# BIORESOURCES INITIATIVES: THAILAND*

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Positioned centrally in Southeast Asia, Thailand has since prehistoric times attracted diverse peoples to its fertile plains and lush, tropical forest. Sukothai was the heart of the first Thai kingdom which flourished 700 years ago. The words of the 13th century, King Ramkhamhaeng are often invoked to illustrate the fruitfulness of the land: in the water there is fish, in the fields there is rice. Nowadays Thailand encounters the problems of environmental degradation, deforestration and increasing a list of endangered species. Presently, the country covers with less than 25% of forest compared to 80% fifty years ago

National parks and reserved forests are essential not only for protecting watersheds and prevention of national disasters like floods. They are also storehouses of a vast biodiversity in plants, animals and microorganisms. Currently, there are 178 protected areas [65 national parks, 18 marine national parks, 44 wildlife sanctuaries and 51 non-hunting areas] in Thailand [Kaeokamnerd, 1998]. Due to its geographical position in the tropics and the climatic variations between north and south, Thailand is a country of high biological diversity reflected by the number of species. The data from World Bank [1992] indicated that there are approximately 35,000 species of vascular plants in China, 26,000 in Indonesia, 19,000 in India and 14,000 species in Thailand. The number of Thai plants is approximately the same as Malaysia. However, only 20% of the Thai plants have been deposited and well documented in "Flora of Thailand".

The high species richness of fungi in the tropics is without dispute [Hawksworth, 1993, Rossman, 1997]. Hawksworth [1991] presented estimates of global fungal biodiversity of 1.5 million species. Given that Thailand usually hosts 7-10% of the world total of biodiversity then we may assume 100,000- 150,000 fungi in Thailand [Hywel-Jones, N.L., personal communication]. Barely 1% of this presumed total has been described from Thailand too date. Watling [1998] said that the Thai macrofungi may ultimately prove more diverse than that of its southern neighbors. The total mycota of Thailand is therefore potentially very large with the country being at the junction of several natural highways linking south and north and thereon westwards. This paper describes how Thailand has developed her biodiversity inventory and sustainable use with emphasis on fungal resources.

**National Center for Genetic Engineering and Biotechnology [BIOTEC] and a Special Program on Biodiversity and Research Training [BRT]**

The present state of the country's biodiversity is not well documented. Very little work has been undertaken to date on identifying and measuring the biological resource that exits in national parks and forest reserves and their interrelationship with humans. BIOTEC has embarked on an ambitious survey campaign to measure these resources and to develop the human resource skills base to buildup more accurate inventories. BIOTEC has placed strong emphasis on public awareness of what this biological resource constitutes, its usefulness and its conservation. Therefore, biodiversity is an area of vital importance to BIOTEC's national program in Thailand. A Special Program for Biodiversity Research and Training [BRT] has been established since November 1995 under the joint sponsorship of the Thailand Research Fund [TRF] and the National Center for Genetic Engineering and Biotechnology [BIOTEC]

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The BRT program is divided into two parts. The first consists of five programs under the broad theme of Research and Training. The second part concerns with the Development of Technology and Policy for Management of Biodiversity which includes 2 programs. The seven programs are as following:

- **Program One** includes inventories, taxonomic research, and studies of the ecological relationships among living organisms in different habitats. An important aspect of the program is the focus of thematic research on relatively unknown tropical forest areas such as Hala Bala Forest in Narathiwat Province, Phu Phan National Park in Sakhon Nakhon and Kalasin Provinces, and Ton Nga Chang Wildlife Sanctuary in Songkhla Province.
- **Program Two** features monitoring of populations and communities, and is specifically intended to promote long-term studies.
- **Program Three** is designed to foster relationships between biodiversity, social and economic activities and local wisdom. Research focuses on the participation of local people in the study and analysis of natural resource conservation for sustainable use.
- **Program Four** encompasses database development, information technology and publications on the biodiversity of Thailand.
- **Program Five** is to support master's and doctoral degree studies from universities representing all regions of the country.
- **Program Six** is "Development of Technological Proficiency for Sustainable Social and Commercial Use of Biological Resources". The projects are linked with laboratories of the BIOTEC. Most of the projects focus on capacity development for sustainable social and commercial use of biological resources, and focus on building of needed infrastructure for the country.
- **Program Seven** involves the study of policy to develop effective management of the country's biodiversity.

**Mycological Study in Thailand**

Although there is a tradition of teaching and research in plant pathology in Thai Universities, mycology has only comparatively recently been introduced. Few records of fungi exist prior to the early 1960's when a Danish Thai expedition reported on the fungi they collected, which were mainly Discomycetes. There is no mycological society in Thailand and mycologists can only therefore participate in plant pathology and general scientific societies. In March, 1992 a meeting was organized at Kasetsart University, with the support of the National Center for Genetic Engineering and Biotechnology [BIOTEC], Thailand and the British Mycological Society. As a result of this meeting, in which some 100 mycologists participated, a greater awareness of mycology developed. Today mycological research is in progress at a number of universities and at the National Center for Genetic Engineering and Biotechnology and these activities are outlined below.

**National Center for Genetic Engineering and Biotechnology [BIOTEC]**

Within BIOTEC, a number of projects are in progress. The mycology laboratory is concerned with the diversity of Thai fungi and is best known for its research of insect pathogenic fungi (their taxonomy, ecology, physiology and their use in biocontrol: Dr Nigel Hywel-Jones); freshwater aquatic lignicolous fungi (Dr Somsak Sivichai); seed fungi (S. Somrithipol); Coelomycetes (the ultrastructure of conidial appendage ontogeny with Prof.Gareth Jones, and Dr Yuwapin Dandusitapunth); molecular phylogeny of marine fungi (Dr Ruud Valyasevi, Prof. Gareth Jones) and Basidiomycetes (taxonomy and ecology: Dr Tim Flegel and Prof. R. Bandoni).

**Thai Universities**

At Burapha University, marine fungi are under investigation with particular interest in omega 3 polyunsaturated fatty acids and bioactive compounds from mangrove fungi. At Chiang Mai University Drs. Saisamon and Pipob Lumyong lead a research group investigating endophytic fungi from a variety of trees and shrubs, their ecology, ability to produce various enzymes and

bioactive compounds. The diversity of macro fungi from soil at Doi Suthep-Pui National Park is also under investigation. Endophytes from teak are another project under the direction of Dr. Nuchnart Jonglaekha. Soil fungi form the major focus for research at Kasetsart University, but projects on endophytes and pathogenic fungi of orchids, rhizosphere fungi and coprophilous are also in progress (Dr Leka Manoch).

King Mongkut's University of Technology Thonburi is more biotechnologically orientated, but a number of mycological projects are in progress: biodiversity of macrofungi (R.Choieklin, R. Dhitaphichit), qualitative and quantitative estimation of lignocellulose breakdown by Xylariaceous fungi and basidiomycetes, ultrastructure of Coelomycetes, morphology, physiology and fatty acid profiles of *Aschersonia* species for identification of related taxa, molecular biology of *Aspergillus oryzae* emphasizing heterologous gene expression, alkaline protease production from *A. oryzae* and identification of mychorrizal fungi using PCR techniques, many of these in collaboration with BIOTEC (Profs. Gareth Jones, Morakot Tanticharoen, Dr. Yuwapin Dandusitapunth). At Chulalongkorn University research on *Turbidomyces* taxonomy and physiology; xylariaceous fungi, and ectomycorrhizal development of *Pinus khesiya* and *Eucalyptus camaldulensis* are in progress (Prof. Sumalee Pichyangkura, Dr Prakitsin Sihanonth). At Prince of Songkla University, a variety of projects are under investigation, including: Biodiversity of freshwater aquatic Hyphomycetes and Basidiomycota at Ton Nga Chang Wildlife Sanctuary; anti-fungal lipopetides and the cultivation of wild mushrooms (*Tricholoma crassum*, *Lentinus* spp.) (Drs V. Petcharat, Souwalak Phongpaichit). At Ramkhamhaeng University there is an active research group on lichen taxonomy, ecology and physiology, describing a number of new species for Thailand (Dr Kansri Boonpragob). On going topics under investigation at the Royal Forestry Department include the macro fungi and Xylariaceae of Thailand (Drs S. A. Chalermpongse, Surang Thienhirun). The Thailand Institute of Scientific and Technological Research also has responsibility for mycological projects: systematics of ballistocondium forming yeasts and molecular phylogeny of fungi, while at Khon Kaen University plant pathology is the major area of research. At a new Suranaree University, the diversity of mushrooms at Tab-lan National Park with Dr. Sureelak Radthang is under investigation as well as molecular studies of commercial edible mushrooms and the bioconversion of cassava waste by *Mucor* sp. and yeasts.

**Thailand National Culture Collection**

There are over 50 identified "collections" of microorganisms in Thailand. Most of these collections are maintained, mainly as working collections, by individual researchers at the universities or by research section of various R&D- related organizations. Major culture collections, which also provide services, include the TISTR Culture Collection at the Thailand Institute of Scientific and Technological Research, the Medical Science Culture Collection at the Department of Medical Sciences [DMST], the Rhizobium Culture Collection and the Mushroom Culture Collection at the Department of Agriculture [DOAC], and the recently established BIOTEC Culture Collection [http://bcc.biotec.or.th] at the National Center for Genetic Engineering and Biotechnology. While the TISTR Culture Collection serves [since 1976] as part of the UNEP/UNESCO Microbial Resource Center for South East Asia [Bangkok MIRCEN], the BIOTEC Culture Collection has been established to provide a central safe deposit facility for the widely scattered individual and/or specialized collections as well as the country's official depository for patent-related microbial cultures.

The BIOTEC Culture Collection [BCC] was established in 1996 at the BIOTEC, NSTDA. This is a specialized culture collection comprising insect pathogenic fungi, wood decayed fungi of Family Xylariaceae and Class Basidiomycetes, fungi from soil, fresh water, marine, and alkaline habitats, seed, dung, palm and lichen sources. The numbers of fungi which had been directly isolated by researchers and collaborators within the Mycology Laboratory at BIOTEC increased to over 10,000 isolates. The total biodiversity of the collection of fungi was about 460 genera and 522 species [including 29 genera and 91 species of insect fungi] at the end of August 2003.

The documentation accompanying the culture being maintained is an essential system in culture collection. To turn microbial data into information, and further become knowledge, BIOTEC has built the computerized Microbial Information Management System (MIMS) to serve the needs for microbial biodiversity preservation and utilization. The system generates species inventories, culture catalogues and species distribution maps. Other major reports the system produces include species summary, locality summary, fermentation and summary for screening of bioactive compounds. The system is installed in and can be accessed from all major laboratories at BIOTEC in particular the mycology, the bioresources, the bioassay and the fermentation laboratories.

The Thailand Network on Culture Collection (TNCC) has been established in late 2000 after over 5 years of preparation. It is operated through the coordination of four major/key organizations which house the qualified culture collections, supported by laboratory/center with necessary equipment, specialized personnel and the mandate to support the culture collection activities of the country. These collections include BIOTEC Culture Collection (BCC), DMST Culture Collection (DMST), DOA Culture Collection (DOAC) and TISTR Culture Collection (TISTR).

The working group comprising the chief curators of these collections has been set up. Each curator is to be rotated in chairing the working group on a one-year term. Initially, the BCC serves as the first secretariat of the network.

*The Network Organization*

- BIOTEC Culture Collection: (Collection of Fungi and Yeasts)
- Department of Medical Science: (Collection of Human Pathogenic Microorganisms)
- Department of Agriculture: (Collection of Plant Pathogenic Microorganisms)
- Thailand Institute of Scientific and Technological Research: (Collection of Waste Recycling and Industrial Important Microorganisms)

*Objective of TNCC*

- To consolidate the management of the country's microbial resources.
- To set up a common standard system in microbial resources management.
- To provide a management tool in supporting conservation utilization of microbial resources.

**Screening for Bioactive Compounds from Bioresources**

Natural products have traditionally provided a rich source of chemical diversity in the search for new biologically active molecules. Plants have been used for thousands of years by numerous civilizations for therapeutic applications but it is only over the last 50 years that microorganisms have been seriously exploited as a source of antibiotics and pharmaceuticals. However, a significant proportion of prescription medicines are microbially derived and include some of the top selling drugs used as antibiotics, serum cholesterol lowering agents and as immunosuppressants. Coupled with organism diversity, the composition of the growth medium and conditions of fermentation are of paramount importance. Productivities can be improved rapidly by medium and process optimization and strain improvement through mutation and other genetic techniques.

In order to exploit the full extent of the chemical diversity of microbial secondary metabolites, it is advantageous to screen very large numbers of samples. A screening strategy and technology should be rapid and effective to detect molecules at low concentrations in which is often a complex mixture. However, screening activities are expensive and risky. To potentially utilize bioresources and to support the work on bioactive compounds in Thailand, the Bioassay Research Facility, a specialized laboratory in Bioresources Research Program at BIOTEC was established to investigate the potential of biological resources of Thailand. The program consists of three laboratories with different activities; Fermentation Technology Laboratory, Bioassay Laboratory

and Natural Product Chemistry Laboratory. Within the research group consisting of over 40 team members, plants and microorganisms are evaluated for their ability to produce secondary metabolites or short peptides with drug-like properties. Fermentation technologists place their efforts on growing microbes and improving growth rate of those that grow slowly in order to efficiently utilize them. Emphasis is also placed on improving the production of secondary metabolites with good biological activities in microorganisms. Regularly, bioassay-guided fractionations are performed, and structures of pure compounds are determined and confirmed via chemical methods such as spectroscopic techniques, NMR and IR spectrophotometry. In certain cases, structure modifications have been undertaken for the structure-activity relationship study and modified compounds are again tested for biological activities and cytotoxicity.

The Bioassay Laboratory also offers services in bioactive compound screening to both private and public sectors. The scientists in the laboratory are constantly modifying existing assays in order to enhance accuracy and sensitivity in addition to developing new assays. Presently, the laboratory offers assays to detect samples with the following properties.

- Anti-cancer
- Anti-fungal (*Candida albicans*)
- Anti-herpes simplex virus type-I
- Anti-inflammatory, the development of rapid throughput screening for anti-inflammatory compounds

The Bioassay laboratory has two unique murine cell lines capable of identifying the selective inhibition of the COX-2 enzyme over COX-1. It is currently developing a solid phase RIA assay to measure prostaglandins, suitable for rapid screening due to its lower cost compared with commercial kits.

- Anti-tuberculosis
- Anti-malaria
- Anti-metatstasis
- Two cytotoxicity testing methods

Samples that test positive in the anti-cancer assay can further be tested to identify the mechanisms involved using the following tests.

- Anti-ras
- Anti-topoisomerase
- Anti-mitotic cell division
- Anti-telomerase

Since the founding of the program in 1996, the bioassay scientists have investigated over 30,000 samples, both crude extracts and pure compounds from plants and microorganisms with some positive results.

Bioassay or activity-guided fractionation of extracts from fungal broth and chemical structure elucidation revealed that insect fungi (entomopathogenic fungi) are an excellent source of chemical versatility. They offer tremendous potential as a source of novel metabolites exhibiting various biological activities ranging from activity against malarial parasites, fungi, virus, mycobacteria and tumor cell lines. For examples, compounds bearing acid anhydride moieties are rare in nature. Cordyanhydrides A and B, two new alkenoic acids bearing two and three maleic anhydride moieties in the linear acid chain, were isolated and identified from a culture broth of the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620 [Isaka *et al* 2000]. A new cyclohexadepsipeptide, named hirsutellide A, was isolated from a cell extract of the entomopathogenic fungus *Hirsutella kobayasii* BCC 1660 and shown to exhibit antimycobacterial

and antimalarial activities with no toxic effects on the Vero cell line [Vongvanich *et al*, 2002]. In addition, other groups of fungi also produce many biological active compounds. For example, a novel secondary metabolite, pughiinin A, together with pycnidione, mevalonolactone, and 7-hydroxy-2-methylchromanone, was isolated from the seed fungus *Kionochaeta pughii* BCC 3878. These compounds show anti-*P. falciparum* and anti-cancer properties [Pittayakhajonwut *et al*, 2002].

The Protein Ligand Engineering Unit, also located at BIOTEC, has collaborated closely with the Bioresources Research Program in identifying new drug targets and understanding the mechanism of drugs on current targets. The main focus is on research into the development of high potential technology for medicine and other areas of biotechnology, with a special emphasis on the rational development of drugs for tropical diseases, especially malaria. New techniques and up-to-date technology such as genome comparison, proteomics and bioinformatics have been employed to elucidate the complex mechanisms. Recently, BIOTEC's scientists and their collaborative partners were able to reveal the crystal structures of both wildtype and drug resistant dihydrofolate reductase from *Plasmodium falciparum*, thus enable a more efficient design of anti-folate drugs in the future [Yuvaniyama et al 2003].

**Developing the software for managing microbial DNA sequence information**

In addition to BIOTEC's Microbial Information Management System (MIMS) which stores and manages various information of microorganisms such as location, morphological and chemical characteristics, and growth conditions, we have developed a **W**eb-based **I**ntegrated **S**ystem for **Se**quence-based identification of microorganisms (**WisSe**). This system generates a centralized gene sequence database of microbial isolates with a connection to their metadata such as ecological or phenotypic data. WisSe also facilitates data sharing and analysis since it can be used with data analysis tools available in the public domain.

**BIOTEC and Bioprospecting Policy**

A national bioprospecting strategy requires both in-country and external considerations. Practical needs and capacity assessment are in-country requirements. BIOTEC, in collaboration with other institutes and agencies, has embarked on an ambitious program of capacity building and human resource development. BIOTEC has brought a number of experts to pass on their knowledge and skills, especially as they relate to field work and data collection. BIOTEC has tried to promote the collaborative researches with public and private institutions outside the country. We realize that biotechnological development cannot be improved without access to, and use of genetic material and knowledge. A material transfer agreement [agreement for release of biological material] between BIOTEC and the scientist is the important policy instruments for the promotion of further academic collaboration. The biological material can be distributed to co-workers under the scientist's direct supervision. Release of the material to colleagues in institutions other than the scientist's organization can be granted after written permission is obtained from BIOTEC. Release of material may be made following the signing of an appropriate copy of this agreement by the third party.

BIOTEC is carefully developing constructive and mutually beneficial links with private sector that will expand screening activities. A few collaborative agreements on bioprospecting have been reached between NSTDA-BIOTEC and foreign companies during the last few years. In all agreements, it should be noted that scientific collaboration and technology transfer are necessary and very important for Thailand. Moreover, any strains, originating from Thailand, to be used in the collaboration must be approved by the BIOTEC Committee on Biological Assessment for International Collaboration.

**Public Information and Education**

- **Service Center for Medicinal Plant Information**

Beginning in 1995, the Service Center for Medicinal Plant Information (SCMPI) was set up jointly by BIOTEC and Mahidol University. SCMPI is based at the Faculty of Pharmacy, Mahidol University. The main tasks of the Center are to assimilate information on medicinal plant from various sources and to render services to government and private agencies, researchers, students and the general public in medicinal plant data search and analysis. Moreover, the Center also disseminates information on medicinal plants in other formats such as short-termed training, production of various teaching media, including books on medicinal plants and Thai traditional medicine, newsletter, journals, video and slides, CD-ROMs. SCMPI renders information service mainly from MedPlant and NAPRALERT database. The MedPlant database is on Thai medicinal plants compiled by the Centers, The Medicinal Plant Information Center (MPIC) at Mahidol University and SCMPI. This database included over 1,157 species of plants reputed to have curative properties as well as those recorded in Thai Herbals as medicinal agents. Information on these plants as well as related species is assimilated according to their biological activities. The data can be accessed by the plant common name (in Thai/English), genus and/or biological activity.

- **Science in Rural schools; SIRs**

BIOTEC has set up a Rural Development Technology Service Unit to perform technology synthesis and technology transfer to rural communities in Thailand. The Unit works closely with the local community to develop technologies appropriate for the local situation. Part of the work has also been dedicated to youth in the area under a sub-program called "Science in Rural Schools – SiRS", launched under the initiative of Her Royal Highness Princess Maha Chakri Sirindhorn. SiRS aims to improve the quality of life and enhance learning capability in science in the rural area covering 7 provinces. Presently, 108 schools have joined the project. SIRs produces educational media such as books, website and CD-ROM, as well as organizes science camp to stimulate science learning. Bala-Hala National Park in Narathiwat province has been developed into an area-based for research in peat swamp and rain forest, with collaboration with the Royal Forest Department and universities. Moreover, several activities have been initiated along the line for educational purpose as well as for the well-being of the community, such as the construction of nature trail in the park (eco-tourism), the organization of science camp, and even the training in tissue culture so that local people can produce ornamental plantlet to earn extra income.

**References**


1. Hawksworth, D.L. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycological Research 95, 641-655.
2. Hawksworth, D.L. 1993. The tropical fungal biota: census, pertinence, prophylaxis, and prognosis. In Aspects of Tropical Mycology [ed. S. Isaac, J.C. Frankland, R. Watling and A.J.S. Whalley], pp. 265-293. Cambridge University Press. Cambridge, UK.
3. Kaeokamnerd, W. 1998. Khao Yai National Park and its biodiversity; the importance of inventory. Proceedings of the Asia-Pacific Mycological Conference on Biodiversity and Biotechnology, July 6-9, 1998, Hua Hin, Thailand.
4. Kittakoop, P., Punya, J., Kongsaeree, P., Lertwerawat, Y., Jintasirikul, A., Tanticharoen, M. and Thebtaranonth, Y., 1999. Bioactive naphthoquinones from *Cordyceps unilateralis* BCC 1869. Phytochemistry; 52, 453-457.
5. Isaka, M., Tanticharoen, M. and Thebtaranonth, Y., 2000. Cordyanhydrides A and B. Two unique anhydrides from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620. Tetrahedron Letters; 41, 1657-1660.
6. Rossman, A.Y. 1997. Biodiversity of tropical microfungi: an overview. In Bioiversity of Tropical Microfungi [ed. K.D. Hyde], pp. 1-10. University of Hong Kong Press. Hong Kong.
7. Watling, R. 1998. Thai National Forest & Nature Reserves: Their Mycodiversity in Relation to Surrounding Countries. Proceedings of the Asia-Pacific Mycological Conference on Biodiversity and Biotechnology, July 6-9, 1998, Hua Hin, Thailand.
8. Isaka M, Kongsaeree P, Thebtaranonth Y. 2001. Bioxanthracenes from the insect pathogenic fungus Cordyceps pseudomilitaris BCC 1620. II. Structure elucidation. J Antibiot (Tokyo) 54(1): 36-43.

9. Vongvanich N, Kittakoop P, Isaka M, Trakulnaleamsai S, Vimuttipong S, Tanticharoen M, Thebtaranonth Y. (2002) Hirsutellide A, a new antimycobacterial cyclohexadepsipeptide from the entomopathogenic fungus Hirsutella kobayasii. J Nat Prod. 65(9): 1346-8.
10. Pittayakhajonwut P, Theerasilp M, Kongsaeree P, Rungrod A, Tanticharoen M, Thebtaranonth Y.(2002) Pughiinin A, a sesquiterpene from the fungus Kionochaeta pughii BCC 3878. Planta Med. 68(11): 1017-9.
11. Yuvaniyama J, Chitnumsub P, Kamchonwongpaisan S, Vanichtanankul J, Sirawaraporn W, Taylor P, Walkinshaw MD, Yuthavong Y. (2003) Insights into antifolate resistance from malarial DHFR-TS structures. Nat Struct Biol. 10(5): 357-65.