BIOTEC
a member of NSTDA

2012 Annual Report

National Center for Genetic Engineering and Biotechnology

Annual Report 2012

National Center for Genetic Engineering and Biotechnology (BIOTEC)

This report was prepared according to the 2012 fiscal year of the Royal Thai Government, from 1 October 2011 – 30 September 2012.

Annual Report 2012

National Center for Genetic Engineering and Biotechnology (BIOTEC)

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Message from the

BIOTEC Executive Director

Thailand endured significant hardships due to a major flood in October-November 2011. Our headquarters and most of our laboratories stopped operating for over two months. During the flood, we put all our efforts into saving the irreplaceable BIOTEC Culture Collection, which houses more than 50,000 strains of microbes isolated within Thailand during the past 20 years. Despite the temporary lost of electricity and difficulty in transporting liquid nitrogen during a flood, we were able to save the entire collection. Our researchers have put in tremendous efforts to compensate for the time lost and fiscal year 2012 turned out to be quite rewarding. We obtained international funding and received several special recognitions both at the local and international levels.

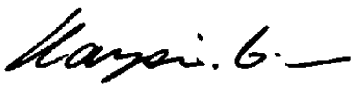
The research team at the Cassava and Starch Technology Research Unit, led by Dr. Kuakoon Piyachomkwan, participated in a collaborative project which was approved for FP7 research funding for 2012-2015. In the "Gains from Losses of Roots and Tuber Crops Project" (Gratitude), BIOTEC research team will use its expertise to make improvements in the use of cassava waste and demonstrate the technology in a Thai SME. The Gratitude project has 16 organizations from Europe, Africa and Asia participating in. Dr. Sithichoke Tangphatsornruang, Head of BIOTEC Genomic Research Laboratory, was selected for the prestigious Young Scientist Award given by the Foundation for the Promotion of Science and Technology under the patronage of His Majesty the King. The dedication of the rice research team has prompted the National Identity Board to present Dr. Apichart Vanavichit, Director of Rice Gene Discovery Unit with the "2012 Person of the Year" award in Science and Technology.

An innovative idea of Dr. Srung Smanmoo to develop a diabetes test kit based on a breath analyzer earned a local True Innovation Award and went on to win an audience award at the Intel–DST Asia Pacific Challenge in India. Enzbleach, an alkaline-tolerant enzyme for pulp bleaching invented by the BIOTEC research team led by Dr. Thidarat Nimchua, was awarded a gold medal at the 2012 Taipei International Invention Show & Technomart (INST2012). KEEEN, a bioremediation agent resulting from a collaboration between Dr. Somkiet Techkarnjanaruk and Hi-Grimm Environmental and Research Co. Ltd., took home awards from several international competitions throughout the year, including the 2012 Taipei International Invention Show & Technomart, ITEX 2012 (International Invention, Innovation and Technology Exhibition) in Malaysia, and the 16th Asian Science Park Association Annual Conference in Vietnam. These accolades demonstrate our commitment to innovative research with real applications.

In addition to excellence in science, BIOTEC strives for excellence in operation. This year, our Center was presented with the Thailand Energy Award 2012 in the category of "Plants and Buildings for Energy Efficiency Improvement". This was the result of introducing new equipment for energy saving, and also a concerted effort on the part of all BIOTEC staff in supporting the energy saving campaign.

The severe flood in 2011 prompted BIOTEC to formulate a business continuity plan for our research and administration operations. A main feature of this plan is to establish a back-up location for our biological resources bank, which includes microbial cultures and biomaterials such as cell lines, and hybridoma cells. Two locations were identified at the NSTDA building in Central Bangkok and Kampaengsaen Campus of Kasetsart University. Renovation and equipment installation have been completed and the preparation of cultures to be deposited in these two facilities is progressing as planned. This will provide considerable assurance that our biological assets will be preserved in the event of unexpected incidents.

In 2013, we will open three new laboratories at the new complex in the Thailand Science Park: a Food and Feed Innovation Center, an Integrative Biorefinery Laboratory, and the Thailand Bioresource Research Center. These three laboratories will serve as a platform for BIOTEC to work more closely with industrial partners. With this expansion, we look forward to continued supports from our partners as well as welcoming new opportunities with new alliances.



Dr. Kanyawim Kirtikara
Executive Director, BIOTEC

FACTS AND FIGURES

1983 BIOTEC is established under the Ministry of Science, Technology and Energy on 20 September.

1984 1985 1986 1987 1988 1989 1990

1991 BIOTEC becomes an NSTDA center operating outside the normal framework of the civil service and state enterprises. This enables BIOTEC to operate more effectively to support and transfer technology for the development of industry, agriculture, natural resources, environment for the social and economic well being of Thai people.

1992

Other centers under the NSTDA umbrella…

NANOTEC	National Nanotechnology Center
NECTEC	National Electronics and Computer Technology Center
MTEC	National Metal and Materials Technology Center
TMC	Technology Management Center

Our activities

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 range of research topics. 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 include policy research, an outreach program, human resource development and international relations.

Our research and development units

BIOTEC Research Units at Thailand Science Park

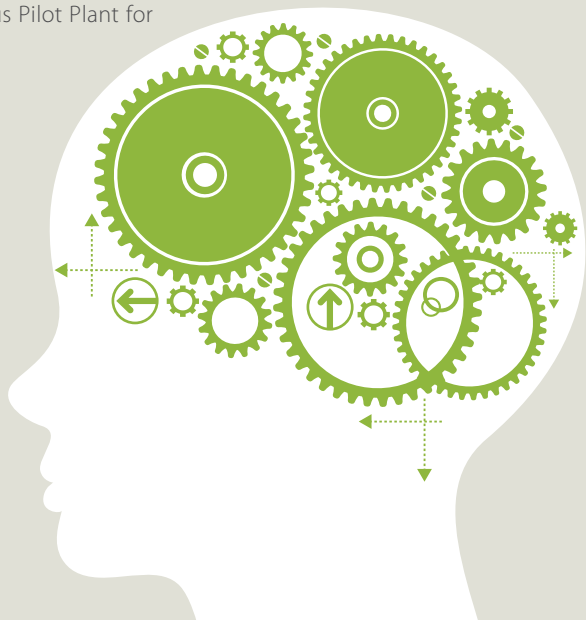
- Bioresources Technology Unit
- Agricultural Biotechnology Research Unit
- Food Biotechnology Research Unit
- Medical Molecular Biology Research Unit
- Genome Institute

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 Siriraj Hospital
- 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 Hala-Bala Rain Forest Research Unit- jointly established with the National Park, Wildlife and Plant Conservation Department and located in Narathiwat Province

Development Units

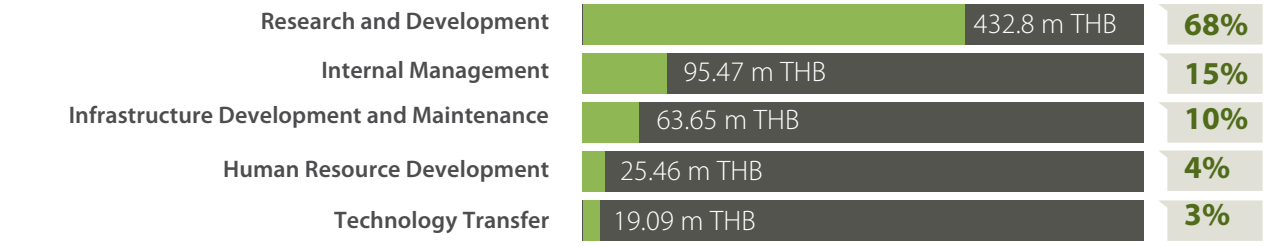
- Shrimp Biotechnology Business Unit (SBBU)
- Dairy Cattle Research and Business Development Project
- Shrimp Genetic Improvement Center (SGIC)
- Nuclear Polyhedrosis Virus Pilot Plant for Insect Pest Control

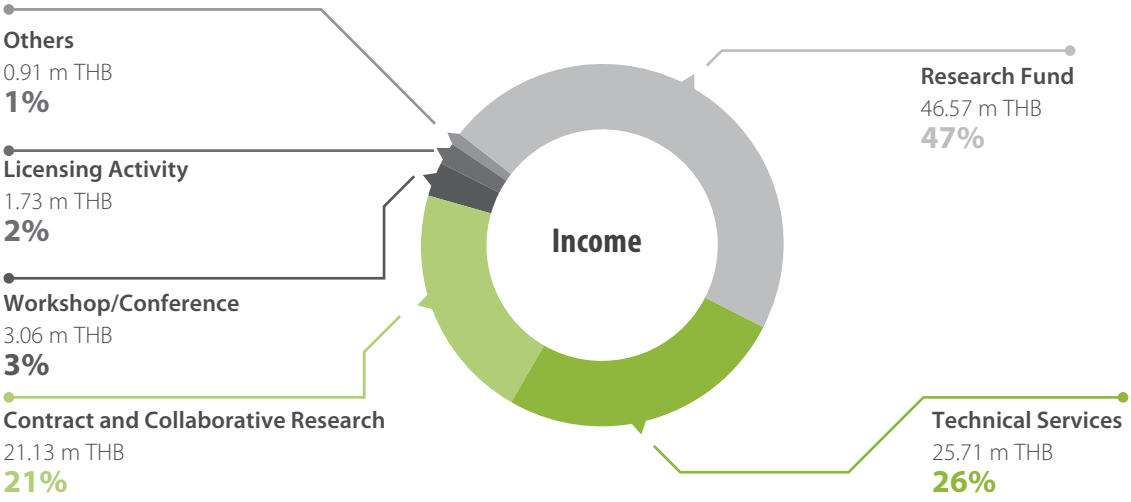


Finances

For fiscal year 2012, BIOTEC's budget totaled **772.68** million Thai Baht (m THB).
Expenditures totaled **636.47** m THB,
and the Center earned an income of **99.11** m THB
from sources outside NSTDA.

Expenditures

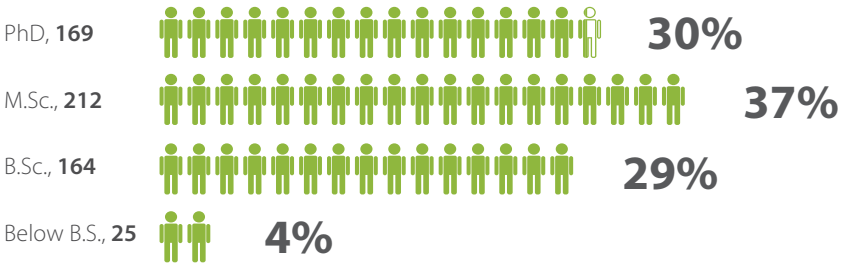




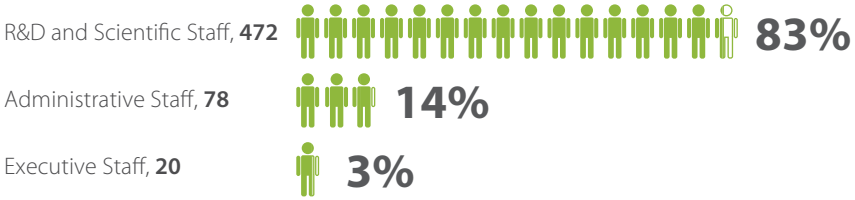
Human Resources

As of 30 September 2012, BIOTEC employed a total of 570 staff.

BIOTEC Staff by Education



BIOTEC Staff by Job Function



Publications

BIOTEC researchers published 216 papers in international journals, 202 of which were in citation index journals. Twenty-nine were published in journals with impact factors greater than 4.

Patents

BIOTEC applied for sixteen patents, two of which were filed internationally under the Patent Cooperation Treaty. Twenty-four petty patents were applied for and two issued in 2012.

Honors and Awards

BIOTEC researchers and affiliated staff were presented with 24 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 foods, 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 emphasizes on 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.

KDML105 CSSL population containing drought tolerance QTL in a yield trial at the Rice Gene Discovery Unit, Nakhon Pathom.

Highlights from Rice Biotechnology

Drought tolerant rice

Drought is a major constraint to rice production in rainfed lowland areas and areas with insufficient irrigation. One strategy to reduce the negative impacts of drought is to further improve drought tolerant varieties.

Quantitative trait loci for primary and secondary traits related to drought tolerance on chromosomes 1, 3, 4, 8 and 9 determined from double haploid lines derived from a cross between CT9993 and IR62266, were introgressed and dissected into small pieces in the genetic background of Khao Dawk Mali 105 (KDML105) to develop a chromosome segment substitution line population (CSSL). The CSSLs were evaluated at the reproductive stage for their agronomic performance and yield components under drought stress. The results were compared with irrigated conditions. The flowering of CSSL lines was 6 to 7 days earlier than KDML105. The mean values of grain yields in the CSSLs were higher than KDML105 under both drought and irrigated conditions. In the irrigated condition, the grain yields of introgression lines carrying drought tolerant quantitative trait loci from chromosomes 4 and 8 were higher than that of KDML105, whereas other traits showed little difference with KDML105. Analysis indicated that grain yield has a positive correlation with plant height, tiller and panicle number per plant, and total grain weight per plant under drought stress, while negatively correlated with days to flowering. CSSLs showing good adaptation under drought stress can be used as genetic materials to improve drought tolerance in the Thai rainfed lowland rice breeding program, and as materials to dissect genes underlying drought tolerance.

This work was a collaboration between BIOTEC Rice Gene Discovery Unit, Kasetsart University, University of Phayao, and Sakon Nakhon Rice Research Center.

Ref: Kanjoo, V., Punyawaew, K., Siangliw, J. L., Jearakongman, S., Vanavichit, A. and Toojinda, T. (2012). Evaluation of Agronomic Traits in Chromosome Segment Substitution Lines of KDML105 Containing Drought Tolerance QTL under Drought Stress. *Rice Science* 19(2), 117-124.

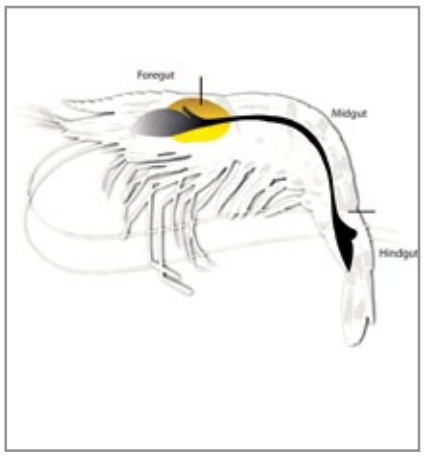
Genetic diversity of rice

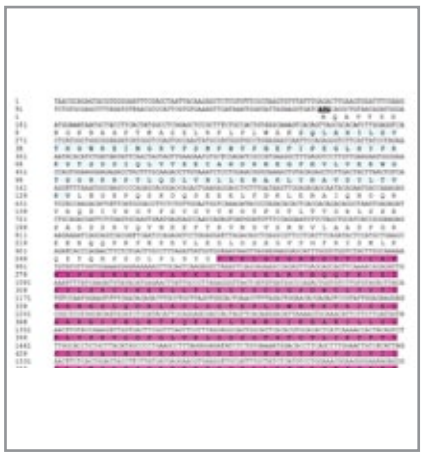
Both Thailand and the International Rice Research Institute (IRRI) have large collections of rice germplasm which could be used to develop breeding lines with desirable traits. This study aimed to investigate the level of genetic diversity and structures of Thai and selected IRRI germplasm.

From the 98 InDel markers tested for single copy and polymorphism, 19 markers were used to evaluate 43 Thai and 57 IRRI germplasm, including improved cultivars, breeding lines, landraces, and 5 other *Oryza* species. The Thai accessions were selected from all rice ecologies such as irrigated, deep water, upland, and rainfed lowland ecosystems. The IRRI accessions were groups of germplasm having agronomically desirable traits, including temperature-sensitive genetic male sterility, new plant type, early flowering, and biotic and abiotic stress resistances. Most of the InDel markers were genes with diverse functions. These markers produced a total of 127 alleles for all loci, with a mean of 6.68 alleles per locus, and a mean polymorphic information content of 0.440. Genetic diversity counts of Thai rice were 0.3665, 0.4479 and 0.3972 for improved cultivars, breeding lines, and landraces, respectively, while genetic diversity of IRRI improved and breeding lines were 0.3272 and 0.2970, respectively. Cluster, structure, and differentiation analyses showed six distinct groups: *japonica*, TGMS, deep water, IRRI germplasm, Thai landraces and breeding lines, and other *Oryza* species. Researchers concluded that Thai and IRRI germplasm were significantly different and can be used to broaden the genetic base and trait improvements.

This study was conducted jointly by BIOTEC Plant Molecular Genetics Laboratory and Thammasart University.

Ref: Chakhonkaen, S., Pitnjam, K., Saisuk, W., Ukoskit, K. and Muangprom, A. (2012). Genetic structure of Thai rice and rice accessions obtained from the International Rice Research Institute. *RICE*, 5, 19.





Shrimp gastrointestinal tract.

Nucleotide sequence of *Pm*IRAK-4.

*Pm*IRAK-4 may play a role in the immune response against bacterial infections in the shrimp intestine.

Highlights from Shrimp Biotechnology

Immunity in black tiger shrimp

Immunity sensors play an essential role in recognizing components of pathogens and then generating a signal that leads to production of host defense molecules. In this study, researchers find an interleukin-1 receptor that may play a role in the immune response against bacterial infections in the intestines of black tiger shrimp.

An interleukin-1 receptor associated kinase-4 (IRAK-4) has been identified as a central signal transduction mediator of the Toll-like receptor (TLR) and Toll/interleukin-1 receptor (TIR) pathways in vertebrate innate immunity. In this study, an IRAK-4 homologue was cloned from the black tiger shrimp (*Penaeus monodon*) (*Pm*IRAK-4). It shares domains and structures with other IRAK-4s. It was found to be mainly expressed in the hemocytes and midgut but also to a lower extent in several other tissues. The *Pm*IRAK-4 responded to bacterial infection in the intestine by an enhancement of its expression level. These results indicate that *Pm*IRAK-4 may play a role in the immune response against bacterial infections in the intestine of *P. monodon*.

This work was done in collaboration between BIOTEC Aquatic Molecular Genetics and Biotechnology Laboratory and Uppsala University, Sweden.

Ref: Watthanasurorot, A., Söderhäll, K. and Jiravanichpaisal, P. (2012). A mammalian like interleukin-1 receptor-associated kinase 4 (IRAK-4), a TIR signaling mediator in intestinal innate immunity of black tiger shrimp (*Penaeus monodon*). *Biochemical and Biophysical Research Communications*, 417(1), 623-629.

Defense against yellow head virus

Shrimp viral diseases are a major constraint in developing a sustainable culture industry. Better understanding of how shrimp respond to viral infections at the molecular level will lead to new methods for disease prevention and control. A number of proteins may be involved in shrimp antiviral defense. Among these are lectins, which have been reported to be involved in several cellular immune responses.

A new shrimp defense molecule named LvCTLD has been discovered. It has a unique primary sequence. Phylogenetic analysis revealed that it is most closely related to but distinct from the antiviral lectin PmAV from *P. monodon*. Recombinant LvCTLD was shown to have encapsulation-promoting activity, to bind to purified yellow head virus particles and to enhance phenol oxidase (PO) activity *in vivo*. It was concluded that a novel shrimp LvCTLD is a host recognition molecule involved in the shrimp defense mechanism against yellow head virus via recruitment of hemocytes, probably at the site of viral infection, and via activation of the proPO system.

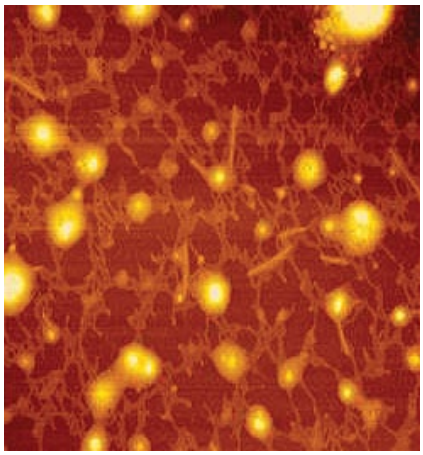
This work was a collaboration between BIOTEC Shrimp Virus Interaction Laboratory, the Center of Excellence for Shrimp Molecular Biology and Biotechnology, and Mahidol University.

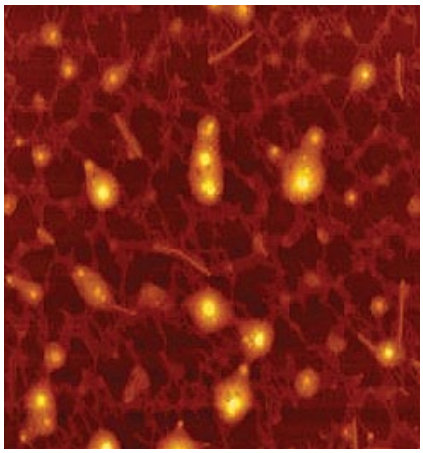
Ref: Junkunlo, K., Prachumwat, A., Tangprasittipap, A., Senapin, S., Borwornpinyo, S., Flegel, T.W. and Sritunyalucksana, K. (2012). A novel lectin domain-containing protein (LvCTLD) associated with response of the whiteleg shrimp *Penaeus (Litopenaeus) vannamei* to yellow head virus (YHV). *Developmental and Comparative Immunology*, 37(3-4), 334-341.

Atomic force microscopy image of chitosan-dsRNA complex

Atomic force microscopy image of QCH4–dsRNA complex

Chitosan and its quarternized derivative (QCH4) are effective dsRNA carriers, with potential for future development of shrimp feed with antiviral activity.





Toward shrimp feed with antiviral properties

RNA interference is a promising strategy for combating viral pathogens, at least in lab-scale experiments. For application at farm level, researchers must develop effective orally delivered agents for double-stranded RNA. In this study, researchers looked at nanoparticles as effective double-stranded RNA carriers.

Since continuous shrimp cell lines have not been established, researchers developed a dsRNA delivery system in *Spodoptera frugiperda* (Sf9) cells for studying *in vitro* RNAi-mediated gene silencing of shrimp virus. Sf9 cells challenged with yellow head virus were used for validating nanoparticles as effective dsRNA carriers. Inexpensive and biodegradable polymers, chitosan and its quarternized derivative (QCH4), were formulated with long dsRNA (>100 bp) targeting yellow head virus. Their morphology and physicochemical properties were examined. When treated with chitosan and QCH4–dsRNA complexes, at least 50% reduction in yellow head virus infection in Sf9 cells relative to the untreated control was evident at 24 hours post infection with low cytoxicity. Inhibitory effects of chitosan and QCH4–dsRNA complexes were comparable to that of dsRNA formulated with Cellfectin®, a commercial lipid-based transfection reagent. The natural and quaternized chitosan prepared in this study can be used for shrimp virus-specific dsRNA delivery in insect cultures, and has potential for future development of dsRNA carriers in shrimp feed with antiviral activity.

This study was a collaboration between the Center of Excellence for Shrimp Molecular Biology and Biotechnology, Mahidol University, and NANOTEC.

Ref: Theerawanitchpan, G., Saengkrit, N., Sajomsang, W., Gonil, P., Ruktanonchai, U., Saesoo, S., Flegel, T.W. and Saksmerprome, V. (2012). Chitosan and its quaternized derivative as effective long dsRNA carriers targeting shrimp virus in *Spodoptera frugiperda* 9 cells. *Journal of Biotechnology*, 160(3-4), 97-104.

Understanding pattern recognition proteins in shrimp immune system

Understanding the biochemistry of the shrimp immune system is the key to developing new and better treatments for microbial and parasitic infections that can cost growers millions of Baht.

The prophenoloxidase (proPO) system is an important component of the immune reaction in the host defense against microbial or parasitic infections in many crustaceans. The first process of the proPO system is to detect the pathogen associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), lipoteichoic acid (LTA), beta-1,3-glucan and peptidoglycan (PGN) molecules. A lipopolysaccharide and beta-1, 3-glucan binding protein (LGBP) is an important pattern recognition protein in crustaceans. The role of shrimp LGBPs in the proPO activating system is still somewhat elusive and poorly elucidated.

In this study, researchers focused on the molecular characterization of a pattern recognition protein in the shrimp *P. monodon* proPO system. The transcript expression profiles in various tissues and in response to the pathogenic bacterium, *Vibrio harveyi*, were examined, as were the binding activity and the proPO activation of the recombinant (r) protein. The protein was named *Pm*LGBP and its involvement in the proPO system was elucidated.

This study was conducted at the Center of Excellence for Molecular Biology and Genomics of Shrimp.

Ref: Amparyup, P., Sutthangkul, J., Charoensapsri, W. and Tassanakajon, A. (2012). Pattern Recognition Protein Binds to Lipopolysaccharide and β-1,3-Glucan and Activates Shrimp Prophenoloxidase System. *Journal of Biological Chemistry*, 287, 10060-10069.

Pre-cooked shrimp with 50˚C core temperature displays browning after 7-day refrigeration.

Pre-cooked shrimp with 80˚C core temperature after 7-day refrigeration.

Highlights from Food Biotechnology

A better method for identifying food poisoning bacteria

PCR or "polymerase chain reaction" is now a common technique used in medical and biological research labs for a variety of applications. In this study, researchers evaluated the ability of a PCR technique to sub-type strains of a common food borne pathogen as an alternative to current methods. Using the new method, a single investigator can analyze hundreds of samples and have results available the following day.

Campylobacter jejuni is a bacterium found in animal feces and is the cause of the most cases of food poisoning after Salmonella. It is also a major concern in veterinary public health, as consumption of poultry products is an important risk factor for human campylobacteriosis. Many molecular methods for clonal identification of *C. jejuni* strains have been reported, among which is multilocus sequence typing (MLST), which is widely used in epidemiological studies for several food borne pathogens. Although MLST is accepted as a suitable technique for bacterial typing because of its high discriminatory power as well as its reproducibility and data portability, the time, cost, and labor associated with the MLST technique may hinder its use in large epidemiologic studies, or in time-sensitive or resource-constrained situations.

The study was a collaboration between Chiang Mai University, Free University Berlin (Germany) and BIOTEC Food Biotechnology Research Unit.

Ref: Patchanee, P., Chokboonmongkol, C., Zessin, K.H., Alter, T., Pornaem, S. and Chokesajjawatee, N. (2012). Comparison of multilocus sequence typing (MLST) and repetitive sequence-based PCR (rep-PCR) fingerprinting for differentiation of *Campylobacter jejuni* isolated from broiler in Chiang Mai, Thailand. *Journal of Microbiology and Biotechnology*, http://dx.doi.org/10.4014/jmb.1112.12049.

Reduced browning in pre-cooked shrimp

Many of the techniques required to kill food pathogens are detrimental to food quality, taste and visual appeal. In this study, researchers found a way to reduce the browning caused by pre-cooking shrimp.

Pre-cooked Pacific white shrimp *(Litopenaeus vannamei)* is a popular shrimp product. Pre-cooking shrimp at boiling temperature for 2 minutes deactivates polyphenoloxidase, the cause of melanosis (browning), but there is a loss in quality and melanosis persists due to the remaining polyphenoloxidase and the presence of protease. Pre-cooking to obtain a core temperature of 80 °C, with a holding time of 30 seconds, prevents severe cooking loss and lowers melanosis during storage.

This study was conducted jointly between Prince of Songkla University and BIOTEC Food Biotechnology Research Unit.

Ref: Manheem, K., Benjakul, S., Kijroongrojana, K. and Visessanguan, W. (2012). The effect of heating conditions on polyphenol oxidase, proteases and melanosis in pre-cooked Pacific white shrimp during refrigerated storage. *Food Chemistry*, 131(4), 1370-1375.

Oil palm plantations have been expanding tremendously in the past decade due to rising demand in food and energy consumption.

Highlights from Plant and Animal Biotechnology

Pre-treatment of cassava for thermoplastic starch production

There is a growing interest in using agricultural produce or byproducts in the plastics industry to minimize the environmental pollution caused by non-degradable polymers produced from fossil oils. This has led to the development of thermoplastic starch. In this study, researchers developed a pre-treatment process for cassava flour, chips and fresh root for thermoplastic starch production.

Pre-treatment involving physical modification with no chemical input yields cassava materials with suitable viscosity for blending with a plasticizer and causes less shear stress in a twin screw extruder. The pre-treatment process can reduce energy consumption and is safe for machinery. The process also maintains the integrity of cassava's natural fiber, which helps strengthen the structure of thermoplastic starch.

A petty patent for this process was filed on 22 July 2011. Based on this process, researchers have started to develop biodegradable film production from cassava flour for agricultural use. Funding from the National Research Council of Thailand was approved in 2012.

Molecular markers for oil palm breeding

The oil palm is now the world's number one source of edible vegetable oil, and the richest dietary source of provitamin A. While new genotypes from traditional breeding programs provide steady yield increases, the long selection cycle (10-12 years) and the large areas required to cultivate oil palm make genetic improvement slow and labor intensive. Molecular breeding programs have the potential to make significant impacts on the rate of genetic improvement, but the limited molecular resources, in particular the lack of molecular markers for agronomic traits of interest, restrict the application of molecular breeding schemes for oil palm.

Researchers identified SSRs in ESTs derived from oil palm to evaluate their use as molecular markers with plant material used in genetic improvement programs. 6,103 non-redundant ESTs derived from cDNA libraries of developing vegetative and reproductive tissues were annotated and searched for SSRs. Primer pairs from sequences flanking 289 EST-SSRs were tested to detect polymorphisms in elite breeding parents and their crosses, of which 230 amplified PCR products. Out of 230 amplified PCR products, 88 were polymorphic within the breeding material tested. A detailed analysis and annotation of the EST-SSRs revealed the locations of the polymorphisms within the transcripts, and that the main functional category was related to transcription and post-transcriptional regulation. SSR polymorphisms were found in sequences encoding AP2-like, bZIP, zinc finger, MADS-box, and NAC-like transcription factors in addition to other transcriptional regulatory proteins and several RNA interacting proteins.

This work was conducted as a collaboration between the Genome Institute, Institut de Recherche pour le Développement (IRD) and Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD).

Ref: Tranbarger, T.J., Kluabmongkol, W., Sangsrakru, D., Morcillo, F., Tregear, J.W., Tragoonrung, S. and Billotte, N. (2012). SSR markers in transcripts of genes linked to post-transcriptional and transcriptional regulatory functions during vegetative and reproductive development of *Elaeis guineensis*. *BMC Plant Biology*, 12, 1.

Understanding fruit ripening and shedding in oil palm

Cell separation that occurs during fleshy fruit abscission and dry fruit dehiscence facilitates seed dispersal, the final stage of plant reproductive development. While our understanding of the evolutionary context of cell separation is limited mainly to the eudicot model systems tomato and Arabidopsis, less is known about the mechanisms underlying fruit abscission in crop species, monocots in particular. Better knowledge of this process would help breeders develop improved species.

Rubber tree plantation.
Rubber tapping.

The polygalacturonase (PG) multigene family encodes enzymes involved in the depolymerisation of pectin homogalacturonan within the primary cell wall and middle lamella. PG activity is commonly found in the separation layers during organ abscission and dehiscence, however, little is known about how this gene family has diverged since the separation of monocot and eudicots and the consequence of this divergence on the abscission process. In this study, researchers aimed to identify polygalacturonases (PGs) responsible for the high activity in the abscission zone during fruit shedding of the oil palm, and to analyze PG gene expression during oil palm fruit ripening and abscission. 14 transcripts that encode PGs were identified, all of which are expressed in the base of the oil palm fruit. One PG transcript (EgPG4) is the most highly induced in the fruit base. The expression pattern of EgPG4 is consistent with the temporal and spatial requirements for cell separation to occur during oil palm fruit shedding. The sequence diversity of PGs and the complexity of their expression in the oil palm fruit tissues contrast with data from tomato, suggesting functional divergence underlying the ripening and abscission processes has occurred between these two fruit species. Furthermore, phylogenetic analysis of EgPG4 with PGs from other species suggests some conservation, but also diversification has occurred between monocots and eudicots, in particular between dry and fleshy fruit species.

This work was conducted as a collaboration between the Genome Institute, Kasetsart University, IRD, CIRAD, The University of Nottingham and PalmElit SAS.

Ref: Roongsattham, P., Morcillo, F., Jantasuriyarat, C., Pizot, M., Moussu, S., Jayaweera, D., Collin, M., Gonzalez-Carranza, Z., Amblard, P., Tregear, J.W., Tragoonrung, S., Verdeil, J.L. and Tranbarger, T.J. (2012). Temporal and spatial expression of polygalacturonase gene family members reveals divergent regulation during fleshy fruit ripening and abscission in the monocot species oil palm. *BMC Plant Biology*, 12, 150.

Transcriptome sequencing of the rubber tree

Natural rubber has a wide range of industrial applications and global demand is increasing. A better understanding of the genome will help breeders improve genetic backgrounds of rubber tree to increase production and resist plant diseases.

To obtain more information on the *Hevea brasiliensis* genome, researchers sequenced the transcriptome from the vegetative shoot apex yielding 2,311,497 reads. Clustering and assembly of the reads produced a total of 113,313 unique sequences, comprising 28,387 isotigs and 84,926 singletons. Also, 17,819 expressed sequence tag (EST)-simple sequence repeats (SSRs) were identified from the data set. To demonstrate the use of this EST resource for marker development, primers were designed for 430 of the EST-SSRs. Three hundred and twenty-three primer pairs were amplifiable in *H. brasiliensis* clones. Polymorphic information content values of selected 47 SSRs among 20 *H. brasiliensis* clones ranged from 0.13 to 0.71, with an average of 0.51. A dendrogram of genetic similarities between the 20 *H. brasiliensis* clones using these 47 EST-SSRs suggested two distinct groups that correlated well with clone pedigree. These novel EST-SSRs, together with the published SSRs, were used for the construction of an integrated parental linkage map of *H. brasiliensis* based on 81 lines of an F1 mapping population. The map consisted of 97 loci, consisting of 37 novel EST-SSRs and 60 published SSRs, distributed on 23 linkage groups and covered 842.9 cM with a mean interval of 11.9 cM and ~4 loci per linkage group. Although the numbers of linkage groups exceed the haploid number, several common markers between homologous linkage groups with the previous map indicated that the F1 map in this study is appropriate for further study in marker-assisted selection.

This study was conducted jointly by researchers from the Genome Institute, Mahidol University and the Rubber Research Institute of Thailand.

Ref: Triwitayakorn, K., Chatkulkawin, P., Kanjanawattanawong, S., Sraphet, S., Yoocha, T., Sangsrakru, D., Chanprasert, J., Ngamphiw, C., Jomchai, N., Therawattanasuk, K. and Tangphatsornruang, S. (2011). Transcriptome Sequencing of *Hevea brasiliensis* for Development of Microsatellite Markers and Construction of a Genetic Linkage Map. *DNA Research*, 18(6), 471-482.

Salt-tolerant sugarcane cultivars in BIOTEC Plant Physiology and Biochemistry Laboratory.

Screening sugarcane genotypes for salt tolerance

Sugarcane is an increasingly popular crop for farmers in Thailand's northeast. One of the constraints on both volume and quality is the high salt content in soils in the northeast plateau. Research on salt tolerant varieties of sugarcane would benefit growers and the national economy.

When cultivated in salt affected soils, sugarcane shows toxic symptoms including low spout emergence, nutritional imbalance and growth reduction, leading to low productivity, especially sugar content. In this study, researchers aimed to discriminate among different genotypes of sugarcane with respect to degree of salt tolerance using the following physiological and biochemical parameters as selection criteria: photosynthetic pigments, chlorophyll a fluorescence, net CO_2 assimilation rate, and proline (a vital osmoprotectant). The experiments were performed on disease-free sugarcane plantlets of 11 cultivars derived from meristem cuttings. Growth parameters including shoot height, root length, fresh weight, dry weight and leaf area in salt stressed plantlets of all genotypes were significantly inhibited. The Na^+ accumulation, pigment degradation, proline accumulation, photosynthetic abilities and growth inhibition in saline regimes were subjected to hierarchical cluster analysis. Eleven sugarcane cultivars were classified into different categories with respect to salt tolerance, i.e., K88-1 and UT94-7 as salt tolerant and K92-2 and LK92-4 as salt sensitive genotypes. The salt tolerant cultivars, K88-1 and UT94-7 may be further studied in terms of yield, sugar content, purity percentage and ratoon recovery rate in salt affected field trials.

This study was conducted jointly between BIOTEC Plant Physiology and Biochemistry Laboratory, Kasetsart University, and the University of Agriculture, Pakistan.

Ref: Cha-um, S., Chuencharoen, S., Mongkolsiriwatana, C., Ashraf, M. and Kirdmanee, C. (2012). Screening sugarcane (*Saccharum* sp.) genotypes for salt tolerance using multivariate cluster analysis. *Plant Cell Tissue and Organ Culture*, 110(1), 23-33.

Insights into influenza A and B viruses

Influenza is an infectious disease of birds and mammals caused by RNA viruses which can be categorized into three types: A, B, and C. Virologists have long known that the co-infection of cells with influenza A and B viruses (FluA and FluB) has been shown to result in suppression of FluA growth. Insights into how FluB impedes FluA have been revealed by researchers from the BIOTEC Virology and Cell Technology Laboratory. Results may lead to new methods of preventing illness and death worldwide caused by FluA strains such as H5N1 and H1N1.

Given the possibility that FluB-specific proteins might hinder FluA polymerase activity and replication, researchers individually determined the effect of each gene of FluB on the FluA polymerase assay. It was found that the nucleoprotein of FluB, NP(FluB), inhibits polymerase activity of FluA in a dose-dependent manner. Moreover, mutational analyses of NP(FluB) suggested that functional NP(FluB) is necessary for this inhibition and slower growth of FluA was also observed in MDCK cells stably expressing NP(FluB). Further analysis of NP(FluB) indicated that it does not affect nuclear import of NP(FluA). All of these findings suggest a novel role of NP(FluB) in inhibiting replication of FluA, providing more understanding into the mechanism of interference between FluA and FluB and the lack of reassortants between them.

Ref: Wanitchang, A., Narkpuk, J., Jaru-ampornpan, P., Jengarn, J. and Jongkaewwattana, A. (2012). Inhibition of influenza A virus replication by influenza B virus nucleoprotein: An insight into interference between influenza A and B viruses. *Virology*, 432(1), 194-203.

Ophiocordyceps unilateralis s.l. growing from a dead ant (*Polyrhachis furcata*) found at Mo Singto, Khao Yai National Park.

Highlights from Biodiversity and Bioresource Use

A platform for bioresources management

In 2011, BIOTEC, the Thailand Institute of Scientific and Technology Research, and the National Science Museum launched a project to create an integrated biological database. The database uses iCollect software developed by BIOTEC Information Systems Laboratory as a platform for data storage and user interface. Thus far, 5,300 items have been entered and verified, consisting of insect pathogenic fungi, soil fungi, freshwater fungi, seed fungi, marine fungi, microalgae, and new animal and amphibian species.

In 2012, the three parties agreed to further expand the database project into Thai2BIO: Thai Bioresources and Biotechnology, a centralized database to include information on biodiversity and bioresources, from fundamental knowledge to the study of its use, allowing an effective management of biomaterials and holotypes and serving as a National Hub for Biotechnology Research in the future. A budget of 25 million Baht has been earmarked by The Ministry of Science and Technology to support Thai2BIO in 2013.

Host-specific divergence of *Ophiocordyceps unilateralis*

This work provides insights into biodiversity on a complex scale. These results can be used in biodiversity education as an example of 'hidden' biodiversity and how organisms evolve. The study also aimed at providing insights into the mechanisms of adaption of entomopathogenic fungi to their hosts, which offers valuable information for applications in biocontrol.

Ophiocordyceps unilateralis (Hypocreales, Ascomycetes) is an entomopathogenic fungus specific to formicine ants (Formicinae, Hymenoptera). Previous works have shown that the carpenter ant *Camponotus leonardi* acts as the principal host with occasional infections of ants from the genus *Polyrhachis* (sister genus of *Camponotus*). Observations were made on permanent plots of Mo Singto, Khao Yai National Park of Thailand according to which *O. unilateralis* was found to occur predominantly on three host species: *C. leonardi, C. saundersi* and *P. furcata*. Molecular phylogenies of the elongation factor 1-α and β-Tubulin genes indicate a separation of *O. unilateralis* samples into three clades, reflecting specificity to each of the three different ant species. Samples collected from *P. furcata* and from *C. leonardi* were found to form sister groups with samples from *C. saundersi* forming an outgroup to the latter. Additional samples collected from unidentified ant species of *Camponotus* and *Polyrhachis* were positioned as outgroups to those samples on identified species. These results demonstrate that *O. unilateralis* is clearly not a single phylogenetic species and comprises at least three species specific to different host ant species. These cryptic species may arise through recent events of speciation driven by their specificity to host ant species.

This study was conducted by BIOTEC Mycology Laboratory.

Ref: Kobmoo, N., Mongkolsamrit, S., Tasanathai, K., Thanakitpipattana, D. and Luangsa-Ard, J.J. (2012). Molecular phylogenies reveal host-specific divergence of *Ophiocordyceps unilateralis sensu lato* following its host ants. *Molecular Ecology*, 21(12), 3022-3031.

Compound discoveries

The aim of compound discovery, or natural product discovery, is to find novel compounds produced in nature. Novel compounds have applications in many industries. The compound discovery program aims to use microbial resources for high value added products such as pharmaceuticals, biocontrol agents, food and feed for the benefit of industry. Bioactive substances produced from various microorganisms are identified by using activity-guided fractionation and structure modification for increased biological activity. Below are a some novel compounds identified in 2012:

- Nine new fungal metabolites, one phthalide derivative, acremonide (1), and eight isocoumarin derivatives, acremonones A-H (2-9), were isolated from the mangrove-derived fungus *Acremonium* sp. PSU-MA70 together with 10 known compounds. Their structures were determined by NMR analysis. The known 8-deoxytrichothecin and trichodermol exhibited moderate antifungal activity against *Candida albicans* and *Cryptococcus neoformanns*, respectively.

Basidiomycete *Stereum ostrea* found in Khao Yai National Park.

Enzbleach is an alkaline-tolerant enzyme for pulp bleaching developed from the metagenomic library of the termite gut.

- Seven new lanostane-type triterpenes, hypocrellols A–G (1–7), and six new hopane-type triterpenes, 7β,15α-dihydroxy-22(29)-hopene (8), 3β,7β-dihydroxy-22(29)-hopene (9), 3β-acetoxy-15α-hydroxy-22(29)-hopene (10), 3β,7β,15α,22-tetrahydroxyhopane (11), 3β-acetoxy-7β,15α,22-trihydroxyhopane (12), and 7β,15α,22-trihydroxyhopane (13), were isolated from the scale insect pathogenic fungus *Hypocrella* sp. BCC 14524. The structures of the new compounds were elucidated by analyses of the NMR spectroscopic and mass spectrometry data.
- Nine new mycotoxins; five xanthones 1e5, hydroxanthone 6, and three anthraquinones 7e9, together with nine known compounds; sterigmatocystin (10), demethylsterigmatocystin (11), dihydrodemethylsterigmatocystin (12), sterigmatin (13), austocystin F (14), averufin (15), aflatoxin B1, paeciloquinone A, and zeorin, were isolated from the scale insect fungus *Aschersonia coffeae* Henn. BCC 28712. The structures of these compounds were elucidated using NMR spectroscopic and MS spectrometric analyses. Compounds 1e3 and 6e9 displayed cytotoxic activity while the xanthone 2 and anthraquinones 8 and 9 also showed antimalarial activity.
- Seven new cyclohexadepsipeptides, beauvenniatins F, G1, G2, G3, H1, H2, and H3 (1–7, resp.), were isolated from cultures of the fungus *Acremonium* sp. BCC 2629. Their structures were elucidated by extensive spectroscopic analyses. The absolute configurations were addressed by HPLC analyses of their acid hydrolysates. Their biological activities were evaluated against *Mycobacterium tuberculosis* H37Ra and *Plasmodium falciparum* K1; the respective MIC and IC50 values were in the micromolar range.
- Sterostreins F-O (1-10), 10 illudalanes and norilludalanes were isolated from cultures of the Basidiomycete *Stereum ostrea* BCC 22955. Their structures were elucidated by analyses of the NMR spectroscopic and mass spectrometry data. Sterostreins M (8), N (9), and O (10) are pyridine-containing illudalanes.

Ref:

1. Rukachaisirikul, V., Rodglin, A., Sukpondma, Y., Phongpaichit, S., Buatong, J. and Sakayaroj, J. (2012). Phthalide and Isocoumarin Derivatives Produced by an *Acremonium* sp. Isolated from a Mangrove *Rhizophora apiculata*. *Journal of Natural Products*, 75(5), 853-858.
2. Isaka, M., Chinthanom, P., Sappan, M., Chanthaket, R., Luangsa-Ard, J.J., Prabpai, S. and Kongsaeree, P. (2011). Lanostane and Hopane Triterpenes from the Entomopathogenic Fungus *Hypocrella* sp. BCC 14524. *Journal of Natural Products*, 74(10), 2143-2150.
3. Kornsakulkarn, J., Saepua, S., Srichomthong, K., Supothina, S. and Thongpanchang, C. (2012). New mycotoxins from the scale insect fungus *Aschersonia coffeae* Henn. BCC 28712. *Tetrahedron*, 68(40), 8480-8486.
4. Bunyapaiboonsri, T., Vongvilai, P., Auncharoen, P. and Isaka, M. (2012). Cyclohexadepsipeptides from the Filamentous Fungus *Acremonium* sp. BCC 2629. *Helvetica Chimica Acta*, 95(6), 963-972.
5. Isaka, M., Srisanoh, U., Sappan, M., Supothina, S. and Boonpratuang, T. (2012). Sterostreins F-O, illudalanes and norilludalanes from cultures of the Basidiomycete *Stereum ostrea* BCC 22955. *Phytochemistry*, 79, 116-120.

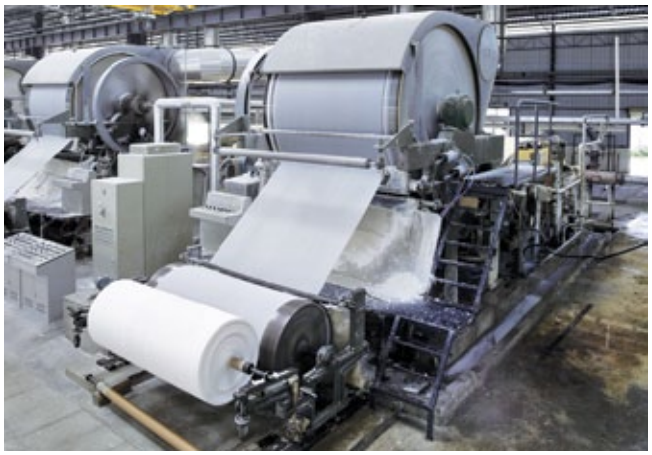
Industrial applications for termite enzymes

Cellulose-degrading enzymes in termites can be used for industrial processes such as pulp biobleaching and denim biostoning.

A metagenomic fosmid library was constructed from genomic DNA isolated from the microbial community residing in hindguts of a wood-feeding higher termite (*Microcerotermes* sp.) collected in Thailand. The library was screened for clones expressing lignocellulolytic activities. Fourteen independent active clones, 2 cellulases and 12 xylanases, were obtained by functional screening at pH 10.0. Analysis of shotgun-cloning and pyrosequencing data revealed six ORFs, which shared less than 59% identity and 73% similarity of their amino acid sequences with known cellulases and xylanases. Conserved domain analysis of these ORFs revealed a cellulase belonging to the glycoside hydrolase family 5, whereas the other five xylanases showed significant identity to diverse families including families 8, 10, and 11. One fosmid clone was isolated carrying three contiguous xylanase genes that may comprise a xylanosome operon. The enzymes with the highest activities at alkaline pH from the initial activity screening were characterized biochemically. These enzymes showed a broad range of enzyme activities from pH 5.0 to 10.0, with pH optimal of 8.0 retaining more than 70% of their respective activities at pH 9.0. The optimal temperatures of these enzymes ranged from 50 degree C to 55 degree C.

This study was conducted by the Bioresources Technology Unit.

Ref: Nimchua, T., Thongaram, T., Uengwetwanit, T., Pongpattanakitshote, S. and Eurwilaichitr, L. (2012). Metagenomic Analysis of Novel Lignocellulose-Degrading Enzymes from Higher Termite Guts Inhabiting Microbes. *Journal of Microbiology and Biotechnology*, 22(4), 462-469.



The refining step in paper-making is energy intensive. The use of enzymes can reduce energy consumption.

Energy efficient refining of recycled paper pulp using an enzyme

The results from this study contribute to the development of an energy efficient pulp industry. Researchers demonstrated the potential of a native fungal enzyme for application in the process of refining recycled pulp. The use of crude enzymes from fermentation with local inexpensive agro-industrial materials could provide a basis for cost-efficient enzyme production. The process led to a substantial reduction in refining energy with no undesirable change in the mechanical properties of the pulp in further processing steps.

Enzymatic modification of pulp is receiving increasing interest for energy reduction at the refining step of the paper-making process. In this study, the production of a multi-fiber modifying enzyme from *Mamillisphaeria* sp. BCC8893 was optimized in submerged fermentation using a response-surface methodology. Maximal production was obtained in a complex medium comprising wheat bran, soybean, and rice bran supplemented with yeast extract at pH 6.0 and a harvest time of 7 days. Treatment of old corrugated container pulp led to reductions in refining energy of 8.5-14.8%. The major physical properties were retained, including tensile and compression strength. Proteomic analysis showed that the enzyme was a complex composite of endo-glucanases, cellobiohydrolases, beta-1, 4-xylanases, and beta-glucanases belonging to various glycosyl hydrolase families, suggestive of cooperative enzyme action in fiber modification, providing the basis for refining efficiency.

This study was conducted jointly between the Bioresources Technology Unit and SCG Paper PLC.

Ref: Laothanachareon, T., Khonzue, P., Rattanaphan, N., Tinnasulanon, P., Apawasin, S., Paemanee, A., Ruanglek, V., Tanapongpipat, S., Champreda, V. and Eurwilaichitr, L. (2011). Production of Multi-Fiber Modifying Enzyme from *Mamillisphaeria* sp. for Refining of Recycled Paper Pulp. *Bioscience Biotechnology and Biochemistry*, 75(12), 2297-2303.

A cellulase-encoding gene from the buffalo rumen metagenomic library

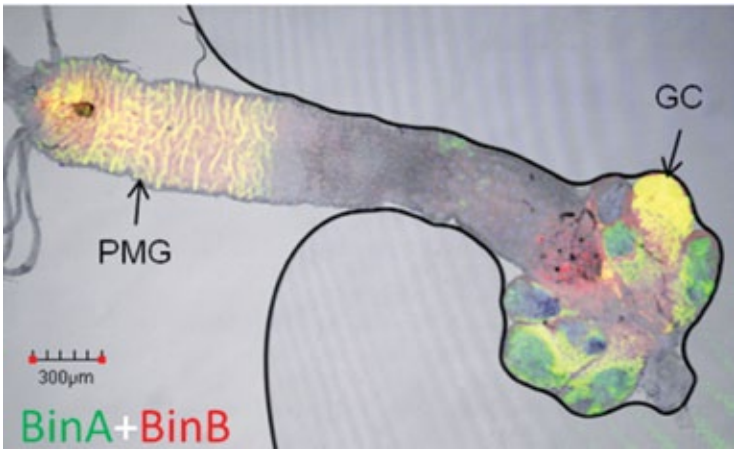
Microorganisms residing in the stomachs of cattle represent a rich source of lignocellulose-degrading enzymes, since their diet consists of plant-based materials high in cellulose and hemicellulose. These enzymes could have useful applications in preparing food supplements and in the biostoning process for denim.

A metagenomic library was constructed from buffalo rumen contents using pCC1FOS fosmid vector. It yielded 12 cellulase and 3 xylanase-positive clones, which showed strong activity against cellulose and xylan substrates respectively. The pyrosequencing technique combined with the metagenomic library also allowed the sequences of glycosyl hydrolases and other enzymes to be obtained and analyzed efficiently. A gene encoding for endoglucanase BT-01 was successfully isolated from the library and expressed in *E. coli*. Along an optimal working pH at 5.5 and stability over acidic pH, an *in vitro* digestibility test suggested that BT-01 can hydrolyze corn-based feedstuff and thus can be used as a feed supplement. In addition, due to its lack of carbohydrate-binding module and its functioning and stability at acidic to neutral pH, BT-01 should prove useful for the biostoning process for denim.

This study was conducted jointly between the Bioresources Technology Unit, Asian Institute of Technology and King Mongkut's University of Technology Ladkrabang.

Ref: Nguyen, N.H., Maruset, L., Uengwetwanit, T., Mhuantong, W., Harnpicharnchai, P., Champreda, V., Tanapongpipat, S., Jirajaroenrat, K., Rakshit, S.K., Eurwilaichitr, L. and Pongpattanakitshote, S. (2012). Identification and characterization of a cellulase-encoding gene from the buffalo rumen metagenomic library. *Bioscience Biotechnology and Biochemistry*, 76(6), 1075-1084.

A mosquito larva fed with fluorescent labeled toxin was dissected and visualized under a fluorescent microscope. Toxin was present on the posterior midgut (PMG) and gastric ceacum (GC) of the larva.



Better, safer microbial agents for controlling mosquitoes and insect pests

Results from this research will contribute to the overall goal of BIOTEC to improve efficacy and safety of microbial agents for controlling mosquitoes and major insect pests.

Bacillus sphaericus produces a binary toxin in the form of crystalline inclusion, during the sporulation phase. The binary toxin is composed of two crystal proteins, BinA and BinB, and both are required at equimolar amounts to exhibit the maximal larvicidal activity. While BinB is expected to bind to a specific receptor on the cell membrane, BinA interacts to BinB or BinB receptor complex and translocates into the cytosol to exert its activity via an unknown mechanism.

Researchers investigated functional roles of aromatic clusters in BinA. Amino acids at positions Y213, Y214, Y215, W222 and W226 were substituted by leucine. All mutant proteins were highly produced and their secondary structures were not affected by these substitutions. All mutants are able to insert into lipid monolayers as observed by a Langmuir-Blodgett trough and could permeabilize the liposomes in a similar manner as the wild type. However, mosquito-larvicidal activity was abolished for W222L and W226L mutants suggesting that tryptophan residues at both positions play an important role in the toxicity of BinA, possibly involved in the cytopathological process after toxin entry into the cells.

This research was done by Bioresources Technology Unit, the Institute of Molecular Biosciences, Mahidol University, Salaya Campus, Nakhon Pathom; and the Department of Physics, Faculty of Sciences, Mahidol University.

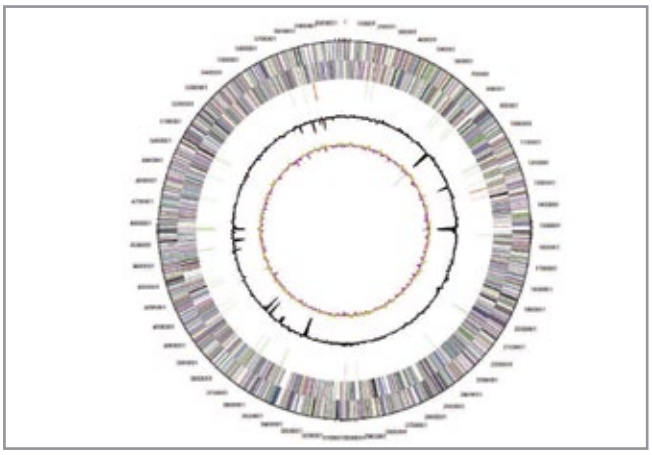
Ref: Kunthic, T., Promdonkoy, B., Srikhirin, T. and Boonserm, P. (2011). Essential role of tryptophan residues in toxicity of binary toxin from *Bacillus sphaericus*. *BMB Reports*, 44(10), 674-679

Garvieacin Q: A novel class II bacteriocin

Peptide bacteriocins play an important role in bacterial competition by giving bacteriocin-producing bacteria considerable survival advantages. Most bacteriocins from lactic acid bacteria (LAB) are small, heat-stable, cationic, amphiphilic, membrane-permeabilizing polypeptides. A large number of strains of *Lactococcus* spp. have been found to produce bacteriocins. Researchers describe the identification of a novel bacteriocin, garvieacin Q (GarQ), from *L. garvieae* BCC 43578 culture medium. The complete garQ, together with three other open reading frames within the vicinity, was cloned and sequenced, enabling the full-length sequence of GarQ to be elucidated. Based on the genetic organization of its gene, its bactericidal activity in the absence of other peptides, and the absence of any modified amino acids, GarQ was designated as belonging to the one-peptide class IId bacteriocins. GarQ had a wide activity range, and is inhibitory against the food borne pathogenic bacterium *L. monocytogenes* ATCC 19115 and various *L. garvieae* strains, as well as *L. lactis, Enterococcus*, and *Pediococcus pentosaceus* but not *E. coli* or *Salmonella*.

This study was conducted jointly by BIOTEC and Kyushu University.

Ref: Tosukhowong, A., Zendo, T., Visessanguan, W., Roytrakul, S., Pumpuang, L., Jaresitthikunchai, J. and Sonomoto, K. (2012). Garvieacin Q, a novel class II bacteriocin from *Lactococcus garvieae* BCC 43578. *Applied and Environmental Microbiology*, doi:10.1128/AEM.06891-11.



Graphical circular map of the chromosome of *Spirulina (Arthrospira) platensis* C1.

Highlights from Algal Biotechnology

Understanding the spirulina genome

Spirulina (Arthrospira) platensis is a well-known filamentous cyanobacterium used in the production of many industrial products, including high value compounds, health food supplements, animal feeds, pharmaceuticals and cosmetics. It has been increasingly studied around the world for scientific purposes, especially for its genome, biology, physiology, and also for the analysis of its small-scale metabolic network. However, the overall description of the metabolic and biotechnological capabilities of *S. platensis* is needed for the development of a whole cellular metabolism model.

In a joint study, researchers describe the complete genome sequence of *A. platensis* C1 strain and its annotation. The *A. platensis* C1 genome contains 6,089,210 bp including 6,108 protein-coding genes and 45 RNA genes, and no plasmids. The genome information has been used for further comparative analysis, particularly of metabolic pathways, photosynthetic efficiency and barriers to gene transfer.

This work was conducted as a collaboration between BIOTEC, King Mongkut's University of Technology Thonburi and Kazusa DNA Research Institute. A summary classification and a set of features of A. platensis C1 together with the complete genomic sequence and its annotation are presented in the journal article.

The availability of the spirulina genome sequence has made system-level studies of this commercial cyanobacterium possible. Researchers presented the genome-scale metabolic network analysis of *Spirulina platensis* C1, *i*AK692, its topological properties, and its metabolic capabilities and functions. The network was reconstructed from the *S. platensis* C1 annotated genomic sequence using Pathway Tools software to generate a preliminary network. Then, manual curation was performed based on a collective knowledge base and a combination of genomic, biochemical, and physiological information. The genome-scale metabolic model consists of 692 genes, 837 metabolites, and 875 reactions. The *i*AK692 model was further used to predict the unique active reactions and essential genes for each growth condition. Additionally, the metabolic states of *i*AK692 during autotrophic and mixotrophic growths were described by phenotypic phase plane (PhPP) analysis.

This work was conducted as a collaboration between Biochemical Engineering and Pilot Plant Research and Development Unit and King Mongkut's University of Technology Thonburi.

Ref:

1. Cheevadhanarak, S., Paithoonrangsarid, K., Prommeenate, P., Kaewngam, W., Musigkain, A.,Tragoonrung, S., Tabata, S., Kaneko, T., Chaijaruwanich, J., Sangsrakru, D., Tangphatsornruang, S., Chanprasert, J., Tongsima, S., Kusonmano, K., Jeamton, W., Dulsawat, S., Klanchui, A., Vorapreeda, T., Chumchua, V., Khannapho, C., Thammarongtham, C., Plengvidhya, V., Subudhi, S., Hongsthong, A., Ruengjitchatchawalya, M., Meechai, A., Senachak, J. and Tanticharoen, M. (2012). Draft genome sequence of *Arthrospira platensis* C1 (PCC9438). *Standards in Genomic Sciences*, 6(1), 43-53.
2. Klanchui, A., Khannapho, C., Phodee, A., Cheevadhanarak, S. and Meechai, A. (2012). *i*AK692: A genome-scale metabolic model of *Spirulina platensis* C1. *BMC System Biology*, 6, 71.

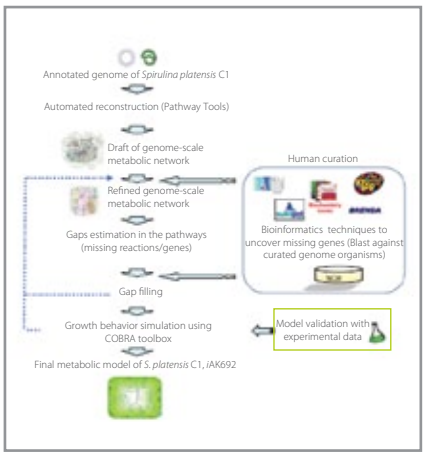
Regulation of *Spirulina* desaturase genes

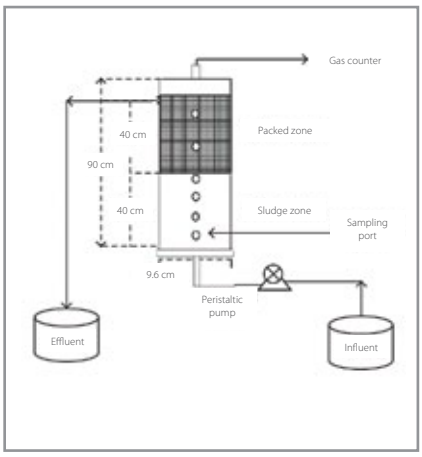
Spirulina is a potential alternative source for commercial gamma-linolenic acid (GLA) production. During the *Spirulina* desaturation process, three enzymes, which are encoded by *desC*, *desA*, and *desD*, respectively, introduce double bonds at the Δ9, Δ12, and Δ6 positions of stearic acid, oleic acid, and linoleic acid. Researchers examined transcriptional and translational expression of the desaturase genes during various growth phases of *Spirulina platensis* Z19/2. The desaturase levels and fatty acids were analyzed in two subcellular locations, the plasma membrane and thylakoid membrane. The results indicated three important points: 1) the regulation level of each *Spirulina* desaturase gene is possibly subcellular location dependent; 2) GLA is important during cell division in the mid-log phase; and 3) vaccenic acid, which is detected at high levels during the lag phase in the plasma membrane, might play a role in the mechanical strength of the cell membrane at low growth rates.

The research was a collaborative study by BIOTEC and King Mongkut's University of Technology Thonburi.

Iterative procedure used to reconstruct a genome-scale metabolic model of *S. platensis* C1.

Schematic diagram of the laboratory-scale anaerobic hybrid reactor.





Ref: Mapaisansup, T., Yutthanasirikul, R., Hongsthong, A., Tanticharoen, M. and Ruengjitchatchawalya, M. (2012). Subcellular localization-dependent regulation of the three *Spirulina* desaturase genes, *desC*, *desA*, and *desD* under different growth phases. *Journal of Applied Phycology*, doi: 10.1007/s10811-012-9880-7.

Highlights from Energy and Environment

Steps towards a viable biorefinery industry

Sacchrification is the process of breaking a complex carbohydrate such as starch or cellulose into smaller components. Systematic formulation of active enzyme systems for biomass saccharification is needed for the development of a viable biorefinery industry.

A synergistic enzyme system for the hydrolysis of alkali-pretreated rice straw was optimized based on the synergy of crude fungal enzyme extracts with a commercial cellulase (Celluclast™). Among 13 enzyme extracts, the enzyme preparation from *Aspergillus aculeatus* BCC 199 exhibited the highest level of synergy with Celluclast™. This synergy was based on the complementary cellulolytic and hemicellulolytic activities of the BCC 199 enzyme extract. A mixture design was used to optimize the ternary enzyme complex based on the synergistic enzyme mixture with *Bacillus subtilis* expansin. Using the full cubic model, the optimal formulation of the enzyme mixture was predicted.

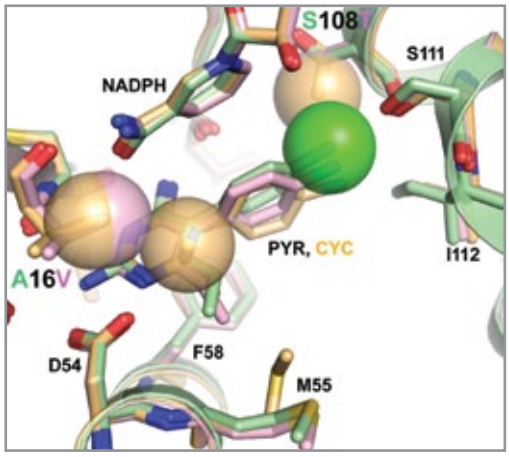
This study was conducted jointly by BIOTEC, the Department of Genetics, Faculty of Science, Kasetsart University; and the Joint Graduate School of Energy and Environment, King Mongkut's University of Technology Thonburi.

Ref: Suwannarangsee, S., Bunterngsook, B., Arnthong, J., Paemanee, A., Thamchaipenet, A., Eurwilaichitr, L., Laosiripojana, N. and Champreda, V. (2012). Optimisation of synergistic biomass-degrading enzyme systems for efficient rice straw hydrolysis using an experimental mixture design. *Bioresource Technology*, 119, 252-261.

Reactor performance and microbial community in anaerobic treatment of palm oil mill effluent

The palm oil industry in Thailand is expanding rapidly. Palm oil is the raw material for production of edible oil, biodiesel, and other applications. The expansion of crude palm oil production will generate wastewater at an increasing rate. Environmental impacts from palm oil mill wastewater are a matter of great concern, as it contains large quantities of high organic pollutants. Biological treatment is the most frequently used treatment method. The following two studies have contributed to this process.

Researchers investigated the effect of organic pollutant concentrations (mainly suspended solids, oil and grease) on the microbial communities and the microbial performance in the sludge and packed zones of an anaerobic hybrid reactor treating palm oil mill effluent. It was found that the process performance and stability, as well as the microbial characteristics, varied according to the organic pollutant concentrations. When the organic pollutant concentrations were increased, the resultant methane potentials were higher, and the methane yield increased to 0.30 L CH_4/g $COD_{removed}$. The increase of organic pollutant concentration affected the eubacterial and archaeal communities, populations, and activity in the sludge and packed zones. The predominant hydrolytic and fermentative bacteria were *Pseudomonas, Clostridium*, and *Bacteroidetes*, whereas the most represented species of methanogens were the acetoclastic *Methanosaeta*, the hydrogenotrophic *Methanobacterium* sp., and the hydrogenotrophic *Methanomicrobiaceae*. Higher levels of archaeal population and activity were found in the packed zone within the microbial biofilm. From the results of the microbial characteristics, this implied that the sludge and packed zones in the AHR acted as acidification and methanation zones, respectively. The work will lead to an understanding of the operational efficiency of an anaerobic hybrid reactor system, depending on the structure of the microbial communities present in the system and the environmental conditions needed to control the system.



Binding of cycloguanil (CYC) and pyrimethamine (PYR) to *Plasmodium falciparum* dihydrofolate reductase (PfDHFR). A major steric clash of A16V and S108T with CYC (orange) but minor with PYR (pink) was observed in the PfDHFR mutant when compared to the binding of PYR (green) in the wild-type PfDHFR.

This research was a collaboration between the Joint Graduate School of Energy and Environment, King Mongkut's University of Technology Thonburi; Excellent Center of Waste Utilization and Management, National Center for Genetic Engineering and Biotechnology at King Mongkut's University of Technology Thonburi; and the Division of Biotechnology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi.

In another study, researchers investigated a microbial adaptation process during a transition from mesophilic to thermophilic treatment of palm oil mill effluent in an upflow anaerobic sludge bed (UASB). It was found that the bacterial population profiles significantly changed with the temperature transition from mesophilic to thermophilic conditions. In addition, the results suggested that even though the thermophilic temperature of 57 °C was suitable for a number of hydrolytic, acidogenic and acetogenic bacteria, it may not be suitable for some *Methanosaeta* species acclimatized from 37 °C. The DGGE analysis predicted that the temperature transition can result in significant methanogenic biomass washout at 57°C. This study demonstrated the importance of the microbial community and of its monitoring. The ability to monitor bacteria and methanogens and to understand their ecology is essential to effectively control the start up and operation of UASB reactors.

This research was conducted by the Joint Graduate School of Energy and Environment, King Mongkut's University of Technology Thonburi; the Research and Technology Center for Renewable Products and Energy, King Mongkut's University of Technology North Bangkok; the Department of Chemical Engineering, King Mongkut's University of Technology Thonburi; and Excellent Center of Waste Utilization and Management (ECoWaste).

Ref:

1. Meesap, K., Boonapatcharoen, N., Techkarnjanaruk, S. and Chaiprasert, P. (2012). Microbial Communities and Their Performances in Anaerobic Hybrid Sludge Bed-Fixed Film Reactor for Treatment of Palm Oil Mill Effluent under Various Organic Pollutant Concentrations. *Journal of Biomedicine and Biotechnology*, 2012, art. ID 902707.
2. Khemkhao, M., Nuntakumjorn, B., Techkarnjanaruk, S. and Phalakornkule, C. (2012). UASB performance and microbial adaptation during a transition from mesophilic to thermophilic treatment of palm oil mill effluent. *Journal of Environmental Management*, 103, 74-82.

Highlights from Malaria Research

Understanding drug resistant malaria

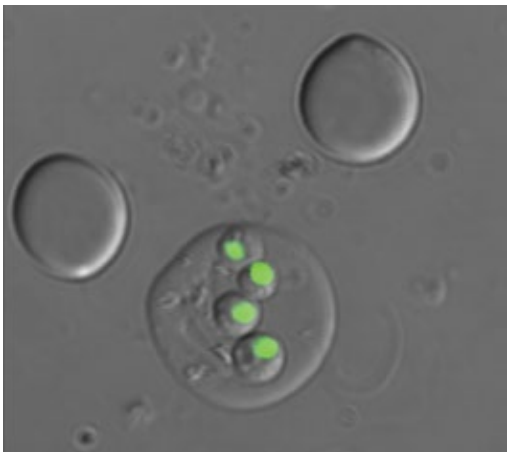
Drug resistance to malaria is becoming a serious concern worldwide. This research offers insights into the mechanisms of drug resistance and may lead to new approaches.

Natural mutations of *Plasmodium falciparum* dihydrofolate reductase (*Pf*DHFR) at A16V and S108T specifically confer resistance to cycloguanil (CYC) but not to pyrimethamine (PYR). To understand the nature of CYC resistance, the effects of various mutations at A16 on substrate and inhibitor binding were examined. Three series of mutations at A16 with or without the S108T/N mutation were generated. Only three mutants with small side chains at residue 16 (G, C, and S) were viable from bacterial complementation assay in the S108 series, whereas these three and an additional four mutants (T, V, M, and I) with slightly larger side chains were viable with simultaneous S108T mutation. Among these combinations, the A16V+S108T mutant was the most CYC resistant, and all of the S108T series ranged from being highly to moderately sensitive to PYR. In the S108N series, a strict requirement for alanine was observed at position 16. Crystal structure analyses reveal that in *Pf*D HFR-TS variant T9/94 (A16V+S108T) complexed with CYC, the ligand has substantial steric conflicts with the side chains of both A16V and S108T, whereas in the complex with PYR, the ligand only showed mild conflict with S108T. CYC analogs designed to avoid such conflicts improved the binding affinity of the mutant enzymes. These results show that there is greater spatial limitation around the S108T/N residue when combined with the limitation imposed by A16V. The limitation of mutation of this series provides opportunities for drug design and development against antifolate-resistant malaria.

This research was a collaboration between BIOTEC Protein-Ligand Engineering and Molecular Biology Laboratory and Department of Chemistry, Faculty of Science, Chulalongkorn University.

Ref: Vanichtanankul, J., Taweechai, S., Uttamapinant, C., Chitnumsub, P., Vilaivan, T., Yuthavong, Y. and Kamchonwongpaisan, S. (2012). Combined Spatial Limitation around Residues 16 and 108 of *Plasmodium falciparum* Dihydrofolate Reductase Explains Resistance to Cycloguanil. *Antimicrobial Agents and Chemotherapy*, 56(7), 3928-3935.

Plasmodium berghei-infected red blood cell stained with SYBR Green I.



Transgenic *Plasmodium* parasites as *in vitro* and *in vivo* models for antifolate screening

Plasmodium vivax is the most prevalent cause of human malaria in tropical regions outside the African continent. The lack of a routine continuous *in vitro* culture of this parasite makes it difficult to develop specific drugs.

To facilitate the development of anti-*P. vivax* drugs, researchers generated bacterial and yeast surrogate models expressing the validated *P. vivax* target dihydrofolate reductase-thymidylate synthase (DHFR-TS). *Plasmodium falciparum* and *Plasmodium berghei* parasites were transfected with DNA constructs bearing *P. vivax dhfr-ts* pyrimethamine sensitive (wild type) and pyrimethamine resistant (mutant) alleles. Double crossover homologous recombination was used to replace the endogenous *dhfr-ts* of *P. falciparum* and *P. berghei* parasites with *P. vivax* homologous genes. The integration of *Pvdhfr-ts* genes via allelic replacement was verified by Southern analysis and the transgenic parasites lines validated as models by standard drug screening assays. With the permanent integration of *Pvdhfr-ts* gene in the genome, the transgenic *Plasmodium* lines expressing *Pv*DHFR-TS are genetically stable and can be useful for screening anti-*P. vivax* compounds targeting *Pv*DHFR-TS. A similar approach could be used to generate transgenic models specific for other targets of interest, thus facilitating the development of anti-*P. vivax* drugs in general.

This research was a collaboration between BIOTEC Protein-Ligand Engineering and Molecular Biology Laboratory and Chiang Mai University.

Ref: Somsak, V., Uthaipibull, C., Prommana, P., Srichairatanakool, S., Yuthavong, Y. and Kamchonwongpaisan, S. (2011). Transgenic *Plasmodium* parasites stably expressing *Plasmodium vivax* dihydrofolate reductase-thymidylate synthase as *in vitro* and *in vivo* models for antifolate screening**.** *Malaria Journal*, 10, 291.

Potential target for new antimalarial drug interventions

An urgent need for new antimalarial drugs has prompted an exploration of potential new targets unique to the parasite or required for its viability to develop new interventions for treating the disease. *Plasmodium* serine hydroxymethyltransferase (SHMT), an enzyme in the dTMP synthesis cycle, is a potential target for such new drugs, but convenient methods for producing and assaying the enzyme are still lacking, hampering the ability to screen inhibitors.

Researchers devised an improvement to the production of SHMT and the screening assay for *Plasmodium* SHMT inhibitors using auto-induction media, the production of recombinant *Plasmodium falciparum* SHMT (PfSHMT), and *Plasmodium vivax* SHMT (PvSHMT). This resulted in a two to three-fold higher yield of Pf- and PvSHMT compared to that produced in cells induced in the conventional Luria Bertani medium with isopropyl thio-β-D-galactoside (LB-IPTG) induction media. As for the assay, researchers proposed a convenient spectrophotometric activity assay coupling *Plasmodium* SHMT and 5, 10-methylenetetrahydrofolate dehydrogenase (MTHFD), in place of the anaerobic assay coupling SHMT and 5,10-methylenetetrahydrofolate reductase (MTHFR). The new procedure gave similar kinetic parameters and is more convenient for inhibitor screening and other studies of the enzyme.

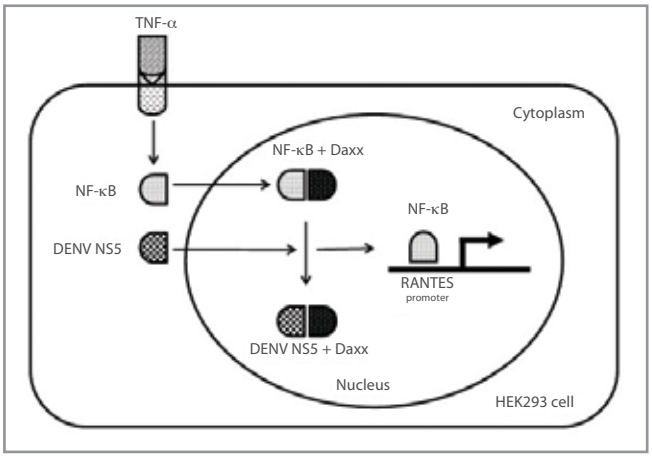
This research was a collaboration between BIOTEC Protein-Ligand Engineering and Molecular Biology Laboratory, Mahidol University and Chulalongkorn University.

Ref: Sopitthummakhun, K., Thongpanchang, C., Vilaivan, T., Yuthavong, Y., Chaiyen, P. and Leartsakulpanich, U. (2012). *Plasmodium* serine hydroxymethyltransferase as a potential anti-malarial target: inhibition studies using improved methods for enzyme production and assay. *Malaria Journal* 11, 194.

Novel tool for high throughput *in vivo* antimalarial drug screening

During the process of antimalarial drug development, active compounds that have shown good antimalarial activity *in vitro* must be confirmed for their activity using *in vivo* models, such as experimental animal hosts infected with Plasmodium parasites. To avoid the conventional counting method under the microscope, a reliable quantitative protocol for flow cytometric enumeration of infected red blood cells stained with SYBR Green I was developed.

Researchers used SYBR Green I, one of the brightest and most sensitive dyes specific to nucleic acid, to stain parasite-infected blood samples. The infected red blood cells are then suitable for counting by a flow cytometer using a bi-dimensional FL-1_{530}/FL-3_{620} detection method. The technique is highly accurate at quantifying the parasite load of infected mouse blood, and the dye stays stable for hours



Proposed model for modulation of RANTES production by dengue virus nonstructural protein 5 (DENV NS5).

after staining. This protocol was validated in an antimalarial assay and the result was comparable to that obtained from conventional microscopic counting.

This work was developed by scientists from BIOTEC Protein-Ligand Engineering and Molecular Biology Laboratory, in collaboration with scientists from Chiang Mai University.

Ref: Somsak, V., Srichairatanakool, S., Yuthavong, Y., Kamchonwongpaisan, S. and Uthaipibull, C. (2012). Flow cytometric enumeration of *Plasmodium berghei*-infected red blood cells stained with SYBR Green I. *Acta Tropica*, 122(1), 113-118.

Highlights from Dengue Research

Steps on the road to a dengue vaccine

Dengue virus infections are still increasing at an alarming rate in tropical and subtropical countries, underlying the need for a dengue vaccine. Although it is relatively easy to generate antibody responses to dengue virus, low avidity or low concentrations of antibody may enhance infection of Fc receptor-bearing cells with clinical impact, posing a challenge to vaccine production.

Researchers characterized a monoclonal antibody, 2H12, which is cross-reactive to all four serotypes in the dengue virus group. Crystal structures of 2H12-Fab in complex with domain III of the envelope protein from three dengue serotypes have been determined. 2H12 binds to the highly conserved AB loop of domain III of the envelope protein that is poorly accessible in the mature virion. 2H12 neutralization varied between dengue serotypes and strains; in particular, dengue serotype 2 was not neutralized. As the 2H12 binding epitope was conserved, this variation in neutralization highlights differences between dengue serotypes and suggests that significant conformational changes in the virus must take place for antibody binding. Surprisingly, 2H12 facilitated little or no enhancement of infection. These data provide a structural basis for understanding antibody neutralization and enhancement of infection, which is crucial for the development of future dengue vaccines.

This work was conducted by scientists from Medical Biotechnology Research Unit, Imperial College London, University of Oxford, Faculty of Medicine Siriraj Hospital and Diamond Light Source Ltd.

Ref: Midgley, C.M., Flanagan, A., Tran, H.B., Dejnirattisai, W., Chawansuntati, K., Jumnainsong, A., Wongwiwat, W., Duangchinda, T., Mongkolsapaya, J., Grimes, J.M. and Screaton, G.R. (2012). Structural Analysis of a Dengue Cross-Reactive Antibody Complexed with Envelope Domain III Reveals the Molecular Basis of Cross-Reactivity. *Journal of Immunology*, 188(10), 4971-4979.

Understanding molecular mechanisms in dengue fever and shock syndrome

Dengue fever, dengue hemorrhagic fever, and dengue shock syndrome, caused by dengue virus (DENV) infection, are important public health problems in the tropical and subtropical regions. Abnormal hemostasis and plasma leakage are the main patho-physiological changes in DHF/DSS. A remarkably increased production of cytokines, the so called 'cytokine storm', is observed in patients with dengue hemorrhagic fever and dengue shock syndrome. A complex interaction between DENV proteins and the host immune response contributes to cytokine production. However, the molecular mechanism by which DENV nonstructural protein 5 (NS5) mediates these responses is not yet fully understood.

A yeast two-hybrid assay was performed to identify host proteins interacting with DENV NS5 and a death-domain-associate protein (Daxx) was identified. The *in vivo* relevance of this interaction was suggested by co-immunoprecipitation and nuclear co-localization of these two proteins in HEK293 cells expressing DENV NS5. HEK293 cells expressing DENV NS5-K/A, which were mutated at the nuclear localization sequences (NLS), were created to assess its functional roles in nuclear translocation, Daxx interaction, and cytokine production. In the absence of NLS, DENV NS5 could neither translocate into the nucleus nor interact with Daxx to increase the DHF-associated cytokine, RANTES (CCL5) production. This work demonstrates the interaction between DENV NS5 and Daxx and the role of the interaction on the modulation of RANTES production.

This work was developed by scientists from Medical Biotechnology Research Unit, Faculty of Medicine Siriraj Hospital and Rangsit University.

Shrimp infected by yellow head virus

Ref: Khunchai, S., Junking, M., Suttitheptumrong, A., Yasamut, U., Sawasdee, N., Netsawang, J., Morchang, A., Chaowalita, P., Noisakran, S., Yenchitsomanus, P. and Limjindaporn, T. (2012). Interaction of dengue virus nonstructural protein 5 with Daxx modulates RANTES production. *Biochemical and Biophysical Research Communications*, 423(2), 398-403.

Highlights from Diagnostic Technology

Screening for thalassemia

Thalassemia syndrome is a group of hereditary disorders causing defects in the synthesis of globin chains of hemoglobin and results in anemia. Two common types, α- and β-thalassemia, are encountered globally. Presently, the conventional screening methods cannot detect the occurrence of α-thalassemia in β-thalassemia and HbE heterozygotes.

Researchers demonstrated the ability of levels of Hb Bart's and epsilon-globin chain quantified by enzyme linked immunosorbent assay (ELISA) in detecting alpha-thalassemia in beta-thalassemia and HbE heterozygotes. This strategy proved to be useful in screening for co-existence of α-thalassemia in β-thalassemia and in HbE heterozygotes, particularly in countries where α-, β-thalassemia and HbE are endemic.

This research was a collaborative project between Biomedical Technology Research Center, Chiang Mai University and Lampang Central Hospital.

Biomedical Technology Research Center has long engaged in the development of antibodies, especially hemoglobin antibodies (Hb Bart's, HbA, HbA2, HbF, HbE and epsilon-globin), antibody production and diagnosis. Examples of past accomplishments include an immune-chromatographic strip test for screening for alpha-thalassemia carriers, which has been patented in Thailand, the USA and Europe, as well as licensed to i+ MED Laboratory Co., Ltd. for commercialization; and a test tube method of screening for hemoglobin E which has been patented in Thailand and is under licensing negotiations.

Ref: Tatu, T., Kiewkarnkha, T., Khuntarak, S., Khamrin, S., Suwannasin, S. and Kasinrerk, W. (2012). Screening for co-existence of α-thalassemia in β-thalassemia and in HbE heterozygotes via an enzyme-linked immunosorbent assay for Hb Bart's and embryonic γ -globin chain. *International Journal of Hematology*, 95(4), 386-393.

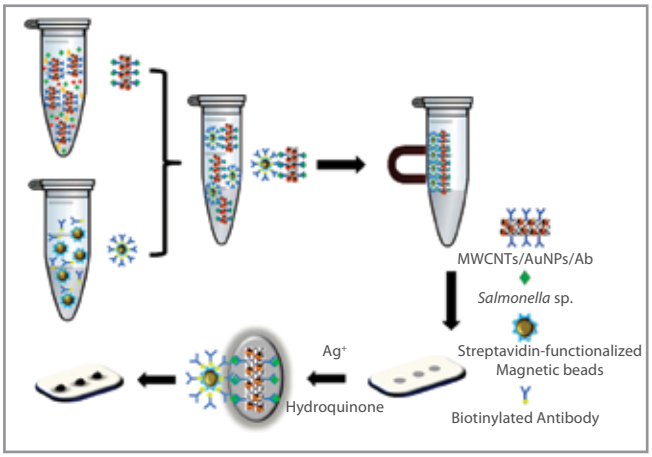
Rapid detection of shrimp yellow head virus using LAMP-AuNP method

One research focus at the Center of Excellence for Shrimp Molecular Biology and Biotechnology, or Centex Shrimp, is the development of diagnostic probes and kits for shrimp diseases. Recently, a research team has been using the loop-mediated isothermal amplification (LAMP) technique as part of the diagnostic development with great success, producing 10 international publications and 4 patent and petty patent applications as well as licensing technologies to industry. For example, LAMP-Gel and LAMP-LFD detection technologies for Taura Syndrome Virus (TSV) were licensed to Shrimp Business Biotechnology Unit, LAMP-LFD detection technology for IMNV (Infectious Myonecrosis Virus) to New World Biotech Co., Ltd. and LAMP-turbidimeter detection technology for shrimp viral diseases to Mobilis Automata Co., Ltd.

Researchers developed and evaluated the performance of a new detection platform, combining the LAMP technique with the use of gold nanoparticles (AuNP), for yellow head virus (YHV). The detection sensitivity of the method was comparable to that of commercial IQ2000™ nested RT-PCR but only required 65 minutes to produce a result, and did not cross-detect other shrimp viruses. As the LAMP-AuNP protocol only requires a heating block, it offers opportunities for rapid detection of yellow head virus.

This research was a collaboration between Center of Excellence for Shrimp Molecular Biology and Biotechnology, or Centex Shrimp, Mahidol University and James Cook University (Australia). The work was reported in the Journal of Virological Methods in December 2012, and applied for patent in Thailand on 26 July 2012. This LAMP-AuNP platform is being adapted for the detection of White-spot Syndrome Virus (WSSV) and Taura Syndrome Virus (TSV).

Ref: Jaroenram, W., Arunrut, N. and Kiatpathomchai. (2012). Rapid and sensitive detection of shrimp yellow head virus using loop-mediated isothermal amplification and a colorogenic nanogold hybridization probe. *Journal of Virological Methods*, 186(1-2), 36-42.



Schematic representation of *S. enterica* serovar Typhimurium detection based on MWCNTs/AuNPs/Ab_1 and MBs/Ab_2 and signal amplified by silver reduction.

Fabrication of nanocapsule for signal amplification in Listeria detection

Listeria monocytogenes is one of the most harmful food borne pathogenic bacteria. A fast and sensitive detection of this pathogen would be instrumental for early detection. Although early detection of bacterial contamination using the classical technique enzyme linked immunosorbent assay (ELISA) is available, the sensitivity of this technique is limited. Accordingly, amplifying the signal to improve the detection limit and accelerate the assay performance is an important analytical goal.

A BIOTEC research team has fabricated a nanocapsule by using polystyrene sulfonate (PSS) to encapsulate gold nanoparticles (AuNPs) bearing adsorbed horseradish peroxidase (HRP), or AuNPs/HRP bioconjugate. The average size of a nanocapsule was in the range 150–400 nm. The efficiency of the capsules to enhance signals in an immunoassay was demonstrated by using ELISA to detect the food borne pathogen *Listeria monocytogenes*. The antibody adsorbed onto the PSS shell of the nanocapsules provided the recognition molecule. For a given quantity of antibody, the bioconjugate nanocapsules showed 30 times greater sensitivity and a shorter assay time (5 minutes) when compared to conventional ELISA using an HRP labeled antibody. This proof-of-concept encapsulation of HRP through PSS nanocapsules paves the way for alternative signal enhancement strategies where sensitivity is a priority.

This study was jointly conducted by Biochemical Engineering and Pilot Plant Research and Development Unit and Microarray Laboratory.

Ref: Oaew, S., Charlermroj, R., Pattarakankul, T. and Karoonuthaisiri, N. (2012). Gold nanoparticles/horseradish peroxidase encapsulated polyelectrolyte nanocapsule for signal amplification in *Listeria monocytogenes* detection. *Biosensors and Bioelectronics*, 34(1), 238-243.

Scano-magneto immunoassay based on MWCNTs/AuNPs nano-composite for Salmonella detection

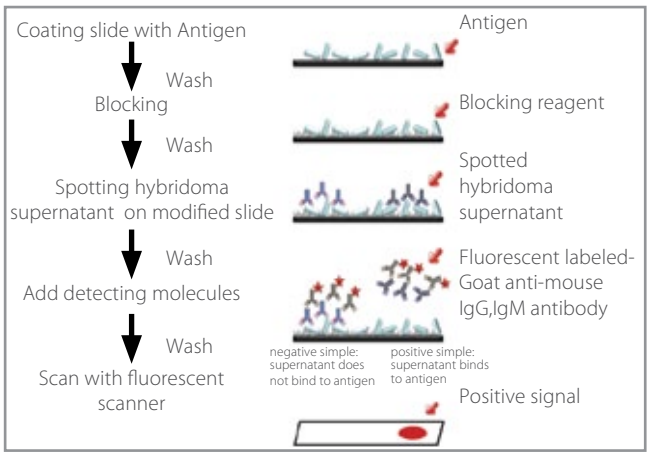
Typically, an enzyme linked immunosorbent assay (ELISA) possesses sensitivity in the pM range, which may be insufficient for the determination of pathogenic bacteria in food. A scanometric assay is an alternative. This scanometric assay has been successfully applied for both DNA and protein detection. To achieve high assay sensitivity, both carbon nanotubes (CNTs) and gold nanoparticles (AuNPs) have been used to amplify electrochemical signal for detection and are gaining popularity.

To improve sensitivity of *S. enterica* serovar Typhimurium detection, multiwalled carbon nanotubes (MWCNTs) and gold nanoparticles (AuNPs) were combined and used as a label to amplify signals in a scanometric based assay. In this study, the MWCNTs/AuNPs nanocomposite was fabricated by direct assemble of Au^{3+} to MWCNTs and allowed growing AuNPs along the MWCNTs surface. This MWCNTs/AuNPs nanocomposite was then attached to an anti-*S. typhimurium* antibody (MWCNTs/AuNPs/Ab_1) and used as a detecting molecule. Upon binding to *Salmonella*, they were pre-concentrated by magnetic beads/antibody (MBs/Ab_2), forming a sandwich immuno-complex which is later spotted on a nitrocellulose membrane coated slide. Silver reduction was applied to amplify the signal. The detection limit of 42 CFU/ml was achieved when 2% BSA was used as a blocking agent. Detection of *Salmonella* in real samples was carried out in different sample matrices. Further investigation on monoclonal antibody selection is required. Nevertheless, this array-based platform could afford simultaneous and low cost detection and enable high throughput screening of large numbers of samples.

This study was jointly conducted by Biochemical Engineering and Pilot Plant Research and Development Unit and King Mongkut's University of Technology Thonburi (KMUTT).

Ref: Amaro, M., Oaew, S. and Surareungchai, W. (2012). Scano-magneto immunoassay based on carbon nanotubes/gold nanoparticles nanocomposite for *Salmonella enterica* serovar Typhimurium detection. *Biosensors and Bioelectronics*, 38(1), 157-162.

Schematic illustration of stepwise hybridoma screening using an antibody array.



Signal amplification of microarray-based immunoassay using nanoliposome

The use of microarray-based immunoassay is often limited by its sensitivity. To increase sensitivities, liposome encapsulation was explored.

Two different liposome formations and several preparation methods were examined to optimize encapsulation and signal-enhancing efficacy for enzyme linked immunosorbent assay (ELISA) and antibody array. Signal amplification by liposome encapsulation was demonstrated through detection for food borne pathogenic *Listeria*. In plate-trapped antigen (PTA) ELISA, horseradish peroxidase (HRP)-loaded liposome increased the signal 9-fold more than the control. Limits of detection of HRP-encapsulated liposome were 6.4×10^5 and 5.5×10^6 CFU/ml in sandwich ELISA and antibody array, respectively. Furthermore, when a chromogenic 4-chloro-1-naphthol (4-CN) substrate was used for signal development in the antibody array, the signal could be detected with the naked eye. These results suggest that the liposome encapsulation technique has great potential for signal amplification and, therefore, for increasing assay sensitivity for various formats of immunoassay, especially the microarray-based format.

This study was a collaboration between BIOTEC Microarray Laboratory and National Nanotechnology Center (NANOTEC).

Ref: Ruktanonchai, U., Nuchuchua, O., Charlermroj, R., Pattarakankul, T. and Karoonuthaisiri, N. (2012). Signal amplification of microarray-based immunoassay by optimization of nanoliposome formulations. *Analytical Biochemistry*, 429(2), 142-147.

Hybridoma screening using an antibody array

Antibodies are important materials for diagnostics. A rapid and simple hybridoma screening method will help in delivering specific monoclonal antibodies.

Researchers systematically developed the first antibody array to screen for bacteria-specific monoclonal antibodies using *Listeria monocytogenes* as a bacteria model. The antibody array was developed to expedite the hybridoma screening process by printing hybridoma supernatants on a glass slide coated with an antigen of interest. This screening method is based on the binding ability of supernatants to the coated antigen. The bound supernatants were detected by a fluorescently labeled anti-mouse immunoglobulin. Conditions for antibody array construction were optimized. To demonstrate its usefulness, the antibody array was used to screen a sample set of 96 hybridoma supernatants in comparison to ELISA. Most of the positive results identified by ELISA and antibody array methods were in agreement except for those with low signals that were undetectable by antibody array. Hybridoma supernatants were further characterized with surface plasmon resonance to obtain additional data on the characteristics of each selected clone. While the antibody array was slightly less sensitive than ELISA, a much faster and lower cost procedure to screen clones against multiple antigens has been demonstrated.

This study was a collaboration between BIOTEC Microarray Laboratory, BIOTEC Monoclonal Production Laboratory and Queen's University Belfast. The result was published in *Analytical Biochemistry* in February 2012, and generated two patent applications in 2011:1) Method for hybridoma screening by antibody array using whole-cell bacteria labeled with fluorescent dye in solution as a reporter and 2) Method for hybridoma screening by antibody array using whole-cell bacteria coated on a solid surface and a fluorescently labeled anti-mouse antibody as a reporter.

Ref: Charlermroj, R., Oplatowska, M., Kumpoosiri, M., Himananto, O., Gajanandana, O., Elliott, C.T. and Karoonuthaisiri, N. (2012). Comparison of techniques to screen and characterize bacteria-specific hybridomas for high-quality monoclonal antibodies selection. *Analytical Biochemistry*, 421(1), 26-36.

Highlights from Proteomics

Identification of reptile egg white proteins

Proteomic analyses of proteins from reptile egg white are used to define qualitative and quantitative differences among reptile species. Inter-species variability may explain particular characteristics concerning functional and biological properties of egg proteins from different reptile origins.

Proteomics of egg white proteins of five reptile species, namely Siamese crocodile (*Crocodylus siamensis*), soft-shelled turtle (*Trionyx sinensis taiwanese*), red-eared slider turtle (*Trachemys scripta elegans*), hawksbill turtle (*Eretmochelys imbricate*) and green turtle (*Chelonia mydas*) were studied using 2D-PAGE. The protein spots in the egg white of the five reptile species were identified by MALDITOF mass spectrometry and LC/MS-MS analysis. Sequence comparison with the database revealed that reptile egg white contained at least seven protein groups: serpine, transferring precursor/iron binding protein, lysozyme C, teneurin-2 (fragment), interferon-induced GTP-binding protein Mx, succinate dehydrogenase iron-sulfur subunit and olfactory receptor 46. This report confirms that the transferrin precursor/iron binding protein is the major component in reptile egg white. In egg white of Siamese crocodile, twenty isoforms of transferring precursor were found. An iron binding protein was found in four species of turtle. In egg white of soft-shelled turtle, ten isoforms of lysozyme were found. This study identified additional reptile egg white proteins, such as the teneurin-2 (fragment), the interferon-induced GTP-binding protein Mx, the olfactory receptor 46 and the succinate dehydrogenase iron-sulfur subunit.

This work was conducted as a collaboration between BIOTEC Proteomics Research Laboratory and Khon Kaen University.

Ref: Prajanban, B.-O., Shawsuan, L., Daduang, S., Kommanee, J., Roytrakul, S., Dhiravisit, A. and Thammasirirak, S. (2012). Identification of five reptile egg whites protein using MALDI-TOF mass spectrometry and LC/MS-MS analysis. *Journal of Proteomics*, 75(6), 1940-1959.

Proteomic analysis of chikungunya virus infected microglial cells

The chikungunya virus is a recently re-emerged public health problem in many countries bordering the Indian Ocean and elsewhere. Chikungunya fever is a relatively self limiting febrile disease, but the consequences of chikungunya fever can include a long lasting, debilitating joint pain, and occasional neurological problems have been reported.

Macrophages have been implicated as an important cell target of the chikungunya virus (CHIKV) with regards to both their role as an immune mediator, as well evidence pointing to long term viral persistence in these cells. Microglial cells are the resident brain macrophages, and so this study sought to define the proteomic changes in a human microglial cell line (CHME-5) in response to CHIKV infection. GeLC-MS/MS analysis of CHIKV infected and mock infected cells identified some 1455 individual proteins, of which 90 proteins, belonging to diverse cellular pathways, were significantly down-regulated at a significance level of $p<0.01$. Analysis of the protein profile in response to infection did not support a global inhibition of either normal or IRES-mediated translation, but was consistent with the targeting of specific cellular pathways including those regulating innate antiviral mechanisms.

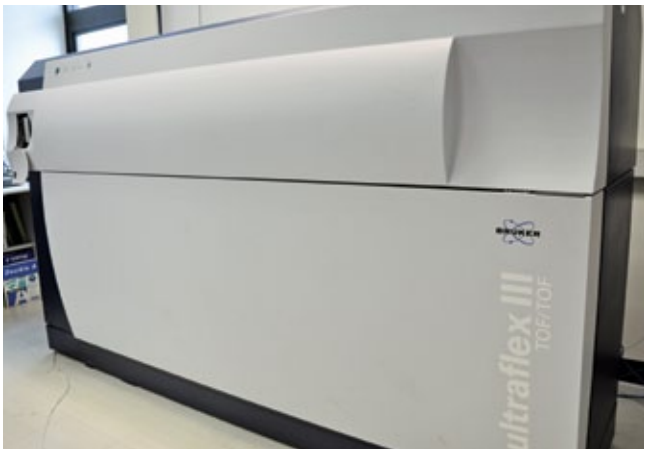
This work was conducted under the collaboration between BIOTEC Proteomics Research Laboratory and Mahidol University.

Ref: Abere, B., Wikan, N., Ubol, S., Auewarakul, P., Paemanee, A., Kittisenachai, S., Roytrakul, S. and Smith, D.R. (2012). Proteomic Analysis of Chikungunya Virus Infected Microgial Cells. *PLoS ONE*, 7(4), e34800.

Proteomic analysis of a unicellular green alga subjected to irradiance and salinity stresses

Oxygenic photosynthetic organisms often suffer from excessive irradiance, which cause harmful effects to chloroplast proteins and lipids. Photoprotection and the photosystem II repair process are the mechanisms that plants deploy to counteract the drastic effects from irradiance stress. Although the protective and repair mechanisms

BIOTEC Proteomics Facility is fully equipped with 2-D gel electrophoresis, image processing and analysis and mass spectrometry.



seem to be similar in most plants, many species confer different levels of tolerance toward high light. Such diversity may originate from differences at the molecular level, i.e., perception of the light stress, signal transduction and expression of stress responsive genes. Comprehensive analysis of overall changes in the total pool of proteins in an organism can be performed using a proteomic approach.

A 2-DE/LC–MS/MS-based comparative proteomic approach was employed to analyze total proteins of the light sensitive model unicellular green alga *Chlamydomonas reinhardtii* in response to excessive irradiance. Results showed that among all the differentially expressed proteins, several heat-shock proteins and molecular chaperones were surprisingly down-regulated after 3–6 hours of high light exposure.

In a separate study, researchers presented a 2-DE-based proteomic analysis of *C. reinhardtii* subjected to 300 mM NaCl for 2 hours. Results showed that, in addition to the protein spots that showed partial up- or down-regulation patterns, a number of proteins were exclusively present in the proteome of the control cells, but were absent from the salinity-stressed samples. Conversely, a large number of proteins exclusively appeared in the proteome of the salinity-stressed samples. Of those exclusive proteins, we could successfully identify, via LC–MS/MS, 18 spots uniquely present in the control cells, and 99 spots specific to NaCl-treated cells. Among the salt-exclusive protein spots, several important housekeeping proteins like molecular chaperones and proteins of the translation machinery were identified, suggesting that they may originate from post-translational modifications rather than from de novo biosynthesis.

This work was conducted as a collaboration between BIOTEC Proteomics Research Laboratory and Mahidol University.

Ref:

1. Mahong, B., Roytrakul, S., Phaonaklop, N., Wongratana, J. and Yokthongwattana, K. (2012). Proteomic analysis of a model unicellular green alga, *Chlamydomonas reinhardtii*, during short-term exposure to irradiance stress reveals significant down regulation of several heat-shock proteins. *Planta*, 235(3), 499-511.
2. Yokthongwattana, C., Mahong, B., Roytrakul, S., Phaonaklop, N., Narangajavana, J. and Yokthongwattana, K. (2012). Proteomic analysis of salinity-stressed *Chlamydomonas reinhardtii* revealed differential suppression and induction of a large number of important housekeeping proteins. *Planta*, 235(3), 649-659.

Highlights from Bioinformatics

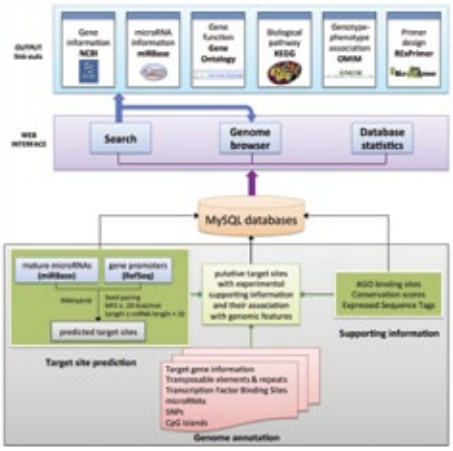
Genetic dating indicates that the Asian–Papuan admixture through Eastern Indonesia corresponds to the Austronesian expansion

Although the Austronesian expansion had a major impact on the languages of Island Southeast Asia, controversy still exists over the genetic impact of this expansion. The co-existence of both Asian and Papuan genetic ancestry in Eastern Indonesia provides a unique opportunity to address this issue.

Researchers estimated recombination breakpoints in admixed genomes based on genome-wide SNP data and date the genetic admixture between populations of Asian versus Papuan ancestry in Eastern Indonesia. Analyses of two genome-wide datasets indicate an eastward progression of the Asian admixture signal in Eastern Indonesia beginning about 4,000-3,000 years ago, which is in excellent agreement with inferences based on Austronesian languages. The average rate of spread of Asian genes in Eastern Indonesia was about 0.9 km/year. The results indicate that the Austronesian expansion had a strong genetic as well as linguistic impact on Island Southeast Asia, and they significantly advance our understanding of the biological origins of human populations in the Asia Pacific region.

This study was conducted by scientists from Shanghai Institutes for Biological Sciences, Max Planck Institute for Evolutionary Anthropology, University Medical Center Rotterdam, Fudan University and The HUGO Pan-Asian SNP Consortium of which BIOTEC bioinformatician is a member.

Ref: Xua, S., Pugach, I., Stoneking, M., Kayser, M., Jin, L. and The HUGO Pan-Asian SNP Consortium. (2012). Genetic dating indicates that the Asian–Papuan admixture through Eastern Indonesia corresponds to the Austronesian expansion. *Proceedings of the National Academy of Sciences of the United States of America*, 109(12), 4574-4579.



A three-tier system overview of the microPIR database displaying the data sources and web interface features.

Understanding transcription factor binding sites

MicroRNAs are important post-transcriptional regulators of gene expression. However, they do not work in isolation, but rather act in concert with other classes of regulatory proteins. In particular, transcription factors, microRNAs and their respective targets form interconnected feedback and feedforward circuits.

Transcription factors are thought to regulate the transcription of microRNA genes in a manner similar to that of protein-coding genes; that is, by binding to conventional transcription factor binding site DNA sequences located in or near promoter regions that lie upstream of the microRNA genes. In the course of analyzing the genomics of human microRNA genes using the UCSC Genome Browser, researchers noticed that annotated transcription factor binding sites commonly lie within 70- to 110-nt long microRNA small hairpin precursor (pre-miR) sequences. This association was characterized in detail, with discussion on several possible explanations for this phenomenon.

This study was conducted by scientists from BIOTEC Bioinformatics and Biostatistics Laboratory, Georgia Institute of Technology and the University of Illinois at Chicago.

Ref: Piriyapongsa, J., Jordan, I.K., Conley, A.B., Ronan, T. and Smalheiser, N.R. (2011). Transcription factor binding sites are highly enriched within microRNA precursor sequences. *Biology Direct*, 6, 61.

microPIR: an integrated database of microRNA target sites within human promoter sequences

The microPIR database is a useful integrated resource of microRNA-promoter target interactions for experimental microRNA researchers and computational biologists to study microRNA regulation through gene promoter.

microRNA-Promoter Interaction Resource (microPIR) is a database hosting predicted microRNA target sites located within human promoter sequences and their associated genomic features. The microPIR database integrates various annotated genomic sequence databases, for example, repetitive elements, transcription factor binding sites, CpG islands, and SNPs. The database enables users to explore relationships among target sites and other genomic features. Furthermore, functional information of target genes including gene ontologies, KEGG pathways, and OMIM associations are provided. The built-in genome browser of microPIR provides a comprehensive view of multidimensional genomic data. A local primer designing tool is also incorporated into microPIR to assist experimental design of microRNA target validation.

This work was developed by BIOTEC Biostatistics and Informatics Laboratory.

Ref: Piriyapongsa, J., Bootchai, C., Ngamphiw, C. and Tongsima, S. (2012). microPIR: An Integrated Database of MicroRNA Target Sites within Human Promoter Sequences. *PLoS ONE*, 7(3), e33888.

Novel heterogeneous ensemble for pre-miRNA classification

MicroRNAs (miRNAs) play a crucial role in post-transcriptional regulation of gene expression of plants and animals. Identification of miRNA genes is one of the most difficult problems in understanding post-translational gene regulation in both normal development and human pathology. Computational methods have been developed to complement experimental approaches to help biologists identify putative miRNA genes. However, key drawbacks of computational methods are 1) their lack of ability to detect novel pre-miRNAs that are not homologous to previously identified miRNAs and 2) they can generate a high false positive rate due to high number of stem-loop structures of non-miRNA sequences in the genome, and 3) a risk of over-fitting of an algorithm to the training data.

Researchers developed a novel heterogeneous ensemble combining various efficient classifiers to the problem of pre-miRNA classification. The method, a cooperative combination of different learning algorithms exposed to different training subsets, can create a high level of diversity and reduce bias that tends to occur when a single individual classifier is used. Consequently, the ensemble provides a more reliable prediction. Additionally, novel robustness features were introduced: the SC-base pair composite features served as promising discriminators in distinguishing real pre-miRNA hairpins from other hairpin sequences with improved sensitivity and specificity from an original SC feature. Moreover, a feature selection method was applied to select only relevant and discriminative features. The problem of imbalanced data was solved by the modified Synthetic

Damage done to the agriculture and food sectors by the 2011 flood was estimated at 90 billion Thai Baht.

Floodwater level at the gate of Thailand Science Park was measured at 1.3 m. on 2 November 2011. The Park, in which BIOTEC headquarters and five research units are located, was closed for two months.

Minority Oversampling Technique bagging method. This enhanced ensemble-based method effectively differentiated pre-miRNA from non-miRNA sequences with higher accuracy and better balanced sensitivity and specificity score across various organisms, making this model a useful tool for finding novel animal, plant and virus pre-miRNAs.

This work was conducted as a collaboration between the Biochemical Engineering and Pilot Plant Research and Development Unit and King Mongkut's University of Technology Thonburi. The program is available at http://ncrna-pred.com/premiRNA.html.

Ref: Lertampaiporn, S., Thammarongtham, C., Nukoolkit, C., Kaewkamnerdpong, B. and Ruengjitchatchawalya, M. (2012). Heterogeneous ensemble approach with discriminative features and modified-SMOTEbagging for pre-miRNA classification. *Nucleic Acids Research*, doi:10.1093/nar/gks878.

Highlights from Policy Research and Development

Roadmap for human resource and network development for diagnostics and sensors

BIOTEC conducted a study on the diagnostics and sensors industry in Thailand and formulated a roadmap to develop this sector with funding provided by the National Science Technology and Innovation Policy Office. Diagnostics and sensors are widely used in major industries for quality control as well as decision making. The market value of diagnostics and sensors was estimated to be worth around 6.7-7 billion Baht. In the next 5 years, the industry should focus R&D on the following topics:

- micro-electronic devices to detect contaminants and pathogens;
- multiple detection devices;
- remote control systems for pollution monitoring; and
- genetic testing.

The study recommended establishing a consortium consisting of professionals from various scientific disciplines and the private sector. The consortium would facilitate translational research, product testing, and setting up standards for domestic and international acceptance. In terms of human resource development, research and scholarship granting agencies should direct funding towards the development of experts in bioelectronics and genetic consultation to prepare for an expansion of genetic testing in the near future. Collaboration with international partners to facilitate technology transfer is also a recommended mechanism.

Assessment of 2011 flood damage and capacity building for the agriculture and food sector in Thailand

In 2011, Thailand faced with one of the most damaging floods of the century. Starting in July when Tropical Storm Nock-ten hit the north and northeast of Thailand, flooding then spread through provinces of Northern, Northeastern and Central Thailand along the Mekong and Chao Phraya River basins and lasted until December in some areas, especially in the Central Plain.

The damage caused by the 2011 floods was estimated to be 1.4 trillion Baht, 90 billion of which were losses in the agriculture and food sectors. 3.55 million acres of rice fields were damaged, accounting for 2.6 million tons or 9% of the total rice production. Nock-ten not only affected Thailand, but also caused damage to rice production throughout Southeast Asia estimated at around 4.5 million tons reduction. Climate change and natural disasters require that Thailand and other Southeast Asian nations build resilience into the agriculture and food sectors through science and technology to ensure food security. In the short-term, Thailand should focus on the deployment of existing technologies to enhance the production process. In the medium to long term, the country needs to build up a critical mass of researchers, increase its research and development budget, as well as establish crucial infrastructure at the national level such as germplasm collections, and food research and development centers. There should be a clear policy to support research and development of transgenic crops, especially field trials.

COMMERCIALIZATION AND PRIVATE SECTOR PARTNERSHIP

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

In fiscal year 2012, BIOTEC worked with 21 companies on 25 projects in the form of collaborative and commissioned research, 12 of which were initiated in 2012. BIOTEC provided consultancy services to nine companies on 12 different projects, seven of which were new in 2012. Examples of collaborators and clients include: SCG Group, Charoen Pokphand Group, Novozyme, Jelly Belly Candy Company (Thailand), Bangkok Seeds Industry, Emsland-Stärke GmbH, Ampol Food Processing, BioNet-Asia and Sherwood Chemicals. Projects include development of DNA markers for plant traits, development of a transformation system for eucalyptus, extraction of soluble fiber from agricultural residue, screening microorganisms that produce alkaline protease and lipase, and providing advice on quality control in vaccine production.

A portable dissolved oxygen test kit offers an accurate reading of dissolved oxygen concentration in a water sample in a simple 3-minute procedure.

WSSV Speedy Color: a colorimetric-LAMP detection technique for shrimp white spot syndrome virus.

Licensing Agreements

Six licensing agreements have been signed:

Licensee	Detail
Sigma Aldrich	The rights to use *Verticillium* spp., an insect pathogenic fungus in the BIOTEC Culture Collection, to produce ascochlorin for commercialization.
Sigma Aldrich	The rights to use *Lentinus* spp., a fungus in the BIOTEC Culture Collection, to produce panepoxydone for commercialization.
Thai Luxe Enterprises	The rights to use the patented tubular denitrification reactor in the company's feed-testing aquaria.
Drew-Bio (Thailand)	The rights to commercialize a system to be connected to Low Pressure Liquid Chromatography (LPLC), for automatic Thalassemia test result interpretation.
Shrimp Biotechnology Business Unit	The rights to commercialize a colorimetric-LAMP detection technique for shrimp white spot syndrome virus.
Thai Herb Tech	The rights to commercialize technology to use herbal oils to control mites (*Suidasia pontifica* Oudemans) in animal feed storage.

Portable Dissolved Oxygen Test Kit

Dissolved oxygen is essential for the maintenance of healthy lakes and rivers, and aquatic plants and animals need oxygen to survive. An easy-to-use and accurate method to measure dissolved oxygen would be a useful tool for monitoring water quality in natural reservoirs, aquariums and aquaculture farms.

To fill this need, BIOTEC Bioresources Research Laboratory developed a portable dissolved oxygen test kit called 'DO-DEE'. DO-DEE offers an accurate reading of dissolved oxygen concentration in a water sample in a simple 3-minute procedure based on a colorimetric reaction with redox dye. The kit is also less expensive than imported test kits.

DO-DEE was one of the five technologies highlighted at NSTDA Investors' Day 2012. NSTDA Investors' Day is an annual event introducing commercially viable research to potential investors.

THAILAND BIOTECH GUIDE 2011/2012

Thailand Biotech Guide 2011/2012 addresses the theme "Sustaining Thailand's Development through Biodiversity". The editorial section features an article on Thailand's biodiversity and exclusive interviews with BIOTEC Executive Director, researchers and CEOs of selected Thai companies engaging in biotech R&D.

Published in English, Thailand Biotech Guide is a source book for foreign investors. The Guide contains articles on up-to-date biotechnology research and investment, a directory of biotech companies, and listings of suppliers for equipment and products supporting bio-industry. Published annually since 2006, Thailand Biotech Guide is produced by Green World Publication Co., Ltd. and Marshall Cavendish Business Information Private Limited, with support from BIOTEC, NSTDA and Thailand Board of Investment.

Open Lab for Industry

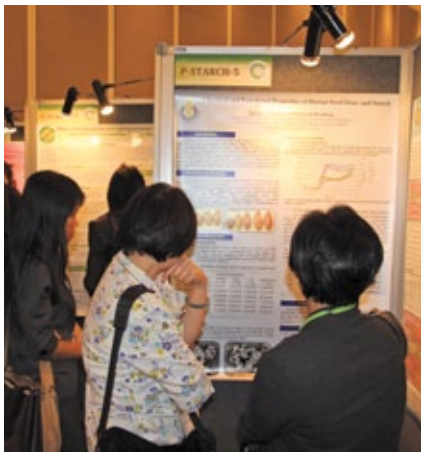
Open Lab is an activity to promote collaboration between laboratories and industries by inviting companies to meet with BIOTEC scientists, learn about BIOTEC expertise, and the potential of biotechnology for improving industrial processes and products. Open Lab often leads to collaborative research, commissioned research, or the provision of analytical services. In 2012, six companies participated in an Open Lab on the topic of nuclear polyhedrosis virus pilot plant for insect pest control.

The pilot plant consists of worm rearing, viral inoculation, and harvesting facilities to produce nuclear polyhedrosis virus using *in vivo* cultures of infected beet army worm (*Spodoptera exigua*). In addition to producing nuclear polyhedrosis virus products for commercialization, the facility is open for research collaboration to produce other viral biocontrol agents from combinations of viral strains and worm types.

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 training workshops for academics and industries, to organizing youth programs.

Starch Update 2011 Conference was held in February 2012. The event was postponed from December 2011 due to the severe flooding.



Post-doctoral Fellowship Program

Established since 2005, the BIOTEC post-doctoral fellowship program aims to foster the development of young scientist in the area of biotechnology. In 2012, two new fellowships were granted, along with nine on-going fellowships.

Training of Post-graduate Students

Most of BIOTEC principle researchers serve as co-advisors to life sciences graduate students from Thai universities. These students take part in on-going research projects at BIOTEC. Some of these students are supported by programs such as the Royal Golden Jubilee PhD Program offered by the Thailand Researcher Fund and the Thailand Graduate Institute of Science and Technology scholarship from the National Science and Technology Development Agency. In 2012, BIOTEC hosted 97 PhD students and 96 MS students. Of these, 10 obtained their Doctorate degrees and 16 obtained their Master degrees.

Workshops and Conferences

In 2012, BIOTEC organized one national conference on biodiversity and one international conference on starch technology. A total of 10 workshops were organized on such topics as entomology, food allergens and biosafety guidelines.

Science in Rural Schools Program (SiRS)

SiRS is a program dedicated to the improvement of science education in rural schools, through activities such as curriculum development, training programs for science teachers, organizing activities such as science project contests, science camps, and science fairs. The program currently has 221 schools in its network. In 2012, SiRS organized 40 training workshops for over 30,000 science teachers and students across the country. Five science camps were organized for a total of 500 participants.

Entomology Workshop
An Entomology Workshop was organized on 5-10 March 2012 with a guest lecturer, Prof. Robert W. Sites, from the Division of Plant Sciences, University of Missouri. The course was designed for researchers and graduate students working in the field of insect ecology, habitats, and behavior to acquire a basic understanding of insect taxonomy and field methodologies.

The workshop was attended by twenty-six participants, one of which was from Oman and the rest from Thailand.

Workshop on Effective Food Allergen Management
The workshop was held on 2-4 April 2012, and co-organized by the Monitoring and Quality Assurance in the Food Supply Chain (MoniQA Association), International Association for Cereal Science and Technology, Food Science & Technology Association of Thailand, and Thammasat University. The workshop targeted professionals working in the food industry as well as food safety authorities. The course, taught mostly by European experts, covered the basics of food allergens, current situations, regulations and management in Thailand and in other countries, as well as food allergen detection methods currently available in the market.

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 bring the public into BIOTEC research facilities where they can meet and talk to scientific staff.

Students study information on insect anatomy and the use of microbes to control insects at the National Science and Technology Fair 2012.

Farmers from Nakhon Nayok Province on a tour of the BIOTEC research laboratory.

Television Programs

BIOTEC has produced a variety of television programs, ranging from 1.5-minute spots, to 3-minute and 40-minute documentaries, for regular broadcast on public channels as part of NSTDA TV. Programs on these topics were produced in 2012:

- Profiles of award-winning scientists designed to create awareness of research as a profession and to inspire young people. Profiles included Dr. Saengchan Senapin (winner of the 2011 L'Oreal Women in Science award) and Dr. Sithichoke Tangphatsornruang (winner of the 2012 Thailand Young Scientist award).
- Products available in the market as a result of BIOTEC research (for example: animal feed enzymes and feed supplements, biocontrol and bioremediation agents, Aqua RASD (a closed recirculating aquaculture system integrated with the hybrid nitrification biofilter tanks for ammonia removal and patented Tubular Denitrification Reactor for nitrate removal), blast-resistant glutinous and other rice strains from the molecular rice breeding program, and a shrimp disease detection kit).
- New products and technologies in development for potential collaborators and investors (for example: enzymes for the pulp and paper industry, biocontrol agent (*Beauveria bassiana*), a breath analyzer for diabetes, a dissolved oxygen test kit, and a mercury test kit).

Biocontrol Exhibition at Thailand National Science and Technology Fair 2012

A range of BIOTEC research work on biological control of pests was presented to visitors at the National Science and Technology Fair, 17-31 August, 2012. BIOTEC took part in organizing this exhibition under the theme "Biocontrol". Exhibits included the use of nuclear polyhedrosis virus to control beet army worms, *Beauveria bassiana* fungus to control insect pests in cassava, brown plant hoppers in rice, and the use of *Bacillus thuringiensis* bacteria to control mosquito larvae.

The National Science and Technology Fair is Thailand's largest annual science and technology festival and aims to promote public understanding and awareness of science and technology to young people. The festival is held every year in August to coincide with National Science Day on 18 August. The fair combines exhibitions, edutainment, and seminars arranged in cooperation with public and private local and international companies and organizations.

Roadshows

BIOTEC organizes exhibitions at established events such as the Agricultural Fair at Walailak University in Nakhon Si Thammarat, the National Dairy Fair in Saraburi, and the Royal Flora in Chiang Mai. Roadshows introduce BIOTEC technologies, products and services. BIOTEC also joined three roadshows organized by the Ministry of Science and Technology to introduce technologies and services to people in the East, Northeast and South of Thailand.

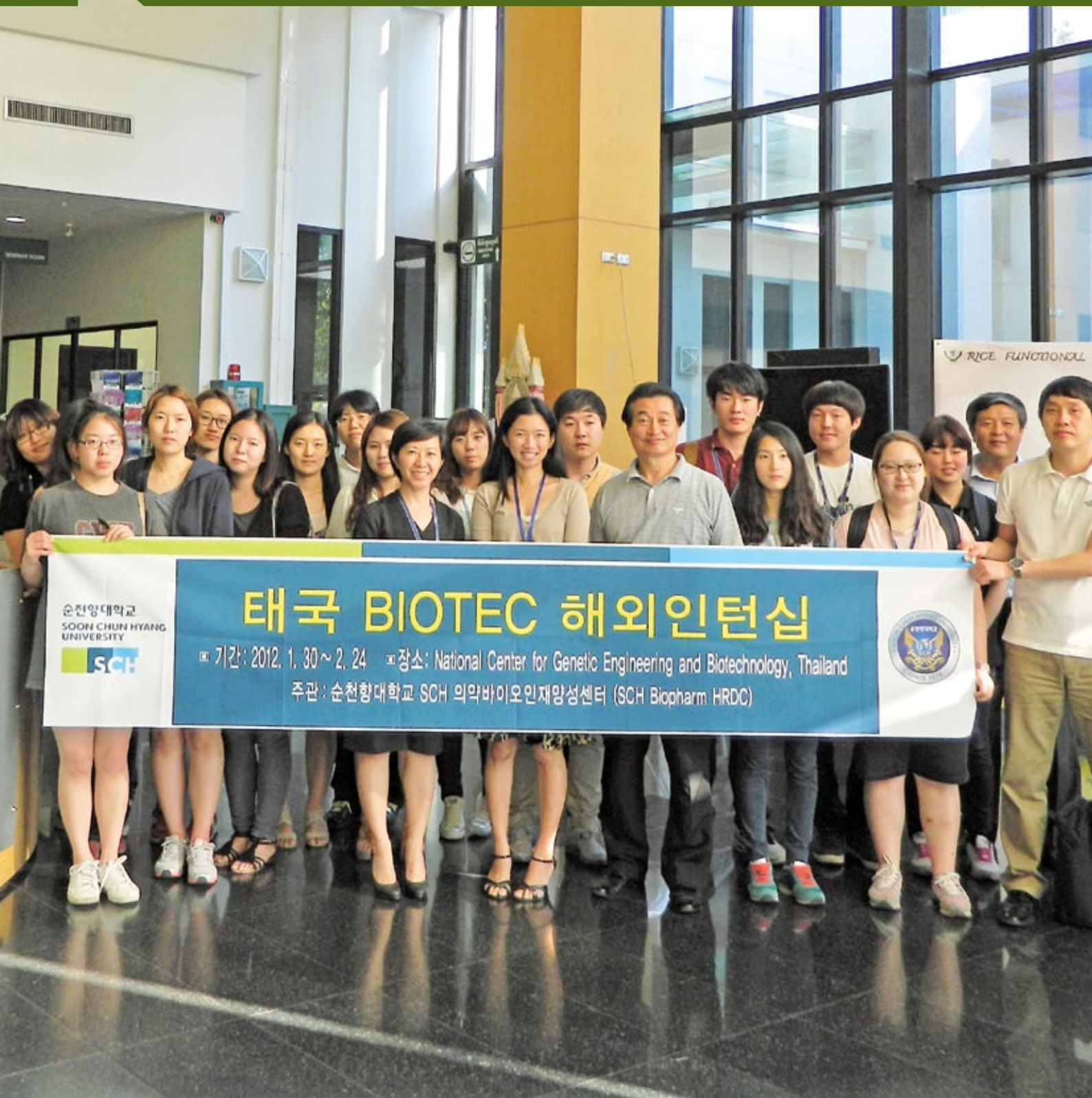
Lab Tours

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. Close interaction with BIOTEC research staff encourages researchers to relate their science to solving everyday problems and to explain their work in easily understandable terms.

In 2012, 120 groups toured BIOTEC laboratories. Visitors included food industry journalists from the US, the management team of Better Pharma (Thailand), farmers from Ayutthaya and Nakhon Nayok provinces, the Deputy Director General of the Rice Department, representatives from food processing companies under the Federation of Thai Industries, and executives from leading companies such as Saha Group (Thailand), Bangchak Petroleum and DuPont (Thailand).

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.



Mycologists from BIOTEC, Vinh University and CRD collect marine fungi at Lang Co Bay in Hue City of Vietnam.

Fostering Collaboration

In fiscal year 2012, BIOTEC signed six Memorandums of Understanding (MOUs) to foster collaboration with the following organizations:

Organization	Detail
Nonglam University, Vietnam	To support collaboration on rice improvement and bio-control
Research Institute, Meijo University, Japan	To foster collaboration on salt tolerance improvement in rice
Cebu Technological University, Barili Campus, The Philippines	To develop collaboration on salinity and drought tolerance improvement of tropical crops through biotechnology
City University of Hong Kong, China	To support the placement of students from City University of Hong Kong in BIOTEC laboratories
Biotechnology and Biological Sciences Research Council, UK and Thailand Research Fund (BIOTEC signed this MOU on behalf of NSTDA).	To promote Thai-UK research collaboration and support PhD scholarships for Thai students in the areas of biotechnology and biological sciences
Vinh University, Vietnam	To promote collaboration on entomopathogenic fungi research
Center for Regional Research and Development (CRD) Vietnam	To promote collaboration on marine fungi research

BIOTEC organized research seminars with foreign institutes to foster the exchange of information on current research work and research interests between Thai and foreign scientists and to promote future collaboration. Seminars with the following institutes were held in the past year:

Organization	Seminar topic
National Collection of Industrial Food and Marine Bacteria and Marine Biodiscovery Centre, University of Aberdeen, UK	Culture collection and microbial use study
Helmholtz Center for Infection Research, Germany	Exploitation of microbial bio- and chemo-diversity
University of Leipzig, Germany	Biocatalytic degradation and modification of synthetic polymers

FP7/Horizon 2020 People Programme Workshop and FP7 Cooperation-Food and Environment Info Day to provide information on the European Union's 7th Framework Programme funding scheme for the Thai research community.

Strategic Japanese-Thai Cooperative Program in Biotechnology 2012

A call for proposals announced in February 2012 resulted in 16 proposal submissions from 20 academic and research institutes in Japan and Thailand. Three projects were selected for funding by the Joint Committee. The scope of the 2012 call for proposals focused on microbial biotechnology, agro-biotechnology, and functional genomic technology, which are the platform technologies through which BIOTEC administers research funds.

BIOTEC and the Japan Science and Technology Agency launched the Strategic Japanese-Thai Cooperative program in Biotechnology in 2012 to support research projects conducted jointly by Thai and Japanese scientists.

The three selected proposals were:

- **Identification of genes conferring high Fe and Fe toxic tolerance in rice,** by Dr. Vinitchan Ruanjaichon from the Rice Gene Discovery Unit, BIOTEC, and Prof. Dr. Naoko Nishizawa from the Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University.
- **A DNA chip for identification and typing of *Mycobacterium tuberculosis* using the DigiTag platform**, by Assoc. Prof. Dr. Angkana Chaiprasert from the Faculty of Medicine, Siriraj Hospital, Mahidol University, and Prof. Dr. Katsushi Tokunaga from the Graduate School of Medicine, University of Tokyo.
- **Bacteriophage biocontrol for sustainable crop production: Application to bacterial wilt**, by Dr. Orawan Chatchawankanphanich of BIOTEC, and Prof. Dr. Takashi Yamada of Hiroshima University.

Financial support for research and staff exchange is provided to scientists in their respective countries by BIOTEC and Japan Science and Technology Agency.

Promotion of the European Union 7th Framework Programme

BIOTEC and Queen's University Belfast, UK, organized the FP7/Horizon 2020 People Program Workshop and FP7 Cooperation-Food and Environment Info Day, 12-13 March 2012 at BIOTEC Thailand Science Park. Financial support was provided by INCONTACT. The objectives of the event were to provide detailed information to the Thai research community about the FP7 People Program and related Marie Curie Actions and to establish a platform for exchange of research staff between Queen's University of Belfast and Thai research organisations.

Experts from Queen's University, Halbert Research, and FP7 National Contact Point (Sweden) gave a training session on consortium formation and proposal writing and preparation. The workshop was attended by more than 50 scientists from research centers and universities in Thailand.

Participation of BIOTEC in an FP7-funded GRATITUDE Project

One way to reduce waste from post-harvest losses of root and tuber crops, particularly cassava and yams, is to turn waste into something of increased value. Technologies and systems developed and validated within the Gratitude project are designed to benefit small-holder farmers and small and medium enterprises. The Gains from Losses of Roots and Tuber Crops Project (Gratitude) is a European Union Framework 7 funded project launched in March 2012.

This project is led by the Natural Resources Institute, University of Greenwich, UK, in collaboration with 15 other organizations from the Netherlands, Portugal, Nigeria, Ghana, Vietnam and Thailand.

The work in Thailand will be conducted by a research team at the Cassava and Starch Technology Research Unit, a collaborative unit between BIOTEC and Kasetsart University. The research subjects include 1) value-chain assessment for cassava; 2) assessment of functional properties and new market developments for high quality cassava flour; 3) technology development to improve the use of waste (peel and pulp); and 4) technology demonstration. The technology demonstration will be performed in close collaboration with Northeastern Starch (1987) Co., Ltd., a Thai SME.

Researchers from neighboring countries participate in BIOTEC's Human Resource Development Program in Biotechnology in 2012.

Collaborative Research on the Flood Aftermath

In late 2011, Thailand experienced one of the most damaging floods of the century. Flooding spread through the provinces of Northern, Northeastern and Central Thailand along the Mekong and Chao Phraya river basins and lasted until December in some areas, especially in the Central Plain. Stagnant flood water led to water quality issues caused by microorganisms. This prompted a joint study launched in early 2012 between BIOTEC, Chulalongkorn University, and international partners funded under the RAPID program of the US National Science Foundation, and the J-RAPID program of the Japan Science and Technology Agency.

The Thai and US teams (North Dakota State University) embarked on an investigation of the type and quantity of sediment and the nature of the microbial community within the sediment. Collaboration with the National Institute of Technology and Evaluation of Japan focused on surveying the distribution of polycyclic aromatic hydrocarbon (PAH-degrading) bacteria in sediments from the two flood-affected rivers, particularly the Chao Praya River and Tha Jin River deltas where intensive sediment deposition was found.

14th A-IMBN Annual Conference: Life Science and Frontiers of Biorefinery Technology

Thailand and several other countries in Asia are major producers of agricultural products and thus have great potential to adopt biorefinery techniques for converting biomass to fuels and other value-added products. Towards this end, BIOTEC organized the 14th A-IMBN Annual Conference: Life Science and Frontiers of Biorefinery Technology on 1-2 March 2012 at the Thailand Science Park. The event was held in collaboration with the Asia-Pacific International Molecular Biology Network and was attended by 122 participants from Thailand and abroad.

The objective of this A-IMBN Annual Conference was to promote the development of scientific and technical excellence amongst scientists and institutions dedicated to molecular biology and genetic engineering research, with a particular focus on Asia and the Pacific Rim. This year the conference included biorefining as an additional theme to the traditional A-IMBN conference which normally focuses on biomedical sciences.

Founded in 1997, The Asia-Pacific International Molecular Biology Network (A-IMBN) brings together the leading minds and institutions in the region to work towards ensuring the economies in Asia and the Pacific Rim will remain at the cutting edge of new developments in biotechnology. BIOTEC is listed among twenty A-IMBN supporting institutes.

International Exchange Programs

Every year, BIOTEC welcomes researchers and students to work and receive training in its laboratories. A total of seventy-one researchers and students from abroad spent time in BIOTEC laboratories in 2012.

An International Internship Program accommodates overseas students allowing them to gain research experience by working with BIOTEC research staff in a professional research setting. Forty-one undergraduate students from Korea, Indonesia, Hong Kong, Taiwan and Singapore were placed in BIOTEC laboratories for periods ranging from 3 weeks to 5 months. This program is implemented regularly with the following academic institutes: Soon Chun Hyang University (Korea), Atma Jaya Catholic University (Indonesia), City University of Hong Kong (Hong Kong), National Taiwan University (Taiwan), Temasek Polytechnic (Singapore) and Nanyang Polytechnic (Singapore). City University of Hong Kong is the newest partner, joining the Program for the first time in 2012. In addition, four graduate students from Slovak University of Technology in Bratislava (Slovak), Kyushu University (Japan), and Chiba University (Japan) were hosted in BIOTEC laboratories during 2012.

Twenty-six researchers from seven countries (India, Vietnam, Pakistan, Sweden, Myanmar, Philippines and Indonesia), were invited to BIOTEC laboratories for collaborative research or training. Of this number, eleven researchers participated in BIOTEC's Human Resource Development Program in Biotechnology, a program dedicated to capacity building for developing countries in the Asia-Pacific region.

IMPACT OF BIOTEC'S OUTPUT

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

Homcholasit rice, a product of Pak Hai Farmers' Cooperative in Ayutthaya, is now widely available in major supermarket chains under the brand name "On-waan"

A rapid strip test for detection of phytoplasma, a cause of white leaf disease in sugarcane.

In 2012, 65 completed projects were selected for impact assessment. An estimated total of 2.5 billion Baht was generated from these projects. This amount was categorized as investment (73 million Baht), revenue generation to licensees or users (1.66 million Baht), cost reductions (732 million Baht) and import replacement (94 million Baht).

Agriculture and Food Cluster

Rice seed production training program. The production training program distributes new rice varieties to farmers to promote seed production. New rice varieties are developed from the molecular rice breeding program and include Thanyasirin (blast-resistant jasmine glutinous rice), Homcholasit (flash-flood tolerant, irrigated jasmine rice) and bacterial-blight-resistant rice.

The seed production training program was implemented in 11 provinces in the central, north and northeast of Thailand. One cooperative in Ayutthaya created a brand for Homcholasit rice and secured the distribution of packaged rice in supermarket chains instead of selling bulk grain. The co-op was able to obtain an additional 9.65 million Baht for machinery investment from the Ministry of Science and Technology for a grain dryer, seed separator machine and grain moisture meter. The rice seed production training program generated a total of 398 million Baht in investment and income to farmers.

Improvement in sugarcane production. Thailand is a key supplier to the international sugar market with approximately five percent of agricultural land under cane production. Good quality cane stalks are in demand to ensure sufficient supply for the growing industry, both sugar and biofuel commodities.

Since 2003, BIOTEC has been working collaboratively with Mitr Phol group, a major Thai sugar miller, to improve sugarcane production through technical consultancy and commissioned work. During 2003-2005, BIOTEC provided technical assistance to the company to establish a tissue culture laboratory for sugarcane. BIOTEC continued to work with Mitr Phol to build up their research capability through training and research collaboration to help the company expand its sugarcane production. In 2011, Mitr Phol established a disease-free sugarcane production unit in Laos, with technical consultancy provided by BIOTEC. These activities have generated employment and produced disease-free sugarcane that serves as a stock parent plant for sustainable production in Thailand and Laos. The economic impact based on employment and sugarcane production is estimated at 93.11 million Baht for 2012.

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 (short for "enzyme-linked immunosorbent assay) uses antibodies and color change to identify a particular substance. 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. *citrulli* (Aac). A rapid strip test for Aac has also been successfully developed. In addition, a strip test to detect phytoplasma in sugarcane was also co-developed with an industry partner. These antibodies and strip tests have already been commercialized, bringing in revenue to the manufacturers of reagents and test kits. Disease detection enables seed farmers and seed companies to produce high quality seed, and thus generate more income for the seed industry. Diagnostic tests allow farmers to screen for disease-free seeds and stalks for plantations, and facilitate disease prevention and mitigation, resulting in improved production and loss prevention. The economic impact from this initiative totalled 195 million Baht.

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

A-Zyme and PentoZyme are commercial feed enzymes produced by Asia Star Animal Health under a licence from BIOTEC/NSTDA.

iLAB αTHAL is an IC Strip Test for Alpha Thalassemia Detection manufactured by i+MED Laboratories under a licence from BIOTEC/NSTDA.

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 Thai Baht. As cows begin lactating, farmers can earn an additional 68,000 Thai Baht per animal. In 2012, training and services resulted in 4,145 pregnant cows with a total estimated impact of 380 million Baht.

Pentosanase production technology for the animal feed industry. Enzymes are biological catalysts. The main benefit of using enzymes in animal diets is a higher feed-conversion rate, i.e. more feed is converted to meat.

In 2009, Asia Star Animal Health Co., Ltd. signed a license agreement to produce pentosanase as a feed enzyme using a local microorganism screened from the BIOTEC Culture Collection. The company has been releasing feed enzyme products under the trade name A-Zyme. In 2011, Asia Star acquired an additional license from BIOTEC for technology to increase the activity of non-starch polysaccharide degrading enzymes, which the company turned into a new product called PentoZyme. The economic impact of these technologies was estimated to be 131.69 million Baht in 2012. Estimates were based on company revenue on product sales, the import substitution value of the enzymes, and the yield improvements passed on to farmers using Asia Star feed enzymes.

Technology service in Sakon Nakhon. BIOTEC facilitated a community enterprise in Sakon Nakhon Province and provided technical assistance through capacity building in agriculture, food processing, business planning and marketing development. In 2012, the community earned 3.24 million Baht from sales of produce and products such as rice seeds, organic rice grains, GABA rice, cassava and natural-dyed fabric.

Health and Medicine Cluster

Diagnostic test for influenza. The outbreak of avian influenza A (H5N1) in Asia in 2003 became a global crisis for animal and human health. Globally coordinated efforts finally brought the pandemic under control, but not before an estimated 160 people died and billions of dollars in damage to the poultry industry. Rapid diagnosis remains a key safeguard against further outbreaks.

BIOTEC collaborated with Innova Biotechnology Co., Ltd., an NSTDA joint venture company, in developing an immuno-chromatographic test kit for influenza type A, and subsequent licensing to Innova for commercial production. Innova Biotechnology also obtained a license to commercialize antibodies to influenza A, influenza B and influenza A virus subtypes H1, H3, H5, H7 and H9. Currently, two products are available: Innova Flu-A (a rapid immunochromatographic test for rapid, qualitative detection of influenza A viruses) and Innova GOLD® H5 (a rapid immunochromatographic test for rapid, qualitative detection of influenza A virus subtype H5). The economic impact of these technologies in 2012 was estimated at 3.46 million Baht. Estimates were based on earnings, import substitution and healthcare savings.

Clove and cinnamon oil formulation for dust mite fumigation. Dust mites are a common cause of asthma and allergic symptoms. The mite's gut contains potent digestive enzymes that are major inducers of allergic reactions such as wheezing. Safe, environmentally friendly remedies are much in demand.

Researchers of King Mongkut conducted a study to develop clove and cinnamon oil formulations for controlling dust mites. The research was conducted by King Mongkut Institute of Technology, Ladkrabang with research funds from Biodiversity Research and Training Program, a joint funding program created by BIOTEC and Thailand Research Fund. The technology was licensed by BIOTEC and King Mongut to Good Guy Group Co., Ltd. and Natural Herb Product (Thailand) Co., Ltd. for commercial application in 2009. The estimated impact of 7.02 million Baht in 2012 was estimated on the sales of products, a canned spray and aromatherapy mist, and the indirect impact in preventing allergies caused by dust mites.

Alpha thalassemia immunochromatographic strip test. Thalassemia is an inherited blood disorder which causes the body to make an abnormal form of red blood cells resulting in anemia. Thalassemia disorders are particularly associated with people of Mediterranean origin, Arabs, and Asians. The conventional test for thalassemia requires specialized equipment, takes four hours and is expensive.

Biomedical Technology Research Center and Chiang Mai University have developed the world's first alpha thalassemia immunochromatographic strip test. The test is convenient to use and takes only 3-5 minutes as opposed to four hours and costs 120 Baht per test, compared to 1,200 Baht per test for the conventional method. This technology was licensed to i+MED Laboratories Co.,

KEEEN is a bioremediation agent manufactured by Hi-Grimm Environmental and Research. The product was co-developed by BIOTEC and Hi-Grimm Environmental and Research.

Ltd. Economic impact in 2012 was estimated at 25.93 million Baht based on sales, cost reductions and healthcare savings from the prevention of thalassemia transmission.

Energy and Environment Cluster

Bioremediation agent. Oil spills range from headline grabbing disasters of sinking supertankers to leaking motor oil on your 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 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 Hi-Grimm Environmental and Research 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 applications in the petrochemical and automobile industries and in hotels and hospitals. The impact of this technology was estimated at 66.11 million Baht in 2012. The estimate was based on company revenue, additional investment of the company towards production, and the value of import substitution.

Innovation technology for phyto-remediation 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 such as eucalyptus. The recovered area expanded from 80 ha (500 rai) in 2009 to 6400 ha (40,000 rai) in 2012. Economic impact, based on the value of produce cultivated, totalled 329.33 million Baht in 2012.

Biogas technology in agro industry. A 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 four cassava starch factories, three fruit canning factories and one palm oil factory. The technology resulted in energy savings of 122.82 million Baht in 2012.

Capacity building for energy and resource efficiency for Thai native starch industry. Starch is a simple chemical compound with scores of commercial, industrial and domestic uses. Thailand is the largest producer of tapioca starch and produces over 2 million tons annually. Thai production technology is the most advanced, and Thai tapioca starch is known for its high quality at competitive prices.

In 2009, 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, fifteen factories participated in the program. A total of 112.52 million Baht in savings was estimated from waste reduction and improvements to machinery.

On-line COD sensor. A chemical oxygen demand (COD) test is commonly used to measure the amount of organic compounds in water. COD is expressed in milligrams per liter (mg/L), which indicates the mass of oxygen consumed per liter of solution. COD is often measured as a rapid indicator of organic pollutants in water. It is normally measured in both municipal and industrial wastewater treatment plants and gives an indication of the efficiency of the treatment process.

Biochemical Engineering and Pilot Plant Research and Development Unit developed a probe and system to accurately measure COD in wastewater. The technology was licensed to Mobolis Automata Co., Ltd. to further develop into an on-line COD sensor called ECO-Sensing COD-EC500 Analyzer. The device is a chemical oxygen demand (COD) sensor employing a stopped-flow thin layer electrochemical cell. The coulometric charge required for exhaustive electrolysis of samples was measured and correlated with the COD evaluated by the standard COD laboratory method. A single measurement took about 15 minutes, much less than the two hours required in the conventional method. The impact of this technology was calculated from the company's revenue and investment, investment by the factories installing the device, and import substitution. Economic impact in 2012 was estimated at 67.36 million Baht.

APPENDICES

- List of Publications
- List of Patents and Petty Patents
- Honors and Awards
- Executives and Management Team

List of Publications

1. Abere, B., Wikan, N., Ubol, S., Auewarakul, P., Paemanee, A., Kittisenachai, S., Roytrakul, S. and Smith, D.R. (2012). Proteomic Analysis of Chikungunya Virus Infected Microgial Cells. *PLoS ONE*, 7(4), e34800.
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List of Patents and Petty Patents

List of Applied Patents and Petty Patents

Title	Filing Date	Ref. No.	Country
Patent			
Lamp-dipstick test kit for *Mycobacterium tuberculosis* detection	25 November 2011	1101003303	Thailand
Method for classification of malaria parasite species based on distribution of chromatin size	2 February 2012	1201000416	Thailand
A bacterial surrogate for testing antimalarials: thy A knockout and FloA knockout bacteria for testing of inhibition of malarial dihydrofolate reductase-thymidylate synthase	9 February 2012	PCT/TH2012/000005	PCT
Anti-folate antimalarials with dual-binding modes and their preparation	9 February 2012	PCT/TH2012/000006	PCT
Detection of *Macrobrachium rosenbergii* nodavirus using nested RT-PCR and chromatographic strip	14 March 2012	1201001101	Thailand
Low-cost process for production of gamma-linolenic acid by fungal submerged fermentation	29 May 2012	1201002434	Thailand
A system for controlling expression of genes in Apicomplexan parasites by means of an inducible ribozyme	29 May 2012	1201002492	Thailand
Enzyme formulation and process for converting biomass to sugar	29 May 2012	1201002493	Thailand
Test kit for silver (I) ion in an aqueous media	20 June 2012	1201002989	Thailand
Biocomposite PLA/modified sawdust film containing antimicrobial peptides for use as antimicrobial food packaging	9 August 2012	1201004042	Thailand
An automated system for screening severity of patients with beta-thalassemia/Hemoglobin E disease from their SNP genotyping interaction profiles	6 September 2012	1201004543	Thailand
A system for automatically mapping pyrosequencing reads to public sequencing database	21 September 2012	1201004891	Thailand
An automated system to identify SNP, insertion, and/or deletion from DNA Sanger sequencing reads	21 September 2012	1201004892	Thailand
Reporter plasmid and its application in anti-tuberculosis agent screening in cell	21 September 2012	1203001020	Thailand
A rapid method for salt tolerant screening in plant species using multivariate parameters of photosynthetic abilities under controlled environments of *in vitro* culture	28 September 2012	1201005091	Thailand
Preparation of soluble recombinant bone morphogenetic protein-2	28 September 2012	1203001093	Thailand
Petty Patent			
Shuttle plamid vectors for *Escherichia coli* and *Lactobacillus casei*	6 February 2012	1203000138	Thailand
Detection of infectious myonecrosis virus using immunochromatographic strip test	22 March 2012	1203000302	Thailand
Screening method for infectious hypodermal and hematopoietic necrosis virus (IHHNV) in shrimp	25 May 2012	1203000518	Thailand
Methanol-inducible expression system for the production of target protein	20 June 2012	1203000594	Thailand
Screening method for white spot syndrome virus (WSSV) in shrimp	26 July 2012	1203000772	Thailand
A method for antioxidant production in white ginger (*Hedychium coronarium* J. G. Koenig) by *in vitro* micro-rhizome induction under controlled environments	9 August 2012	1203000833	Thailand

Title	Filing Date	Ref. No.	Country
Pulp bleaching process using alkaline-tolerant xylanase obtained from metagenomic library of sugarcane bagasse compost	9 August 2012	1203000834	Thailand
Inoculum preparation of methanol utilization slow (Mut^S) *Pichia pastoris* to improve fermentation efficiency	17 August 2012	1203000856	Thailand
Fungal fermentation process for omega-6 fatty acid production	17 August 2012	1203000857	Thailand
Simultaneous desizing and scouring process for natural fiber fabric using multi-activity enzymes	23 August 2012	1203000885	Thailand
Fermentation process of cassava feedstock with high solid content for the production of biofuels and chemicals	27 August 2012	1203000892	Thailand
Gene expression system for the production of target protein at high temperature without induction using thermotolerant *Pichia thermomethanolica*	27 August 2012	1203000894	Thailand
Protocol and method for measuring dissolved oxygen	30 August 2012	1203000917	Thailand
A method for increasing the germination rate of hard-shelled seeds by high-temperature water and high concentration of acid	6 September 2012	1203000942	Thailand
Method to screen and classify *Plasmodium vivax* in blood sample	6 September 2012	1203000943	Thailand
Method to screen and classify *Plasmodium falciparum* in blood sample	6 September 2012	1203000944	Thailand
Method to induce mutation in plant using the combination of controlled atmospheric pressure and mutagen	13 September 2012	1203000960	Thailand
Method to induce mutation in plant using the combination of ultrasonic energy and mutagen	13 September 2012	1203000961	Thailand
Primer for the specific detection of *Vibrio harveyi*	13 September 2012	1203000962	Thailand
Process for storage of rice pollen under controlled environment: low temperature and high relative humidity	21 September 2012	1203001021	Thailand
A system for identifying genetic susceptible genes from genotyping SNP array data	21 September 2012	1203001027	Thailand
Preservation technique for fungal spores used as biocontrol agent, its preparation and usage	28 September 2012	1203001089	Thailand
Method to minimize released water and weight losses in fermented meat products under acidic condition using heat-modified whey protein isolates	28 September 2012	1203001090	Thailand
Recombinant *Beuveria bassiana* for biocontrol application	28 September 2012	1203001096	Thailand

List of Issued Petty Patents in Thailand

Title	Granting Date	Petty Patent No.
Preparation of hyaluronan binding protein labeled with biotin and its application	29 November 2011	6725
Preservation technique for rambutan in syrup in an intermediate moisture condition	2 February 2012	6921

HONORS AND AWARDS

Assoc. Prof. Apichart Vanavichit

Rice Gene Discovery Unit

Outstanding Person of the Nation (Science and Technology) 2012, awarded by the National Identity Board, the Prime Minister's Office.

Dr. Sithichoke Tangphatsornruang

Genome Institute

Young Scientist Award 2012 for the work on "Application of genomic technology in plant improvement", awarded by the Foundation for the Promotion of Science and Technology under the Patronage of His Majesty the King.

Dr. Nitsara Karoonuthaisiri

Agricultural Biotechnology Research Unit

Young Technologist Award 2011 for the work on "Capacity building in microarray production in Thailand", awarded by the Foundation for the Promotion of Science and Technology under the Patronage of His Majesty the King.

Dr. Somvong Tragoonrung

Genome Institute

Biotechnologist Award, awarded by Biogenomed Co., Ltd.

Dr. Kuakoon Piyachomkwan, Dr. Sunee Chotineeranat, Dr. Jackapon Sunthornvarabhas, Mr. Sittichoke Wanlapatit and Ms. Rungtiva Wansuksri

Cassava and Starch Technology Research Unit

Grant for a collaborative project on "Gains from Losses of Root and Tuber Crops", awarded by the European Union Framework Programme 7

Dr. Pattareeya Ponza

Agricultural Biotechnology Research Unit

Grant for a research project on "Molecular effects of photoperiods on expression of genes functional related to reproductive maturation of the giant tiger shrimp *Penaeus monodon*", awarded by the International Foundation for Science (IFS)

Dr. Sissades Tongsima

Genome Institute

The Meritorious Service Award, awarded by the Asia-Pacific Bioinformatics Network (APBioNet)

Ms. Wansika Kiatpathomchai

Center of Excellence for Shrimp Molecular Biology and Biotechnology

Invention Award 2012 for "New platform for detection of shrimp viruses by loop-mediated isothermal amplification (LAMP) technique and a portable multichannel turbidimeter", awarded by the National Research Council of Thailand

Dr. Satinee Suetrong

Bioresources Technology Unit

Dissertation Award 2011 (Agriculture and Biology) for "Molecular systematics of the marine *Dothideomycetes"*, awarded by the National Research Council of Thailand

Dr. Noppol Kobmoo

Bioresources Technology Unit

Outstanding Poster Presentation Award for the work titled "Molecular phylogenies reveal cryptic speciation of *Ophiocordyceps unilateralis* through specificity to its host ants", presented at the 8th International Conference on Ants, 17-21 October 2011, Songkhla, Thailand

Dr. Warangkhana Songsungthong

Medical Molecular Biology Research Unit

Poster Presentation Award for the work titled "Role of Plasmodium Glutathione Biosynthesis in Antimalarial Sensitivity", presented at Keystone Symposia: Drug Discovery for Protozoan Parasites, 15-20 January 2012, New Mexico, USA

Dr. Kunruedee Sangseethong

Cassava and Starch Technology Research Unit

Poster Presentation Award (second runner-up) for the work titled "Paste and film properties of oxidized cassava starch", presented at Starch Update 2011: The 6th International Conference on Starch Technology, 13-14 February 2012, Bangkok, Thailand

Dr. Jarunee Vanichtanankul

Medical Molecular Biology Research Unit

Dissertation Award 2011 (Science and Applied Science) for "Trypanosomal Dihydrofolate Reductase Reveals Natural Antifolate Resistance", awarded by Mahidol University

Ms. Jiranan Techaprasan and Dr. Jana Leong-Skornickova

Bioresources Technology Unit and Singapore Botanic Gardens

Tem Smitinand Poster Award for the work titled "*Kaempferia candida* (Zingiberaceae): *Curcuma* in Disguise", presented at the 15th Flora of Thailand Meeting, 7-11 November 2011, Chiang Mai, Thailand

Dr. Srung Smanmoo

Bioresources Technology Unit

Gold Prize (Idea Seed) of True Innovation Award 2011 for the best initiative idea on, "Breath Analyzer for Diabetes", awarded by True Corporation Public Company Limited (PCL)

Dr. Srung Smanmoo

Bioresources Technology Unit

Audience Choice Award for the work "G-breath: a sensor device to diagnose diabetes from breath biomarkers", presented at at the Intel-DST Pacific Challenge 2012, 8-9 August 2012, Bangalore, India.

Dr. Apiradee Hongsthong, Ms. Pavinee Rakruangdet, Dr. Jittisak Senachak, Ms. Matura Sirijuntarut, Ms. Rayakorn Yutthanasirikul, Ms. Phuttawadee Phuengcharoen, Ms. Wattana Jeamton, Dr. Supapon Cheevadhanarak and Dr. Sittiruk Roytrakul

Biochemical Engineering and Pilot Plant Research and Development Unit and Genome Institute

Paper titled "Comparative analysis of the *Spirulina platensis* subcellular proteome in response to low- and high-temperature stresses: uncovering cross-talk of signaling components" was hightlighted on A-IMBN Research, selected by the Asia-Pacific International Molecular Biology Network (A-IMBN)

Mr. Chumpol Ngamphiw, Dr. Anunchai Assawamakin, Dr. Sissades Tongsima and Dr. Philip J. Shaw

Genome Institute and Medical Molecular Biology Research Unit

Paper titled "PanSNPdb: The Pan-Asian SNP genotyping database" was hightlighted on A-IMBN Research, selected by the Asia-Pacific International Molecular Biology Network (A-IMBN)

Dr. Nitsara Karoonuthaisiri, Mr. Ratthaphol Charlermroj, Dr. Oraprapai Gajanandana, Dr. Orawan Himananto, Ms. Mallika Kumpoosiri, Prof. Christopher Elliott and Dr. Michalina Oplatowska

Agricultural Biotechnology Research Unit and Queen's University Belfast

Paper titled "Comparison of techniques to screen and characterize bacteria-specific hybridomas for high-quality monoclonal antibodies selection" was hightlighted on A-IMBN Research, selected by the Asia-Pacific International Molecular Biology Network (A-IMBN)

Dr. Chairat Uthaipibull, Dr. Sumalee Kamchonwongpaisan and Prof. Yongyuth Yuthavong

Medical Molecular Biology Research Unit

Paper titled "Flow cytometric enumeration of Plasmodium berghei-infected red blood cells stained with SYBR Green I" was hightlighted on A-IMBN Research, selected by the Asia-Pacific International Molecular Biology Network (A-IMBN)

Dr. Jittima Piriyapongsa, Mr. Chaiwat Bootchai, Mr. Chumpol Ngamphiw and Dr. Sissades Tongsima

Genome Institute

Paper titled "microPIR: an Integrated database of microRNA target sites within human promoter sequences" was hightlighted on A-IMBN Research, selected by the Asia-Pacific International Molecular Biology Network (A-IMBN)

Dr. Kallaya Sritunyalucksana, Dr. Amornrat Tangprasittipap, Dr. Saisunee Chouwdee, Prof. Timothy William Flegel, Dr. Saengchan Senapin, Ms. Kornsunee Phiwsaiya and Ms. Seansook Laiphrom

Agricultural Biotechnology Research Unit, Center of Excellence for Shrimp Molecular Biology and Biotechnology, and Shrimp Genetic Improvement Center

Paper titled "Structure and expression of a shrimp prohormone convertase 2" was hightlighted on A-IMBN Research, selected by the Asia-Pacific International Molecular Biology Network (A-IMBN)

Dr. Anan Jongkaewwattana and research team from Virology and Cell Technology Laboratory

Agricultural Biotechnology Research Unit

Paper titled "Inhibition of influenza A virus replication by influenza B virus nucleoprotein: An insight into interference between influenza A and B viruses" was hightlighted on A-IMBN Research, selected by the Asia-Pacific International Molecular Biology Network (A-IMBN)

National Center for Genetic Engineering and Biotechnology

Thailand Energy Awards 2012 (in the category of plants and buildings for energy efficiency improvement), awarded by the Department of Alternative Energy Development and Efficiency, Ministry of Energy.

Executives and Management Team

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Mr. Thosapol Tantiwong	Chairman of Biotech Industry Club, Federation of Thai Industries (FTI)
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Prof. Gerald T. Keusch	Professor of International Health and of Medicine, Boston University School of Public Health, USA
Prof. Lene Lange	Director of Research, Aalborg University, DENMARK
Prof. Sangkot Marzuki	Director, Eijkman Institute for Molecular Biology, INDONESIA
Dr. Jean-Marcel Ribaut	Director, Generation Challenge Programme (GCP)

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Deputy Executive Director

Dr. Suvit Tia
Deputy Executive Director

Ms. Kruawan Potisombat (retired on 31 May 2012)
Assistant Executive Director

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Dr. Somvong Tragoonrung
Director, Genome Institute

Dr. Sumalee Kamchonwongpaisan
Director, Medical Molecular Biology Research Unit

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