1E32; ^a: hVISA (Heterogenous Vancomycin Intermediate *Staphylococcus aureus*) 22900; ^b: MRSA (Methicillin resistant *S. aureus*) ST544; ^c: VISA (Vancomycin intermediate *S. aureus*) 35403; ^d: *S. aureus* NCIMB 9518: '+' = positive. '-' = negative; n.d. not determined. *

Adapted from Kennedy *et al.*, 2009.

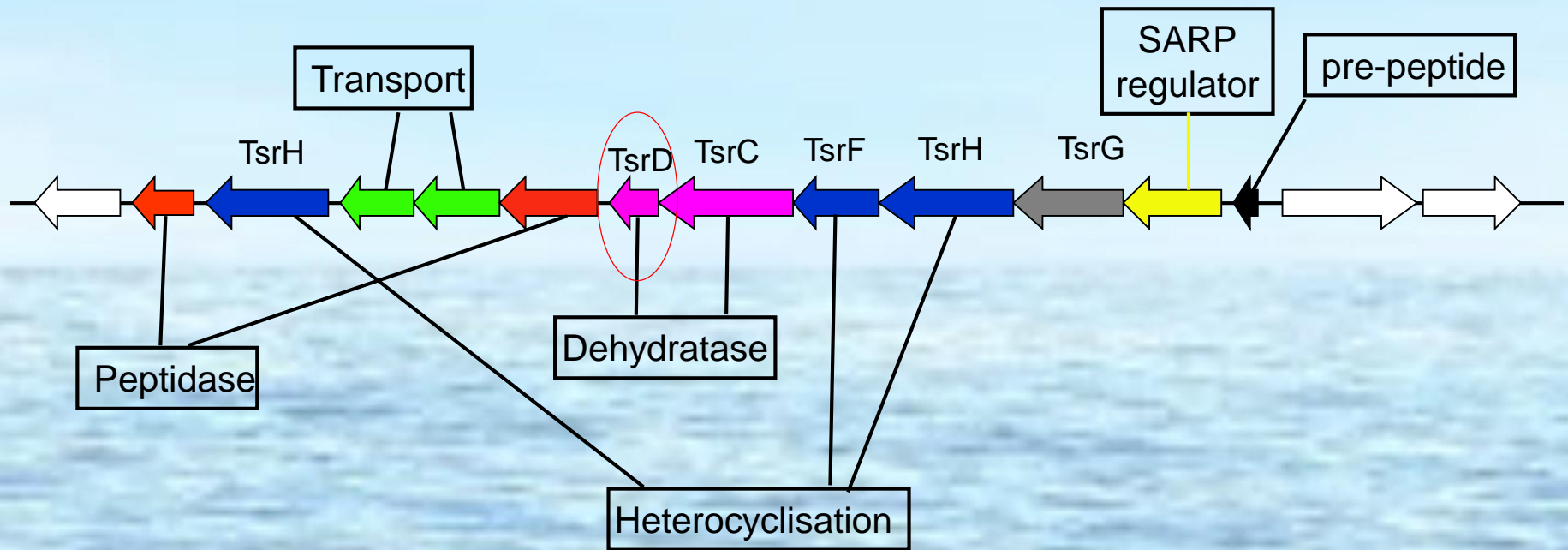
Group A isolates SM11, SM16, B188M101 and B226SN101) appear enriched for adenylation and condensation domains of NRPS.

The majority of predicted protein domains from the deep sea isolates (B188M101 and B226SN101) are more similar to each other than to similar genes in shallow water or terrestrial isolates.



Log2 heatmap of predicted secondary metabolism protein domains of interest from marine *Streptomyces* spp. isolates and from select reference terrestrial genomes.

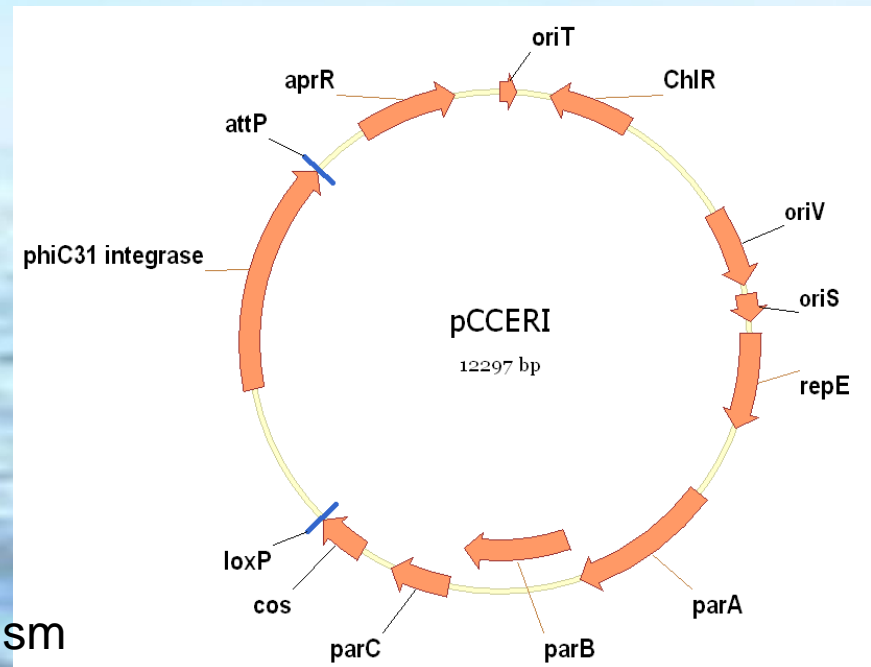
Novel thiopeptide gene cluster from *Streptomyces* SM2



- Related proteins from thiostrepton biosynthesis cluster are indicated (30-40% identity)
- Contains all 'core' thiostrepton *tsr* genes except *tsrE*
- Functional predictions are based on thiostrepton cluster
- Pre-peptide sequence indicates novel thiopeptide core

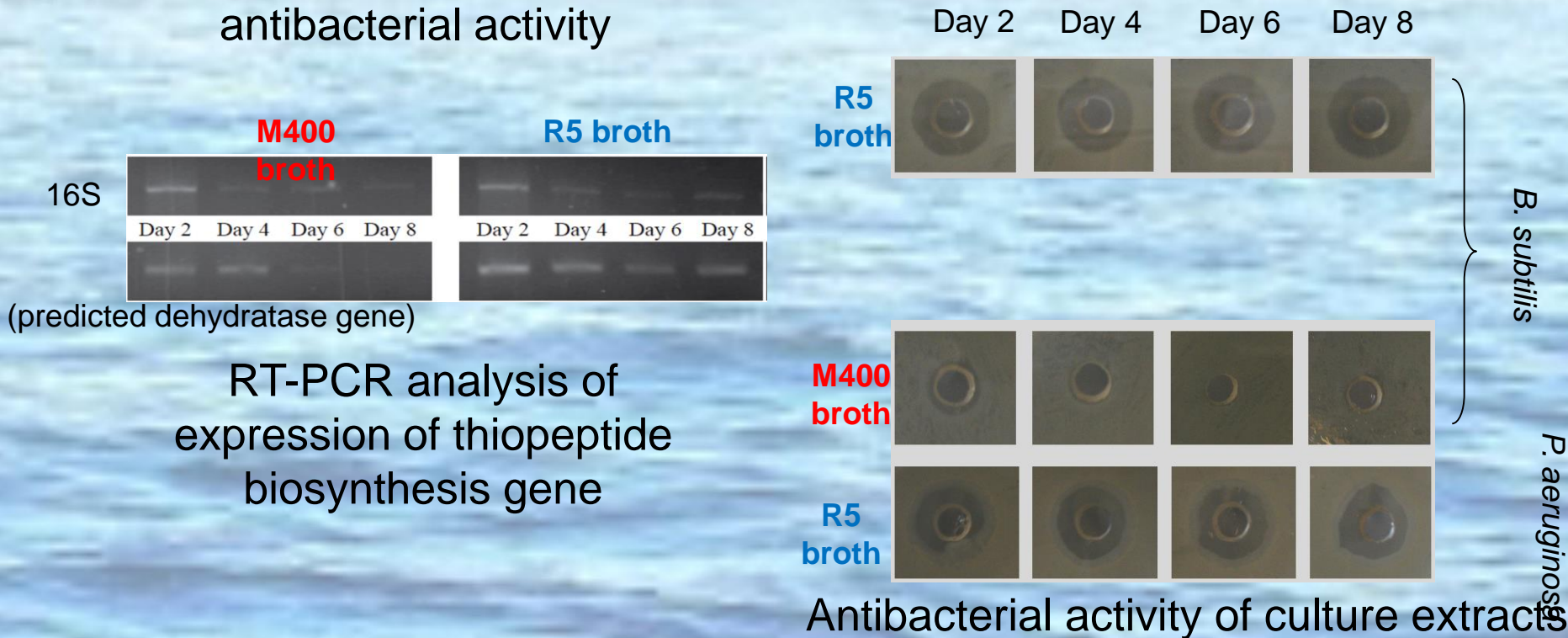
Heterologous expression of silent gene clusters

- SM2 genomic library prepared in *E.coli* – *Streptomyces* shuttle fosmid/BAC pCC-ERI
- Select clones with clusters
- Heterologous hosts
 - *S. coelicolor* M1152 & M1154
 - No antibiotic activity
 - Up-regulated for secondary metabolism



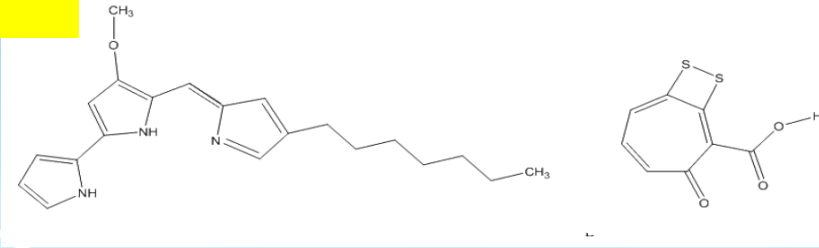
Novel thiopeptide antibiotic cluster from *Streptomyces* SM2

- Expression not detected in native host
- Heterologous expression:
 - Fosmid conjugated into *S. coelicolor* M1152
 - Expression of predicted dehydratase gene detected
 - Expression correlates with production of broad spectrum antibacterial activity



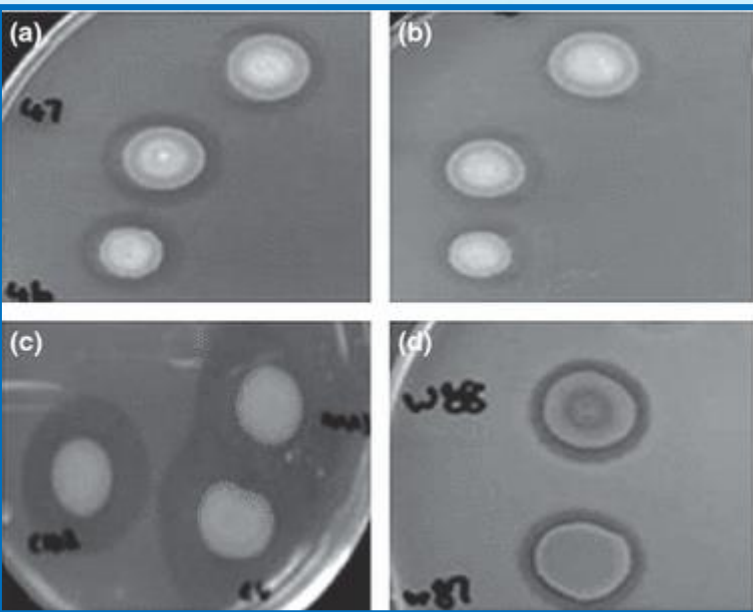
Pseudovibrio

Members of this genus are from the Rhodobacteracea family, Rhodobacterales order and alphaproteobacteria class.

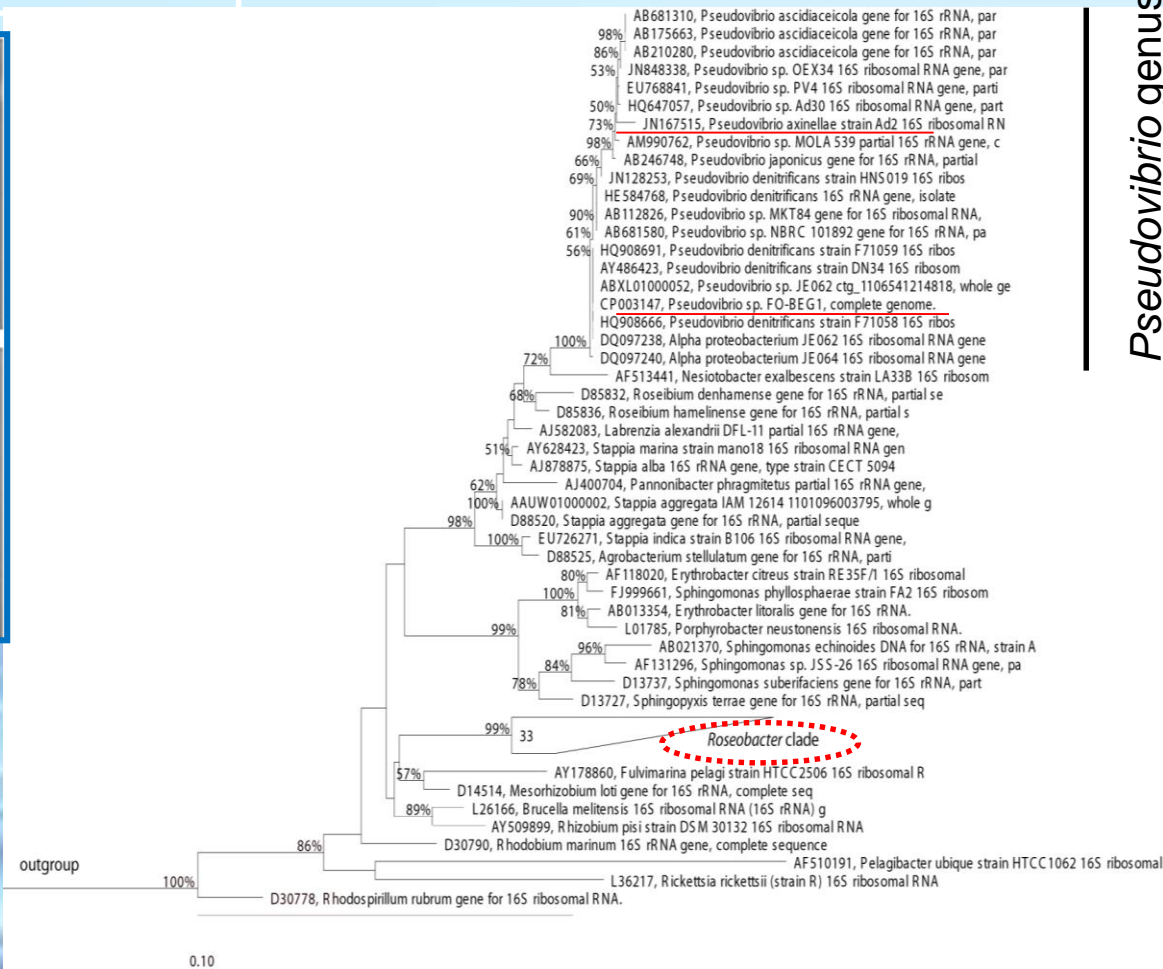


Heptylprodigiosin

Tropodithietic acid

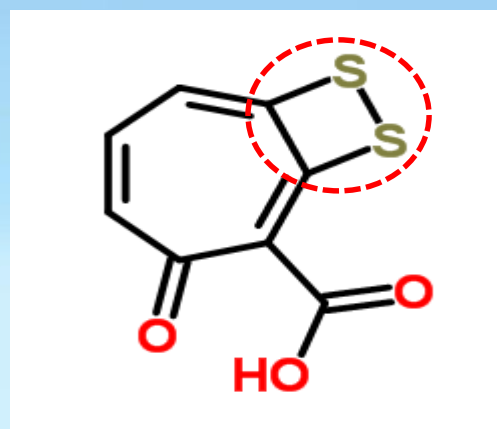


Antimicrobial activity of *Pseudovibrio* isolates against (a) *E. coli*, (b) *S. Typhimurium*, (c) *C. difficile* and (d) methicillin-resistant *Staphylococcus aureus* using deferred antagonism assays.



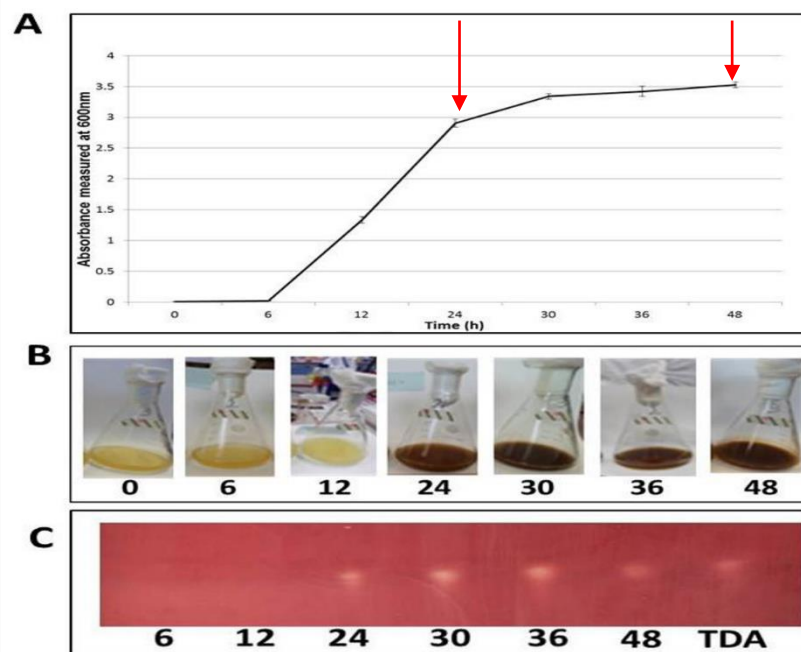
Pseudovibrio genus

Tropodithietic acid (TDA) is a sulphur-containing compound with a unique structure consisting of a dithiete moiety fused to tropone-2-carboxylic acid.

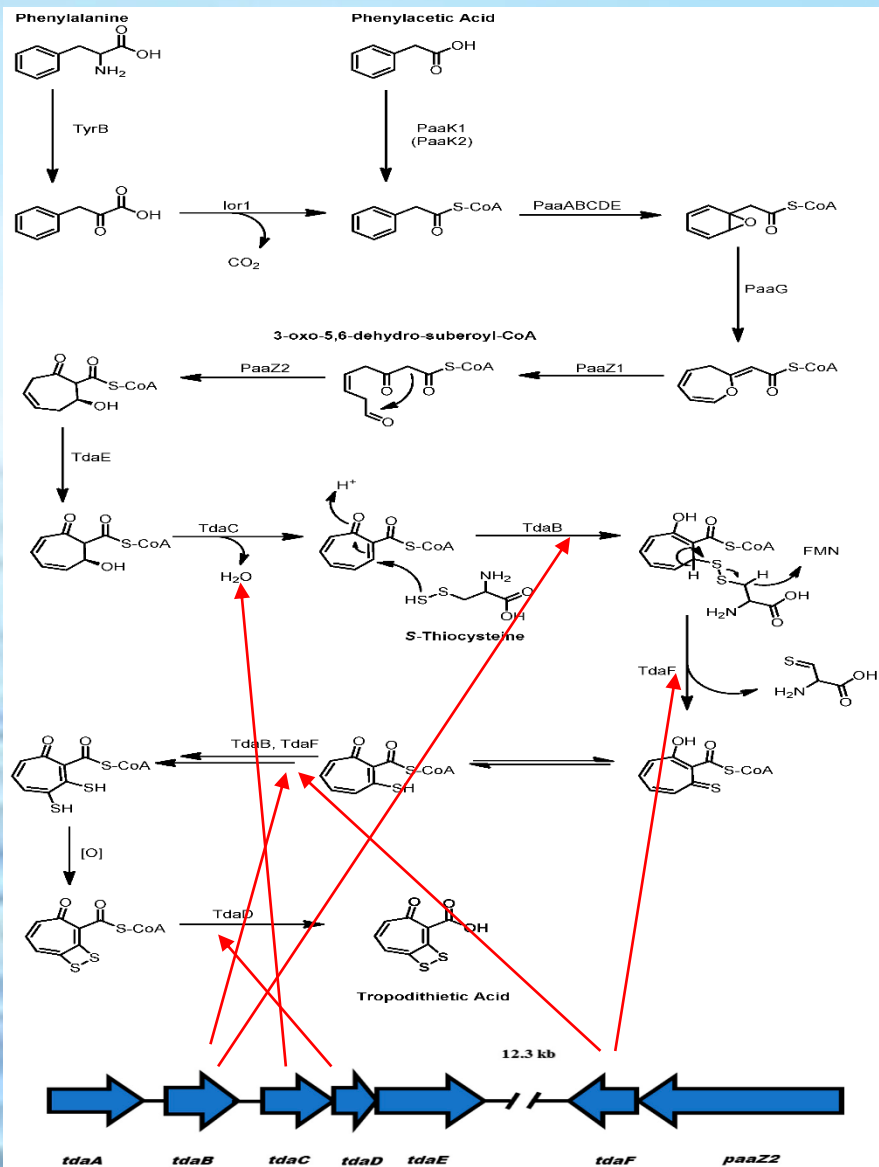


TDA has a strong inhibitory activity against a range of marine bacteria, such as *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes*, the fish pathogens *Vibrio anguillarum* and *Vibrio splendidus*, as well as marine algae and a range of human pathogenic bacteria.

Timecourse for TDA production in *Pseudovibrio* W74

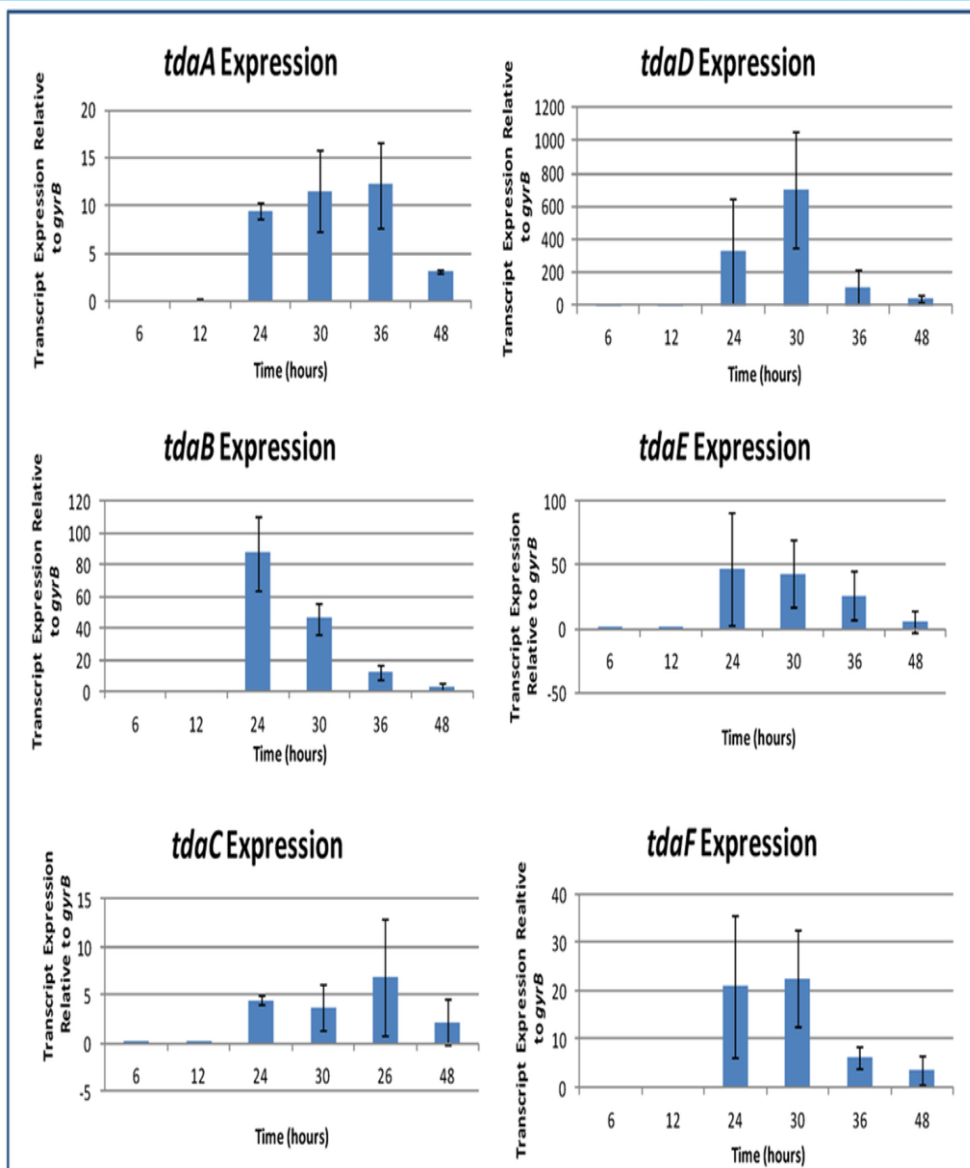


Proposed TDA Biosynthetic pathway in *Pseudovibrio* W74



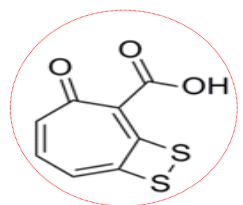
Timmermans *et al.*, 2017 Mar Drugs 15, 235-271.

Induction of TDA genes is linked to bioactivity in *Pseudovibrio* W74

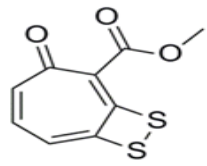


Harrington *et al.*, 2014 Mar Drugs 12, 5960-5978.

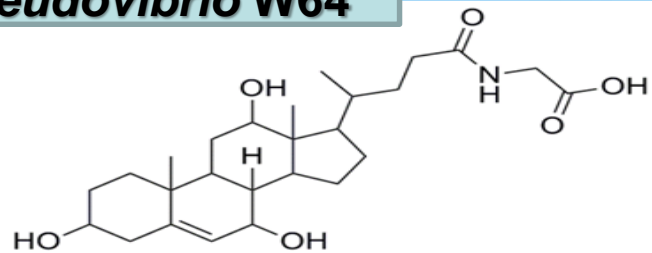
Other metabolites in *Pseudovibrio* W64



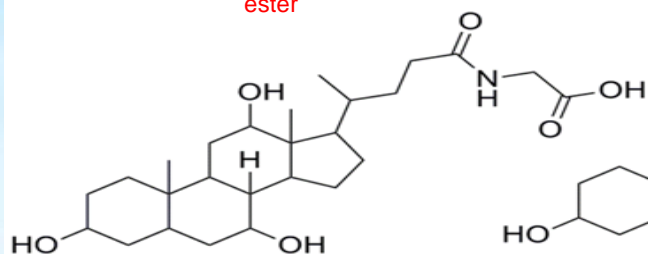
(1) TDA Methyl ester



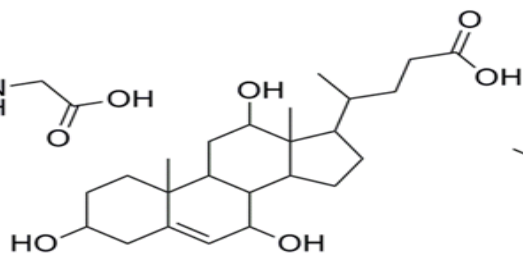
(2) TDA



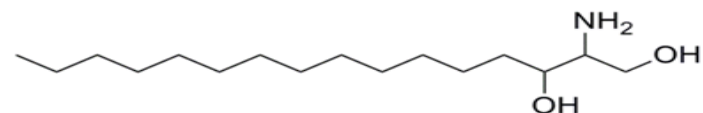
(3) 3,7,12-trihydroxychol-5-enoylglycine



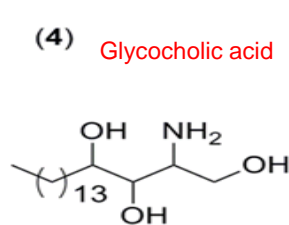
(4) Glycocholic acid



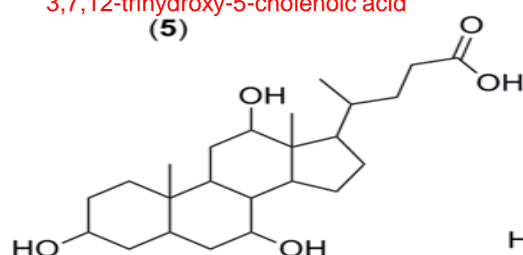
(5) 3,7,12-trihydroxy-5-cholenoic acid



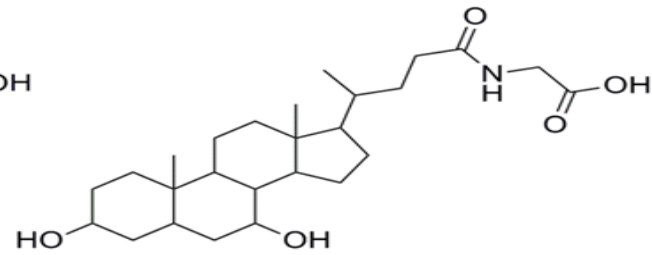
(6) Hexadecasphinganine



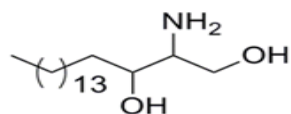
(7) 4-hydroxysphinganine



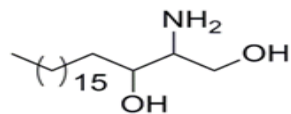
(8) Cholic acid



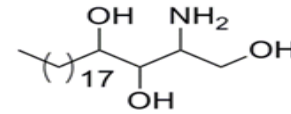
(9) Glycodeoxycholic acid



(10) Sphinganine



(12) C₂₀ sphinganine



(13) 4-hydroxyl C₂₂ sphinganine

A unique analogue of TDA, methyl-TDA, and a number of cholic acid derivatives together with amino diols and triols have been identified in *Pseudovibrio* W64 strain. These metabolites have previously been reported to possess antimicrobial activity.

Novel marine enzymes/bioactives: *Bioprocessing*

- World industrial enzyme market to be worth \$6.2 billion by 2020
- Biostimulant market grow to \$3 billion by 2018
- Fastest growth in developing economies
- Demand rising 6.8% pa
- Supporting small molecule production/processing

DRIVERS:

- Demand for natural products
- Novel food grade enzymes
- Recognition of unique properties
- Search for novelty
- Low capital investments

APPLICATIONS:

- Animal feed
- Food and beverages
- Agriculture biocontrol
- Horticulture

Amylase
Pullanase

} Hydrolysis of starch - Food and beverages

Glucosidase
Phytases

} Animal feed, human food supplements

Inulinases

Fructose/fructo-oligosaccharides -
sweeteners/prebiotics

Xylanases

Food and feed applications, baking, brewing

Proteases

Baking, brewing, dairy, fish and seafood, fruit juices, meat,
functional foods, nutraceuticals

Lipase/Esterase

Flavour enhancement, structured lipid synthesis/baby formula,
nutraceuticals

UNIQUE PROPERTIES:

- Salt & pH Tolerance
- Hyperthermostability
- Barophilicity
- Novel Chemically & Stereochemically
- Cold Adaptivity

The deep-sea is a particularly harsh environment with high pressures, low temperatures, high salinity and low light and oxygen concentrations

Novel Enzymes

- **Approx. over 3,500 microbial enzymes have to date been isolated.**
- **The majority have been derived from either mesophilic bacteria or fungi; predominantly sourced from terrestrial environments.**

Lipases

- **Important biocatalysts**
 - **Multiple families of enzymes**
 - **Hydrolyse triacyl glycerols**
 - **Short chain and long chain**
 - **Applications**
 - **Biodiesel, laundry, food, paper, pharmaceutical**
 - **3rd most important enzyme group (market value- **\$590M by 2020**)**
 - **Desired characteristics**
 - **Substrate specificity, stability, activity, temperature, pH, halotolerance.**

Metabolic Potential

- Bacteria can achieve densities of up to 10^6 per of ml seawater (Azam, 1998)
- assuming 3,000 genes per single genome
- could be up to 3×10^9 genes mediating up to 1.2×10^9 putative reactions in that sample
(assuming that 40% of these genes have catalytic activity) (Dinsdale *et al.*, 2008)

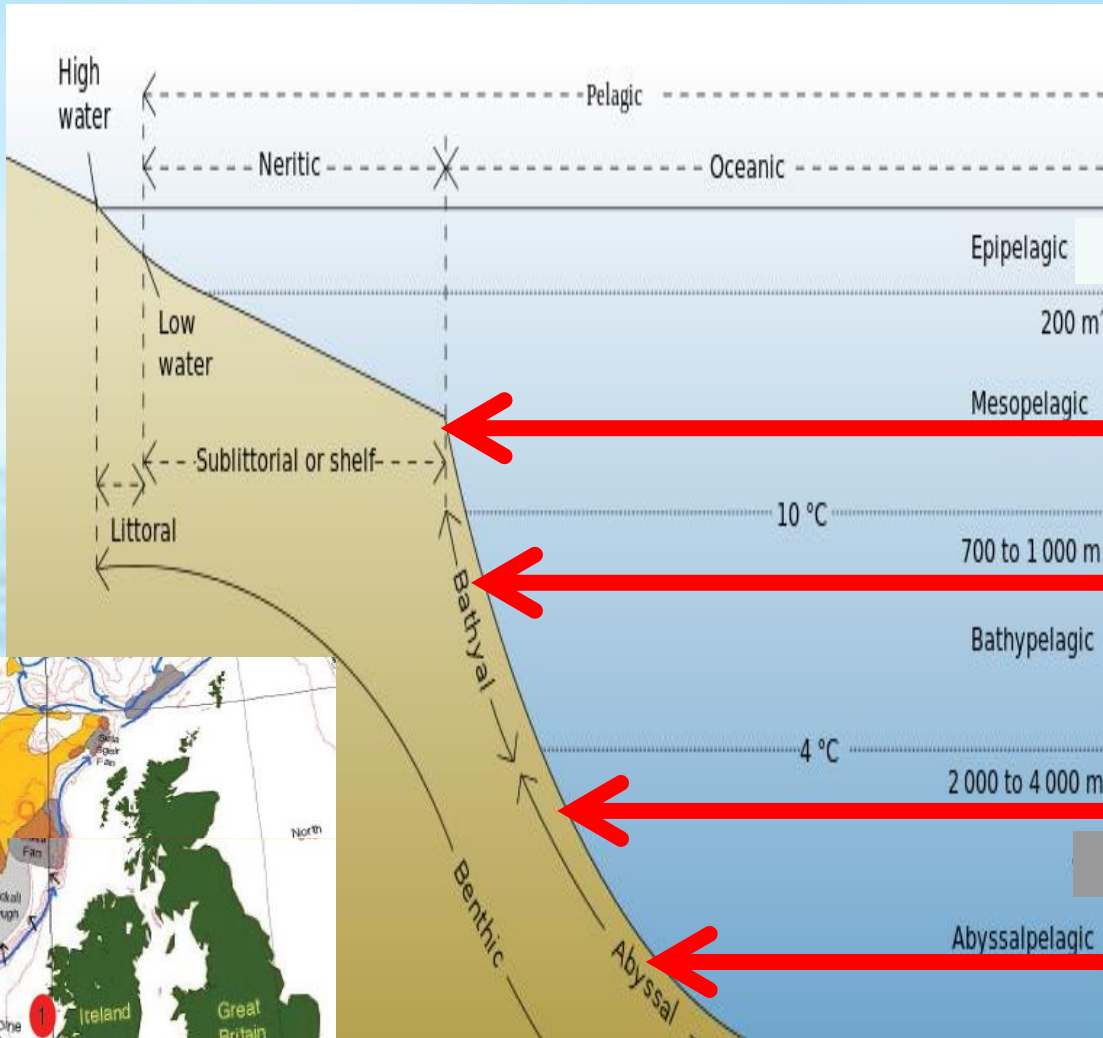
Definitions

- **Metagenome:** The collective genomes of an assemblage of microorganisms.
- **Metagenomics:** The genetic and functional analysis of the metagenome.
- The culture independent study of microbial communities.

Typically used to determine:-

- * What organisms are there, and where they are distributed within an ecosystem.
- * What they are doing.
- * Their interactions with each other and with their ecosystem.

Metagenome of deep-sea sponges



RV Celtic Explorer



ROV Holland I

Inflatella pellicula
(750 m)



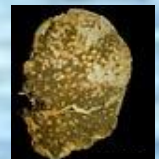
Lissodendoryx diversichela
(1300 m)



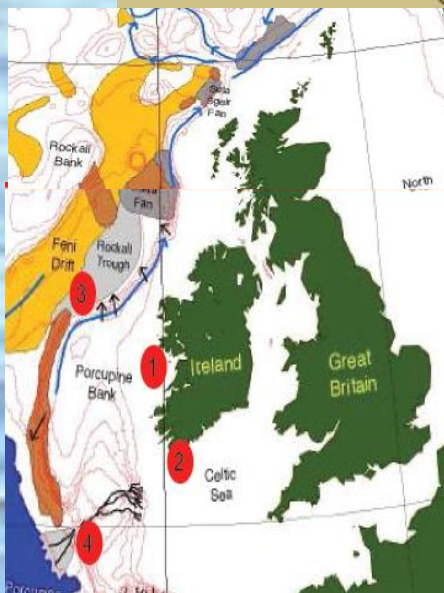
Stelletta normani
(1300 m)



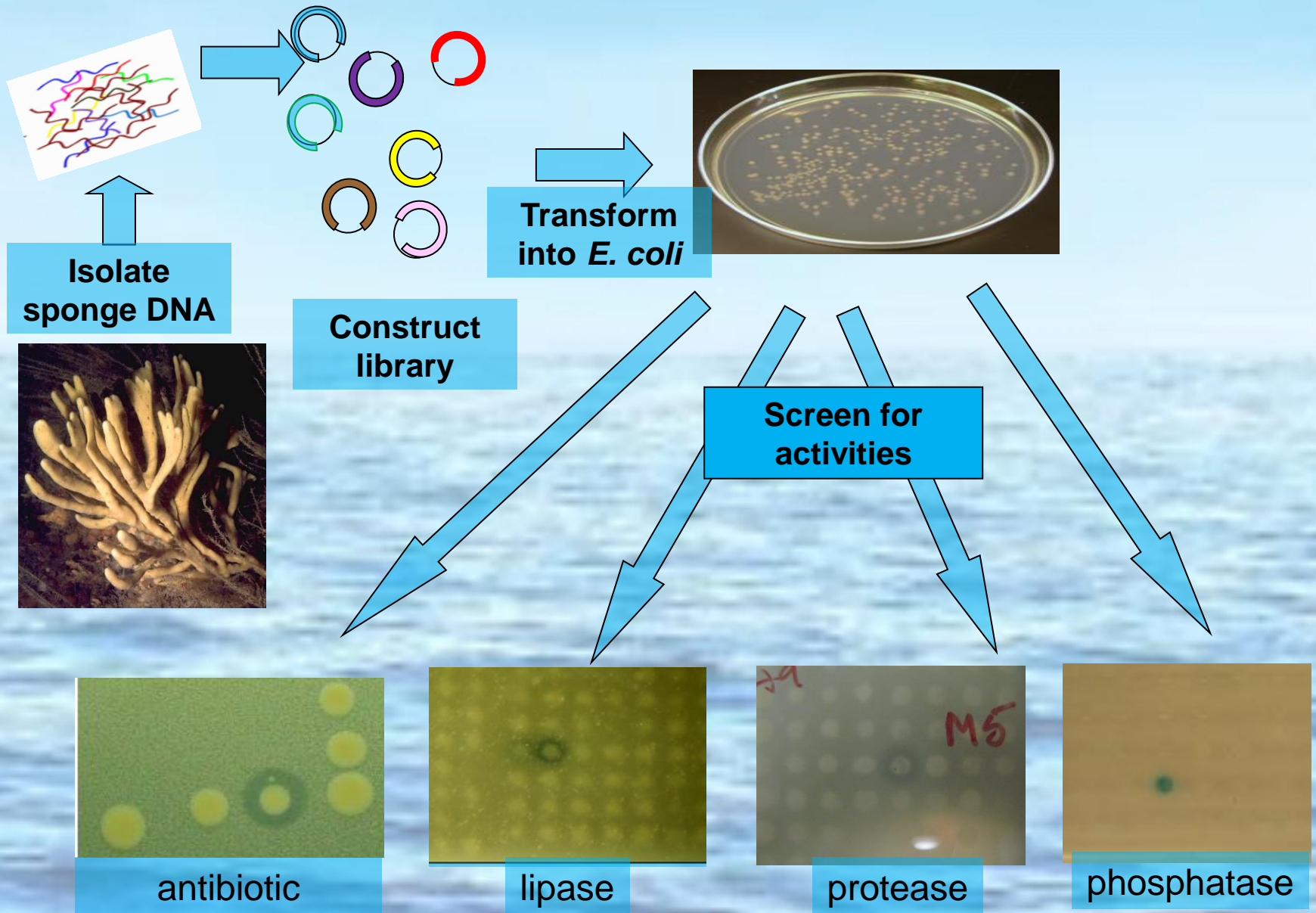
Poecillastra compressa
(2100 m)



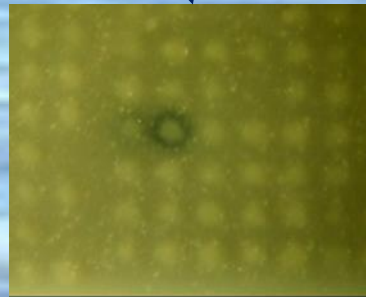
Inflatella pellicula
(2900 m)



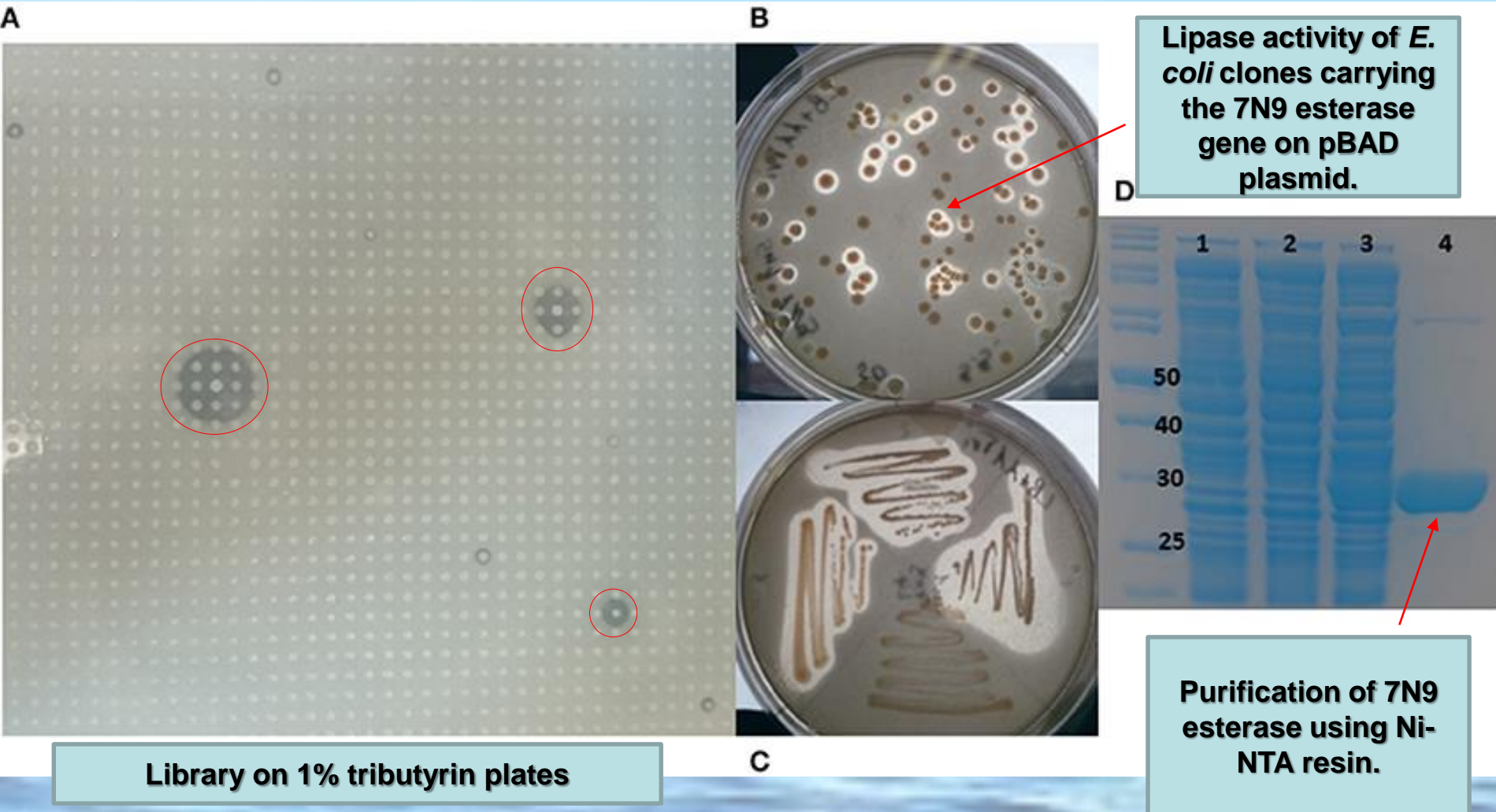
Functional Metagenomics



**High throughput screening with
automated colony picker.**



Lipase from *Stelletta normani* library



Multiple Sequence Alignment of closely related esterase sequences

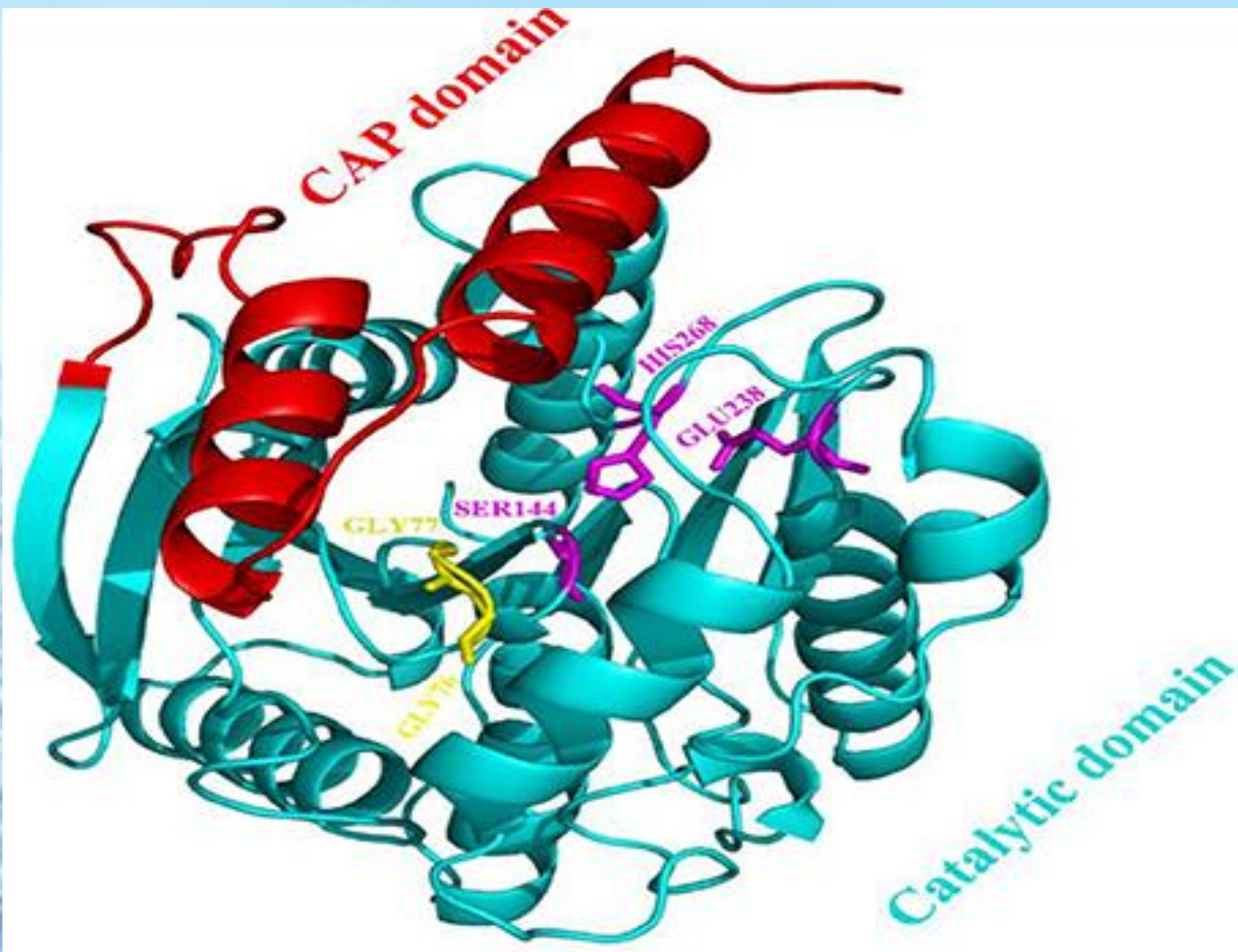
```

SN1_7N9_esterase      --MASPELDTVFQMIKEWGENF--GTTIEDNRLAYEKLVEPLPWVDDVKTERVAGSAPAE
E40_esterase          MAKSPFLDRVIGMIRERAATP--RKTDDDDRRLYETMLGSMPLDDDIQTERLGVNGVPAE
AJZ73156.1            --MASPQLQTAIEAFKATGEKIAKASDMKGMRAVMEE--MVMPVFPDDVKCTPVNAGGVPAAE
ACL67845.1            --MASQQLQAIIQALRSTPGQH--GADLEQRRALMEAVTWMPVFPDDVKREPVDAAGVPGE
ADM67446.1            -----MMSRSGPNFA--GLSVEEQRKGMEEIASQFFPADDDVKCEPVDAAAGVPAQ
ETW98043.1            MAQSPQLDRVIGMIKARAQAT--RKTDDEDRASYNMLASMPMADDIETERVAGGVPAAE
ETX02836.1            MAQSPQLDRVIGMIKARAQAT--RKTDDEDRASYNMLASMPMADDIETERVAGGVPAAE
                        ::          *          *          *          *          *          *          *          *
SN1_7N9_esterase      WIIAPGAEDGPILLYIHGGGYVMGSMRTHRVMLAHSRAAGARVLGLDYRLAPEFVFPAPQ
E40_esterase          WIYAPGARDDQVFLYIHGGGYVIGSMRTHRVMLSHIARAAGCRVLGLDYRLAPETFPFAP
AJZ73156.1            WIVAPGAAEDRFLLYIHGGGYVMGSIKTHREMVSRISRMAGVRALALDYRLAPESPFPAA
ACL67845.1            WIAAPGAAPERVIIYIHGGGYVIGSINSHRQMVSHLSRAAGARALAI DYRLAPENFPFPA
ADM67446.1            WISAPDADPERVIYIHGGGYVIGSVSTHRDIISRIARASGARALAI DYRLAPEHPFPAA
ETW98043.1            WIRAPGARADRVMLYIHGGGYVVGSMRTHRTMLSHISRASGFSVLGLDYRLAPENFPFAP
ETX02836.1            WIRAPGAQADRVMLYIHGGGYVVGSMRTHRTMLSHISLASGFSVLGLDYRLAPENFPFAP
                        ** ** *          * :*****:*** :** :***** :*          * :***** **
SN1_7N9_esterase      VEDAVAAAYRWLLANGSDPKKIVIGSDSAGGGLMVATLVALRVLGEPMPAAGVGLSPWVDM
E40_esterase          VEDTVAAYRWLLAHGYDPSRIALGSDSAGGGLVVAALVALRYIGEPMPAAGVCLSPWIDM
AJZ73156.1            VDDATAAYRWLLAQGAKPARTAIGSDSAGGGLALATLVAIRDGKQLPAAAGVCLSPWADM
ACL67845.1            VEDATAAYRWLLSTGVDPAVVVIGSDSAGGGLTVAATLVALRDAGDPLPAAAVCLSPWVDM
ADM67446.1            VEDSTAAAYRWLLSTGADPARTVIGSDSAGGGLTVAATLVALRDAGDPLPAAAVCLSPWIDM
ETW98043.1            VEDALAAAYRWLLDQGTESGNIVLIGSDSAGGGLVVSILVAIRYAGEPMPAAGVCISPWVDM
ETX02836.1            VEDALAAAYRWLLDQGTESGNIVLIGSDSAGGGLVVSALVAIRYAGEPMPAAGVCISPWVDM
                        *:* :***** *          * :***** :*:*:* * :***** * * *
SN1_7N9_esterase      EGTGETFITNAEVDPMVQKDLILQIAGVYLGKDPRAPLASPIHADLTGLPPLLQVGS
E40_esterase          EATGESFTTNATMDPSVKNRVMISAALYLGGKNPQAPLASPLYADLQGLPPLVQVGGI
AJZ73156.1            EGVGASMTSKAKEDPIVQKEMLLGMAKLYLGGADPKTPLAAPLHADLRGLPPLLQVGS
ACL67845.1            EGLGESMTTKADLDPMIQPGDILEGAKAYLGGADPRTPLAAPPLYADLTGLPPLLIHVGT
ADM67446.1            EALGESMTTKADADPTVERQGLLOMAKAYLGDHPRTPLAAPPLYADLTGLPPLLIHVGT
ETW98043.1            EGTGESFTTNADVDPVSKERIVNIAKVYLGKKNRAPLASPLHADLHELPLLSIVGSI
ETX02836.1            EATGESFTTNADVDPVSKERILNIAKVYLAGKHPRAPLASPLHADLHELPLLSIVGSI
                        * . * : : * * * . : : * * * . . :*****:***** ***** **
SN1_7N9_esterase      ETLLDDSNQLARLAKADGVEVKVEVWDDMPHVQDFAPILPEALQAIIDGIGEFIKKHTG-
E40_esterase          ETLLDDARALTTAKAAGVDADLEVWDDMPHVWQHFAPILPEGQQAIAIRIGEFLRKQIG-
AJZ73156.1            ETLLDDSTRVTERAKAAGVNVLDLEIWPDMIHVFQLFAPILPEGQEAIVAKIGKFIRESHTS-
ACL67845.1            ETLLDDSTRLAERAKAAGVNVLLQVWDEMIHVFQFFAAMLPEGQQAIDRIGEFIREHTGA
ADM67446.1            ETLLDDSTRLAERASAAAVDVTLEPWDDMIHVVQYFAAMLPEGQQAIERIAGFIREHTIGA
ETW98043.1            ETLLDDARVITERAQAAGVEAVLEVWDDMPHVWTHFAPILPEGQQAIVDRIGDFMRHQVG-
ETX02836.1            ETLLDDARAITERAQAAGVEAVLEVWDDMPHVWTHFASILPEGRQAVDRIGDFMRHQVG-
                        ***** : : * . * . . : : * : * * : * : * * : * : * . : : .
SN1_7N9_esterase      -----
E40_esterase          -----
AJZ73156.1            -----
ACL67845.1            ARGAVFEAAA
ADM67446.1            RAAAR-----
ETW98043.1            -----
ETX02836.1            -----

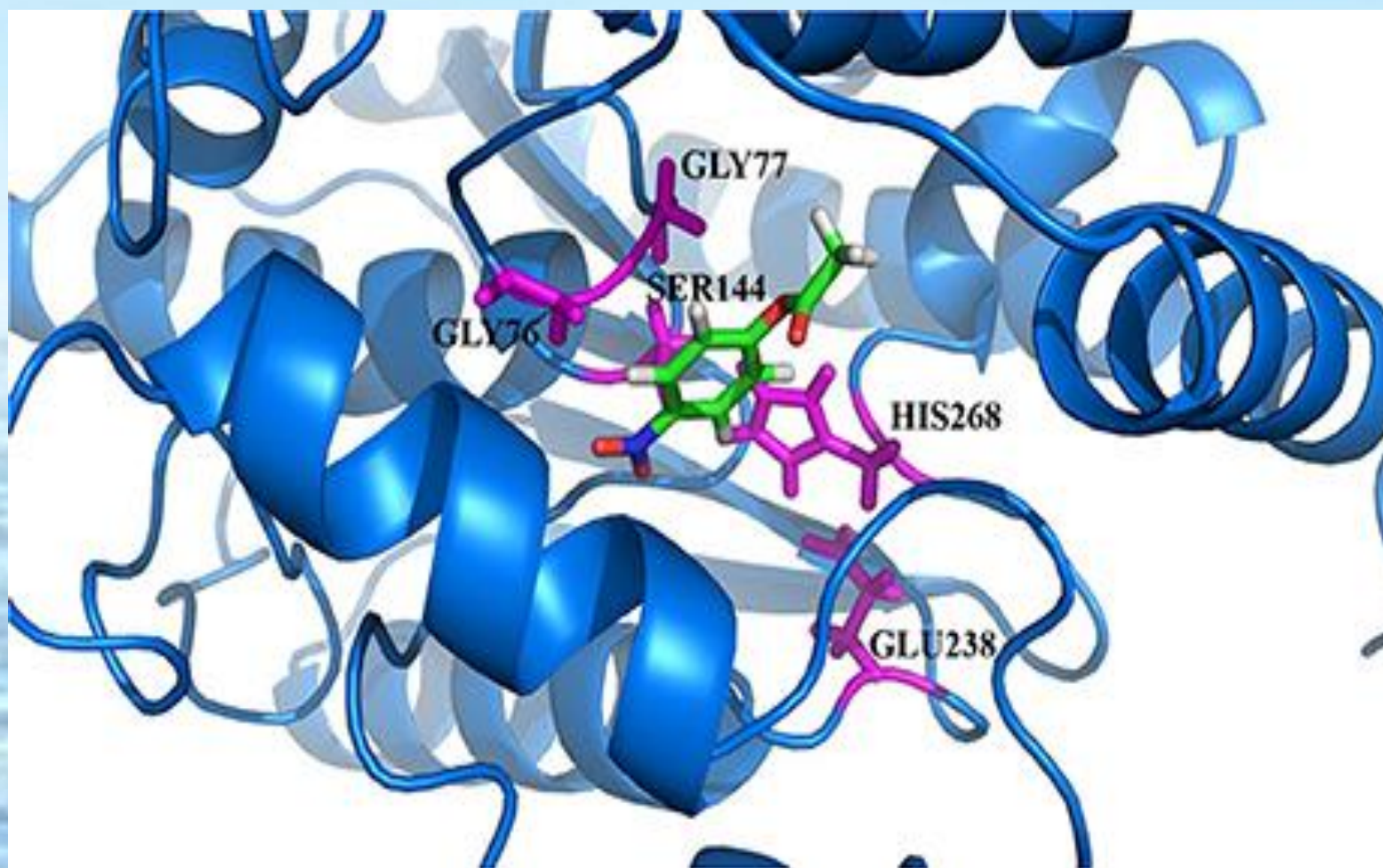
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GXSXG

Domain architecture of the 7N9 Esterase



3D Docking model of the preferred substrate 4-Nitrophenol Acetate



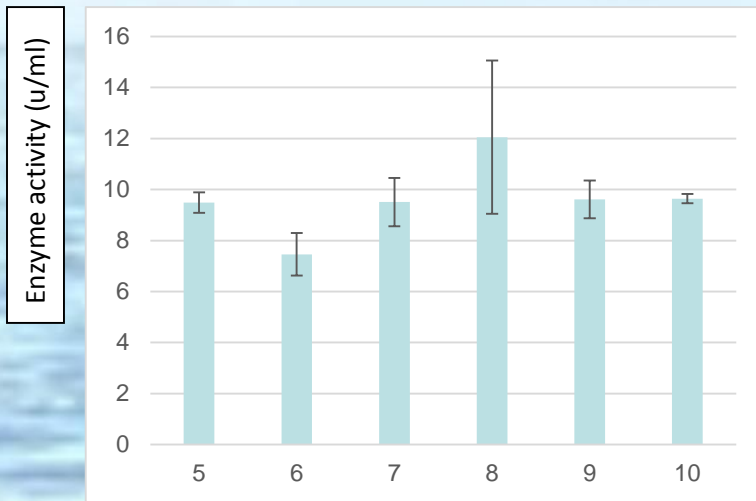
Enzyme characteristics

pH	Enzyme activity (U/ml)
5	9.49
6	7.46
7	9.51
8	12.05
9	9.61
10	9.64

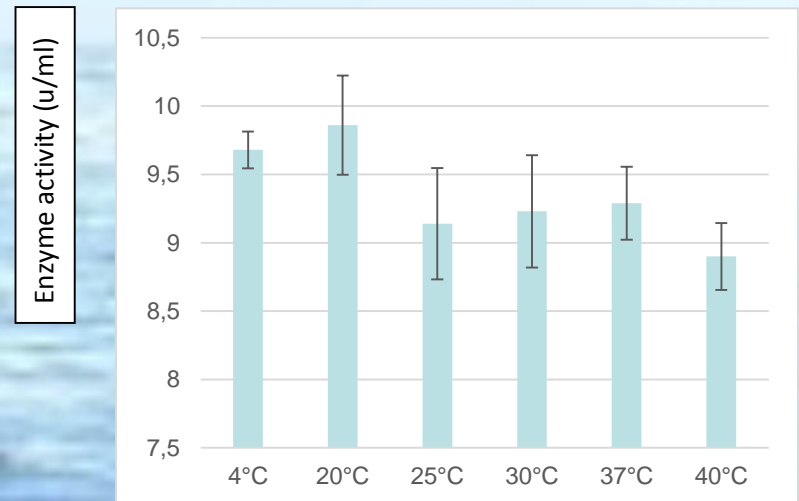
Optimum at pH 8

temperature	Enzyme activity (U/ml)
4°C	9.68
20°C	9.86
25°C	9.14
30°C	9.23
37°C	9.29
40°C	8.9

Cold adapted



pH

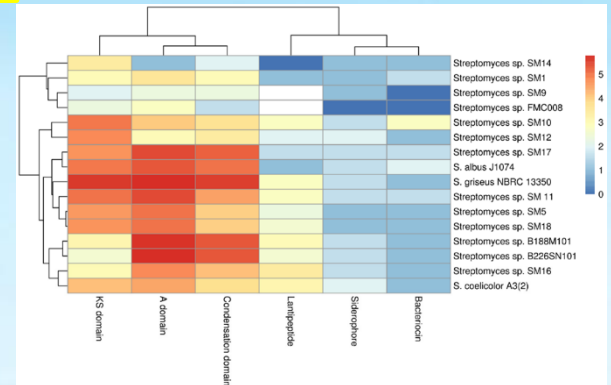


Temperature

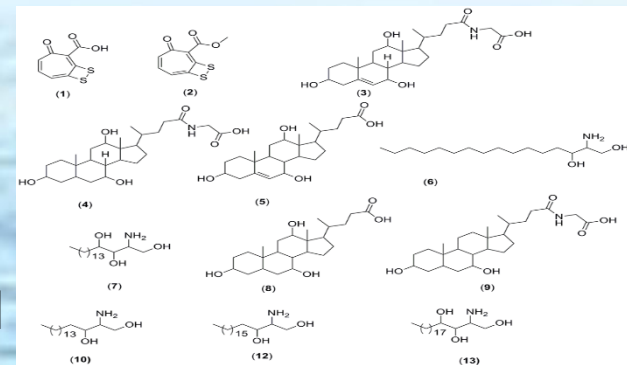
These properties would allow use of this enzyme in industrial related low temperature applications such as the manufacture of food ingredients or thermo-labile pharmaceutical products or the production of cold-wash detergents.

Summary

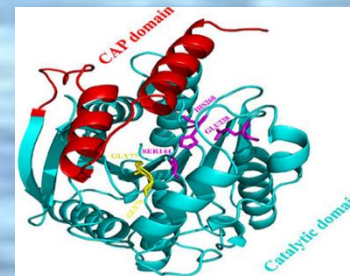
Marine sponge derived *Streptomyces spp.* isolates contain a diverse range and number of secondary metabolism biosynthetic gene clusters. Some of these may encode novel bioactive compounds, with potential biopharmaceutical applications.



Marine sponge derived *Pseudovibrio* species potentially produce a range of bioactive metabolites other than Heptylprodigiosin and Tropodithetic acid.



Functional metagenomics approaches can lead to the identification of enzymes with novel biochemical characteristics. (Cold adapted lipase).



Acknowledgements

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Teagasc Ashtown

Dilip Rai
Alka Choudhary



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Beaufort Marine Research Award**



**Thank
You**