การจำแนกชนิดแบคทีเรียในสกุล *Asaia* Yamada et al. 2000 โดยการวิเคราะห์รูปแบบดีเอนเอที่ได้จากการตัดดีเอนเอบริเวณ 16S rDNA ด้วยเอนไซม์ตัดจำเพาะ

Identification of strains assigned to the genus *Asaia* Yamada et al. 2000 based on 16S rDNA restriction analysis

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Thirteen representative strains assigned to the genus *Asaia* were examined for restriction analysis of 16S rDNA. On digestion of 16S rDNA PCR products with three restriction endonucleases, *Sty*I, *Bsa*JI, and *Sna* BI, four restriction groups were recognized by combination of the resulting restriction patterns. Of the four restriction groups, eight strains of Group A were identified as *A. bogorensis*. Two strains each of Group B and Group C were respectively identified as *A. siamensis* and *A. krungthepensis*. The exceptional one strain, which was grouped into Group D, was identified as *A. bogorensis*. The 16S rDNA restriction analysis using three restriction endonucleases, which was discussed taxonomically and phylogenetically in comparison with the 16S-23S rDNA ITS restriction analysis using two restriction endonucleases previously reported, was useful for identifying *Asaia* strains at the species level as well.

การศึกษาราน้ำเบื้องต้นจากแหล่งน้ำธรรมชาติที่อุณหภูมิแตกต่างกันในประเทศไทย

Preliminary study of freshwater fungi in natural habitat at different temperature of Thailand

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This project has been undertaken to study and carry on the diversity of freshwater lignicolous fungi in natural habitat at different temperature of Thailand. Two hundred natural submerged woods from four sites (Saphan Yung hot stream; 43˚C, Wang Chumpee waterfall; 25˚C, Klong Ploo waterfall; 27˚C and Than Bokorani waterfall; 25˚C) were collected and incubated in plastic boxes. Their included 112 Mitosporic fungi (70.8%), 33 Ascomycetes (20.8%), 5 Coelomycetes (3.2%) and 8 unidentified (5.2%) of the total taxa. While 158 strains of freshwater fungi were isolated in to pure culture and preserved in BIOTEC Culture Collection for screening of bioactive compound.

ลักษณะทางชีววิทยาบางประการของราน้ำ *Achlya ambisexualis* ที่แยกจากไข่ปลานิล (*Oreochromis niloticus* Linn.)

Some biological characteristics of *Achlya ambisexualis* isolated from Tilapia Eggs (*Oreochromis niloticus* Linn.)

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Fungal infected tilapia eggs (Oreochromis niloticus Linn.) were collected from private fish farms (Kalasin, Khon Kaen and Sakon Nakhon provinces), an Inland Fisheries Station (Kalasin province) and Inland Fisheries Research and Development Centers (Khon Kaen, Mahasarakham and Sakon Nakhon provinces) during June-July, October-November 2005 and March-April 2006. Several fungal species have been isolated and identified. The effects of temperature, pH and NaCl on mycelial growth were studied. Achlya ambisexualis was isolated from fish hatcheries (Khon Kaen and Sakon Nakhon provinces). Biological characteristic examination of the fungus showed that the optimum temperature and pH for mycelial growth were 15-35°C and 4-11, respectively A. ambisexualis could grow on glucose yeast extract agar containing various concentrations of NaCl and it was able to tolerate up to 15 ppt NaCl.

การศึกษาความสัมพันธ์ของราน้ำในสกุล *Hymenoscyphus*

The significance of the anamorph in the mega genus *Hymenoscyphus*

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The mega genus *Hymenoscyphus* is in the order Helotiales and known from a broad range of substrata and habitats in both terrestrial and aquatic environments. A group of aquatic hyphomycetes as the anamorph stage in *Hymenoscyphus* (*Tricladium* spp. and *Varicosporium* spp.) is included for study of their relationships and classification. The result of a molecular phylogenetic study based on the ITS region of *H. varicosporoides* and their anamorph connections have proven this taxon to be polyphyletic. While rooted in the 18S rDNA in the phylogram, the neighbourhood of their parsimonious clade is a sister taxon to the order Helotiales, and is accommodated within the same Leotiomycetes Class. Hence, it was found that three chosen species, namely *V. giganteum* SS3012, *V. delicatum* SS3008, and *T. terestre* SS3011 are not monophyletic. In addition, other anamorphic stages of *H. varicosporoides* SS76.01 and two strains of *Cudoniella indica* (SS708, SS3005) are clustered together. More related species will be added for further study of this mega genus.

วิวัฒนาการลงสู่ทะเลของรา Dothideomycetes

Multiple invasion of Dothideomycetes (Ascomycota) lineages into the sea

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It has been suggested from numbers of studies that the broadly defined fungal group such as halosphaerialean ascomycetes also evolved more than once independently from terrestrial ancestors. Therefore, the lineages of marine Dothideomycetes were of interest, in order to reveal their ordinal assignment and the inter-relationships between marine and terrestrial counterparts. Some genera of marine Dothideomycetes cannot be assigned based on their morphology to an appropriate order with certainty. In the current study, small subunit (SSU) ribosomal DNA of *Aigialus grandis*, *Aigialus parvus*, *Decasinella formosa*, *Julella aviceniae*, *Lineolata rhizophorae*, *Platystomum scabridisporium*, *Massarina velatospora*, *Massarina thalassiae* and *Verruculina enalia* were sequenced and subjected to phylogenetic analysis. These taxa grouped consistently with many terrestrial and freshwater taxa within the Pleosporales. Our study revealed that they have invaded into the sea several times and may have originated from terrestrial ancestors.

สายสัมพันธ์เชิงวิวัฒนาการของราซีโลไมสีส 2 สกุล: *Infundibulomyces* species และ *Satchmopsis brasiliensis* ที่มีลักษณะโคนิดิโอมาตาเหมือนกัน โดยใช้ข้อมูลลำดับเบสดีเอ็นเอ

Phylogeny of two coelomycete genera with cupulate conidiomata based on rDNA sequence analysis: *Infundibulomyces* species and *Satchmopsis brasiliensis*

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This project sets out to make a major contribution to the classification of the anamorphic fungi, coelomycetes, which produce asexual spores (conidia) and play an important role in terrestrial ecosystems. Three species of interest comprising 2 genera presented in this poster are *Infundibulomyces cupulata*, *Infundibulomyces* sp. SFC 981 (a new species) and *Satchmopsis brasiliensis*. The genus *Infundibulomyces* resembles *S. brasiliensis* in its nidulariaceous-like conidiomata but differs in having holoblastic conidiogenesis and appendaged conidial morphology. Our phylogenetic analyses based on small subunit and large subunit ribosomal DNA sequences revealed that *I. cupulata* and *Infundibulomyces* sp. SFC 981 are monophyletic with high bootstrap support, and well placed (100%) within the Chaetosphaeriales (Sordariomycetes). Although these two species are congeneric they differ in conidial morphology: *I. cupulata* with longer narrower conidia, while the new taxon has shorter conidia. Moreover, the genus *Infundibulomyces* is distantly related to *S. brasiliensis*, which positioned in the discomycete order Helotiales (Leotiomycetes) with moderate support (64%). Therefore, this molecular result suggests that the nidulariaceous-like conidiomata have arisen more than once because *Infundibulomyces* and *Satchmopsis* are distantly placed and share few other common morphological features.

งานบริการของห้องปฏิบัติการเก็บรักษาสายพันธุ์จุลินทรีย์ไบโอเทค

BIOTEC culture collection services

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Major role of BIOTEC Culture Collection (BCC) is to collect and preserve microbial cultures and their relevant data for BIOTEC's in-house research and public. Recently, BCC holds more than 20,000 strains, majority of which is fungi isolated from several sources such as insects, decayed wood, plant seeds, fresh water, soil and leaf litter, lichens and alkaline sources. Almost all strains in the collection are cryopreserved at –80^{o}C waiting for screening of bioactive compounds and enzymes. Freeze-drying or storage in vapor phase of nitrogen is used for safe and for strains with special characteristics such as those that produce bioactive compounds. Strain data as well as data regarding storage and supply of cultures are recorded in BCC's database. Based on data available from BCC's database, some thousand strains are made available for public trough the internet (http://bcc.biotec.or.th). BCC also offers other services including safe deposit, patent deposit, technical service (freeze-drying and freezing), identification service (eubacteria, sporulating *in vitro* fungi and yeasts) and personalized training.

การดำเนินการเก็บรักษาตัวอย่างแห้งราทำลายแมลง

The management of insect fungal herbarium

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The BIOTEC Bangkok Herbarium (BBH) was established in 1999 when registered with the New York Botanical Garden (NYBG). In 2004 the BRT (Grant BRT D_547001) and BIOTEC (BT-B-02-XG-BC-4702) provided support for the infrastructure and development of the BBH. During the last two years a standardised system has been developed for the management of the collection. A website and brochure for the collection has also been produced for in-house and outside parties. In the last two years a backlog of 11,000 specimens has been processed. 88% of this collection has been identified at least to genus level. Within this we have five fungal phyla, 12 divisions, 51 orders, 147 families, 525 genera and 1160 species. The collection now contains the holotypes of 61 new species. Within these 61 new species there are four new genera: *Flammispora* U. Pinruan, J. Sakayaroj, K.D. Hyde & E.B.G. Jones, gen. nov. *Infundibulomyces* Plaingam, Somrith. & E.B.G. Jones, (2003) gen. nov., *Phruensis* Pinruan, (2004), and *Unisetosphaeria* Pinnoi, E.B.G. Jones, McKenzie & K.D. Hyde, (2003) gen. nov. There is also one new fungal order – the Jahnulales. The BBH collection had 31 recorded depositors during this period.

การจัดจำแนกและความหลากหลายของราเอนโดไฟท์จากพืชสกุล *Garcinia*

Molecular identification and diversity of endophytic fungi from *Garcinia* spp.

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A total of 1,979 endophytic fungi were isolated from leaves and branches of five healthy *Garcinia* plants from southern Thailand; *Garcinia atroviridis*, *G. dulcis*, *G. mangostana*, *G. nigrolineta* and *G. scortechinii.* Three hundred and seventy seven isolates (19.0%) were randomly selected according to colony morphology to screen for their antimicrobial activities against seven human pathogens. Seventy out of 377 isolates (18.6%) demonstrated antimicrobial activity against at least one human pathogen; *Staphylococcus aureus* ATCC29523, methicillin-resistant *S. aureus* (MRSA), *Candida albicans*, *Cryptococcus neoformans*, and *Micrsoporum gypseum*. Molecular identification based on Internal Transcribed Spacers (ITS1-5.8S-ITS2, ITS) ribosomal DNA sequences was performed. Our endophytic fungal sequences were compared and aligned with other related sequences retrieving from GenBank database using BioEdit V. 7.0.5. The phylogenetic trees were constructed using PAUP* V.4.10b. The result showed that selected 22 active endophytes belong to 6 orders 10 genera; *Aspergillus* sp., *Aspergillus aculeatus*, *Penicillium* sp., *Penicillium paxilli* (Eurotiales), *Botryosphaeria* sp., *Fusicoccum* sp., *Guignardia mangiferae*, (Dothideomycetes *et* Chaetothyriomycetes *incertae sedis*), *Curvularia* sp. (Pleosporales), *Fusarium* sp. (Hypocreales), *Phomopsis* sp. (Diaporthales), *Eutypella* sp. and *Xylaria* sp. (Xylariales). The results indicate that endophytic fungi from *Garcinia* spp. are diverse and ITS region is efficient for fungal molecular identification.

ความหลากหลายของเชื้อรากลุ่มซีโลมัยซีทในประเทศไทย

Diversity of coelomycete fungi in Thailand

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Coelomycete is unique anamorphic fungi, which is poorly surveyed and documented. This group plays an important role in the ecosystem as saprophytes, parasites, endophytes and mutualist organisms. Many species of coelomycetes possess the ability in producing bioactive compounds with pharmaceutical potential and are used in biological control. This study was proposed to isolate coelomycete fungi from the Thai forests and deposit them into the BIOTEC Culture Collection and to determine their ecology. Any new fungi encountered during the study would be described and published. Thirty-nine coelomycetes (21 species) were isolated from 340 samples of fallen leaves, twigs, fruits and seeds. *Chaetospermum camelliae* was the most abundant species and occurred in a wide range of habitats. Eight new fungi were also collected during this study. Three of them have been described, and submitted to publish in the international journals. Further studies are to survey for fungi throughout the rainy months, and to do the ecological analysis.

ความหลากหลายของราภกลุ่ม Xylariaceae ในประเทศไทย

Diversity of Xylariaceous fungi in Thailand

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Xylariaceae is known for its ability to produce bioactive compounds and enzymes with some industrial potential. *Xylaria* is the largest genus within the *Xylariales* family and many species have been discovered habitats in all types of forests, including mangroves, due to their ability to degrade various plant materials e.g. wood, twigs, leaves, seeds and also dung. Since 2005, diversity of Xylariaceous fungi in Thailand have been surveyed and isolated into axenic culture, we found total of 260 samples some of which have been categorized into 10 genera, *Annulohypoxylon*, *Biscogniauxia, Daldinia*, *Entoneama*, *Hypoxylon*, *Kretzschmaria*, *Nemania*, *Stilbohypoxylon*, *Xylaria* and *Whalleya*. *Xylaria* remain a dominant genus. All cultures were collected and preserved at BIOTEC Culture Collection (BCC). They were meant for the screening of many useful bioactive compounds under the bioresources utilization programe.

ความหลากหลายของราบนปาล์ม

Biodiversity of fungi on palms

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The biodiversity of fungi on selected palms: *Calamus* sp., *Licuala spinosa*, and *Elaeis guineensis* were studied. Four field collections were made for saprophytic fungi: September, October, and November 2005 and January 2006; and one for endophytic (November 2005). On *Elaeis guineensis* a total of 118 taxa including 28 Ascomycota, 28 anamorphic fungi and 62 Basidiomycota were recorded. *Annulatascus velatispora*, *Stilbohypoxylon* sp., *Falciformispora* sp. and *Vanakripa* sp. were common on this palm. On *Calamus* sp. a total of 78 taxa (176 records), including 40 Ascomycota, and 38 anamorphic fungi were recorded. Samples were collected from 4 parts of the palm: with 61% of the fungi recorded from petioles; 37.5% from rachis and 1.5% from the trunk. Palm material collected from different habitats were also sampled: dry aerial material yielded 66% of the fungi and damp/moist material 34%. On this palm, a number of species: AOM 318, *Morenoina palmicola* and *Diaporthe* sp., were common. In this work we document the occurrence of endophytic fungi within petioles and leaves of the fan palm, *Licuala spinosa* from Khuan Khang Hotspring, Trang Province. One thousand two hundred and twenty nine isolates were made. Cultures on PDA and CMA were examined periodically for reproductive structures and identified as they sporulated. Many cultures did not sporulate but their distinctive colony morphology and production of sterile stromata, suggested they were xylariaceous species, with 75 morpho types. Two hundred and nineteen axenic morpho strains were characterised and deposited in the BIOTEC Culture Collection (BCC).

การหาสภาวะที่เหมาะสมของเชื้อรา ในการผลิตไซลาเนสโดยใช้แหล่งคาร์บอนจากเศษวัสดุเหลือทิ้งทางการเกษตร

Optimization of conditions for xylanase production of fungi from agricultural waste

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Aspergillus foetidus TISTR 3159 and *Fusarium moniliforme* TISTR3175 were studied for xylanase production. The results showed that *F. moniliforme* TISTR3175 produced higher amounts of xylanase and activity than *A. foetidus* TISTR 3159. When rice straw, rice bran, narrow-leaved cattail and water hyacinth were used as carbon sources, it was found that *F. moniliforme* TISTR3175 and *A. foetidus* TISTR 3159 produced the highest activity of xylanase at 917 and 908 units/g substrate on rice straw, respectively. Pretreatment of rice straw with 1% sodium hydroxide for 0.5 h at room temperature before growing *F. moniliforme* TISTR3175 on it yielded xylanase at 1,480 units/g substrate. When ammonium sulphate and urea were compared as nitrogen sources, *F. moniliforme* TISTR3175 produced higher xylanase yield on ammonium sulphate than urea..Some properties of xylanase production from *F. moniliforme* TISTR3175 were studied. After xylanase was precipitated with ammonium sulphate (40-50% saturation), and undergone dialysis and ultrafiltration, the xylanase enzyme was more pure (57.5 times) and the specific activity was 938 units/mg protein. The optimum temperature and pH were 40^{o}C and 7.0, respectively. The xylanase was stable at temperatures of 30-40^{o}C with relative xylanase activity at 100 %. The K_m and V_{max} values of xylanase with oat spelt xylan were 14.88 mg/ml and 212.22 ug/ml/min, respectively.

Bipolar Budding Yeasts สายพันธุ์ใหม่จำนวน 4 สายพันธุ์ ในจีนัส *Hanseniaspora* และ anamorph *Kloeckera* ที่คัดแยกได้ในประเทศไทย

Four new species of bipolar budding yeasts of genus *Hanseniaspora* and its anamorph *Kloeckera* isolated in Thailand

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Among yeast strains isolated from the natural environment of Thailand, 15 strains proliferated by bipolar budding. In the phylogenetic tree based on the D1/D2 domain sequencesof 26S rDNA, they were located in the cluster where species of the genus *Hanseniaspora* and its anamorph counterpart *Kloeckera* were located. Based on the morphological, physiological characteristics, and sequences of D1/D2 domain, 7 strains of bipolar budding yeasts are considered to represent 4 new species namely, *Hanseniaspora thailandica* sp. nov. (ST-250 and ST-306), *Kloeckera siamensis* sp. nov. (ST-464, ST-493 and ST-613), *Kloeckera songkhlaensis* sp. nov. (ST-476) and *Kloeckera tradensis* sp. nov. (ST-391).

การศึกษาจุลินทรีย์ในกระบวนการผลิตปุ๋ยอินทรีย์ขององค์การบริหารส่วนตำบลท่าข้าม อำเภอหาดใหญ่ จังหวัดสงขลา

Study on microorganisms involving in organic fertilizer production processes from biofertilizer plant at Takam subdistrict administration organization Amphoe Hat Yai, Songkhla province

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The objectives of this work are to study the microbial diversity involving to the organic fertilizer production processes from pilot plat of biofertilizer at Takam subdistrict, Amphoe Hat Yai. The production mainly rely on their own agricultural waste materials such as chicken manure and rice coat residue which are augmented with various microbial inocula, such as “Por Dor 1” (from office of Land development) and fermented juice that mixed microbial inocula “Por Dor 2”. These raw materials are processed in pellet form. From this study, we used both culture and non-culture-based techniques to investigation the presence of useful microorganisms and their roles from different production processes. The isolation of actinomycetes was conducted and it was found some potential strains that can inhibit the growth of some fungi causing plant disease. In addition, the 16S rRNA clone library analysis technique has being conducted to obtain the bacterial community structure from various steps of fertilizer production.

การประยุกต์ใช้คุณสมบัติการต้านยาปฏิชีวนะในการตรวจสอบผลกระทบของการปนเปื้อนโลหะหนักต่อความหลากหลายของจุลินทรีย์จากน้ำทิ้งชุมชนและการเพาะเลี้ยงสัตว์น้ำ

Application of antibiotic resistance to monitor heavy metal contamination impacts to microbial diversity from domestic and aquaculture wastewaters

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This led to the objective of the present study which aims to isolate various bacteria to use as indicators of heavy metal contamination by using antibiotic resistance characteristics in conjunction with chemical analysis method. Water samples were collected from 4 sites of domestic hospital aquaculture and old tin mines during September 2005 and April 2006. It was found that the percentage of antibiotic resistance was significantly correlated with metal resistance, namely in Kanamycin and Zn and Kanamycin and Fe ($P<0.05$). It was found that the bacterial strains isolated from domestic hospital wastewater and aquaculture ponds had a high resistance to antibiotics and heavy metals. However, there was a very low diversity among those bacterial strains which were found resistant to the heavy metals and it showed a positive relationship with heavy metal contamination Analyses by Inductively Coupled Plasma Optical Emission Spectrometry method (ICP-OES). In September 2005, it was found that some samples contained higher heavy metals concentrations than surface water quality standards (WHO). From this study we conclude that antibiotic resistance characteristics can be used to monitor heavy metal contamination which affects the microbial diversity in other environments.

นโยบายของประเทศไทยเรื่องสิ่งมีชีวิตตัดแต่งพันธุกรรม

National policy for Thailand on genetically modified organisms

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Genetically Modified Organisms have a tremendous potential in the national economic development. However, the global market acceptance is presently yet quite limited. Intellectual property, ecological risk, organization, rule and regulation as well as economic analyses indicated that there is no scientific evidence confirmed that GMOs do not pose higher risk to human and ecosystem than their ordinary counterpart. Moreover, patents owned by foreign entities may protect each transgenic plant variety. Thus, the use of GMOs would affect the national ecosystem as well as the national food security. Thailand should then impose the so-call “Safe Use of Forefront Technology” policy on GMOs issue. Prior to the acceptance of the technology for the enhancement of the country competitiveness in an open system, the enacting process to have a complete legal system on biosafety must be urgently executed. To effectively implement the law, a regulating organization on biosafety must be established evaluate and monitor the impact of GMOs. This will give an assurance to Thai society. In addition, capacity building on biosafety management and on the GMOs technology that not cause genetic pollution must be immediately implemented. Labeling must be performed on all goods (except pure chemical) produced from GMOs. The responsibility for strict liability and redress are the burden of the applicant for the GMOs public release.