
A new species, *Gluconobacter kanchanaburiensis* sp. nov., proposed for strains isolated from Thong Pha Phum, Kanchanaburi, Thailand

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Two isolates, AD92^T and AD93, were isolated from fermented fruits of *Artocarpus heterophyllus* (common name: Jackfruit) collected at Thong Pha Phum, Kanchanaburi, Thailand. In phylogenetic trees based on 16S rRNA gene sequences, the two isolates were included within a lineage comprising *Gluconobacter oxydans*, *Gluconobacter albidus*, *Gluconobacter kondonii*, *Gluconobacter roseus* and *Gluconobacter sphaericus* and formed an independent cluster along with the type strains of *Gluconobacter cerinus*, *Gluconobacter frateurii* and *Gluconobacter thailandicus*. Pair-wise sequence similarities of isolate AD92^T with the type strains of the eight *Gluconobacter* species were calculated to be 97.4-99.5%. DNA base composition was 59.4-59.5 mol% G+C with a range of 0.1 mol%. Labeled DNA of isolate AD92^T presented levels of DNA-DNA hybridization of 100, 32, 10, 16, 27, 20, 39, 47, 44 and 7% to DNA respectively from isolate AD93 and the type strains of *G. oxydans*, *G. cerinus*, *G. frateurii*, *G. albidus*, *G. thailandicus*, *G. kondonii*, *G. roseus*, *G. sphaericus* and *Acetobacter aceti*. The two isolates were phenotypically discriminated from *G. oxydans*, *G. albidus*, *G. kondonii*, *G. roseus* and *G. sphaericus* by growth without nicotinic acid. The two isolates had Q-10. The unique phylogenetic, genetic and phenotypic characteristics obtained indicate that the two isolates were sufficiently different to be classified into a separate species, and *Gluconobacter kanchanaburiensis* sp. nov. is proposed. The type strain is isolate AD92^T (= BCC 15889^T, = NBRC 103587^T), which has a DNA G+C content of 59.5 mol%.

Biodiversity of waste lubricating oil-degrading bacteria in soil

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Consortia of waste lubricating oil-degrading microorganisms were isolated from oil contaminated soil collected from garages and petrol stations in Nakhon Si Thammarat, Songkhla and Suraj Thani Provinces. An enrichment culture technique was used for the isolation of microorganisms responsible for the biodegradation of waste lubricating oil. One gram of soil sample was added into mineral salt medium containing 1% waste lubricating oil as the sole carbon source. Waste lubricating oil degradation activity was measured by a weight loss method. The most active consortium in the assimilation of waste lubricating oil was SC-9. The SC-9 consortium showed 40.46% oil degrading activity within 5 days. Oil concentration affected degradation by the SC-9 consortia. Degrading activity was decreased from 40.46% to 15.05% when waste lubricating oil concentration was increased from 1% to 10%. The SC-9 consortia contained four bacterial isolates, two isolates were Gram-positive with rod-shapes and the others were Gram-negative, with cocci and rod shapes. Analysis of nucleotide sequences of the gene encoding 16S rDNA resulted in the identification of the four bacterial strains as *Chryseobacterium* sp., *Bacillus cereus*, *Sphingobacterium multivorum* and *Agrobacterium tumefaciens*.

Diversity of marine gliding bacteria in Thailand

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Eighty-four marine gliding bacteria were isolated from specimens collected in the Gulf of Thailand and the Andaman Sea. All exhibited gliding motility and swarm colonies on cultivation plates. Phylogenetic analysis indicated that the represented isolates could be separated into six different clades (gr. 1 - gr. 6) within the *Cytophaga-Flavobacterium-Bacteriodes* (CFB) group. Group 1 formed a remote line with only 90% sequence similarity with *Flavobacteriaceae bacterium*, which indicated a potentially novel taxonomic group. Groups 2 and 3 were identified as the recently proposed *Tenacibaculum mesophilum* and *Fulvivirga kasyanovii*, respectively. Groups 4, 5 and 6, consisting of the largest number of members, were identified as *Rapidithrix thailandica*, *Aureispira marina* and *Aureispira maritima*, respectively.

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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Two hundred and twenty-one isolates from the Thai medicinal plants, *Pueraria mirifica*, *Derris elliptica* Benth., and *Indigofera tinctoria* Linn., grown in 16 Provinces in Thailand 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 geographic areas. The total of 54 representative strains was characterized using a numerical analysis of 113 phenotypic features including utilization of 52 carbon sources, utilization of 30 nitrogen sources, requirements for 10 vitamins, tolerances to 10 antibiotics, growth at pH's 5.0, 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. Partial sequences (approx. 500 bp) of the 16S rRNA gene of the strains DASA57009, DASA57020 and DASA57038 from *I. tinctoria* showed 69%-72% homology with strains in the *Ralstonia/Cupriavidus* group.

Molecular phylogeny of selected coelomycetes with fusiform conidia, *Robillarda*, *Pseudorobillarda* and *Xepiculopsis*, based on nuclear ribosomal DNA sequences

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Conidial appendages and conidiogenesis are key characters in the identification of coelomycete genera and species. The genera *Robillarda*, *Pseudorobillarda* and *Xepiculopsis* possess ellipsoidal conidia with appendages but differ in conidial appendage ontogeny. No teleomorphs are known for *Pseudorobillarda* and *Xepiculopsis*, while the putative teleomorph of *Robillarda* is a member of the Amphisphaeriaceae. *Robillarda* and *Pseudorobillarda* are often confused as they both possess septate conidia with polar and or basal appendages. Because of the lack of clarity in identifying *Robillarda*, *Pseudorobillarda* and *Xepiculopsis*, a taxonomic study was undertaken to resolve their familial and ordinal statuses using nuclear SSU and LSU rDNA sequences. The phylogenetic analysis demonstrated that these three coelomycetes fall into two distinct lineages. Firstly, *Robillarda* and *Xepiculopsis* are well placed within the Sordariomycetes, and the ordinal position of these two genera fall within the Hypocreales and Xylariales, respectively. Secondly, *Pseudorobillarda* species have a phylogenetic affinity with the Dothideomycetes, although their ordinal and familial levels remain unresolved. Although these three genera morphologically have similar fusiform conidia, they show no consistency in their phylogenetic relationships. This study has enabled the identification of the putative teleomorphs for these three coelomycete genera.

A multigene phylogeny of the marine ascomycetes: *Biatriospora marina*, *Decaisnella formosa* and *Platystomum scabridisporum*

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A multigene phylogeny of the marine ascomycetes, *Biatriospora marina*, *Decaisnella formosa* and *Platystomum scabridisporum*, was investigated using the combined nucleotide sequences of SSU, LSU rDNA, RPB2 (RNA polymerase II second largest subunit) and Elongation factor 1-alpha (EF-1-alpha). 234 sequences of these gene regions from 71 species from GenBank were aligned with *Hypocrea lutea*, *Xylaria acuta* and *Neurospora crassa* as outgroups. The combined data set was analysed phylogenetically using maximum parsimony and Bayesian analyses.

The results showed that *Biatriospora marina*, *Decaisnella formosa* and *Platystomum scabridisporum* grouped within the Pleosporales in all analyses supported by 100% bootstrap values and 1.00 posterior probabilities. Sequence analyses indicated that *Decaisnella formosa* and *Platystomum scabridisporum* clustered within the Lophiostomataceae subclade 4 (59% bootstrap values and 0.99 posterior probabilities) with *Lophiostoma arundinis*, *L. crenatum*, *M. bipolaris* and *Trematosphaeria heterospora* as sister taxa. *Biatriospora marina* has an affinity with the Testudinaceae, but its taxonomic position within the family remains unresolved and it does not appear to have any close evolutionary relationship with any known testudinaceaeous genera. Our molecular data also support the previous phylogenetic hypotheses that the Lophiostomataceae is polyphyletic.

Comparative fungal diversity studies of palms in Thailand

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The biodiversity of fungi on selected palms, *Calamus* sp. and *Elaeis guineensis*, was studied. Endophytic fungi were collected following 2 field surveys in November 2006 and May 2007. Ten field collections were made for saprophytic fungi in September, October, November, December 2006 and March 2007 and one for endophytic isolation experiments (November 2006). Saprophytic fungi from *Elaeis guineensis* comprised 65 taxa: 28 Ascomycota, 28 anamorphic fungi and 9 Basidiomycota. *Annulatascus velatispora*, *Stilbohypoxyton* sp., *Falciformispora* sp., *Vanakripa* sp., *Grammothele fuligo*, *Schizophyllum commune* and *Marasmius* sp. were common on this palm. On *Calamus* sp. a total of 98 saprophytic taxa (224 records), consisting of 40 Ascomycota, 12 Basidiomycota and 46 anamorphic fungi, were recorded. 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% of fungi were collected from the leaves. On this palm, a number of species, namely AOM 318, *Morenoina palmicola* and *Circinoconis paradoxa*, were common.

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

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One-hundred and eighty-two species of freshwater fungi were collected from submerged wood at 27 subsites in Thailand, which ranged in water temperatures between 21 and 95°C. Two-hundred and fifty-eight strains were isolated and are preserved in the BIOTEC Culture Collection (BCC). These include 48 Ascomycetes (36.3%) and 45 Mitosporic fungi (56.6%) of the total taxa. They belong to 2 classes, 10 orders, 13 families and 47 genera. Tat Ta Phu Waterfall (25°C) at Khao Yai National Park and Sirindhorn Waterfall (25°C) at Hala-Bala Wildlife Sanctuary possessed the highest fungal diversity (10% and 9.2%) while Malika Hot-spring (68°C) had the highest number of fungal species of all hot-springs (3.8%). This study showed that the number of fungal species is not correlated to the temperature of the habitat in which they were found. The reason may be due to the thermo-tolerance characteristics of freshwater fungi. Moreover, water from a marine hot-spring (68°C) (0% fungal diversity), which is based on powdery sand rock with accumulated salt and CaCO₃ crystals, may have influenced the efficiency of fungal growth and colonization on this substratum. The most common species in streams with normal temperature profiles (e.g. <35°C) were *Ellisembia opaca* and *Phaeoisaria clematidis*. However, our study requires more information in order to completely understand the relationship between temperature and freshwater fungal biodiversity.

Relationship of the genus *Savoryella* (teleomorph ascomycete) and its anamorph *Canalisporium* as inferred by multiple gene phylogenies

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The taxonomic placement of selected freshwater *Savoryella* species and some marine *Savoryella* species as well as putative *Canalisporium* species that originated from submerged woods in aquatic habitats have not been classified into any family or order with certainty. Results based on individual molecular data analyses of the partial small subunit (SSU) sequence (SSU data), indicate that *Savoryella* form a monophyletic clade and group within the subclass Hypocreomycetidae, Sordariomycetes. The genus *Savoryella* shows no affinities with the Hypocreales despite earlier assignment to that order. In addition, we can confirm using the large subunit rRNA gene (28S rDNA) the taxonomic position within Hypocreomycetidae, which is in good agreement with the 18S rDNA gene. Further analyses will be conducted including more strains of these taxa, and combining molecular analyses, such as ITS, RPB1, RPB2 and EF1- α , for determining the precise taxonomic placement of these genera.

Biodiversity of *Xylaria* species associated with termite nests or that emerge from soil in Thailand

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Thailand supports a rich diversity of fungi. The Xylariaceous fungi are very interesting to study especially the termite-associated *Xylaria* species. In the past, *Xylaria* species associated with termite nests were too poorly known to be described. Only 25 *Xylaria* species associated with termite nests have been described. This study aimed to survey and collect *Xylaria* species associated with termite nests or that emerge from soil in Thailand. More than 10 species were found and a further 5 species await identification and description. The following species were recognized: *Xylaria acuminatilongissima*, *X. atrodivaricata*, *X. brunneovinosa*, *X. cirrata*, *X. escharoidea*, *X. intraflava*, *X. kedahae*, *X. nigripes*, *X. ochraceostroma*, *X. piperiformis* and *X. reinkingii*.

Fungal diversity 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 been shown to produce bioactive compounds with pharmaceutical potential. The objectives of this study are to record fungi colonizing seeds of the family Dipterocarpaceae, which is the dominant plant family of tropical forests in Asia, and to isolate them into pure culture for further utilization. Methods used in the study included: collection of seeds on the forest floor, incubation of seeds in moist chambers, seed examination using dissection and compound microscopes, and fungal isolation with a single spore technique. Collection sites included: Khao Yai National Park, Khao Soi Dao Wildlife Sanctuary, and Phu Phan National Park. This study has yielded over 40 fungal species from 300 seed samples. Seeds of each collection site supported 8-16 fungal species with 3-5 species overlapping with the other sites. The similarity of fungi on different seed species ranged between 20-25%, thus indicating that different seed species support different fungal communities.

Comparitive study between blastospore and conidia preservation of insect pathogenic fungi using the L-Drying technique

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Insect pathogenic fungi are important regulators of insect populations in nature. In addition, many of the species known to date have been reported as a group of potential candidates for novel secondary metabolites useful for medical and agricultural applications. However, insect pathogenic fungi are a group of fungi which are difficult to grow and maintain under laboratory conditions. Long term storage of most cultures using standard procedures such as freeze-drying of spores often results in the loss of valuable properties, genetic stability and, finally, viability of the strains. Moreover, induction of spore formation of insect pathogenic fungi is one of the obstacles. Therefore, an efficient and inexpensive method for long-term preservation of insect pathogenic fungal isolates especially those that cannot produce asexual spores under laboratory conditions will be investigated in this study. Approximately 50 isolates of important insect pathogenic fungi will be investigated for their ability to produce different types of spores such as blastospores and conidia. Optimization of induction conditions will be studied for all isolates in order to maximize yield. Then, preservation protocols using the liquid-drying (L-drying) technique for each isolate will be determined and the viability obtained will be compared with a commonly used preservation method. By the end of the study, the most effective and economical means for long-term preservation of valuable species of insect pathogenic fungi will be established which could be used as a standard protocol for culture collections dealing with insect pathogenic fungi.

Diversity of fungi in water, plants and fish in the Chi River

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The fungi from water, plants and fishes in the Chi River in Chaiyaphum, Khon kaen and Maha Sarakham Provinces, were bimonthly isolated during April 2007 to February 2008. Two hundred and ninety-four fungal isolates were found. The most dominant fungi were imperfecti (206 isolates), especially the genus *Fusarium* (79 isolates). Eighty-four isolates of Oomycetes were also investigated, mostly belonging to the Order Saprolegniales, Family Saprolegniaceae. Within this family, the genus *Achlya* obtained from the studied sites was dominant (33 isolates). Other genera such as *Aphanomyces* (27 isolates), *Saprolegnia* (21 isolates) and *Leptolegnia* (3 isolates) were found as well as 4 isolates of *Pythium*. Only few isolates were identified through to species because sexual reproductive organs were not produced. In addition, the results revealed that only *Aphanomyces*, *Saprolegnia*, *Achlya*, *Fusarium* and some imperfect fungi could be isolated from fishes, whereas other fungi were isolated from water, sand and plants (leaves, twigs and roots) in the Chi river. These would need to fulfill Koch's postulates by artificial infection of normal fish. However, the results suggested that temperature and geographical differences rather than infected fish species has played an important role in the species diversity of water moulds.

Biological characteristics of 4 genera including effects of temperature, salinity (sodium chloride, NaCl) and pH on hyphal growth showed that the fungi grew at 10-30°C, 1-2.5% NaCl, and pH 5-10. Artificial infections of 6 fungal genera to Nile tilapia *Oreochromis niloticus* and Silver barb *Puntius gonionotus* showed only *Aphanomyces* sp. was pathogenic to the fish but others were secondary infections.

Yeast diversity in water of mangrove forest at Laem Son National Park, Ranong Province

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The diversity of yeast in water of a mangrove forest in Laem Son National Park, King Amphoe Suksamran, Ranong Province, was investigated by isolation of yeasts using a membrane filtration technique and identification based on the analysis of the D1/D2 domain of 26S rDNA sequences by similarity and phylogeny. Among seventy-five strains belonging to the Phylum Ascomycota, 44 strains were identified as 17 described species, namely *Candida butyri*, *Candida parapsilosis*, *Candida pinguabensis*, *Candida rugosa*, *Candida silvae*, *Candida thaimueangensis*, *Candida tropicalis*, *Debaryomyces nepalensis*, *Galactomyces geotrichum*, *Issatchenkia occidentalis*, *Issatchenkia orientalis*, *Issatchenkia siamensis*, *Issatchenkia terricola*, *Kodamaea ohmeri*, *Pichia burtonii*, *Pichia galeiformis* and *Pichia kluyveri*, and 12 strains were found to be five undescribed yeast species, similar to *Candida* sp. NRRL Y-27127, *Candida* sp. NRRL Y-27665, *Hanseniaspora* sp. CS-2008b, *Hanseniaspora* sp. ST-250 and *Hanseniaspora* sp. YS DN19. The remaining 19 strains were found to represent four novel species. On the basis of polyphasic taxonomy including molecular taxonomy, phylogenetic analysis, conventional taxonomy and chemotaxonomy, *Kluyveromyces siamensis* sp. nov. was described.

Diversity of yeast in water and sediments of mangrove forests along the upper coast of the Gulf of Thailand

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Mangrove forest is a unique ecosystem which possesses much biodiversity. In Thailand, mangrove forests are distributed over both saline coasts of the Gulf of Thailand and the Andaman Sea. Due to the lack of investigation of yeast diversity in Gulf of Thailand coastal mangrove forests, this study was proposed to determine the diversity of culturable yeasts in water and sediments in mangrove forests on the east and west coasts of the Gulf of Thailand. A total of 100 strains of yeast were isolated from water and sediment samples collected from mangrove forests in the upper coast of the Gulf of Thailand and were subjected to identification procedures. Molecular taxonomy, based on the analysis of the D1/D2 domain of 26S rDNA sequences using similarity and phylogenetic analyses revealed that 105 strains were known species and four strains were undescribed species. Among the strains of known species, 87 strains belonged to 31 species of 13 genera of ascomycetous yeast, namely *Brettanomyces*, *Candida* (39% of all known species), *Debaryomyces*, *Hanseniopsis*, *Issatchenkia*, *Kloeckera*, *Kluyveromyces* (15% of all known species), *Kodamaea*, *Metschnikowia*, *Pichia*, *Saccharomyces*, *Torulaspora* and *Williopsis*, and 19 strains belonged to *Rhodotorula mucilaginosa* (17% of all known species), a basidiomycetous yeast. Three were found to represent novel species in three genera of ascomycetous yeast, namely *Candida*, *Issatchenkia* and *Saturnispora*.

Development of microbial diversity as biological indicators for soil quality in sustainable organic rice farming

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This study is aimed at developing and using microbial diversity in rice soil as indicators of soil quality for sustainable organic rice farms. An experiment was carried out using an existing rice field (Kao Dok Mali 105 variety) in Surin Rice Research Center, Thailand. Four plots of rice receiving different management practices were investigated in this study. They include: (1) conventional farming (CF) with a normal rate of chemical fertilizer applied; (2,3) two organics, one with green manure (GM) and the other with rice straw (RS); and (4) a control plot (CT) without an external source of plant nutrients. Soil quality in the four treatments was assessed using selected physical, chemical and biological parameters. Relevant findings were that a typical low pH (4.2-5.2) was observed in soil at harvest from all treatments, likewise inadequate phosphorus was detected (Bray II P with a range 2-5 ppm) except in the CF treated soil, and available P was enough for growing rice with values ≥ 10 ppm. In addition, soil respiration (1.75-7.76 mg/kg/day) as well as microbial biomass carbon and nitrogen (0.041-2.169 and 0.027-0.571 g/kg, respectively) were quite low for all treatments. Because of mineralized N and because other plant nutrients were small, there was not enough to meet the demand of rice plants. Low yields of rice (≥ 500 kg/rai) and perhaps low quality too are expected from organic rice (GM and RS) soils. Nitrogen seemed to be a limiting factor and should be provided to all plots including the CF plot. Determination of microbial community diversity in paddy soil is still on-going with denaturing gradient gel electrophoresis of polymerase chain reaction-amplified 16S rDNA partial sequences (PCR-DGGE). Fluorescence *in situ* hybridization analysis (FISH) and the BIOLOG Ecoplate method are included in this study.

Impact of land management on soil bacterial diversity at Thong Pha Phum District, Kanchanaburi Province

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Variations in soil properties and bacterial diversity in soil samples were observed for different types of land management, namely a chemically-intensive farm, an organic farm, and a forest at Thong Pha Phum District, Kanchanaburi Province. Significant differences in soil physical factors and nutrients were found at sampling sites. Analysis of soil bacterial community structure showed that the bacterial communities obtained from soils in the 3 different sites were dominated by *r*-strategist bacteria. No significant differences were found in bacterial numbers both between sites and seasons. However, colony forming unit (CFU) values were positively correlated with soil pH and soil nutrients. Ecophysiological index (EPI) values were not significantly different between sites and seasons. Finally, impacts of land management on bacterial diversity were analyzed. Total soil DNA samples were isolated from soil collected from 3 sites. The 16S rDNA was amplified and cloned into plasmid vectors. Among 70 clones obtained, the majority belonged to unidentified, uncultured bacteria (57.14%). Moreover, *Alpha*- (8.57%), *Beta*- (2.86%), and *Gamma*- (7.14%) *Proteobacteria*, *Firmicutes* (12.86%), *Actinobacteria* (8.57%), *Bacteriodes* (1.43%), and *Planctomycetes* (1.43%) were also found in this study. From the results of bacterial diversity study, different land management could affect bacterial diversity because there were large numbers of unique phylotypes in each site. Furthermore, impacts of land management were related to other factors, such as chemical residues remaining in the area. Long-term studies should be implemented to examine absolute changes caused by land management.