Annual Report 2013

National Center for Genetic Engineering and Biotechnology (BIOTEC)

BIOTEC
a member of NSTDA

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from 1 October 2012 - 30 September 2013.

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National Center For Genetic Engineering and Biotechnology

Message from the BIOTEC Executive Director

In March 2013 the Thai government's Rice Department announced the certification of flood-tolerant jasmine rice and named it "RD51". It was the first rice variety developed from BIOTEC's molecular rice breeding program to ever be certified. In addition, another variety, RD18, a blast-resistant glutinous rice, was recently certified in August. Both varieties and many more in the pipeline are the result of over two decades of dedication by our rice research team in implementing marker-assisted selection technology in this breeding program.

In September, the Agricultural Research Development Agency (Public Organization) unveiled oil palm plant lines that were developed by a BIOTEC research team, in collaboration with Kasetsart University, Prince of Songkla University, the Department of Agriculture and Golden Tenera Co., Ltd. These elite lines were developed for the Thai climate, especially that of the north and northeastern regions where humidity and rainfall are limited. Production of seedlings is underway, with 100,000 seedlings expected to be released in the next few years. These lines are expected to make a significant impact on oil palm production and make a major contribution to the food and fuel sectors of the Thai economy.

BIOTEC also celebrated the 30th anniversary of its establishment in September of this year. The Center has come a long way, from having two staff and a budget of less than one hundred thousand US$ to promote biotechnology research -- to an organization with 570 staff members and an operating fund of over US$ 20 million annually. Several major contributions have been made to the nation during these past 30 years. BIOTEC established the first DNA testing laboratory to serve the agriculture and food industry of Thailand during a time when GMO-free products were in need of verification. The laboratory has likewise made a significant impact on the Thai rice industry by authenticating Thai jasmine rice to enable it to command premium prices in domestic and international markets. With the expertise of our research team on shrimp disease, we were able to protect the shrimp farming industry from disease outbreaks in the late 1990s through the introduction of molecular diagnostic tools for important pathogens. With regard to scientific excellence, Prof. Yongyuth Yuthavong, the former BIOTEC Executive Director and an avid scientist in antimalarial drug development, and his team made a breakthrough discovery of the crystal structures of a primary malarial drug target, dihydrofolate reductase, in 2003, opening new frontiers for malarial drug design. Now one of the compounds against this drug target designed by the team, P218, is being pre-clinically tested.

These are just a few examples of past success stories which we hope will serve as a motivating force for our staff for the next chapter of BIOTEC.

Dr. Kanyawim Kirtikara
Executive Director, BIOTEC

30th Anniversary BIOTEC a member of NSTDA

Celebrating 30 Years of BIOTEC

In 1982, the Thai Government tendered a bid to host a UNIDO international centre for genetic engineering and biotechnology. The attempt was unscuccesful. Nevertheless, recognizing the potential of biotechnology, the Government decided to found the National Center for Genetic Engineering and Biotechnology (BIOTEC) in 1983.

1983

Following a cabinet resolution, the National Center for Genetic Engineering and Biotechnology (BIOTEC) is established under the Ministry of Science, Technology and Energy on 20 September 1983 with the aim, at that time, to support biotechnology research in various public organizations.

1985-1986

To promote and strengthen biotechnology research, BIOTEC sets up four collaborative research units jointly with universities:

- Plant Genetic Engineering Unit at Kasetsart University, Kamphaengsaen Campus;
- Microbial Genetic Engineering Unit at Mahidol University, Salaya Campus;
- Biochemical Engineering and Pilot Plant Research and Development Unit (BEC) at King Mongkut's Institute of Technology Thonburi (now King Mongkut's University of Technology Thonburi); and
- Marine Biotechnology Research Unit at Chulalongkorn University.

1990

The Royal Thai Government launches the Science and Technology Scholarship Program, supporting talented students to study in foreign countries from undergraduate level to doctoral degree in five areas: biotechnology, materials science, computer science, nanotechnology and technology management.

1991

- As the interest in biotechnology grew, it was realized that the country still lacked the convenience of procuring biological materials and services necessary for research activities. BIOTEC thus establishes the BioService Unit to provide services in biological analysis and synthesis using modern, advanced instruments and technologies.
- The National Science and Technology Development Agency (NSTDA) is established and BIOTEC becomes a member of NSTDA.

1992

BioSafety Program is established to build up the nation's infrastructure and technical capability in safety assessment of products derived from genetic modification technology, as well as enhancing biosafety management and regulation. National biosafety guidelines for both laboratory work and field testing are released, marking the first in Thailand and the region.

1995

- DNA Fingerprint Laboratory is established in collaboration with Kasetsart University to develop and set the standards for DNA fingerprinting, a technique vital to breeding programs, genetic identification, seed purity tests and forensic science. In 1999, the unit is renamed DNA Technology Laboratory, providing services based on DNA fingerprinting and DNA sequencing techniques such as genetic identity determination, genetic authentification, DNA marker development, DNA sequencing of plants, animals and microorganisms and also GMO testing of food and feed.
- In collaboration with the Thailand Research Fund (TRF), BIOTEC founds the Biodiversity Research and Training Program (BRT) to provide support and funding for research into, and management of, Thailand's biodiversity resources.

1996

- BIOTEC Yothi Research Unit, the first in-house research unit, is founded.
- BIOTEC Culture Collection (BCC) is established to serve as a central facility to collect and maintain microorganisms and their relevant data, as well as a repository of patent-related microorganisms of Thailand Department of Intellectual Property.
- BIOTEC launches Biotechnology for Rural Development Program, to use biotechnology to enhance the development of rural communities.
- BIOTEC, in collaboration with several organizations, pushes forward the establishment of the National Food Institute (NFI) under the purview of the Ministry of Industry.

1997

- The first patent titled "Methodology for immobilization of cells, enzymes or bioreactive substances to fiber threads" is issued to a BIOTEC-funded project on 18 June 1997.
- Thailand Tropical Diseases Research Programme (T-2) is established under the inter-agency cooperation and participation of BIOTEC, the Thailand Research Fund (TRF) and The Special Program for Research and Training in Tropical Diseases (TDR/WHO).

1999

BIOTEC joins the International Rice Genome Sequencing Project (IRGSP), a consortium of publicly funded laboratories from 10 countries, paving the way to the development of Thai rice utilizing genomic data.

2000

To facilitate the coordination and management of bioresources in Thailand, the Thailand Biodiversity Center (TBC) is established under a decree approved by the Prime Minister, within the organizational framework of NSTDA. TBC activities are transferred to the newly-established Ministry of Natural Resources and Environment in 2002.

2001

The Human Resource Development Program in Biotechnology is lauched, providing training scholarships to young researchers from Cambodia, Laos, Myanmar and Vietnam (CLMV).

2002

- BIOTEC headquarters and in-house laboratories are relocated to Thailand Science Park, Pathum Thani.
- The first transgenic papaya resistant to ringspot virus is achieved through the collaboration of BIOTEC and Kasetsart University.

2003

- BIOTEC research team discovers the crystal structures of a primary malarial drug target, dihydrofolate reductase, providing new approaches for the anti-malarial drug design.
- The National Biotechnology Policy Committee is established with the Prime Minister serving as the chairperson and BIOTEC as the secretariat.

2004

Thailand's first National Biotechnology Policy Framework is announced, setting a roadmap for biotechnology R&D and bio-business promotion from 2004 to 2009.

First Decade (1983-1992): In the initial stage, BIOTEC's role was to establish basic infrastructure in biotechnology for the country. To achieve its goals, the Center built up a network of biotech experts and supported the development of laboratories in academic institutions. BIOTEC also strongly supported the human resource development in the biotechnology field by granting scholarships for Master's and Doctoral degrees.

Second Decade (1993-2002): As new technology quickly evolved, BIOTEC worked in collaboration with several leading institutions in Thailand to achieve biotechnology excellence. In preparing the country for biotechnology, notable successes included: Biotechnology Policy Development, Intellectual Property Management, Biosafety Guidelines, raising public awareness and educating the public about biotech and its applications.

2005

BIOTEC-Novartis Drug Discovery Partnership is launched, with the aim to find potential use of microorganisms and natural compounds derived from microorganisms, as sources for innovative medicines.

2006

Submergence-tolerant KDML 105 becomes the first rice variety achieved through marker-assisted selection technique.

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2008

The US Patent Office grants the protection to a patent titled "Transgenic rice plants with reduced expression of Os2AP and elevated levels of 2-acetyl-1-pyrrolin", marking the first international patent for BIOTEC.

2009

Due to the re-structure of NSTDA, BIOTEC's mandate shifts to conducting research and building up capacity in platform technology.

2010

- HRH Princess Maha Chakri Sirindhorn bestows the name "Thanyasirin" to blast-resistant glutinous rice, developed from marker-assisted selection technique.
- Hom-cholasit rice, flash-flooding-tolerant rice, helps prevent farmers in Phak Hai district, Ayutthaya province, from massive loss caused by major floods.
- P218, anti-malarial compound designed by BIOTEC research team, enters preclinical stage of Medicines for Malaria Venture (MMV).

2011

Chimeric dengue vaccine candidate, co-developed by BIOTEC, Chiang Mai University and Mahidol University, is licensed to BioNet-Asia Co., Ltd. to further develop into a commercial product.

2012

New dwarf glutinuous rice with resistance to both blast and bacterial blight is introduced.

2013

Two rice varieties, flood-tolerant jasmine rice and blast-resistant glutinous rice, are granted certification from the Rice Department.

Third Decade (2003-2013): Thailand's National Biotechnology Policy Framework was implemented with emphasis on healthcare services, modern biotechnology, education, training, biotechnology business and intellectual property rights. While new technologies and discoveries have been transferred to the private sector and for the benefit of the public, the Center has also made good progress in collaborating with international partners, both advanced and neighboring countries.

FACTS AND FIGURES

BIOTEC was first set up under the Ministry for Science, Technology and Energy on 20 September 1983. After the establishment of the National Science and Technology Development Agency (NSTDA) on 30 December 1991, BIOTEC became one of the NSTDA centers, operating outside the normal framework of civil service and state enterprises. This enabled the Center to operate more effectively to support and transfer technology for the development of industry, agriculture, natural resources, environment and consequently the social and economic well-being of Thai people. Other centers under the NSTDA family include National Metal and Materials Technology Center (MTEC), National Electronics and Computer Technology Center (NECTEC), National Nanotechnology Center (NANOTEC) and Technology Management Center (TMC).

As a premier research institute in Thailand and Asia, BIOTEC operates research units located at Thailand Science Park and specialized laboratories hosted by various universities, covering a wide spectrum of research topics from agricultural science to biomedical science and environmental science. In addition to research units, development units have been established for activities with high commercial potential. These are full scale business and production operations designed to demonstrate the commercial viability of technologies to prospective investors.

Apart from research and commercialization, BIOTEC activities also include policy research, an outreach program, human resource development and international relations.

Research Units

BIOTEC Research Units at Thailand Science Park

- Bioresources Technology Unit
- Agricultural Biotechnology Research Unit
- Food Biotechnology Research Unit
- Medical Molecular Biology Research Unit
- Genome Institute

Collaborative Research Units at universities and government organization

- Biochemical Engineering and Pilot Plant Research and Development Unit - at King Mongkut's University of Technology Thonburi (KMUTT)
- Excellent Center of Waste Utilization and Management (ECoWaste) - at King Mongkut's University of Technology Thonburi (KMUTT)
- Cassava and Starch Technology Research Unit - at Kasetsart University
- Rice Gene Discovery Unit - at Kasetsart University
- Medical Biotechnology Research Unit - at Faculty of Medicine Siriraj Hospital and Chiang Mai University
- Biomedical Technology Research Center - at Chiang Mai University
- Center of Excellence for Marine Biotechnology - at Chulalongkorn University
- Center of Excellence for Molecular Biology and Genomics of Shrimp - at Chulalongkorn University
- Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp) - at Mahidol University
- Peat Swamp and Rain Forest Research Unit- jointly established with the National Park, Wildlife and Plant Conservation Department and located in Narathiwat Province

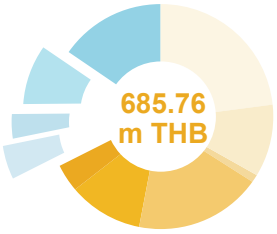
Translational Research Facilities

- Shrimp Genetic Improvement Center (SGIC)
- Nuclear Polyhedrosis Virus Pilot Plant for Insect Pest Control
- National Biopharmaceutical Facility (in collaboration with King Mongkut's University of Technology Thonburi)

Finances

Expenditures

17% Internal Management
11% Infrastructure
3% Technology Transfer
5% Human Resource Development

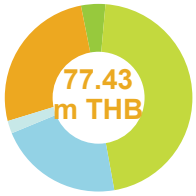


64% Research and Development

21% Agriculture and Food
10% Health and Medicine
2% Energy and Environment
16% Bioresources and Community
1% Cross-cutting Technologies
11% Platform Technologies
3% Research Management

Income from sources outside NSTDA

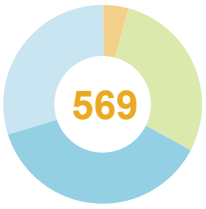
2% Others
4% Workshop/Conference
11% Licensing Activity
20% Technical Services



35% Research Fund
28% Contract/Collaborative Research

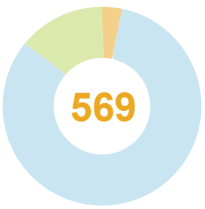
Human Resources

By education



PhD, 167 (29%)
M.Sc., 221 (39%)
B.Sc., 155 (27%)
Below B.S., 26 (5%)

By job function



R&D and Scientific Staff, 471 (83%)
Administrative Staff, 75 (13%)
Executive Staff, 23 (4%)

Publications

BIOTEC researchers published 222 papers in international journals, 206 of which were in citation index journals. Thirty-four were published in journals with impact factors greater than 4.

Intellectual Properties

BIOTEC applied for nine patents in Thailand, one patent in the US, twenty-four petty patents and three trade secrets. Two patents and eight petty patents were granted in Thailand. A patent for antimalarial compounds with flexible side-chains was granted in the US.

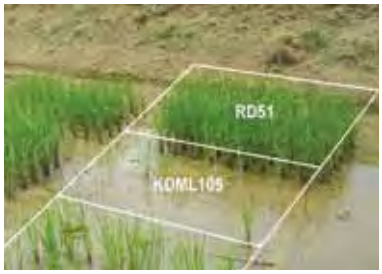
Honors and Awards

BIOTEC researchers and affiliated staff were presented with 30 awards, both locally and internationally.

RESEARCH AND DEVELOPMENT

BIOTEC's R&D and technical program covers a wide range of topics. Plant biotechnology focuses on three economically important plants: rice, cassava, and oil palm. Animal biotechnology focuses on shrimp and dairy cows, whereas food biotechnology aims to improve and upgrade the processing and quality of fermented food, including topics such as food safety and risk assessment, food chemistry, and starter culture technology. Medical biotechnology focuses on tropical and emerging diseases such as malaria, tuberculosis, dengue fever and influenza. On environmental issues, BIOTEC gives emphasis to the study of microbial diversity, and the preservation, use and conservation of bioresources. Biogas and other renewable energies are the focus in the energy research theme.

Rice infected by blast disease (left) compared to blast-resistant RD18 (right).



Flood-tolerant RD51 alongside KDML105 during field testing on day 14 of flooding.

Dwarf glutinous rice with blast and bacterial blight resistance in a farmer field test in Bueng Kan province.

Highlights from Rice Biotechnology

New certified rice varieties

The research team at the Rice Gene Discovery Unit, a joint laboratory between BIOTEC and Kasetsart University, has employed marker-assisted selection (MAS) in developing rice lines to hasten the transfer of gene/QTL more efficiently. Two famous varieties namely glutinuous rice RD6 and the non-glutinous rice Khao Dawk Mali 105 (KDML105) are the main focus for improvement, as both are predominantly grown by farmers in the rain-fed lowlands and mainly intended for consumption and commercial purposes. Both have excellent cooking qualities, but are susceptible to disease and insects and stress from floods, drought and soil salinity.

The MAS has resulted in successful rice variety improvement and yielded several breeding lines of improved KDML105 and RD6. In 2013, two rice varieties, RD51 and RD18, have been certified by the Rice Department, marking for the first time rice derived from MAS being granted certification. Once certified, the Rice Department produces seeds for distribution to farmers in the various areas recommended for each variety.

RD51 is a new version of KDML105, or jasmine rice. It is derived from a marker-assisted backcrossing (MAB) using KDML105 as the recipient and IR49830 (submergence-tolerant rice from the International Rice Research Institute) as the donor. It was developed for flash flooding tolerance, while maintaining the excellent cooking quality that the jasmine rice is well-known for. RD51 has been tested in multiple locations and conditions before finally being granted a variety certification from the Rice Department on 12 March 2013.

RD18, a new glutinous rice, is a cross between RD6 and Jao Hom Nin rice using molecular markers in the backcross breeding process. It is photoperiod-sensitive and resistant to blast disease, especially during the seedling stage. Suitable for rainfed lowland areas in the upper north and northeast Thailand where blast disease is prevalent, it provides an average yield of 3.8 tons/hectare (609 kg/rai). RD18 maintains the excellent grain and cooking qualities of RD6. This variety was certified on 19 August 2013.

New dwarf glutinous rice with blast and bacterial blight resistance

New glutinuous rice with resistance to both blast and bacterial blight was introduced to the public in December 2012. The new improved variety was achieved through marker-assisted selection technology, with RGD05219-12-12-B (KDML105/IR62266), a bacterial-blight resistant rice, as a donor and RGD04069-1-179-1 (RD6*5/Jao Hom Nin) *3///(KDML105*5/IR1188) as a recipient. Key features of this photoperiod-sensitive rice include resistance to blast and bacterial blight diseases and a strong stem. Its short form, 130 cm in height, facilitates the harvest by machine. The variety also provides an average yield of 4.38-5 tons/ha (700-800 kg/rai). This new rice is now cultivated in several provinces in the north and northeast such as Nan, Chiang Mai, Chiang Rai, Lampang, Sakon Nakhon and Beung Kan.

This new rice variety is part of a long-term collaborative project between Rice Gene Discovery Unit and other institutes such as Kasetsart University, Rajamangala University of Technology Lanna and the Rice Department to improve rice varieties suitable for rainfed lowland ecosystems.

Bacterial-blight resistant jasmine rice.

Damage to the rice field caused by brown planthopper.

Breeding bacterial-blight resistant jasmine rice variety KDML105

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most devastating diseases of rainfed lowland rice in Thailand. Although the gene *Xa21* shows broad-spectrum resistance and has been widely utilized to improve BB resistance in rice worldwide, it is not fully expressed in the seedling stage.

To breed the Thai jasmine rice variety KDML105 to obtain non-age-related broad-spectrum resistance to BB, the *Xa21* gene and seedling resistance genes from rice variety IR1188 were introgressed into KDML105 through marker-assisted backcrossing (MAB) and phenotypic selection. KDML105 backcross introgression lines (KBILs) with *Xa21* and QTLs for seedling resistance were successfully developed over three generations. These KBILs are fragrant, exhibit broad-spectrum resistance against a diverse range of bacterial blight isolates and show broad adaptability to the target environments, as revealed in multi-location trials. The established non-age-related broad-spectrum BB-resistant KDML105 is currently recommended by Kasetsart University for planting in farmers' fields where the crop is vulnerable to BB and serves as a breeding stock for the further improvement of bacterial blight resistance in Thai rice breeding programs.

This project is part of a long-term collaboration between Rice Gene Discovery Unit, Kasetsart University, Rajamangala University of Technology Lanna, the Rice Department and the Department of Agricultural Research of Myanmar.

Ref: Win, K.M., Korinsak, S., Jantaboon, J., Siangliw, M., Lanceras-Siangliw, J., Sirithunya, P., Vanavichit, A., Pantuwan, G., Jongdee, B., Sidhiwong, N. and Toojinda, T. (2012). Breeding the Thai jasmine rice variety KDML105 for non-age-related broad-spectrum resistance to bacterial blight disease based on combined marker-assisted and phenotypic selection. *Field Crops Research*, 137(1), 186-194.

Identification of gene responsible for brown planthopper resistance

Bph3, a major brown planthopper (BPH) resistance locus, has been used as a stable donor of traits that improve highly susceptible aromatic rice varieties in Thailand. Researchers conducted a study to identify the gene responsible for *Bph3*. Map-based cloning was initiated using a set of isogenic lines (ILs) harboring the major *Bph3* locus on chromosome 6. IL genomes were scanned with a 57 K Affymetrix Rice GeneChip. An expression analysis of 15 selected candidate genes in the aromatic rice cultivar KDML105 (KD) and the ILs under normal conditions revealed two differentially expressed sequences. Following hopper feeding, only one candidate gene, Os04g27430, was differentially expressed. Os04g27430 encodes a putative *sesquiterpene synthase* (*STPS*) gene that was induced by BPH feeding in ILs. Os04g27430 is the second known rice *STPS* induced by BPH. The gene may involve an antixenosis BPH resistance mechanism. The combination of the *STPS* and the *Bph3* locus was more effective than *Bph3* alone in the tested ILs.

This research was conducted by Rice Gene Discovery Unit in collaboration with Kasetsart University.

Ref: Kamolsukyunyong, W., Sukhaket, W., Ruanjaichon, V., Toojinda, T. and Vanavichit, A. (2013). Single-feature polymorphism mapping of isogenic rice lines identifies the influence of terpene synthase on brown planthopper feeding preferences. *RICE*, 6, 18.

Salt-sensitive Pathumthani 1 used in an experiment to study salt-stress effects on starch metabolism.

Oil palm elite line producing high yield and drought tolerance.

Effect of salt stress on starch metabolism in rice

Soil salinity is one of the major abiotic stresses affecting plant growth and development and leading to a reduction of crop productivity. Soluble sugars have been identified as playing a key role in salt-tolerance defense mechanisms, specifically in osmoregulation and detoxification as a chelating agent to bound sodium ions with starch granules. However, the details regarding the transcriptional profiling of starch metabolism genes under salt stress is largely lacking.

Researchers investigated the transcriptional expression of starch metabolism related genes, soluble sugar, physiological changes and productivity in two contrasting *indica* rice genotypes, salt-tolerant Homjan (HJ) and salt-sensitive Pathumthani 1 (PT1) in response to salt stress. The study concluded that in the HJ genotype, enhancement of starch metabolism related genes, *AGPL1*, *AGPS2b* and *SBEIIb*, and photosynthetic abilities act as triggers resulting in sugar accumulation for salt defense mechanism, and lead to maintaining water use efficiency, growth and yield traits under salt stress. In contrast, the expression of *AGPL1*, *AGPLS2b*, *SBEIIb*, and *GWD* genes in salt-stressed PT1 was up-regulated relating to enriched soluble sugar levels. Therefore, the photosynthetic abilities in salt-stressed PT1 declined significantly leading to growth inhibition and yield reduction.

This work is a collaborative effort between the Agricultural Biotechnology Research Unit, Mahidol University and Meijo University (Japan).

Ref: Boriboonkaset, T., Theerawitaya, C., Pichakum, A., Cha-um, S., Takabe, T. and Kirdmanee, C. (2012). Expression levels of some starch metabolism related genes in flag leaf of two contrasting rice genotypes exposed to salt stress. *Australian Journal of Crop Science*, 6(11), 1579-1586.

Highlights from Plant Biotechnology

Development of oil palm elite lines and mass propagation technology

Palm oil is now the largest source of edible oil in the world, and cultivation of oil palm has expanded enormously in recent years, with worldwide yearly increases in demand being driven by dietary and biofuel applications. Oil palm cultivation in Thailand is faced with problems of quality seedlings that can provide high yield and be grown in northern and northeastern regions, where rainfall is relatively limited. Crop improvement of oil palm is proved to be challenging, as it is a perennial crop species with a long breeding cycle.

With funding from the Agricultural Research Development Agency (Public Organization), or ARDA, researchers teamed up to develop oil palm elite lines well adapted to climates of various regions of Thailand. Several elite lines have been achieved using biomarkers in the selection process of Tenera, a hybrid of a thick-shell Dura and a shell-less Pisifera. These elite lines have been tested in research stations in the north (Nan province), the northeast (Khon Kaen, Sisaket and Nong Khai provinces), and the south (Krabi and Narathiwat provinces) of Thailand. The productivity is approximately 31.25 tons/ha (5 tons/rai), with an average oil yield of 25%, and bearing 15-20 fruit bunches annually. They can survive drought up to 90 days. Their slow vertical growth of 40-50 cm/year makes it easy for harvesting.

Apart from developing elite lines, a somatic seed production method has also been developed to efficiently propagate oil palm. Researchers were able to develop a process to accelerate the generation of somatic embryos using inflorescences as the explant source to prevent parental tree damage. This provides cost-effectiveness and reduces somaclonal variation of mass propagation of oil palm elite lines. The method has been filed for PCT patent by ARDA in 2011.

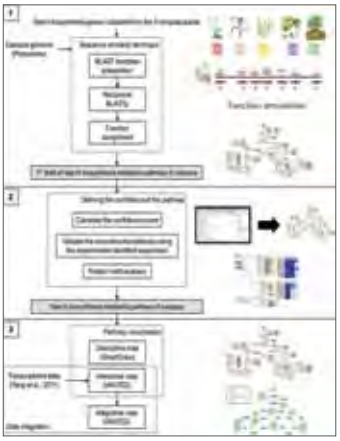


Diagram depicts methodology to reconstruct metabolic pathway of cassava starch biosynthesis.

Production of elite Tenera seedlings started in 2012 at Kasetsart University and was released to interested farmers starting in October 2013. ARDA expected to release a total of 100,000 seedlings.

This work involved collaboration between the Genome Institute, Kasetsart University, Prince of Songkla University, Department of Agriculture and Golden Tenera Co., Ltd.

Effect of drought on cassava starch synthesis

Due to the identified new role for cassava (*Manihot esculenta* Crantz) as a possible biofuel crop, as well as its current usage as a food crop, understanding on the correct time to plant and harvest cassava in order to maximize starch quantity and quality is crucial.

To gain more insight into the metabolic fate of starch, researchers conducted the investigation of expression profiling of the three genes involved in the cassava sucrose synthesis. Molecular approaches were conducted to investigate expression profiling of sucrose phosphate synthase (SPS), sucrose phosphatase (SPP) and 14-3-3, which are important as regulatory points to modulate availability of sucrose in cassava. In source organs, expression of cassava sucrose phosphate synthase (*MeSPS*) and cassava sucrose phosphatase (*MeSPP*) varied depending on developmental stage and day/night cycle. In sink organ, the levels of *MeSPS*, *MeSPP* and *Me14-3-3* transcripts were affected to a much greater degree by different planting season than by root developmental stage. In response to fluctuating levels of rainfall, a correlation between *MeSPS* and *Me14-3-3* expression patterns was observed. This finding suggests the role of these enzymes and the importance of sucrose metabolism in seasonal drought stress response in cassava.

This research involved collaboration between the Agricultural Biotechnology Research Unit, Mahidol University and the Rayong Field Crops Research Center.

Ref: Netrphan, S., Tungngoen, K., Suksangpanomrung, M., Boonseng, O. and Narangajavana, J. (2012). Differential Expression of Genes Involved in Sucrose Synthesis in Source and Sink Organs of Cassava Plants Undergoing Seasonal Drought Stress. *Journal of Agricultural Science*, 4(11), 171-185.

Metabolic pathway of cassava starch biosynthesis

Cassava tubers are an important source of starch and increasing demand has lead to studies for improving starch content and quality. The knowledge on starch biosynthesis is essential in developing new cassava varieties with desired properties.

Researchers explored the starch biosynthesis pathway in cassava using the comparative genomic approach. The recently released genome information, along with the post-genomic approaches, was used to reconstruct the metabolic pathway of starch biosynthesis in cassava using multiple plant templates. The reconstructed pathway was presented in the form of an informative map, which describes all important information of the pathway, and an interactive map, which facilitates the integration of omics data into the metabolic pathway. Additionally, microarray gene expression data was integrated into the reconstructed pathway of starch biosynthesis to illustrate the advantage of the resulting pathway to increase comprehension of the studied system. The interactive map of the reconstructed starch biosynthesis pathway is available for download at http://sbi.pdti.kmutt.ac.th.

This work was conducted by King Mongkut's University of Technology Thonburi, in collaboration with the Agricultural Biotechnology Research Unit.

Ref: Saithong, T., Rongsirikul, O., Kalapanulak, S., Chiewchankaset, P., Siriwat, W., Netrphan, S., Suksangpanomrung, M., Meechai, A. and Cheevadhanarak. S. (2013). Starch biosynthesis in cassava: a genome-based pathway reconstruction and its exploitation in data integration. *BMC System Biology*, 7, 75.

3-year-old salt-tolerant hybrid eucalypt genotype H4 grown in field test at Ban Haet district, Khon Kaen province.

Greenhouse at Kasetsart University, Nakorn Pathom province, is currently one of the six facilities in Thailand designed for performing risk assessment of genetically-modified plants.

Salt-tolerant classification of eucalypts

Saline land in the northeastern Thailand poses serious problems for agriculture. Eucalypts (*Eucalyptus* spp.) are one of the most bio-economic plant species for phytoremediation due to their high potential to grow in salt-affected soils with arid climate, as well as their economic value to the pulp and paper industry.

Researchers investigated the responses of *Eucalyptus camaldulensis* provenances and their hybrid (*E. camaldulensis* × *Eucalyptus urophylla*) to NaCl salt stress and to subject multivariate parameters as effective indices for salt-tolerant classification. Five genotypes of *E. camaldulensis* (T5, BD4, 1-7-1, H1, and SH4) and three genotypes of the *E. camaldulensis* × *Eucalyptus urophylla hybrid* (H4, 58H2, and 27A2) were screened for salt tolerance. The reduced growth, restricted photosynthetic activity and proline accumulation in eucalypt populations acted as multivariate indices for salt tolerance classification. The hybrid eucalypt genotypes H4, 58H2, and 27A2 were identified as salt tolerant while the selection genotypes of *E. camaldulensis*, T5, BD4, 1-7-1, H1, and SH4, were classified as salt susceptible.

This work was conducted collaboratively between the Agricultural Biotechnology Research Unit and Siam Forestry Co. Ltd.

Ref: Cha-um, S., Somsueb, S., Samphumphuang, T. and Kirdmanee, C. (2013). Salt tolerant screening in eucalypt genotypes (*Eucalyptus* spp.) using photosynthetic abilities, proline accumulation, and growth characteristics as effective indices. *In Vitro Cellular and Developmental Biology-Plant*, 49, 611-619.

Biosafety guidelines

Established in 1992, the Biosafety Program of BIOTEC aims to strengthen the nation's capability in biosafety through conducting research on risk assessment (environmental and food safety assessment) of genetically-modified organisms (GMOs). The Center also acts as the secretariat of the national Technical Biosafety Committee (TBC) whose role is to catalyze the formation of, and provide technical assistance to, the Institutional Biosafety Committee (IBC).

In 2013, two new guidelines have been developed and released.

- **Guidelines for Risk Assessment of Transgenic Plants with Stacked Genes or Traits.** At present, there are a growing number of plants with stacked transgenic traits which are produced through the cross-breeding of two transgenic plants. These guidelines emphasize points that need particular consideration when assessing risks which may result from the combination of genetic elements from two or more parental transgenic plants.
- **Guidelines on containment and control measures for the use of GM plants in greenhouse facilities.** The Guidelines contain information on construction materials, design and building of a greenhouse, as well as the procedures for waste and GM material disposal, prevention of seed and pollen dispersal, and control of environmental conditions.

Thailand was the world's top exporter of shrimp products in 2012, with export value of US$3.12 billion, accounting for 20% of the world market share.

Highlights from Shrimp Biotechnology

Role of *Br-c* gene in ovarian development of the giant tiger shrimp

Farming of giant tiger shrimp (*Penaeus monodon*) relies almost entirely on wild-caught broodstock for supply of juveniles because reproductive maturation success of captive *P. monodon* females is extremely low. The basic knowledge on molecular and hormonal mechanisms controlling reproductive maturation of *P. monodon* in captivity could be applied to assist the domestication and genetic improvement of this species.

In this study, researchers examined the molecular involvement of the *Broad-complex* (*Br-c*) gene in ovarian development of *P. monodon*. The full-length cDNA of the *Br-c* gene of the giant tiger shrimp (*Penaeus monodon*) was identified. *PmBr-c* was 1897 bp in length containing an ORF of 1329 bp deducing to a polypeptide of 442 amino acids. *PmBr-c* was more abundantly expressed in ovaries than testes of *P. monodon* broodstock. The expression levels of *PmBr-c* mRNA in ovaries of juveniles was significantly greater than that in stages II (vitellogenic), IV (mature) and V (post-spawning) ovaries of intact broodstock. The expression level of *PmBr-c* was significantly increased in stage III (nearly mature) ovaries of intact wild broodstock and in stage IV ovaries of eyestalk-ablated broodstock. In domesticated broodstock, ovarian *PmBr-c* was expressed lower in 18-month-old shrimp compared to 6-month-old shrimp. *In situ* hybridization revealed that *PmBr-c* mRNA was localized in ooplasm of previtellogenic oocytes in various ovarian stages of *P. monodon* broodstock. Serotonin and progesterone injection significantly promoted the expression level of *PmBr-c* in ovaries of domesticated broodstock at 24 and 48-72 h post injection. The expression levels of *PmBr-c* in ovaries of juvenile *P. monodon* were significantly increased following 20-hydroxyecdysone treatment for 168 hpi.

This work was conducted by researchers from the Agricultural Biotechnology Research Unit, the Center of Excellence for Marine Biotechnology and Chulalongkorn University.

Ref: Buaklin, A., Sittikankaew, K., Khamnamtong, B., Menasveta, P. and Klinbunga, S. (2013). Characterization and expression analysis of the *Broad-complex (Br-c)* gene of the giant tiger shrimp *Penaeus monodon*. *Comparative Biochemistry and Physiology-Part B: Biochemistry and Molecular Biology*, 164(4), 280-289.

Bacterial lipopolysaccharide (LPS) as an immunostimulant for a black tiger shrimp

The application of natural immune stimulants in aquaculture has been shown to increase survival of aquatic species in the presence of pathogens. Lipopolysaccharides (LPS) have been increasingly investigated as a potential immunostimulant in aquaculture.

Researchers investigated the effect of bacterial LPS as a feed supplement to improve immunity of the black tiger shrimp (*Penaeus monodon*). LPS was coated to commercial feed pellets and given to the shrimp. The immunity was tested by exposing shrimp to pathogenic bacterium *Vibrio harveyi*. From the experiments, while the LPS containing diet did not have an effect on growth rate, it resulted in significantly higher survival rates under exposure to *V. harveyi* than the normal diet. The LPS supplement also showed induction of some crucial immune-related transcripts such as *ALF3*, *C-lectin* and *mucin-like PM* in shrimp digestive tracts. These findings suggest that LPS is able to activate the immune system at a molecular level in the digestive tract of the host and enhanced disease resistance in black tiger shrimp. LPS supplement is therefore a promising candidate to increase disease resistance in black tiger shrimp farming.

This work was conducted by Agricultural Biotechnology Research Unit.

Ref: Rungrassamee, W., Maibunkaew, S., Karoonuthaisiri, N. and Jiravanichpaisal, P. (2013). Application of bacterial lipopolysaccharide to improve survival of the black tiger shrimp after *Vibrio harveyi* exposure. *Developmental and Comparative Immunology*, 41(2), 257-262.

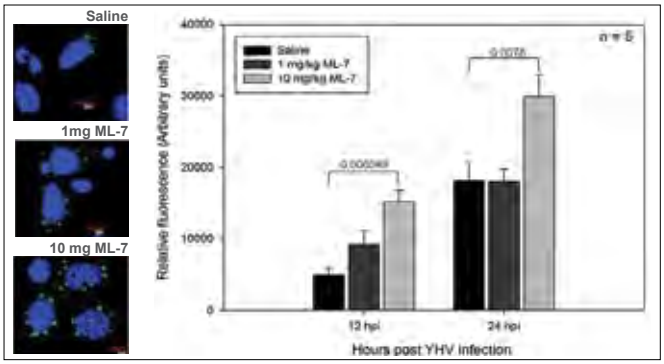


Figure shows the inhibition of shrimp MRLC phosphorylation by its inhibitor (ML-7) resulting in higher replication of YHV. YHV replication level in granular hemocyte is shown by fluorescence intensity (green, left). Graph shows the relative fluorescence intensity of YHV replication in MRLC suppressed shrimp (right). The inhibitory effect of ML-7 inhibitor is shown in a dose–dependent manner.

Yellow head virus infection in shrimp

Yellow head disease, caused by yellow head virus (YHV), is one of the major causes of severe shrimp mortality. In the giant or black tiger shrimp (*Penaeus monodon*), granule-containing hemocytes (i.e., semi-granular and granular cells) have been reported to be targets of several viruses including YHV. Analysis of hemocyte proteins and their posttranslational modifications such as phosphorylation is considered important for identification of disease markers and targets for therapeutic drug design.

Researchers studied the phosphorylation of *Penaeus monodon* myosin regulatory light chain (*Pm*MRLC) that is induced at an early hour post YHV infection and is concomitant with cellular actin remodeling. They were able to demonstrate that *Pm*MRLC phosphorylation increases after YHV infection in shrimp and that inhibition of the phosphorylation leads to increased YHV replication, reduced hemocyte phagocytic activity, probably through actin remodeling, and subsequent shrimp death. Thus, further studies on the myosin light chain kinase (MLCK) activation pathway can lead to new strategies in development and implementation of therapy for YHV infection in shrimp.

This work was conducted by scientists from the Center of Excellence for Shrimp Molecular Biology and Biotechnology, Agricultural Biotechnology Research Unit, Genome Institute, National Taiwan University and Mahidol University.

Ref: Taengchaiyaphum, S., Havanapan, P., Roytrakul, S., Lo, C.F., Sritunyalucksana, K. and Krittanai, C. (2013). Phosphorylation is required for myosin regulatory light chain (*Pm*MRLC) to control yellow head virus infection in shrimp hemocytes. *Fish and Shellfish Immunology*, 34(5), 1042-1049.

Shrimp defense mechanism

Serine proteinases (SPs) participate in various biological processes and play a vital role in immunity. Scientists investigated the function of *Pm*ClipSP2 from shrimp *Penaeus monodon* in defense against bacterial infection. *Pm*ClipSP2 was identified as a clip-domain SP and its mRNA increased in response to infection with *Vibrio harveyi*. *Pm*ClipSP2-knockdown shrimp displayed significantly reduced phenoloxidase (PO) activity and increased susceptibility to *V. harveyi* infection. Injection of LPS and/or ß-1,3-glucan induced a dose-dependent mortality and a significant decrease in the number of total hemocytes, with clear morphological changes in the hemocyte surface of the *Pm*ClipSP2-knockdown shrimp. Recombinant *Pm*ClipSP2 was shown to bind to LPS and ß-1,3-glucan and to activate PO activity. These results reveal that *Pm*ClipSP2 acts as a pattern-recognition protein, binding to microbial polysaccharides and likely activating the proPO system, whilst at the same time it may play an essential role in the hemocyte homeostasis by scavenging LPS and neutralizing its toxicity.

This work was conducted by Center of Excellence for Molecular Biology and Genomics of Shrimp and Chulalongkorn University.

Ref: Amparyup, P., Promrungreang, K., Charoensapsri, W., Sutthangkul, J. and Tassanakajon, A. (2013). A serine proteinase *Pm*ClipSP2 contributes to prophenoloxidase system and plays a protective role in shrimp defense by scavenging lipopolysaccharide. *Developmental and Comparative Immunology*, 41(1), 597-607.

Understanding the role of SPI*Pm*2 on the viral replication process

White spot syndrome (WSS) is a viral disease caused by the white spot syndrome virus (WSSV) which leads to severe mortality in cultured penaeid shrimp. In response to WSSV infection in *Penaeus monodon*, a Kazal serine proteinase inhibitor SPI*Pm*2, normally stored in the granules of granular and semi-granular hemocytes, has been up-regulated and found to deter the viral replication.

By using yeast two-hybrid screening, researchers have identified a viral target protein, namely WSV477. Instead of being a proteinase, the WSV477 was reported to be a Cys2/Cys2-type zinc finger regulatory protein having ATP/GTP-binding activity. *In vitro* pull down assay confirmed the protein-protein interaction between rSPI*Pm*2 (the bait protein) and rWSV477. Confocal laser scanning microscopy demonstrated that the SPI*Pm*2 and WSV477 were co-localized in the cytoplasm of shrimp hemocytes. Using RNA interference, the silencing of WSV477 resulted in down-regulated of viral late gene VP28, the same result obtained with SPI*Pm*2. In this instance, the SPI*Pm*2 does not function as proteinase inhibitor but inhibit the regulatory function of WSV477.

This work was conducted by the Center of Excellence for Shrimp Molecular Biology and Biotechnology, Center of Excellence for Molecular Biology and Genomics of Shrimp, Chulalongkorn University and Mahidol University.

Ref: Ponprateep, S., Phiwsaiya, K., Tassanakajon, A. and Rimphanitchayakit, V. (2013). Interaction between Kazal serine proteinase inhibitor SPI*Pm*2 and viral protein WSV477 reduces the replication of white spot syndrome virus. *Fish and Shellfish Immunology*, 35(3), 957-964.

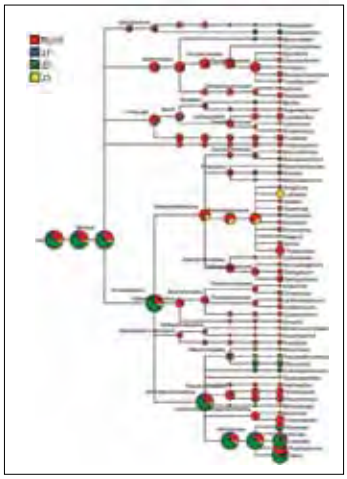
Novel feed supplement to enhance shrimp growth

The monodon slow growth syndrome (MSGS) in cultured black tiger shrimp (*Penaeus monodon*) leads to economic loss in the shrimp culture industry, with Laem-Singh virus (LSNV) identified as a possible causative agent. To date, there is no effective farm-scale treatment, and the current means to control LSNV relies primarily on routine detection and removal of LSNV-positive batches of post-larvae.

Double-stranded (ds)RNA-mediated gene silencing or RNA interference (RNAi) has been known as a tool for controlling shrimp viral diseases. The research team had earlier developed a method to produce dsRNA in a bacterial system for simple and low cost production of large quantities of dsRNA targeting specific genes of interest. In this study, the team investigated efficacy of LSNV-specific dsRNA (dsRNA-LSNV) in mediating inhibition of LSNV in Thai *P. monodon*. Oral administration of dsRNA-LSNV was shown to eliminate LSNV-associated MSGS in *P. monodon*. It can be concluded that feed with dsRNA-LSNV can enhance the overall growth of *P. monodon*, most likely due to a decrease in LSNV infection.

This research involved collaboration between scientists from the Center of Excellence for Shrimp Molecular Biology and Biotechnology, Shrimp Genetic Improvement Center, Mahidol University and Prince of Songkla University, Surat Thani Campus.

Ref: Saksmerprome, V., Thammasorn, T., Jitrakorn, S., Wongtripop, S., Borwornpinyo, S. and Withyachumnarnkul, B. (2013). Using double-stranded RNA for the control of Laem-Singh Virus (LSNV) in Thai *P. Monodon*. *Journal of Biotechnology*, 163(4), 449-453.



Comparison of the bacterial compositions in shrimp intestines of various growth stages.

Bacterial population in shrimp intestine

The intestinal tract is a complex ecosystem that harbors a diverse bacterial community and this bacterial population has been shown to have profound influence on immunity, nutrient processing and protective processes in the host animal. This concept is applied to black tiger shrimp, a major cash crop in Thailand, as it is speculated that bacterial community in shrimp intestines may play protective roles as natural barriers against pathogens. Knowledge on the diversity of the bacterial population in shrimp intestines will further research on disease protection.

Researchers conducted a study on intestinal bacterial populations in the black tiger shrimp (*P. monodon*) at different growth stages using a pyrosequencing approach and the results were validated by additional methods of real-time PCR and denaturing gradient gel electrophoresis (DGGE). Four developmental stages of shrimp were studied: 15-day-old post larvae (PL15), 1-month-old juvenile (J1), 2-month-old juvenile (J2), and 3-month-old (J3) juveniles. A total of 25,121 pyrosequencing reads were obtained. Bacteria in the phyla *Bacteroides*, *Firmicutes* and *Proteobacteria* were found in intestines at all four growth stages. There were 88, 14, 27, and 20 bacterial genera associated with the intestinal tract of PL15, J1, J2 and J3, respectively. Pyrosequencing analysis revealed that *Proteobacteria* (class *Gammaproteobacteria*) was a dominant bacteria group in all growth stages. Intestinal bacterial communities from the three juvenile stages were more similar to each other than that of the PL shrimp based on PCA analyses of pyrosequencing results and their DGGE profiles.

This study was a joint effort by the Agricultural Biotechnology Research Unit and the Genome Institute.

Ref: Rungrassamee, W., Klanchui, A., Chaiyapechara, S., Maibunkaew, S., Tangphatsornruang, S., Jiravanichpaisal, P., Karoonuthaisiri, N. (2013). Bacterial Population in Intestines of the Black Tiger Shrimp (*Penaeus monodon*) under Different Growth Stages. *PLOS ONE*, 8(4), e60802.

Circadian regulation in crustaceans

Daily, circadian rhythms influence essentially all living organisms and affect many physiological processes from sleep and nutrition to immunity. The hematopoietic process of the crayfish *Pacifastacus leniusculus* is under circadian control and is tightly regulated by astakines, a new family of cytokines sharing a prokineticin (PROK) domain. The expression of AST1 and AST2 are light-dependent, and this suggests an evolutionarily conserved function for PROK domain proteins in mediating circadian rhythms. Vertebrate PROKs are transmitters of circadian rhythms of the suprachiasmatic nucleus (SCN) in the brain of mammals, but the mechanism by which they function is unknown.

Researchers provided evidence that melatonin regulates AST2 expression and thereby affects the core clock of the crustacean brain. This process may be very important in all animals that have AST2 molecules, i.e. spiders, ticks, crustaceans, scorpions, several insect groups such as Hymenoptera, Hemiptera, and Blattodea, but not Diptera and Coleoptera. The findings further revealed an ancient evolutionary role for the prokineticin superfamily protein that links melatonin to direct regulation of the core clock gene feedback loops.

This work was conducted by Uppsala University (Sweden), in collaboration with Prince of Songkla University, the Genome Institute and the Agricultural Biotechnology Research Unit.

Ref: Watthanasurorot, A., Saelee, N., Phongdara, A., Roytrakul, S., Jiravanichpaisal, P., Söderhäll, K. and Söderhäll, I. (2013). Astakine 2-the Dark Knight Linking Melatonin to Circadian Regulation in Crustaceans. *PLOS Genetics*, 9(3), e1003361.

Thai fermented pork sausage is a major industry using starter cultures.

Highlights from Food Science and Biotechnology

Food additives to improve shelf-life of shrimp

Seafood products are sensitive to quality deterioration during storage. Generally inorganic salts, especially phosphate compounds, are used in seafood products to improve the qualities such as bound water and cooking yield. However, excess treatment with phosphate may cause not only phosphate imbalance in the diet, which poses health concerns, but also decrease quality. Alternative additives are needed, especially those from natural products.

The application of water-based nano-sized chitin and chitosan as food additives was examined on Pacific white shrimp (*Litopenaeus vannamei*). By simply soaking fresh shrimp in the additives, the shrimp quality, especially, the color and the texture can be maintained for as long as 48 h under storage at 4°C. The treatment by chitosan whiskers (CSWK) 0.25% (w/v) in combination with 2.5% NaCl and 1% $NaHCO_3$ at pH 8 is an optimal condition leading to weight gain and cooking yield, which is similar to the treatment by using mixed phosphate compounds. CSWK swells the gaps inside the myofibrillar fibers of the shrimp flesh, resulting in a layer structure similar to that of the fresh shrimp.

This was a collaborative study involving scientists from the Food Biotechnology Research Unit, Chulalongkorn and Kasetsart Universities.

Ref: Chantarasataporn, P., Yoksan, R., Visessanguan, W. and Chirachanchai, S. (2013). Water-based nano-sized chitin and chitosan as seafood additive through a case study of Pacific white shrimp (*Litopenaeus vannamei*). *Food Hydrocolloids*, 32(2), 341-348.

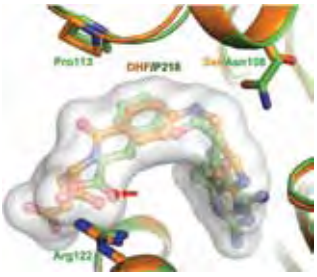
Development of auxotrophic starter cultures

In Thailand, *Lactobacillus plantarum* is used as a starter culture for the production of a Thai fermented sausage product called Nham. At present, Nham is manufactured using commercial starter bacteria, yet these bacterial starters can readily be sub-cultured from the fermented sausage product. Therefore, an auxotrophic starter for Nham would be of high economical value for the starter culture provider.

A genome-scale metabolic model was employed as a strategy to identify potential auxotrophs used as a starter for Nham production. A published genome-scale model of *Lactobacillus plantarum* WCFS1 was adopted for studying the *L. plantarum* BCC9546 characteristics. Single gene deletion analysis is performed to determine the genes essential for cell growth. Strains lacking such essential genes are considered potential auxotrophic mutants. Metabolite supplement analysis was then introduced to determine a list of metabolite supplements for each mutant required to restore its growth. Nine potential auxotrophs were proposed for use in Nham fermentation, along with their metabolite supplements. Simulation studies showed that the secreted fluxes of organic acids, as well as amino-derived flavor compounds of these auxotrophs, are similar to those of the wild-type, indicating that Nham fermented by these auxotrophs would have similar taste and flavors as Nham fermented by the wild-type. This systemic identification of auxotrophs can serve as a powerful computational platform for future auxotroph development and improvement of Nham starter culture. In addition, it could become a standard protocol for the generation of auxotrophs used for other fermented food products.

This research was conducted collaboratively by scientists from the Food Biotechnology Research Unit, Biochemical and Pilot Plant Research and Development Unit and King Mongkut's University of Technology Thonburi.

Ref: Chiewchankaset, P., Srimarut, Y., Klanchui, A., Kurdi, P., Plengvidhya, V. and Meechai, A. (2012). Systematic identification of *Lactobacillus plantarum* auxotrophs for fermented Nham using genome-scale metabolic model. *Journal of Biotechnology*, 162(2-3), 327-335.



Superposition of P218 (green) and the dihydrofolate substrate envelope (orange) bound to quadruple mutant and wild-type PfDHFR-TS, respectively, showing the fit of the P218 in the substrate envelope.

Correlation of meat texture and pork breeds

Cooked emulsified meat products or sausages are widely consumed and emulsion-type sausages are mainly dependent upon the state of meat proteins and their water-binding and emulsifying properties. A number of factors govern the quality of sausages, e.g., species of meat (beef, pork or chicken), the basic condition of the meat at the time of use, fat source, fat particle size, non-meat additives, pH of emulsions, chopping or emulsifying temperature and time, and cooking method, etc. Although pork has been often utilized for sausage production, information regarding the effects of pig breeds on quality of cooked pork emulsion is scarce.

Researchers conducted an investigation to study the quality of cooked pork emulsion as influenced by the variation of muscle composition and structure among meats derived from different pig breeds. The influences of composition and structure of meats from different pig breeds, including Duroc (D), Large White (LW), Landrace (LR), two-way cross (LR×LW) and three-way cross (D×[LR×LW]) on stability and textural characteristics of cooked meat emulsions were studied by using partial least squares (PLS) regression. Compared to other pig breeds, cooked meat emulsion from LW exhibited superior properties as indicated by lower water and fat released, as well as higher chewiness, gumminess, cohesiveness, resilience, springiness and hardness. The univariate analyses of those selected properties indicated a significant correlation with higher contents of myofibrillar and sarcoplasmic proteins, smaller muscle fiber diameter and lower myofibril fragmentation of LW meat, as compared to other breeds.

This was a collaborative study between the BIOTEC Food Biotechnology Research Unit and Prince of Songkla University.

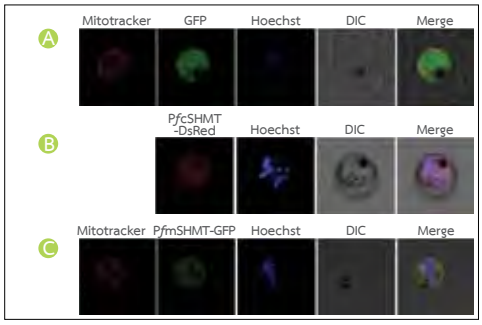
Ref: Sorapukdee, S., Kongtasorn, C., Benjakul, S. and Visessanguan, W. (2013). Influences of muscle composition and structure of pork from different breeds on stability and textural properties of cooked meat emulsion. *Food Chemistry*, 138(2-3), 1892-1901.

Highlights from Malaria Research

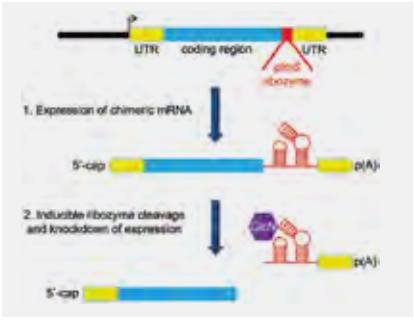
Designing an antimalarial drug candidate, P218

Malarial dihydrofolate reductase (DHFR) is the target of antifolate antimalarial drugs such as pyrimethamine and cycloguanil, the clinical efficacy of which have been compromised by resistance arising through mutations at various sites on the enzyme.

The research team described the use of cocrystal structures with inhibitors and substrates, along with efficacy and pharmacokinetic profiling for the design, characterization, and preclinical development of a selective, highly efficacious, and orally available antimalarial drug candidate that potently inhibits both wild-type and clinically relevant mutated forms of *Plasmodium falciparum* (Pf) DHFR. Important structural characteristics of P218 include pyrimidine side-chain flexibility and a carboxylate group that makes charge-mediated hydrogen bonds with conserved Arg122 (PfDHFR-TS amino acid numbering). An analogous interaction of P218 with human DHFR is disfavored because of three species-dependent amino acid substitutions in the vicinity of the conserved Arg. P218 binds to the active site of PfDHFR in a substantially different fashion from the human enzyme, which is the basis for its high selectivity. Unlike pyrimethamine, P218 binds both wild-type and mutant PfDHFR in a slow-on/slow-off tight-binding mode, which prolongs the target residence time. P218, when bound to PfDHFR-TS, resides almost entirely within the envelope mapped out by the dihydrofolate substrate, which may make it less susceptible to resistance mutations. The high *in vivo* efficacy in a SCID mouse model of *P. falciparum* malaria, good oral bioavailability, favorable enzyme selectivity, and good safety characteristics of P218 make it a potential candidate for further development.



Confocal micrographs demonstrate localization of *P. falciparum* SHMT isoforms. Parasites were transfected with (A) pGFP, (B) pPfcSHMT-DsRed and (C) pPfmSHMT-GFP plasmids expressing fluorescent signals from green fluorescence protein (GFP) or DsRed (red fluorescent protein), which indicate localizations of GFP in cytoplasm, PfcSHMT in cytoplasm, and PfmSHMT in mitochondria, respectively.



Schematic of the *glmS* ribozyme reverse genetic tool

This work was conducted by scientists from various institutes, namely BIOTEC, Chulalongkorn University, Mahidol University, Monash University (Australia), University of London (UK) and Medicines for Malaria Venture.

Ref: Yuthavong, Y., Tarnchompoo, B., Vilaivan, T., Chitnumsub, P., Kamchonwongpaisan, S., Charman, S.A., McLennan, D.N., White,K.L., Vivas, L., Bongard, E., Thongphanchang, C.,Taweechai, S., Vanichtanankul, J., Rattanajak, R., Arwon, U., Fantauzzi, P., Yuvaniyama, J., Charman, W.N. and Matthews, D. (2012). Malarial dihydrofolate reductase as a paradigm for drug development against a resistance-compromised target. *Proceedings of the National Academy of Sciences of the United States of America*, 109(42), 16823-16828.

Study on *Plasmodium* serine hydroxymethyltransferase

Serine hydroxymethyltransferase (SHMT), a pyridoxal phosphate-dependent enzyme, plays a vital role in the *de novo* pyrimidine biosynthesis pathway in malaria parasites. Two genes have been identified in *Plasmodium* spp. encoding a cytosolic SHMT (cSHMT) and putative mitochondria SHMT (mSHMT); however their roles have not been fully investigated.

Researchers performed localization studies of *Plasmodium* SHMT isoforms by transfection of fluorescent-tagged gene constructs into *P. falciparum*, and observed expressions of fluorescent fusion proteins in parasites using a laser scanning confocal microscope. Genetic targeting through homologous recombination was used to study the essentiality of SHMT in *Plasmodium* spp. The results provided the genetic evidence confirming the two distinct compartmental localizations of SHMT isoforms and demonstrate the indispensable role of cSHMT in growth and development of *Plasmodium* parasites, indicating it as a valid target for development of novel anti-malarials.

This work was conducted by the Medical Molecular Biology Research Unit.

Ref: Pornthanakasem, W., Kongkasuriyachai, D., Uthaipibull, C., Yuthavong, Y. and Leartsakulpanich, U. (2012). *Plasmodium* serine hydroxymethyltransferase: indispensability and display of distinct localization. *Malaria Journal*, 11, 387.

glmS ribozyme reverse genetic tool for study of essential genes in the malaria parasite

Conventional reverse genetic approaches for study of *Plasmodium* malaria parasite gene function are limited. Hence, new inducible systems are needed.

Researchers devised a method to control *P. falciparum* gene expression in which target genes bearing a *glmS* ribozyme in the 3′ untranslated region are efficiently knocked down in transgenic *P. falciparum* parasites in response to glucosamine inducer. Using reporter genes, the *glmS* ribozyme cleaves reporter mRNA *in vivo* leading to reduction in mRNA expression following glucosamine treatment. Glucosamine-induced ribozyme activation led to efficient reduction of reporter protein, which could be rapidly reversed by removing the inducer. The *glmS* ribozyme was validated as a reverse-genetic tool by integration into the essential gene and antifolate drug target dihydrofolate reductase-thymidylate synthase (*Pf*DHFR-TS). Glucosamine treatment of transgenic parasites led to rapid and efficient knockdown of *Pf*DHFR-TS mRNA and protein. *Pf*DHFR-TS knockdown led to a growth/arrest mutant phenotype and hypersensitivity to pyrimethamine. The *glmS* ribozyme may thus be a tool for study of essential genes in *P. falciparum* and other parasite species amenable to transfection.

This work was conducted through collaboration between the Medical Molecular Biology Research Unit and MRC National Institute for Medical Research (UK).

Ref: Prommana, P., Uthaipibull, C., Wongsombat, C., Kamchonwongpaisan, S., Yuthavong, Y., Knuepfer, E., Holder, A.A. and Shaw, P.J. (2013). Inducible Knockdown of *Plasmodium* Gene Expression Using the *glmS* Ribozyme. *PLOS ONE*, 8(8), e73783.

Automated equipment allows a digital camera connected to a computer to take high-definition images of malaria parasites in thick blood films. The high-quality images allow detection of structural differences between parasites — such as *P. falciparum* and *P. vivax* — leading to quick species identification.

Automatic device for detection and classification of malaria parasite

Current malaria diagnosis relies primarily on microscopic examination of Giemsa-stained thick and thin blood films. This method requires vigorously trained technicians to efficiently detect and classify the malaria parasite species such as *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv) for appropriate drug administration.

Researchers developed an automatic device for both detection and classification of malaria parasite species on thick blood film. The system is based on digital image analysis and features motorized stage units, designed to easily be mounted on most conventional light microscopes used in the endemic areas. The constructed motorized module could control the movements of objective lens and microscope stage at high precision for effective acquisition of quality images for analysis. The analysis program could accurately classify parasite species, into Pf or Pv, based on distribution of chromatin size. The prototype was able to detect parasite-positive and parasite-negative blood films at the rate of 95% and 68.5% accuracy, respectively. For classification performance, the thick blood films with Pv parasite was correctly classified with the success rate of 75% while the accuracy of Pf classification was 90%.

This device was developed by scientists from BIOTEC and the National Electronics and Computer Technology Center (NECTEC).

Ref: Kaewkamnerd S., Uthaipibull C., Intarapanich A., Pannarut M., Chaotheing S. and Tongsima S. (2012). An automatic device for detection and classification of malaria parasite species in thick blood film. *BMC Bioinformatics*, 13(Suppl 17), S18.

Highlights from Dengue Research

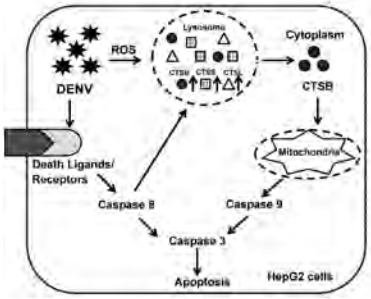
Therapeutic potential of CpdA in dengue virus infection

Dengue Virus (DENV) infection is an important mosquito-borne viral disease and its clinical symptoms range from a predominantly febrile disease, dengue fever (DF), to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Increased levels of cytokines-the so-called ‘cytokine storm’, contribute to the pathogenesis of DHF/DSS.

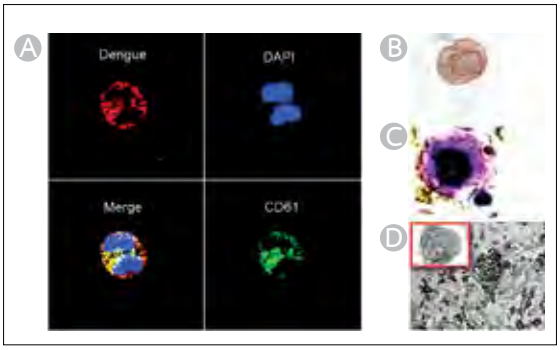
Comparison was made on the expression of cytokine genes between mock-infected and DENV-infected HepG2 cells using a real-time PCR array and several up-regulated chemokines and cytokines were revealed, including CXCL10 and TNF-α. Compound A (CpdA), a plant-derived phenyl aziridine precursor containing anti-inflammatory action and acting as a dissociated nonsteroidal glucocorticoid receptor modulator, was selected as a candidate agent to modulate secretion of DENV-induced cytokines. CpdA is not a glucocorticoid, but has an anti-inflammatory effect with no metabolic side effects as steroidal ligands. CpdA significantly reduced DENV-induced CXCL10 and TNF-α secretion and decreased leukocyte migration indicating for the first time the therapeutic potential of CpdA in decreasing massive immune activation during DENV infection.

This work was conducted by the Medical Biotechnology Research Unit and Faculty of Medicine Siriraj Hospital.

Ref: Suttitheptumrong, A., Khunchai, S., Panaampon, J., Yasamut, U., Morchang, A., Puttikhunt, C., Noisakran, S., Haegemana, G., Yenchitsomanus, P. and Limjindaporn, T. (2013). Compound A, a dissociated glucocorticoid receptor modulator, reduces dengue virus-induced cytokine secretion and dengue virus production. *Biochemical and Biophysical Research Communications*, 436(2), 283-288.



The proposed model of the lysosomal pathway of apoptosis after dengue virus infection in HepG2 cells.



Monkey and human bone marrow were infected with dengue virus *in vitro* and assessed for cell lineages containing dengue virus. Immunostaining demonstrated the presence of dengue virus antigens in CD61⁺ cells (A) with the characteristics of diploid and multilobulated megakaryocytes (B and C) in the bone marrows. Large numbers of dengue virus particles were also observed inside the cytoplasm of infected megakaryocytes as evidenced by electron microscopy (D).

Role of cathepsin B in dengue virus-mediated apoptosis

Patients with dengue hemorrhagic fever (DHF) generally present hemorrhagic tendencies, plasma leakage, thrombocytopenia, and hemoconcentration. Hepatic dysfunction is also a crucial feature of dengue virus (DENV) infection. Hepatic biopsy specimens obtained from fatal cases of DENV infection show cellular apoptosis, which apparently relates to the pathogenesis. Cathepsins, which are cysteine proteases inside the lysosome, were previously reported to be up-regulated in patients with DHF. However, their functions during DENV infection have not been thoroughly investigated.

Researchers demonstrated for the first time that DENV induces lysosomal membrane permeabilization. The resulting cytosolic cathepsin B and S contributed to apoptosis via caspase activation. The activity of caspase 3 was significantly reduced in DENV-infected HepG2 cells treated with cathepsin B or S inhibitors. Treatment with cathepsin B inhibitor also reduced the activity of caspase 9, suggesting that cathepsin B activates both caspase-9 and caspase-3. Reduced cathepsin B expression, effected by RNA interference, mimicked pharmacological inhibition of the enzyme and confirmed the contribution of cathepsin B to apoptotic events induced by DENV in HepG2 cells.

This research was conducted by the Medical Biotechnology Research Unit and Faculty of Medicine Siriraj Hospital.

Ref: Morchang, A., Panaampon J., Suttitheptumrong, A., Yasamut, U., Noisakran, S., Yenchitsomanus, P, and Limjindaporn, T. (2013). Role of cathepsin B in dengue virus-mediated apoptosis. *Biochemical and Biophysical Research Communications*, 438(1), 20-25.

Dengue virus infection in bone marrow

Depression of the peripheral blood platelet count during acute infection is a hallmark of dengue. This thrombocytopenia has been attributed, in part, to an insufficient level of platelet production by megakaryocytes that reside in the bone marrow (BM). It was observed that dengue patients experience BM suppression at the onset of fever. However, few studies focus on the interaction between dengue virus (DENV) and megakaryocytes and how this interaction can lead to a reduction in platelets.

Researchers utilized BM cells from normal healthy rhesus monkeys (RM) and humans to identify the cell lineage(s) that were capable of supporting virus infection and replication. A number of techniques were employed, including the use of viral RNA quantification, nonstructural protein and infectivity assays, phenotypic studies utilizing immunohistochemical staining, anti-differentiation DEAB treatment, and electron microscopy. Cumulative results revealed that cells in the BM were indeed highly permissive for DENV infection, with human BM having higher levels of viral production compared to RM. DENV-like particles were predominantly observed in multi-nucleated cells that expressed CD61+. These data suggest that megakaryocytes are likely the predominant cell type infected by DENV in BM, which provides one explanation for the thrombocytopenia and the dysfunctional platelets characteristic of dengue virus infection.

This work was conducted by the Medical Biotechnology Research Unit, Faculty of Medicine Siriraj Hospital, Yerkes National Primate Research Center, Emory University School of Medicine (USA) and National Cheng Kung University (Taiwan).

Ref: Clark, K.B., Noisakran, S., Onlamoon, N., Hsiao, H., Roback, J., Villinger, F., Ansari, A.A. and Perng, G.C. (2012). Multiploid CD61+ Cells Are the Pre-Dominant Cell Lineage Infected during Acute Dengue Virus Infection in Bone Marrow. *PLOS ONE*, 7(12), e52902.

A simple system for the generation of dengue pseudoinfectious virus

To facilitate studies of dengue virus replication and vaccine development, researchers developed a simple system for the generation of pseudoinfectious particles of the virus. Selected clones of the C6/36 mosquito cell line expressing an anchored form of the dengue virus capsid protein served as host cells for the *trans*-complementation of partially capsid-deleted viral RNA generated *in vitro*. Transfection of the partially capsid-deleted viral RNA into the anchored capsid-expressing C6/36 cells resulted in moderate titers of infectious virus. Progeny viruses multiplied in the capsid *trans*-complementing C6/36 cells for up to three weeks, but only initiated single rounds of replication in Vero cells lacking the capsid protein. Employing this *trans*-complementation system, it was found that nearly all of the capsid-coding sequence in the viral RNA was dispensable for the generation of pseudoinfectious dengue virus particles in mosquito cells.

This work was undertaken collaboratively between the Medical Biotechnology Research Unit, Biomedical Technology Research Center, Chiang Mai University and the Faculty of Medicine Siriraj Hospital.

Ref: Sangiambut, S., Suphatrakul, A., Sriburi, R., Keelapang, P., Puttikhunt, C., Kasinrerk, W., Malasit, P. and Sittisombut, N. (2013). Sustained replication of dengue pseudoinfectious virus lacking the capsid gene by *trans*-complementation in capsid-producing mosquito cells. *Virus Research*, 174(1-2), 37-46.

Genome diversity of dengue virus quasispecies

Dengue is the world's most common mosquito-borne viral disease. Poor proofreading by RNA polymerase during its replication results in the accumulation of mutations in its genome. This leads to a diversity of genotypes in the viral population termed quasispecies. Quasispecies play an important role in disease severity. The study of quasispecies in dengue has been hindered because of the requirement for large amounts of cloning and sequencing.

Researchers demonstrated the feasibility of using 454 pyrosequencing to study genome diversity of dengue virus quasispecies by sequencing a pool of known dengue viral strains. The obtained sequences covered the whole genome of the dengue virus. More than 99% of reads could be aligned back to the correct serotypes by BLAST. The method could reliably detect a dengue genotype present in a mixture with a frequency of more than 1%. The calculated ratios were in agreement with the input ratios.

This work was done under collaboration between the Genome Institute, Medical Biotechnology Research Unit, Faculty of Medicine Siriraj Hospital and Mahidol University.

Ref: Chin-inmanu, K., Suttitheptumrong, A., Sangsrakru, D., Tangphatsornruang, S., Tragoonrung, S., Malasit, P., Tungpradabkul, S. and Suriyaphol, P. (2012). Feasibility of using 454 pyrosequencing for studying quasispecies of the whole dengue viral genome. *BMC Genomics*, 13(Suppl 7), S7.

Highlights from Tuberculosis Research

Clarithromycin resistance in *Mycobacterium tuberculosis*

The emergence of extensively drug-resistant tuberculosis (XDR-TB) makes the control of tuberculosis (TB) more difficult. As an alternative to developing new drugs, drugs that have already been used in humans as anti-infectives and later found to have antitubercular activity might be useful as anti-TB drugs, particularly against drug-resistant TB. Clarithromycin (CLR) has potent activity against most mycobacterial infections, except *Mycobacterium tuberculosis*.

To gain insight into the mechanisms of innate CLR resistance in *M. tuberculosis*, CLR-susceptible *M. tuberculosis* H37Rv mutants were generated by transposon mutagenesis. One mutant, designated as Tn-196, was further investigated and it was found that *ksgA* (Rv1010) was inactivated by the transposon. The *ksgA* gene encodes a 16S rRNA adenine dimethyltransferase that methylates A1518 and A1519 (*Escherichia coli* numbering) of 16S rRNA and plays an important role in ribosome biogenesis. Complementation of the Tn-196 mutant with a wild-type *ksgA* gene restored the resistant phenotype (MIC of 8-16 µg/mL), corroborating the association of *ksgA* with intrinsic CLR resistance in *M. tuberculosis*.

This work was conducted by the Medical Molecular Biology Research Unit, Mahidol University and King Mongkut's Institute of Technology Ladkrabang.

Ref: Phunpruch, S., Warit, S., Suksamran, R., Billamas, P., Jaitrong, S., Palittapongarnpim, P., Prammananan, T. (2013). A role for 16S rRNA dimethyltransferase (*ksgA*) in intrinsic clarithromycin resistance in *Mycobacterium tuberculosis*. *International Journal of Antimicrobial Agents*, 41(6), 548-551.

Active site loop of MtFBA

Class II fructose 1,6-bisphosphate aldolases (FBAs, EC 4.1.2.13) comprise one of two families of aldolases. Instead of forming a Schiff base intermediate using an ε-amino group of a lysine side chain, class II FBAs utilize Zn(II) to stabilize a proposed hydroxyenolate intermediate (HEI) in the reversible cleavage of fructose 1,6-bisphosphate, forming glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (DHAP). As class II FBAs have been shown to be essential in pathogenic bacteria, focus has been placed on these enzymes as potential antibacterial targets. Although structural studies of class II FBAs from *Mycobacterium tuberculosis* (MtFBA), other bacteria, and protozoa have been reported, the structure of the active site loop responsible for catalyzing the protonation-deprotonation steps of the reaction for class II FBAs has not yet been observed.

Researchers utilized the potent class II FBA inhibitor phosphoglycolohydroxamate (PGH) as a mimic of the HEI- and DHAP-bound form of the enzyme and determined the X-ray structure of the MtFBA–PGH complex to 1.58 Å. Well-defined electron density for the previously elusive active site loop of MtFBA trapped in a catalytically competent orientation was observed. Utilization of this structural information and site-directed mutagenesis and kinetic studies conducted on a series of residues within the active site loop revealed that E169 facilitates a water-mediated deprotonation-protonation step of the MtFBA reaction mechanism. Also, solvent isotope effects on MtFBA and catalytically relevant mutants were used to probe the effect of loop flexibility on catalytic efficiency. Additionally, the structure of MtFBA in its holoenzyme form was also revealed.

This work was conducted by the University of Denver, Purdue University and the University of Illinois at Chicago (USA), in collaboration with the Medical Molecular Biology Research Unit.

Ref: Pegan, S.D., Rukseree, K., Capodagli, G.C., Baker, E.A., Krasnykh, O., Franzblau, S.G. and Mesecar, A.D. (2013). Active Site Loop Dynamics of a Class IIa Fructose 1,6-Bisphosphate Aldolase from *Mycobacterium tuberculosis*. *Biochemistry*, 52(5), 912-925.

Highlights from Virology Research

Pivotal role of N-terminal region in the nuclear localization of BNP

The nucleoprotein of the influenza B virus (BNP) shares several characteristics with its influenza A virus counterpart (ANP), including localization in the host's nucleus. However, while the nuclear localization signal(s) (NLS) of ANP are well characterized, little is known about those of BNP.

Researchers were able to demonstrate that the fusion protein bearing the BNP N-terminus fused with the green fluorescent protein (N70–GFP) is exclusively nuclear, and identified a highly conserved KRXR motif spanning residues 44-47 as a putative NLS. In addition, residues 3-15 of BNP, though not an NLS, are also crucial for nuclear import. Results from mutational analyses of N70-GFP and the full-length BNP suggest that this region may be required for protection of the N-terminus from proteolytic cleavage. Altogether, the findings indicated that the N-terminal region of BNP contains the NLS and cleavage-protection motif, which together drive its nuclear localization.

This work was conducted by scientists from the Virology and Cell Technology Laboratory.

Ref: Wanitchang, A., Narkpuk, J. and Jongkaewwattana, A. (2013). Nuclear import of influenza B virus nucleoprotein: Involvement of an N-terminal nuclear localization signal and a cleavage-protection motif. *Virology*, 443(1), 59-68.

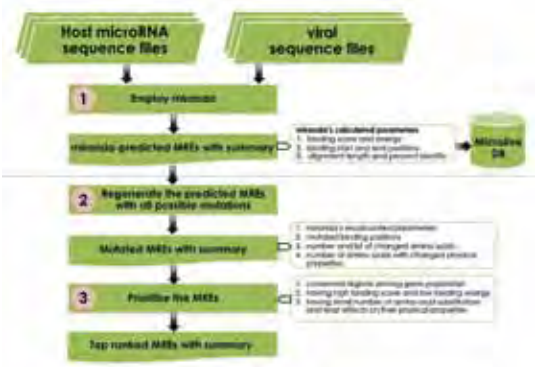
Molecular interactions between oseltamivir and N1 NA variants

Pandemic influenza A/H1N1 (2009) and avian influenza A/H5N1 neuraminidase (NA) differ at two critical residues, positions 149 and 347. Researchers investigated the impact of amino acid differences between A/H1N1 (2009) NA and A/H5N1 NA at two highly conserved residues, Val149 (in the 150-loop region) and Asn347 (in the active site), which were hypothesized to be involved in modulating oseltamivir sensitivity.

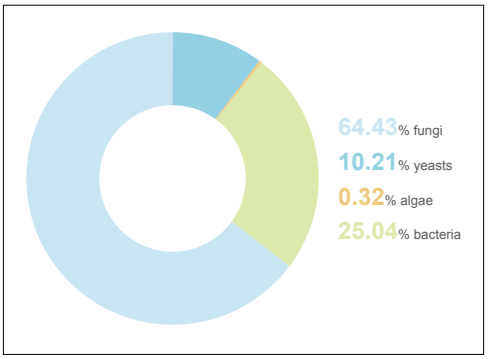
Recombinant influenza A viruses were constructed in which these two residues in pandemic influenza A/H1N1 (2009) NA were changed to the corresponding amino acids of avian influenza A/H5N1 NA, and vice versa. Recombinant viruses bearing N1 NA with the oseltamivir resistance mutation H274Y in combination with mutations at residues 149 and 347 were also constructed. Recombinant viruses grew normally in allantoic fluid and were subsequently studied for viral infectivity ($TCID_{50}$), substrate binding (K_m) and sensitivity to oseltamivir (K_i). The data demonstrated that infectivity of mutant viruses in Madin Darby canine kidney cells was comparable to, or even greater than, the infectivity of the parental viruses harboring wild-type N1 NA. Furthermore, mutations at NA residues 149 and 347 altered K_m and K_i values, and thus modulated oseltamivir sensitivity. Although these mutants have yet to be observed among natural isolates, the minimal costs to the growth of recombinant viruses indicate their possible viability. Reassortment between pandemic influenza A/H1N1 (2009) and avian influenza A/H5N1 viruses may therefore generate new influenza A viruses with increased infectivity and oseltamivir resistance, and continued surveillance will be crucial for public health preparedness.

This study was conducted jointly by scientists from the Virology and Cell Technology Laboratory, Protein-Ligand Engineering and Molecular Biology Laboratory and Thammasat University.

Ref: Yongkiettrakul, S., Nivitchanyong, T., Pannengpetch, S., Wanitchang, A., Jongkaewwattana, A. and Srimanote, P. (2013). Neuraminidase amino acids 149 and 347 determine the infectivity and oseltamivir sensitivity of pandemic influenza A/H1N1 (2009) and avian influenza A/H5N1. *Virus Research*, 175(2), 128-133.



Overall workflow for the computational identification of potential miRNA response elements (MREs).



Distribution of microorganisms in BIOTEC Culture Collection.

microRNA-controlled live attenuated virus vaccine design tool

The microRNA-based gene-silencing machinery has been recognized as a promising approach to control viral replication and being used to improve the safety of live attenuated virus vaccines. Effective host microRNA response elements (MREs) have been incorporated into a virus sequence mainly based on the experimental trials for identifying both microRNA binding sites and effective mutations. The design of MREs for viral genomes or with multiple host microRNAs of interest, then, will be time consuming and costly.

Researchers developed a computational flow that helps virologists design the potential miRNA response elements (MREs) for constructing safer live attenuated virus vaccines. The designed MREs of selected human miRNAs within Influenza A H1N1 virus demonstrated the usefulness of the proposed computation to reduce the experimental time and cost. The MRE design tool on the MicroLive web server enables users to interactively find and optimize the potential MREs for a specific human miRNA within user input of viral sequences of interest. The database provided allows users to explore the miranda-predicted MREs for several RNA viruses and try the MRE design tool based on the system-provided sequences. While the demonstration and implementation were based on human miRNAs and Influenza A H1N1 virus, the proposed computation can be flexibly applied to design MREs for engineering tissue-specific oncolytic viruses or for constructing live attenuated virus vaccines for other hosts of interest such as animals. The MicroLive web server is freely accessible at http://www3a.biotec.or.th/microlive/.

This tool was designed by the Information Systems Laboratory and the Virology and Cell Technology Laboratory.

Ref: Wichadakul, D., Mhuantong, W., Jongkaewwattana, A. and Ingsriswang, S. (2012). A computational tool for the design of live attenuated virus vaccine based on microRNA-mediated gene silencing. *BMC Genomics*, 13(Suppl 7), S15.

Highlights from Biodiversity Research and Utilization

Collection of biological materials

BIOTEC established the BIOTEC Culture Collection (BCC) in 1996, to be a depositary and distribution center for microbes. The facility is also designated by the Department of Intellectual Property as a repository of patent-related microorganisms. At present, a total of 63,792 strains are preserved at the BCC, of which 64.06% are identified strains. Among these, 292 strains are patent-related and safe-deposited. These strains are maintained by freezing and/or freeze drying to ensure their genetic stability for long term storage. Many precautionary steps have been taken to ensure the survival and integrity of the preserved cultures; these preventative measures have included automated temperature control and regulator units, temperature monitoring with alert systems, and back-up power-storage units. BCC has also been certified with ISO 9001:2000 as a service provider since 2005.

BIOTEC has also collected and preserved other biological materials for both research and industrial utilization. A total of 153 biological materials are currently being preserved in the collection, comprising 42 vectors, 21 hosts, and 80 recombinant clones. BIOTEC Bangkok Herbarium (BBH) preserves 34,681 dried specimens of fungi.

Scanning electron micrographs of (from left to right) *Actinoplanes siamensis*, *Planobispora siamensis* and *Acrocarpospora phusangensis*.

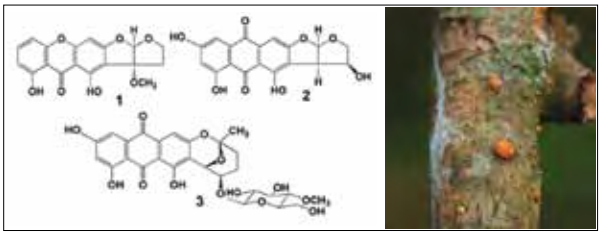
Microbial diversity study

Microbial diversity study at BIOTEC involves the collection, isolation and identification of microorganisms from a variety of natural habitats. Examples of novel species discovered in 2013 are:

- Two novel species of the ascomycetous anamorphic yeast were isolated from industrial wastes in Thailand. Strain JP52^T represent a novel species which was named *Cyberlindnera samutprakarnensis* sp. nov. Strain JP59^T and JP60 were assigned as a single novel species which was named *Candida thasaenensis* sp. nov.
- A new yeast species (KKU-FW10) belonging to the *Candida* genus was isolated from *Jasminum adenophyllum* in the Plant Genetic Conservation Project under The Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn area, Chulabhorn Dam, Konsan district within Chaiyaphum province in Thailand. The name *Candida konsanensis* is proposed for this yeast.
- A novel actinomycete strain, A-T 4600(T), which developed cylindrical sporangia containing a longitudinal pair of motile spores forming singly or in bundles on short ramifications of the aerial mycelium, was isolated from soil collected from an evergreen forest in Thailand. It was named *Planobispora siamensis* sp. nov.
- A novel actinomycete, strain PS33-18(T), that formed club-shaped and spherical structures borne on the tip of the aerial mycelia was isolated from a temperate peat swamp forest soil in Phu-Sang National Park, Phayao province, Thailand. This strain represents a novel species, for which the name *Acrocarpospora phusangensis* sp. nov. is proposed.
- An actinomycete strain, S3-1(T), was isolated from a marine sponge sample collected from the Gulf of Thailand. The name *Micromonospora spongicola* sp. nov. is proposed.
- An actinomycete strain, SP03-05T, was isolated from a marine sponge sample (*Xestospongia* sp.) collected from Phuket province of Thailand. The name *Verrucosispora andamanensis* sp. nov. is proposed.
- A Gram-positive filamentous bacterial strain that developed large campanulate sporangia at the ends of sporangiophores on substrate mycelium was isolated from bamboo forest soil in Thailand. Following an evaluation of phenotypic, chemotaxonomic and genotypic studies, the isolate is proposed to represent a novel species to be named *Actinoplanes siamensis* sp. nov.

Ref:

1. Poomtien, J., Jindamorakot, S., Limtong, S., Pinphanichakarn, P. and Thaniyavarn, J. (2013). Two new anamorphic yeasts species, *Cyberlindnera samutprakarnensis* sp. nov. and *Candida thasaenensis* sp. nov., isolated from industrial wastes in Thailand. *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology*, 103(1), 229-238.
2. Sarawan, S., Mahakhan, P., Jindamorakot, S., Vichitphan, K., Vichitphan, S. and Sawaengkaew, J. (2013). *Candida konsanensis* sp. nov., a new yeast species isolated from *Jasminum adenophyllum* in Thailand with potentially carboxymethyl cellulase-producing capability. *World Journal of Microbiology and Biotechnology*, 29(8), 1481-1486.
3. Ngaemthao, W., Suriyachadkun, C., Chunhametha, S., Tamura, T. and Sanglier, J.J. (2013). *Planobispora siamensis* sp. nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, 63(7), 2649-2654.
4. Niemhom, N., Suriyachadkun, C., Tamura, T. and Thawai, C. (2013). *Acrocarpospora phusangensis* sp. nov., isolated from a temperate peat swamp forest soil. *International Journal of Systematic and Evolutionary Microbiology*, 63(Pt6), 2174-2179.
5. Supong, K., Suriyachadkun, C., Pittayakhajonwut, P., Suwanborirux, K. and Thawai, C. (2013). *Micromonospora spongicola* sp. nov., an actinomycete isolated from a marine sponge in the Gulf of Thailand. *Journal of Antibiotics*, 66(9), 505-509.
6. Supong, K., Suriyachadkun, C., Suwanborirux, K., Pittayakhajonwut, P. and Thawai, C. (2013). *Verrucosispora andamanensis* sp. nov., isolated from the marine. *International Journal of Systematic and Evolutionary Microbiology*, 63(Pt 11), 3970-394.
7. Suriyachadkun, C., Ngaemthao, W., Chunhametha, S., Thawai, C. and Sanglier, J.-J. (2013). *Actinoplanes siamensis* sp. nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, 63(Pt 8), 3037-3042.



Three new mycotoxins isolated from the scale insect fungus *Aschersonia marginata* BCC 28721.

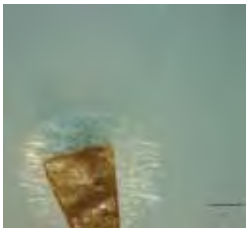
Akanthomyces novoquineesis on a spider.

Gibellula dimorpha on a spider.

Bioactive compound discoveries

BIOTEC natural product discovery program aims to explore microbial resources for bioactive compounds that have potential for pharmaceutical and agricultural product development. Bioactive substances produced from various microorganisms are identified by activity-guided fractionation and subjected to spectroscopic analyses to determine chemical structures. In 2013, a total of 114 active compounds were identified, 39 of which were novel compounds. Below are examples of microbial-derived compounds that have been identified in 2013:

- Five new picolinic acid derivatives, penicolinates A–E, together with four known compounds including phenopyrrozin, *p*-hydroxyphenopyrrozin, gliotoxin, and *bis*dethio*bis* (methylthio) gliotoxin were isolated from endophytic *Penicillium* sp. BCC16054. The chemical structures were established from spectroscopic data-1D and 2D NMR experiments, IR, UV, and HRESIMS. These compounds were evaluated for antimalarial and antitubercular activities and for their activity against *Bacillus cereus* and *Candida albicans* along with cytotoxicity against both cancerous and non-cancerous cells.
- Three new mycotoxins, together with 14 known compounds were isolated from the scale insect fungus *Aschersonia marginata* BCC 28721. The structures were elucidated on the basis of NMR spectroscopic and MS spectrometric data. Two of the new mycotoxins exhibited moderate cytotoxic activity.
- In search of new bioactive compounds from invertebrate-pathogenic fungi infecting spiders, one hundred and sixty-five crude extracts from *Akanthomyces* and *Gibellula* were screened for their antimicrobial activity against nine human pathogens. Twenty-one extracts out of 165 from 16 isolates exhibited antimicrobial activity against at least one test strain. The most activity was against *Staphylococcus aureus* American Type Culture Collection (ATCC 25923) (8.48%). The ethyl acetate extract of mycelia from *Gibellula pulchra* EPF083 had the strongest broad spectrum antimicrobial activity with a minimum inhibitory concentration (MIC) value of 16 µg/ml against *S. aureus* ATCC 25923, MRSA SK-1, *C. neoformans* (ATCC 90112 and ATCC 90113) and *P. marneffei* and exhibited fungicidal activity against *C. neoformans* ATCC 90112 and *P. marneffei* with minimum fungicidal concentration (MFC) values of 16 and 32 µg/ml, respectively. These preliminary data show that invertebrate-pathogenic fungi could be a potential source of antimicrobial agents. This study was conducted by researchers from the Bioresources Technology Unit and Prince of Songkla University.
- Torrubiellins A and B, two new dimeric anthraquinones were isolated from the leafhopper pathogenic fungus *Torrubiella* sp. BCC 28517. The structures of the new compounds were elucidated by analyses of the NMR spectroscopic and mass spectrometry data. Torrubiellin B exhibited a broad range of biological activities.
- Cordylactam, a new lactam-fused 4-pyrone was isolated from the spider pathogenic fungus *Cordyceps* sp. BCC 12671. The structure was elucidated on the basis of NMR spectroscopic and mass spectrometry data, which was further confirmed by the spectroscopic analyses of a semisynthetic derivative. This is the first report of a new compound from spider pathogenic *Cordyceps* species.



Endophytic fungi emerging from seagrass leaf segment.

Seagrass *Halophila ovalis* (left) and *Thalassia hemprichii* (right).

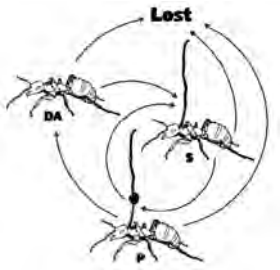
- Seven lanostane triterpenoids, ganorbiformins A-G, together with twelve known compounds, were isolated from cultures of the mushroom fungus *Ganoderma orbiforme* BCC 22324. Ganorbiformin A is an unusual rearranged analog, whereas the other compounds share the same lanostane skeleton with known ganoderic acids. The C-3 epimer of ganoderic acid T also exhibited significant antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra.

- Endophytic fungi from three commonly found sea-grass species in southern Thailand were explored for their ability to produce antimicrobial metabolites. One hundred and sixty endophytic fungi derived from *Cymodocea serrulata* (Family Cymodoceaceae), *Halophila ovalis* and *Thalassia hemprichii* (Family Hydrocharitaceae) were screened for production of antimicrobial compounds by a colorimetric broth microdilution test against ten human pathogenic microorganisms. Sixty-nine percent of the isolates exhibited antimicrobial activity against at least one test strain. Antifungal activity was more pronounced than antibacterial activity. Among the active fungi, seven isolates including Hypocreales sp. PSU-ES26 from *C. serrulata*, *Trichoderma spp.* PSU-ES8 and PSU-ES38 from *H. ovalis*, and *Penicillium* sp. PSU-ES43, *Fusarium sp.* PSU-ES73, *Stephanonectria sp.* PSU-ES172 and an unidentified endophyte PSU-ES190 from *T. hemprichii* exhibited strong antimicrobial activity against human pathogens with minimum inhibitory concentrations (MIC) of less than 10 µg/ml. The inhibitory extracts at concentrations of 4 times their MIC destroyed the targeted cells as observed by scanning electron microscopy. These results showed the antimicrobial potential of extracts from endophytic fungi from sea-grass. This study was conducted jointly by the Bioresources Technology Unit and Prince of Songkla University.

- Three new angucyclinones, saccharosporones A, B and C, together with (+)-ochromycinone, (+)-rubiginone B2, tetrangulol methyl ether and fujianmycin A, were obtained from fermentation of the terrestrial actinomycete of the genus *Saccharopolyspora* BCC 21906 isolated from soil collected in Chanthaburi province, Thailand. Structures of the new compounds and their relative configurations were assigned by NMR spectral data interpretation. Saccharosporones A and B exhibited antimalarial activity against *Plasmodium falciparum* K1. Both metabolites also possessed cytotoxic activities against cancer cell lines and nonmalignant Vero cell, while saccharosporone C only showed cytotoxic activity against NCI-H187.

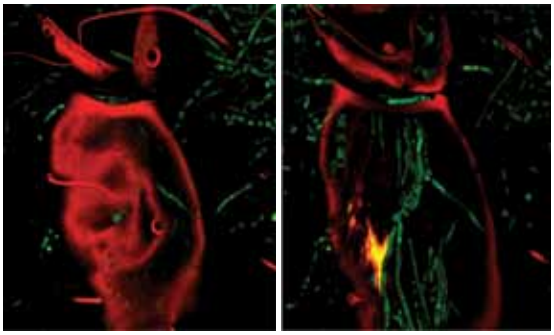
Ref:

1. Intaraudom, C., Boonyuen, N., Suvannakad, R., Rachtawee, P. and Pittayakhajonwut, P. (2013). Penicolinates A-E from endophytic *Penicillium* sp. BCC16054. *Tetrahedron Letters*, 54(8), 744-748.
2. Kornsakulkarn, J., Saepua, S., Laksanacharoen, P., Rachtawee, P. and Thongpanchang, C. (2013). Xanthone and anthraquinone-type mycotoxins from the scale insect fungus *Aschersonia marginata* BCC 28721. *Tetrahedron Letters*, 54(29), 3813-3815.
3. Kuephadungphan, W., Phongpaichit, S., Luangsa-ard, J.J. and Rukachaisirikul, V. (2013). Antimicrobial activity of invertebrate-pathogenic fungi in the genera *Akanthomyces* and *Gibellula*. *MYCOSCIENCE*, http://dx.doi.org/10.1016/j.myc.2013.06.007.
4. Isaka, M., Palasarn, S., Tobwor, P., Boonruangprapa, T. and Tasanathai, K. (2012). Bioactive anthraquinone dimers from the leafhopper pathogenic fungus *Torrubiella* sp. BCC 28517. *Journal of Antibiotics*, 65(11), 571-574.
5. Isaka, M., Chinthanom, P., Kongthong, S., Srichomthong, K. and Choeyklin, R. (2013). Lanostane triterpenes from cultures of the Basidiomycete *Ganoderma orbiforme* BCC 22324. *Phytochemistry*, 87(1), 133-139.
6. Supaphon, P., Phongpaichit, S., Rukachaisirikul, V. and Sakayaroj, J. (2013). Antimicrobial Potential of Endophytic Fungi Derived from Three Seagrass Species: *Cymodocea serrulata*, *Halophila ovalis* and *Thalassia hemprichii*. *PLOS ONE*, 8(8), e72520.
7. Boonlarppradab, C., Suriyachadkun, C., Rachtawee, P. and Choowong, W. (2013). Saccharosporones A, B and C, cytotoxic antimalarial angucyclinones from *Saccharopolyspora* sp. BCC 21906. *Journal of Antibiotics*, 66(6), 305-309.



Description of *Ophiocordyceps unilateralis* life cycle. The life cycle of *O. unilateralis* is composed of different stages: dead ants (DA) infected by the fungus are typically found on the underside of leaves in tropical rainforests; a fungal stroma (S) then grows from the back of the head of the dead ants; and finally, a perithecia pad (P) develops from the fungal stroma for spore dispersion.

Ophiocordyceps camponoti-saundersi on an ant.



Proliferation of the insect pathogen *Beauveria bassiana* expressing green fluorescent protein in the cassava mealybug at 10 days after inoculation. Photos were taken by a high resolution confocal microscope. The technique revealed the fungal growth inside the insect leg (right), which was not readily seen at the surface (left).

Life cycle of an ant-infected fungus, *Ophiocordyceps unilateralis*

The parasitic fungus *Ophiocordyceps unilateralis* infects ants, modifies their behavior, and is found in many countries around the world. One unifying concept of all such parasitic associations is that both the parasite and the host adapt to maximize their fitness and reproductive output. In the case of *O. unilateralis*, infected ants develop erratic behaviors that include leaving their nests, climbing trees and hanging themselves from leaves by their jaws to disperse fungal spores. Understanding the life cycle of this pathogen and its temporal pattern of infection could have important impacts on the ant population and will provide a foundation for further study of the host-parasite association.

In an attempt to understand the life cycle of this pathogen, researchers surveyed a permanent plot in Khao Yai National Park and recorded the presence of dead ants infected by this fungus. Each dead ant was studied, and new dead ants were recorded monthly over the course of two years. It was found that out of thirty seven species of live ants found on site, only 10 species of ants in the genera *Camponotus* and *Polyrhachis* were infected with *O. unilateralis*/*H. formicarum*. Researchers were able to describe the life cycle of *O. unilateralis* as consisting of three distinct stages: 1) dead ants (DA) infected by the fungus are typically found on the underside of leaves in tropical rainforests; 2) a fungal stroma (S) then grows from the back of the head of the dead ants; and 3) a perithecia pad (P) develops from the fungal stroma for spore dispersion. Researchers also reported that *O. unilateralis* occurrence on ants has a seasonal pattern with peaks in both the rainy and dry seasons.

This work was conducted by the Bioresources Technology Unit.

Ref: Mongkolsamrit, S., Kobmoo, N., Tasanathai, K., Khonsanit, A.,Noisripoom, W., Srikitikulchai, P., Somnuk, R. and Luangsa-ard, J.J. (2012). Life cycle, host range and temporal variation of *Ophiocordyceps unilateralis/Hirsutella formicarum* on Formicine ants. *Journal of Invertebrate Pathology*, 111(3), 217-224.

Infection and colonization of insects by a fungus

Studies of infection mechanisms and pathogenesis of insects by biocontrol fungi should lead to a better understanding of the underlying mechanisms and thus to better control methods.

In this study, researchers used light, electron and confocal laser scanning microscopy (CLSM) to investigate in detail infection routes by *Beauveria bassiana* on and below the host cuticle layers and subsequent development in the body cavity of two intact insects, the aphid *Myzus persicae* and the highly destructive cassava mealybug *Phenacoccus manihoti*. Important events in aphids included fungal penetration of the integument of the less-resistant leg intersegmental membrane and invasion of natural openings, formation of hyphal bodies in live aphids by three days post-inoculation (PI), and extensive hyphal colonization of the two leg segments closest to the insect body at death of the aphids. Confocal microscopy of green fluorescent protein-labeled *B. bassiana* in live mealybugs indicated the fungus penetrated the host through the legs and mouthparts. The fungus was scarce in live mealybugs at 1-5 days PI, formed hyphal bodies by six days PI, and growth was limited to parts of dead hosts at 6-7 days PI. In dead mealybugs, hyphal bodies were near solid tissue. Blastospores were in the hemolymph.

This work was conducted jointly by scientists from the Bioresources Technology Unit and King Mongkut's University of Technology Thonburi.

Ref: Amnuaykanjanasin, A., Jirakkakul, J., Panyasiri, C., Panyarakkit, P., Nounurai, P., Chantasingh, D., Eurwilaichitr, L., Cheevadhanarak, S. and Tanticharoen, M. (2013). Infection and colonization of tissues of the aphid *Myzus persicae* and cassava mealybug *Phenacoccus manihoti* by the fungus *Beauveria bassiana*. *Biocontrol*, 58(3), 379-391.

Red yeast rice acquires its color from being fermented with red mould, *Monascus*.

Photo courtesy of Asian-ingredients.com

Gene expression in *Aspergillus oryzae* producing new pyrone compounds

Fungi from the genus *Xylaria* produce a wide range of polyketides with diverse structures, which provide important sources for pharmaceutical agents. At least seven polyketide synthase (PKS) genes, including *pksmt*, were found in *Xylaria* sp. BCC 1067.

Researchers investigated the function and direct product of the *pksmt* gene by heterologous expression of the gene in *Aspergillus oryzae*. To identify the gene function, *pksmt* was fused with a gene encoding green fluorescent protein (GFP) and introduced into a surrogate host, *A. oryzae*, and expressed under the control of a constitutive *gpdA* promoter. In the transformant, the *pksmt* gene was functionally expressed and translated as detected by a green fluorescence signal. This transformant produced two new 2-pyrone compounds, 4-(hydroxymethyl)-5,6-dihydro-pyran-2-one and 5-hydroxy-4-methyl-5,6-dihydro-pyran-2-one, as well as a previously identified 4-methyl-5, 6-dihydro-pyran-2-one. The results suggested that *pksmt* from *Xylaria* sp. BCC 1067 represents a family of fungal PKSs that can synthesize 2-pyrone-containing compounds.

This work was conducted jointly by scientists from the Bioresources Technology Unit and King Mongkut's University of Technology Thonburi.

Ref: Punya, J., Tachaleat, A., Wattanachaisaereekul, S., Haritakun, R., Boonlarppradab, C. and Cheevadhanarak, S. (2013). Functional expression of a foreign gene in *Aspergillus oryzae* producing new pyrone compounds. *Fungal Genetics and Biology*, 50(1), 55-62.

Production and degradation of a metabolite monacolin K

Red yeast rice is rice fermented with red mould, *Monascus*. It has been used as a natural dietary supplement for controlling serum cholesterol as it contains a metabolite monacolin K or lovastain. Monascus also produces beneficial pigments, as well as a mycotoxin called citrinin.

Researchers attempted to optimize solid state fermentation of *Monascus purpureus* TISTR 3541 by statistical methodology to obtain high production of monacolin K and yellow pigment, along with a low level of citrinin. Fractional factorial design was applied, and five parameters (i.e., glycerol, methionine, sodium nitrate, cultivation time, and temperature) were identified as having significantly influenced monacolin K, yellow pigment, and citrinin production. A central composite design was further employed to investigate the optimum level of these five factors. The maximum production of monacolin K and yellow pigment of 5,900 mg/kg and 1,700 units/g, respectively, and the minimum citrinin concentration of 0.26 mg/kg were achieved in the medium containing 2% glycerol, 0.14% methionine, and 0.01% sodium nitrate at 25°C for 16 days of cultivation. The yields of monacolin K and yellow pigment were about 3 and 1.5 times higher than the basal medium, respectively, whereas citrinin was dramatically reduced by 36 times.

In a related study, researchers investigated the effects of storage temperature (4, 20, 30, 40, 50°C) and atmospheric conditions in packages (vacuum, atmospheric) on degradation kinetics of monacolin K in red yeast rice powder using multiresponse modeling approach. Storage of red yeast rice powder at 4°C under vacuum package was found to enhance retention of monacolin K. Multiresponse modeling revealed the degradation path of monacolin K acid forms into their dehydromonacolin K and unknown product under vacuum package, while oxidized product was also formed under atmospheric package. Monacolin K lactone form and precursor were degraded into dehydromonacolin K lactone form, while degradation of dehydromonacolin K lactone form to unknown was more pronounced at temperatures higher than 30°C. Oxidized product was also generated from monacolin K lactone form in atmospheric package. High activation energy of monacolin K degradation in acid form indicated that degradation of monacolin K to their dehydromonacolin K was more susceptible to temperature change as compared to lactone form.

This work involved collaboration between scientists from the Biochemical Engineering and Pilot Plant Research and Development Unit and King Mongkut's University of Technology Thonburi.

Ref:

1. Jirasatid, S., Nopharatana, M., Kitsubun, P., Vichitsoonthonkul, T. and Tongta, T. (2013). Statistical Optimization for Monacolin K and Yellow Pigment Production and Citrinin Reduction by Monascus purpureus in Solid-State Fermentation. *Journal of Microbiology and Biotechnology*, 23(3), 364-374.
2. Jirasatid, S., Nopharatana, M., Kitsubun, P. and Tongta, T. (2013). Degradation kinetics of monacolin K in red yeast rice powder using multiresponse modeling approach. *Journal of Food Engineering*, 116(2), 436-443.

High-level production of thermotolerant ß-xylosidase from fungal origin

In the conversion of plant biomass, comprised mostly of cellulose and hemicellulose, into its sugar components, xylanase is required. To obtain xylan-degrading enzymes, ß-xylosidase was sought from fungal collections in Thailand's BIOTEC Culture Collection (BCC), and the *Aspergillus* sp. BCC125 strain was identified as a strong producer of ß-xylosidase.

A thermotolerant ß-xylosidase of *Aspergillus* sp. BCC125 was produced as a secreted protein using *P. pastoris* expression system. The high yield production (156 unit/mg protein) of secreted enzyme without a major contaminant in the culture supernatant obviated enzyme purification and thus simplifies enzyme preparation. This enzyme has kinetic properties similar to other fungal ß-xylosidases of the same family but its efficiency was superior to others identified to date. This ß-xylosidase was demonstrated to synergize with xylanase and is suitable for simultaneous saccharification and fermentation together with *P. stipitis* to maximize ethanol production efficiency from xylan.

This work was conducted by the Bioresources Technology Unit.

Ref: Wongwisansri, S. Promdonkoy, P., Matetaviparee, P., Roongsawang, N., Eurwilaichitr, L. and Tanapongpipat, S. (2013). High-level production of thermotolerant ß-xylosidase of *Aspergillus* sp. BCC125 in *Pichia pastoris*: Characterization and its application in ethanol production. *Bioresource Technology*, 132, 410-413.

Rice straw is one of the most abundant agricultural wastes in Thailand.

Highlights from Biorefinery

Pre-treatment and saccharification of cassava pulp

Cassava pulp has been identified as having the potential to be a long-term sustainable resource for biological refining. To obtain fermentable sugars and other bioproducts, cassava pulp has to first be pretreated or saccharified.

In the study, cassava pulp was pretreated by the Star Burst System (SBS) involving a super high pressure water jet system, and was subsequently saccharified by two types of hyperthermophilic cellulases (endoglucanase and ß-glucosidase) and thermophilic α-amylase. The SBS can improve the saccharification efficiency of a multi-enzyme system by more than 1.5-fold. In addition to fermentation sugars, cellulose nanofibers are produced from the trapped starch granules from the fibrous cell wall structure of cassava pulp.

This work involved collaboration between scientists from BIOTEC, the National Institute of Advanced Industrial Science and Technology (Japan), Sugino Machine Limited (Japan) and Kangwon National University (Korea).

Ref: Chaikaew, S., Maeno, Y., Visessanguan, W., Ogura, K., Sugino, G., Lee, S.-H. and Ishikawa, K. (2012). Application of thermophilic enzymes and water jet system to cassava pulp. *Bioresource Technology*, 126(1), 87-91.

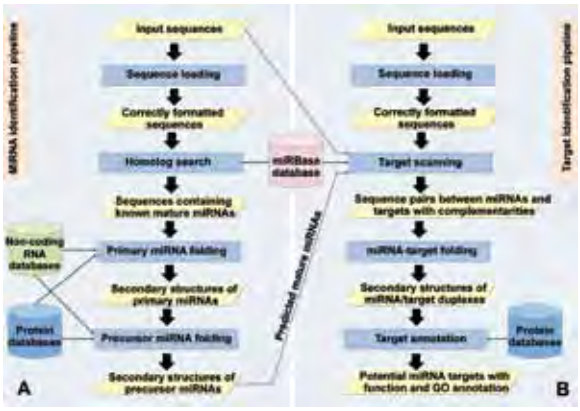
Simultaneous saccharification and co-fermentation of rice straw

Biofuels are considered potential clean alternatives, which can be produced as liquid, gas and/or solid fuels from lignocellulosic biomass. Lignocellulosic ethanol production generally requires several steps including pretreatment of biomass feedstock; saccharification process to release fermentable sugars; fermentation of released sugars and distillation step for ethanol separation. Since enzymatic hydrolysis and fermentation process of lignocelluosic hydrolysates requires multiple process parameters optimization to simultaneously determine the individual and interactive effects of many factors affecting ethanol production efficiency, researchers have optimized ethanol production from rice straw by simultaneous saccharification and co-fermentation (SSCF). Hydrolysis and fermentation process of *Saccharomyces cerevisiae* and *Scheffersomyces stipitis* co-culture were investigated.

The optimal saccharification solid loading was 5%. Key fermentation parameters for co-culture including cell ratio, agitation rate and temperature was rationally optimized using design of experiment (DoE). Optimized co-culture conditions for maximum ethanol production efficiency were at *S. cerevisiae*:*S. stipitis* cell ratio of 0.31, agitation rate of 116 rpm and temperature of 33.1°C. The optimized SSCF process reached ethanol titer of 15.2 g/L and 99% of theoretical ethanol yield. SSCF process under high biomass concentration resulted in high ethanol concentration of 28.6 g/L. This work suggests the efficiency and scalability of the developed SSCF process which could in turn provide an important basis for the economic feasibility of ethanol production from lignocelluloses.

This work involved collaboration between scientists from the Bioresources Technology Unit, Biochemical Engineering and Pilot Plant Research and Development Unit and the Joint Graduate School of Energy and Environment (JGSEE) at KMUTT.

Ref: Suriyachai, N., Laosiripojana, N., Champreda, V. and Unrean, P. (2013). Optimized simultaneous saccharification and co-fermentation of rice straw for ethanol production by *Saccharomyces cerevisiae* and *Scheffersomyces stipitis* co-culture using design of experiments. *Bioresource Technology*, 142, 171-178.



Workflow of C-mii: (A) miRNA identification pipeline and (B) target identification pipeline.

Optimization of fed-batch fermentation for ethanol production

Bioethanol can be produced from the fermentation of lignocellulosic biomass feedstock, which is composed of a mixture of hexose and pentose sugars. *Scheffersomyces stipitis*, an ascomycetous yeast, has been of potential interest as a biological catalyst for the production of lignocellulosic ethanol due to its ability to ferment a wide variety of sugars into ethanol. In fed-batch fermentation, microbial cells were allowed to grow at their maximum specific growth rate, which is often not optimized for ethanol production. The trade-off relationship between cell growth and ethanol production has been an apparent limitation in obtaining high ethanol production. Thus, the design of fed-batch fermentation conditions with optimized cell growth rate is essential for maximizing ethanol production performance by *S. stipitis*.

In this study, researchers investigated the effect of feed medium composition on sugar utilization and ethanol production. The process strategy was aimed at controlling the *S. stipitis* cultivation at an optimal growth rate through a fed-batch kinetic model to achieve high yield, high productivity, and high titer of ethanol during fed-batch cultivation. Substrate feed rates were calculated from the necessary parameters, such as optimized growth rate, specific sugar uptake rate, and yield of ethanol, which was obtained from chemostat and batch fermentation. The results of efficient ethanol fed-batch fermentation by *S. stipitis* from a mixture of glucose and xylose were reported. The information could be useful for improvement of ethanol fermentation from lignocellulosic biomass by *S. stipitis* at the industrial scale.

This work was conducted by the Biochemical Engineering and Pilot Plant Research and Development Unit.

Ref: Unrean, P and Nguyen, N.H.A. (2013). Optimized Fed-Batch Fermentation of *Scheffersomyces stipitis* for Efficient Production of Ethanol from Hexoses and Pentoses. *Applied Biochemistry and Biotechnology*, 169(6), 1895-1909.

Highlights from Information Systems and Bioinformatics

C-mii: Software for computational miRNA identificaion

MicroRNAs (miRNAs) play an important role in several biological processes in both animals and plants. Although several tools for miRNA and target identification are available, the number of tools tailored towards plants is limited, and those that are available have specific functionality, lack graphical user interfaces, and restrict the number of input sequences.

C-mii was developed in order to overcome these limitations and programming complexities. It is a ready-made software package for both plant miRNA and target identification. C-mii was designed and implemented based on established computational steps and criteria derived from previous literature with the following distinguishing features. Firstly, the software is easy to install with all-in-one programs and packaged databases. Secondly, it comes with graphical user interfaces (GUIs) for ease of use. Users can identify plant miRNAs and targets via step-by-step execution, explore the detailed results from each step, filter the results according to proposed constraints in plant miRNA and target biogenesis, and export sequences and structures of interest. Thirdly, it supplies a bird's eye view of the identification results with infographics and grouping information. Fourthly, in terms of functionality, it extends the standard computational steps of miRNA target identification with miRNA-target folding and GO annotation. Fifthly, it provides helper functions for the update of pre-installed databases and automatic recovery. Finally, it supports multi-project and multi-thread management. With these features, C-mii is a very useful software package that can help accelerate the study of plant miRNAs and targets by plant biologists. C-mii is freely available at http://www.biotec.or.th/isl/c-mii.

This software was developed by the Bioresources Technology Unit.

Ref: Numnark, S., Mhuantong, W., Ingsriswang, S. and Wichadakul, D. (2012). C-mii: a tool for plant miRNA and target identification. *BMC Genomics*, 13(Suppl 7), S16.

ChemEx: A tool for chemical information extraction

Accurate chemical data curation is essential for cheminformatics. Researchers or exploration software can access internal or external public databases to retrieve necessary information. Unfortunately, information in the literature is unstructured or semi-structured, and written in natural language. Chemical structures are often embedded in reports, journals, and patents in the form of images. These cannot be input into chemical databases or chemistry software directly. Manual reproduction of the information is time-consuming and liable to errors. Furthermore, rapid growth of publications results in difficulty to maintain up-to-date data sets. Automatic information extraction can help overcome these problems.

ChemEx is a software package developed to assist the chemical data curation process. While it can be used with general chemical information extraction, ChemEx is designed for extracting information of natural products which are a major source of novel bioactive compounds or structures. It provides a framework to integrate optical structure recognition and chemical text-mining software. The extracted information can then be visualized and exported to a database. Enormous chemical libraries become available with minimum time and effort. The software and corpus can be downloaded from http://www.biotec.or.th/isl/ChemEx.

This software was developed by the Bioresources Technology Unit.

Ref: Tharatipyakul, A., Numnark, S., Wichadakul, D. and Ingsriswang, S. (2012). ChemEx: information extraction system for chemical data curation. *BMC Bioinformatics*, 13(Suppl 17), S9.

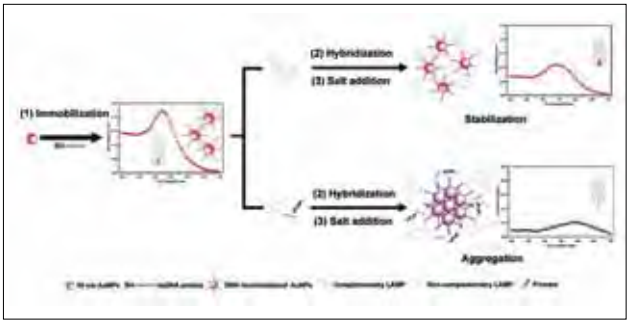
MetaSel: A metaphase selection tool

Identification of good metaphase spreads is an important step in chromosome analysis for identifying individuals with genetic disorders. The process of finding suitable metaphase chromosomes for accurate clinical analysis is, however, very time consuming since they are selected manually. The selection of suitable metaphase chromosome spreads thus represents a major bottleneck for conventional cytogenetic analysis.

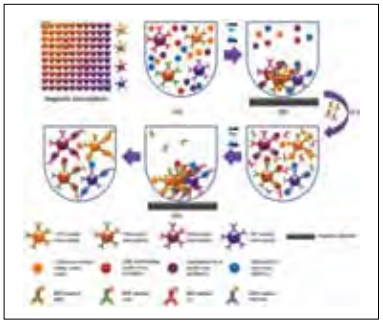
A software tool, termed MetaSel, was developed. Using the Gaussian-based rules, the tool can be used to quickly rank hundreds of chromosome spread images so as to assist cytogeneticists to perform karyotyping effectively. Furthermore, MetaSel offers an intuitive, yet comprehensive, workflow to assist karyotyping, including tools for editing chromosome (split, merge and fix) and a karyotyping editor (moving, rotating, and pairing homologous chromosomes). The program can be freely downloaded from http://www4a.biotec.or.th/GI/tools/metasel.

This software was co-developed by scientists from the Genome Institute, Mahanakorn University of Technology, the National Electronics and Computer Technology Center, Food and Drug Administration and Mahidol University.

Ref: Uttamatanin, R., Yuvapoositanon, P., Intarapanich, A., Kaewkamnerd, S., Phuksaritanon, R., Assawamakin, A. and Tongsima, S. (2013). MetaSel: a metaphase selection tool using a Gaussian-based classification technique. *BMC Bioinformatics*, 14(Suppl 16), S13.



Schematic illustration for the detection of WSSV using DNA-functionalized gold nanoparticles as probes.



Schematic of magnetic microsphere immunoassay.

Highlights from Diagnostic Technology

Shrimp disease detection based on LAMP and colorimetric assay

In the shrimp farming industry, the ability to detect diseases accurately, timely and conveniently is crucial as it enables farm operators to effectively manage the farm to minimize the spread of disease. White spot syndrome is one of the major diseases affecting the shrimp farming industry. Molecular techniques such as polymerase chain reaction (PCR) and nested PCR have been widely used and recommended as standard methods for the detection of WSSV. However, due to their complexity, time consumption, labor-intensiveness and the requirement for lab equipment, the techniques are not suitable for resource-limited areas and non-laboratory environments such as at the pond or station sites.

The research team had earlier developed an ultrasensitive assay based on the loop-mediated isothermal amplification (LAMP) reaction to detect WSSV under isothermal conditions using a simple heat block. In the latest study, researchers further developed a novel colorimetric assay for the detection of WSSV-LAMP amplified product, by employing gold nanoparticles (AuNPs). The principle of the LAMP–AuNPs relies on the stability characteristics of the DNA-functionalized AuNPs upon hybridization with their complementary target DNA against salt-induced aggregation. The results obtained could be detected qualitatively by visualization using the naked eye. In addition, an ultraviolet–visible spectroscopy can be used for more precise and quantitative results.

This work was conducted by research team at the Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp) and Mahidol University.

Ref: Seetang-Nun, Y., Jaroenram, W., Sriurairatana, S., Suebsing, R. and Kiatpathomchai, W. (2013). Visual detection of white spot syndrome virus using DNA-functionalized gold nanoparticles as probes combined with loop-mediated isothermal amplification. *Molecular and Cellular Probes*, 27(2), 71-79.

A multiplex microsphere immunoassay for plant pathogens

The seed industry is a primary industry which has a strong impact on the quantity and quality of agricultural products down the value chain. Important to this industry is an ability to detect and identify plant pathogens which is essential to epidemiological and disease management studies, selective breeding programs and disease-free certification for import and export. Conventional detection methods are time-consuming, laborious and require special skills such as taxonomy to identify the pathogen responsible for the particular disease.

Researchers have developed a novel multiplex detection method based on microsphere immunoassays to simultaneously detect four important plant pathogens: a fruit blotch bacterium *Acidovorax avenae* subsp. *citrulli* (Aac), chilli vein-banding mottle virus (CVbMV, potyvirus), watermelon silver mottle virus (WSMoV, tospovirus serogroup IV) and melon yellow spot virus (MYSV, tospovirus). An antibody for each plant pathogen was linked on a fluorescence-coded magnetic microsphere set which was used to capture the corresponding pathogen. The presence of pathogens was detected by R-phycoerythrin (RPE)-labeled antibodies specific to the pathogens. The assay was able to detect all four plant pathogens precisely and accurately with substantially higher sensitivity than enzyme-linked immunosorbent assay (ELISA). The assay time of the microsphere immunoassay is 1 hour, as opposed to 4 hours required by ELISA. This system was also shown to be capable of detecting the pathogens in naturally infected plant samples. The capacity of this microsphere immunoassay technique could be further expanded to higher throughput such as detecting up to 50 targets simultaneously. The system could also become fully automatic if dealing with a larger volume of routine testing.

The work was a collaborative effort between scientists from the Agricultural Biotechnology Research Unit and Queen's University Belfast (UK).

Ref: Charlermroj, R., Himananto, O., Seepiban,C., Kumpoosiri, M., Warin, N., Oplatowska, M., Gajanandana, O., Grant, I.R., Karoonuthaisiri, N. and Elliott, C.T. (2013). Multiplex Detection of Plant Pathogens Using a Microsphere Immunoassay Technology. *PLOS ONE*, 8(4), e62344.

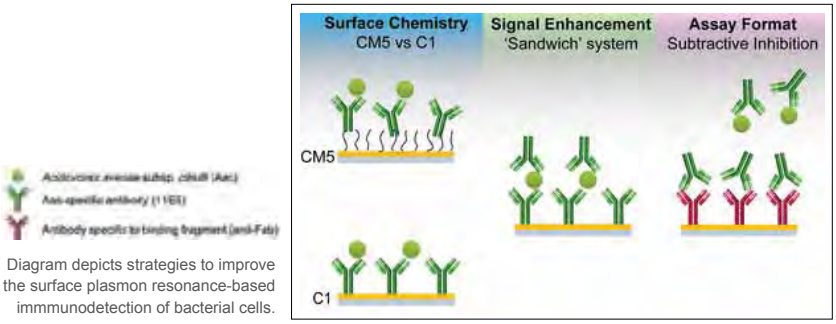


Diagram depicts strategies to improve the surface plasmon resonance-based immmunodetection of bacterial cells.

SPR immunosensor for the detection of a seedborne pathogen

ELISA methodology is commonly used for bacteria screening, however, this technique tends to be time-consuming and labor intensive. In contrast, SPR (Surface Plasmon Resonance) as a technique is an alternative which provides label-free, highly automated, real-time monitoring with rapid detection capabilities.

Researchers employed a number of different strategies to improve the sensitivity of SPR-based detection of bacteria. Two different sensor surfaces (carboxymethylated dextran CM5 and carboxymethylated C1) were compared. Direct detection, sandwich system and subtractive assay were investigated. A pathogenic seedborne bacterium *Acidovorax avenae* subsp. *citrulli* (Aac), the causative agent of fruit blotch in watermelon and cucurbits, was used as a model target in the assay development work. The study found that the combination of a carboxymethylated sensor chip C1 with the direct assay format resulted in the highest sensitivity for Aac, with a limit of detection of 1.6×10^6 CFU /mL. This SPR immunosensor was applied to the detection of Aac in watermelon leaf extracts spiked with the bacteria, and the lower limit of detection is 2.2×10^7 CFU /mL.

The work was a collaborative effort between scientists from the Agricultural Biotechnology Research Unit and Queen's University Belfast (UK).

Ref: Charlermroj, R., Oplatowska, M., Gajanandana, O., Himananto, O., Grant, I.R., Karoonuthaisiri, N. and Elliott, C.T. (2013). Strategies to improve the surface plasmon resonance-based immmunodetection of bacterial cells. *Microchimica Acta*, 180(7-8), 643-650.

Highlights from Policy Research

Status and direction of Thai shrimp industry

An analysis of Thailand's competitiveness throughout the value chain of the shrimp industry was made. The results revealed factors that lend Thailand to having certain advantages over other shrimp-exporting countries, especially countries concentrating on processed products. Some of these advantages are; geographical location, expertise of Thai shrimp farmers, advanced food processing technologies, reputation for food quality and food safety, experienced exporters and strong marketing supporters in major markets. The recent increase in minimum wage and shortage of labor in the manufacturing sector are preventing an expansion of labor-intensive export products. To sustain in this sector, Thailand needs to 1) allow market demand to dictate production, as over-production will drive the market price down; 2) use the nation's strengths in marketing, cultivation and processing technologies to be proactive in product innovation, to increase production of a premium-grade giant tiger shrimp, to develop business in ASEAN countries as a base for cultivation, processing and exporting; and 3) enhance capabilities in the following technologies: shrimp breeding program for both giant tiger shrimp and white shrimp, improvement in hatchery farms, develop a successful model to showcase holistic science-based shrimp cultivation.

This study was conducted by the BIOTEC policy research team in collaboration with experts from the Department of Fisheries, Kasetsart University, Thai Frozen Foods Association and NSTDA. Their report was adopted by the National Shrimp Cluster Board.

Shrimp farm in Phang Nga Province.

Biogas plant at Cholcharoen cassava starch factory.

Economic and social impact assessment of biogas technology and opportunities for expansion within ASEAN

This study focused on a campaign to promote biogas technology that was run by the Energy Policy and Planning Office (EPPO) from 2008-2110. It was found that the campaign had stimulated investment of 9.4 billion Baht and created 704 jobs, of which one third were engineers and scientists. From interviews conducted with 20 factories participating in the campaign, 88 million m^3 of biogas was produced yearly, accounting for 85% of the goal and worth approximately 570 million Baht per year. Biogas technology was credited for reducing odor problems, improving the livelihood of the surrounding communities, as well as resolving conflicts between the factory and the surrounding community. The study also investigated the potential of Thai industries for producing biogas, and the results revealed that only 63% of waste produced by the agriculture and food industry was utilized to produce biogas, with the sector producing wastes such as cassava pulp and oil palm empty fruit-bunches lagging behind other industries.

From analysis of return on the investment as well as current technology, it was found that industries such as palm oil and ethanol production, producing a high volume of waste with high COD, would take about 4-5 years to recover the investment and that it is more cost effective to use biogas for fuel oil substitution, rather than electrical production. Industries with a low volume of waste and containing materials toxic to microorganisms, such as some food industries and rubber latex, do not demonstrate good return in investment, due primarily to technological limitations.

Thailand has potential to promote this biogas technology to other ASEAN countries, specifically the oil palm industry in Malaysia and Indonesia; cassava processing industry in Indonesia and Vietnam; and sugar mill and bioethanol industry in Indonesia, the Philippines and Vietnam. Different models of promotion are proposed in the report, for each case, which are grouped into established high-growth industry/country and emerging industry/country.

Recommendations put forward to EPPO were: 1) increase the adoption of technology as well as assist factories that currently utilize biogas technology to make technological improvement; 2) support R&D to make improvement on efficiency of the technology; 3) review incentive measures; and 4) support technology transfer to other ASEAN countries.

COMMERCIALIZATION AND PRIVATE SECTOR PARTNERSHIP

BIOTEC places strong emphasis on the industrial application of biotechnology with both Thai and overseas companies. The main mechanisms used to promote bio-business in Thailand are technology and product licensing, along with collaborative and commissioned research with the private sector.

Licensing Agreements

Five licensing agreements have been signed:

Licensee	Technology
Mitr Phol Cane and Sugar Research Co., Ltd.	Process for humidity control and air exchange in-vitro
Mitr Phol Cane and Sugar Research Co., Ltd.	Method for in-vitro screening of rice blast-resistance under environmental control
Mobilis Automata Co., Ltd.	Detection of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) in shrimp by loop-mediated isothermal amplification (LAMP) combined with a lateral flow dipstick
Mobilis Automata Co., Ltd.	Detection of Yellow Head Virus (YHV) in shrimp by loop-mediated isothermal amplification (LAMP) combined with nanogold probe
The Peace Canning (1958) Co., Ltd.	Starter culture for industrial fermentation of pickled mustard greens

Collaboration with Companies

In fiscal year 2013, BIOTEC worked with 38 companies on 50 projects in the form of collaborative and commissioned research, as well as consultancy services. Of these, 28 projects were initiated in 2013. Collaborations and services covered a range of topics, such as screening for specific microorganisms, feasibility study of algal technology for fuel production, diagnostic development for detection of plant pathogens, production of antibodies, and the development of an industrial-scale production system for enzymes. Following are some of the highlights:

An agreement between BIOTEC and
Bioscience Animal Health signed on 18 September 2013.

Working with Bioscience Animal Health on Animal Feed Nutrition

BIOTEC and Bioscience Animal Health Co., Ltd. entered into an agreement to study a fermentation process of biomass to improve the level of nutrients in animal feed. The project aims to find an alternative source of protein for animal feed production from biomass, as animal protein becomes increasingly costly. Studies have shown that fermentation can improve protein content in biomass. A combination of expertise in microbial utilization and the rich resources of the BIOTEC Culture Collection will be exploited to develop a fermentation process for selected biomass, the result of which will contribute to the development of commercial feed supplement.

Bioscience Animal Health Co., Ltd. is a Thai company, providing innovative products, services and technologies related to animal health.

Working with KEEEN on Bioremediation Solution

BIOTEC and KEEEN Limited reached an agreement to jointly develop a small-scale mobile microbial reactor for on-site bioremediation. This reactor will allow for the on-site production of microorganisms used in the treatment of oil-contaminated wastewater. It will eliminate the cost of packaging and transportation of bioremediation agents, making it suitable for industrial sites that need to regularly manage oil-contaminated wastewater in high volume.

A 100-L reactor will be designed and equipped with automatic aerator as well as a suitable culture medium to produce oil-degrading microorganisms used for treating high-oil-content wastewater.

BIOTEC and KEEEN previously collaborated on the screening for effective oil-degrading bacteria for a commercial bioremediation product. KEEEN successfully launched bioremediation products in 2010, with products and services for various industries such as petrochemical industry, automobile industry, food industry, hotels and hospitals.



Open lab to industry consists of research presentation, lab tour and one-on-one discussion with research team.

Working with DMF (Thailand) to Serve Food Industry

An agreement between BIOTEC and DMF (Thailand), an expert company in designing functional and sensorial egg fractions, was reached to develop a customer-oriented test-validation protocol (CVP). The CVP is used to determine, optimize and standardize the functionalities of the egg fractions produced in a designated egg processing plant, to serve the local food market. Physical and chemical parameters of pasteurized liquid egg fractions will be investigated.

Open Lab for Industry

Open Lab is an activity to promote collaboration between laboratories and industries by inviting companies to meet with BIOTEC scientists, learn about BIOTEC expertise, and the potential of biotechnology for improving industrial processes and products. Open Lab often leads to collaborative research, commissioned research, or the provision of analytical services. In 2013, a total of 5 open lab events were organized:

NSTDA Open House: NSTDA Annual Conference (NAC) is a 3-day open-to-public event consisting of seminars and an exhibition. In 2013, part of the focus of NSTDA Open House included activities dedicated to introducing NSTDA as a solution provider to industry. NSTDA technologies encompass biotechnology and life sciences, materials technology, information technology and computer science, and nanotechnology. Two hundred companies -- in various industries such as food and health food, agriculture, IT, chemicals and energy -- participated in NSTDA Open House.

Open Lab to Shrimp Biotechnology Business Unit: This event introduced products (e.g., shrimp disease test kits and diatom culture unit) and services (e.g., aquaculture feed trials and shrimp disease detection services) to operators of cold storage and exporters of shrimp and other aquaculture products. Seven companies participated in this event.

Open Lab for Food Industry: Two events were organized targeting local food industry. A total of 39 companies took part in the two events.

Open Lab for the Federation of Thai Industries: The management of Thailand Science Park, which is also the location of BIOTEC headquarters and research units, invited members of the Federation of Thai Industries to visit selected NSTDA laboratories and tenant companies. Enzyme Technology Laboratory was introduced in this event due to its vast application to diverse industries. Eleven companies joined the activity.

HUMAN RESOURCES DEVELOPMENT

BIOTEC places a high priority on capacity building through increasing the quantity and quality of human resources in biotechnology as well as upgrading and educating the workforce. Several activities were designed to assist different segments of the workforce as well as address a variety of objectives, ranging from providing fellowships, to training post-graduate students, to organizing scientific conferences and training workshops for academics and industry, as well as organizing youth programs.

In 2013, BIOTEC granted 9 post-doctoral fellowships, 4 of which are new fellowships. A total of 27 events, including three international conferences, two national conferences, and twenty-two training workshops and seminars, were organized on various topics, such as LAMP technique, yeast biotechnology, biosafety of GM microorganisms in industry, drug design and diagnostic technology for pathogen detection.

The International Conference on Bioinformatics (InCoB) 2012 was held from 3-5 October 2012, with over 200 participants.

Training Course on Drug Development and Neglected Tropical Infectious Diseases was attended by 43 participants from 6 countries.

International Conference on Bioinformatics (InCoB) 2012

On 3-5 October 2012, the International Conference on Bioinformatics (InCoB) 2012 under the theme "From Biological Data to Knowledge to Technological Breakthroughs" was held at Central Plaza Ladprao Bangkok, Thailand. The local hosts of this event comprised NSTDA and BIOTEC, as well as King Mongkut's University of Technology (KMUTT). The conference aimed to promote the advancement of bioinformatics and computational biology and encourage the exchange of scientific breakthroughs amongst participants on the development of bioinformatics in the Asia Pacific region.

The keynote speakers in InCoB2012 included Professor Emeritus Jurg Ott (Head of Statistical Genetics Laboratory, Rockefeller University, USA), Dr. Tatsuhiko Tsunoda (Director of Medical Informatics Research Group, RIKEN Center for Genomic Medicine, Japan), Prof. Yongyuth Yuthavong (Senior Research Fellow, BIOTEC Medical Molecular Biology Research Unit, Thailand) and Prof. Sandra Leah Baldauf (Head of Systematic Biology Program, Uppsala University, Sweden).

Besides keynote lectures, InCoB 2012 also offered panel discussions and presentations from experts from governmental and private sectors. The content focused on the latest bioinformatics and related topics on agroinformatics, population genetics, drug design and discovery, systems biology, biological sequence analysis, expression data analysis, scalable data storage and other related subjects. In addition, there were pre-conference tutorials, special sessions and technical visits to BIOTEC and Thailand Science Park. The Conference was attended by 240 participants from 15 countries. It was announced on the last day of the Conference that InCoB 2013 will be held in China.

Other sub-programs held at this event include the 3rd International Conference on Computational Systems-Biology and Bioinformatics (CSBio2012), the main topic of which covered engineering sciences related to the pharmaceutical industry, in both material and manufacturing sciences, and the International Neural Network Society-Winter Conference 2012 (INNS-WC2012) featuring invited plenary talks by world-renowned speakers in the areas of neural network theory and applications, computational neuroscience, robotics, and distributed intelligence.

International Training Course on Drug Development and Neglected Tropical Infectious Diseases

A Training Course on Drug Development and Neglected Tropical Infectious Diseases was held on 27-31 May 2013. This five-day training course consisted of a series of presentations covering all aspects of the design of new medicines for neglected tropical infectious diseases. The course was conducted by Prof. Paul A. M. Michels from the Universite Catholique de Louvain, Belgium, Prof. Wim G. J. Hol and Assoc. Prof. Christophe L.M.J. Verlinde from the University of Washington, USA. The course was divided into two parts; lecture and computer lab training. The lecture covered the topics of parasitology, biochemistry, protein crystallography and structure-based drug design, while the computer lab training, held on the last day, focused on structure-based drug design using computer programs.

There were 43 participants attending this training course, 15 of whom were young scientists from China, Indonesia, Pakistan, the Philippines, Thailand and Vietnam who received financial support from the Asia-Pacific International Molecular Biology Network (A-IMBN) and BIOTEC.

Packaging room for organic vegetables produced by the Royal Project.

The First Biosafety and Bioethics Course in Thailand

BIOTEC and the Center for Agricultural Biotechnology (CAB), Kasetsart University, jointly developed a “Biosafety and Bioethics” course. This course was launched as an elective subject for a Doctoral Program in Agricultural Biotechnology in 2013 at CAB. The course includes the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, international and national biosafety regulations, biosafety guidelines for work related to modern biotechnology and genetic engineering, including risk assessment and management. BIOTEC researchers and technical officers took part in the teaching of this course.

Training Course on Curation and Management of Microbial Resources

Recognizing the importance of microorganisms, especially those isolated from natural environments in Thailand, a training course on “Curation and Management of Microbial Resources” was conducted in order to provide professional development for curators of culture collections in academic, research institutes, government agencies and industries, as well as to promote collaboration between the various culture collections and strengthen the human capacity of the participating culture collections in Thailand.

The course covers comprehensive topics such as microbial identification and preservation; database management, as well as legal framework on microbial research and management. The first Course was held on 14-17 January 2013 at BIOTEC, Thailand Science Park, with over 100 attendants.

This “Curation and Management of Microbial Resources” Course was the result of collaboration between BIOTEC, NITE Biological Resource Center (NBRC) of Japan and World Data Centre for Microorganisms (WDCM). Instructors consisted of experts from Thailand, Japan and China. The course will be offered annually and expanded to include international participants in the near future.

Capacity Building in GMP/GHP in Factories under the Royal Project

An initiative of His Majesty King Bhumibol Adulyadej, the Royal Project was conceptualized in 1969 with one intention to help develop occupation and income earning for hilltribe villagers, as an alternative to their traditional cultivation of opium poppies. Good agricultural practice and temperate crops suitable for growing in highlands were introduced. Over the years, the Royal Project has established a number of facilities to collect, distribute, and sell highland produce. Some facilities also include food processing activity. There are a total of 38 centers at present.

In 2013, BIOTEC, in collaboration with King Mongkut’s University of Technology Thonburi, launched a project to build up capacity in Good Hygiene Practice (GHP) and Good Manufacturing Practice (GMP) among staff working in the Royal Project’s facilities to ensure the safety of produce and products, as well as the 5S Process to ensure a safer, more efficient, and more productive operation. The activities include facility inspection, training sessions and regular follow-up. Inspection has been completed in 17 centers and the training was organized for staff in 7 centers in the 2013 fiscal year.

Buddhist novice monks during science workshop.

Buddhist novice monk presents his science project to HRH Princess Maha Chakri Sirindhorn during one of her regular visits to Nan province.

Organic vegetable garden and diet buddy contest are among the activities of "Safe Food and Good Health" in schools.

Promotion of Science Education in Monastic Schools

Under an initiative of HRH Princess Maha Chakri Sirindhorn to improve the quality of life in remote areas, one of the focuses is the support of education in monastic schools. In the rural area, a monastic school is perhaps the only option for poverty-stricken children to receive basic education.

BIOTEC supported the Princess's initiative by implementing activities to enhance science education in 52 monastic schools situated in Nan, Phrae, Phayao and Chiang Rai provinces. The activities include workshops for science teachers to improve teaching skills and organizing science contests and science camps. These monastic schools are also integrated into an eDLTV project, a NSTDA distance-learning project that provides a platform and creates learning materials in digital format freely accessible through internet and satellite television, made available at learning centers in remote areas.

Safe Food and Good Health in Schools

With funding provided by the Thai Health Promotion Foundation, BIOTEC launched a campaign to promote safe food and good health in schools in October 2012. The campaign supports schools to implement activities that will 1) increase fruit and vegetable consumption; 2) minimize food poisoning incidents; 3) increase physical exercise; and 4) reduce childhood obesity. A total of 32 selected schools, from northern, northeastern, central and eastern regions of Thailand and Bangkok Metropolitan, participate in the campaign. Activities are proposed by each school, to be implemented for one year throughout 2013. Examples of activities are vegetable garden and daily 10-minute aerobic routine in the morning.

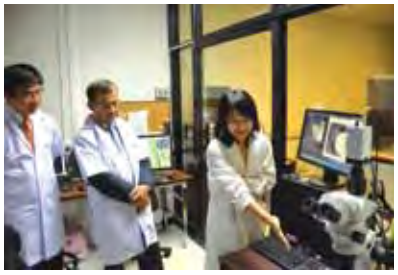
In parallel, BIOTEC also enlisted local universities in each region to provide technical advice to schools to safely operate facilities such as school kitchens, drinking water supply and waste disposal.

PUBLIC AWARENESS

One of BIOTEC's missions is to raise public awareness of how biotechnology and life sciences relate to everyday life. This is part of the larger goal of making Thailand a knowledge-based economy. The mission is carried out in part by channeling information through popular media such as the internet and television. BIOTEC also organizes exhibitions at various events, ranging from scientific conferences, as well as science and technology, agricultural and industry fairs. Lab tours are also organized to bring the public into BIOTEC research facilities where they can meet and talk to scientific staff.

BIOTEC invited journalists to visit farmers in the North who participated in the Thanyasirin seed production training program (left). An article in a local newspaper featured the story of Thanyasirin rice and farmers in the North (right).

HRH Princess Maha Chakri Sirindhorn paid a visit to the BIOTEC exhibition at the National Dairy Fair on 18 January 2013.



Science and Technology Minister Dr. Phiraphan Phalusuk visited BIOTEC laboratories.

Television Programs

In 2013, BIOTEC produced three television programs, ranging from a 1.5 minute spot to a 5 minute show, for regular broadcast on public channels as part of NSTDA TV. A total of 41 shows were produced and went on air, as well as made available for viewing on BIOTEC Youtube (http://www.youtube.com/biotecthailand). These television programs serve several purposes, from raising awareness of science, educating the public about the benefit of technologies developed locally, showcasing successful inventions that have reached the market, and introducing products and technologies to potential investors or collaborators.

Exhibitions

BIOTEC participated in a number of exhibitions and displays at major events, reaching out to several demographics and industrial sectors. Examples of key events were ECO Innovation and Solution in October 2012, Conference of the International Association of Science Park Asia-Pacific and West Asia Divisions (IASP Asia 2012) in November 2012, National Dairy Fair 2013 in January 2013, NSTDA Annual Conference in April 2013, National Rice and Farmers Day in June 2013, National Science and Technology Fair in August 2013, S&T Exhibition at the Parliament in August 2013 and TRF IP Expo in September 2013.

Open Lab

Thailand Science Park, home of BIOTEC and its five research units, is considered the largest R&D community in Thailand, with government-funded research labs and also private labs of several companies. With such a concentration of facilities, the Science Park makes an ideal study site for schools, universities, government and private organizations, as well as the general public. BIOTEC has an open-door policy and welcomes the public to tour our laboratory facilities on a regular basis.

In 2013, 130 groups toured BIOTEC laboratories. Visitors included the Institute of Forensic Training and Research, the Minister of Science and Technology, Members of Parliament, Thailand Board of Investment, journalists, students from Fukuoka Prefectural Jonan High School in Japan, participants in Asia Young Scientist and Engineer Advanced Study Program 2013 and the Agriculture Minister of Kelantan State in Malaysia.

INTERNATIONAL COLLABORATION

The BIOTEC International Cooperation Program aims to capitalize on international links to help BIOTEC and Thailand become a regional leader in the field of biotechnology. In so doing, the Center has developed close relations with overseas organizations at the bilateral, multilateral and regional levels. These relations are developed through formal collaborative agreements, organizing joint scientific seminars with international partners, hosting foreign scientists and students in laboratories, and organizing an annual meeting of the BIOTEC International Advisory Board.

Thailand Pavilion at the 2013 BIO International Convention in Chicago, USA.

Fostering Collaboration

In fiscal year 2013, BIOTEC signed three new Memorandums of Understanding (MOUs) and renewed four MOUs to foster collaboration with the following organizations:

Organization	Detail
Graduate School of Agriculture, Meiji University, Japan	To develop collaboration on agriculture and biosciences.
Institute of Microbiology and Biotechnology, Vietnam National University Hanoi, Vietnam	To collaborate on bioresource research, utilization and management.
Graduate School/School of Engineering, Osaka University, Japan	To promote collaboration on microbial biotechnology.
National Institute of Technology and Evaluation (NITE), Japan	To promote collaboration on the conservation and sustainable use of biological resources.
Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Slovakia	To foster collaboration on fermentation technology and bio-processing.
Queen's University of Belfast, Northern Ireland	To foster collaboration on food and agricultural biotechnology.
Atma Jaya Catholic University of Indonesia	To support the placement of undergraduate and graduate students from Atma Jaya Catholic University in BIOTEC laboratories.

Exhibitions in International Events

BIOTEC took part in an exhibition at two international events: 2013 BIO International Convention and Kelantan International BIO-Carnival Conference and Exhibition 2013.

The 2013 BIO International Convention was held on 22-25 April 2013 in Chicago, Illinois, USA. It is one of the largest biotechnology conventions with participation of industry leaders and research scientists from both government and the private sector from around the world. Thailand Pavilion was organized at the 2013 BIO International Convention with the leadership of Thailand Center of Excellence for Life Sciences (TCELS), and BIOTEC was among Thai organizations taking part in the Thailand Pavilion. BIOTEC showcased the new facilities to support translational research such as the National Biopharmaceutical Facility, Integrative Biorefinery Laboratory, Food and Feed Innovation Center and Thailand Bioresource Research Center.

Kelantan International BIO-Carnival Conference and Exhibition 2013 was held from 28 February-2 March 2013 in Kelantan, Malaysia. Kelantan is a Malaysian state that shares a border with Thailand in the south and therefore is a strategic partner in trade and development. In this connection, the BIOTEC exhibition displayed technologies related to agriculture and microbial utilization, which include: phytoremediation technology for saline land, the use of geographic information systems (GIS) for mapping soil salinity potential and BIOTEC Microbe Bank, which is a rich source for developing new products for agriculture and pharmaceutical industries. The display also showcased a number of commercial products developed by Thai scientists such as diagnostic tests for shrimp and plant diseases, animal feed, bioremediation agents, biocontrol agents (NPV) and enzymes for green pulp and paper bleaching process. Other agencies under Thailand's Ministry of Science and Technology joining this event included: National Innovation Agency, Thailand Institute of Scientific and Technological Research and the Institute of Nuclear Technology.

The 9th Annual Meeting of the Asian Consortium for the Conservation and Sustainable Use of Microbial Resources (ACM) was held on 25-27 October 2013 in Chiang Mai, Thailand.

Ratthaphol Charlermroj, PhD student under a joint project between BIOTEC and Queen's University Belfast, on the cover of Belfast Telegraph's graduation supplement (left). Ratthaphol is holding a multiwell-plate antibody array for simultaneous detection of multiple plant pathogens (right).

The 9th ACM Annual Meeting

BIOTEC hosted the 9th Annual Meeting of the Asian Consortium for the Conservation and Sustainable Use of Microbial Resources (ACM) on 25-27 October 2013 in Chiang Mai, Thailand. The 3-day meeting consisted of a conference on "Value Added Culture Collection" and a management meeting of member institutes.

The conference on "Value Added Culture Collection" addressed the applications of bioresources in various industries, as well as regulation and policy. Key speakers included experts from government institutes and private companies, such as the National Institute of Technology and Evaluation, Japan; the World Data Centre for Microorganisms; Esperanza Medicine Foundation; PTT Global Chemical and Institute for Innovation, Ajinomoto Co., Inc. The conference was open to the public and attended by 85 participants.

The ACM Annual Meeting provided an update on the activities of three Task Forces under the ACM: Biological Information Management, Human Resources Development, and Management of Material Transfer. This year, the ACM approved the membership of four new institutes, namely: Japan Collection of Microorganisms (JCM) of Japan, Biodiversity-Based Economy Development Office (BEDO) of Thailand, Microbial Type Culture Collection and Gene Bank (MTCC) of India and University of Santo Tomas of the Philippines.

ACM was established in 2004 by representatives of 12 Asian countries (Cambodia, China, Indonesia, Japan, Korea, Laos, Malaysia, Mongolia, Myanmar, the Philippines, Thailand and Vietnam). The Consortium aims to promote collaboration among government or public organizations in Asian countries in order to enhance the conservation and sustainable use of microbial resources in Asia. BIOTEC is one of the founding members and serves as a co-chair, with Japan, on the Task Force on Management of Material Transfer. The Center also hosted the 2nd ACM Annual Meeting back in 2005 in Bangkok.

Collaboration with Queen's University of Belfast

BIOTEC entered into a Memorandum of Understanding with Queen's University of Belfast (QUB) in 2010. Since the establishment of collaboration, considerable progress has been made as follows:

- A joint research project was set up at the onset of collaboration in 2010 to develop multiplex detection of plant diseases using antibody array technology and multiplex SPR. The project was conducted by scientists from BIOTEC Microarray Laboratory and the Institute for Global Food Security of QUB, combining QUB's expertise in SPR and assay development with BIOTEC's competence in microarray technology and unique collection of highly specific antibodies. Additionally, a sandwich PhD scholarship was obtained for a Thai student to work on this project. A multiwell-plate antibody array has been developed to simultaneously detect multiple plant pathogens. The new technology can test up to 96 samples and up to 4 pathogens all at the same time, offering convenience to testing at an industrial scale. It also consumes less reagent (antibody), and thus reduces the cost by 5 times compared to the conventional method. This multiwell-plate antibody array test is being validated in collaboration with a seed company before it can be released to the market. The Thai PhD student under this project graduated from the Institute for Global Food Security in 2012.

BIOTEC hosted a total of 28 researchers and 57 students from 12 countries in its laboratories in 2013.

- A joint project entitled "Pathfinder-Rapid and Reliable Detection of Foodborne Pathogens by Employing Multiplexing Biosensor Technology" commenced in 2011. The project focuses on the development of innovative means of detecting important pathogenic organisms associated with food poisoning using biosensor technology capable of detecting many different pathogens simultaneously. The project received funding from the European Union FP7 under the Marie Curie International Incoming Fellowship, awarded to a BIOTEC researcher to conduct the work at the Institute for Global Food Security of QUB from 2011-2013. The project has resulted in the identification of phage-based binders specific to *Salmonella* spp. and *Listeria monocytogenes* which can be used as alternative bioreceptors to the more expensive antibodies. The alternative phage-derived binders were successfully applied in various biosensor platforms such as SPR and bead array.
- A new project was launched in January 2013 to establish metabolomics technology in Thailand using known traits of Thai rice as a model. The project involved the transfer of expertise on metabolomics technology from the Institute for Global Food Security of QUB to BIOTEC. Specifically, the study focuses on generating metabolic profiles of important rice traits namely, brown planthopper resistance and aroma; identifying metabolite markers underlying those traits; and establishing a rice metabolomic database and customized analytical tools for data processing and analysis of the metabolomic data.

The MOU with Queen's University Belfast was renewed in March 2013 to continue and expand the collaboration in the field of agriculture and food sciences.

International Exchange Programs

A number of programs have been established to host foreign researchers and students in BIOTEC laboratories. These are:

- **Human Resource Development Program in Biotechnology for Asia Pacific.** Operating since 2001, this program provides fellowships to young researchers from developing countries in the Asia-Pacific region to undergo research-based training in BIOTEC laboratories for a period of 3-6 months. Approximately 10-15 fellowships are granted annually.
- **TWAS-UNESCO Associateship Scheme.** Under this scheme, BIOTEC becomes one of 100 Centers of Excellence in the South to offer fellowships to foreign researchers from the South for training at and fostering collaboration with BIOTEC.
- **International Student Internship Program.** BIOTEC offers internship placements in laboratories for undergraduate and graduate students from overseas. This includes students sent from our partner institutes, under an established agreement, as well as students applying directly to the program. Current partner institutes consist of Atma Jaya Catholic University, Temasek Polytechnic, National Taiwan University, Nanyang Polytechnic, University of Kent, Soon Chun Hyang University and City University of Hong Kong.

In 2013, BIOTEC hosted a total of 28 researchers and 57 students from 12 countries (Vietnam, Laos, Indonesia, the Philippines, Malaysia, Singapore, China, Taiwan, Japan, Korea, the UK and Nigeria).

IMPACT OF BIOTEC'S OUTPUT

Every year, a number of technology transfer projects are selected for detailed impact study. Impact is measured in terms income generated by our clients from products and technologies, and where appropriate, other factors such as import substitution and employment generation.

In 2013, 60 completed projects were selected for impact assessment. An estimated total of 2.3 billion Baht was generated from these projects. This amount was categorized as investment (432 million Baht), revenue generation to licensees or users (1.63 billion Baht), cost reductions (207 million Baht) and import replacement (76 million Baht).

Thanyasirin field in Nan province.

Homcholasit seed production site in Lampang province in collaboration with SCG.

Agriculture and Food Cluster

Homcholasit: flash-flooding tolerant rice. Homcholasit is an irrigated non-photoperiod sensitive rice, derived from a cross between KDML105 (jasmine rice) and IR57514. It was developed for flash flood tolerance, while maintaining the aromatic and good eating quality of jasmine rice, through marker-assisted selection technology.

In 2010, BIOTEC and NSTDA started working with Phakhai Agricultural Cooperative, providing Homcholasit seeds and training on rice seed production technology to member farmers. Phakhai district in Ayutthaya province is an area prone to flooding. The economic impact is calculated from the value of seed and grain produced from this seed which is sold as packed rice in major supermarket outlets. In 2013, the economic benefit is estimated at 18.6 million Baht, based on 213 tons of rice produced by 36 members on 468 rai (75 ha) of land.

The major flood in 2011 has prompted two major companies in Thailand, Siam Cement Group and Farm Chokchai Group to seek ways to assist rice farmers. Homcholasit was selected, leading to the establishment of collaboration between the companies, BIOTEC, NSTDA and Rajamangala University of Technology Lanna, to transfer seed and seed production technology to seed production sites, 174 rai (28 ha) in total. Seed was distributed to flood-effected farmers in Lampang, Nakhon Ratchasima, Saraburi, Chaiyaphum, Ayutthaya and Lopburi provinces. A total of 21.13 million Baht was estimated for economic benefit/impact from this activity in 2013.

Thanyasirin: blast-resistant glutinous rice. Thanyasirin is the RD6 cultivar, jasmine glutinous rice, with blast resistance added into its genome using molecular markers in the backcross breeding. While maintaining the superb cooking quality of RD6, Thanyasirin shows drastic improvement in leaf and neck blast resistance to a broad range of spectrum in the rainfed lowland areas.

Seed and seed production technology has been transferred to various sites in the north and northeast of Thailand, covering a total area of 151 rai (24 ha) in 2013. Approximately 600 tons of seed were produced. The value of this seed, as well as the grain produced, indicates the clear benefits of Thanyasiri, which is estimated to be 76 million Baht.

Plant disease detection. Pests and pathogens reduce both quantity and quality of Thai agricultural products. A fast, accurate detection system is also useful for selective breeding programs for pathogen-resistant plants and for certification of disease-free seeds for import and export. Development of a rapid, accurate and cost effective diagnosis system is therefore essential for crop production in Thailand. An ELISA test uses antibodies and color change to identify a particular substance, whereas an immunochromatographic strip test is a more user-friendly detection test.

In collaboration with academic partners, BIOTEC developed antibodies for ELISA tests for various plant pathogens such as geminiviruses, tospovirus, potyviruses and *Acidovorax avenae* subsp. A rapid strip test to detect *Acidovorax avenae* subsp. in water melon has also been developed and licensed to a company for evaluation. The antibodies are produced and sold to major seed companies. Easier disease detection enables seed farmers and seed companies to produce high quality seed. Diagnostic tests allow farmers to screen for disease-free seeds for plantations, and facilitate disease prevention and mitigation, resulting in improved production and loss prevention. It is also used for disease-free certification. These have resulted in better yields, and thus have generated more income for the seed industry sector. The economic impact from this initiative totalled 230 million Baht in 2013.

Immunochromatographic strip test for detection of *Acidovorax avenae* subsp. *citrulli*.

KEEEN bioremediation agent comes in a variety of formulas for various applications.

Improvement in dairy cattle reproduction rate. Only pregnant cows give milk and if a farmer can synchronize pregnancies in the herd the overall operation is much more efficient and profitable.

BIOTEC implemented projects to provide training, services and consultancies on hormone-based technology for ovulation synchronization and artificial insemination for both large and small-scale dairy farmers. The technology helps induce pregnancy in cows with reproductive system difficulties, increasing the value of each cow by up to 23,000 Baht. As cows begin lactating, farmers can earn an additional 68,000 Baht per animal. In 2013, training and services resulted in 4,145 pregnant cows with a total estimated impact of 324 million Baht.

Starter culture for the production of pickled mustard greens. Conventional production of pickled mustard greens depends on naturally-existing unidentified microorganisms which can create inconsistency in the product quality. To overcome this problem, starter culture can be used.

BIOTEC collaborated with Peace Canning Co., Ltd. to develop starter culture for industrial production of pickled mustard greens, which is the flagship product of the company. The research involved the screening for effective lactic acid bacteria for the fermentation and development of large scale production of this starter culture. The use of starter culture provides quality consistency and reduces losses from sub-standard products. Savings resulting from this technology in 2013 were estimated to be 3.05 million Baht.

Technology service for community enterprises. BIOTEC, in collaboration with alliances and local organizations, facilitated community enterprises and provided technical assistance through capacity building in agriculture, food processing, business planning and marketing development. As a result of the training, some communities were able to obtain food safety certification from FDA for their products or were able to develop new products. In 2013, economic impact on three sites was assessed, resulting in an estimated 36 million Baht from an increment of revenue and employment, as well as additional investment made into each enterprise.

Health and Medicine Cluster

Vaccine development and cell technology. BIOTEC/NSTDA and partner universities have successfully engineered the chimeric live-attenuated dengue viruses, which have good potential to be developed further as vaccine candidates for the safety and efficacy studies in a non-human primate model. In 2011, the chimeric strains were licensed to Bionet-Asia Co., Ltd. to further develop and produce the vaccine for testing in pre-clinical and clinical stages of vaccine development into a commercial product. BIOTEC also collaborated with Siam Bioscience Co., Ltd. to develop mammalian cell culture for the production of biologics.

These technologies have generated further investment from the private sector, reducing the cost of importing technology. Economic impact was estimated to 367 million Baht in total.

Energy and Environment Cluster

Bioremediation agent. Oil spills range from headline grabbing disasters of sinking supertankers to leaking motor oil on the garage floor. Cleaning up spills has always been messy, time consuming and expensive. Some microbes, mostly bacteria and fungi, can break down oil into carbon dioxide and water. Microbial cleaning agents are becoming increasing popular as an easy and effective cleaning method for oil spills large and small.

BIOTEC and KEEEN Co. Ltd. (previously known as Hi-Grimm Environmental and Research Co. Ltd.) collaborated on a joint research project to develop a commercial bioremediation product based on oil-degrading microbes. The technology was subsequently licensed to KEEEN for commercialization. In 2010, the company launched a blend of oil-degrading microbes under the trade name “KEEEN”. KEEEN comes in a variety of formulas for application in the petrochemical and automobile industries and in hotels and hospitals. The impact of this technology was estimated at 26 million Baht in 2013, based on company revenue and the value of import substitution.

Cholcharoen cassava starch factory.

Innovation technology for phytoremediation on saline land. Phytoremediation is the use of plants and good agricultural practices for improving saline soils. Using an *in vitro* environmental control system, a research team identified a number of salt-tolerant varieties of tropical plants, as well as some economic crops like rice and forest trees, such as eucalyptus. The remediation technology is a combination of salt-tolerant crops from BIOTEC Laboratory and soil treatment with organic and mineral supplements.

The project was implemented in collaboration with SCG, Pimai Salt Co. Ltd., Department of Mineral Resources, Land Development Department and Royal Forest Department. The project was able to turn saline land into arable land for rice and other crops in four provinces -- Sakon Nakhon, Udon Thani, Nakhon Ratchasima and Khon Kaen. Economic impact, based on the value of produce cultivated, totalled 115 million Baht in 2013.

Biogas technology in agro industry. Biogas is produced during the anaerobic processing of organic matter such as manure, plant matter, or even municipal waste materials. Biogas technology is thus a powerful technology to convert organic waste to a useable energy source.

Biogas technology for wastewater treatment and methane production was developed by EcoWaste, a joint lab between BIOTEC and King Mongut's University of Technology. The technology has been implemented in several agro-industry factories. In 2013, the economic impact of this technology in four cassava starch factories was 58 million Baht, based on energy savings.

Capacity building on efficiency for Thai native starch industry. Although Thailand is the world's largest cassava starch exporter, most of the factories still conduct their work based on individual on-the-job skills, causing repeated operational performance problems. This results in low productivity and a higher level of waste during the manufacturing process.

From 2009-2011, BIOTEC, NSTDA and GTZ launched a project to further increase the competitiveness of Thailand's tapioca starch industry through practical training and advice to starch factory owners. The project aimed to make improvements in production processes, pollution control mechanisms, waste recycling/re-use, and energy efficiency. In 2012, BIOTEC and NSTDA partnered with the Department of Industrial Promotion to implement the program in more factories and in 2013, nine factories participated in the program. A total of 166.12 million Baht in savings was estimated from waste reduction and improvements to machinery/process.

Near-zero waste cassava starch factory. Having built up the knowledge capacity in efficiency improvement in cassava starch industry, EcoWaste introduced the near-zero waste concept to the industry. Chol Charoen factory participated in the project to create a model of near-zero waste cassava factory. Improvements to the existing machinery were made to increase the production and resource efficiency, and biogas technology was used to treat waste, as well as generate energy for the factory. In 2013, the savings and profit increase were 80.74 million Baht in total.

Appendices

- List of Publications
- List of Intellectual Properties
- Honors and Awards
- Executives and Management Team

List of Publications

1. Aksoy, M., Pootakham, W., Pollock, S.V., Moseley, J.L., González-Ballester, D. and Grossman, A.R. (2013). Tiered Regulation of Sulfur Deprivation Responses in *Chlamydomonas reinhardtii* and Identification of an Associated Regulatory Factor. *Plant Physiology*, 162(1), 195-211.

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List of Intellectual Properties

List of Issued Intellectual Properties

Title	Granting Date	Ref. No.	Country
Patent			
Shrimp pond with water treatment system	2 November 2012	34215	Thailand
Production of human growth hormone in *Pichia pastoris*	3 June 2013	35877	Thailand
Antimalarial compounds with flexible side-chains	10 September 2013	US8530591	USA
Petty Patent			
Detection method for white spot syndrome virus, WSSV	15 October 2012	7611	Thailand
Detection method for Infectious hypodermal and hematopoietic tissue necrosis virus, IHHNV	15 November 2012	7607	Thailand
Detection method for Monodon Baculovirus, MBV	15 November 2012	7608	Thailand
Detection method for Hepatopancreatic parvo-like virus,HPV	15 November 2012	7609	Thailand
Detection method for *Macrobrachium rosenbergii* nodavirus, *Mr*NV	15 November 2012	7610	Thailand
Method to induce spore germination in wild mushroom	31 January 2013	7812	Thailand
Pre-treatment process of cassava materials for thermoplastic starch production	8 February 2013	7846	Thailand
Novel detection method for Hepatopancreatic parvo-like virus,HPV	6 June 2013	8071	Thailand

List of Applied Intellectual Properties

Title	Filing Date	Ref. No.	Country
Patent			
Method for preparing feed with double-stranded RNA to reduce slow growth in black tiger shrimp	18 January 2013	1301000289	Thailand
DNA identification method from biological trace evidence	6 March 2013	1301001122	Thailand
Confidential			USA
Process to extract lignin from pulp mill black liquor	12 September 2013	1301005102	Thailand
An assistive cytogenetic tool for automatically sorting good metaphase images for karyotyping	13 September 2013	1301005121	Thailand
Process to increase air bubble in solid media for tissue culture	19 September 2013	1301005228	Thailand
Medium formulation for fungal cultivation and process for arachidonic acid production by solid state fermentation	26 September 2013	1301005402	Thailand
DNA detection using gold nanoparticles to amplify SPR signal	30 September 2013	1301005534	Thailand
Methods for simultaneous identification of tospoviruses and thrips species in an individual thrips	30 September 2013	1301005546	Thailand
Confidential			Thailand
Petty Patent			
Detection of white spot syndrome virus, WSSV, with LAMP-LFD technique	2 November 2012	1203001275	Thailand
Production process for enzyme - resistant rice starch type III	17 January 2013	1303000062	Thailand
Direct screening for selected clones from BAC and fosmid libraries preserved in frozen glycerol tocks using PCR method	28 March 2013	1303000340	Thailand
Monoclonal antibodies specific to *Listeria monocytogenes*	22 May 2013	1303000538	Thailand
Detection of YHV/GAV virus in shrimp	30 May 2013	1303000584	Thailand
Detection of 6 genotypes of yellow head virus	30 May 2013	1303000585	Thailand
Culture medium for growth of fungi isolated from marine and mangrove habitats	30 May 2013	1303000586	Thailand
Modified DigiTag2 conditions, primers, and probes set for screening species of *Mycobacterium tuberculosis* complex (MTBC) and genotyping *Mycobacterium tuberculosis*	13 June 2013	1303000644	Thailand

Title	Filing Date	Ref. No.	Country
Primers specific to lysogenic bacteriophages of *Bacillus* spp. and their applications	11 July 2013	1303000765	Thailand
Primers specific to lytic bacteriophages of *Bacillus* spp. and their applications	11 July 2013	1303000769	Thailand
Production of bolete spawn (Thaeogyroporus porentosus (berk. ET. Broome)) on semi-solid culture media	30 July 2013	1303000839	Thailand
Species-specific DNA probes for nucleocapsid gene of CaCV, MYSV, TNRV and WSMoV	30 July 2013	1303000840	Thailand
Universal primers for nucleocapsid gene of CaCV, MYSV, TNRV and WSMoV	30 July 2013	1303000841	Thailand
Method for multiplex detection and identification of CaCV, MYSV, TNRV and WSMoV using RT-PCR-ELISA	30 July 2013	1303000842	Thailand
Immunomagnetic separation method for separation of *Listeria monocytogenes*	8 August 2013	1303000874	Thailand
Gene expression system for construction of recombinant yeasts directly produce ethanol from cellulose and hemicellulose	22 August 2013	1303000946	Thailand
Method to induce mutation in plant	22 August 2013	1303000943	Thailand
Breeding flood-tolerant rice by mutation	22 August 2013	1303000944	Thailand
Method to induce mutation in plant tissue culture under controlled environment	29 August 2013	1303000993	Thailand
Primers and internal amplification control DNA fragment for detection of *Listeria monocytogenes* by real-time PCR	5 September 2013	1303001027	Thailand
Culture media to increase sporulation of blast pathogen	26 September 2013	1303001183	Thailand
Immunoassay based on flocculation of graphene oxide particles	30 September 2013	1303001235	Thailand
Specific primers for identification of thrips species found in pepper, tomato and cucurbits fields in Thailand	30 September 2013	1303001236	Thailand
Non-autoclave tissue culture media	30 September 2013	1303001238	Thailand
Trade Secret			
The use of *Bacillus* sp. BCC 27846 for animal feed supplement production	5 October 2012		Thailand
Enzyme production process for plant biomass hydrolysis	15 March 2013		Thailand
Industrial-scale production of pickled mustard greens using starter lactic acid bacteria	12 July 2013		Thailand

Honors and Awards

Dr. Wanilada Rungrassamee
Agricultural Biotechnology Research Unit

Individual Research Grant for a project on "Study of relevance of heat shock protein 90 to the female reproduction of the black tiger shrimp (*Penaeus monodon*)", awarded by the International Foundation for Science (IFS).

Dr. Vanvimon Saksmerprome
Center of Excellence for Shrimp Molecular Biology and Biotechnology

Individual Research Grant for a project on "Formulation of shrimp feed for prevention and inhibition of Lame-Singh virus infection in the black tiger shrimp (*Penaeus monodon*)", awarded by the International Foundation for Science (IFS).

Dr. Alongkorn Amnuaykanjanasin
Bioresources Technology Unit

Individual Research Grant for a project on "Chemotypic and physiological analysis of mutants disrupted in insect-specific clade III polyketide synthase gene for identification of the crucial factor for insect pathogenesis", awarded by the International Foundation for Science (IFS).

Dr. Sansanee Noisakran
Medical Biotechnology Research Unit

L'Oreal Thailand-UNESCO "For Women in Science 2012" fellowship (life science) for the work on "The investigation of the mechanisms of dengue virus infection and host cellular responses against the infection, awarded by L'Oreal Thailand and the Thai National Commission for UNESCO.

Assoc. Prof. Apichart Vanavichit
Rice Gene Discovery Unit

NSTDA Research Chair Grant 2012 for a project on "Whole genome mutagenesis to enhance rice breeding potential for climate change", awarded by the National Science and Technology Development Agency (NSTDA)

Biotechnology Researcher Award 2013, for outstanding achievements in applying research to industrial and societal benefit, presented by Biogenomed Co., Ltd.

Assoc. Prof. Nopporn Sittisombut
Medical Biotechnology Research Unit

Outstanding Technologist Award 2012 for achievements in dengue vaccine development, awarded by the Foundation for the Promotion of Science and Technology under the Patronage of His Majesty the King.

Rice improvement through biotechnology project
Rice Gene Discovery Unit

"Excellence in Science" Award 2012 by the Committee on Science, Technology, Communications and Telecommunications of the Senate.

Transfer of Biogas Technlogy Utilizing Waste from Agro-Industry Project
Excellent Center of Waste Utilization and Management

"Excellence in Science" Award in 2013, awarded by the Committee on Science, Technology, Communications and Telecommunications of the Senate.

Prof. Yongyuth Yuthavong
Medical Molecular Biology Research Unit

"Excellence in Science" Award 2013 for his accomplishment in the development of anti-malarial drug, awarded by the Committee on Science, Technology, Communications and Telecommunications of the Senate.

Dr. Thidarat Nimchua, Dr. Verawat Champreda, Dr. Lily Eurwilaichitr, Mr. Phitsanu Pinmanee and Mr. Nakul Rattanaphan
Bioresources Technology Unit

"Excellence in Science" Award 2013 for her accomplishment in applying enzyme technology for pulp and paper industry, awarded by the Committee on Science, Technology, Communications and Telecommunications of the Senate.

Dr. Satinee Suetrong
Bioresources Technology Unit

Taguchi Award 2012 for excellent Ph.D. dissertation an "Molecular systematic of the marine *Dothideomycetes*", awarded by the Thai Society for Biotechnology

Dr. Thidarat Nimchua, Dr. Verawat Champreda, Dr. Lily Eurwilaichitr, Mr. Phitsanu Pinmanee, Ms. Benjarat Bunterngsook, Ms. Supattra Kitikhun and Mr. Nakul Rattanaphan
Bioresources Technology Unit

Gold Medal for "ENZbleach: alkaline-tolerant enzyme for pulp bleaching process", presented at the 2012 Taipei International Invention Show & Technomart (INST2012), 20-23 September 2012, Taiwan.

Gold Medal (Environmental Protection Field) for "ENZbleach: alkaline-tolerant enzyme for pulp bleaching process", presented at the 41st International Exhibition of Inventions Geneva, 12-14 April 2013, Switzerland.

Ms. Wansika Kiatpathomchai and Mr. Narong Arunrut
Center of Excellence for Shrimp Molecular Biology and Biotechnology
In collaboration with Faculty of Medicine, Srinakharinwirot University and Innovative Learning Center, Srinakharinwirot University

Two Silver Medals (Health Field) for two types of TB-DNAsensor kits for diagnosis of *Mycobacterium tuberculosis* (MTB), presented at the 41st International Exhibition of Inventions Geneva, 12-14 April 2013, Switzerland.

Gold Prize for "DNA-dipstick test kit for diagnosis of *Mycobacterium tuberculosis",* presented at the Seoul International Invention Fair 2012, 29 November-2 December 2012, Seoul, Korea.

Dr. Phanramphoei Namprachan Frantz
Agricultural Biotechnology Research Unit

Winner of EURAXESS Science Slam ASEAN 2013, a competition for a researcher to present his/her research project to their peers and the wider public.

Dr. Jarunee Vanichtanankul
Medical Molecular Biology Research Unit

Outstanding Poster Presentation for "Investigation of Innate-antifolate Resistance of *Trypanosoma Brucei* ", presented at the 13th FAOBMB International Congress of Biochemistry and Molecular Biology, 25-29 November 2012, Bangkok, Thailand.

Dr. Sissades Tongsima
Genome Institute

Best Paper Award for "METASEL: A metaphase selection tool using Gaussian-based classification technique", presented at the International Conference on Bioinformatics 2013, 20-22 September 2013, Taicang, China.

Dr. Wonnop Visessanguan
Food Biotechnology Research Unit

NRCT Research Award 2012 (Agricultural Science and Biology Category) for "Development of Biological Process for Histamine Degradation in Fish Sauce by Histamine Dehydrogenase from *Natrinema gari* BCC 24369", awarded by the National Research Council of Thailand.

Dr. Oraprapai Gajanandana
Agricultural Biotechnology Research Unit

NRCT Invention Award 2012 (Agricultural Science and Biology category) for "Diagnostic tests for detection of bacterial fruit blotch in cucurbits", awarded by the National Research Council of Thailand.

Dr. Chunya Puttikhunt
Medical Biotechnology Research Unit

NRCT Invention Award 2012 (Medical Science Category) for "Serotyping-NS1-ELISA: A method for detection of dengue NS1 protein and serotyping of dengue virus simultaneously", awarded by the National Research Council of Thailand.

Dr. Thidarat Nimchua
Bioresources Technology Unit

NRCT Invention Award 2012 (Engineering and Industry Category) for "ENZbleach: alkaline-tolerant enzyme for pulp bleaching process", awarded by the National Research Council of Thailand.

Mr. Sombat Rukpratanporn
Agricultural Biotechnology Research Unit
In collaboration with Srinakharinwirot University

NRCT Research Award 2012 (Agricultural Science and Biology Category) for "Development of Shrimp Viral Disease Detection using Monoclonal Antibodies", awarded by the National Research Council of Thailand

Dr. Pornkamol Unrean
Biochemical Engineering and Pilot Plant Research and Development Unit

NRCT Dissertation Award 2013 (Engineering and Industry Category) for "Strain Optimization Through Theoretical and Experimental Tools", awarded by the National Research Council of Thailand

Dr. Piti Amparyap
Center of Excellence for Molecular Biology and Genomics of Shrimp

NRCT PhD Dissertation Award 2012 to Dr. Walaiporn Charoensapsri, for her thesis titled "Characterization and Functional Analysis of Prophenloxidase System-associated Genes from the Black Tiger Shimp *Penaeus monodon*", with Dr. Piti Amparyap as a co-advisor.

Dr. Chairat Uthaipibull and Dr. Sissades Tongsima
Medical Molecular Biology Research Unit and Genome Institute

Paper titled "An automatic device for detection and classification of malaria parasite species in thick blood film" was hightlighted on A-IMBN Research, selected by the Asia-Pacific International Molecular Biology Network (A-IMBN).

Dr. Sutipa Tanapongpipat, Ms. Peerada Promdonkoy, Dr. Niran Roongsawang and Dr. Lily Eurwilaichitr
Bioresources Technology Unit
In collaboration with Mahidol University and National Institute of Advanced Industrial Science and Technology (Japan)

Paper titled "Heterologous protein expression in *Pichia thermomethanolica* BCC16875, a thermotolerant methylotrophic yeast and characterization of *N*-linked glycosylation in secreted protein" was hightlighted on A-IMBN Research, selected by the Asia-Pacific International Molecular Biology Network (A-IMBN).

Ms. Suchada Mongkolsamrit, Dr. Noppol Kobmoo, Ms. Kanoksri Tasanathai, Mr. Artit Khonsanit, Ms. Wasana Noisripoom, Mr. Prasert Srikitikulchai, Mr. Rathasart Somnuk and Dr. Janet Jennifer Divinagracia Luangsa-ard.
Bioresources Technology Unit

Paper titled "Life cycle, host range and temporal variation of *Ophiocordyceps unilateralis /Hirsutella formicarum* on formicine ants" was hightlighted on A-IMBN Research, selected by the Asia-Pacific International Molecular Biology Network (A-IMBN).

Prof. Yongyuth Yuthavong, Dr. Bongkoch Tarnchompoo, Dr. Penchit Chitnumsub, Dr. Sumalee Kamchonwongpaisan, Ms. Supannee Taweechai, Dr. Jarunee Vanichtanankul, Ms. Roonglawan Rattanajak, Mr. Uthai Arwon and Dr. Chawanee Thongpanchang
Medical Molecular Biology Research Unit and Bioresources Technology Unit
In collaboration with Chulalongkorn University, Mahidol University, Monash University (Australia), University of London (UK), Medicines for Malaria Venture (Switzerland)

Paper titled "Malarial dihydrofolate reductase as a paradigm for drug development against a resistance-compromised target" was hightlighted on A-IMBN Research, selected by the Asia-Pacific International Molecular Biology Network (A-IMBN).

Executives and Management Team

Executive Board

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Naksitte Coovattanachai	Advisor to Secretary-General, National Science Technology and Innovation Policy Office (STI)
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Sermpol Ratasuk	Expert on organization planning and environmental impact assessment

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Vice Chairman

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Pachok Pongpanich	Executive Committee, Thai Seed Trade Association -THASTA Managing Director, PacThai (Pacific Seeds Co., Ltd.)
Rutjawate Taharnklaew	Assistant Vice President, R&D Center, Betagro Group
Kittiphong Limsuwannarot	Vice President of Green Chemicals, PTT Global Chemical Public Company Limited
Kanyawim Kirtikara	Executive Director, BIOTEC
Dussadee Siamhan	Deputy Executive Director, BIOTEC

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Chairman

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Members

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Gerald T. Keusch	Professor of International Health and of Medicine, Boston University School of Public Health, USA
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Jens Nielsen	Department of Chemical and Biological Engineering, Chalmers University of Technology, SWEDEN
Sang-Ki Rhee	Executive Director, SCH Center for BioPharmaceutical Research and Human Resources Development and Dean, College of Medical Sciences, Soon Chun Hyang (SCH) University, KOREA
Jean-Marcel Ribaut	Director, Generation Challenge Programme (GCP), MEXICO

Management Team

Kanyawim Kirtikara
Executive Director

Dussadee Siamhan
Deputy Executive Director

Suvit Tia
Deputy Executive Director

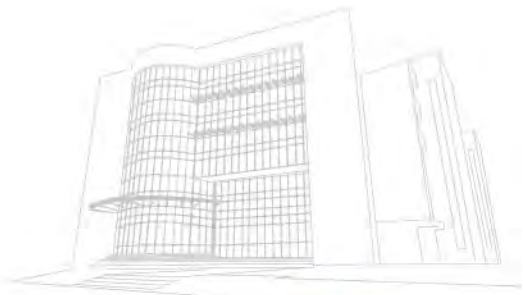
Lily Eurwilaichitr
Deputy Executive Director and
Director, Bioresources Technology Unit

Somvong Tragoonrung
Director, Genome Institute

Sumalee Kamchonwongpaisan
Director, Medical Molecular Biology Research Unit

Sirawut Klinbunga
Director, Agricultural Biotechnology Research Unit

Wonnop Visessanguan
Director, Food Biotechnology Research Unit



BIOTEC
a member of NSTDA

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