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จุลินทรีย์

Biodiversity of waste lubricating oil-degrading bacteria in soil

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Waste or used lubricating oils have become a serious environmental problem. Once in the environment, waste oil can bind to organic matter, mineral particles and organisms. This can play an important role in the persistence and toxicity of oil components. Hydrocarbon assimilation, however, can be conducted using many methods. Bioremediation is a promising method because it is effective, is of lower cost than other technologies, and the final products are water and carbon dioxide. Hydrocarbon microbiology research has supported the hypothesis that hydrocarbons and its derivatives are amenable to microbial degradation; microorganisms possessing this potential are widespread in many environments. The ability of microbial communities to adapt to contaminants is evident from contaminated zones. Accordingly there is a good possibility to use bioremediation to eliminate spilled oil or waste lubricating oil in the environment. Therefore, this research aims to isolate and screen waste lubricating oil-degrading bacteria from soil. Bacterial strains which exhibit high degradation activity will be selected and identified, and the selected strains will be kept for use in the future.

Biodiversity of marine gliding bacteria in Thailand

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This investigation of marine gliding bacterial diversity in Thailand has led to the discovery of at least 2 new genera and 3 novel species based on the analysis of 16s rDNA sequences and the biochemical characteristics of the isolates. The newly proposed taxa include *Aureispira marina* gen. nov. sp. nov., *Aureispira maritima* gen. nov. sp. nov. and *Rapidithrix thailandica* gen. nov. sp. nov. The remaining unidentified isolates have 16s rDNA sequences close to *Cytophaga* sp. and alpha-proteobacteria but the percentages of similarity are lower than 95, suggesting that these can be also classified as novel genera or species. Identification of these isolates is currently being investigated.

Pyrrole derivatives from a marine gliding bacterium

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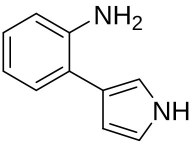
A methanolic extract of *Rapidithrix thailandica* TISTR1749 showed anticandida activity at 150 mg/mL with low cytotoxicity (against cancer cell lines not higher than 15% at 25 μg/mL). Further investigation led to the isolation of a new pyrrole derivative, 3-(2′-aminophenyl)-pyrrole **(1)**, along with its isolation artifact, 2,2-dimethyl-pyrrolo(1,2)-dihydroquinoline **(2)**. The isolation and structural elucidation of both compounds will be presented.



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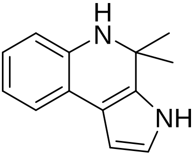

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Numerical analysis and random amplified polymorphic DNA (RAPD) analysis of root-nodule bacteria isolated from the Thai medicinal plants, *Pueraria mirifica*, *Derris elliptica* Benth., and *Indigofera tinctoria* Linn.

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Root-nodule bacteria were isolated from some Thai medicinal plants, including *Pueraria mirifica*, *Derris elliptica* Benth., and *Indigofera tinctoria* Linn, growing in 16 provinces in Thailand. The 221 isolates were analyzed for DNA polymorphisms using a randomly amplified polymorphic DNA (RAPD) method. The results indicated that there was significant genetic diversity among strains from distinct geographical areas. A total of 54 representative strains were characterized by a numerical analysis of 113 phenotypic features including utilization of 50 carbon sources, utilization of 30 nitrogen sources, requirements for 10 vitamins, tolerance to 10 antibiotics, growth at pH 5.5, 6.5 and 8.0, growth on media supplemented with NaCl at concentrations of 0.2 and 1.0 M, growth at 20, 30, and 40°C, acid or alkaline production, colony morphology, doubling time, indole-3-acetic acid (IAA) production and melanin production. The strains could be differentiated and the phenotypic diversity among these strains was revealed.

Quality of bacterial strains after collection by the freezing method

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Bacterial strains are well recognized for their extremely wide applications. One of the most important processes supporting the sustainable uses of all microorganisms is to maintain all those useful strains in the form of culture collections with high stability and good quality. The objective of this work is to evaluate the stability and quality of some medically important bacterial strains after they have been maintained in a culture collection for 2-12 years. A total of 300 strains of 15 bacterial species were analyzed for their quality according to their viability, purity, phenotypic characters and genotypic characters after a period of collection ranging from 2 to 12 years. The results showed a mean viability of about 5-11 log10 CFU/ml that was not significantly different among species except for some fastidious bacteria such as *S. pneumoniae* and *H. influenzae*, in which viabilities of from 5-7 log10 CFU/ml were observed. All cultures were pure with phenotypic characters corresponding to the typical characters of each. Genotypic characters were investigated using the PCR-RFLP technique with a universal primer pair to amplify a portion of the 16S rRNA gene, followed by restriction analysis of the PCR products. The results also exhibited good genotypic quality. It can be concluded that all bacterial strains are in good condition for any potential uses.

Phylogenetic analyses of Ingoldian anamorphs in the genus *Hymenoscyphus*

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Evolutionary trees based on 18S rDNA (or SSU) and ITS1-5.8S-ITS2 sequences were generated by using Neighbor-Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses to clarify the anamorph-teleomorph connections in 69 taxa including both in-group and out-group taxa using fungal reference species from GenBank. Molecular phylogenies clustered the chosen taxa and their related fungi into the classes Dothideomycettes, Leotiomycetes and Mitosporic Ascomycota. The NJ tree showed similar topology to the trees obtained by MP and ML analyses. The separation of the Class Dothideomycettes from the Class Leotiomycetes in the trees is well supported by bootstrap values. *Beltrania rhombica* (SS 3562), *Speriopsis pedatospora* (SS 2236), *S. pedatospora* (SS 2229) and *Brachiosperia tropicalis* (SS 2152.1) were clustered in the Dothideomycetes whereas *Variocladium giganteum* (SS 3012), *Cudoniella indica* (SS 708), *Varicosporium delicatum* SS3008, *Tricladium terestre* (SS 3011), H*ymenoscyphus varicosporoides* (SS 76.01) and *Cudoniella indica* (SS 3005) were grouped in the Leotiomycetes.

Diversity and distribution of freshwater fungi in natural habitats with different temperatures in Thailand

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One-hundred and forty-seven species of freshwater fungi were collected from the south and east of Thailand in a range of water temperatures between 25 and 63°C. Two-hundred and nine strains were isolated and preserved in the BIOTEC Culture Collection (BCC). These included 63 Ascomycetes (43% of total taxa) and 79 Mitosporic fungi (54%). They belonged to 2 classes, 8 orders, 13 families and 42 genera. Sirindhorn Waterfall (25°C) at Hala-Bala National Park possessed the highest fungal diversity (60%) while Saphan Yung Hot-stream (43°C) and Khuankhang Hot-springs (51°C) had the lowest (4%), with 0% at Marine Hot-springs (45°C) and Wat Than Nam Ron (63°C). This study showed that the number of fungal species is not correlated to the temperature of the habitat where fungi were found. The reason may be due to the thermo-tolerance characteristics of freshwater fungi. Moreover, water from Marine Hot-springs and Wat Than Nam Ron, which is based on powdery sand rock with salt and $CaCO_3$ crystal accumulations, may influence the efficiency of fungal growth and colonization of substrata. The most common species in streams with normal temperature profiles (e.g. <35°C) were *Ellisembia opaca, Phaeoisaria clematidis* and *Sporoschisma uniseptatum*. *Monodictys* sp.1 was recorded from both normal temperature streams and hot-spring habitats. However, our study requires more information before conclusions concerning the relationship between temperature and freshwater fungal biodiversity can be made.

Palmicolous fungi in Thailand

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The biodiversity of fungi on selected palms, *Calamus* sp. and *Elaeis guineensis*, was studied. One field collection of endophytic fungi was made in November. Ten field collections were made for saprophytic fungi in September, October, November, and December 2006, and March 2007, and one for endophytic isolation experiments (November 2006). Saprophytic fungi on *Elaeis guineensis* comprised a total of 65 taxa: 28 Ascomycota, 28 anamorphic fungi and 9 Basidiomycota. *Annulatascus velatispora*, *Stilbohypoxylon* sp., *Falciformispora* sp. and *Vanakripa* sp., *Grammothele fuligo*, *Schizophyllum commune* and *Marasmius* sp. were common on this palm. On *Calamus* sp., a total of 98 taxa (224 records) were found: 40 Ascomycota, 12 Basidiomycota and 46 anamorphic fungi. Samples were collected from 4 parts of the palm with 61% of the fungi recorded from petioles, 38% from rachises and 1% from trunks. Palm material collected from different habitats was also sampled. Dry aerial material yielded 68.5% of the fungi and damp/moist material 31.5%. On this palm, a number of species, AOM 318, *Morenoina palmicola* and *Circinoconis paradoxa*, were common. Endophytic fungi within petioles and leaves of the oil palm, *Elaeis guineensis*, from an oil palm plantation in Trang Province were sampled. One thousand and seventy-two 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 54 morpho types. Two hundred and eighty-seven axenic morpho strains were characterised and deposited in the BIOTEC Culture Collection (BCC).

Morphological and molecular characteristics of a poorly known marine ascomycete, *Manglicola guatemalensis* (Jahnulales: Pezizomycotina; Dothideomycetes, *Incertae sedis*): A new lineage of marine ascomycetes

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Collections of the poorly known marine ascomycete *Manglicola guatemalensis* from Trang and Trat Provinces, Thailand, were made in 2005 and 2006 from the brackish water palm *Nypa fruticans*. This fungus is only known from two previous collections. This paper reports on the morphological characteristics and molecular phylogeny of this unique marine bitunicate ascomycete. *Manglicola guatemalensis* has large clavate to obtusely fusiform ascomata, wide ostioles, bitunicate asci, cylindrical, thick-walled, unequally one-septate ascospores, constricted at the septum, apical cell larger, chestnut-brown and a smaller light brown basal cell. Ascospores germinate readily, always from the basal cell. Four isolates from different locations were selected for the phylogenetic study. Different regions of the rDNA gene, including SSU and LSU, were sequenced. The molecular data places *M. guatemalensis* in the Jahnulales with high bootstrap support, all strains are monophyletic. In the combined SSU and LSU analyses the Jahnulales comprise two subclades, A and B. In subclade A, *Manglicola* strains form a sister group to the *Jahnula* species, *Jahnula appendiculata, J. australiensis, J. bipolaris* and *J. sunyatsenii*. Subclade B comprises *A. khaoyaiensis, J. siamensiae* and *Patescospora separans.*

Putative basidiomycete teleomorphs and phylogenetic placement of the coelomycete genera *Chaetospermum, Giulia* and *Mycotribulus* based on nuclear rDNA sequences

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Few basidiomycetes are known to have a coelomycete anamorph. The partial small subunit and large subunit of nuclear rDNA of three coelomycete genera (*Chaetospermum*, *Giulia*, *Mycotribulus*) were sequenced to determine their phylogenetic relationships. *Mycotribulus mirabilis* was well placed in the marasmioid clade (Marasmiaceae and Physalacriaceae), Agaricales, while *Giulia tenuis* clustered with the Corticiaceae, Corticiales. *Chaetospermum camelliae* and *Chaetospermum artocarpi* formed a close relationship with the Sebacinaceae, Sebacinales. Although morphologically these coelomycetes are pycnidial and with appendaged conidia, they show no consistency in their phylogenetic relationships, belonging to disparate major taxonomic groups with putative teleomorphs in the Agaricales, Corticiales and Sebaciniales. Further molecular studies of coelomycetes may be rewarding to evaluate their phylogenetic affinities.

Diversity of Thai Coelomycetes

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Coelomycetes are anamorphic fungi that produce asexual spores (conidia) within a flask-shaped (pycnidium) or a shield-shaped structure (pycnothyrium), or by a combination of fungus and host tissue (acervulus), or on a mass of vegetative mycelium (stromata). They play important roles in terrestrial ecosystem functioning as decomposers, endophytes, and parasites. Previous studies of Thai coelomycetes have focused on pathogenic taxa of agricultural importance with *Colletotrichum* species being dominant. The objectives of this study were to extend our knowledge of this fungal group to the colonization of substrata in non-agricultural ecosystems and to isolate them into axenic culture for further utilization. Incubation and examination of fallen leaves, dead twigs, decaying fruits and seeds collected from various natural forests and urban parks have yielded over forty coelomycete species. *Chaetospermum camelliae* was the most abundant species and common to a wide range of habitats. The other frequent species included *Coniella castaneicola*, *Pseudorobillarda sojae*, *Satchmopsis brasiliensis* and various *Pestalotiopsis* species. Ecological study has focused on the coelomycete communities in two forest types of Khao Yai National Park, an evergreen forest and hill evergreen forest, and in an urban park of Bangkok. The results have shown that the urban park possessed the highest species richness while the evergreen forest possessed the highest species diversity and offered some new taxa. Similarities of coelomycetes between the two natural forests were higher than those between the natural forest and the urban park.

Fungal communities on decaying seeds of the Dipterocarpaceae

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Fungi on naturally decaying seeds play an important role in recycling nutrients in the forest ecosystem. They have also shown ability in producing bioactive compounds with pharmaceutical potential. Trees of the family Dipterocarpaceae are of ecological and economic significance. They predominate over a wide area of tropical forest in Asia. A study on fungi colonizing seeds of this family is therefore proposed in order to obtain ecological information on fungi in relation to the major trees of the tropical forest, and to isolate these fungi into axenic culture. Any new fungi collected during the study will be described and published. Cultures isolated from this study will support further biotechnological utilization whereas data on fungal ecology will contribute basic knowledge for natural resource management in this country.

A preliminary study on termite-associated *Xylaria* in Thailand

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The *Xylaria* species associated with termite nests are poorly known. It has been a common belief that all belong to three or four species. It is now known that a wide range of species are involved but many species appear to be undescribed. This project is a preliminary project with the aim to study fungi associated with termite nests and to study the biodiversity of Xylariaceous fungi in Thailand, especially *Xylaria* species, in order to fully understand the diversity of this group of taxa. Previous surveys and collections have resulted in more than 10 variable morphological characters being found. Molecular results show there are three separate clades of *Xylaria* species associated with termite nests.

Aquatic fungi developing on eggs of tilapia, *Oreochromis niloticus* Linn., and their control

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Fungal-infected eggs of tilapia (*Oreochromis niloticus* Linn.) were collected from Kalasin, Khon Kaen, Maha Sarakham and Sakon Nakhon Provinces during June 2005-April 2006. The fungi were identified to the family Saproegniaceae (*Achlya* spp., *A. ambisexualis*, *A. bisexualis*, *Aphanomyces* spp., *Saprolegnia diclina*) and family Pythiaceae (*Pythium* sp.). Some biological characteristic studies were conducted. The optimal temperatures for vegetative growth of *Achlya*, *Aphanomyces*, *Saprolegnia* and *Pythium* were 20-35 ºC, whereas the optimal temperature for zoospore production was 25 ºC. The optimum pH for vegetative growth and zoospore germination of all genera were 6.0-9.0 and 4.0-11.0, respectively. Histopathological examination of fungal-infected eggs showed numerous hyphae spread over the outer layers of egg envelopes and penetrating egg envelopes. The anti-fungal effect of sodium chloride on zoosporic and vegetative stages was 2.5 and 3.0 % exposed for 2 and 24 hours, respectively, whereas 25 and 200 µg/mL potassium permanganate was effective in killing the zoosporic stage at 30 minutes and the vegetative stage at 24 hours, respectively. Toxicity of 2.0 % sodium chloride or higher reduced the percent hatching rate of treated groups of eyed eggs ($P<0.05$). Treatment of eyed eggs with 25, 50, 100, 150 and 200 µg/mL potassium permanganate against fungal activity showed 0 % hatching rate within 1 and 24 hours exposure. Therefore, it may be possible to use 3.0 % sodium chloride or higher concentrations to prevent fungal infection of tilapia eggs by bathing with less than 1 hour exposure.

Biodiversity of water moulds in water and fungal infection in fish in the Chi River

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Isolation of water moulds from water and fish in the Chi River in Chaiyaphum, Khon Kean and Mahasarakham Provinces was conducted during April to June 2007. Fungi were mostly isolated from water; in total there were 48 strains. Only 2 were isolated from diseased fish because fungal infection was lacking due to high temperatures (31-34°C). Isolated fungi were classified as Class Oomycetes and most belonged to the Order Saprolegniales, Family Saprolegniaceae. In this family, the Genus *Achlya* obtained from the study sites was dominant. Other genera, such as *Saprolegnia* and *Aphanomyces* and *Leptolegnia*, were also found as well as several isolates of imperfect fungi, which have not been identified yet. Moreover, some unidentified strains of Oomycetes were not characterized yet because neither asexual or sexual reproduction has occurred. However, the results indicated that geographical differences, rather than infected fish species have played an important role in the species diversity of water moulds. In further study, fungal collection and biological characteristics as well as molecular characteristics based on internal transcribed spacer genes will be determined to evaluate fungal evolution and construct phylogenetics. In addition, determination of pathogenicity capacity of the fungi to snakehead and nile telapia, histopathological examination of diseased fish and the development of prophylactic and curative fungicides for the isolated fungi will be carried out.

Species diversity of antagonistic fungi, *Trichoderma* spp., in tomato seed production fields and their application for biological control of plant diseases

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One hundred and eighty-six isolates of soil fungi derived from tomato and cucurbit seed production fields in Khon Kaen and Maha Sarakam provinces were tested for antagonistic properties against the Fusarium wilt pathogens, *Fusarium oxysporum* f.sp. *lycopersici* (FOL), *F. oxysporum* f.sp. *melonis* (FOM) and *F. oxysporum* f.sp. *cucumerinum* (FOC). Sixty-two isolates were effective against FOL, 28 against FOM and 20 against FOC. Twenty isolates were classified in the genus *Trichoderma* and coincidently showed antagonistic properties against all wilt pathogens. Based on morphological characteristics, *Trichoderma* spp. were identified as *Trichoderma harzianum*, *T. koningii*, *T. piluliferum*, *T. aureoviride* and *T. reesei* for 5, 10, 1, 3 and 1 isolate, respectively. The *T. aureovirid*e (TKK2701) and *T. koningii* (TKK4301) showed higher cellulolytic activities than other isolates. The *T. aureoviride* (TKK2701), *T. koningii* (TKK2602, TKK4301, TKK2203), and *T. harzianum* (TKK3803) isolates expressed high protease activity. Chitinolytic activity was detectable for most isolates except *T. koningii* (TKK3702) and *T. harzianum* (TKK3803). However, *T. harzianum* (TKK2801), *T. aureoviride* (TKK1001) and *T. koningii* (TKK3501) produced high activity of β-1,3 glucanase. The results of fungicide tests revealed that all isolates of fungi were tolerant toward carboxin, captan, and slightly tolerant to mancozeb. However, there was sensitivity to carbendazim. The high potential isolates for integrated use with fungicides for control of wilt of tomatoes and cucurbits were *T. aureoviride* (TKK1001), *T. harzianum* (TKK2501), and *T. koningii* (TKK2602, TKK3501, TKK4701). This study showed the species diversity of antagonistic fungi, *Trichoderma* spp., in vegetable seed production fields for export, their potential and their integrated use for control of Fusarium wilt of tomatoes and cucurbits.

A Study of microorganisms involved in organic fertilizer production processes at the biofertilizer plant of Takam Subdistrict Administration Organization, Hat Yai District, Songkla Province

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This study aims to compare enumeration methods for microorganisms involved in organic fertilizer production processes at the biofertilizer plant at Takam Subdistrict Administration Organization, Hat Yai district, Songkla province. A culture-dependent method and 16S rDNA clone library analysis technique were used. The results of the culture-dependent method showed that numbers of microorganisms varied among different production processes. Most mesophilic microorganisms were found to be dominant groups in raw material mixtures. 16S rDNA clone library analysis was conducted on microorganisms from the raw material mixture and the finished fertilizer product. Three clone libraries were studied, two clones for bacterial communities and one for actinomycetes. The results showed that halophilic bacteria (such as *Salinicoccuss*, *Salinicoccus*) were the most dominant group consisting of 93% and 61% of total bacterial clones in the raw material mixture sample and the finished fertilizer product, respectively. *Dietzia* in the actinomycetes clone library (71% of total clones) was the dominant group. Some uncultured bacteria were also found in all clone libraries which had not been obtained by the culture-dependent method.