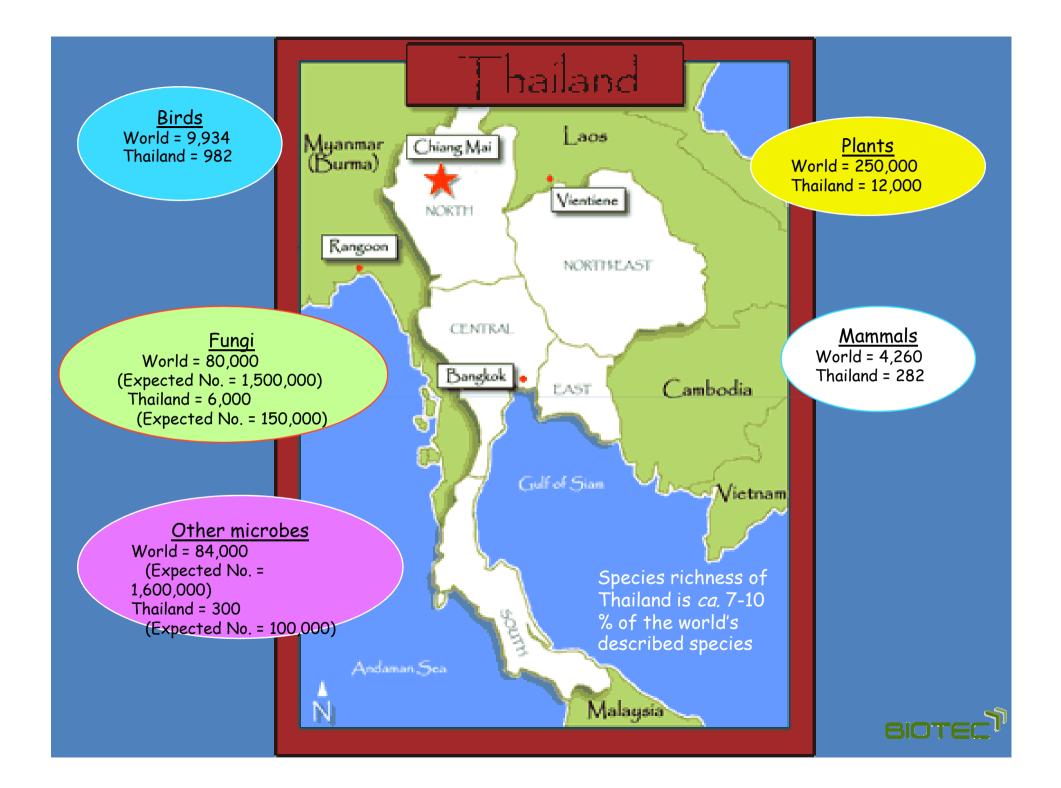
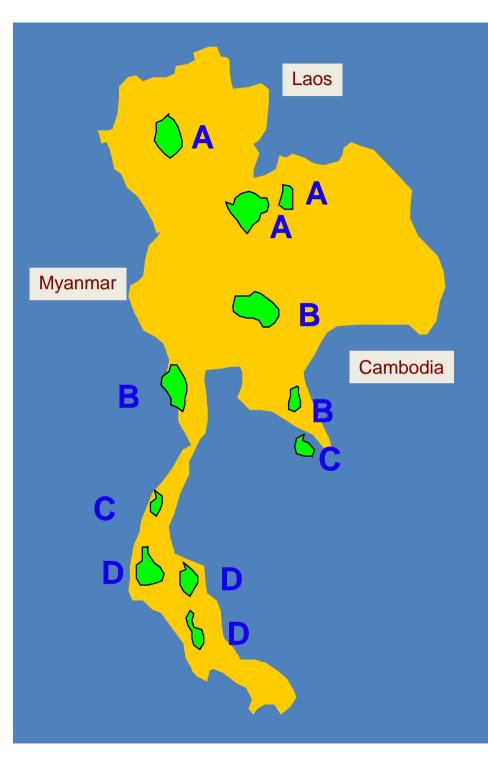
ดวามหลากหลายทางชี่วภาพของจุลินทรีย์ในประเทศไทย"

Diversity of microbes in the BIOTEC Culture Collection

Janet Jennifer Luangsa-ard jajen@biotec.or.th 1 April 2013 NAC 2013





FOREST TYPES

- A. Cool deciduous teak and pine forest
- **B. Evergreen monsoon forest**
- **C.** Coastal evergreen rain forest
- **D. Hill evergreen rain forest**



Survey and

collection

Wildlife Sanctuaries National Parks















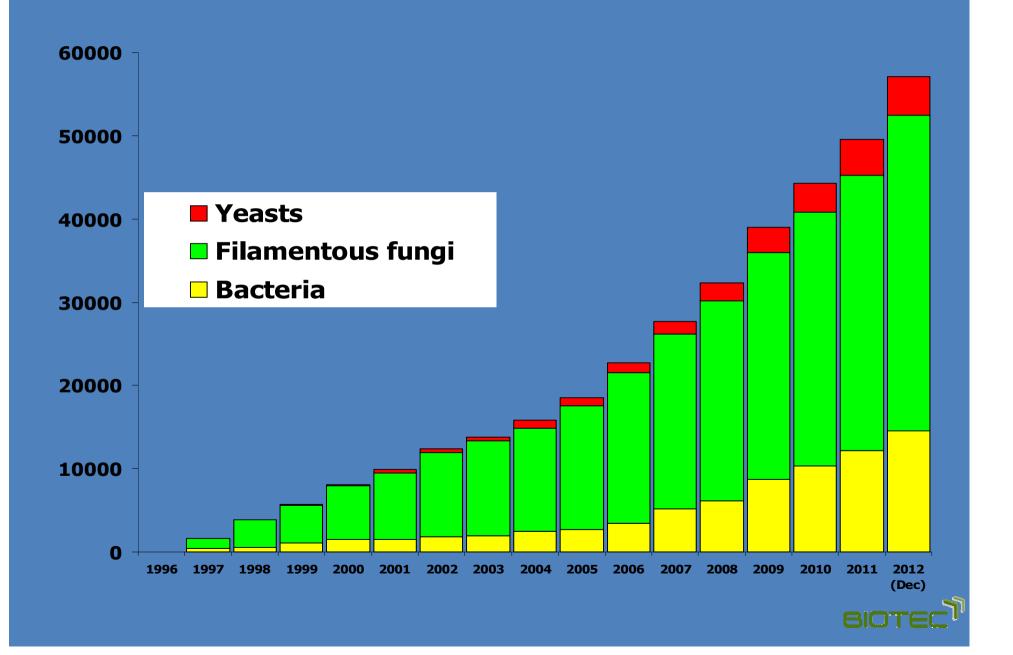
Ramong



Thailand Network on Culture Collections (TNCC) was established since 2000. The network consists of four major collections specialized in different groups of microorganisms:

- 1) **BIOTEC Culture Collection** (BCC) holds more than **57,000** strains (1,896 species of 984 genera) of filamentous fungi, yeasts and bacteria isolated from natural environments in Thailand.
- 2) DMST Culture Collection (DMST) has in the collection 32,000 strains of filamentous fungi, yeasts, bacteria and cell lines related to human disease control and prevention.
- 3) DOA Culture Collection (DOA) houses more than 6,000 strains of agriculturally important algae, bacteria and filamentous fungi.
- 4) TISTR Culture Collection (TISTR) collects more than 5,500 strains of agriculturally and industrially useful algae, bacteria, filamentous fungi and yeasts.

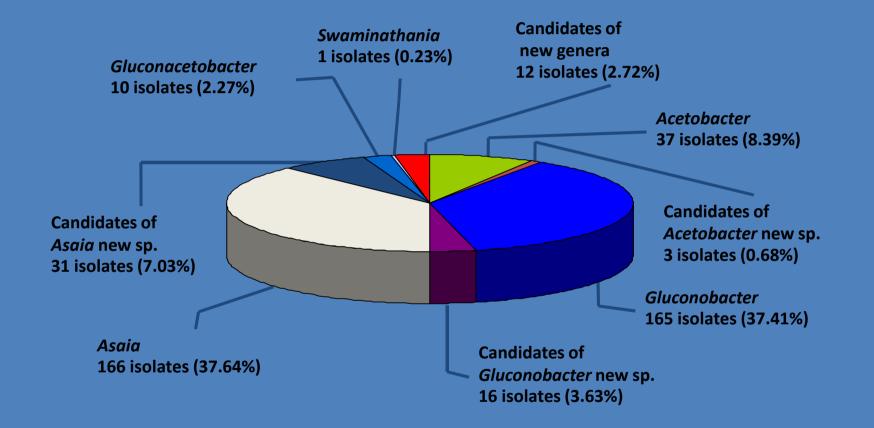
The number of microorganisms at BCC (57,079 samples)



BCC holdings

Group	No. strains	No. genera	No ₋ species
Bacteria	14,561	155	424
Filamentous fungi	38,012	767	1,158
Yeasts	4,621	62	314
Total	57,194	984	1,896

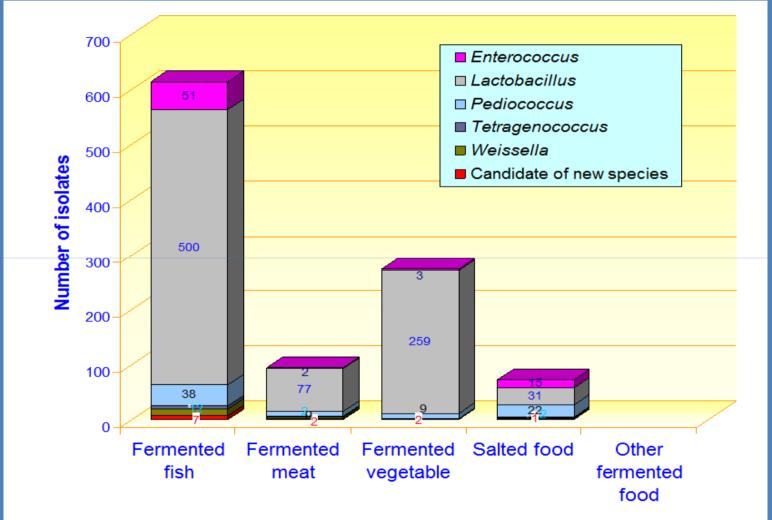
Taxonomic study of acetic acid bacteria in Thailand



441 strains of acetic acid bacteria from natural sources collected in Thailand

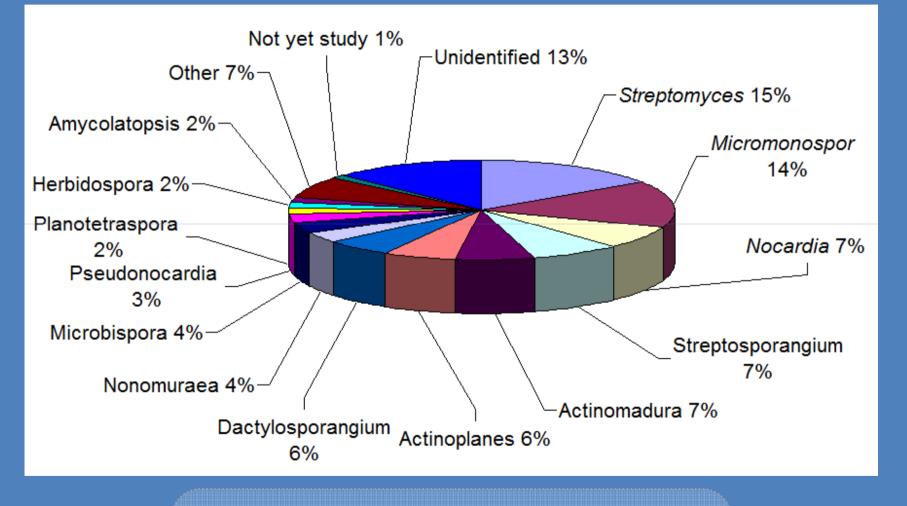


Taxonomic and ecological study of lactic acid bacteria from fermented foods in Thailand



1,053 strains of lactic acid bacteria from fermented food isolated since 2008

Filamentous actinomycetes isolated from Thailand for bioactive compound screening



7,984 strains of 43 genera isolated since 2005



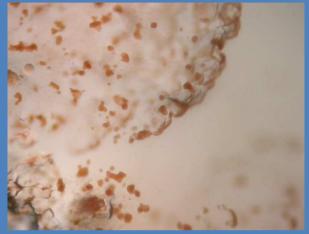
Myxobacteria isolated from Thailand for bioactive compound screening



Stigmatella



Condromyces





Myxococcus



Cystobacter



Corallococcus





Angiococcus

105 strains of 7 genera isolated since 2008



Fungi





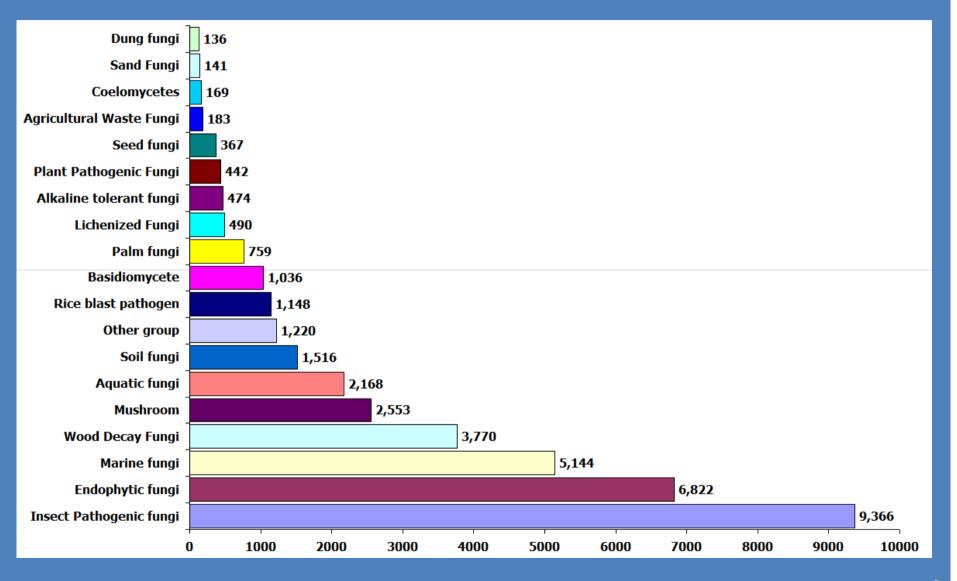


In 1989, BIOTEC started a research on insectpathogenic fungi. This became a BIOTEC program in 1993 which now concentrates on several taxonomic and ecological fungal groups:

- Alkalinophilic fungi
- Basidiomycetes
- Coelomycetes
- Endophytes
- Dothideomycetes
- Dung fungi
- Freshwater and Aquatic fungi
- Invertebrate-pathogenic fungi
- Lichens
- Marine fungi
- Palm fungi
- Plant pathogenic fungi
- Seed decay and leaf litter fungi
- Thermophilic fungi
- **Yeasts**

A total of 500-700 species of fungi had been recorded by the late 1980's while this figure now stands at around 6,000 species (Jones *et al.* 2004).

Group of Fungi in BIOTEC Culture Collection (38,012 Samples)



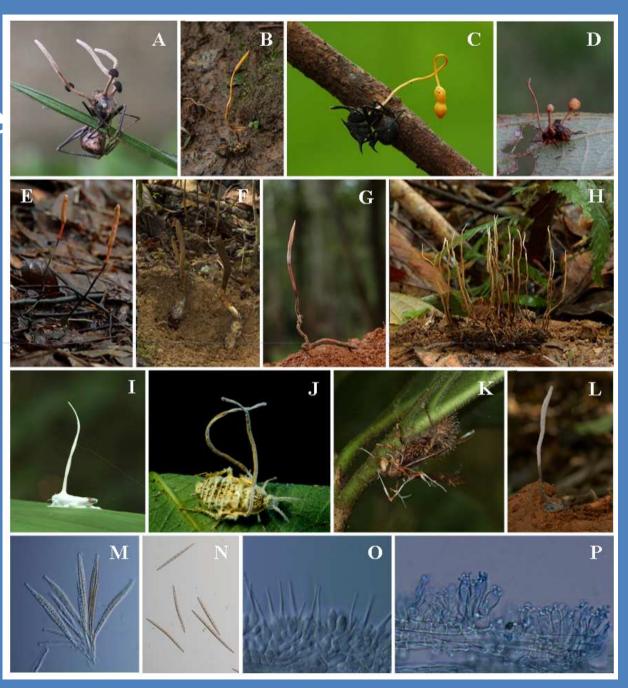
BIOTECI

Cordycipitaceae



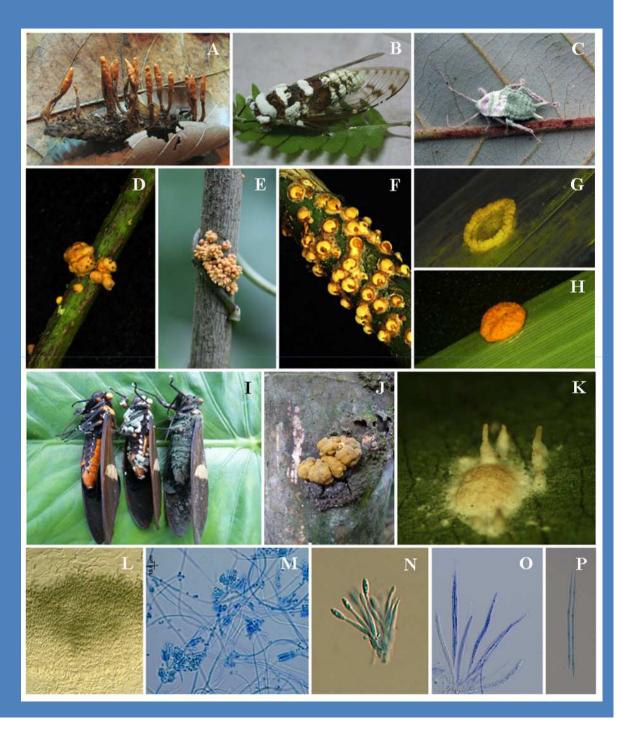


Ophiocordycipitaceae





Clavicipitaceae



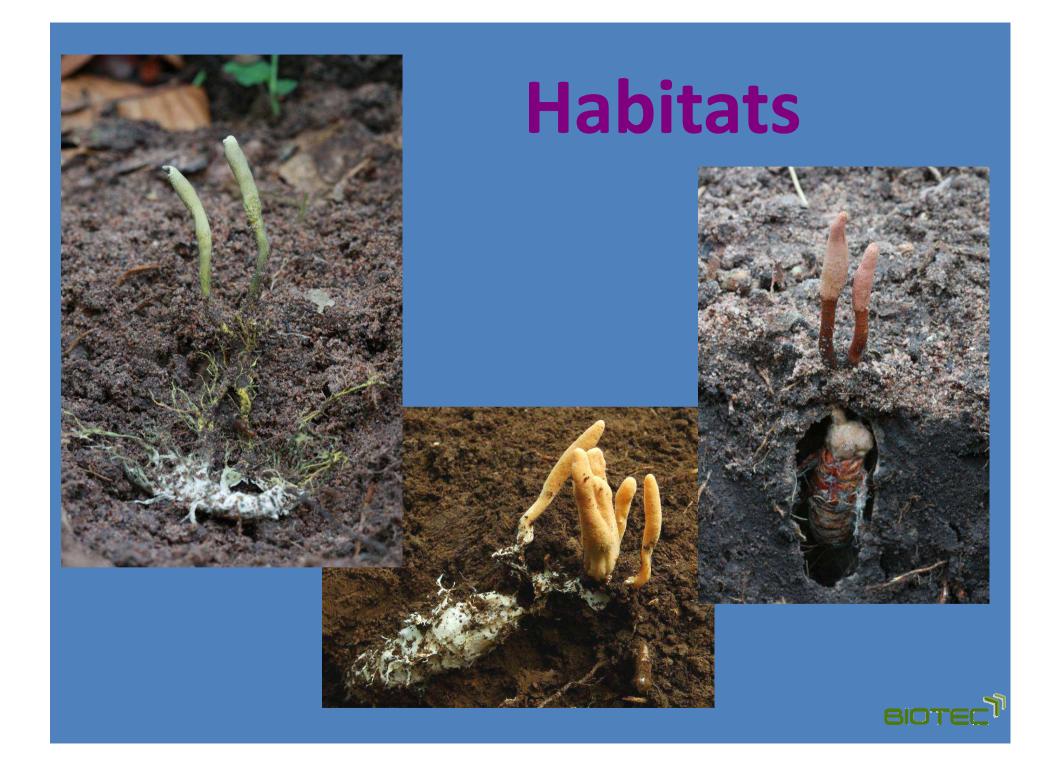


Host specificity

- General opportunistic pathogens
- Fastidious pathogens







Habitats





Habitats







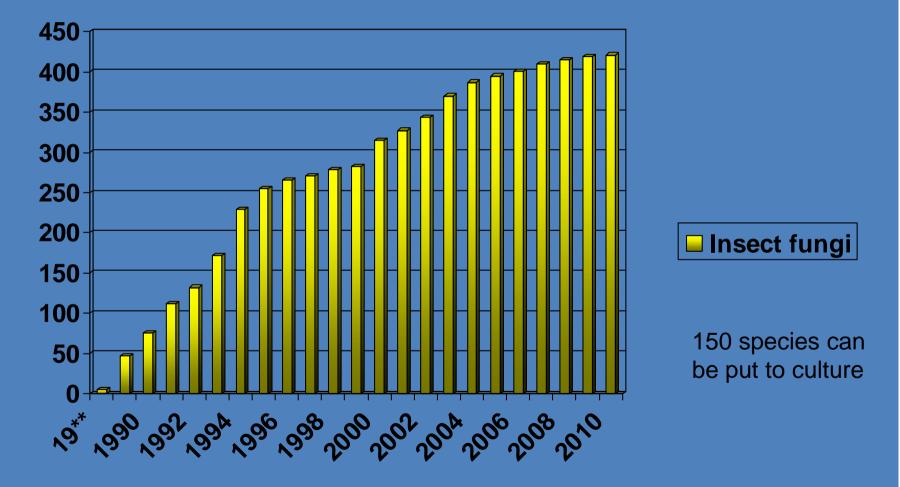
Habitats







Numbers of Insect Fungi in Thailand





Acc. Chem. Res. 2005, 38, 813-823

Bioactive Substances from Insect Pathogenic Fungi

MASAHIKO ISAKA, PRASAT KITTAKOOP, KANYAWIM KIRTIKARA, NIGEL L. HYWEL-JONES, AND YODHATHAI THEBTARANONTH*

National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Klong Luang, Pathumthani 12120, Thailand



Bioactive Substances from Insect Pathogenic Fungi Isaka et al.

FIGURE 3. Excavation revealing the larval host of Cordyceps sinensis.



FIGURE 4. Cordyceps sinensis stroma on Lepidoptera larvae.



FIGURE 5. Cordyceps unilateralis on an ant.

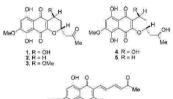
derivatives, 1-6. Interestingly, these naphthoquinones exhibited antimalarial activity with ICsg values of 2.5–10.1 $\mu g/mL$ (Table 1). The above naphthoquinones show a

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FIGURE 6. Cordyceps nipponica on ant lions.

deep red color under acidic conditions but intense purple in basic environments; such color characteristics are attractive to the pigment industry. Production of naphthoquinones by *C. unilateralis*, after optimization of fermentation conditions, can attain yields up to 3 g/L of culture broth.²⁸



Cordyceps nipponica was originally described from cicadas in Japan and is found infecting both cicadas and ant lions (Neuroptera) in Thailand. Two N-hydroxy-2pyridones, cordypyridones A (7) and B (8), and two tricyclic N-methoxy-2-pyridones, cordypyridones C (9) and D (10), were isolated from Cordyceps nipponica BCC 1389 (collected from Khao Yai National Park, central Thailand, Figure 6).²⁹ Cordypyridone A (7) is identical to 8-methyl-pyridoxatin, previously isolated from an unidentified fungus OS-F61800,30 while its atropisomer, cordypyridone B (8), was shown to be a metabolite of BCC 1389. A careful study indicated that interconversion between compounds 7 and 8 occurred upon heating the solution, and the absolute configuration of cordypyridone 7 (and hence its atropisomer, 8) was later determined using chemical means. Epoxidation of compound 11 (1-Omethyl derivative of 7) and subsequent cyclization gave the major product 12, which is the 14-hydroxy derivative of cordypyridone C (9). X-ray analysis of 13, the pbromobenzolate derivative of 12, revealed the proposed absolute configuration. Cordypyridones A (7) and B (8) exhibited potent antimalarial activity with respective IC50

Received March 7, 2005

ABSTRACT

Insect pathogenic fungi have opened up a relatively untapped area of natural product research which, unfortunately, has not received much attention to date. Found in wild abundance in wet tropical Thailand, the insect fungi are shown to contribute not only as controllers of insect populations but also as rich sources of structurally novel biologically active substances.

- Phomalactone common in various family and genera
- Oosporein common in various family and genera
- Beauvericin 7 strains, Coleopterans and lepidopterans
- Cordytropolone only from Cordyceps



Conoideocrellide A



pubs.acs.org/jnp

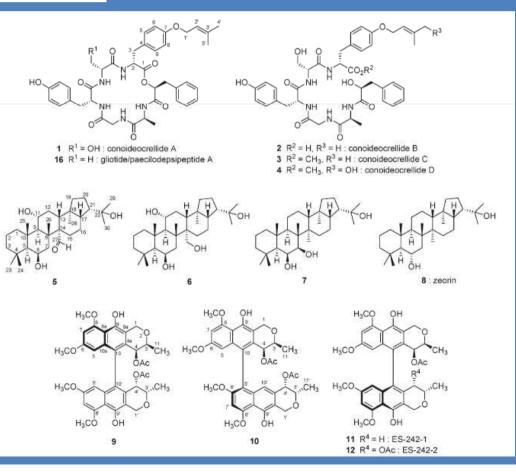
ARTICLE

Bioactive Compounds from the Scale Insect Pathogenic Fungus Conoideocrella tenuis BCC 18627

Masahiko Isaka,* Somporn Palasarn, Sumalee Supothina, Somjit Komwijit, and J. Jennifer Luangsa-ard

National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phaholyothin Road, Klong Luang, Pathumthani 12120, Thailand





NRPS screening

Cordycipitaceae:

Beauveria bassiana Cordyceps ninchukispora Isaria tenuipes Gibellula sp.





Clavicipitaceae:

Aschersonia samoensis Metarhizium flavoviride Hypocrella discoidea

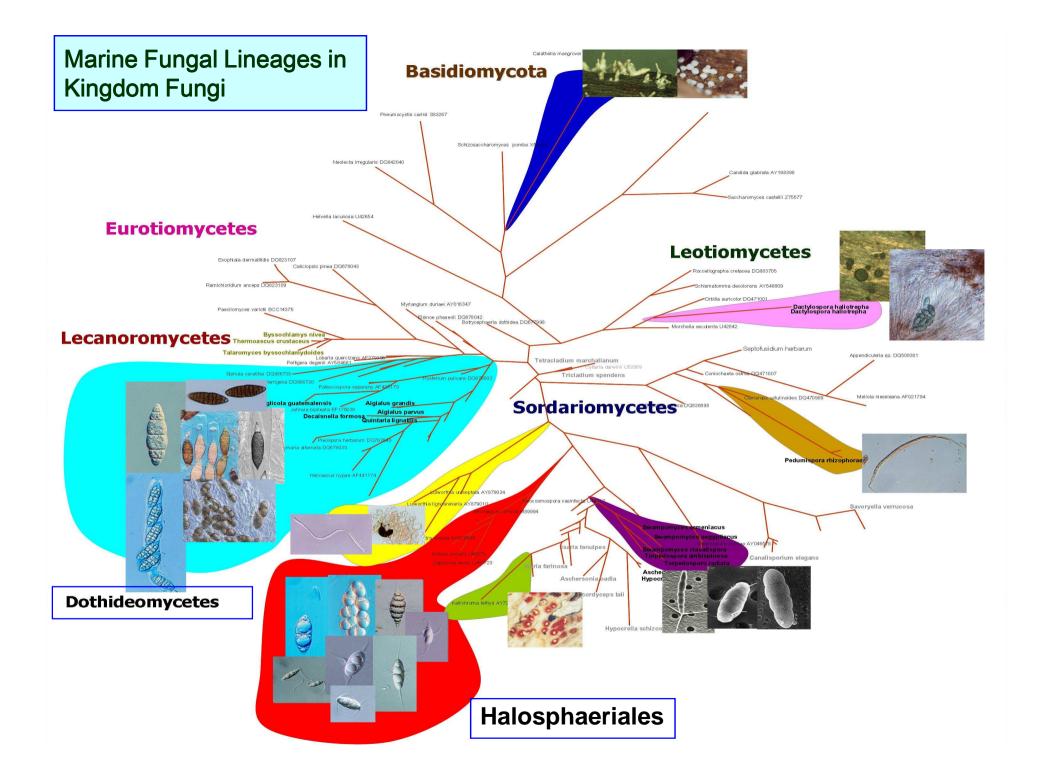


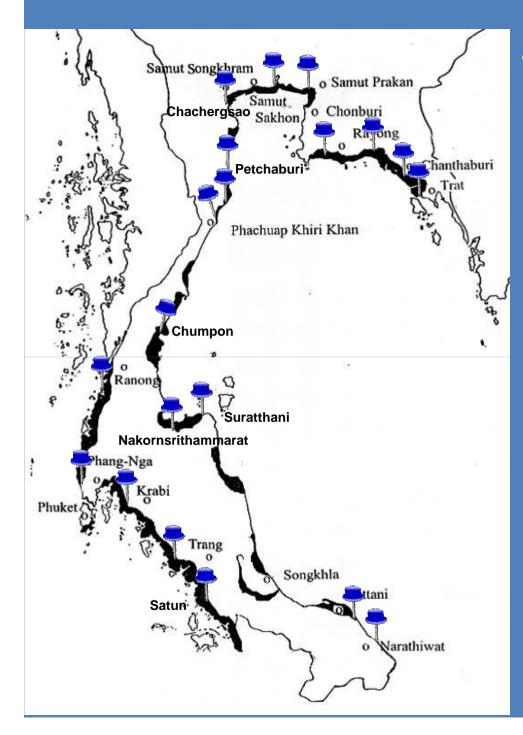


Marine Fungi

- Marine fungi are an ecological group that are able to grow and sporulate in marine habitats (Kohlmeyer & Kohlmeyer, 1979)
- Marine fungi grow on a variety of substrata.
- Marine fungi are mostly saprobes, and produce a wide range of enzymes. They play a major role in decaying woody materials in the marine habitats.







Thai marine fungal diversity

• Studies of marine fungi in Thailand have been restricted to sandy beaches, sea shores, mangroves and estuarine *Nypa* palm forests along Thai Gulf and Andaman sea.

•Marine and mangrove fungi documented for Thailand: total 163 (Jones *et al.* 2006, Sakayaroj et al 2011).

•A comprehensive classification of the filamentous marine fungi worldwide has outlined 530 sp .(from 321 genera) (Jones et al. 2009)

NEW RECORDS for Thailand

Lindra thalassiae

Swampomyces cf aegyptiacus

Lautospora simillima

Mauritiana rhizophorae

Manglicola guatemalensis Patellaria atrata





Carinispora nypae

Aigialus rhizophorae

Aigialus mangrovei

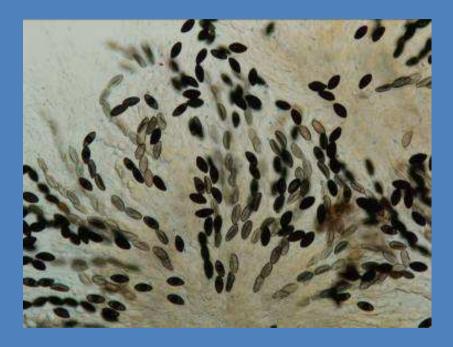
Marine fungi as source of bioactive metabolites



- Marine fungi are of particular interest to the pharmaceutical industry
- The most successful marine fungi in producing new compounds are those isolated from mangrove habitats.
- They grow under extreme/stressful conditions and thus may produce a wider range of bioactive compounds.

Aigialus parvus BCC5311

Halorosellinia oceanica BCC 5149



• Produced halorosellins A and B,

4,8-dihydroxy- 6-methoxy-4,5-dimethyl-3methyleneisochroman-1-one, 3-acetyl-7-hydroxy-5-methoxy-3,4-dimethyl-3*H*isobenzofuran-1-one and 17-dehydroxyhalorosellinic acid

Exhibited anti mycobacterial activity



Aigialomycins A and Hypothemycin showed significant antimalarial and antitumor properties

Isaka et al., 2002, J.Org. Chem. 67:1561-1566. Isaka et al 2009, Tetrahedron 65 (2009) 4396-4403. Vongvilai et al., 2009. J. Nat. Prod. 67: 457-460 Chinworrungsee, M., et. al. J. Chem. Soc., Perkin Trans. 2002; 2004

Thraustochytrids: A potential PUFA source

• Thraustochytrids are a group of nonphotosynthetic, heterotrophic marine organisms classified under the Labyrinthulomycetes, Kingdom Straminipila.

• Recently, attention has been given to this group of organisms because of their high lipid contents, particularly Poly Unsaturated Fatty Acids (PUFAs).

• With the continuously decreasing resources for traditional fish oils as PUFA sources for aquaculture, thraustochytrids offer a promising alternative and stable source for heterotrophic fatty acid production.





Marine and estuarine habitats: seawater, marine sediments, littoral algae, seaweeds, invertebrates, fallen mangrove leaves

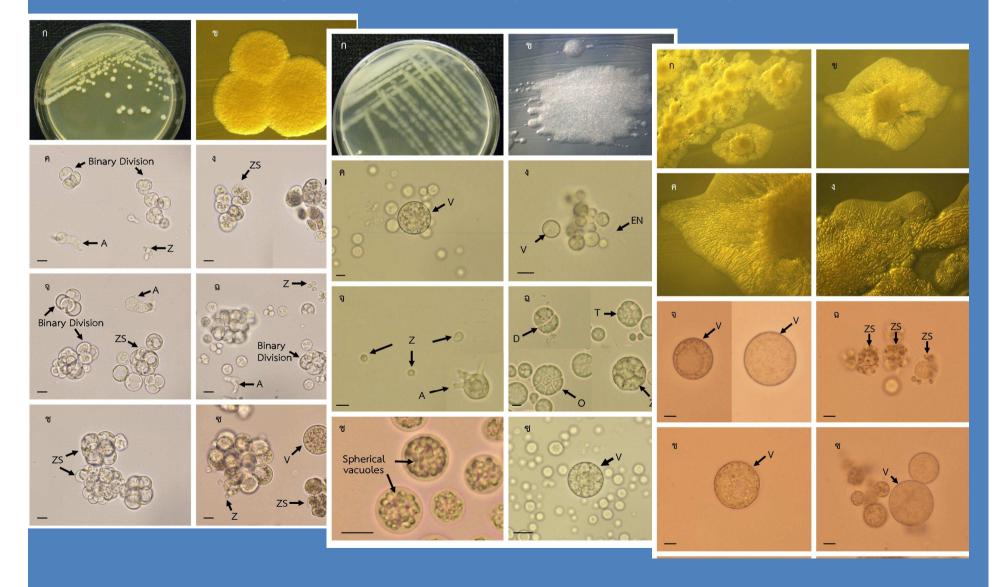


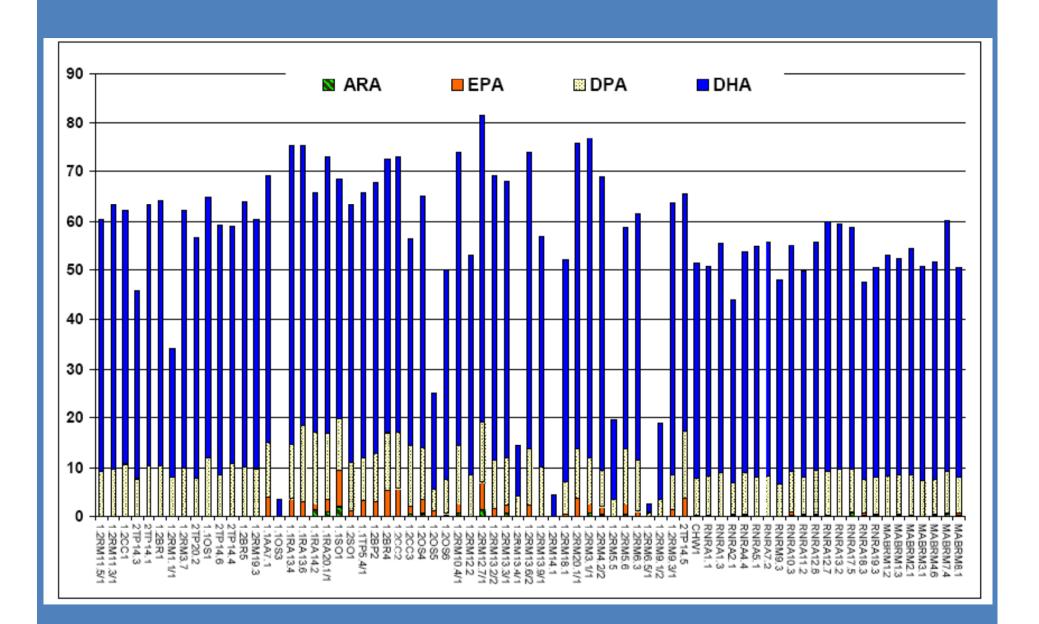


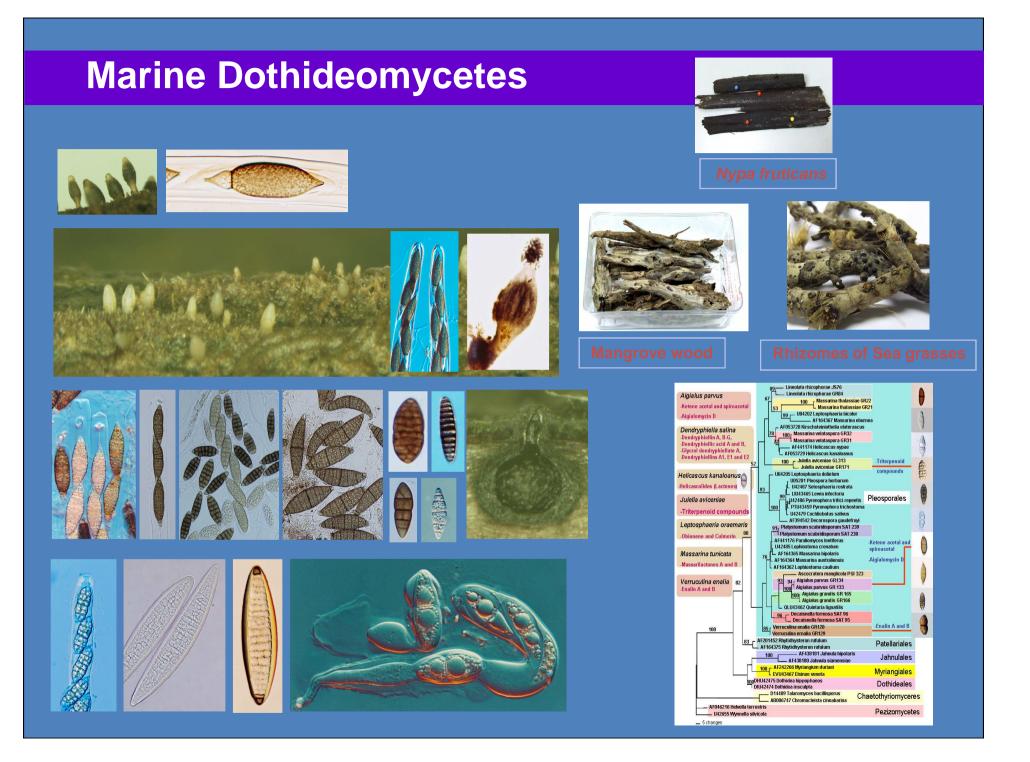


Colony appearance, dividing cells, zoosporangium, zoospores and mature cells of thraustochytrids.

(The most common genera are *Aurantiochytrium* and *Schizochytrium*.)

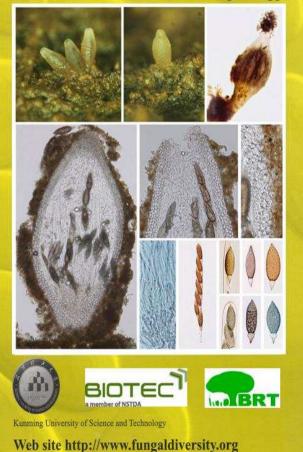






Fungal Volume 35, March 2009 Diversity

An International Journal of Mycology

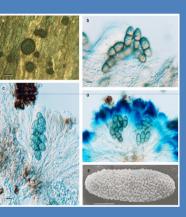


Classification of marine Ascomycota, anamorphic taxa and Basidiomycota

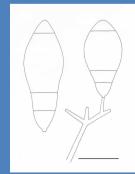
Jones, E.B.G.^{1*}, Sakayaroj, J.¹, Suetrong, S.^{1,3}, Somrithipol, S.¹ and Pang, K.L.²

¹Bioresources Technology Unit, Phylogenetics Laboratory, National Center for Genetic Engineering and Biotechnology, 113 Paholyothin Road, Khlong 1, Khlong Luang, Pathum Thani 12120, Thailand ²Institute of Marine Biology, National Taiwan Ocean University, No. 2 Pei-Ning Road, Keelung 20224, Taiwan ³Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand.









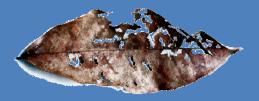
Fungal Diversity

Examples of bioactive compounds from Thai marine fungi

Species	Activity	Chemical structure	References
Aigialus parvus	Anti-malarial	Aigialomycin D, Aigialone and aigialospirol	Isaka et al 2002; Vongvilai et al 2004
Halorosellinia	Weak anti-	Halorosellinic acid	Chinworrungsee et al,
oceanica	mycobacterial		2001
Halorosellinia	Mild anti-	Halorosellins A, B	Chinworrungsee et al,
oceanica	mycobacterial		2004

FUNGI ON DECAYING SEEDS AND LEAF LITTER





Leaf litter fungi (Twelve new described species)

- Berkleasmium typhae
- Calcarisporium phaeopodium
- Dictyoarthrinium synnematicum
- Digitoramispora lageniformis
- Falcocladium turbinatum
- Infundibulomyces cupulata
- Infundibulomyces oblongisporus
- Lauriomyces cylindricus
- Lauriomyces ellipticus
- Melanogrophium proliferum
- Pseudoacrodictys dimorphospora
- Pseudorobillarda siamensis

One fungus produces a new bioactive compound.

 Kionochaeta ramifera → Ramiferin A



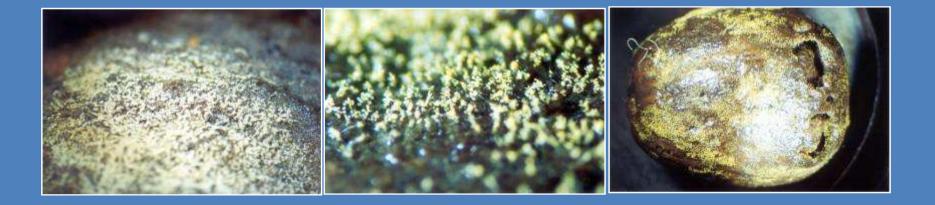
Fungi on decaying seed (Two new described species)

- Cirrenalia nigrospora
- Lauriomyces sakaeratensis

Two fungi produce 2 new bioactive compounds.

- Kionochaeta pughii
 → Pughiinin A
- Menisporopsis theobromae
 → Menisporopsin A

Fungal characteristics under dissection microscope

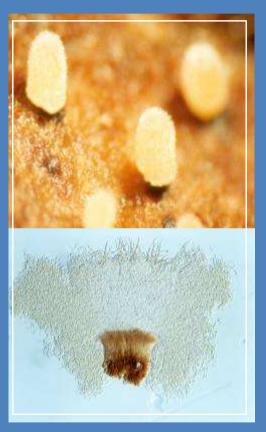




Common species on decaying fruits and seeds



Dictyochaeta sp.



Thozetella nivea





Cryptophialoidea secunda

Dinemasporium lanatum

Anamorphic fungi on decaying fruits and seeds



Somrithipol, S., Hywel-Jones, N.L. and Jones, E.B.G., and 2004. Seed fungi. In Thai Fungal Diversity. (E.B.G Jones, M. Tanticharoen and K.D. Hyde, eds.). BIOTEC, Thailand. pp. 129-140.

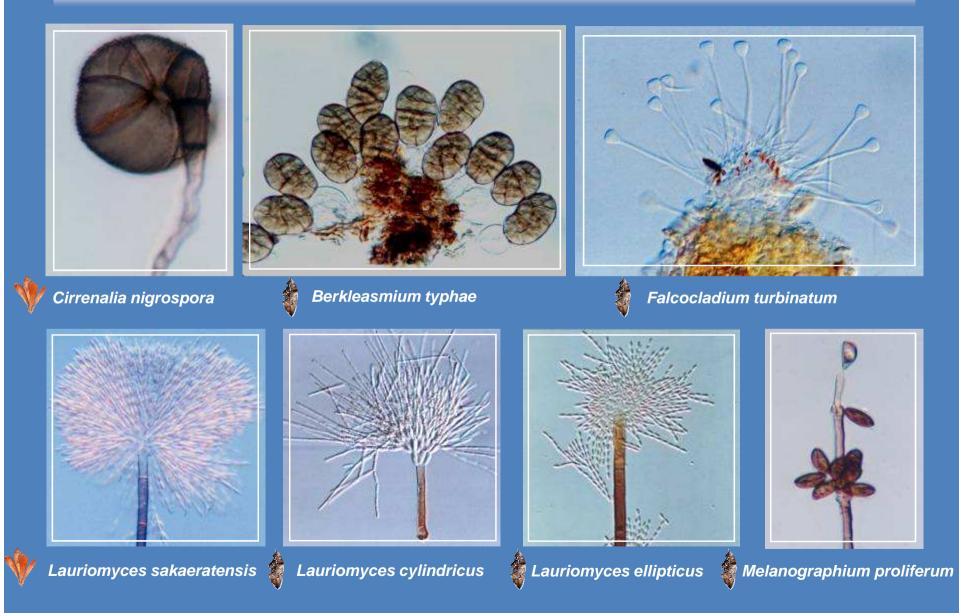
Teleomorphic fungi on decaying fruits and seeds







Some new fungi on seed and leaf litter



Lichens







Lichen herbarium

- 3,515 specimens comprising
- 3 orders
- 14 families
- 33 genera and ~ 250 species

Lichenized fungi

- 152 strains
- 36 strains showed the bioactive screening against anti-*Candida albicans* (30%), anti-*Herpes simplex* virus (50%), anti-tuberculosis (25-50 μ g/ml), anticancer (5%) and anti-malaria at ED₅₀ = 0.84-12.68 μ g/ml
- 2 new compounds were found

Freshwater fungi

Lignicolous Freshwater fungi





Groups encountered

- Ascomycota
- Basidiomycota
- Mitosporic (excluding Ingoldian fungi) : >300 spp.
- Aquatic & aero-aquatic fungi

:~4500 specimens : >400 spp. : ~ 15 spp.

- - : ~ 200-300 spp.





Aquatic & aero-aquatic fungi





WEEK





Identification of Xylariaceae by inducing the formation of fruiting bodies



Palm Fungi

Peat

swamp

palms

Terrestrial

palms

Selected palms to study

- 1. Licuala longicalycata
- 2. Nenga pumila
- 3. Eleiodoxa conferta
- 4. Metroxylon sagu
- 5. Calamus spp.
- 6. Arenga sp.
- 7. Elaeis guineensis

Potential application of fungal isolates from palms

- Weight loss from basidiomycetes
 - Antagonistic fungi from palms
 - Edible mushroom cultivation from oil palm waste

Results

Saprophytes Ascomycetes 251 taxa Basidiomycetes 298 collections Anamorphic fungi 293 taxa

Endophytes

717 morpho types (4 different trips)

New Taxa

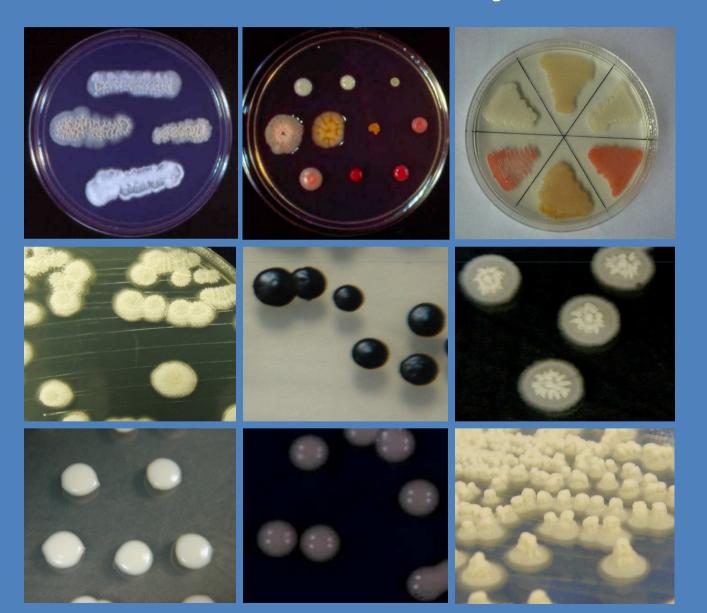
- 18 new species were described including 13 genera (5 new genera, 13 new species)
- ✤ 15 new species await descriptions



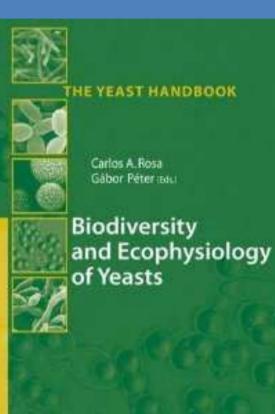
Sources of yeasts



Yeasts colony



Biodiversity of Yeasts in Thailand and Asia



Springer

Yeast biodiversity in tropical forest of Asia

by T. Nakase and S. Jindamorakot published in The Yeast Handbook "Biodiversity and Ecophysiology of Yeasts"

Edited by Carlos A. Rosa and Gábor Péter Springer January 2006

http://www.cplbookshop.com/contents/C2426.htm

New Yeast Species Found in Thailand

be rich in microbial biodiversity. Since the recognized, 34 ascomycetous yeasts and first report describing new yeast species, 25 of basidiomycetous yeasts. These Kockovaella thailandica and K. imperatae studies suggested that a vast number of from Thailand (Nakase et al., 1991), the unknown yeast species are living in the describing new yeast taxa have been natural environment of Thailand. increased year and year and 59 new yeast

List of new yeast species found in Thailand

Candida asiatica (Limtong et al, 2010b) Candida asiauca (Linitori et al., 2010) Candida easanensis (Jindamorakot et al., 2004) Candida chanthaburiensis (Linitong et al., 2010a) Candida golubevii (Rosa et al., 2009) Candida jaroonii (Imanishi et al., 2008) Candida jaroonii (Imanishi et al., 2008) Candida gosingica (Chang et al. 2010) Candida kasegawae (Nakase et al, 2007)
Candida kasenaburiensis (Nakase et al, 2008)
Candida khaoyaiensis (Jidamorakot et al, 2008) Chiang Mai 10. Candida kungkrabaensis (Limtong et al. 2010) Candida kratjensis (Lintong et al, 2004)
Candida kratjensis (Lintong et al, 2004)
Candida kazuoi (Nakase et al, 2007)
Candida lignicola (Jindamorakot et al, 2007) 14 Candida morakotiae (Nakase et al. 2009) Candida nakhorratchasimensis (Jindamorakot et al, 2009)
Candida nonsorbophila (Nakase et al, 2009)
Candida pattaniensis (Jindamorakot et al, 2004) North Candida paragnersis (Limtong et al. 2004)
Candida phangnersis (Limtong et al. 2008)
Candida ratchasimensis (Jindamorakot et al. 2008)
Candida sekii (Limtong et al. 2009b)
Candida sanitii (Limtong et al. 2009b) 22. Candida saraburiensis (Nitivon et al. 2010 16***** 22* Candida siamensis (Boonmak et al, 2009)
Candida sithepensis (Limgtong et al, 2004)
Candida songkhlaensis (Imanishi et al, 2008) North East 22 26. Candida stellimalicola (Suzuki et al. 1994) Candida suratensis (Limtong et al, 2010)
Candida suvanaritii (Limtong et al, 2009b 29. Candida thialandica (Jindamorakot et al. 2007 21 34 Candida thaimueargensis (Limtong et al. 2007b)
Candida wancherniae (Nakase et al. 2009)
Candida xylanilytica (Boonmak et al. 2010) 214,33+ 40441442 33. Citeromyces siamensis (Nagatsuka et al. 2002) ² Central Geotrichum phurueaensis (Kaewwichian et al, 2010)
Geotrichum siamensis (Kaewwichian et al, 2010)
Hanseniaspora singularis (Jindamorakot et al, 2009) 37. Hanseniaspora thailandica (Jindamorakot et al. 2009) Kazachstania siamensis (Limtong et al. 2007c)
Kloeckera hatyaiensis (Jindamorakot et al. 2009)
Kluyveromyces siamensis (Am-In et al. 2008) 41. Millerozyma phetchabunensis (Tammawong et al, 2010) Ogataea chonburiensis (Limgtong et al, 2008)
Ogataea nakhonphanomensis (Limtong et al, 2008)
Pichia jaroonii (Limtong et al, 2009a) 45 Pichia koratensis (Nakase et al. 2007 Pichia Vateriala (randos et al. 2007)
Pichia nongkratonensis (Limtong et al. 2004)
Pichia nongkratonensis (Nakase et al. 2005)
Pichia thermomethanolica (Limtong et al. 2005) 49. Tetrapisispora namnaonensis (Sumpradit et al. 2005) Torulaspora malenae (Limbrais (Sumpran et al. 2007a)
Vanderwaltozyma tropicalis (Nakase et al. 2010)
Wickerhamomyces edaphicus (Limtong et al. 2009) S. Bullera arundinariae (Fungsin et al. 2002b)
Bullera koratensis (Fungsin et al. 2006)
Bullera lagerstroemiae (Fungsin et al. 2006)
Bensingtonia musae (Takashima et al. 1995) 57. Bullera panici (Fungsin et al, 2003) 58. Bullera penniseticola (Takashima et al, 1998) 59. Bullera sakaeratica (Fungsin et al, 2003b) 11 4 24 4 50. Bullera siamensis (Fungsin et al. 2003a) Bensingtonia thailandica (Fungsin et al. 2003)
Bensingtonia thailandica (Fungsin et al. 2001)
Fellomyces thailandicus (Prillinger et al. 1997)
Kockovaella barringtoniae (Fungsin et al. 2001)
Kockovaella imperatae (Nakase et al. 1991) 33 20 ▲ 13 ▲ 65 Kockovaella thailandica (Nakase et al. 1991 Kockovaella inaliandica (Nakase et al, 1991)
Kockovaella sacchari (Takashima et al, 1998)
Moniliella fonsecae (Rosa et al, 2009) South 68. Pseudozyma parantarctica (Sugita et al, 2003) dior Besudozyma thailandica (Sugita et al. 2003)
Speciozyma thailandica (Sugita et al. 2003)
Sporobolomyces nylandi (Takashima and Nakase, 2000)
Sporobolomyces poursokiae (Takashima and Nakase, 2000)
Sporobolomyces blumeae (Takashima and Nakase, 2000) 2 31* 154+ Basi 17 3. Sporobolomyces vermiculatus (Takashima and Nakase, 2000) Tilletiopsis derxii (Takashima and Nakase, 2001)
Tilletiopsis oryzicola (Takashima and Nakase, 2001) 6# Iletiopsis penniseti (Takashima and Nakase, 2001) 2 12 59 77. Trichosporon siamense (Nakase et al. 2006) Mushroom Water from mangrove Sediment Soil Other 21A Insect frass Elower Fruit Exudate + Moss

Thailand have long been considered to species from Thailand are currently

26

11*

References d (2008) FEMS Venet Rev. 8, 823-82

FEMS Yeast Res. 9, 504-11 FEMS Yeast Res. 10(1): 11

mak et al (2009) FEMS Yeast Research. 9, 668-67. mak et al (2010) IJSEM (DOI:ijs.0.021873-0)

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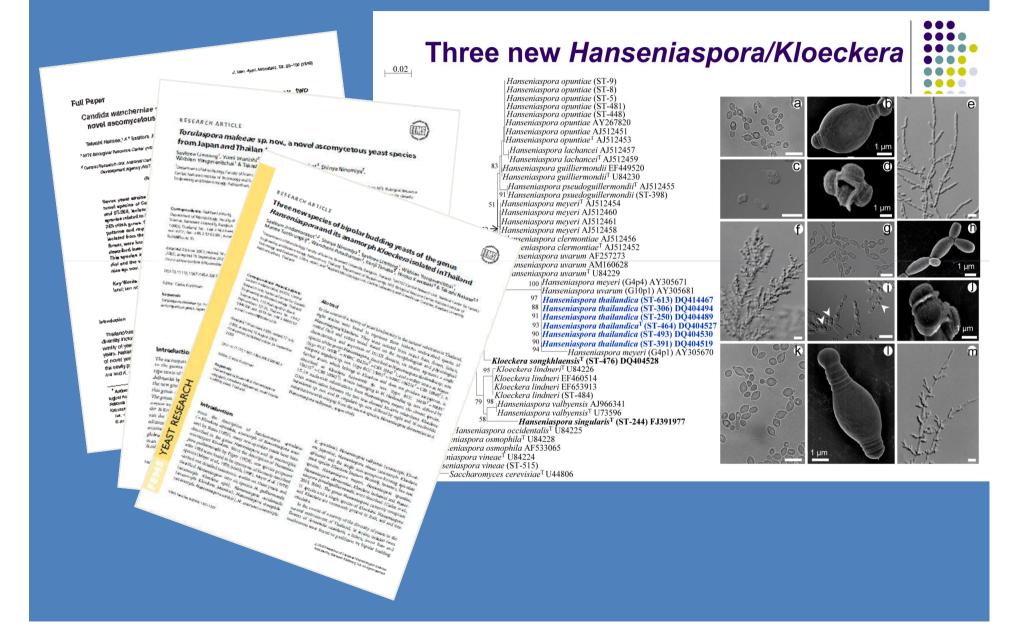
7 .38

Diversity of yeasts in Thailand

Many new species of yeasts have been isolated from Thailand and described in recent years.

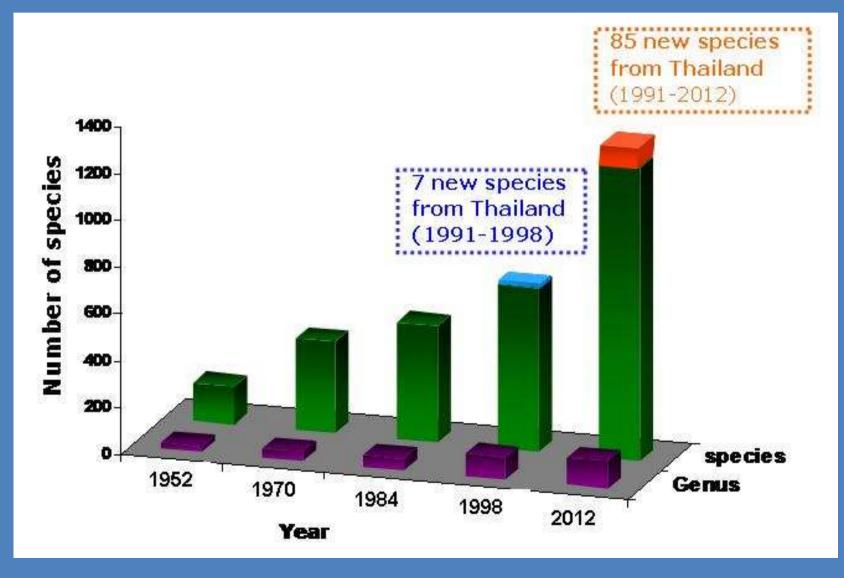
More than 80 species of ascomycetous yeasts have been described as new species from various kinds of substrates in Thailand from 1991-2012.

Many new yeast species published from Thailand



The increasing number of recognized species

(The Yeast, A Taxonomic study 5th edition)



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Thank you for your attention

