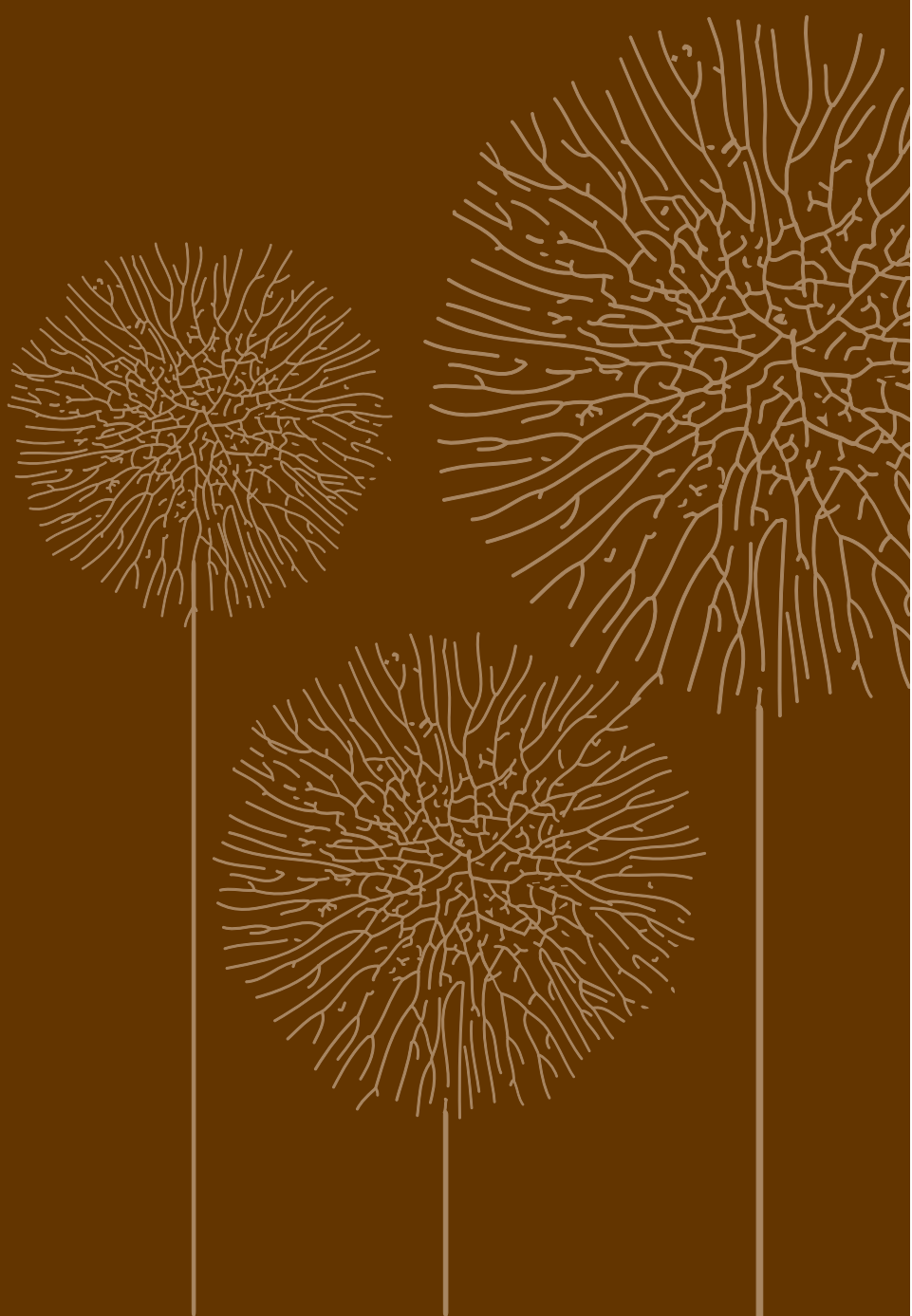
Book of Abstracts

International Conference on Fungal Evolution and Charles Darwin: From Morphology to Molecules



BIOTEC
a member of NSTDA

9-11 July 2009

Sirindhorn Science Home, Thailand Science Park, THAILAND

International Conference on Fungal Evolution and Charles Darwin: From Morphology to Molecules

9-11 July 2009
Sirindhorn Science Home,
Thailand Science Park,
Thailand

Organized by:

National Center for Genetic Engineering and Biotechnology (BIOTEC)
National Science and Technology Development Agency (NSTDA)
Ministry of Science and Technology (MOST)

ISBN : 978-616-120-000-8

Sponsored by:

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About the Conference

The year 2009 marks the 200th anniversary of the birth of Charles Darwin. The theory of evolution, thanks to him, is no longer a theory but has placed life in the domain of natural laws; completely transforming the way we think about the natural world. Life evolves, it started out small and slow and our very form of existence today is the product of progress upon nature over millions and millions of years.

Due to the fact that the fungi comprise a significant proportion of the diversity of species on Planet Earth, this conference aims to examine the contributions that mycology has made over the years to our understanding of evolution, natural selection and the concept of species since Darwin. It will also focus on the progress of taxonomy, classification and applications of fungi after Darwin. The outcome of this conference will be an initiation of fruitful partnership for evolutionary relationships and classification of fungi. The activities of the events include scientific conferences, poster presentations and exhibition.

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Keynote and Plenary Lecture

Adaptation examined through comparative genomics in experimental speciation

Linda M. Kohn

Ecology and Evolutionary Biology, University of Toronto

Understanding how speciation occurs is a central goal of evolutionary biology. By inciting speciation in experimental populations of the yeast *Saccharomyces cerevisiae*, we demonstrated the link between divergent selection in isolated populations and the onset of reproductive isolation (Dettman *et al*. 2007 Nature). In this system, reproductive isolation had two independent origins, ecological isolation evident as phenotypic mismatch of hybrids to the environments in which selection occurred and Muller-Dobzhansky interactions evident as inherent genetic conflict among adaptive mechanisms, independent of environment. Still lacking in any system is a genome-wide identification of the determinants of adaptation and reproductive isolation. We have deployed whole-genome sequencing to identify candidate mutations in two haploid representatives from yeast populations evolved for 500 generations in a high-salt environment and two from a low-glucose environment. From these candidates, we have determined the mutations responsible for the adaptive increases in mitotic fitness and for the reproductive isolation in hybrids. Current research focuses on systems interactions, the likely field upon which reproductive isolation plays out.

Notes:

Species concepts of *Fusarium* and possible examples of its speciation

Takayuki Aoki

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Fusarium is the genus name of well-known anamorphic fungi belonging to the Hypocreales, Ascomycota. The phrase, “Species concept of *Fusarium*” may lead us to an image that the group is a representative of chaotic classification. As a truth, very severe debates among different schools of *Fusarium* taxonomists, e.g. “splitter” vs. “lumper”, have continuously been performed over the decades. It is because the classification of *Fusarium* species has traditionally been based on “Morphological species concepts”, as similarly observed in other groups of fungi. For the “Morphological species concepts”, recognition of phenotypic characters to differentiate species from species would have a main role of taxonomic practices. “Biological species concepts” on the basis of cross experiments and considering sexuality of species have also been introduced in some groups of *Fusarium*. From viewpoints of their teleomorph formation, i.e. ascomycetous structures, species recognition/delimitation has been challenged. Recently, application of “Molecularphylogenetic species concepts” proceeds very rapidly and species become defined by comparative analyses of DNA nucleotide sequences of different gene regions. Scientific names of *Fusarium* based on different species concepts have often been applied like a mosaic in the current taxonomic discussions. For the re-construction of overall taxonomic system of *Fusarium*, detailed comparison and an equivalence process would be always required among the different species concepts. Species concepts applied to the *Fusarium* taxonomy would be briefly introduced in the present talk. Various taxonomic systems as the results of different species concepts would be contrasted, with examples of representative species groups of *Fusarium*.

The famous monograph, “Die Fusarien (the fusaria, in English)” published by Wollenweber & Reinking from Berlin, Germany in 1935 is considered in general as the substantial starting point of modern taxonomy of the genus *Fusarium*. Sixteen sections, 65 species, 55 varieties and 22 forms, in total, were recognized in their taxonomic system based on morphological characters like conidial shapes and sizes, as most important phenotypes. Before their compilation, no special cares had been taken even in morphology, and more than 600 species of *Fusarium* were enumerated simply according to their hosts, habitats and geographical difference of collection sites. Wollenweber & Reinking started applying culture media such as agar plates or sterilized plant pieces like cut potatoes to stabilize morphological phenotypes. The German school and its successors/followers were called “splitters” and, on the contrary, the American school and its successors/followers were called “lumpers”. Snyder & Hansen, the American school of *Fusarium* taxonomy, considered most of diagnostic morphological differences used by the German school only as cultural variations of the same species. The latter school, then, recognized only 9 species within the genus (therefore, it was called “the nine species system”). For the practical purpose, the latter school suggested intraspecific criteria of forma specialis (f. sp.) for the plant pathogenic species related to their hosts. Among them, species concepts of middle sizes, such as the Canadian-British system and the Russian-Israeli system, were also introduced. Very severe debates had, therefore, been continuously made over the decades among different schools of *Fusarium* taxonomy.

Introduction of biological species concept based on pairing experiments using tester strains was also challenged around 1970s, by discovering corresponding mating types in each of “biological species” or “mating populations”. However, its application was limited only to some heterothallic species that form teleomorphs (sexual stages) easily as their sexual reproduction.

Currently the most influential technique for the *Fusarium* taxonomy is molecular-phylogenetic analysis based on DNA nucleotide sequencing, gradually but rapidly introduced into the research area since 1990s. Molecular-phylogenetics may re-evaluate application of current morphological phenotypes and resulted taxonomic systems from the view-point of molecular evolution independently. As estimation, existence of more than 300 phylogenetic species has also been suggested, including many so-called “cryptic species” within given species criteria. Correspondently, molecular-phylogenetic species concepts or GCPSR (genealogical concordance phylogenetic species recognition) would also be placed as the basis of taxonomic studies of *Fusarium*. Therefore, it would probably be a natural consequence for us to see shortage of phenotypic characters to describe, diagnose or identify species morphologically. Exploration for additional stable characters would be an urgent requirement, when *Fusarium* taxonomists/workers would still like to use diagnostic phenotypes for the (new) species definitions.

In the present talk, species related to the diseases, soybean SDS (sudden death syndrome) and *Phaseolus* root rot, within the *Fusarium solani* species complex (formerly called as the section *Martiella*) will be introduced and contrasted with species related to the head scabs of wheat and barley within the *Fusarium graminearum* species complex (formerly called as the section *Discolor*), as good examples to consider what could be the real unit of species of *Fusarium* in relation to their possible mode of speciation.

Notes:

Complexities in naming fungal species resulting from the inability to define fungal genera, species, and individuals, and our mycological history

Scott Redhead

Agriculture and Agri-Food Canada, Canada

150 years have passed since ***The origin of species*** first was published in 1859. Educated persons worldwide might assume that Darwin would have defined his concept of a 'species' in the book, but part of the brilliance that allowed him to articulate his hypothesis was in skilfully avoiding a definitive circumscription of a species definition because he knew it could not be done. Modern science is hardly further ahead but our ability to resolve taxa is improved. In 1859 as of now we speak of species using binomial names standardized by Linnaeus 106 years earlier, but in fact pre-dated by a classification of genera lacking species names. The recognition of species, and hence the decision to adopt or synonymize multiples of species' names remains subjective. Fungi further challenge the intellectual envisagement of 'species'. In part it is impossible in almost all cases to define or to delimit fungal individuals, and in many cases to disentangle individuals from one another or other fungi. The classical classification of fungi is fleshed out on a skeletal framework that is based upon gross sexual morphology and detailed anatomy on which species were grouped into genera that were originally defined morphologically. Many fungi largely or wholly reproduce asexually expressing vastly different morphology making taxonomic links tenuous. For fungi, the availability of, and competition between published names continues to be guided and regulated by the International Code of Botanical Nomenclature that relies upon type designations. However, neither the morphology, nor anatomy of phylogenetically linked taxa may match that of the type for a generic or a specific name. To facilitate discussion and description of fungal spcies discovered reproducing asexually, mycologists allowed for temporary form names, and in order to match type morphology, such names were restricted in usage. Now these names compete with those upon which the sexual classification was founded. Mycologists still do not know how to handle taxa that cannot be defined morphologically. This is a widespread problem across biology, leading in part to the development of the Phylocode, but for fungi the inability to morphologically distinguish taxa goes down to the species or even the level of individual. Fungal nomenclatural problems are unique even compared to bacterial nomenclature because fungal thalli range from unicellular all the way up to species with highly complex morphological structures while close phylogenetic relatives also may have collapsed lifecycles. Fungal nomenclatural problems span all of the inherent problems, including labelling of species, in both of the other Codes of nomenclature. It is little wonder that Darwin did not mention a single fungus in ***The origin of species*****.** For that matter, one might ask, 'what is a single fungus'?

Notes:

Evolution of marine fungi, speciation by adaptation

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Many theories have been advanced to account for the origin of marine fungi, in particular the ascomycetes. The Floridean hypothesis stated that ascomycetes evolved from parasitic red algae (phylum Rhodophyta, family Florideae) or a red algal-like ancestor. However, evidences from recent molecular data did not support this hypothesis. Over the last decade, the use of molecular techniques has been applied to examine the inter-relationships of a number of marine taxa at different taxonomic levels. Spatafora et al. (1998) were first to demonstrate that marine ascomycetes are being independently derived from terrestrial ancestors. Recently, our study has also contributed to the knowledge of marine fungal evolution. We found that at least seven lineages within the unitunicate ascomycetes have migrated into the sea. Halosphaeriales is the largest order of marine fungi with thin-walled deliquescing asci and appendaged ascospores. A great diversity of ascospore appendage developments has been observed for a wide range of genera and species in this order. They may have arisen as an adaptation in aquatic environment, e.g. aiding in spore dispersal and attachment to the substrata. Furthermore five lineages of marine bitunicate ascomycetes are also indicated from analyses of molecular data, and may be intermediate forms with many retaining active ascospore discharge. A comprehensive classification of the filamentous marine fungi is outlined in the recently published monograph. The classification of higher marine fungi includes 530 species (in 321 genera): 424 species of Ascomycota (in 251 genera), 94 species of anamorphic fungi (in 61 genera) and 12 species of Basidiomycota (in 9 genera).

Notes:

Biogeography of macrofungi: Not everything is everywhere

Gregory M. Mueller
Chicago Botanic Garden, USA

Biogeography was central to the development of Darwin's ideas. The realization that unique, but seemingly related species occurred on adjacent islands or different parts of the globe supported the theory that species arose from common ancestors that diverged and dispersed over time and space. While animal and plant biogeography has been an active field of study since the time of Darwin and Alfred Russell Wallace, until recently, the prevailing notion wasthat ancient organisms with easily dispersed spores like fungi would be broadly distributed with few interesting biogeography patterns. There were exceptions, such as the distribution of *Cytaria* with *Nothofagus* and potential temperate East Asian / Eastern North American disjuncts. But, for the most part, distribution patterns of fungi were listed only as secondary information in monographs or discussed with a pathogen's host distribution. This was in large part due to comfort with accepting highly morphologically and ecologically plastic species concepts, coupled with limited collecting outside of Europe and North America and the acceptance of the paradigm that long-distance dispersal is rampant through spores carried by wind. But now, greatly increased global sampling, interactions among mycologists, and new technologies have revolutionized ideas of fungal biogeography. With the exception of some polypore taxa, most examined species of macrofungi show discrete distribution ranges and many "cosmopolitan" species have been shown to be species complexes. These studies of fungal distributions are revealing fascinating geographic patterns and are providing insights into fungal evolution.

Notes:

Comparative biogeography of closely related, ectomycorrhizal and saprotrophic basidiomycetes: Hysterangiales and Geastrales (Phallomycetidae)

Kentaro Hosaka

Department of Botany, National Museum of Nature and Science, Japan

Although fungi are ubiquitous and play an important role in terrestrial ecosystems, fungal biogeography (or mycogeography) has not been extensively studied within a phylogenetic framework. The cryptic nature of many fungi makes them difficult to sample, and thus hindering global scale biogeographic studies. In this talk, I will examine my preliminary results from multigene analyses of Hysterangiales and Geastrales, two of the four major groups within Phallomycetidae (Basidiomycota), to understand the comparative biogeography of closely related, but ecologically distinct groups. For example, unlike Hysterangiales, which is characterized by ectomycorrhizal habit and hypgeous fruiting bodies with spore dispersal by animal (rodents and small marsupials) mycophagy, Geastrales is characterized by saprobic habit and above - or below-ground fruiting bodies with spore dispersal by wind. Comparative biogeography of four closely related groups within Phallomycetidae will provide exciting insight into the fungal biogeography, which is still in a developing stage as compared to plant and animal studies. No such comprehensive biogeographic studies for macrofungi are yet available.

Notes:

Adding leaves to the Fungal Tree of Life: DNA barcoding mushrooms

Jean-Marc Moncalvo, Bryn T.M. Dentinger. Emma Harrower, and Simona Margaritescu.

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In a letter to T.H. Huxley in 1857 Charles Darwin wrote “The time will come […] when we shall have very fairly true genealogical trees of each great kingdom of nature.” Darwin’s prediction is becoming true. Biologists are now in the process of unfolding the evolutionary Tree of Life, thanks to recent advances in molecular biology and bioinformatics methods. For Fungi, Deep Hypha, AFTOL, and, several other initiatives have recently identified many monophyletic groups and have tentatively inferred their relative order of origin; the DNA barcoding project is attempting to survey and document species diversity at a global scale using molecular data; and DNA-based environmental samplings are unraveling many new and mysterious taxa at both species and higher taxonomic levels. A comprehensive reconstruction of the Fungal Tree of Life is therefore well underway, but there is still a long way to go to fulfill Darwin’s dream. While the succession of many diverging branches in the fungal evolutionary tree is now relatively well established, much less is known about how many leaves - or species - these branches actually support, and how fungal species distribute geographically and ecologically. DNA barcoding is an approach to rapidly identify species using short, standard genetic markers. One of the several purposes behind this simple method of species identification is to speed up the discovery and documentation of biodiversity. In this presentation we use examples from mushrooms (Agaricomycotina) to review and discuss methods and logistic challenges for a global, DNA-based survey of fungal species diversity. Problems include (1) the choice of molecular markers for species identification; (2) field and laboratory techniques; (3) quick dissemination of information about new “molecular” species discoveries and geographic distributions; and (4) how to attach species - or leaves - on branches of the fungal tree of life.

Notes:

Progress and challenges with circumscribing species in lichen-forming fungi

H. Thorsten Lumbsch

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Lichen-forming fungi have generally larger distribution ranges than most vascular plants with numerous cosmopolitan or pantropical species. This has led to a widespread notion that distribution of lichens is primarily shaped by ecological conditions rather than explained by historical events. The few exceptions include the impact of glaciation on the European lichen flora for the explanation of Eastern North American – Eastern Asian disjunctions in several genera and also continental drift has been invoked to explain current distribution patters of some tropical and southern Hemisphere. While classical biogeographical studies were descriptive, recently some studies analyzed distribution patterns in a phylogenetic framework, with, however, only a few using statistical methods to address ancestral range evolution of clades. Molecular data have challenged the current mostly morphology-based species concept in lichenology. An increasing number of studies reveal that some of the widely distributed, morphologically circumscribed species in fact consist of several distinct lineages. This presentation will summarize recent progress in our understanding of circumscription of species in lichenized fungi and its impact on our changing ideas about the biogeography of these organisms. Further challenges with using molecular phyogenies to circumscribe species are discussed and the application of phylogenetic or cohesion species concepts. Specifically, the problem of distinguishing distinct lineages and structured populations and the non-monophyly of species due to founder effects in peripatric speciation will be addressed.

Notes:

Fungi in the Tree of Life: From plants to their rightful realm

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The phylogenetic position of Fungi in the Tree of Life was debated for several centuries. Traditionally Fungi have been studied as plants. Over this time continuing assessment of morphological and biochemical traits contributed to a late 20th Century understanding that Fungi are indeed an independent monophyletic group of organisms. The advent of universal molecular characters that could be analyzed and polarized brought agreement that Fungi are a separate groupof organisms, more closely related to animals than to plants. Recent emphasis in the Fungal Tree of Life has been on filling out the tree by sampling all major lineages, particularly those from the under sampled basal groups. The inclusion of additional fungi from basal lineages gives a view of higher than previously realized diversity among these lineages. In addition to sampling more lineages there also is progress in obtaining structural and biochemical data to support branches of the tree. Such efforts also are supported by the increase in DNA genomes and sequences available in public databases. More than a hundred fungal systematists from around the world participated in development of an ordinal level phylogenetic classification based on a fungal phylogeny.

Notes:

From phylum to population: The molecular evolution of mycorrhizal fungi

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Symbioses between plants and fungi have had a profound influence on terrestrial ecosystems. Evidence has accumulated that mycorrhizae have accompanied land plants since the original colonization of land.

Mycorrhizal interactions arose multiple times independently in the evolution of land plants, involving different groups of symbionts in the plant as well as on the fungal side. The oldest and most widespread mycorrhiza is arbuscular mycorrhiza (AM). As in other fungal groups, molecular phylogenetic research has contributed considerably to clarify the evolutionary history of the fungal symbionts of AM, which are now placed in the phylum Glomeromycota.

Likewise, molecular identification methods have revolutionized ecological studies of mycorrhizal fungi in natural and human-influenced ecosystems. They helped to better understand diversity and dynamics of comunities of fungal communities, the influence of environmental factors, host specificity and fungal colonization strategies. Molecular markers of high resolution for studying populations of mycorrhizal fungi have become available and allow to elucidate evolutionary processes at the population level.

Notes:

Evolution of hypocrealean pathogens of arthropods: A Cretaceous explosion

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New outlooks of evolution have increasingly taken a cosmological approach. At the chemical level stars are seen as factories for the evolution of new atoms. The coalescence of atoms into planets resulted in an explosive evolution giving rise to molecules and the World of Chemistry. Unique characteristics of the carbon atom resulted in planets evolving a whole new carbon-based 'biochemistry' which brought forth 'Life'. Such 'Life on Earth' arose about three billion years ago. About three million years ago hominids arose. And about 10,000 years ago mankind stumbled upon Agriculture. However, some 600 million years before human-agriculture fungi appeared in the picture.

Fungi have had a long history of association and evolution with plants. So much so that for long they have been taught under botany. The revolution brought about in phylogenetics by the use of DNA/RNA/protein/metabolite fingerprinting has altered the way we look at much life – especially the fungi, which we no longer consider plants.

It is this association with plants that had (and still has) damned fungi to the World of Botany. But in one small corner of the Kingdom fungi have fought back on their own terms through evolution. The Hypocreales is an order with (in)famous plant pathogens (e.g. Nectria and Fusarium). Such fame gained prominence in the last 10,000 years as mankind evolved to a level requiring agriculture – and consequently evolving to the level of being able to understand the workings of other carbon-based life. The Hypocreales has demonstrated a tremendous plasticity in the way that it makes use of metabolic pathways for the engineering of hypocrealean-beneficial compounds. Such compounds have impinged increasingly on mankind thanks to our usage and abusage of agriculture.

Through the plasticity of their metabolic pathways the hypocrealean fungi have branched out beyond the Plant Kingdom to make use of animals and even other fungi as sources of energy and nutrition. Consequently, this has resulted in an explosion of evolution for the clavicipitaceous members of the Hypocreales. This explosion was so dramatic that morphology gave good grounds for erecting this group as a separate order – Clavicipitales. However, molecular phylogenetics leads us to consider this as merely a dynamically evolving assemblage within the order Hypocreales. This clavicipitaceous assemblage is founded on animal/fungal associates – although the order of acquisition id not clear. This is in contrast to the rest of the fungal Kingdom which is clearly founded upon and depends upon members of the plant Kingdom.

We postulate, here, that it is this nutrition change that has driven an explosive evolution of the clavicipitaceous assemblage within the Hypocreales to a point where the Fungal Kingdom is taking new directions in evolution. Not least in the evolution of the metabolites that result from such new associations.

Notes:

Extrolites, ecology and evolution: Functional molecular features are the leaders, genes are followers

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Extrolites, functional molecules produced by living organisms, that are outwards directed, have played a major role in ecology and evolution. Other important outwards directed features of anyone organism are morphology, nutrition, ecophysiology and resistance; but often extrolites are clear-cut families of signal molecules that are used in the chemical reactions to the abiotic and biotic environment, and therefore of immense value for the systematist. Yet extrolites are hardly used in general biosystematics, rather nucleotide sequences of house-hold genes are used in conjunction with "traditional" morphological features. Even though they are used in practically all modern biosystematic studies and species descriptions, house-hold gene sequences are only indirect molecular clock measurements, and have in many cases been rather useless at the species level. For example the Cox1 gene and ITS or other rDNA sequences are similar in quite different species and are therefore not suitable for bar-coding, cladistic studies etc. This is not surprising, as selection does not work on internal introvert house-hold genes at all. However, calmodulin, beta-tubulin and other sequences do contain some phylogenetic information, probably because they are, after all, indirect molecular clocks. Extrolites, in contrast, are the features selection and niche construction can act upon or use, thereby also being extremely important in co-evolution and ecology. They include secondary metabolites (ecological expression: aristolites), hormones, overproduced organic acids, hydrophobins, extracellular enzymes, biotransforming enzymes, outwards directed lipids and carbohydrates etc. They are secreted or placed on the outer cell wall, and it is therefore hard to imagine that they are not functional. When they have been applied in biosystematics, they have given very clear results at the species level, even in cases where only aristolites have been used. In general a polyphasic approach to taxonomy is recommended, based on all outwards directed features, but avoiding individuality based features (the tokotype, "fingerprinting") whenever possible. Such approaches have been used in the important Trichomaceae members *Byssochlamys (Paecilomyces)*, *Eupenicillium (Penicillium)*, *Eurotium*, *Neosartorya*, *Petromyces*, *Chaetosartorya*, *Emericella* etc. *(Aspergillus)*, talaromcyes (s/g *Biverticillium*) and many other genera. These taxonomic studies have always resulted in clear-cut well-circumscribed species. Examples of such studies will be presented.

Notes:

Molecular and chemical ecology of the Xylariaceae: Importance of secondary metabolites for classification and phylogeny

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The Xylariaceae is one of the largest and most diverse families of the Ascomycota, demonstrating their highest biological diversity in the tropics (Rogers, Mycol Res 104: 1412f., 2000). However, they are ubiquitous and predominant among both the wood-degrading mycobiota and the fungal endophytes of plants in all forests of the Earth. Their immense phenotypic plasticity is expressed by an extremely high variability of morphological features, as wellas by a rather diversesecondary metabolism. We have evaluated the secondary metabolism in stromata and cultures of these fungi extensively, involving studies of over 5000 specimens and several hundreds of culturesby metabolomic methodology. In the course of this work, over 100 secondary metabolites, many of which proved to be chemotaxonomically significant, were isolated and identified, revealing over 30 novel natural products. The data generated by HPLC (mass spectra, Diode array spectra,. and retention times in standardised systems) have been stored in a comprehensive database which serves for on-line identification of the constituents in crude extracts, using minute quantities of material.

The chemotaxonomic data were mapped against a molecular phylogenetic tree and compared with the classical morphology-based concepts. Based on this data matrix, it appears feasible to predict phylogenetic relationships from HPLC profiles of newly studied taxa and clarify their taxonomic affinities, since rDNA-based phylogenies often concurred with the secondary metabolite profiles. For instance, cultures of the genera *Daldinia*, *Entonaema*, *Phylacia*, and *Thamnomyces* produce several common classes of polyketides and also appear related with respect to rDNA-based phylogenies, despite they strongly deviate in their stromatal morphology (Bitzer et al., Mycol Res 104: 1412f., 2008; Stadler *et al*., subm. to Mycoscience, 2009). On the other hand, certain species were transferred to different genera as chemotaxonomic and molecular evidence revealed that certain morphological and anatomic features have arisen in convergence (Stadler *et al*, Mycol. Progr. 7: 53-73).

On the other hand, some classes and types of secondary metabolites of the teleomorph, such as the stromatal azaphilone pigments of *Hypoxylon* and related taxa, are chemotaxonomic markers at the level of species or species group.

Most of the above mentioned phylogenetically significant metabolites from cultures as well as the stromatal pigments, have shown antibiotic, phytotoxic, insecticidal or nematicidal activity, indicating that their biological function relates to chemical defence, or the involvement of the compounds in the relationships to their hosts. A comparison of their chemical structures suggests that these metabolites have co-evolved along with biological speciation, as a means of adaptive radiation. The metabolite production may be induced in the presence of mycophilic parasites. Moreover, some compounds that are overproduced by freshly isolated endophytic Xylariaceae as well as isolates from ascospores, may decrease substantially or even cease altogether upon frequent subculturing in nutrient-rich culture media. These features, as well as the fact that up to 20% of the stromata may be comprised of secondary metabolites in some species of Xylariaceae, are discussed in scope of Darwin's evolutionary theories. Some hitherto unknown chemotaxonomic correlations, and some new genera and species that were recently discovered from our polyphasic taxonomic approach will also be presented.

Notes:

Evolution and regulation of secondary metabolite biosynthesis in filamentous fungi

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Fungi produce a diverse array of secondary metabolites with a range of activities. Epipolythiodioxopiperazines (ETPs) are an important class of toxic metabolites produced by filamentous ascomycetes. The toxicity of ETPs is due to the presence of a disulphide bridge in a diketopiperazine ring, which can inactivate proteins via reaction with thiol groups, and to the generation of reactive oxygen species by redox cycling. The best known ETP is gliotoxin, which causes apoptosis and is implicated in aspergillosis of humans caused by *Aspergillus fumigatus.* Another ETP, sirodesmin PL, is produced by *Leptosphaeria maculans*, a pathogen of oilseed rape. These molecules are produced by enzymes encoded by genes clustered in the genome. Production is regulated by a pathway-specific transcription factor in the cluster, which in turn is regulated by upstream genes including cpcA, a cross pathway control gene. Under conditions of amino acid starvation, cpcA is transcribed in *L. maculans* at high levels and sirodesmin PL is not produced. In nutrient –rich media, cpcA is transcribed at low levels and the fungus produces high levels of sirodesmin PL. Thus production of these secondary metabolites is dependent on nutrient status of the fungus. With the availability of complete fungal genome sequences and efficient gene-disruption techniques for fungi, approaches are now feasible to delineate biosynthetic pathways for ETPs and to gain insights into the evolution of such gene clusters. Analysis of ETP clusters in more than 25 ascomycete genomes suggests that the gene clusters have a common origin and that the inheritance and distribution of clusters is consistent with horizontal gene transfer and loss of cluster within the ascomycetes.

Notes:

Secondary metabolites of insect pathogenic fungi

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The most representative insect pathogenic fungus, *Cordyceps sinensis* (recently renamed as *Ophiocordyceps sinensis*), has been used in China and many Asian countries as traditional medicine and as health food for good health and “eternal youth”. This fungus is rare, occurring only in highlands of Himalaya, and the production of synnemata by cultivation has been unsuccessful. Several other species of *Cordyceps* and related genera have been mass produced either by cultivation on insect larvae or solid media in forms of synnemata or by fermentation in liquid media as mycelia, and the extracts have been commercialized as health foods. Although most of insect pathogenic fungi are specific to their host insect, some of them are strongly pathogenic to various insects. Such species have been commercialized as insecticides. However, the potential of insect pathogenic fungi as sources of bioactive compounds, particularly as lead compounds of modern medicines, has not yet well demonstrated, when compared to other groups of fungi.

As part of the research program on utilization of bioresources in Thailand, conducted at the National Center for Genetic Engineering and Biotechnology (BIOTEC), we have been searching for novel bioactive secondary metabolites from fungi, especially focusing on insect pathogenic fungi. The research results in the last 12 years include the isolation of 60 new natural products from fermented insect pathogenic fungi, many of them possess unique chemical skeleton and/or exhibit significant biological activities. Three representative compounds are cordypyridone A (anti-malarial, IC_{50} 0.66 □g/mL) from *Cordyceps pseudomilitaris* BCC 1389, hirsutellone A (anti-TB, MIC 0.78 □g/mL) from *Hirsutella nivea* BCC 2594, and ascherxanthone B (antifungal activity against the rice-blast pathogen Magnaporthe grisea, IC_{90} 0.58 □g/mL) from *Aschersonia luteola* BCC 8774. Systematic analysis of the extracts from insect pathogenic fungi also provided information of commonly occurring secondary metabolites that have been applied to chemotaxonomy.

Notes:

An emerging opportunistic pathogen, *Penicillium marneffei* in southern China and its pathogenesis study

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Penicillium marneffei is an emerging pathogenic fungus that can cause a life-threatening systemic mycosis in immunocompromised hosts, especially in the patients with AIDS. This infection is endemic in South-east Asian. With the prevalence of AIDS in China recently, the number of patients is increasing dramatically in southern part of China. In Hong Kong, about 10% of AIDS patients developed penicilliosis, while in Guangdong, about 16% HIV-infected patients developed penicilliosis.

P. marneffei is the only known *Penicillium* species that exhibits temperature dependent dimorphic growth, and has the ability to grow as mycelium at 25°C and as yeast at 37°C. Morphogenic transition plays a key role in the pathogenicity of *P. marneffei.* In healthy hosts, *P. marneffei* can be cleared within 2 to 3 weeks, whereas in immunocompromised hosts, *P. marneffei* infection is fatal.

We had isolated *P. mameffei* from the internal organs of bamboo rat captured from Guangdong and bordering area, and found that the carrier rate of *P. mameffei* in rat was about 80%. This finding indicates that *P. mameffei* may exist in nature in Guangdong province of SE China. As yet the source in nature for this organism is not established and represents one of the many mysteries relative to this new disease. Although this infectious disease is very severe and difficult to heal, we know only little about the mechanisms of host-fungus interaction and host immune response in *P. marneffei* infection. More studies on the factors influencing pathogenesis of *P. marneffei* focused on the biology of it, particularly in areas pertaining to its dimorphic nature and interaction with the host defense. In recent study, we identified differentially expressed genes and proteins between yeast and mycelium cells in whole genome with Suppression Subtractive Hybridization (SSH) and proteome with Differential In-gel Electrophoresis (DIGE) technique respectively, 43 differently genes and 26 differently proteins had been identified between two phases. We also isolated and characterized a novel Ras small monomeric GTPase Rsr1 gene, designated *PmRsr1*, from yeast-form *P. marneffei*. The differential expressions of *PmRsr1* in different phases (conidia, mycelia and yeast cells) were also analyzed by sensitive real-time RT-PCR. HSP70, Catalase and Isocitrate acid lyase (ICL) had been confirmed to be differently expression gene in the two phases of *P. marneffei* by RT-PCR and Western blotting. The function research of Isocitrate acid lyase (ICL) by RNAi interference illustrated that it plays a very important role in *P. marneffei* infection and colonize in host.

Although much more attention had been paid to the penicilliosis in the field of medical mycology due to the obscurity of pathogenesis, many unsolved problems exist in geography, clinical epidemiology and pathogenicity of *P. marneffei.* Further researches are progressing in our group now.

Notes:

Medical mycology in Thailand and unique mycosis; pythiosis

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Thailand is a member country of ASEAN and located in tropical rain forest area. There is a high diversity of fungal species and also various mycoses have been found and reported. Common mycoses are pityriasis versicolor, dermatophytosis, candidosis and cryptococcosis. Rare mycoses consist of tinea nigra, black piedra, white piedra, chromoblastomycosis, mycetoma, subcutaneous zygomycosis, phaeohyphomycosis, hyalohyphomycosis, sporotrichosis, aspergillosis and histoplasmosis. Some mycoses are mainly reported from Thailand such as penicilliosis marneffei and pythiosis which occur mainly in AIDS patients as opportunistic infection and thalassemia patients, respectively.

Pythiosis is caused by a water mold *Pythium insidiosum*, which is recently classified in the Kingdom Chromista (Straminopila). The disease has been reported worldwide in mammals such as horses, cows, dogs, cats, and humans. Human pythiosis is mainly reported from Thailand in patients with some haemogloninopathy such as thalassemia, aplastic anemia and paroxysmal nocturnal haem oglobinuria. There are three types of clinical manifestations namely; cutaneous/subcutaneous, ocular and disseminated forms. Laboratory diagnosis can be accomplished by microscopic examination of infected tissue or thrombus and isolation of *P. insidiosum* on Sabouraud dextrose agar plus chloramphenical. Detection of antibody against *Pythium insidiosum* by immunodiffusion test in patient's sera is helpful for diagnosis and monitoring of human pythiosis. Treatment of choice is an amputation of infected tissue/organs because antifungal drugs therapy is usually failed to cure the disease. Only a few cases have been reported on successful treatment with terbinafine plus itraconazole and/or echinocandin. An immunotherapy with *Pythium's* antigens revealed 50% success rate in human pythiosis.

Molecular study of *P. insidiosum* isolates obtained from animals, humans and environment based on ITS1, 5.8 S rDNA and ITS2 nucleotide sequences showed the separation of all strains in 3 clades with geographic correlation.

Clade 1 is mainly found in the United States, central and south America.

Clade 2 contains strains from animals, humans and environment and predominantly found in Asia and Australia.

Clade 3 is specific to Thailand. Almost of the strains in this clade are obtained from Thai patients and water reservoirs in Thailand.

On going researches on pythiosis are dealing with its pathogenesis, improvement of antigen-mix for immunotherapy and molecular epidemiology of patients', animals' and environmental isolates.

Notes:

Ecological fitting and pathogenic evolution in *Exophiala dermatitidis*

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Exophiala dermatitidis is defined as asexual fungi potentially able to produce melanized budding cells in any stage of its life cycle. The fungus is able to pass through the stomach and the intestinal tract of laboratory animals without decrease of vitality, probably aided by its ability to convert to meristematic growth at low pH. The fungus occurs consistently, though at a low incidence, in the digestive tract of otherwise mostly asymptomatic humans. Using rDNA ITS sequencing, two main genotypes ***A*** and ***B*** are known. The group of genotype ***A*** strains relatively contains more clinical isolates, whereas, genotype ***B*** strains are preponderantly found in the (natural and man-made) environment. With multilocus analysis, the result on ITS, TUB1 showed recombination, nevertheless no evidence in EFα-1. Intraspecific variation was moderate, but phylgenetically informative sites was found I ITS and TUB1. Comparing different partitions, standardized index of association (IAS) demonstrated recombination between main ITS genotypes ***A*** and ***B***, but there was also a significant degree of clonality. Recombination networks based on concatenated sequences also shared moderate evidence of recombination. Phenetically the genotypes responded differentially on incubation at 37˚C. These differentiations, ultimately leading to ecological speciation processes, are likely to mark the species's transition from its natural habitats as an aymtomatic associate of furgivorous animals in the tropical rain forest to the human-dominated environment. We hypothesize that it is transported to this new environment by humans, after ingestion of contaminated tropical fruits and berries and further are transferred into the human community by occasional ingestion of wild berries, further dissemination must be brought about by mechanisms different from those found in nature. Human-carriage and growth in the steam bath might promote the selection of adapted genotypes. A set of 178 strains from natural and human-dominated environments in Thailand with a worldwide selection of strains from CBS collection were analyzed by AFLP fingerprinting method to established the haplotype distribution. It is concluded that virulence is likely to be linked to individual clones. The main genotypes ***A*** and ***B*** may be viewed as clusters of haplotypes that on average show different ecological trends.

Notes:

Allergenic fungi of North America and aeroallergen index studied with Burkard Volumetric Spore Trap

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We standardized the use of Burkard Volumetric Spore Trap (UK) to study the fungal aeroallergen. Experimental trials at the Allergy AARTS Clinic and West Texas A&M University revealed that the Allergic Rhinitis patients showed susceptibility to air spores, including *Alternaria*, *Cladosporium*, *Curvularia*, and *Pithomyces*. Airborne spores are usually present in outdoor air throughout the year in high numbers and frequently exceed pollen concentrations by 100- to1,000-fold depending on environmental factors such as water and nutrient availability, temperature, and wind. Most fungi commonly considered allergenic, such as *Alternaria* spp.,*Cladosporium* spp., *Epicoccum* nigrum, *Fusarium* spp., or *Ganoderma* spp. Generally, indoor fungi are a mixture of those that have entered from outdoors and those from indoor sources. *Aspergillus* spp. and *Penicillium* spp. are less common outdoors and are usually considered the major indoor fungi. We collected the samples of fungi from different outdoor and indoor environments including the school buildings and cultured them on agar petri-plates that were examined with an SZ-40 stereo-scope to observe and count the colonies. We used digital and fluorescent microscopy using Olympus BX 40 microscope attached to DP-70 digital camera, FITC and TRITC filters to study the aeroallergens collected from air. The data were analyzed using the attached computer to the microscope equipped with Image pro 6.0 software. Our study showed significant correlation between the aeroallergen indices with the clinical symptoms like allergic rhinitis and asthma.

Notes:

Cooperation and conflict between domesticated fungi and insect societies

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If Charles Darwin would have been alive today, it would probably be easier to explain modern social evolution theory to him than the principles of molecular genetics. Where the latter would involve more than a century's worth of molecular biology and technological development, the former merely requires combining Darwin's own type of phenotypic reasoning with William D. Hamilton's (1936-2000) gene's eye view of evolution. This genetic abstraction and its mathematical elaborations have allowed evolutionary biologists to understand the deep principles of cooperation and conflict. A crucial insight has been that it is impossible to understand the nature of cooperation without understanding how natural selection has regulated or suppressed the potential reproductive conflicts that can corrupt and destabilize social arrangements and mutualistic interactions between species. I will use a decade of work on fungus-farming in ants and termites by our Copenhagen Centre and collaborators to illustrate these principles and the ways in which they can be investigated by experiments and molecular techniques. I will show that fungal symbionts can indeed be highly beneficial to their insect hosts because of the cooperative services that they provide, but that these mutualisms are only evolutionary stable when the fungal symbionts can secure their own reproductive interests.

Notes:

Has classic field based natural history studies and taxonomy a role to play in current mycology?

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The paper focus on the lack of basic knowledge of fungal biodiversity almost across the globe but in particular in tropical countries, and the lack of funding for large scale inventories in the remaining unspoiled part of our nature rather than among fungarium specimens with a limited quality. In particular the lack of core staff in major collections of fungal material is stressed. Examples, on how past and modern day explorations have lead to invaluable new insights are given. Furthermore, the educational crisis is highlighted, and the internet revolution is emphasized.

Notes:

Algicolous marine fungi and their associations

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Algicolous marine fungi exhibit a range of associations with their hosts that fall into three broad categories: saprophytes growing on dead algal thalli, symbionts living in lichen-like associations with the algae and parasites. Fungal pathogens do exist but it is often difficult to distinguish them from parasitic forms. The number of acknowledged algal-inhabiting marine fungi is relatively low, with the majority being characterised as parasites, although a great number of ascomycete, deuteromycetes and sterile mycelia isolates can be recovered, and detected molecularly, from healthy algal tissue. Do any of these fungi represent uncharacterised groups that have adapted to co-exist with algae? In this paper, the relationship between fungi associated with healthy and decayed thalli of *Fucus seratus* is reported and discussed.

A fungal culture collection, comprising over 300 isolates, was constructed from samples of healthy and decayed *F. seratus* thalli. Over a one-year period, molecular signals were extracted from these thalli so that they could be compared to those from the isolates using 18S and 28S rRNA-DGGE-PCR, molecular cloning, real time PCR, as well as analysed phylogenetically. The pre-dominant molecular sequences from healthy thalii matched those belonging to *Lindra, Lulworthia, Sigmoidea/ Corollospora*, and *Emericellopsis/Acremonium* species, whereas sequences from decayed thalli represented members from the Dothideomycetes. Two sequence ribotypes were present throughout the sampling period, and these matched those from *Sigmoidea* marina and *Acremonium fuci*. Both of these species exhibited physiological adaptations suggesting that they have an intimate relationship with *F.seratus.*

Notes:

Chemical interactions of endophytes and their hosts – from parasitism to mutualism

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Endophyte-host plant symbioses represent a broad continuum of interactions, from pathogenic to mutualistic, even within the lifespan of an individual microorganism and its host plant. It has been hypothesized that the interaction between fungal endophytes and their host plants is characterized by a finely tuned equilibrium between fungal virulence and plant defense, in which disease symptoms may develop if the association is disturbed by either a weakening of plant defense or an increase in fungal virulence.

In numerous cases, chemical interactions are thought to play an important role in host-endophyte symbiosis. Experimental data obtained in the literature so far indicates both overtly negative and positive effects. For example, calli of certain host plants became necrotic and died when exposed to a culture filtrate or isolated secondary metabolites of their own endophytes. On the other hand, symbioses between grasses and their endophytic fungi represent the best characterized example for positive effects. Physiologically active alkaloids produced by endophytes have been demonstrated to protect the host plants from grazing livestock, invertebrate herbivores and pathogenic microorganisms, or to inhibit germination and growth of other competing grasses. However, for other plants outside grasses, the detection *in planta* of secondary metabolites produced by endophytic fungi has only rarely been reported. Recent studies by our group clearly demonstrated that various fungal secondary metabolites, initially characterized from a culture of the respective endophyte, can be detected within the tissues of healthy host plants, implying a contribution of the fungal endophyte to the chemical composition of the host plant, and lending support to a mutualistic interaction between both partners.

Notes:

Poster Abstracts

Phylogenetic analyses of *Ramaria* and its allies in Japan

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The genus *Ramaria* is one of the large genera belonging to Agaricomycetes and primarily characterized by having coral-shaped basidiomata. Several species, including *R. campestris*, are edible but some species cause nausea, vomiting and diarrhea to human. This genus has been divided macro- and micromorphologically into four subgenera, *Echinoramaria*, *Laeticolora, Lentoramaria and Ramaria*, and more than 300 species have been described under these subgenera (Marr and Stuntz, 1973). However, recently several phylogenetic analyses have suggested that the *Ramaria* is not monophyletic (Humpert et al., 2001, Hosaka et al. 2006). We conducted phylogenetic analyses of the *Ramaria* and its allied genera in Japan based on nuclear large subunit ribosomal DNA (nucLSU rDNA), mitochondrial ATPase subunit 6 *(ATP6)* and mitochondrial small subunit ribosomal DNA (mtSSU rDNA). In the maximum-parsimony and neighbor-joining trees, *Ramaria* subgenus *Ramaria* and subgenus *Laeticolora* formed a *Ramaria-Gomphus* clade together with *Gomphus* spp. In addition, member of subgenus *Ramaria* were phylogenetically separated into several groups, and *Ramaria botrytis* species complex and *Gautieria* spp. formed a monophyletic group. The topology of molecular phylogenetic trees indicates that coralloid basidioma is an ancestral form of *Gautieria* species producing gasteroid basidiomata and that gasteromycetation occurs at least once in *Ramaria-Gautieriaclade*. Maximum parsimonious trees based on *ATP6* revealed that *Ramaria* subgenus *Echinoramaria* is not monophyletic. The clade containing subgenus *Echinoramaria* is different from *Ramaria-Gomphus* clade, and contains species not only of subgenus *Lentoramaria* but also of *Lentariaceae* and *Clavariadelphaceae.* These results suggest that taxonomic revisions, including establishment of new genera or families, are needed for *Ramaria* and its allied genera. In addition, during the present molecular phylogenetic and morphological analyses, we found several new taxa and previously unreported taxa from Japan.

Notes:

Species diversity of the sequestrate genus, *Octaviania* in Japan revealed by molecular phylogenetic and ultrastructural studies and its evolutionary inference

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The sequestrate (truffle-like) genus, *Octaviania*, is characterized by a marbled pattern of the gleba and dextrinoid or non-amyloid basidiospores with coarse, conical to pyramidal ornamentation. *Octaviania* spp. are considered ectomycorrhizal as well as most members of other sequestrate fungi. The genus accommodates ca. 15 species that suit its generic concept of today. A recent molecular study placed the genus within the *Leccinum–Chamonixia* clade in the Boletaceae (Binder & Hibbett 2006). However, intrageneric phylogeny of *Octaviania*, which includes several Asian species, has not been focused on so far, and taxonomic study of the genus, especially in Asia, has not been fully investigated. Thus, molecular phylogenetic studies of the Asian *Otaviania* species coupled with morphological and ecological approaches could reveal great species diversity of the genus and the detailed evolutionary sequence of the genus than ever. Accordingly, we conducted phylogenetic analyses of *Octaviania* specimens and the related taxa collected throughout Japan based on two nuclear rDNA regions (ITS and nuc-LSU) and one mitochondrial gene *(ATP6)*, and made their detailed morphological observation including transmission electron microscopy (TEM). The resulted Maximum Likelihood and Bayesian trees showed that Japanese *Octaviania* sequences were divided into at least seven lineages, either of which was not conspecific with the type species, *O. asterosperma.* These seven lineages roughly reflected their presumed host specificity. Furthermore, TEM observation revealed that there is considerable variation in the number and form of cavities inside the basidiospore ornaments among these lineages. These differences could be helpful to delimit species among morphologically similar *Octaviania* spp. In addition, one of the lineages was further divided into three sublineages that lack any distinct morphological characteristics. This suggests that the presence of several cryptic taxa within the lineage. TEM observation of the basidiospore ornamentation of *Octaviania* and the related sequestrate genera (*Chamonixia* and *Rhodactina*) further showed their morphological similarities, implying their close evolutionary relationships, although their monophyly was not supported by the molecular analyses. In conclusion, our results show that there is much greater species diversity within *Octaviania* in Japan than considered, and suggest that TEM observation of the basidiospores could help not only to delimit species but also to infer the evolutionary sequence among *Octaviania* and the related taxa.

Notes:

Phylogenetic studies of some *Scleroderma* spp.

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The fungal genus *Scleroderma* comprises about 25 species and has a world wide distribution in temperate and tropical regions. Several species have been shown to form ectomycorrhizal associations with at least 39 genera of phanerogams. To delimit phylogenetic *Scleroderma* species and aid in their identification, we selected 43 basidiomes from different geographical locations and their rDNA internal transcribed spacer (ITS) were sequenced. Phylogenetic analyses of these sequences together with additional sequences retrieved from the GenBank EMBL identified 11 taxa. A strong phylogenetic pattern indicates that basidiospore ornamentation together with ITS sequences can distinguish three distinctive groups within the genus.

Keywords: Ectomycorrhizal fungi, Ecology, Gasteromycete, Taxonomy, Phylogenetic species

Notes:

Using molecular data to understand patterns of chemical variation in the *Cladia aggregata* complex (Cladoniaceae)

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Cladoniaceae is a family of lichenized ascomycetes that includes 13 genera and has a worldwide distribution. Within the family, the small genus *Cladia* has received attention of taxonomists, since the species circumscription has always been difficult based on enormous morphological and chemical plasticity. Furthermore, the unique morphology of the genus has led to the description of its own family Cladiaceae, which has been rejected by molecular data and hence the genus is recognized within the Cladoniaceae. Currently,14 species are accepted in the genus, most of them restricted to New Zealand and temperate Australia. *Cladia* is characterized by the presence of numerous perforations along the vertical thalli, called pseudopodetia. Within the genus, the *Cladia aggregata* complex is considerably difficult and different authors accepted one to seven species to classify the diversity in this group. Therefore, the aims of this study were to (i) test the phylogenetic placement of the *Cladia aggregata* complex within the Cladoniaceae and (ii) investigate patterns of the chemical variation observed in the *Cladia aggregata* complex using molecular data. Specimens from several countries, including Cuba, India, Thailand, the Philippines, Australia and New Zealand were studied. These specimens encompassed the *Cladia aggregata* complex with four recognized chemotypes, and other members within the Cladoniaceae. Genes targeted included the partial GAPDH gene, partial RPB2 gene and ITS region. In a preliminary analysis only ITS sequences were employed. Based on different analyses (maximum parsimony and Bayesian approach) of the ITS region, *Cladia* was supported as monophyletic. Within the *Cladia aggregata* complex the phylogeny was not congruent with the chemical data, indicating that some of the accepted chemotypes do not reflect independent lineages.

Notes:

Environmental influence on genomic diversity in Chinese isolates of AMF

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The D2 domain of the LSU rRNA gene has a high level of genetic variability in the Glomeromycota. Multiple sequences have been shown to occur within individual spores, isolates as well as within and between species. *Glomus mosseae* (BEG185), isolated from China, showed similar sequence diversity, but was then subcultured under different conditions to monitor if this influenced the pattern of genomic diversity recorded. The original pot culture of the *G.mosseae* isolate had a baseline sequence frequency of 12 different sequences, 3 of which were dominant, and nine of which occurred at low frequency only. Preliminary results indicate that frequency changes in the three dominant sequences were observed in samples kept under a high light intensity, with the possible emergence of a new dominant sequence. A much more intensive study will be needed to obtain statistically significant results, but these observations indicate that environmental conditions may influence genomic frequencies within cultures of AMF.

Notes:

ITS analyses and species identification of *Ochroconis* and *Scolecobasidium*

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Ochroconis and *Scolecobasidium* are closely related melanized hyphomycetes, which have an isolated position in the fungal Kingdom. Most of species are saprobes, but some are able to cause diseases in both cold- and warm-blooded animals including humans. Repeated cerebral infections in immunocompromised humans caused by *O. gallopava* have been reported, which make the species be one of the most virulent black fungi. To study the relationships between two genera through their ITS rDNA sequences, sixty eight strains were cultured and DNAs were extracted and sequenced. These sequences, including some from GenBank were analyzed using BioNumeric version 4.61, BioEdit, MrBayes version 3.1.2 and TreeFinder version 2007. The ITS of *Ochroconis* species had remarkably longer lengths and higher %GC contents than those of *Scolecobasidium.* The similarity between two genera was low (<60%), while similarities between strains of each described species were variable: *S. salinum* (97.7%), *S. terreum* (87.6%), *O. tshawytschae* (99.1%), *O. gallopava* (97.90%) and three species clusters of *O. humicola-constricta* (80.80%, 94%, 99.2%). Phylogenetic distances between taxa appeared to be very large, despite the morphological and ecological unity of the group. *Scolecobasidium terreum* seemed to be more closely related to *Ochoconis* than to other species originally described in *Scolecobasidium.* The positions of three distinct clusters of *O. constricta-O. humicola* were strongly confirmed; new taxa might be considered. In conclusion, ITS rDNA sequences can support the close relationships between *Ochroconis* and *Scolecobasidium*, and the large gaps among their species make ITS be an excellent tool for identification and laboratory diagnosis.

Notes:

Morphology, cross-infectivity and PCR-RFLP in identification of *Erysiphe* spp. on alfalfa and clover

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Alfalfa and clover are two important forage crops of the family Fabaceae which are extensively cultivated in Iran. More over them have useful effect on soil structure and have a fundamental role in feeding domesticated animals. As a consequence, they influence on meat and milk production, which influences on human nutrition. The powdery mildew of alfalfa and clover is one of the diseases in Arid and semi-arid regions of Iran. Sixteen isolates were morphologically identified as *Oidium* spp., as they had no mature ascocarp or even lack it. Two isolates on alfalfa recognized as *Erysiphe pisi* and three others on clover as *E. trifolii* var. *trifolii.* One isolate of clover was identified as *E. trifolii* var. *desmanthi* which is reported for first time from Iran. Five infected weeds (*Glycyrrhiza glabra*, *Polygonum persicaria*, *Polygonum rottboellioides*, *Convolvulus arvensis* and *Potentilla* sp.) were collected from inside of alfalfa and clover fields and were used for their ability of infectivity on alfalfa and clover, cross inoculation was done in greenhouse and only isolates of clover and alfalfa could infect clover and alfalfa and no infection from weed sites to clover and alfalfa was observed. Six methods of DNA extraction have been tested and crushing of mycelia between two glass slides gave sufficient amount of DNA. Internal transcribed spacer ITS1-5.8s-ITS2 regions of rDNA was amplified using primer pairs of ITS5 and P3 and in nested PCR assay ITS1 and P3 primer pairs was used. A 522 bp fragment was amplified in *E. pisi* and 558 bp fragment was amplified in *E. trifilii*. PCR-RFLP profiles were obtained using *Taq*I, *Alu*I, *Msp*I, *Hin*fI restriction enzymes. The profiles from *Alu*I confirmed that different isolates are identical and the cluster analysis based on three other enzymes showed variability among different isolates. PCR-RFLP results were concordant with geographical distribution of isolates and could separate the two species *E. pisi* and *E. trifolii.*

Notes:

Species concepts of *Fusarium oxysporum* f.sp. *cubense* in Thailand

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Morphological Species Concept (MSC) of *Fusarium oxysporum* f.sp. *cubense* has been investigated by collecting 117 isolates from banana growing area for 25 provinces in 6 parts of Thailand. Morphological characteristics have been studied in vitro under light microscope. The result indicated that all isolates were classified to be FOC and four morphological variations have been existed among FOC population such as gross colonial color, pigmentation of culture, colonial growth on medium and sporodochium production. Biological Species Concepts (BSC) of FOC has been identified by using the vegetative compatibility groups among 117 isolates. The result indicated that 117 isolates of FOC could be identified into 6 VCGs, as following, VCG 0123 for 89 isolates (76.0%) VCG 0124 for 9 isolates (7.7%), VCG 0125 for 6 isolates (5.0%) VCG 0124/1025 for 8 isolates (7.0%) VCG 01218 for 3 isolates (2.6%) and VCG 01221 for 2 isolated (1.7%). Physiological Species Concept (PSC) was identified by inoculating 117 isolates to four banana differential varieties. The result indicated that all isolates were classified to race 1 that they could infect Klaui Namwa but they did not infect Klaui Hom Thong is belonged to Gros Michel, Klaui Khai and Klaui Bour, respectively. The result exhibited the difference on pathogenecity existing among isolates of FOC. VCG 0123 represented dominated population of FOC in Thailand showed a higher disease severity, 67.8% of crown tissue discoloration and VCG 01221 showed the lowest disease severity, 38.7% of crown tissue discoloration.

Chemotaxonomy Species Concept (CSC) has been proposed by studying the secondary metabolite profile of some FOC isolates. Crude extracts were extracted from culture filtrate and they were spoted on TLC to observe the compounds under UV lamp. The result revealed the different profile exhibited among isolates of FOC and it indicated that it might be used to differentiate the variation among population of FOC within physiological species.

Notes:

A study of systematic position of *Phaeoisaria clematidis*

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Phaeoisaria clematidis is a common anamorph species of fungi in freshwater. It can be found worldwide on submerged wood and recently an isolate has been reported as an opportunistic pathogen human. It therefore is potentially significant to human health. In Thailand, the teleomorph of *P. clematidis* has been discovered is differed from teleomorphs of other *Phaeoisaria* species. This teleomorph-anamorph connection was further confirmed by culture studies. Until now the teleomorph of *Phaeoisaria* had not been reported and its relationship to other fungal genera is unknown. The aim of this investigation is to evaluate the phylogenetic position of this teleomorph-anamorph link based on both molecular and morphological data.

Notes:

Species concepts in fungi and the Darwinian theory

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The fungal world is so unique that it is sometimes considered to be a 'parallel universe' (Becker, 1976), and this is not an overstatement. The combinatorial space of characters in many groups of fungi is so extensive that all possible combinations of specific characters within a genus or generic characters within a family can be observed irrespective of the environments within which they are found. These combinations seem to have no adaptive meaning, but each is associated with a certain type of substratum and occupies a particular area. Ecologically and biogeographically, each species combination conforms to the Darwinian concept of adaptive radiation, although the direct connection between features and habits is not always apparent. For example, it is unclear why *Hypoxylon fragiforme* occurs only on *Fagus*, whereas *Hypoxylon truncatum* prefers *Quercus.*

Although Darwin's principal work is titled "The Origin of Species", it is mainly about the causes responsible for the diversification of life. He was correct in stating that "the forms of life throughout the universe become divided into groups subordinate to groups", but Darwin also dismissed this *hierarchical pattern* of nature in a number of *taxonomically* erroneous phrases. Thus, he spoke of the absence of essential *boundaries between* 'varieties' and 'species', although there cannot be 'boundaries' between parts and wholes. He wrote that "if a variety were to flourish so as to exceed in numbers the parent species, it would then rank as the species, and the species as the variety". That is clearly improbable, since the number of individuals is not a basis for recognizing a group as 'species' or 'variety'. In general, Darwin did not appear to be overly concerned about 'species' per se; he expressed hope for a "considerable revolution in natural history" when systematists "will not be incessantly haunted by the shadowy doubt whether this or that form be a true species" and "the endless disputes whether or not some fifty species of brambles are good species will cease". However, the most interesting question is knowing just how many species of brambles or fungi actually exist.

In mycology, the species concept has most often been discussed in traditional terms associated with sexual reproduction and isolation, but such a concept does not give us a clue on how to segregate 'good species' in fungi, since many examples lack sexual reproduction or demonstrate genetically different mating types in the same populations. The *taxonomic* problem of fungal species concerns the *method of estimation* of a 'hierarchical value' of characters. Since we have a tentative hierarchy of characters defining large groups (Ascomycota, Basidiomycota, etc.) and smaller groups within them (classes, orders), the main task is to determine whether 'species' or 'genera' segregated on the basis of chaotically chosen characters are really equal in rank. To answer this question, an important rule should be followed: *taxa could be of equal rank only when they are described by the states of the same character set.* After testing the tentative 'species' and 'genera' for rank equality, we can obtain combinatorial arrangements of high prognostic qualities that show the possible diversity of taxa at different levels.

Notes:

Morphological and phylogenetic species concepts of *Marasmius* (Basidiomycota) *sensu stricto* from northern Thailand

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Sixty-four taxa of *Marasmius* are reported from northern Thailand. They were described based on morphological characteristics and were found to represent 57 species and 7 forms. Molecular phylogenetic hypotheses were constructed based on internal transcribed spacer (ITS) and 5.8S nuclear ribosomal DNA sequences using Bayesian, Maximum Likelihood and Parsimony analyses. Phylogenetic data are strongly correlated with morphological data and are useful to aid in delimiting species and distinguishing among closely related species. ITS sequences were of limited use, however, in recognizing currently circumscribed infrageneric taxa at the series rank and higher.

Keywords: Agaricales, ITS, Marasmiaceae, phylogeny, taxonomy

Notes:

Sirodesmin PL: Role in virulence of *Leptosphaeria maculans* and phylogeny of epipolythiodioxopiperazine gene clusters

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Sirodesmin PL is a secondary metabolite toxin produced by *Leptosphaeria maculans*, which causes blackleg disease of oilseed rape, *Brassica napus*. Its biosynthesis involves a cluster of 18 co-regulated genes and disruption of the two module non-ribosomal peptide synthetase gene prevents the production of sirodesmin PL. The non-ribosomal peptide synthetase mutant has less antibacterial and antifungal activity than the wild type and is half as effective in colonising stems, as shown by quantitative PCR analyses, indicating sirodesmin's role as a virulence factor in stems. The expression pattern of promoter GFP-fusions of the non-ribosomal peptide synthetase and an ABC transporter *in planta* is consistent with the distribution of sirodesmin PL as revealed by mass spectrometry experiments.

Sirodesmin is a member of the epipolythiodioxopiperazine (ETP) class of toxins, which are involved in animal and plant diseases. Surveys of genome sequence data show putative ETP gene clusters in 14 taxa; these clusters are discontinuously distributed amongst ascomycete lineages. Such clusters appear to have a single origin and have been inherited relatively intact rather than assembling independently in the different lineages. Movement of entire clusters by horizontal gene transfer is the most parsimonious hypothesis to explain the discontinuous distribution of clusters. The ability of fungi to transfer gene clusters that encode toxins or virulence factors presents important implications, particularly for fungi that are plant and animal pathogens.

Notes:

Phylogenetic relationships within the genus *Marasmius sensu stricto* inferred from two ribosomal encoding loci

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Current infrageneric classifications of *Marasmius* based on cellular morphology and histological architecture are historically controversial. While several molecular studies have demonstrated that fungal genera based on homoplastic morphological characters are artificial in the phylogenetic sense, the degree of infrageneric paraphyly within the genus *Marasmius* has never been appropriately addressed. Previous studies addressing the monophyly of infrageneric circumscription in this large genus based on a single rapidly evolving locus have fallen short due to apparent mutational saturation. We present the first study examining phylogenetic relationships within the genus *Marasmius sensu stricto* based on two loci known to be evolving at significantly different rates with high probability of phylogenetic resolution. Two ribosomal-RNA encoding loci (ITS and nuLSU) were sequenced from approximately one-tenth of the described biodiversity of *Marasmius sensu stricto* worldwide, including representatives of all current subgeneric divisions. Molecular sequence data were evaluated using multiple phylogenetic approaches, with members of the genus *Crinipellis* as outgroup taxa. Preliminary results show that the current infrageneric classifications are paraphyletic, with members of sections *Sicci* and *Globulares* interspersed within one lineage. Pilipellis anatomy in species of *Marasmius* appear to have convergently evolved, and do not represent a phylogenetically informative character. Members of section *Marasmius* fall into polyphyletic clades indicating multiple evolutionary origins of the collarium in this genus. By plotting morphological characters used in taxonomic delimitation on this phylogenetic hypothesis, we begin to assess the evolutionary plasticity of the characters currently used to circumscribe infrageneric groupings, and determine suitable morphological characters to inform classification. This study is presented to stimulate discussion on the reevaluation of current subgeneric circumscriptions within the genus *Marasmius.*

Notes:

Distinct reducing polyketide synthases (PKSs) and their insect specificity in entomopathogenic fungi

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Polyketide is one of the most important classes of secondary metabolites found in filamentous fungi. According to phylogenomic analysis, microbial polyketide synthase (PKS) genes are classified into three major groups. Two of these consist solely of fungal PKSs, and synthesize two different structural classes of polyketides: reduced and unreduced. The reducing PKSs are of considerable interest since they are known to produce many biological active compounds. These PKSs are subdivided into four subclades (I-IV). We reported here, by using a degenerate PCR specific to fungal reducing PKSs, 127 PKS genes identified from 39 isolates (representing 12 genera and 26 species) of entomopathogenic fungi. Phylogenetic analysis of these PKSs was carried out using their corresponding amino acid sequences, including also PKSs from non-entomopathogenic fungi. The resulting cladogram showed that 123 of the 127 entomopathogenic fungal PKSs were members of fungal reducing group, whereas the other four were similar to non-reducing group. The distribution pattern of entomopathogenic fungal PKSs clades showed no significant relation to their insect host. Interestingly, PKSs within the reducing subclade III (identified by our degenerate primers) from entomopathogenic fungi were grouped together to form an insect-specific branch, separated from PKSs from fungi in other habitats (marine, lichen). The clustering of the entomopathogenic fungal PKSs within reducing subclade III hints that these orthologous genes may represent a distinct group of PKSs synthesizing closely related compounds that play a role in the insect pathogenicity or might be functionally linked to the interaction between these fungi and their insect hosts. Moreover, the reducing subclade III PKSs were always found as a single copy in each fungal isolate. In contrast, those in the reducing subclades I, II, and IV were found to be present more than one copy.

Notes:

The cryptic complexity of *Lactifluus gerardii*

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Milkcaps are a group of mushrooms that can be found on almost every continent. Their name refers to the remarkable feature of exuding a milk-like substance when damaged. Like all Russulaceae, they are strictly ectomycorrhizal and always live in close association with specific trees or shrubs. Recently, the Milkcaps have been split up into two closely related genera: *Lactarius* and *Lactifluus.*

Lactifluus gerardii Peck is described from eastern North America, where it is a rather common species. Its appearance is quite characteristic: a dark brown, velutinous cap and stipe contrasting with the whitish and widely spaced lamellae. *Lactarius gerardii* has also been recorded all-over Asia, making us wonder wether this is truly a case of intercontinental conspecificity.

Using three molecular markers (ITS,LSU,rpb2) and including American and Thai specimens, it soon became clear that, not only were American and Thai specimens different species, but also that the Thai *L. gerardii* comprised more than one species. This incited us to extend our sampling and set out new goals: (1) Can more species be discovered in Asia? (2) How are specimens from different regions related to one another? (3) Are there yet morphological differences to be found between them? (4) Which known species are included in this group surrounding *L. gerardii*? This resulted in a phylogeny comprising specimens from Costa Rica, Mexico, the U.S.A., Canada, Japan, South Korea, China, Nepal, India, Sri Lanka, Thailand, Malaysia, Indonesia, Australia and New Zealand.

The analyses shows that the clade of *L. gerardii* s.l. is well-supported and contains at least 30 distinct species, including some already described species such as *L. subgerardii* Hesler & A.H. Sm., *L. bicolor* Massee, *L. ochrogalactus* Hashiya, *L. petersenii* Hesler & A.H. Sm., *L. reticulatovenosus* Verbeken & Horak, *L. wirrabara* Grgur. and *L. sepiaceus* McNabb. All share the brown cap and stipe, the more or less distant lamellae and the white spore print. Detailed microscopic study revealed that most clades have specific spore characteristics, though several clades cannot be distinguished based on their spores. Remarkable is the inclusion of *L. uyedae* Singer, a small, white, pleurotoid (with lateral stipe) species. The phylogeny does not allow for any straightforward hypotheses to explain the geographical distribution because several clades contain species from different continents, and most regions harbour several, similar but therefore not closely related species. In other words, there is intercontinentality of closely related species and sympatry of distantly related species. So far, no European or African representatives have been found. The astonishing diversity revealed within *L. gerardii* s.l. confronts us with new questions concerning its biogeography and in that way, *L. gerardii* demonstrates how common species are not always the easiest to understand.

Notes:

Candida visutii sp. nov., *Candida insecticola* sp. nov. and *Candida muscusicola* sp. nov., three anamorphic yeast species isolated in Thailand.

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Five strains of anamorphic yeasts obtained from natural substrates collected in Thailand were found to represent three novel *Candida* species closely related to *C. fructus*/ *C. musae* on the basis of morphological, biochemical, physiological and chemotaxonomic characteristics and the sequence analysis of the D1/D2 domain of the large subunit (LSU) rRNA gene. Two strains (ST-302 and ST-1169) isolated from mosses and sea water, respectively, were assigned to a single novel species which was named *Candida visutii* sp. nov. The type strain is ST-302^T (BCC 15048^T). Two strains (ST-249 and ST-337) from insect frass were assigned to a novel species which was named *Candida insecticola* sp. nov. The type strain is ST-249^T (BCC 11775^T). Strain ST-451 was named *Candida muscusicola* sp. nov. The type strain is ST-451^T (BCC 15142^T). In the D1/D2 of 26S rRNA gene, *Candida visutii* sp. nov., differ from *C. fructus*/*C. musae*, the closest relatives by 38 nucleotide substitutions (7.1%) and 19 indels. *Candida insecticola* sp. nov., differ from *C. fructus*/*C. musae*, the closest relatives by 38 nucleotide substitutions (7.1%) and 37 indels. *Candida muscusicola* sp. nov., differ from *C. fructus*/ *C. musae*, the closest relatives by 23 nucleotide substitutions (4.3%) and 15 indels.

Notes:

Application of zymogram analysis in identification and genetic variation of fungi

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Isozyme electrophoresis has been used to characterize strains within natural population of fungi. In particular, it has been valuable for identification of microorganisms in epidemiological, ecological and systematic studies. A number of species isolates of different genera; *Rhizoctonia*, *Aspergillus*, *Penicillium* and *Rhizopus* originating from different hosts was examined by pectic zymogram electrophoresis. Several zymogram phenotypes were produced for the species studied. Polygalacturonase and pectin esterase isozyme were observed in all isolates tested. Zymogram profiles occurred at different frequencies throughout the species sampled tested. The similarity among the electrophoretic phenotypes were calculated for the isolates of each species and pair wise comparison were made between all isolates of each species to generate similarity matrixes. The genetic similarity based on band frequency was calculated. The data were subjected to UPGMA analyses and express in dendrograms to show genetic similarity between isolates of each species. The zymogram patters were grouped according to consistent diagnostic polygalacturonase and pectin esterase bands. Zymogram analyses demonstrated that variation was considerable among the isolates of the species. Pectic zymogram variability may provide a useful tool for analyzing fungi population and taxonomic composition.

Notes:

Bistorta vivipara and its mycobionts: Changes in ECM community structure along a primary successional gradient

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Ectomycorrhizal (ECM) fungal communities are likely to change during the ecosystem succession process, and common mycelial networks are known as important determinants of seedling establishment and plant community structure during early primary succession. As one of few herbaceous plants, *Bistorta vivipara* forms ECM relationships with various fungi. The small and condensed root system makes it an ideal model organism for ECM community studies, since the entire fungal assemblage associated with the roots easily can be analyzed simultaneously. Hence, using *B. vivipara* as a model system for ECM associations may overcome many of the challenges we face when working with the large root systems of slow-growing trees. In this project we aim to explore the importance of ECM during primary succession and to what degree the ECM community changes along a primary successional gradient.

Three transects were placed from a glacier foreland to fully established vegetation in an alpine region in southern Norway, Finse in Hordaland County. Due to the occurrence of various recession moraines, which previously have been studied by glaciologists, data about the maximum age of the vegetation along transects were available. Hence, we can relate ECM community to time since vegetation established. Plants were sampled randomly along transects, and the vegetation was mapped. Soil samples were also taken in order to analyze differences in abiotic factors along the transects. Furthermore, phenotypic characteristics of the sampled plants were measured and will be implemented as co-variables in the analyses. DNA was extracted from the entire root systems of *B. vivipara*, and the internal transcribed spacer 1 was amplified using fungal specific primers. The ITS1 amplicons were analyzed further using 454 sequencing, which will yield an extensive coverage of the ECM root community, both in qualitative and quantitative terms.

Notes:

Biodiversity and taxonomy of foliose lichen from Phu Luang Wildlife Sanctuary, Loei province

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This study is the first investigation of foliose lichens throughout well-reserved areas of Phu Luang Wildlife Sanctuary. Two hundred and thirty-four specimens were collected in June – November 2008 from four forest types. One hundred and thirteen specimens were identified into two families, eight genera and twenty species of *Erioderma mollissimum*, *E. sorediatum*, *Bulbothrix geoebelii*, *B. goebelii*, *B. hypocrea*, *B. isidiza*, *B. tabacina*, *Everniastrum vexan*, *Hypotrachyna adducta*, *H. kingii*, *H. osseoalba*, *Parmelinella chozoubae*, *P. wallichiana*, *Parmelinopsis expallida*, *Parmotrema maclayanum*, *P. poolii*, *P. reticulatum*, *P. sancti-angelii*, *P. tinctorum* and *Relicinopsis intertexta*. The most widely distributed species are *Parmotrema tinctorum* and *Parmelinella wallichiana*. Seven species are new record and three species expected to be new species. Ten metabolic substances were mainly found in this family Parmeliaceae namely atranorin, consalazinic acid, gyrophoric acid, lecanoric acid, menegazziaic acid, norstictic acid, protocetraric acid, protolichesterinic acid, salazinic acid and usnic acid. These lichen substances will be used for testing antibiotic activity, antitumor activity, and potential in pharmacological, medicinal research in the near future.

Notes:

Endophytic fungi in mangrove plants of Thailand

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The distribution of fungal endophytes in the leaves of mangrove forest trees growing in three different locations (Kung krabaen bay; Chanthaburi Province, Pranburi forest park; Prachuapkhirikhan Province and Ranong Biosphere Reserve; Ranong Province) in Thailand were studied. Two thousand and seven hundred leaf segments from nine different hosts belonging to seven families, Rhizophoraceae (*Rhizophora apiculata*, *R. mucronata*), Sonneratiaceae (*Sonneratia alba*), Combretaceae (*Lumnitzera littorea*) Avicenniaceae (*Avicennia alba*), Acanthaceae (*Acanthus ilicifolius*), Meliaceae (*Xylocarpus granatum* and *Xylocarpus moluccensis*) and Malvaceae (*Thespesia populneoides*), were screened for the presence of fungal endophytes. The dominant endophytes differed for the different host types. *Phyllosticta* was the most frequently fungal isolated from plants in all sites. The common fungal endophyte genera were *Cladosporium*, *Colletotrichum*, *Phomopsis* and *Xylaria*. Most endophytic isolates in mangrove leaves were recovered from Ranong Province. The antimicrobial potential of 71 endophytic fungi isolated from mangrove plants towards selected bacteria (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*) was tested using ethyl acetate extracts of fungi cultivated under static conditions. All test bacteria were inhibited by a *Cladosporium* sp. isolated from the leaves of *T. populneoides* and endophytic *Xylaria* spp. isolated from *A. ilicifolius* leaves showed considerable inhibition to Gram-positive and Gram-negative bacteria. Additionally the crude extracts of 84 endophytic fungi were tested for anticancer activities by the MTT assay against A375 (Human malignant melanoma), SW620 (Human colorectal adenocarcinoma), Kato Ⅲ (Human gastric carcinoma), HepG2 (Human liver hepatoblastoma) and Jurkat (Human acute T cell leukemia). Most of extracts displayed cytotoxicity against some cancer cell lines.

Notes:

Preliminary study on diversity of Aero-aquatic fungi in the rainy season at Khao Yai and Doi Inthanon National Parks, Thailand

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Aero-aquatic fungi are an ecological group characterized by producing distinctive asexual spores (conidia) exposed to the air and by inhabiting in submerged leaves/woods in static to slow-flowing freshwater environments. Fungal diversity was investigated and compared during the rainy season at two main sites, composed of three sub-sites in each area as follows: Khao Yai National Park (Km. 29, Tat Ta Phu Waterfall, and Wang Champi Waterfall) and Doi Inthanon National Park (Ang Ka Nature Trail, Pha Doksiao Waterfall and Wang Muang Waterfall). More than 19 fungal taxa, derived from 120 woody litter/submerged leaves, however, fungal community from six sub-sites, do not different among these six sub-sites. The common genera from both locations were found and classified by morphological characteristics, including *Candelabrum*, *Helicomyces* and *Helicosporium*, while genus *Helicoon* was recorded only at Doi Inthanon National Park. Interestingly, one taxon (BCC 31904) is described as a new species from Doi Inthanon National Park. All strains of aero-aquatic fungi are preserved at BCC and NBRC under collaborative project. Further studies of those fungi will be compared with other seasons in Thailand (mild and summer seasons) and those strains in Japan on the diversity, ecology and community.

Keyword: Aero-aquatic fungi

Notes:

Diversity of marine fungi from twigs and sponges in Chonburi province

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Study of marine fungi was carried out by isolating fungi from twigs and marine sponges including *Chalinula* sp., *Clathria reinwardti*, *Haliclona* sp., *Mycale armata* and *Xestospongia testudinaria*. The twigs were colleted from Bangsarai Beach, Amphur Sattahip while sponges were kept at Bangsaen Beach, Amphur Muang. Direct isolation and half strength potato dextrose agar with 70% sea water were used to isolate microfungi from twigs. For isolation of marine sponge-associated fungi, sponge tissues were rinsed three times with sterile seawater. The tissue was cut into 0.5 x 0.5 cm. and placed on malt extract agar with 70 % sea water. They were incubated at 28oC, for 7 days. A total of 131 marine fungal isolates from twigs were found, comprising 26 genera and 31 species: *Algialus parvus*, *Antennospora quadricornuta*, *Arenariomyces trifurcatus*, *Camerosporium* sp., *Clavatospora bulbosa*, *Cladobotryum* sp., *Cladosporium* sp., *Corollospora pulchella*, *Dactylospora haliotrepha*, *Emericella nidulans*, *E. variecolor*, *Eurotium* sp., *Halosphaeria quadricornuta*, *H. quadriremis*, *H. hamata*, *Herpotrichella* sp., *Kallichroma tethys*, *Lulworthia grandispora*, *Lulworthia* sp., *Marinosphaera mangrovel*, *Massarina* sp., *Nais inornata*, *Pestalotia* sp., *Pontoponae* sp., *Savoryella paucispora*, *Spirodesmium eupatoriicola*, *Torpedospora radiata*, *Varicosporina ramulosa*, *Verruculina enalia*, *Zalerion varium and* unidentified sp.1. Microfungi isolated from sponges were *Acremonium* sp., *Arthrinium* sp., *Curvularia lunata*, *Chaetomiun globosum*, *C. minutum*, *Clodosporium clodosporioides*, *Cladobotyum varium*, *Curvularia lunata*, *Emericella variecolor*, *Eurotium cristatum*, *Eupenicillium parvum*, *Fusarium semitectum*, *F. solani*, *Menmoniella echinata*, *Nigrospora* sp., *Nodulisporium* sp., *Pestalotiopsis* sp., *Pithomyces* sp., *Penicillium* sp., *Stemphylium* sp. *Speggazzinia tessarthra*, *Trichoderma* spp. and *Xylaria* sp. Pure cultures are being maintained in a culture collection at the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok for further investigation.

Notes:

Diversity of *Neosartorya* species and Antagonistic Test Against Plant Pathogenic Fungi *in vitro*

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Study on diversity of *Neosartorya* species from 94 forest and agricultural soil samples from 7 provinces in Central, Eastern, Northern and Northeastern Thailand: Angthong, Nakhon Pathom, Rayong, Chiang Mai, Kamphaeng-Phet, Uttaradit, Ubon Ratchathani, and Loei provinces was conducted. For isolation methods, the alcohol and heat treatment techniques and Gochenaur's glucose ammonium nitrate agar were used. Identification was based on macroscopic characteristics as colony growth pattern on Czapek's agar, Czapek yeast autolysate agar and malt extract agar incubated at 28˚C for 7-14 days. Microscopic features were observed under stereo, compound and scanning electron microscopes. A total of 106 isolates of Neosartorya species were found, including *Neosartorya fischeri*, *N. glabra*, *N. spinosa*, *N. takakii* and *Neosartorya* spp. For antagonistic activity test, *Neosartorya* sp. (KUFC 6301) collected from forest soil in Loei province was tested against five plant pathogenic fungi *in vitro.* They were cultivated on potato dextrose agar at 25˚C for 14 days. The results revealed that *Neosartorya* sp. (KUFC 6301) could inhibit 30% mycelium growth of *Bipolaris maydis*, *Colletotrichum capsici* and *C. gloeosporioides*, but failed to control *Rhizoctonia solani* and *Sclerotium rolfsii.*

Notes:

Clonality and recombination in panmictic population of *Sclerotinia scleortiorum* in Iran

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Population genetic structure of 252 isolates of *Sclerotinia sclerotiorum* from canola fields in four regions (provinces) of Iran was studied by using SSR markers and mycelial compatibility grouping. Six microsatellite loci were analysed. To estimate the level of admixture and infer the number of clusters present in the Iranian *S. sclerotiorum* populations, a Bayesian genotypic clustering analysis was conducted in STRUCTURE ver. 2.2 (Falush *et al*., 2007). Clonal fraction was calculated for each regional population (Zhan *et al*., 2002). To recognize the occurrence of recombination in populations, the proportion of compatible pairs of loci and $\bar{r}_d$, an index of linkage disequilibrium, were estimated using MULTILOCUS ver. 1.3 (Agapow and Burt, 2001). Multilocus genotypic diversity was calculated (Shannon and Weaver, 1949). To compare evenness of genotypes among populations, E_5 was calculated (Ludwig and Reynolds, 1988) for all populations. Mycelial compatibility groups were identified by crossing isolates on PDA amended with 80 µL of red food dye (Wilton, USA) per litre. The results of molecular analysis revealed 80 haplotypes among studied populations. Although 32 haplotypes (40%) occurred in low frequencies represented by only one isolate, clonality was also evident. Clones were identified not only at regional populations but also at the smallest scale of analysis within 25 out of 37 fields analysed. Only one large panmictic population could be inferred (ln P(D) = -1548; Var[Ln*P*(D)] = 12.1), with a low within population differentiation for the populations (F_{ST} = 0.066). Clonal fractions were large ranging from 0.39 to 0.67. Haplotypes were distributed evenly within regional populations (E_5 = 0.77 to 0.86). Genotypic diversities ranged from 2.7 to 3.3. According to a *t*-test, genotypic diversities were not significantly different in pairwise comparisons of the four populations. Neither $\bar{r}_d$ values nor the proportions of compatible pairs of loci were significant demonstrating the occurrence of recombination in populations. All isolates were grouped in 12 MCGs. Common MCGs were identified among four populations. The lack of association between two different grouping systems and high occurrence of intransitivity within MCGs were also other indicators of the occurrence of recombination in populations.

Keywords: *Sclerotinia sclerotiorum*, SSR, MCG, panmictic population, recombination.

Notes:

Electrophiretic karyotype of *Pleurotus eryngii* and *Lyopyllum ulmarium*

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Molecular karyotypes of *Pleurotus eryngii* (king oyster mushroom) and *Lyopyllum ulmarium* (buna-shimeji) have been obtained by contour-clamped homogeneous electric field(CHEF) gel electrophoresis. Eleven and ten chromosomal bands were separated from nuclei of *Pleurotus eryngii* and *Lyopyllum ulmarium*, respectively. Compairing with chromosomes of *Schizosaccharomyces pombe* and *Hansenula wingei* as size standards, we estimate the sizes of the chromosomes of the mushrooms could be from 3.2 to 7.2 megabase pairs(Mbp), and from 2 to 5.2 Mbp for *Pleurotus eryngii* and *Lyopyllum ulmarium*, respectively. A certain chromosome was often losted in monokaryotic progenies and chromosome number were varied in the basidiosores.

Keywords: *Pleurotus eryngii*, *Lyopyllum ulmarium*, karyotype, contour-clamped homogeneous electric field (CHEF) gel electrophoesis

Notes:

Yeasts in traditional fermented foods in Thailand

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Three hundred isolates of yeast were obtained from 105 samples of fermented pork, fermented fishes, fermented beef, and fermented shrimp. Among them, 147 isolates had alcohol-fermenting ability from glucose. Based on the analysis of the D1/D2 domain on 26S rDNA sequencing, they are belonging to ascomycetous yeast. One hundred and thirty five strains (91.8%) were identified as 10 known species in 8 genera. *Candida tropicalis* (50 strains, 34%) and *Issatchenkia orientalis* (50 strains, 34%) are the dominant species, fallow by *Candida glabrata* (9 strains, 6.1%) and the remaining strains identified as *Candida parapsilosis* (1 strains), *Candida rugosa* (5 strains), *Hanseniaspora guilliermondii* (3 strains), *Kluyveromyces marxianus* (2 strains), *Kodamaea ohmeri* (8 strains), *Pichia rhodanensis* (5 strains), *Torulaspora globosa* (2 strains) and *Zygosaccharomyces pseudorouxii* (1 strains). Among identified strains, 10 strains (6.8%) belonging to sister species which show 1-4 nucleotide substitutions from any know species. The remaining two strains (1.4%) were found to represent 2 novel species of *Candida* and *Issatchenkia* by 8-10 nucleotide substitutions (2% substitutions) from nearest species. The polyphasic taxonomy including molecular taxonomy, phylogenetic analysis, conventional taxonomy and chemotaxonomy are required for complete identification for the later two groups. It is concluded that yeasts associated with fermented foods in Thailand are not rich in biodiversity and that most of them belong to already described species which are common to other southeastern Asian and European countries.

Notes:

Diversity of saprobic fungi on woody litter of *Manglietia garrettii* in Phitsanulok

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The diversity of fungi found on woody litter of *Manglietia garrettii*, during the wet and dry season (June 2008 to May 2009) is reported and the communities are compared. Saprobic fungi were investigated from 100 samples of decaying woody litter of *M. garrettii* collected in Phitsanulok Province. Based on morphological characteristics, more than 60 taxa were identified in this study, comprising ascomycetes, basidiomycetes and anamorphic fungi. In terms of the numbers of taxa recovered, dry season samples supported more diverse fungal community than samples from the wet season, although the common genera of fungi obtained from woody litter of each season were similar. It is worth noting that samples from this study provided higher numbers of fungi (especially lichens) than the previous study in Doi Suthep-Pui National Park, with relatively few species overlap.

Keywords: Lignicolous fungi, Magnoliaceae, *Manglietia*, Saprobic fungi

Notes:

Diversity of endophytic fungi from Phu Luang Wildlife Sanctuary and their antagonistic effects against plant pathogenic fungi *in vitro*

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Healthy stem and leaf samples of *Bacckea frutescens* (Myrtaceae), *Dufrenoya sessilis* (Santacaceae), *Lithocarpus truncatus* (Fagaceae), *Oroxylum indicum* (Bignoniaceae), *Schefflera actinophylla* (Araliaceae), *Sambucus simpsonii* (Caprifoliaceae), *Ternstroemia gymnanthera* (Theaceae) were collected from Phu Luang Wildlife Sanctuary, Loei province in June and November 2008. The leaf sample was cut into small pieces and placed in sodium hypochlorite-ethanol for surface sterilization. The stem was thoroughly treated with 95% ethanol and flamed until the alcohol was eliminated and the bark removed with a sterilized sharp blade. The treated bark as well as the inner part of the stem, containing the cambium, phloem, and xylem tissues, were placed on water agar (WA) and glycerol arginine media (GAM). Identification of endophytic fungi was based on growth rate and colony pattern on potato dextrose agar incubated at 28°C for 7-30 days. Microscopic characters were examined under stereo and light microscopes. Camera lucida drawings were employed. A total of 60 isolates of endophytic fungi were found including 7 species of Ascomycetes: *Chaetomium globosum*, *Eupenicillium* sp., *Gelasinospora* sp., *Glomerella cingulata*, *Hamigera* sp., *Talaromyces trachyspermus* and *Talaromyces* sp. ; 7 species of Coelomycetes: *Chaetomella raphigera*, *Colletotrichum gloeosporioides*, *C. dermatum*, *Cryptosporiopsis* sp., *Pestalotiopsis* sp. *Phomopsis* sp., *Pyllosticta* sp. ; 6 species of Hyphomycetes: *Arthrinium phaeospermum*, *Curvularia pallescens*, *Fusarium semitectum*, *Nigrospora oryzae*, *Periconia* sp., *Pteroconium pterospermum* ; 5 isolates of synematous Hyphomyctes ; 9 isolates of Xylariaceae and 15 isolates of non-sporulating fungi.

For antagonistic activity test, five isolates of endophytic fungi was cultivated on PDA at 28°C for 10-14 days and plant pathogenic fungi were placed on the agar media at 0.5, 1.0 and 1.5 cm. apart from the endophytic fungus at 3 points. The results revealed that 4 isolates of non-sporulating endophytic fungi from *Bacckea frutescens*, *Lithocarpus truncates*, *Schefflera actinophylla* and *Ternstroemia gymnanthera* could inhibit mycelium growth of *Bipolaris maydis*, *Rhizoctonia solani*, *Pythium aphanidermatum* and *Phytopthora palmivola*, but failed to inhibit mycelium growth of *Sclerotium rolfsii* and *Lasiodiplodia theobromae*.

Notes:

Clavaria and *Clavulinopsis* from Nam Nao National Park, with two new records of *Clavaria* to Thailand

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Coral fungi from Nam Nao National Park, Thailand were collected during wet seasons (May to September 2008) in order to investigate their species diversity. Forty-six samples were collected, photographed and examined morphologically at both macroscopic and microscopic levels. From the collected samples, seven samples were showed simple club shaped. They were confined to 2 genera (*Clavaria* and *Clavulinopsis*) and 7 species as following: *Clavaria acuta* sowerby, *C. rosae* Fr., *C. vermicularis* Sw., *C. fumosa* Persoon, *Clavulinopsis fusiformis* (Sowerby) Corner, *C. helvola* (Pers.) Corner and *C. miyabeana* (S. Ito) S. Ito. Two species of these, *C. acuta* and *C. fumosa* are new records of *Clavaria* to Thailand. From this study, a total of 15 species of simple club shaped coral fungi are record in Thailand and brings up number of coral fungi to 34 species.

Keywords: new records, *Clavaria, Clavulinopsis*, Thailand

Notes:

Diversity of heat resistant ascomycetes from soil

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An investigation on diversity of microfungi from soil was carried out. The soil samples were collected from various locations such as agricultural, forest, termite mound in Chiang Rai, Chiang Mai, Loei, Ubol Ratchathani, Sakon Nakhon, Singburi and Chonburi Provinces. The alcohol and heat treatment techniques and Gochenaur's glucose ammonium nitrate agar were used to isolate microfungi. Identification of the fungal isolates were based on morphological characteristics as colony growth on agar media, fruiting bodies and spore ornamentation using light and scanning electron microscopes. The results revealed that 11 genera 23 species of Trichocomaceae and 7 genera 15 species of Pyrenomycetes were found including *Anixiella indica*, *Bionectria* sp., B*yssochlamys fulva, B. nivea, Dichotomomyces cepjii, Echinopodospora spinosa, Emericella nidulans, Eupenicillium javanicum, E. parvum, E. stolkiae, Eurotium amsterodami, E. cristatum, Fennellia flavipes, F. niveus, Gelasinospora indica, G. hapsidophora, G. dictyophora, G. udagawae, G. hippopotami, Gelasinospora* sp.1,*Gelasinospora* sp.2, *Hamigera avellanea, Nectria viridescens, Neosartorya delicate, N. multiplicata, N. quadricincta, Neurospora lineolata, N. africana, N. dodgei, Sagenoma* sp., *Talaromyces bacillisporus, T.s flavus, T. luteus, T. stipitatus, T. trachyspermus, T. wortmannii, Thermoascus aurantiacus* and *Trichodelitschia* sp.

Notes:

Morphological and molecular study of *Hyphodermella* (Basidiomycota) in Western Mediterranean area

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The genus *Hyphodermella* J. Erikss. & Ryvarden is a well defined genus with resupinate, effuse and crustose basidioma; the hymenophore is irregularly odontioid with scattered aculei apically provided with projecting cystidioid hyphae. This cosmopolitan genus includes four species: *H. corrugata* (Fr.) J. Erikss. & Ryvarden mostly from Southern Europe (also reported from Great Britain, Central and Northern Europe), *H. densa* Melo & Hjortstam described from Portugal, *H. rosea* (Bres.) Nakasone from Italy, and *H. maunakeaensis* Gilbertson & Hemmes described from Hawaii. Thus, in the Mediterranean area three species are recognized, although to some authors, due to its morphological variability, *H. corrugata* could represent a species complex more that a single species.

The aims of this paper are to analyse, in the light of morphological and molecular data, the taxonomic characters of *H. corrugata*, *H. densa* and *H. rosea*, as well as to revaluate the distribution of the genus in the Western Mediterranean area (France, Spain, Portugal and Morocco).

Thirty five specimens were studied and are preserved in the international herbaria MA-Fungi (Madrid, Spain), LISU (Lisboa, Portugal) and UPS (Uppsala, Sweden).

Macro- and micromorphology were studied to every specimen. Measurements and drawings were made from microscopical sections mounted in 3% KOH and examined at magnifications up to x 1,250 using an Olympus BX51 microspore. Thirty spores and 10 basidia were measured from each sample. Drawings were made with aid of a drawing tube.

Genomic DNA was extracted from 20 specimens. Primer pair ITS1F and ITS4 were used to obtain amplifications of both ITS regions, including the 5.8S of the ribosomal RNA gene cluster and small flanking parts of the SSU and LSU genes. The sequence alignment was analyzed under parsimony and bayesian approaches.

Both phylogenetic analyses segregate the *Hyphodermella* specimens in two clades, well correlated with morphological characters.

This study has been supported by the Project Flora Mycologica Iberica (CGL2006-12732-CO2-01/BOS).

Notes:

First species report of the genus *Samuelsia* on scale insects from Thailand (old world)

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The genus *Hypocrella* Sacc. *sensu lato* (anamorph *Aschersonia* Mont. s. l.) are pathogenic to scale insects (Coccidae and Lecaniidae, Homoptera) and whiteflies (Aleyrodidae, Homoptera). This genus is a discrete genus within the family Clavicipitaceae *sensu stricto* (Hypocreales, Ascomycota). A recent revision based on molecular and morphological characters supported and segregated the genus into three genera: *Hypocrella s. str*., *Moelleriella* Bres., and *Samuelsia* gen. nov. These two genera *Hypocrella s. str*. and *Samuelsia* gen. nov. are ancestral and produced whole ascospores. *Samuelsia* gen. nov. was described from the Neotropics comprising five species: *S. chalalensis*, *S. rufobrunnea* and *S. geonomis* and *S. intermedia* were collected from south America and *S. sheikhii* from central America. Surveys and collection of insect fungi were from various National Parks of Thailand throughout the year. A Thai specimen producing whole ascospores was collected from Khao Soi Dao Wildlife Sanctuary (Chantaburi province). In a combined multi-gene analyses using the large subunit of the nuclear ribosomal DNA (LSU), translation elongation factor 1-α (EF1-α), and RNA polymerase II subunit (RPB1), this specimen clearly belongs in the genus *Samuelsia* clade. Our results show that *Samuelsia* is distributed in the new and the old world.

Notes:

Mode of vegetative reproduction of *Wickerhamomyces pijperi* and related yeast strains found in Thailand, Japan, South Africa and French Guiana

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In 1957, van der Walt and Tscheuschner isolated a new yeast species from buttermilk in South Africa and placed it in the genus *Pichia* because it produced a pellicle in liquid media and described as *Pichia pijperi.* Later, Ditlevson and Hjort (1964) transferred this species to the genus *Hanseniaspora* because it proliferates by bipolar budding. In the 2nd edition of The Yeasts, a Taxonomic Study (1970), however, Kreger van-Rij retained it in the genus *Pichia* based on the observation of bud scars under transmission electron microscope (TEM) though she did not show the pictures. Recently, Kurtzman et al. (2008) transferred *P. pijperi* to a newly proposed genus *Wickerhamomyces.*

During the course of a survey of yeasts living in the natural substrates in Thailand, we isolated five strains of yeasts closely related to *Wickerhamomyces pijperi* and found that these strains proliferate by bipolar budding like *Hanseniaspora* strains. Based on this observation, we studied the mode of vegetative reproduction of all of available strains of *W. pijperi* and related yeasts by scanning electron microscope (SEM) and staining of bud scars with Fungiflora Y (Biomate Inc., Japan).

Fifteen strains of *W. pijperi* and related yeasts were classified into 2 groups based on the mode of vegetative reproduction. In group 1, 37-69% of cells proliferated exclusively by bipolar budding and produced layered bud scars like *Hanseniaspora* strains (type 1), 29-60% of cells produced the first bud at a pole and succeeding buds were produced at the same position or at the opposite pole, or near bud scars at poles, namely at shoulders (type 2), and the remaining cells (0-8%) produced the first bud at a pole then produced succeeding buds randomly on the cell surface (type 3). The ratio of three types varied from strain to strain. Twelve strains including the type strain of *W. pijperi*, four CBS strains (three from French Giana, one from Thailand), two NBRC strains from Japan, and five new isolates from Thailand, were included in this group. In group 2, only 3-10% of cells proliferated by type 1, 25-57% of cells by type 2 and 33-72% cells by type 3. Three NBRC strains from Japan were included in group 2. In a neighbour-joining tree based on the D1/D2 domain sequences, strains of group 1 constituted a clade and connected with a clade comprising strains of group 2 with high bootstrap support, then connected with *Candida solani*, the nearest multilateral budding species of *W. pijperi* group in the D1/D2 domain sequence of LSU rRNA genes. Type 3 of budding belongs to the category of multilateral budding but is somewhat different from typical one found in species such as *Saccharomyces cerevisiae* and resembles that of *C. solani*. Among strains of group 1, up to 8 nucleotides differed from one another, and strains of group 2 differed by 23 nucleotides from *W. pijperi* CBS 2887^T. Therefore, the strains examined in the present study may represent several different species.

Notes:

Biodiversity of entomopathogenic fungi in Vietnam

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Entomopathogenic fungi are an unique group of fungal kingdom which has received very little concern from most mycologists not only in Vietnam but also around the world. However, over the last two year, Center for Research and Applying Biotechnology in Agriculture, Vinh University has been studying this fungal group. Result of this survey shown that there are 18 recorded genera with 82 species in a total of 770 collected specimens in Pu Mat National Park and others place, Vietnam. The most popular genus is *Aschersonia* with 16 species in 175 collected specimens, followed by *Paecilomyces* with 5 species in 159 collected specimens. The diversity of insect pathogenic was presented by 10 popular species such as: *Paecilomyces cinamomeus*; *Ophiocordyceps unilateralis*; *Paecilomyces* sp1.; *Aschersonia* sp2.; *Aschersonia calendulina*; *Gibellula* sp1.; *Hypocrella discoidea* and *Hypocrella raciborskii*. These are 7 insectan orders and 1 spider order belonging to 771 specimens which were collected in Pu Mat National Park, Vietnam. The most common order was *Homoptera* with 11 genera in 399 collected specimens, followed by *Hymenoptera* with 2 general in 113 collected specimens.

Keywords: biodiversity, entomopathogenic fungi

Notes:

Isolation and identification of mycorrhizal fungi from roots of three commercial orchid genera, *Paphiopedilum*, *Dendrobium* and *Cymbidium*

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Thailand is the one of the world largest exporters of orchids. However, orchid mycorrhizal research in Thailand is still very limited. This study is aimed to investigate the diversity of mycorrhizal fungi associated with three commercial orchid genera, *Paphiopedilum*, *Dendrobium* and *Cymbidium*. Twenty four isolates of *Rhizoctonia*-like fungi were obtained from root sections of *P. sukhakulii*, *P. charlesworthii*, *P. villosum*, *P. exul, P. callosum, D. anosmum, D. crystallinum, D. friedericksianum, C. tracyanum, C. lowianum, C. sinense,* a *Dendrobium* hybrid, and two *Cymbidium* hybrids collected from several ecologically diverse sites in Chiang Mai and Chiang Rai provinces of Thailand. Morphological characters and a preliminary study on the ITS-5.8S rDNA sequences indicated that *Epulorhiza repens* was the most common species found in roots of various species of the three orchid genera, whereas, *E. calendulina*-like isolates were strictly found in roots of three *Paphiopedilum* species. Seven other isolates were tentatively identified as anamorphs of *Tulasnella*. The ITS-5.8S rDNA sequence of one isolate did not show high homology with high sequence coverage to any known cultured mycorrhizal species but the 5.8S rDNA sequence showed 89% sequence identity to *T. asymmetrica* (GenBank accession no. DQ388047). This fungal isolate is potentially a new species in the genus *Tulasnella.* Further phylogenetic analysis is underway.

Notes:

Study on the host preference of xylariaceous endophytes

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In the course of studies on the diversity and ecology of xylariaceous endophytes in Khao Yai National Park in Thailand, several clades that were considered to be a single species each were recognized in the phylogeneic analyses based on rDNA sequences. Four clades of them consisted of many strains isolated from phylogenetically diverse plants. Two of these four clades were considered *Xylaria cubensis*, which were isolated from healthy leaves of 23 plant species (of 22 gen. in 20 fam.), and *Nemania bipapillata*, which were found from those of 11 plant species (of 10 gen. in 9 fam.), respectively. Another two clades, which also consisted of many strains isolated from various plants, have been still unclear in their species level identification, while they are likely to belong to *Xylaria* and the relatives.

In this study, the four species clades were studied based on sequence analyses of beta-tubulin gene coding regions in addition to rDNA ITS regions in order to know the host preference among strains in each clade. As a result, strains isolated from the same host plant were not aggregated into a subclade within a single species clade. The four xylariaceous fungi were clarified to inhabit phylogenetically diverse plants as endophytes. However, they are not likely to possess ability to infect specific hosts in the endophytic phase, that is, “the specialization of parasitism” was not found, though it has been reported in some of plant pathogenic fungi. These xylariaceous fungi probably can penetrate living tissues of various plant as endophytes and quiescently inhabit the tissues without the induction of host resistant reaction against pathogens, and moreover, they can be pleioxeny: the condition of plurivorous parasitism, in the endophytic phase.

Further studies on anatomical features and on viability in plant tissues are also needed to presume the ecological strategy for the plurivorous parasitism of xylariaceous endophytes and their ecological functions as components of forest ecosystem. Further isolation test from fallen leaves and field survey for detecting sporulation form of the fungi are necessary to reveal whether the lifestyle of such xylariaceous fungi are categorized as the foraging ascomycete’s lifestyle, in which the fungi can reproduce somehow on the tissues of plants other than the primary host plants, or the dead ends of the evolution.

Notes:

Studies of Lecanoraceae (Lacanorales: Ascomycota) in Thailand

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Studies of Lecanoraceae in Thailand were found five species of *Lecanora*; *Lecanora achroa*, *L. helba, L. phaeocardia, L. subimmergans* and *L. tropica* and only one species of *Ramboldia* (*R. russula*). *Lecanora subimmergans* is first reported from Thailand. This study is identified all collection used by morphology, anatomy and chemistry. Some of *Lecanora* is very closely on morphology, however can be recognized by different chemistry. Most of collection can be found in dry dipterocarp forest in Thailand, dry evergreen forest and lower mountain forest, respectively. Lecanoraceae can be found many secondary metabolizes also used for identified to species. The phylogeny of Lecanoraceae are necessary in progress in Thailand.

Notes:

Diversity of microscopic and macroscopic fungi in Kaeng Krachan National Park

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Kaeng Krachan National Park is the largest national park in Thailand and composes of many kinds of forest, such as evergreen forests and deciduous forests. It locates in western region of Thailand and its territory closes to forests of Tung-Yai-Naresuan Wildlife Sanctuary and in Myanmar where are recognized as the complex ecosystem in South-East Asia. Therefore, forests in Kaeng Krachan National Park may be showed high biodiversity of microbial resources, especially fungal diversity. Fungi are an important group of forest microorganisms that plays a role in biodegradation and nutrient recycling in forest ecosystem. Moreover, several fungal species were applied to biotechnological applications, including agriculture, environment, industry and medicine. However, data of fungal diversity in Thai forest are limited. Therefore, Forest Entomology and Microbiology Group, Forest and Wild Plant Conservation Research Office, National Parks, Wildlife and Plant Conservation Department and Faculty of Medical Science, Naresuan University, were generated the cooperative research program to analyse microscopic and macroscopic fungal diversity in Kaeng Krachan National Park, by culture-dependent and culture-independent approaches. Factor affecting on fungal diversity and distribution, including physical, chemical and biological factors, were also studied. The completed data obtained are essential for forest management, bioresource conservation, and understanding of distribution and ecological roles of fungi found in this national park. Furthermore, fungal strains isolated were detected and measured the activities of several types of extra- and intracellular enzymes as same as their biotechnological potentials determined.

Notes:

Litter fungi diversity in Piranmalai Forest of Eastern Ghats, India

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The amount and nature of litter which is the major source of a variety of organic matter has an important bearing of soil formation and its fertility. It is now well established that the decomposition of plant litter on the soil surface is brought about by a variety of microorganisms including bacteria, fungi and actinomycetes. Among these, fungi are the chief colonizers and decomposers. There is a dearth of information on the fungi associated with decomposition of litters of tree species grown in natural forest and plantations in India. In the present study, some ecological attributes and the succession of fungi on decomposing litters of various altitudes of Piranmalai forests of Eastern Ghats were analysed.

A total of 15, 20, 24 and 21 fungal genera were isolated from the decomposing litter of study sites of Vellaimalai, Esarivalanthan malai, Mottamalai and Piranmalai forests respectively. The total number of fungal species recorded during decomposition varied across the study forests and they were 30 in Vellaimalai litter, 29 in Esarivallanthan malai litter, 36 in Mottamalai litter and 34 Piranmalai litter.

Our study indicates that Hypomycetes play a predominant role in forest litter degradation and there is a successional pattern of fungal flora. The primary colonizers on forest litter were mostly saprophytes. The secondary colonizers were those fungi with the ability to utilize lignin and cellulose for growth. Notably in the study the role of *Alternania* sp., *Aspergillus niger*, *Cladosporium* sp., *Cladosporioides* sp., *Curvularia lunata*, *Fusarium solani*, *Mucor racemosus*, *Nigrospora* sp., *Pennicillium* sp., *Phoma* sp. and *Trichoderma* sp. are considered to be indispensable in litter degradation due to their high level of occurrence and greater survivability during the fungal stages of decomposition.

Notes:

The genus *Akathomyces* on moths and spiders: A case of convergent evolution?

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The genus *Akanthomyces* are pathogens of insects and spiders. *Akanthomyces* is characterized by producing white, cream or flesh-colored cylindrical, attenuated synnemata covered with a hymenium of phialides. These conidiogenous cells are either ellipsoidal, cylindrical, or narrowly cylindrical and gradually or abruptly tapering to a more or less distinct neck. Conidia are unicellular, hyaline, in short or long chains.

The genus *Akanthomyces* was established by Lebert in 1858 for a species, A. aculeatus, found on a moth in Europe. There are 24 species of *Akanthomyces* known worldwide, not only on moths but also invertebrate. Hywel-Jones (1996) monographed the genus *Akanthomyces* on spiders in Thailand, including three new species; *A. koratensis*, *A. cinereus* and *A. wedsteri*. He also provided a key to the known species of *Akanthomyces* on spiders. Many authors have found *Akanthomyces* on spider; A. ampullifer, *A. aranearum*, *A novoguineensis*, *A. ovalongatus*, *A. arachnophilus* and *A. longisporus.*

Strains of *Akanthomyces* were collected throughout Thailand. Specimens were found on underside of living leaves of forest plants. Molecular studies using multigene analyses based on the nuclear ribosomal small and large subunits (*nrSSU* and *nrLSU*), the elongation factor 1α (*tef1*). Showed 7 species of *Akanthomyces* in Thailand. The genus *Akanthomyces* comprises three clades A, B, and C. Clade A comprises *Akanthomyces arachnophilus*, *A. novoguineensis* and *A. cinereus* infecting spiders. Clade B comprises *A. aculeatus* and *A. pistillariiformis* infecting moths. Clade C comprises *A. websteri* and *A. koratensis* infecting spiders.

Keywords: *Akanthomyces*, Molecular phylogeny

Notes:

Development of REMAP markers for the fingerprinting of mushrooms

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The retrotransposon, marY1, is a gypsy family retroelement which is detected ubiquitously within the fungal taxonomic groups in which mushrooms are included. In order to utilize marY1 as a molecular marker for the DNA fingerprinting of mushrooms, two oligonucleotides, marY1-LTR-L and marY1-LTR-R, were designed on the basis of the highly conserved regions from the multiple sequence alignment of 30 marY1 sequences, which were retrieved from a nucleotide sequence database. In accordance with Retrotransposon Microsatellite Amplified Polymorphism (REMAP) classification methodology, the two oligonucleotides were utilized together with UBC807 and UBC818, which are two short sequence repeat (SSR) primers, for PCR using templates from different mushroom genomic DNAs. Among the tested oligonucleotides,the marY1-LTR-L and UBC807 primer set yielded the greatest amount of abundance and variation in terms of DNA band numbers and patterns. This method allowed us to verify 10 different mushroom species. Furthermore, the primer set was successfully employed to discriminate between different commercial mushroom cultivars of the same strains of 14 *Pleurotus ostreatus* and 16 *P. eryngii*. In this study, it was also demonstrated that REMAP evidenced superior reproducibility compared to other popular DNA fingerprinting methodologies, including the random amplified polymorphic DNA (RAPD) method.

Notes:

Collection of microorganisms at BIOTEC

Sarawut Saidaengkham, Suwanee Chunhametha, Sasitorn Jindamorakot, Pattaraporn Rattanawaree, Winai Chaipitakchonlatarn, Wipaporn Ngaemthao, Chanyanut Sathitkunanon, Watcharapong Taksadate, Wanchern Potacharoen and Kanyawim Kirtikara

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BIOTEC Culture Collection (BCC) was established in 1996 at the National Center for Genetic Engineering and Biotechnology (BIOTEC). The primary objective of BCC is to collect and preserve microbial cultures and their relevant data for BIOTEC's in-house research. Currently, BCC holds approximately 34,000 strains of microorganisms including approximately 24,700 strains of filamentous fungi (72.64%, 991 species of 714 genera), 7,000 strains of bacteria (20.59%, 223 species of 102 genera), 2,100 strains of yeasts (6.18%, 261 species of 58 genera) and 200 strains of algae (0.59%, 74 species of 38 genera). The strains were isolated from various sources; for example, insects, seeds, leaves, flowers, decayed woods, soils, fresh water and seawater. Seventy-three species are new species found in Thailand, 10, 43 and 20 species of bacteria, filamentous fungi and yeasts, respectively. Strains in the collection are preserved by freeze-drying and freezing at -80°C, and in vapor phase of nitrogen. Approximately 42% of fungal strains have been supplied for the screening of novel metabolites and almost 30% of the fungal strains tested produced bioactive substances. Some strains are made available for public access (http://www.biotec.or.th/bcc).

Notes:

Direct microscopic investigation on infection process of brown spot fungus (*Bipolaris oryzae*) on rice leaf

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This research is to investigate the infection process of *B. oryzae* on rice leaves using light and scanning electron microscopes. At the early stage, the fungal conidia successfully attached to rice leaf began germinate. Multiple germ-tubes developed randomly and grew in any direction across the leaf surface. Appressorium formation at the ends of germ-tubes was also observed. It is assumable that appressoria is an infection structure used by the fungus in order to penetrate the host cuticle in early stage. At the later stage of infection, the fungus directly penetrates rice leaves mainly via hyphae. Extracellular matrix (ECM) was observed around hyphae growing over the leaves surface. Epicuticular wax, the most external covering structure of the leaves, appeared lysed at the points attached with the fungal ECM. In this stage, hyphae of *B. oryzae* were present mostly around the collapsed epidermal cells showing signs of necrosis. No evidence for direct stomatal penetration was observed. These observations suggested that *B. oryzae* represents two different behavior when infect the host plant, biotrophic infection in early stage follow by necrotrophic infection in the later stage. This lead to the conclusion that *B. oryzae* should be a hemibiotroph on infected rice leaves rather than an obligate necrotroph.

Keywords: Brown Spot Diseases, *Bipolaris oryzae*, Hemibiotroph

Notes:

Biodiversity of termite-associated *Xylaria* species in Thailand

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This is a preliminary project with the aims to study fungi associated with termite nests and to study biodiversity of *Xylaria*ceous fungi in Thailand, especially *Xylaria* species, in order to fully understand the diversity of this group of the taxa. In the past, *Xylaria* species associated with termite nests were poorly known. Only 25 species of them were described. The termite-associated *Xylaria* were found more than 10 morphological different characters were recognized and described: *Xylaria acuminatilongissima*, *X. atrodivaricata, X. brunneovinosa, X. cirrata, X. escharoidea, X. intraflava, X. kedahae, X. nigripes, X. ochraceostroma, X. piperiformis* and *X. reinkingii*. In addition, more than 5 species are waiting for identification and description.

Phylogenetic analysis of the ITS region revealed four dominant species of termite associated *Xylaria*ceae belong to *Xylaria escharoidea*, *Xylaria brunneovinosa*, *Xylaria nigripes* and *Xylaria* sp.IV. There are many unidentified *Xylaria* species from termite nests that formed independent groups and some of them are expected to be described as new species.

Notes:

Isolation and identification of associated pathogens, in particular to *Fusarium* spp., of mulberry root rot disease in northeastern Thailand

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This study was conducted to determine the associated agents of mulberry root rot disease with particularly *Fusarium* spp. Four hundred and nine isolates of fungi were obtained from 5 mulberry plantations during 2007 to 2008 and all the fungal obtained were identified. All species were identified morphologically with particular emphasis on macroconidia, microconidia, the size and types of conidiophores, and the presence or absence of chlamydospores. The species associated with mulberry roots and surrounding rhizosphere were identified mostly *Fusarium* spp. including 102 isolates of *Fusarium solani*, 26 isolates of *Fusarium moniliforme*,16 isolates of *Fusarium oxysporum*, 3 isolates each of *Fusarium phaseoli* and *Fusarium dlamini*, 2 isolates of *Fusarium culmorum*, 1 isolates each of *Fusarium dimerum*, *Fusarium graminearum*, *Fusarium beomiforme*, *Fusarium scirp*i, *Furarium anthophilum*. Other associated fungal genera were as following: 127 isolates of *Aspergillus* spp., 71 isolates of *Penicillium* spp., 25 isolates of *Aureobasidium* sp., 13 isolates of *Rhizopus* sp., 10 isolates of *Phoma* sp., 2 isolates of *Phytophthora* sp., and one isolate each of *Pythium* sp., *Botryoderma* sp., *Peyronellaea* sp., and *Sphaeropsis* sp. *Fusarium* spp. is most likely to be the most troublesome for mulberry growing in Thailand. Pathogenicity test of eleven species of *Fusarium* spp., showed no symptoms developed on all plants. When recovering isolates of *Fusarium* spp. from roots, 30 days after inoculation *F. solani* resulted in significantly (P = 0.05) greater percentage recovery than other species (52.5% recovery) suggesting high possibility of the fungus to be the main causal agent.

Keywords: mulberry root rot, associated pathogens, *Fusarium*

Notes:

Phylogenetic analysis of selected *Annulohypoxylon* and *Hypoxylon* taxa assessed with ITS and partial LSU nrDNA sequences

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The variability of ITS nrDNA and nrLSU sequences from selected *Annulohypoxylon* and *Hypoxylon* taxa (Xylariaceae, Ascomycota) was investigated to assess phylogenetic relationships. Thirty-five isolates from 14 different fungal taxa were amplified and sequenced. The ITS sequences ranged from 462 to 613 bp, while partial LSU sequences ranged from 839 to 881 bp. The ITS sequences, particularly ITS1, showed high variation among taxa. The phylogenetic trees constructed based on ITS and LSU sequences were compared. Both trees topology were similar and revealed clear segregation of each taxon including problematic taxa investigated and also showed the relationships between closely related taxa. Nevertheless, both phylogenetic trees did not support the fungal classification at genus level between genera *Annulohypoxylon* and *Hypoxylon*. They could not separate all *Hypoxylon* taxa from *Annulohypoxylon* taxa.

Notes:

Collection and isolation study of unculturable insect fungi

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Previous studies of insect pathogenic fungi in Thailand have revealed that only 30% of the collected fungi had been successfully isolated into pure cultures. These cultures have many advantages for further studies; for examples, molecular phylogeny, and the screening for bioactive compounds. Therefore, this study has the purpose to improve the appropriate media for isolation of the fungi which could not be previously isolated. In the first part, we pay the attention to *Hymenostilbe ventricosa* on cockroaches.

The results show that factors influencing the blastosporation of *Hymenostilbe ventricosa* are nitrogen sources and the growth inducing substances. The appropriate media from this study is GICM with 3% (w/v) maltose as the carbon sources, 1% (w/v) malt extract as the nitrogen sources, and 0.1% (v/v) multivitamins as the growth inducing substances. This medium will be further used to investigate for the appropriate carbon and nitrogen sources, the growth inducing substances, and also the appropriate physical factors for the blastosporation.

Keywords: *Hymenostilbe ventricosa*, blastosporation

Notes:

The distribution of *Cordyceps* spp. in Thailand

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Cordyceps is the most diverse genus in terms of number of species and host range, comprising over 45 species, based on cylindrical asci, thickened ascus apices and filiform ascospore, which often disarticulate into part-spore (Gi-Ho Sung *et al*. 2007).The distribution is cosmopolitan, including all terrestrial regions except Antarctica, with the height of known species diversity occurring in subtropical and tropical region, especially East and Southeast Asia (Kobayasi 1941, 1982, Samson et al. 1988).

The distribution of insect-associated fungus *Cordyceps* spp. was collected mainly from forest habitats in difference region of Thailand. These regions differ from each other with respect to altitude, climate, amount of rainfall, humidity, temperature, soil type, and geography. In each region, four different locations were sampled: Khao Yai National Park in Nakhon Ratchasima Province (Hill Evergreen forest), Doi Inthanon National Park in Chiang Mai Province (Dry Dipterocarp Forest), Kaeng Krachan Nationl Park in Phetchaburi Province (Tropical Rain Forest), Khlong Nakha Wildlife Sanctuary in Ranong Province (Mixed Deciduous Forest).

A total of 104 samples were isolated and identified by morphological characteristics and molecular techniques by analysis of ITS rDNA sequences. Species diversity on Lepidoptera was highest, followed by pathogen on Coleoptera and termites. Samples from Khao Yai National Park had higher frequencies of occurrence than other locations (61.54%).In Doi Inthanon National Park there is 23.08%, 12.5% in Kaeng Krachan National Park, and only 2.88% in Khlong Nakha Wildlife Sanctuary.

Keywords: *Cordyceps,* Molecular phylogeny

Notes:

Characterization and distribution of long chain unsaturated fatty acids in lower filamentous fungi isolated from Thailand

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Lower filamentous fungi isolated from various locations of Thailand were extracted and analyzed for fatty acid composition by gas chromatography. The strains accumulated long chain unsaturated fatty acid (LCUFA) containing gamma linolenic acid (GLA; C18:3n6), arachidonic acid (ARA; C20:4n6) and eicosapentaenoic acid (EPA; C20:5n3). Morphological and molecular analyses showed that most strains belong to *Mucor, Pythium* and *Saprolegnia*. The major LCUFA found in *Mucor* was GLA whereas ARA present in *Saprolegnia*. *Pythium* produced both ARA and EPA. The results showed distribution of long chain unsaturated fatty acids in lower filamentous fungi from Thailand.

Notes:

Mating type allele analysis and existence of genetic heterogeneity among clonal population of pathogenic *Cryptococcus neoformans* species complex

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Introduction: In an era where an emerging threat due cryptococcosis is encountered among the immunocompromised patients especially in those AIDS and in immunocompetent patients attributed to the virulent *C. gatti*, routine surveillance and application of molecular typing methods are crucial to know the baseline and existing pattern of Cryptococcosis. Population genetic studies of *C. neoformans* have indicated a clonal structure, which may either influence or be influenced by the imbalance of mating types. A clonal population results when sex is absent or negligible, as might happen, for example, if conditions promoting sexual union do not occur or if one mating partner is absent. Such a population could consist of a single clonal lineage or many different lineages. In a recombining population, asexual reproduction may still predominate but the clonal structure is disrupted by the swapping of genes between members of different clonal lineages.

Objectives: In this study we try to understand the diversity among the global population of *C. neoformans* serotype A and to investigate mode of reproduction and degree of clonality in the population structure of native pathogenic Cryptococcus in the geographic settings of Indian subcontinent.

Methods: In-vitro crossing experiments were performed using the congenic strains JEC20 (serotype D, MATa) and JEC21 (serotype D, MATα) are used as testers. These two strains differ only at the mating type locus. The determination of mating type was analysed using the two gene loci STE12 and STE20 PCR primer pairs, specific for mating type α and a.

Results: Clinical *C. neoformans* isolates belong to an unprecedented proportion of *MATa* isolates. In addition the population exhibits evidence of recombination characterized by linkage equilibrium among the alleles: Index of association (I_A) ≤ 0 and the incongruence among the genealogies of unrelated genes.

Conclusion: Different regions of the genome have different phylogenetic histories with improved hybrid fitness. The study facilitates generation of a baseline data of the predominant mating type allele pattern among pathogenic cryptococci.

Keywords: *Cryptococcus*, Mating Allele, Clonal lineage, Genetic diversity

Notes:

Anastomosis Groups Related to Potato and Survey of Somatic Compatible Groups by Morphological Characters and RAPD Marker

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In this study, 58 isolates of *Rhizoctonia solani* were obtained from infected subterranean potato stems and tuber collected in Hamedan and Kordestan provinces and their variability were studied by using morphological and molecular markers. In morphological studies, some characters comprising number of nuclei, anastomosis groups, somatic compatibility groups, and pathogenicity severity were used as well in molecular study randomly amplified polymorphism DNA marker was used. Nuclear condition examination showed that all isolates were multinucleate and according to anastomosis group determination, 65 isolates were belonging to AG-3, one isolate belonging to AG-4 and one isolate did not anastomose to any of available anastomosis group tester isolates in this study. In somatic compatible group determination, AG-3 isolates were paired on PDA amended with 1% charcoal. Out of 56 isolates, 43 somatic compatible groups were obtained in which 35 groups possessed an isolates alone, one group comprising four isolates all collected from fields of Qorveh as largest group (EE). and seven remaining groups each one was comprising two or three isolates. Pathogenicity severity of isolates belonging to AG-3, as a group, on potato sprouts, were higher than AG-4 isolate, while unknown isolate did not produce any lesion or symptom of disease on potato sprouts. Finely, genetic diversity of four selected somatic compatible groups including E, F, T and EE were studied by RAPD. Among 12 primers used in this study, 11 primers based clearance and numbers of bands were chosen and data were analyzed by NTSYS software using UPGMA method and Jaccard coefficient. Isolates were divided in four clusters with a minimum similarity coefficient of 0.42. Results of molecular study supported and verified morphological results. Therefore according this study, more attention is needed in seed tubers exchange among studied area.

Keywords: *Solanum tuberosum*, Anastomosis, Hamedan.

Notes:

Creation of a non-ribosomal peptide library from BIOTEC fungal culture collection

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Background: The pharmaceutical sector is again interested in exploring the potential on natural products as new drug leads. A group of these compounds which are of potential interest are the peptides derived from microorganisms e.g. bacteria and fungi. All fungal peptides are synthesised non-ribosomally involving enzyme complexes known as peptide synthetases. These peptides are characterised by the inclusion of two or more moieties derived from amino acids which are associated with biological activities of these molecules. The BIOTEC Culture Collection (BCC) provides a bioresource for screening of organisms, which produce bioactive peptides, based on chemical analysis and molecular approaches.

Aims: To develop chemical approaches for detecting fungal non-ribosomal peptides. To identify bioactive fungal peptides and establish a library of their chemical profiles.

Methods and Results: Initially, 14 commercially available peptide antibiotics were used to optimise the conditions for their separation by HPLC with a 10 cm C18 reversed phase column. UV profiles were obtained at 200 – 220 nm and molecular mass profiles were determined by an ESI-mass spectrometer. The optimal separation profiles depended on size, polarity and the structures of functional groups associated with these compounds.

To establish a non-ribosomal peptide library, 11 representative fungi including some known producers of peptides, were extracted with different methods. XAD7 resin adsorption gave the best recovery of active compounds. The previously determined optimal HPLC conditions provided a starting step of separation. Individual peaks in the samples analysed, particularly putative peptides, have been to identify potentially interesting molecules. The profiles collected in this way are being used to create a non-ribosomal peptide library. Biological activities of partially purified peptide molecules and the biosynthetic genes (non-ribosomal peptide synthetase, *NRPS*) in the fungal genomes were also investigated. Working with more fungi will be challenging and should enable the discovery of new active peptides. Fungi from different habitats have been chosen and will be grown in different media to expand the variety of compounds identified and thereby the peptide library held at BIOTEC.

Conclusion: Utilisation of fungal bioresources in the discovery of active peptides is relevant to the mission of BIOTEC. The creation of a non-ribosomal peptide library is in progress and simultaneously expertise in the fermentation technology associated with production, and also a molecular biology approach is being developed. These will play a critical role in the discovery of new bioactive peptide metabolites.

Notes:

Quantitative analysis of beauvericin in natural specimen and cultivated synnemata of the lepidopteran pathogen *Isaria tenuipes*

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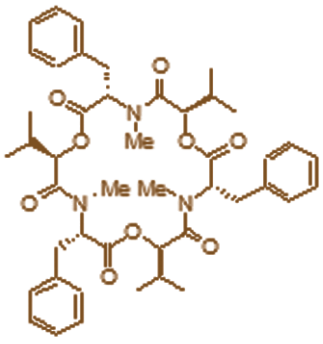
Isaria tenuipes (also known as *Paecilomyces tenuipes* or *Isaria japonica*) is a pathogen of Lepidoptera - larvae or pupa. Its cultivated synnemata have been used in health foods in East Asian countries such as China and Japan. On the other hand, we often isolate the cyclodepsipeptide antibiotic, beauvericin, from liquid fermentation of *I. tenuipes*, in our continuing chemical studies on insect pathogenic fungi. Beauvericin is known to possess broad range of biological activities, and it could also be considered as a toxin. To compare the chemical constituents of natural specimen, cultivated synnemata, and fermentation mycelia of this fungus, we have established an analytical method to detect beauvericin. Because of the very small quantity of natural specimen, we have employed LC-MS method for analyses.

In our methods, trace of beauvericin was successfully detected both in the natural specimen and cultivated synnemata (on rice). For example, synnemata and an insect cadaver of *I. tenuipes* BCC 31640 natural specimen contained 0.036 and 0.058 mg/g of beauvericin, respectively. Synnemata of this strain cultivated on rice contained 0.92 mg/g of beauvericin, whereas fermentation mycelia of the same strain included much higher composition (14.1 mg/g).



beauvericin

Notes:

Inhibitory effects of plant extract from clove (*Syzygium aromaticum*) and sweet-flag (*Acorus calamus*) on some fungi isolated from hydroponically grown vegetables

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Fungi isolated from the symptoms plants (including leaf spot and root rot) grown in hydroponics were tested on culture media containing with clove or sweet-flag's extract at several concentrations. The results showed that crude extract of clove at the concentration range between 500-1,000 ppm could inhibit fungal growth significantly. The mycelial growth of *Alternaria* sp., *Curvularia* sp., *Helminthosporium* sp., *Cercospora* sp., *Pythium aphanidermatum* and *Pythium myriotylum* were inhibited around 41-72%, 62-77%, 60-100, absolutely 100%, 66-100% and 53-100%, respectively. Clove's extract at concentration of 500 ppm also reduced sporulation of *Alternaria* sp., *Curvularia* sp. and *Helminthosporium* sp. significantly compared with control whereas the concentration to inhibit conidial germination of *Cercospora* sp. was found at over 1,000 ppm. For crude extract of sweet-flag, the effective concentrations to inhibit mycelial growth were mostly found at 500-5,000 ppm, which could inhibit fungal growth around 40-100% depend on each fungus. The concentration of 500 ppm also reduced sporulation of *Alternaria* sp., *Curvularia* sp. and *Helminthosporium* sp. but did not affect on conidial germination of *Cercospora* sp. Since *Cercospora* sp., *P. aphanidermatum* and *P. myriotylum* had been reported as the serious pathogens of hydroponic crops, the development of effective plant extracts for controlling diseases in this growing system might be possible.

Notes:

Taxol producing new endophytic fungus *Pestalotiopsis mangiferae*

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The need for new and useful compounds to provide assistance and relief in all aspects of the human condition is ever growing. Natural products still remain the most important source for discovery of new and potential drug molecules. Over the last few years there has been increasing interest in the investigation of endophytic fungi, which live asymptomatically within plant tissues, as a source of novel anticancer compounds.

Pestalotiopsis species are important group endophytic fungi, some of which produce secondary metabolites with a great potential for antimicrobial and antitumor medicinal applications. In the present study, endophytic fungus was isolated from the medicinal plant *vitex negundo* L. and identified as *Pestalotiopsis mangiferae* based on its morphological character. The crude ethyl acetate extract of endophytic fungus showed strong antibacterial activity against gram positive (*Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*) and gram negative (*E. Coli*, *Proteus* sp., *Klebsiella pneumonia*, *Salmonella typhii*, *Pseudomonas aeruginosa*) bacterial pathogens. Fungal extract also showed significant antifungal activity against *Candida albicans* and *Aspergillus niger*. Minimum inhibitory concentration (MICs) of the crude fungal extract was also determined against bacterial and fungal pathogens. HPLC analysis of the filtered culture extract of endophytic fungus *Pestalotiopsis mangiferae* revealed the presence of taxol compound.

Notes:

Antimicrobial and anticancer of endophytic fungi from *Mitragina javanica* leaves

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A total of 106 endophytic fungal isolates were obtained from *Mitragina javanica* leaves from Ayuthaya, Prathumthani and Amnatchareon provinces in Thailand. These endophytic fungi were identified as *Colletotrichum* spp., *Fusarium* spp., *Phomopsis* spp., *Alternaria* spp., *Phyllostica* spp. *Xylaria* spp., *Penicillium* spp., a *Cladosporium* sp. and the majority were mycelia sterilium. The ethyl acetate extracts of all the endophytes were evaluated for antimicrobial and anticancer activities. The antimicrobial activities were tested by a paper disc diffusion assay against six reference microorganisms including *Bacillus subtilis*, *Staphylococus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Saccharomyces cerevisiae* and *Candida albicans*. The crude extracts of 65.43% showed antimicrobial activities against at least one tested microorganism with inhibition zones ranging from 8-3.5 mm. Out of these isolates 5.66 % displayed a broad antimicrobial spectrum and 12.15 % inhibited the pathogenic yeast *C. albicans*. Moreover, the endophytic fungus PT11 exhibited the strongest antimicrobial activity against all test microorganisms. Anticancer activities were examined with six human cancer cell lines including A375 (malignant melanoma), KatoIII (gastric cancer), SW620 (colorectal cancer), HepG2 (liver cancer), BT474 (breast cancer), and Jurkat (T-cell leukemia)) by the MTT method. The crude extracts of the endophytic isolates exhibited 58.49%, 60.38%, 58.49%, 54.28%, 61.32% and 13.13% cytotoxicity (cell viability < 40%) against A375, Kato III, SW620, BT474, HepG2, and Jurkat, respectively. The endophytic fungal isolates AY16, AY34, and PT1 which had the most specific activity against the cell lines were evaluated for their potential to induce apoptotic cell death in SW620, BT474 and Jurkat, respectively using Hoechst 33258 fluorescence stain. The results showed that ethyl acetate crude extracts of AY16, AY34, and PT1 could induce DNA fragmentation and the characteristic morphological features of apoptosis toward the target cancer cells. These results of antimicrobial and anticancer screening support the view that endophytic fungi from *M. javanica* are a potential source of bioactive compounds. Further analysis of secondary metabolites from the most active endophytic fungi may lead to the discovery of novel bioactive compounds.

Keywords: endophyte, endophytic fungi, *Mitragina javanica*, antimicrobial activities, anticancer activities

Notes:

Effects of crude extract from physic nut to inhibit plant pathogenic fungi *in vitro*

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Physic nut fruits (*Jatropha curcas* Linn.), Family Euphorbiaceae, were collected from Kamphaengsaen, Nakhon Pathom province. Fine dry powder samples from the fruit were extracted in water and methanol (CH3OH) solvent at the ratio of 1:2 by weight and volume. The samples were macerated for 30 days at 28°C. They were filtrated by Whatman filter paper and then they were evaporated by rotary evaporator at 50-55°C to obtain a dark brown viscous of water and methanol crude extract for 15 g and 16 g, respectively. Crude extracts were tested for anti mycelial growth to seven plant pathogenic fungi including *Colletotrichum gloeosporioides* (anthracnose of mango), *Rhizoctonia solani* (leaf blight of durian), *Pythium aphanidermatum* (damping-off of mungbean), *Curvularia lunata* (leaf spot of corn), *Fusarium oxysporum* (wilt of tomato), *Lasiodiplodia theobromae* (fruit rot of banana) and *Selerotium rolfsii* (basal and root rot of taro). They were cultivated on potato dextrose agar containing crude extract at concentrations of 100,000, 10,000, 1,000, 100 and 0 ppm (control) for 14 days at 28°C. The results indicated that water crude extract of physic nut at 1,000 and 10,000 ppm concentrations inhibited more than 50% mycelial growth of *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium oxysporum* and *Lasiodiplodia theobromae in vitro*.

Notes:

Bioactive metabolites of endophytic fungus *Alternaria alternata* isolated from the medicinal plant *Vitex negundo*, L.

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Fungal secondary metabolites are an importance source of lead structures for new drug compounds. Many endophytic fungi have the ability to produce antimicrobial metabolites. The species of *Alternaria* have been demonstrated to be rich sources of bioactive secondary metabolites with diverse structural features. In the present study, bioactive metabolites producing endophytic fungus was isolated from the leaves of the medicinal plant *Vitex negundo*, L. The fungus was identified as *Alternaria alternate* based on its morphological characteristics. The crude ethyl acetate extract of fungal culture exhibited antimicrobial activity against bacterial and fungal pathogens. The ethyl acetate extract was partially purified by TLC and Column chromatography methods. Out of the six fractions, fourth and sixth fraction of the extract inhibited the growth of all the tested gram positive (*Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*) and gram negative (*E.Coli*, *Proteus* sp., *Klebsiella pneumonia*, *Salmonella typhii*, *Pseudomonas aeruginosa*) bacterial pathogens with minimal inhibitory concentration (MIC) ranging from 50 to 200µg/mL. The fourth and sixth fractions exhibited potent antifungal activity against the pathogens *Candida albicans* and *Aspergillus niger*. The partially purified fourth fraction of endophytic fungal culture extract was analysed by UV, IR, ^{1}H and ^{13}C NMR spectroscopy.

Notes:

5-Hydroxyramulosin, a cytotoxic compound from an endopythic fungus in *Cinnamomum* sp.

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Cinnamomum spp. is a plant which has been widely used for medicinal purposes. Among the medicinal values are to cure bronchitis, cold, cough, fever, indigestion, diarrhoea, some cancerous tumors and to ease inner muscular pain. In this study, different plant parts of *Cinnamomum partheoxylon* had been sampled in order to isolate endophytic fungi. Fungal isolates emerging from these plant parts were tested for biological activity. Extracts from isolate CB007 (WA) had significant and consistent biological activity against microbial pathogens. Therefore compounds from ethyl acetate extracts of this isolate were characterized. One of the compounds identified was 5-Hydroxyramulosin, a compound which formed crystals at low temperature. It was cytotoxic to P388 murine leukemic cells with an IC_{50} value of 2.102µg/ml and active against *B.subtilis*, *A.fumigatus* and *A.niger*. The structure of 5-Hydroxyramulosin crystal was determined by x-ray crystallography. This compound was previously identified in a marine derived fungus, *Phoma tropica.*

Notes:

Inhibition of the oil palm pathogen, *Ganoderma boninense* by endophytic fungi from the palm *Licuala spinosa*

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The biodiversity of endophytic fungi from the fan palm, *Licuala spinosa* in Kuan Kang Hot Spring, Kantang, Trang province, was investigated yielding 195 and 182 morpho types (in two studies), with 75 and 68 xylariaceous morpho types, 3 and 10 coelomycetes, respectively. Then 420 strains were selected and grown on Yeast Extract Agar (YEA) to measurement their radial growth rate for 10 days and then re-selected from fast growth (5.91-7.20 cm), moderate growth (2.50-2.90 cm) and slow growth (0.01-2.49 cm) amount 300 strains and tested for their antagonistic ability against a selected oil palm pathogen, *Ganoderma boninense* by dual culture test. A 5 mm. diameter disc from the growing edge of *G. boninense* was placed at one side of a Petri dish containing YEA while a 5 mm. diameter disc from the growing edge of the endophytic fungal isolate were placed on the other side of the dish.For the control a disc from the growing edge of *G. boninense* was placed alone at one side of a Petri dish. Each test was replicated on three plates.The plates were incubated at 25°C and a dimeter of fungal colonies recorded daily until 15 days to determine antagonistic activity. Eighty six isolates tested had a high antagonistic activity (> 60% inhibition the growth of *G. boninense*) with 17 isolates had a greatest antagonistic activity (> 80% inhibition). These seventeen isolates will be further test for their bioactive compound.

Keywords: *Ganoderma boninense*, endophytic fungi, antagonist

Notes:

Optimization of synnemata production on solid medium of Isaria isolated in Thailand

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The optimum medium and condition for synnemata production of 3 Isaria strains isolated in Thailand including *Isaria tenuipes* BCC 23112, *Isaria* amoeneroseus BCC 19494 and *Isaria* sp. BCC 25098 were studied by using a Packatte Burman design. In this study, there are eight factors including a substrate, silk worm, yeast extract, fish meal, vitamin complex, pH and cold shock that may affect the production of synnemata of these 3 strains on solid medium. The results showed that the important factor for synnemata production of all strains was the substrate, while the yeast extract and vitamin complex affected only synnemata production of *Isaria tenuipes* BCC 23112.

Notes:

Biological activities of extracts from fungal strains isolated in Thailand

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Over 23,000 strains of fungi have been isolated from various natural habitats in Thailand and collected at the BIOTEC Culture Collection. To evaluate the value of this microbial resource, approximately half of these strains have been screened for biological activities against human and agricultural disease pathogens. Crude extracts prepared from fungal cultures were tested against a non-cancerous Aftrican green monkey kidney (Vero) cell line; three cancer cell lines including KB (oral cavity cancer), MCF-7 (breast cancer) and NCI-H187 (small cell lung cancer); malarial parasites (*Plasmodium falciparum*, K1); tuberculous bacterium (TB) (*Mycobacterium tuberculosis*, H37RA); herpes simplex virus type 1; four bacterial strains including *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*; yeast (*Candida albicans*); and two plant pathogens including *Magnapothe grisea* and *Curvularia lunata*. The fungal strains that have been tested were classified into 20 groups according to their distinct habitats and taxonomy. Each of the group that contains more than 500 strains; including insect pathogenic fungi, Xylariaceae, entophytic and marine fungi, exhibited a different range of activities. Insect pathogenic fungi showed a broad spectrum of cytotoxicity against cancer cell lines (KB, 5.48%; MCF7, 10.72%; and NCI-H187, 11.52 %) and Vero cell line (7.81 %), anti-malarial activity (5.64 %), anti-TB (8.39 %), anti-*C. albicans* (5.04 %) and anti-fungal activities (*M. grisea*, 2.66 %). Similarly, marine fungi showed cytotoxicity against Vero cell line (7.82 %) and anti-*C. albicans* activity (7.67 %). On the other hand, endophytic fungi showed anti-malarial (4.59 %) and anti-TB activity (10.42 %), while Xylariaceae showed only cytotoxicity against Vero cell line (14.02 %). The numbers of positive strains among the fungal groups that contain fewer than 500 strains were insignificant. These groups of fungi, including alkaline tolerant, aquatic, dung, palm, sand, saprobic, seed, soil, wood decaying fungi, fungi on bamboo, Ascomycete, Basidiomycetes, Coelomycetes, Hyphomycetes, mushroom and unidentified, remain to be further evaluated whether they have potential as a source of bioactive compounds. These screening results demonstrated the diversity of microbial resource in Thailand, their strong potential as a source of bioactive compounds and, hence, the possibility of future development of value-added products from natural resources.

Notes:

Preliminary screening of hydrolase enzymes from cold adapated fungi from Fildes Peninsula, King George Island, Antarctica

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A sample collection was done during a summer expedition to Fildes Peninsula, King George Island, Antarctica in February 2007. Samples were collected from various parts of the Island such as ornithogenic site, human impacted area and area surrounded by moss. Isolation of fungal species were done using Warcup's soil plating method and classified into three thermal classes: mesophilic, psychrotrophic and psychrophilic. 13 mesophilic species which comprised 12 deuteromycete and 1 ascomycete; 2 psychrotrophic species which comprised of a zygomycete and a deuteromycete; and 13 psychrophilic species including 4 ascomycete, a basidiomycete, 4 deuteromycete and 4 yeast. Screening of enzyme from hydrolase group were carried out on 13 psychrophilic species and 2 psychrotrophic species. Enzymes we chose for screening were cellulase, beta galactosidase, amylase and protease. These all four enzymes are from hydrolase group. Each species was prepared in 3 replicates for each enzyme and incubated in 4ºC refrigerator for 15 days. Cellulose was screened using R2A agar plates supplemented with carboxymethyl cellulose and trypan blue, beta galactosidase was screened using R2A agar plates supplemented with lactose and X-gal, amylase screened by flooding the R2A agar plates with lugol solution which was supplemented with starch while protease activity screened by flooding R2A agar plates supplemented with skim milk with coomassie blue. The experiment is still on going. Species that shows clear zone around the colony has enzyme activity. We expect to get a few species that gives positive results.

Notes:

Biosorption of lead (II) by *Penicillium* sp. MRF-1 isolated from South Korean mine soil sample

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A potential heavy metal resistant fungus *Penicillium* sp. MRF-1 was isolated from the mine soil sample. The soil sample was acidic in pH 5.41 and highly contaminated with Pb 356.96 mg/kg and CN 14.567 mg/kg. Four different fungal species were isolated from the mine soils sample. Among the 4 fungal species, *Penicillium* sp. MRF-1 was identified as the best lead resistant fungus based on weight loss with increasing concentration of lead in the triple filtered distilled water solution. *Penicillium* sp. MRF-1 was characterized based on morphological features and molecular sequences. ITS regions sequences of *Penicillium* sp. MRF-1 were used for molecular identification. ITS regions sequences of *Penicillium* sp. MRF-1 were showed 99.794% similarity with *Penicillium janthinellum* based on GenBank BLASTn search. The experimental data's of effect of pH, contact time and effect of contact time on biosorbent *Penicillium* sp. MRF-1 were used for the biosorption studies. pH 4 was identified as favorable condition for lead adsorption and adsorption of lead was gradually increased with increasing temperature. Adsorption capacity of *Penicillium* sp. MRF-1 was described using two-parameter isotherm models (Langmuir and Freundlich models). Adsorption kinetics of biosorbent was studied using Pseudo-first order and Pseudo-second order kinetics. Adsorption data was well analyzed by using both Langmuir and Freundlich isotherms, based on the analysis results, Langmuir isotherm showed better fit than Freundlich isotherm. The results of kinetic studies showed that Pseudo-first order kinetic model highly correlated with experimental data compare than Pseudo-second order model. Biosorbent *Penicillium* sp. MRF-1 showed better desorption in alkali condition. In addition, in presence of lead, the surface structural change of *Penicillium* sp. was analyzed using SEM. In conclusion, the new biosorbent *Penicillium* sp. MRF-1 may be used as potential, inexpensive, easily cultivatable material for removal of lead from aqueous solution.

Notes:

Biocontrol of aflatoxigenic fungi and aflatoxin B_1 production in cold storage chillies

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Chilli *(capsicum annum L)* is one of the important crops of India. Virudhunagar region in Tamilnadu and Guntur region in Andhra Pradesh are the largest chilli producing area in India. After harvest, the dried chillies are kept in cold storage as the product gives premium price due to excellent retention of the colour. During cold storage period, chillies are infected by molds and produces aflatoxin which affects the quality. Aflatoxins are potent toxic, carcinogenic, mutagenic and immuno-suppressive agent. They are produced as secondary metabolites by the infected fungi.

In the present study, the microflora of the cold storage chillies were analyzed. The fungi such as *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp. were dominant in the chillies. Aflatoxin producing fungi were isolated from the storage chillies and identified as *Aspergillus flavus* and *Aspergillus parasiticus* based on their morphological characteristics. The fungal isolates were found to produce B_1 toxin. Chilli samples kept in cold storage for various periods were collected and analyzed for the aflatoxin B_1 content by HPLC method. HPLC analysis revealed that the production of aflatoxin B_1 by the infected fungi in chillies ranges between 10-30 ppb. According to US FDA the minimum acceptable level of aflatoxin B_1 in all foods other than milk is 10 ppb.

Among the various methods of control of aflotoxigenic fungi, the use of microbial antagonists as biological control agents seems to be a promising way to control the infection of chillies. In the present study, an antagonistic fungus was isolated and its effect on the inhibition of growth of aflatoxigenic fungi was studied.

Notes:

Identification and Characterization of Retrotransposons in the Mushroom Pleurotus eryngii

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Retrotransposons (RTNs) are mobile genetic elements, which amplify via RNA transcripts, in eukaryotic genomes. They comprise 3% and 43% of *Sacchomyces cerevisiae* and human genomes, respectively. We recently identified 22~26 signatures of novel RTNs from the basidiomycete fungus *Pleurotus eryngii*, which is one of the most popular edible mushrooms worldwide, while seeking for the cause of mushroom strain degeneration. In order to further characterize in terms of activity, copy number, types of these RTNs, we initially approached the expression of RTN RNA via RT-PCR and northern blot analysis. RT-PCR analysis revealed that 18 out of 26 RTNs produced active RNA, suggesting that they are functionally active. Some of these results were also confirmed through the northern blot analysis. Additionally, we screened the RTNs from fosmid library of *P. eryngii* genome to determine whole RTN DNA sequences. More than 10 fosmid clones were isolated and the determination of their insert sequences was conducted.

Notes:

Optimization of enzyme production from BCC fungi collection

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Microbial enzymes have been applied widely in various industries such as animal feed, pulp & paper. For specific industrial process, each enzyme is selected based on their natural catalytic actions and its optimally working conditions. For example, extreme alkali enzymes are required for pulp and paper industry whereas acidic one is preferred for animal feed. From the previous work at Enzyme Technology Laboratory, extreme enzymes have been screened, identified, and characterized from fungi in BCC collection. Three fungal strains, namely *Aspergillus niger, Syncephalastrum racemosum,* and *Marasmius* sp. produced high level of xylanase and cellulase working at wide pH range. This study aims to determine media and growth conditions that promote enzyme production of these three fungi. Different media recipes were compared while effect of trace elements was tested as well as various ratios of inducers. To lower production cost, optimization was carried out in comparison using solid cultivation. This study will therefore give essential information applicable for production of enzyme in industrial level.

Notes:

Identification of fungal strains producing β-mannanases potential for animal feed industry

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Animal feed contains various types of polysaccharides including soybean meal and palm kernel cake which contain high amount of mannan, galactomannan, and xylan. The presence of these polysaccharides causes high viscosity and anti-nutritional properties in feed. β-mannanase is able to degrade target polysaccharides into a low-molecular oligosaccharide, thus, plays an important role to increase the nutritional value of animal feed. In feed industries, a large amount of β-mannanase has been imported annually, thus, it would be of great interest to screen for potential β-mannanase produced from microorganisms isolated in Thailand for local industrial uses. Therefore, the aim of the study is to identify fungal strains that produce β-mannanase with the optimal working condition at pH range of 3.5-5.5.

The total of 680 various fungal strains were selected for the ability to produce β-mannanase. Using mannanase plate assay, 300 strains were found to qualitatively produce the enzymes. Quantitative enzyme assay was then performed and 40 strains with highest mannanase activities were selected for further investigation. When cellobiose was used as an inducer, 8 strains exhibited high level of mannanase activity. The enzymes also functioned at condition found in animal gut. These fungal strains also exhibited thermostability and they were found to be stable at both acidic and alkaline condition. Other enzymes including cellulase, xylanase, amylase and β-glucanase, were detected from selected 6 fungal strains, albeit at low level. Further optimization to obtain high level of these enzymes would be beneficial for the feasibility study of these enzymes as animal feed supplement.

Notes:

The efficiency of Xtreme 3000 and InductACT air purifiers on eradication of fungal and bacterial pathogen in the laboratory conditions with special reference to *Staphylococcus aureus*

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The air surrounding us plays an extremely important role in our well being and efficiency. Breathing pure and clean air allows us to think more clearly, sleep more soundly, and stay healthier. Studies show that we receive 56% of our energy from the air we breathe, more than from water and food combined. On average we breathe 37 pounds of air a day (equivalent to volume of an Olympic sized pool). In previous experimental studies utilizing the Xtreme 3000 air purifier, it has been established that the use of negative ions in a purification system is an effective means of eradicating aeroallergens such as mold and microbes in room air. Studies conducted by the United States Department of Agriculture (USDA) have illustrated the ability negative ion air purifiers to significantly reduce the airborne amounts of *Salmonella enteriditis* in rooms containing infecting cage laying chickens. The removal of bacterial pathogens from the air, especially in diseases that are transmitted by droplets like *Mycobacterium tuberculosis*, is a promising way to reduce disease transmission in the clinical healthcare setting. In this study, the efficiency of negative ion purifiers such as the Xtreme 3000 air purifier and the InductACT air purifier were evaluated in the microbiology and mycology room of Baptist Saint Anthony's Hospital (BSA) laboratory in Amarillo, Texas. This department processes around 85,000 bacterial and fungal cultures per calendar year, and the staff also performs many various types of serological testing for bacterial pathogens and routine parasitological examinations on various types of clinical specimens. We evaluated the efficiency on the net reduction of bacteria in a negative pressure laboratory and the specific effect on isolates indentified to be methicillin resistant *Staphylococcus aureus*, MRSA. We evaluated 2 units, the Air Oasis Xtreme 3000 Air Purifier and the Induct Air Purifier. The Xtreme 3000 is designed to sanitize air, as well. The majority of bacteria isolated from the room air exposure was gram positive bacilli such as *Bacillus* sp. and *Coryneform* (diptheroids) sp., coagulase negative *Staphylococcus* sp., *Micrococcus* sp., and encapsulated gram negative bacilli. In order to determine if net reduction in bacteria in room occurs with the operation of the Xtreme 3000 and the InductACT air purification systems, we used laboratory rooms of different sizes. In each and every case there was reduction in airborne pathogen. Although, this experiment confirmed that infections such as *Staphylococcus aureus* infections, particularly MRSA, are not caused by airborne transmission. We devised a new technique to assess and evaluate the air purifiers in reducing the growth of the MRSA isolates. Xtreme 3000 air purifier kills surface mold, bacteria, and viruses in areas up to 3000 square feet. It has been proven to reduce colony growth in petri-dishes in one, two and four foot distances. The effectiveness of the Xtreme 3000 air purifier was evaluated in the 80 square feet mycology room. In this experimental study the re-evaluation of the square footage of the microbiology room and the layout of the air ducts were taken into consideration. The four InductACT units were evaluated in the 960 square feet microbiology room. These units use an Advanced Hydrated Photocatalytic Oxidation filtration system that is placed directly into the air circulation system via a hole cut into the ventilation system. It is a broad spectrum high intensity UV light targeted on a quad metallic catalyst in a low-level ozone and moist atmosphere. The peak hours of activity of culture evaluation and clinical specimen setup in the microbiology department are from 0600 to 1630 daily. The BSA Microbiology department handles a large variety of clinical specimens from the inpatient cases, emergency room, two urgent care centers, a dozen long term care facilities. In order to establish growth patterns in both rooms, tryptic soy agar plates with 5% sheep blood were placed out in the microbiology and mycology rooms at 15, 30, 60, 120, 180, 240, 300, 360, 420, and 480 minutes of exposure to room air during peak times of activity. The plates were evaluated for the total number of bacterial and

fungal colonies observed at 24 and 48 hrs of incubation at 37° in a CO_2 incubator. The reduced numbers of gram negative bacteria from the isolates does suggest that the air purifiers do have a greater effect on this type of bacteria. Finally, safety measures and protective equipment used in a BSL-2 lab such as laminar flow hoods, disposable culture loops, and other protective measures may help reduce the number of potential bacterial pathogens from the room air. To culture the methicillin resistant *Staphylococcus aureus*, MRSA in both rooms, tryptic soy agar plates with 5% sheep blood were inoculated with the strain after serial dilutions as follows. 5 ml of initial culture was diluted to 10^{-4} after an incubation at 37° C for 24 hours. The isolates were added to the petri plates containing medium (tryptic soy agar plates with 5% sheep blood) and allowed them to grow for 24 hours. One set consisting of 10 petri plates were kept at room temperature without any air purifier. The other set consisting of 10 petri plates were kept in front of the air purifier with a constant distance from the air purifier Xtreme 3000. The same experiment was repeated with the constant distance from the air purifier (6 feet) using InductACT air purifier. After 24, 48 and 72 hours the plates were secured over a numbered map of the plate marking on each plate where the top counter was positioned. The summarized data showed the reduction in the number of colonies produced in the inoculated TSA agar plates with 5% sheep blood when any of the air purifier was used. From collected data from the agar plates it can be concluded that the InductACT air purifier is more efficient than the Xtreme 3000 in reducing the bacterial colonies. Since the methicillin resistant *Staphylococcus aureus* did not grow on the agar plates in the original experimentation we plated them from the stock culture after dilution 10^{-4} to investigate the effect of the Xtreme 3000 and the InductACT air purifier on the production of number of colonies of the methicillin resistant *Staphylococcus aureus*. The air purifiers were effective in reducing the number of emerged colonies on agar plates at different intervals of 24, 48 and 72 hours. InductACT was the most effective one which was able to minimize the number of bacterial colonies. The Xtreme 3000 was the next effective one. We reported that Xtreme 3000 air purifier as efficient in eradication of microflora over a longer period of time at the “high” setting by using the negative ion emission at the World Allergy Congress 2007. In the present investigation we observed the reduction in the number of the bacterial colonies emerged on the inoculated agar plates because of the negative ion emission that has a negative effect on bacterial growth. Negative Ions clean the air of impurities like dust, pollen, animal dander, mold spores, odors, smoke and even bacteria. Due to these qualities negative ions are termed as the vitamins of air. Negative ions get attached to pollution particles (e.g. dust, pollen and other impurities in the air). When that happens, both the ions and the pollutant particles tend to be swept out of the air by the electric field that exists naturally near the earth’s surface. Hence, polluted or impure surroundings tend to reduce the count of negative ions in the air. Bactericidal effects of negative air ions on airborne and surface *Salmonella enteritidis* from an artificially generated aerosol are well documented by USDA and other agencies. We found that the colonies produced on the agar plates inoculated from the diluted culture could not grow further when subjected to the two air purification systems that we are evaluating because the negative ions generated by the air purifiers killed the bacterial population on agar plates restricting their further growth.

Notes:

Hyperparasitic behavior in *Cordyceps* related fungi

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Cordyceps (Fr.) Link s. l. (Clavicipitaceae s. l., Ascomycota) has been known as pathogen to 10 orders of arthropods and truffles. On the hosts, they develop one to several stromata, which are composed of stipes and perithecial fruiting part at their apex and lateral part. Anamorphic synnemata sometimes occur on the stipe of the stroma. Such synnemata are often considered as the asexual stage of the *Cordyceps* species constituting the stroma. However, it has been sometimes revealed that unrelated anamorph fungus grows on the stroma of *Cordyceps* species. In the cause of our taxonomic study of insect fungi accompanying with cultural study and molecular analysis, such phenomena of hyperparasitism have been found in many case as follows;

1) *Hirsutella nutans* is known as a hyperparasitic fungus forming marking-pin-like synnemata on the stipe of *Oph. nutans.* This species has been recorded only from Oph. nutans, shaowing high specificity to the host.

2) An anamorphic fungus forming white synnemata with *Hirsutella*-like phialides occur on the stipe of immature or postmature stroma of *Oph. prolifica.* We revealed this anamorphic fungus and *Oph. prolifica* are unrelated species. Phylogenetic analysis shows this hyperparasitic fungus is clustered as a species with other strains isolated from the stroma of *Cordyceps* species on cicada and Lepidopteran larva.

3) An anamorphic fungus (*Polycephalomyces formosus*-like) forming marking-pin-like synnemata on the stroma of *Oph. heteropoda* is also a hyperparasite. (The true anamorph of *Oph. heteropoda* is *Tolypocladium*-like.) This species was found to attack *Oph. nigrella*, *Oph. ryogamiensis*, undescribed *Cordyceps* sp. and directly Coleopteran larva.

4) A teleomorphic fungus, *Oph. cuboidea* sometimes produces their stomata on the stipe of *Oph. stylophora* and other *Cordyceps* spp. as well as on the larva of Coleoptera. But the anamorph of *Oph. cuboidea* has not been found as a hyperparasite of other *Cordyceps* species in nature.

These hyperparasitic behaviors can be categorized as follows;

A. Parasitic to a specific *Cordyceps* species

B. Parasitic to a wide range of *Cordyceps* spp.

C. Parasitic to both insects and a wide range of *Cordyceps* spp.
   - by anamorphic state
   - by teleomorphic state

Notes:

Endophytic fungi associated with seagrass (Enhalus acoroides, Hydrocharitacea) at Had Khanom - Mu Ko Thale Tai National Park, southern Thailand

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Seagrasses are flowering plants inhabiting coastal and marine environments, with a worldwide distribution in temperate and tropical regions. They serve as feeding, breeding and nursery grounds for economically important marine organisms, and including endangered species. Little information is available of fungi associated with seagrasses, especially fungal endophytes. Therefore, the tropical eelgrass *Enhalus acoroides* (Family Hydrocharitacea) was collected from two sites at Had Khanom-Mu Ko Thale Tai National Park, as it is the most common species in this study area. The objectives of this project were to investigate for the presence of endophytes in *E. acoroides* and test for their antimicrobial activity. This study yielded 42 fungal assemblages, isolated from four collections over one year. Our results confirm that the seagrass *E. acoroides* harbored fungal endophytes, and is the first report of endophytes associated with seagrass from Thailand. Molecular identification of endophytes based on LSU and ITS1, 2, 5.8S rRNA sequences revealed a diversity of fungal groups including two Phyla: Ascomycota (98%) and Basidiomycota (2%). Three major Ascomycota classes including the Eurotiomycetes, Sordariomycetes and Dothideomycetes were determined. Eight genera and two species were fully identified while others remain to be characterized. The predominant 12 isolates (29%) were members of the Hypocreales, followed by the Eurotiales and the Capnodiales, respectively. However, identification of some strains was not fully resolved at this stage, due to lack of known reference species for comparison in the GenBank database. This study provides a baseline for further monitoring of fungal communities respect to habitat changes or to monitor the effects of pollution. Fermentation broths, from selected fungal endophytes, were tested for their antimicrobial activity by agar well diffusion. Approximately 16% displayed antimicrobial activity against at least one pathogen with significant inhibition zones. Therefore, our study has opened up the potential use of marine endophytes as a good source of natural antimicrobial compounds.

Notes:

Origin and evolution of hydnoid hymenophore among holobasidiate agaricomycetes

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The himenophore has been defined as plecto that supports the hymenium, and such structure has had great importance in the taxonomy of holobasidiate agaricomycetes (formerly Homobasi diomycetes). Traditional classifications at the order or family of this group were strongly influenced by the macroscopical configurations of himenophore. However, this importance decayed as advanced in study about micromorphology and taxonomy of agaricomycetes. Recent phylogenetic evidence suggests that the evolution of the himenophore among agaricomycetes represents multiple morphological convergences. Without exception all of different types of himenophore (i.e. porus, lamellas, teeth, etc.) apparently have originated several times among major clades of agaricomycetes. Hydnoid (toothed) hymenophore is probably one of the most recurrent macroscopical configurations within this group; however, it is rare at the species level. In this study, we conducted detailed observations of micromorphological attributes of hydnoid hymenophora in different genera of agaricomycetes. Trough the use of phylogenetic comparative methods, we investigated the distinct origins of hydnoid hymenophora, and their transformation into other types of hymenophora. The results support the independent evolution of this macroscopic configuration in several instances along the evolutionary history of agaricomycetes. In addition, our morphological study show that the hymenophore of most hydnoid fungi is plectologically similar, and in all cases it display some distinctive morphological attributes as the presence of sterile hyphae in the apex, the subapical distribution of the hymenium, a regular to subregular hymenophoral trama, and tramal origin for hymenophore. This suggests that, in addition to convergence in macroscopic shape, hydnoid fungi also could share an underlying morphogenetic development.

Notes:

Morphological and molecular differentiation of two closely related species: *Phellinus gilvus* and *Phellinus torulosus*

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Phellinus gilvus has been used widely as a traditional medicinal mushroom. Recent studies revealed that this species has several important biological activities such as antitumor, free radical scavenging, and proliferation of the human fibroblast cells. *P. gilvus* has similar external morphology with *P. torulosus*. In northeastern Thailand they are also use the same host plant species (i.e. *Shorea obtuse* Wall. ex Blume) thus make it difficult for species identification based only on external morphology. In this study we use ultra-morphological characters using scanning electron microscope (SEM) and molecular marker based on RAPD-PCR to differentiate these species. SEM revealed that size and shape of the spore and pore are the characters that could be distinguished these species. RAPD-PCR based on five primers clearly differentiated *P. gilvus* from *P. torulosus*. There are eight bands that fixed difference between these two species. Thus, RAPD is useful molecular marker to identify these species.

Keywords: *Phellinus gilvus, Phellinus torulosus*, PCR-RAPD, molecular marker

Notes:

A simple and efficient method to separate the fungal conidia from the infected insect larvae

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Entomopathogenic fungi synthesize and up regulate “virulence” genes upon encountering the target insect. The majority of these genes were identified as differentially expressed genes in transcriptomic analysis. However, these experiments were all performed *in vitro*. This is because it is almost impossible to separate fungal conidia/mycelium from infected insect tissues. Thus, it is most likely that not all pathogenicity genes have been identified. Here, a simple mechanical technique was developed to increase the pool of virulence genes so that the *in vivo* interaction between the insect larva *(Spodoptera exigua)* and its fungal pathogen *(Beuveria bassiana)* can be studied.

Keywords: *Beauveria bassiana* / subpressive subtractive hybridization / PCR select cDNA subtraction

Notes:

Category 9

Potential use of acidophilic *Trichoderma koningii* as antagonistic fungi for controlling *Rhizoctonia solani* and *Sclerotium rolfsii*

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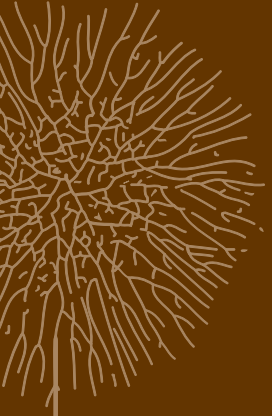
An acidophilic fungus, *Trichoderma koningii* (MC6/2) was obtained from the rhizosphere soil and root of two kinds of plant namely khlongkhleng; *Melastoma candideum* (Melastomataceae), and Lumtheng; *Stenochlaena palustris* (Pteridaceae) in acid sulfate soil, Narathiwat Province. This fungi was isolated using the soil dilution plate method and Gochenaur's glucose ammonium nitrate agar at 30°C for 3 days (pH 4.5 and 7.0). Antagonistic test of *T. koningii* on potato dextrose agar against two plant pathogenic fungi, *Rhizoctonia solani* and *Sclerotium rolfsii* was conducted. The results showed that this fast growing *T. koningii* could inhibit 46% mycelium growth of the two plant pathogenic fungi after 4 days incubation at 28°C. For enzyme activity analysis, *T. koningii* produced the highest filter paperase and protease 79.14 and 86.29 milliunit /milliliter at 3 and 4 days incubation respectively. Application of *T. koningii* inoculum and *R. solani* and *S. rolfsii* in non – sterile acid sulfate soil for nine days, the number of *R. solani* and *S. rolfsii* were reduced from 5.72 and 6.17 to 2.96 and 2.83 log no. / gm of soil as compared with the control (non – additional of *T. koningii*).

Notes:



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