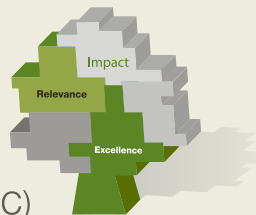




ANNUAL REPORT 2016



Annual Report 2016

National Center for Genetic Engineering and Biotechnology (BIOTEC)

This report is prepared according to the 2016 fiscal year of the Royal Thai Government, from 1 October 2015 – 30 September 2016

Annual Report 2016

National Center for Genetic Engineering and Biotechnology (BIOTEC)

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MESSAGE FROM THE BIOTEC EXECUTIVE DIRECTOR

It is my great privilege to present the BIOTEC Annual Report 2016 as a new Executive Director of BIOTEC. I took up the directorship in May after Dr. Kanyawim Kirtikara, who guided this Center through remarkable developments, especially her achievement in creating a support system for research staff to work efficiently and her keen interest to foster the growth of young staff. I would like to thank Dr. Kanyawim and all my predecessors for their dedication in transforming BIOTEC into an exceptional research center and I hope to carry on this legacy.

BIOTEC has developed core competencies in food science and biotechnology and this has helped to serve the Thai food and feed industry. 2016 was an exciting year for BIOTEC. We celebrated the opening of the Food and Feed Innovation Center in April. This newly established Food and Feed Innovation Center will elevate our service to industrial clients as it is fully equipped with advanced instruments necessary to perform cutting-edge research to create innovation. Its spacious laboratory can accommodate scientists from clients and partner institutes to work side-by-side with our team. Another important feature is the pilot-scale production infrastructure to assess the feasibility of the various technologies, ensuring smooth transition from lab-scale to industrial scale. We hope that this Food and Feed Innovation Center will be a major driving force in providing viable, forward- and global-looking solutions for the Thai food and feed industries.

We always take pride in our illustrious people and research excellence. In December 2015, our Director of Medical Biotechnology Laboratory, a joint lab between BIOTEC and the Faculty of Medicine, Siriraj Hospital, Prof. Prida Malasit, won a prestigious NSTDA Research Chair Grant. The 20 million Baht grant will be used towards the exploration of the complex mechanism of the dengue virus infection to uncover biomarkers associated with the infection severity which can then be applied to the development of novel predictive diagnostics and better guidelines for clinical care and public health intervention.

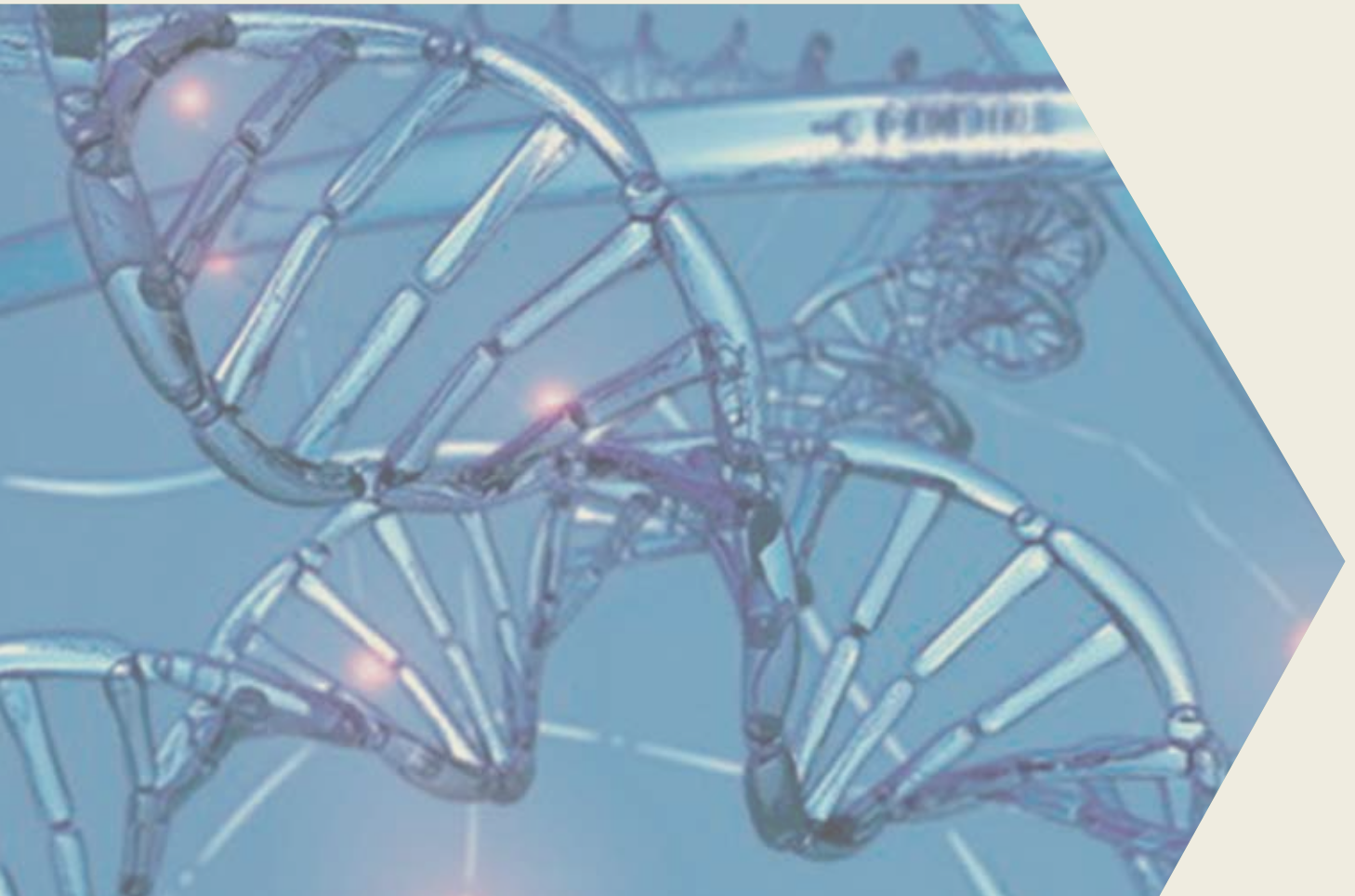
It is nice to see our staff have developed skills recognized around the world. We were ecstatic that two of our vibrant researchers won the awards at the first FameLab Thailand, a science communication competition organized by the British Council. Dr. Khaow Tonsomboon was named the winner and people's choice, while Dr. Wanarit Wimuttisuk was the second runner up.

In 2016, the Thai Government made an announcement on Thailand 4.0 policy to transform the country to a more creative and innovative economy. Therefore, science, technology and innovation will take center stage as they are key and provide the engine to enable new growth for the economy. BIOTEC is committed to bringing our research excellence to support this movement and support the future creativity and innovations of the economy of Thailand.

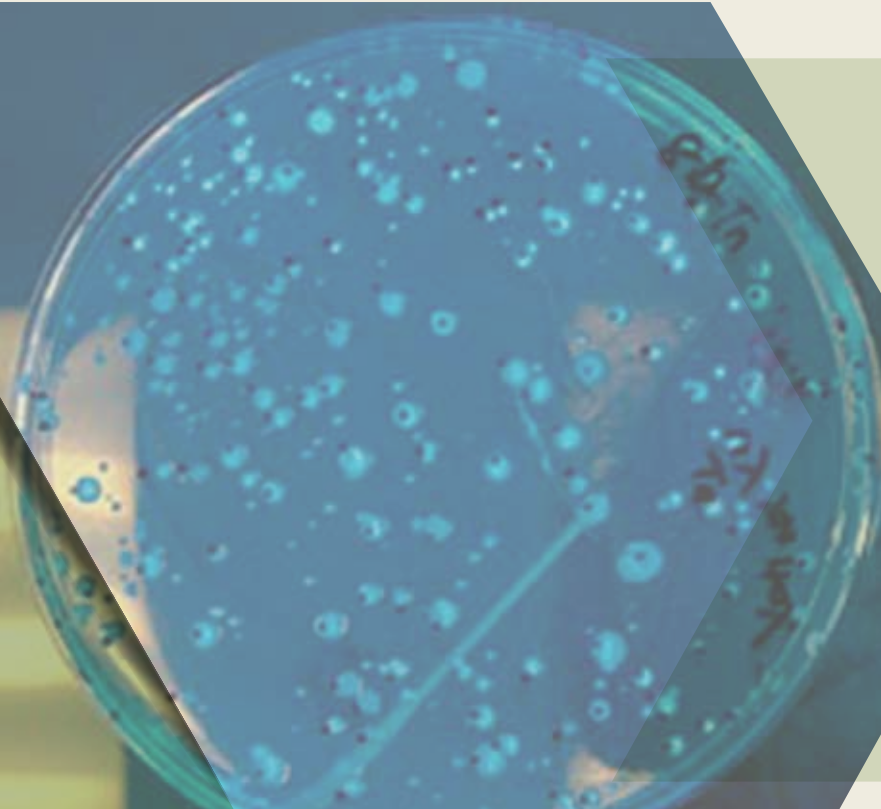


Somvong Tragoonrung, Ph.D.
Executive Director, BIOTEC





FACTS AND FIGURES



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

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

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

► Organization

BIOTEC Research Units at Thailand Science Park

- Bioresources Technology Research Unit
- Animal Biotechnology Research Unit
- Food Biotechnology Research Unit
- Medical Molecular Biology Research Unit
- Genome Technology Research Unit
- Biosensing Technology Research Unit

Collaborative Research Laboratories at universities and government organization

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

Translational Research Facilities and Multi-disciplinary Laboratory

- Shrimp Genetic Improvement Center
- Nuclear Polyhedrosis Virus Pilot Plant for Insect Pest Control
- Thailand Bioresource Research Center
- Food and Feed Innovation Center
- Integrative Biorefinery Laboratory
- National Biopharmaceutical Facility (in collaboration with King Mongkut's University of Technology Thonburi)

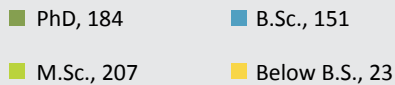
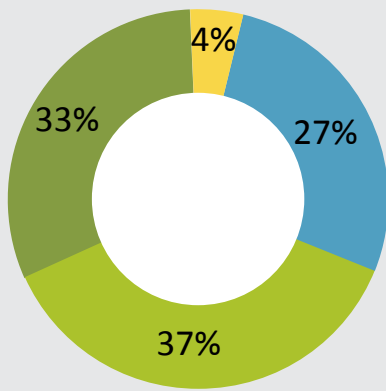
Administration

- Policy Study and Biosafety Unit
- Rural Development Technology Service Unit
- Biotechnology Business Development Division
- Human Resources and Platform Technology Development Division
- Strategic Planning and Organization Development Division
- Evaluation and Monitoring Division
- Research Unit Coordination Division
- Management Information System Division
- International Cooperation and Public Relations Division
- Building Management and Equipment Service Division
- General Management Division

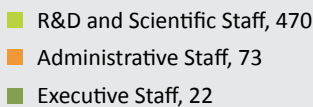
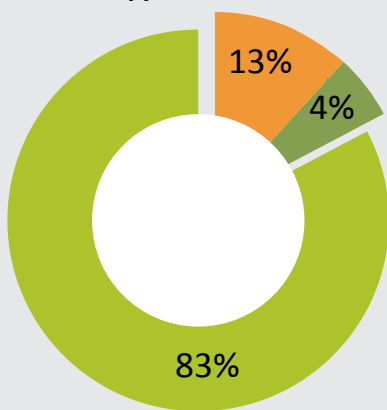


Human Resources, 565

By education



By job function



24 awards

Honors and Awards
24 awards and honors

Publications
223 papers



223 papers

Intellectual Properties

Granted patent in Thailand

1

Granted petty patent in Thailand

30

16

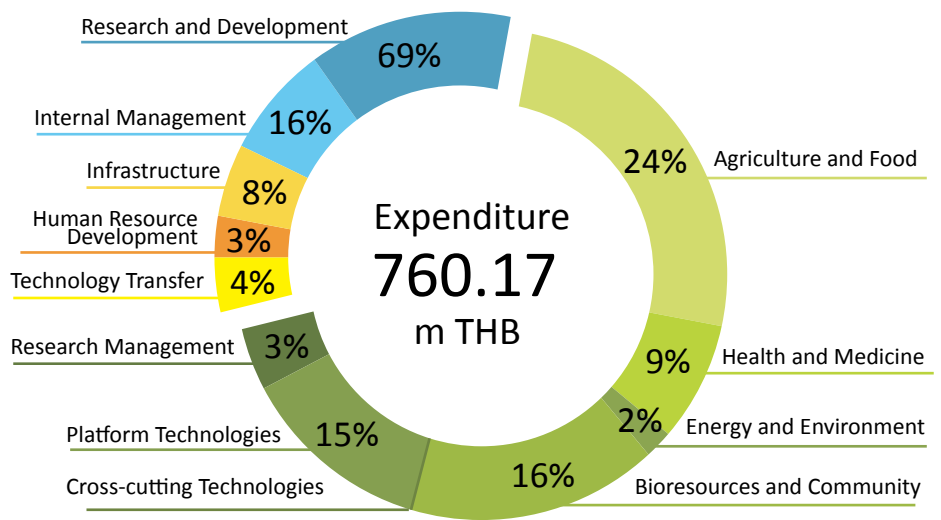
Applied patents in Thailand

13

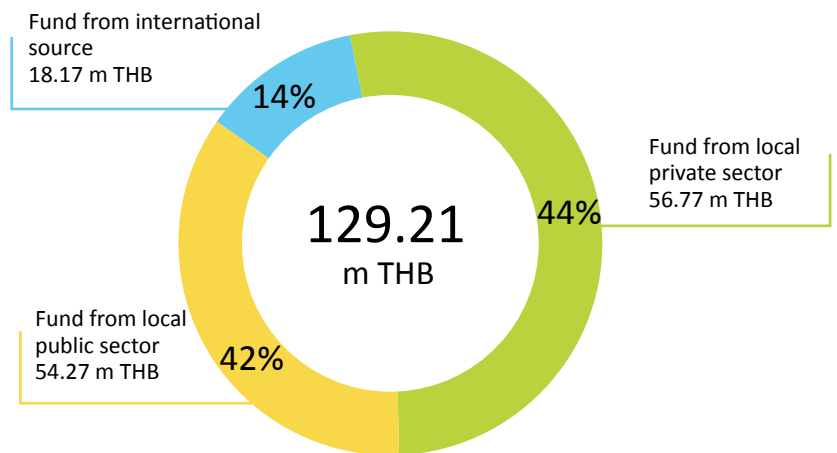
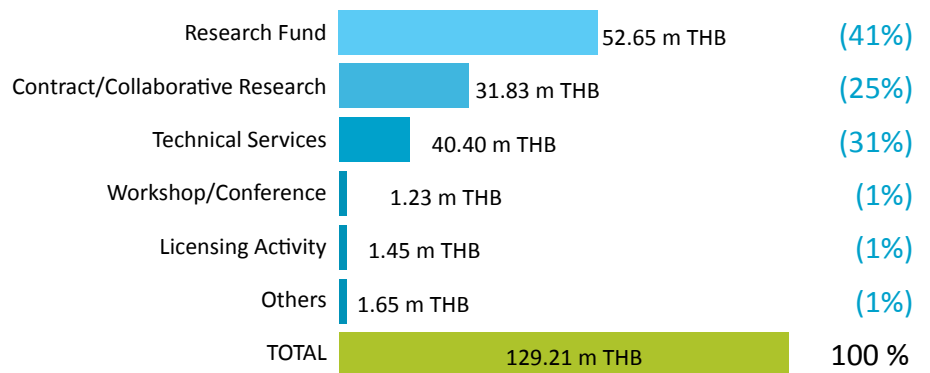
Applied petty patent

16

Applied trade secret

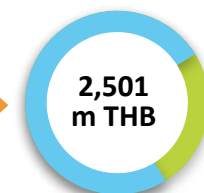


Income from sources outside NSTDA



generated by completed project

Socio-economic impact of





RESEARCH AND DEVELOPMENT



BIOTEC's R&D and technical program covers a wide range of topics, spanning from fundamental to applied research. The research is designed to address the clusters and programs set by NSTDA. In Agriculture and Food Cluster, the focus is to develop technologies to improve crop, animal and food production, taking into account the impact of climate change. Energy and Environment Cluster targets enabling technologies in support of sustainable development such as energy and resource efficiency as well as renewable and alternative energy. Tropical diseases, namely malaria, dengue and tuberculosis, are the focus of Health and Medicine Cluster.

Bioresources management and sustainable utilization are the major theme of Bioresources Cluster. Cross-cutting Technology Program places an emphasis on diagnostic and sensor technology, whereas Platform Technology Program aims to develop core competencies in genomics, bioinformatics, proteomics to support long-term biological research and development. In addition, BIOTEC also engages in policy research directed towards strategic planning and establishing future R&D direction in the areas that biotechnology can benefit the country.

Highlights from Agriculture and Food Cluster

Improvement of the submergence tolerance and the brown planthopper resistance of the Thai jasmine rice

Shifts in weather patterns and increases in the frequency and magnitude of extreme weather events resulting from unfavorable climate change have led to frequent flooding and brown planthopper outbreaks in rice fields, particularly those in rainfed lowland areas in Thailand. The traditional jasmine rice cultivar KDML105 popularly grown in rainfed lowland areas in north and northeast Thailand is intolerant to flash flooding and is very susceptible to the brown planthopper.

Scientists have pyramided *Sub1* and *Qbph12* in the genetic background of KDML105 via marker-assisted selection coupled with stringent phenotypic selection for plant type and physical grain quality traits. In total, sixty-four pyramid lines (PLs) were developed and characterized for submergence tolerance, brown planthopper resistance, important agronomic traits and physical and chemical grain quality. Evaluation of the PLs indicated their significantly enhanced submergence tolerance and brown planthopper resistance compared with the original KDML105 line. All PLs satisfied the KDML105 grain and cooking quality standards, although their agronomic characteristics showed significant variations in days to flowering, plant height and grain yield. These variations might allow breeders to select new versions of KDML105 that are suitable for different locations in rainfed lowland environments.

This study was jointly conducted by researchers from the Rice Gene Discovery Laboratory, Kasetsart University, Phitsanuloke Rice Research Center, Ubon Ratchathani Rice Research Center, Bureau of Rice Research and Development and Chiang Rai Rice Research Center.

Ref: Korinsak, S., Siangliw, M., Kotcharerk, J., Jairin, J., Siangliw, J.L., Jongdee, B., Pantuwan, G., Sidthiwong, N. and Toojinda, T. (2016). Improvement of the submergence tolerance and the brown planthopper resistance of the Thai jasmine rice cultivar KDML105 by pyramiding *Sub1* and *Qbph12*. *Field Crops Research*, 188, 105–112.

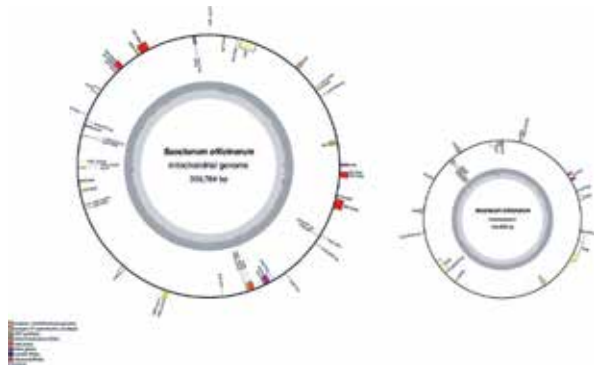
Using PacBio reads to sequence and assemble mitochondrial genome of a sugarcane cultivar

Sugarcane is the major source of processed sugar in the world and therefore an important crop species. Modern commercial cultivars are complex hybrids of *S. officinarum* and several other *Saccharum* species. Historical records identify New Guinea as the origin of *S. officinarum* and that a small number of plants originating from there were used to generate all modern commercial cultivars. The mitochondrial genome can be a useful way to identify the maternal origin of commercial cultivars.

The PacBio RSII, a sequencer that is able to sequence single molecules in real time without any sample amplification, was employed to sequence and assemble the mitochondrial genome of a South East Asian commercial cultivar, known as Khon Kaen 3. The long read length of this sequencing technology allowed for the mitochondrial genome to be assembled into two distinct circular chromosomes with all repeat sequences spanned by individual reads. Comparison of five commercial hybrids, two *S. officinarum* and one *S. spontaneum* to our assembly reveals no structural rearrangements between our assembly, the commercial hybrids and an *S. officinarum* from New Guinea. The *S. spontaneum*, from India, and one sample of *S. officinarum* (unknown origin) are substantially rearranged and have a large number of homozygous variants. This supports the record that *S. officinarum* plants from New Guinea are the maternal source of all modern commercial hybrids.

The study was conducted by researchers from the BIOTEC Genomic Research Laboratory.

Ref: Shearman, J.R., Sonthirod, C., Naktang, C., Pootakham, W., Yoocha, T., Sangsakru, D., Jomchai, N., Tragoonrung, S. and Tangphatsornruang, S. (2016). The two chromosomes of the mitochondrial genome of a sugarcane cultivar: assembly and recombination analysis using long PacBio reads. *Scientific Reports*, 6, 31533.



Chromosomes of the sugarcane mitochondrial genome

Effects of the combined stress on biochemical compounds in a commercial cyanobacterium

In nature, several abiotic stresses occur simultaneously, leading to retarded growth and biochemical changes in microalgae, including the commercial cyanobacterium, *Arthrospira platensis*. Since high temperature was an uncontrollable factor in outdoor cultivation of microalgae, the knowledge from changes in biomass and biochemical compositions under the combined stress should provide a better strategy for the nutrient management of microalgae outdoor cultivation to overcome low biomass productivity under nitrogen depletion and high temperature stress.

To gain more understanding of stress response, researchers investigated the integrative effects of nitrogen depletion and high temperature stress on physiological changes of *A. platensis* C1. The results revealed that the combination of nitrogen depletion and high temperature stress showed stronger effects on the photosynthetic activity than the individual stress of nitrogen depletion. Although nitrogen depletion caused a decrease in protein content, including phycocyanin (PC) and an increase in carbohydrate contents, the accumulation of carbohydrates, especially the increase in the levels of polysaccharides, seemed to be growth temperature-dependent. The fatty acid profiles were changed only under the combination of nitrogen depletion and high temperature stress. Although nitrogen depletion highly increased the production of carbohydrates and polysaccharides in *A. platensis* C1, the growth of stressed cells decreased at 24 h of

incubation. In order to utilize carbohydrates as a source for pharmacological applications and the production of biofuels, high productivity of carbohydrates under stress conditions should be considered concomitant with cell growth in further study. In addition, nutrient management, as a factor that can be controlled, should be regarded as one of the essential requirements for the improvement of microalgal mass cultivation under stresses.

This study was a combined effort of researchers from the Biochemical Engineering Department and Pilot Plant Research and Development Institute at King Mongkut's University of Technology Thonburi.

Ref: Panyakampol, J., Cheevadhanarak, S., Senachak, J., Dulsawat, S., Siangdung, W., Tanticharoen, M. and Paithoonrangarid, K. (2016). Different effects of the combined stress of nitrogen depletion and high temperature than an individual stress on the synthesis of biochemical compounds in *Arthrospira platensis* C1 (PCC 9438). *Journal of Applied Phycology*, 28, 2177-2186.

Bacterial dynamics in shrimp intestines during pathogen exposure

The intestinal microbiota play important roles in the health of their host, contributing to maintaining the balance and resilience against pathogens. To investigate effects of pathogens to intestinal microbiota, the bacterial dynamics upon a shrimp pathogen, *Vibrio harveyi*, exposures were determined in two economically important shrimp species; the black tiger shrimp and the Pacific white shrimp.

In this study, intestinal bacterial populations under a time course *V. harveyi* exposure in the black tiger shrimp and the Pacific white shrimp were determined by using a pyrosequencing-based method to provide a comprehensive coverage of bacterial community structure. The bacterial population patterns of the black tiger shrimp and the Pacific white shrimp were also compared using denaturing gradient gel electrophoresis (DGGE) of 16S rRNA fragments. The findings showed that bacterial populations were altered after the pathogen invasion, and the host's ability to regain stability and resilience in their bacteria compositions might contribute to invasion

resistance. This work will lead to biological control strategies such as gut microbial management as preventive and treatment measures against pathogen invasion in future aquaculture production.

This study was performed by the BIOTEC Microarray Laboratory.

Ref: Runggrasamee, W., Klanchui, A., Maibunkaew, S. and Karoonuthaisiri, N. (2016). Bacterial dynamics in intestines of the black tiger shrimp and the Pacific white shrimp during *Vibrio harveyi* exposure. *Journal of Invertebrate Pathology*, 133, 12–19.

A new, highly sensitive method for detection of IHNV in shrimp tissues

Infectious hypodermal and hematopoietic necrosis virus (IHNV) is listed as a reportable crustacean disease by the World Organization for Animal Health (OIE), many countries, therefore, require testing for IHNV in shrimp stocks imported for aquaculture. Rapid and highly sensitive detection methods to guarantee and maintain absence of IHNV in traded stocks is in need.

A new, highly sensitive method for detection of IHNV in shrimp tissues has been developed. It involves *in situ* loop-mediated DNA amplification (IS-LAMP) employing digoxigenin-11-dUTP (DIG-dUTP) as part of the deoxynucleotide triphosphate (dNTP) mix. This *in situ*, DIG-labeling LAMP (ISDL) method gave no cross-reactions with tissues of shrimp infected with other common pathogens, and the total time needed to carry out ISDL was one-third of that needed for conventional *in situ* hybridization. This method can be used as a confirmatory test carried out with tissue sections to confirm the results from molecular assays, especially when immune-histochemical methods are not available to do so. ISDL is especially important for molecular detection from a new or unexpected location and for molecular detection in newly suspected carriers to determine whether they are actually infected or just mechanical carriers.

This investigation was undertaken by researchers from BIOTEC and Mahidol University.

Ref: Jitrakorn, S., Arunrut, N., Sanguanrut, P., Flegel, T.W., Kiatpathomchai, W. and Saksmerprome, V. (2016). *In situ* DIG-labeling, loop-mediated DNA Amplification (ISDL) for highly sensitive detection of infectious hypodermal and hematopoietic necrosis virus (IHNV). *Aquaculture*, 456, 36–43.

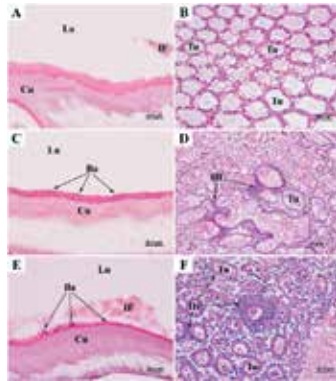
Differentially expressed transcripts in shrimp stomach in response to AHPND infection

Acute Hepatopancreatic Necrosis Disease (AHPND) is an emerging disease in aquacultured shrimp caused by a pathogenic strain of *Vibrio parahaemolyticus*. As with several pathogenic bacteria, colonization of the stomach appeared to be the initial step of the infection for AHPND-causing *Vibrio*. To better understand the host immune response to pathogenic AHPND bacteria, the cDNA transcripts in the stomach of black tiger shrimp (*Penaeus monodon*) were examined.

Scientists investigated differentially expressed transcripts (DETs) in the stomach during *V. parahaemolyticus* strain 3HP (VP3HP) infection using Ion Torrent sequencing. From the total 42,998 contigs obtained, 1585 contigs representing 1513 unigenes were significantly differentially expressed with 1122 and 391 unigenes up- and down-regulated, respectively. Among the DETs, there were 141 immune-related unigenes in 10 functional categories: antimicrobial peptide, signal transduction pathway, proPO system, oxidative stress, proteinases/proteinase inhibitors, apoptotic tumor-related protein, pathogen recognition immune regulator, blood clotting system, adhesive protein and heat shock protein. Expression profiles of 20 of 22 genes inferred from RNA sequencing were confirmed with the results from qRT-PCR. Additionally, a novel isoform of anti-lipopolysaccharide factor, PmALF7 whose transcript was induced in the stomach after challenge with VP3HP was discovered. This study provided fundamental information on the molecular response in the shrimp stomach during the AHPND infection that would be beneficial for future research.

This study was a collaborative effort between BIOTEC, Chulalongkorn University and Auburn University (USA).

Ref: Soonthornchai, W., Chaiyapechara, S., Klinbunga, S., Thongda, W., Tangphatsornruang, S., Yoocha, T., Jarayabhand, P. and Jiravanichpaisal, P. (2016). Differentially expressed transcripts in stomach of *Penaeus monodon* in response to AHPND infection. *Developmental and Comparative Immunology*, 65, 53-63.



Histological analysis of representative tissues (H&E stain). Control shrimp (*Artemia*-fed) at 24 h post feeding: stomach.

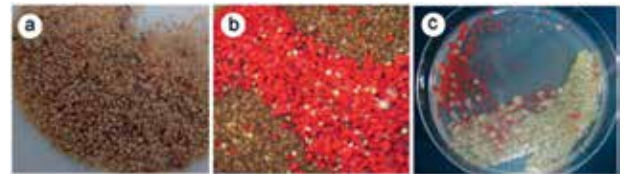
Uncovering a causative agent of a novel red egg disease in tilapia

Tilapia (*Oreochromis* spp.) is a highly valuable freshwater fish which is commercially farmed in Thailand as well as other Asian countries. Intensification of tilapia production has seen an increase in the number of infectious disease outbreaks. For years, red egg disease has been observed in Thai tilapia hatcheries. Infected eggs turned to red and eventually failed to hatch. The problem has been considered as an emerging infectious disease in tilapia eggs, especially during the cold season in Thailand from early December to mid-February.

Scientists have identified a red pigmented, Gram negative bacterium, *Hahella chejuensis* as the cause of “red egg syndrome” and proposed the novel disease to be called *Hahellosis*. In addition, PCR protocols were developed for detection of *H. chejuensis* in tilapia samples including eggs and ovaries and testes of tilapia broodstock. Positive signals were obtained in the ovaries and testes of tilapia broodstock with *in situ* DNA hybridization using probes specific for *H. chejuensis*, suggesting possible vertical transmission of the red pigmented bacteria from broodstock to eggs.

This study was jointly conducted by researchers from BIOTEC, Mahidol University, Chulalongkorn University and Nam Sai Farms Company Limited.

Ref: Senapin, S., Dong, H.T., Meemetta, W., Siriphongphaew, A., Charoensapsri, W., Santimanawong, W., Turner, A.W., Rodkhum, C., Withyachumnarnkul, B. and Vanichviriyakit, R. (2016) *Hahella chejuensis* is the aetiological agent of a novel red egg disease in tilapia (*Oreochromis* spp.) hatcheries in Thailand. *Aquaculture*, 454, 1-7.



Tilapia eggs and isolated bacteria. (a) Normal looking tilapia eggs; (b) red eggs (c) bacterial colonies streaked from red eggs grown on TSA plate at 30 °C overnight.

Evaluation of a pumice bottom substrate nitrification-denitrification tank

Recirculating aquaculture systems (RAS) have been developed to serve sustainable, environmentally friendly aquaculture. Nitrification is the major treatment process in RAS to remove ammonia and nitrite, which are toxic to aquatic animals and eventually convert them to the lower toxic nitrate; thus, the water can be reused for a longer period. The accumulation of nitrate derived from nitrification treatment is a common problem in most RAS. The use of anaerobic denitrification to remove nitrate is not yet widely applied in commercial RAS due to its level of efficiency, complexity and cost.

With a previous study demonstrating that pumice could be used as media for integrated nitrification-denitrification treatment for aquaculture, researchers investigated the efficiency of a pumice stone biofilter tank for nitrogen removal from a recirculating aquaculture system. It was found that the pumice stone could perform as a nitrification biofilter under aerobic conditions. When applying methanol as the external carbon source at a COD:N ratio of 5:1 and then covering the tank with a plastic sheet to reduce gas exchange, pumice stone could remove nitrate through denitrification. When tested with a fish recirculating tank, the nitrification

treatment performed by the pumice tank could control ammonia and nitrite concentrations within the required safety range throughout the 121-day culture period. Moreover, the pumice bottom substrate tank with methanol supplement had no negative effect on growth and survival of fish in the recirculating system.

This study was performed by researchers from BIOTEC Marine Biotechnology Laboratory and Chulalongkorn University.

Ref: Punggrasmiya, W., Phinitthanaphaka, P. and Powtongsook, S. (2016). Nitrogen removal from a recirculating aquaculture system using a pumice bottom substrate nitrification-denitrification tank. *Ecological Engineering*, 95, 357–363.

Nitrite-oxidizing communities and kinetics in a brackish environment after nitrite enrichment

Nitrite accumulation is usually a concern in shrimp ponds that lack sediment, as sediment is the major source of nitrifying microorganisms, including nitrite-oxidizing bacteria (NOB). Bioaugmentation with NOB inocula is considered an attractive solution to nitrite accumulation in shrimp ponds.

Researchers investigated the effects of nitrite concentrations on microbial communities and the kinetics of NOB enrichments, which can then be further applied to develop NOB inocula suitable for aquaculture ponds. The results from NOB population analysis with quantitative polymerase chain reaction (qPCR) showed that only *Nitrospira* were detected in the sediment from the shrimp ponds. The NOB enriched at high nitrite concentrations resulted in the predominance of *Nitrobacter* with high q_{max} and K_s . In contrast, the NOB enriched at low nitrite concentrations led to the coexistence of *Nitrospira* and *Nitrobacter* with low K_s values. The results suggested that the selection of nitrite concentrations for the enrichment of NOB inocula can significantly influence NOB populations and kinetics, which could affect the effectiveness of their applications in brackish shrimp ponds.

This research was conducted by the BIOTEC Marine Biotechnology Laboratory and Chulalongkorn University.

Ref: Tangkitjawisut, W., Limpiyakorn, T., Powtongsook, S., Pornkulwat, P. and Suwannasilp, B.B. (2016). Differences in nitrite-oxidizing communities and kinetics in a brackish environment after enrichment at low and high nitrite concentrations. *Journal of Environmental Sciences*, 42, 41-49.

Deciphering the biology of porcine epidemic diarrhea virus

Emergence of the porcine epidemic diarrhea virus (PEDV) as a global threat to the swine industry underlies the urgent need for deeper understanding of this virus. However, the mechanisms behind PEDV pathogenesis remain largely unknown due to the challenges in propagating field isolates in cell culture and the lack of genetic tools for virus manipulation.

Researchers discussed the reverse genetics approaches that have been successfully used to engineer infectious clones of PEDV, as well as other potential and complementary methods that have yet to be applied to PEDV. The importance of proper cell culture for successful PEDV propagation and maintenance of disease phenotype are addressed in their survey of permissive cell lines. Highlights were given to areas of particular relevance to PEDV pathogenesis and disease that have benefited from reverse genetics studies and pressing questions that await resolution by such studies. In particular, the team examined the spike protein as a determinant of viral tropism, entry and virulence, ORF3 and its association with cell culture adaptation, and the nucleocapsid protein and its potential role in modulating PEDV pathogenicity. The review concluded with an exploration of how reverse genetics can help mitigate the global impact of PEDV by addressing the challenges of vaccine development.

This review was conducted by the BIOTEC Virology and Cell Technology Laboratory.

Ref: Teeravechyan, S., Frantz, P.N., Wongthida, P., Chailangkarn, T., Jaru-ampornpan, P., Koonpaew, S. and Jongkaewwattana, A. (2016). Deciphering the biology of porcine epidemic diarrhea virus in the era of reverse genetics. *Virus Research*, 226, 152–171.

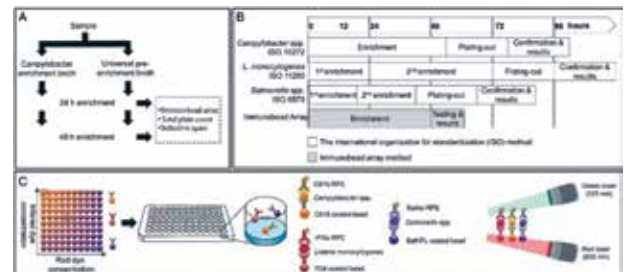
Validation of an immunobead array technique for multiplex detection of foodborne pathogens

In 2015, scientists from BIOTEC and Queen's University Belfast reported the development of an immunobead array method to simultaneously detect three important foodborne pathogens, *Campylobacter jejuni*, *Listeria monocytogenes*, and *Salmonella* spp., for application in routine food testing. While the sensitivities of detection of this technique were better than a sandwich ELISA method when using the same sets of antibodies, the limits of detection were not low enough to detect the pathogens at the concentrations required by food safety regulations. To make this developed technique applicable for food safety testing, enrichment steps to increase bacterial numbers are required and validation with actual food samples must be undertaken.

In the new study, scientists evaluated commercial enrichment culture media to ensure sufficient numbers of pathogens were present at time of testing and validated the immunobead array by testing chicken products. The findings showed that the immunobead array method was capable of detecting as low as 1 CFU of the pathogens spiked in the culture media after being cultured for 24 h for all three pathogens. The immunobead array method was evaluated for its pathogen detection capabilities in ready-to-eat (RTE) and ready-to-cook (RTC) chicken samples and proven to be able to detect as low as 1 CFU of the pathogens spiked in the food samples after being cultured for 24 h in the case of *Salmonella* spp., and *L. monocytogenes* and 48 h in the case of *C. jejuni*. The method was subsequently validated with three types of chicken products -- RTE, RTC and raw chicken -- and was found to give the same results as the conventional plating method. These findings demonstrated that the previously developed immunobead array method could be used for actual food testing with minimal enrichment period of only 52 h, whereas the conventional ISO protocols for the same pathogens take 90–144 h. The technique is an inexpensive, rapid and simple method for food testing.

This study was conducted by researchers from BIOTEC and Queen's University Belfast (UK).

Ref: Charlermroj, R., Makornwattana, M., Grant, I.R., Elliott, C.T. and Karoonuthaisiri, N. (2016). Validation of a high-throughput immunobead array technique for multiplex detection of three foodborne pathogens in chicken products. *International Journal of Food Microbiology*, 224, 47–54.



The multiplex detection of *Campylobacter* spp., *Listeria monocytogenes*, and *Salmonella* spp. using the immunobead array method.

Highlights from Health and Medicine Cluster

Role of *Plasmodium vivax* dihydropteroate synthase polymorphisms in sulfa drug resistance

Dihydropteroate synthase (DHPS) is a known sulfa drug target in malaria treatment, existing as a bifunctional enzyme together with hydroxymethyldihydropterin pyrophosphokinase (HPPK). Polymorphisms in key residues of *Plasmodium falciparum* DHPS (*PfDHPS*) have been characterized and linked to sulfa drug resistance in malaria. Genetic sequencing of *P. vivax dhps* (*Pvdhps*) from clinical isolates has shown several polymorphisms at the positions equivalent to those in the *Pfdhps* genes conferring sulfa drug resistance, suggesting a mechanism for sulfa drug resistance in *P. vivax* similar to that seen in *P. falciparum*.

In this present study, researchers carried out a study to characterize the role of polymorphisms in the *PvDHPS* in sulfa drug resistance. The results showed that mutations at *PvDHPS* affect the enzyme kinetic properties and susceptibility to sulfa drugs and that the accumulation of mutations is associated with reduced sensitivity to sulfa drugs. Moreover, due to the lack of a continuous *in vitro* culture system for *P. vivax* parasites, a surrogate *P. berghei* model expressing *Pvhppk-dhps* genes was established to demonstrate the relationship between sequence polymorphisms and sulfa drug susceptibility and to

test the activities of PvDHPS inhibitors on the transgenic parasites. These enzyme inhibition assays and the *in vivo* screening system can be useful tools for screening new compounds for their activities against PvDHPS.

This study was jointly conducted by researchers from Biosensing Technology and Medical Molecular Biology Research Unit.

Ref: Pornthanakasem, W., Riangrunroj, P., Chitnumsub, P., Ittarat, W., Kongkasuriyachai, D., Uthaipibull, C., Yuthavong, Y. and Leartsakulpanich, U. (2016). Role of *Plasmodium vivax* Dihydropteroate Synthase Polymorphisms in Sulfa Drug Resistance. *Antimicrobial Agents and Chemotherapy*, 60(8), 4453-63.

A new approach to counter drug resistance in *Plasmodium*

Plasmodium falciparum, one of the species of *Plasmodium* that cause malaria in humans, has become resistant to all front-line antimalarial drugs in current use. Thus, new strategies are needed to counter drug resistance in *Plasmodium*. Glutathione plays a central role in maintaining cellular redox homeostasis and modulations to this status may affect malaria parasite sensitivity to certain types of antimalarials. However, the effect of glutathione on modulating *Plasmodium* sensitivity to other antimalarials is unclear.

In this study, researchers demonstrated that inhibition of glutathione biosynthesis in the *Plasmodium berghei* ANKA strain through disruption of the γ -glutamylcysteine synthetase (γ -GCS) gene, which encodes the first and rate-limiting enzyme in the glutathione biosynthetic pathway, significantly sensitizes parasites *in vivo* to pyrimethamine and sulfadoxine, but not to chloroquine, artesunate, or primaquine, compared with control parasites containing the same pyrimethamine-resistant marker cassette. Treatment of mice infected with an antifolate-resistant *P. berghei* control line with a γ -GCS inhibitor, buthionine sulfoximine, could partially abrogate pyrimethamine and sulfadoxine resistance. The role of glutathione in modulating the malaria parasite's response to antifolates suggests that development of specific inhibitors against

Plasmodium γ -GCS may offer a new approach to counter *Plasmodium* antifolate resistance.

This study was conducted by researchers from Medical Molecular Biology Research Unit.

Ref: Songsungthong, W., Koonyosying, P., Uthaipibull, C. and Kamchonwongpaisan, S. (2016). Inhibition of Glutathione Biosynthesis Sensitizes *Plasmodium berghei* to Antifolates. *Antimicrobial Agents and Chemotherapy*, 60(5), 3057-3064.

Role of *gyrB* mutations in quinolone resistance in Thai clinical isolates

Quinolones are considered to be the cornerstone of the current treatment for multidrug-resistant tuberculosis (MDR-TB); however, its use has caused concerns about emerging resistance in *M. tuberculosis*. Resistance to quinolones can pave the way for the emergence of extensively drug-resistant tuberculosis (XDR-TB), a potentially life-threatening form of tuberculosis. DNA gyrase mutations are a major cause of quinolone resistance in *M. tuberculosis*, resulting primarily from mutations in *gyrA* and *gyrB*.

A comprehensive study was conducted to determine the diversity of gyrase mutations in pre-XDR and XDR Thai clinical tuberculosis isolates. All pre-XDR-TB and XDR-TB isolates carried at least one mutation within the quinolone resistance-determining region of GyrA or GyrB. MIC and DNA gyrase super-coiling inhibition assays were performed to determine the role of gyrase mutations in quinolone resistance. Compared to the MICs against *M. tuberculosis* H37Rv, the levels of resistance to all quinolones tested in the isolates that carried GyrA-D94G or GyrB-N538D were significantly higher than those in isolates bearing GyrA-D94A or GyrA-A90V. Intriguingly, GyrB-E540D led to a dramatic resistance to later-generation quinolones, including moxifloxacin, gatifloxacin and sparfloxacin. However, GyrB-E540D caused low-level resistance to early-generation quinolones, including ofloxacin, levofloxacin and ciprofloxacin. In this study, DC-159a was the most active antituberculosis agent and was little affected by the gyrase mutations described above. The findings suggest that although they are rare, *gyrB* mutations have a notable role in

quinolone resistance, which may provide clues to the molecular basis of estimating quinolone resistance levels for drug and dose selection.

This work was a collaborative study by researchers from the Faculty of Medicine Siriraj Hospital, Mahidol University, Siriraj Foundation, the Research Institute of Tuberculosis (Japan) and Medical Molecular Biology Research Unit.

Ref: Disratthakit, A., Prammananan, T., Tribuddharat, C., Thaisuttikul, I., Doi, N., Leechawengwongs, M. and Chaiprasert, A. (2016). Role of *gyrB* Mutations in Pre-extensively and Extensively Drug-Resistant Tuberculosis in Thai Clinical Isolates. *Antimicrobial Agents and Chemotherapy*, 60(9), 5189-5197.

Role of SB203580 in dengue virus-induced liver injury

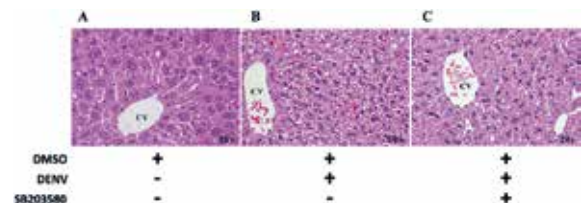
Dengue virus (DENV) infection causes organ injuries and the liver is one of the most important sites of DENV infection, where viral replication generates a high viral load. The molecular mechanism of DENV-induced liver injury is still under investigation. The mitogen activated protein kinases (MAPKs), including p38 MAPK, have roles in the hepatic cell apoptosis induced by DENV. However, the *in vivo* role of p38 MAPK in DENV-induced liver injury is not fully understood.

The role of SB203580, a p38 MAPK inhibitor, in a mouse model of DENV infection was investigated. Both the hematological parameters, leucopenia and thrombocytopenia, were improved by SB203580 treatment and liver trans-aminases and histopathology were also improved. A real-time PCR microarray was employed to profile the expression of apoptosis-related genes. Tumor necrosis factor α , caspase 9, caspase 8, and caspase 3 proteins were significantly lower in the SB203580-treated DENV-infected mice than that in the infected control mice. Increased expressions of cytokines including TNF- α , IL-6 and IL-10, and chemokines including RANTES and IP-10 in DENV infection were reduced by SB203580 treatment. DENV infection induced the phosphorylation of p38MAPK, and its downstream signals including MAPKAPK2, HSP27 and ATF-2. SB203580 treatment did not decrease the

phosphorylation of p38 MAPK, but it significantly reduced the phosphorylation of MAPKAPK2, HSP27, and ATF2. Therefore, SB203580 modulates the downstream signals to p38 MAPK and reduces DENV-induced liver injury.

This study was jointly conducted by researchers from Medical Biotechnology Research Laboratory and the Faculty of Medicine Siriraj Hospital, Mahidol University.

Ref: Sreekanth, G.P., Chuncharunee, A., Sirimontaporn, A., Panaampon, J., Noisakran, S., Yenchitsomanus, P. and Limjindaporn T. (2016). SB203580 Modulates p38 MAPK Signaling and Dengue Virus-Induced Liver Injury by Reducing MAPKAPK2, HSP27, and ATF2 Phosphorylation. *PLoS ONE*, 11(2): e0149486. doi:10.1371/journal.pone.0149486.



SB203580 treatment reduces DENV-induced liver pathology in DENV-infected mice.

Serological cross-reaction between Zika and dengue viruses

Zika virus is an arbovirus that belongs to the family flaviviridae and is transmitted to man by *Aedes* mosquitoes. It was discovered in 1947 and was thought to lead to relatively mild disease. The recent explosive outbreak of Zika virus in South America has led to widespread concern, with reports of neurological sequelae ranging from Guillain Barré syndrome, a disorder in which the body's immune system attacks part of the peripheral nervous system, to microcephaly. Zika virus infection has occurred in areas previously exposed to dengue virus, a flavivirus closely related to Zika virus.

The serological cross-reaction between Zika and dengue viruses was investigated. Plasma immune to dengue virus showed substantial cross-reaction to Zika virus and was able to drive antibody-dependent enhancement of Zika virus infection. It was found that most antibodies that reacted to dengue virus

envelope protein also reacted to Zika virus. Antibodies to linear epitopes, including the immunodominant fusion-loop epitope, were able to bind Zika virus but were unable to neutralize the virus and instead promoted antibody-dependent enhancement. The data indicated that immunity to dengue virus might drive greater Zika virus replication and had clear implications for disease pathogenesis and future vaccine programs for Zika virus and dengue virus.

This work is an ongoing collaborative effort among researchers from the BIOTEC Medical Biotechnology Research, Mahidol University, Imperial College London (UK), the Institut Pasteur (France), and the Institut Louis Malardé (French Polynesia).

Ref: Dejnirattisai, W., Supasa, P., Wongwiwat, W., Rouvinski, A., Barba-Spaeth, G., Duangchinda, T., Sakuntabhai, A., Cao-Lormeau, V.-M., Malasit, P., Rey, F.A., Mongkolsapaya, J. and Screaton, G.R (2016). Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. *Nature Immunology*, 17, 1102–1108.

Highlights from Energy and Environment Cluster

An on-site microbial reactor for high oil-content wastewater

Previously, BIOTEC and KEEEN Company Limited collaborated on a project to screen for local oil-degrading bacteria and develop this technology into a commercial bioremediation product. The project was a success resulting in bioremediation products and services for various industries such as petrochemical industry, automobile industry, food industry, hotels and hospitals. In 2013, KEEEN and BIOTEC started to develop a small-scale microbial reactor. This reactor will allow on-site production of microorganisms used in the treatment of oil-contaminated wastewater. It will eliminate the cost of packaging and transportation of bioremediation agents, making it suitable for industrial sites that need to regularly manage oil-contaminated wastewater in high volume.

The project was completed in 2016. The 10L reactor is equipped with an automated system controlling

aeration and influent/effluent for the production of oil-degrading microorganisms within 24-48 hours. It comes with starter (specially formulated oil-degrading microbes) and concentrated culture medium.

Efficiency improvement in cassava starch processing

BIOTEC has been engaging in research, development and technology demonstration in efficiency improvement in cassava starch processing since 1996. From 2008-2011, BIOTEC implemented a project to build up capacity in resource and energy efficiency among business operators in the cassava starch industry with the sponsorship from NSTDA and German International Cooperation (GTZ). The project was later carried on with funding from NSTDA and the Department of Industrial Promotion. Overall, the project has been successfully implemented in 6 factories, resulting in the cost savings of 223.7 million Baht/year

In 2016, parameters and values for benchmarking resource efficiency for the cassava starch processing industry were established. A total of 21 factories participated in the capacity building program. A manual and case studies have been published for dissemination. Cost saving has been estimated at 147.2 million Baht.

Highlights from Bioresources Cluster

Collection of biological materials

BIOTEC established the BIOTEC Culture Collection (BCC) in 1996 as a depository and distribution center for microbes. The facility is also designated by the Department of Intellectual Property as a repository of patent-related microorganisms. As of September 2016, a total of 80,210 strains are preserved at the BCC, of which 50,541 are fungi, 22,248 are bacteria, 7,212 are yeast and 209 are algae. There are 41,337 dried specimens of fungi preserved at BIOTEC Bangkok Herbarium (BBH). In addition, BCC provided depository services for 241 biological materials, comprising 61 vectors, 25 hosts and 155 recombinant clones, in 2016.

BIOTEC Hybridoma Bank preserves hybridoma clones for the production of antibodies specific to plant pathogens as well as hormones and antigens associated with the cattle reproductive system. BIOTEC Hybridoma Bank currently has 644 monoclonal antibody-secreting hybridomas and two types of myeloma cells.

Thailand Bioresource Research Center (TBRC) was established in 2015 with the main mission to promote accessibility to, and utilization of, biological materials, operating within a concept of a value co-creation and co-innovation approach through network interaction. TBRC on-line catalogue contains biological materials from its in-house collections as well as selected materials from its network. The network currently contains five collections: 1) BCC, 2) Thailand Institute of Scientific and Technological Research (TISTR), 3) Sustainable Development of Biological Resources Lab (SDBR) of Chiang Mai University, 4) Fermentation Research Center for Value Added Agricultural Products (FerVAAP) of Khon Kaen University, and 5) Department of Industrial Microbiology of Chulalongkorn University. As of September 2016, TBRC on-line catalogue contained 10,608 microbial strains. These are categorized as 2,477 strains of bacteria, 3,765 strains of fungi and 4,366 yeast strains. In addition, services on 15 molecular materials and 15 antibodies have been made available. Information on TBRC services can be viewed at <http://www.tbrcnetwork.org/>.

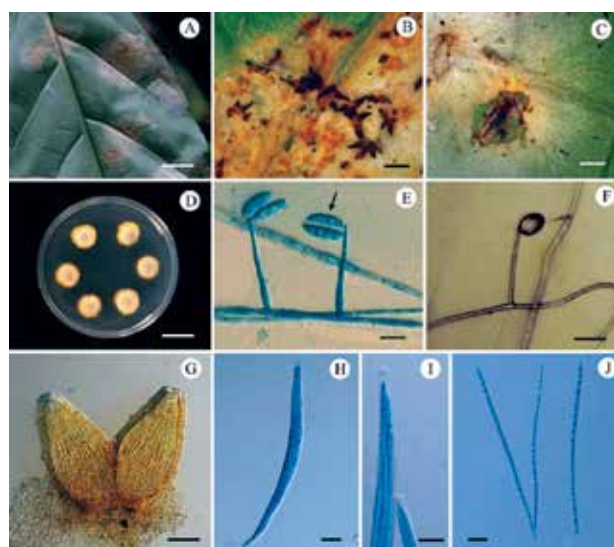
Microbial diversity study

Microbial diversity study at BIOTEC involves the collection, isolation and identification of microorganisms from a variety of natural habitats. A total of nineteen novel species were discovered in 2016 and many of these discoveries were made in collaboration between researchers from BIOTEC and other academic and research institutes.

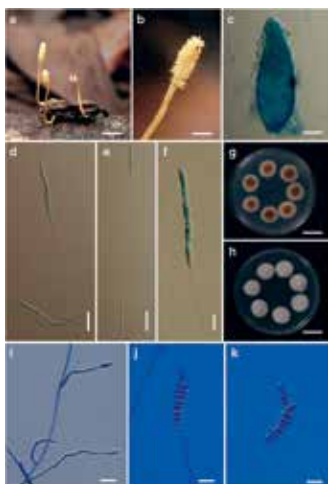
- **Five new actinomycete species:** *Kutzneria chonburiensis* sp. nov. SMC 256^T (isolated from soil), *Phytohabitans kaempferiae* sp. nov. (isolated from *Kaempferia larsenii* leaf), *Streptomyces verrucosisorus* sp. nov. (isolated from marine sediments), *Streptomyces phyllanthi* sp. nov. (isolated from the stem of *Phyllanthus*

amarus) and *Planomonospora corallina* sp. nov. (isolated from bamboo rhizospheric soil).

- **Two new bacterial species:** *Acetobacter suratthanensis* sp. nov. (isolated from a fruit) and *Actinoplanes subglobosus* sp. nov. (isolated from mixed deciduous forest soil).
- **Seven new insect-pathogenic fungal species:** *Cordyceps chiangdaoensis* (isolated from Coleoptera larvae), *Cordyceps morakotii* (isolated from *Odontomachus* ant pupae), *Conoideocrella krungchingensis* sp. nov. (isolated from armored scale insects on a dicotyledonous plant), *Beauveria gryllotalpidicola* (found in Khao Yai National Park), *Beauveria loeiensis* (discovered on a farm in Loei Province), *Moelleriella pongdueatensis* (discovered in Chiang Mai Province) and *Moelleriella phukhiaensis* (discovered in Chaiyaphum Province).
- **Two new fungal species:** *Shrungabeeja longiappendiculata* (isolated from bamboo) and *Durotheca macrostroma* (isolated from *Castanopsis acuminatissima* wood).
- **Three new poisonous mushroom species:** *Amanita gleocystidiosa* (discovered in Phetchabun Province), *Amanita pyriformis* (discovered in Chiang Mai Province) and *Amanita digitosa* (discovered in Sisaket Province).
- **One new family of aquatic fungus:** Tirisporellaceae (in the Order Diaporthales).



Conoideocrella krungchingensis (BBH 27004)



Cordyceps morakotii

Bioactive compound discoveries

BIOTEC natural product discovery program aims to explore microbial resources for bioactive compounds that have potential for pharmaceutical and agricultural product development. Bioactive substances produced from various microorganisms are identified by activity-guided fractionation and subjected to spectroscopic analyses to determine chemical structures.

Types of organisms	No. of new compounds	No. of known compounds	Total no. of compounds
Actinomycetes	9	28	37
Marine fungi	13	12	25
Endophytic fungi	12	24	36
Insect-pathogenic fungi	7	10	17
Fungi	13	27	40
Mushroom	31	38	69
Total	85	139	224

In 2016, a total of 224 active compounds were identified, 85 of which were novel compounds. Below are examples of microbial-derived compounds that have been identified in 2016:

- Two new and two known anthraquinone derivatives, along with the previously reported compound, steffimycin B were isolated from *Streptomyces* sp. BCC27095. The chemical

structures were determined on the basis of NMR spectroscopic data. These compounds exhibited significant antimalarial and antimycobacterial activities. They were also evaluated for antibacterial activity against *Bacillus cereus* and for cytotoxicity against both malignant and non-malignant cells.

- Three polycyclic tetrahydroxanthones, chrestoxanthones A C, together with known albofungin and chloroalbofungin were isolated from the actinomycete *Streptomyces chrestomyceticus* BCC 24770. Their structures were elucidated by extensive spectroscopic analyses. Chrestoxanthone A was active against *Curvularia lunata* and *Alternaria brassicicola*, while all other isolated metabolites displayed broad anti-fungal activities against *C. lunata*, *A. brassicicola*, *Colletotrichum capsici* and *Colletotrichum gloeosporioides*.
- Three macrolides, efomycins M, G and oxohydrogrolidin, along with two polyethers, abierixin and 29-O-methylabierixin were isolated from endophytic *Streptomyces* sp. BCC72023 isolated from rice. All compounds exhibited antimalarial activity. These compounds were evaluated for biological activity against *Mycobacterium tuberculosis*, *Bacillus cereus*, *Colletotrichum gloeosporioides* and *Colletotrichum capsici*, as well as cytotoxicity against both cancerous and non-cancerous cells.
- Two new actinoquinolines A and B were isolated from *Streptomyces* sp., strain CNP975 found in a sediment sample in the US. These compounds exhibited significant inhibition of the arachidonic acid pathway enzymes cyclooxygenases-1 and -2.
- Nineteen compounds including two cyclic peptides, five macrolides, new naphthoquinone, nahuic acid C, geldanamycin derivatives, cyclooctatin, germicidins A and C, actinoramide A, abierixin, and 29-O- methylabierixin were isolated from *Streptomyces* sp. BCC71188. These isolated compounds were evaluated for antimicrobial activity, such as antimalarial, anti-TB and antibacterial activities, and for cytotoxicity against both cancerous and non-cancerous cells. Fifteen compounds (two cyclic peptides, five macrolides, geldanamycin derivatives and

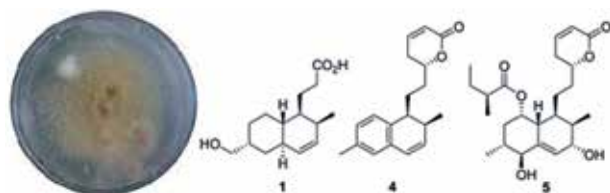
- cyclooctatin) exhibited antimalarial, and elaiophylin analogs displayed anti-TB and *B. cereus* activities. Two cyclic peptides, cyclooctatin and abierixin displayed weak cytotoxicity, indicating a potential for antimicrobial agents.
- Ten compounds including nine phenalenone derivatives (five bipolarides and four bipolarols) and a sesterterpene acid (bipolarenic acid), were isolated from a marine isolate of the fungus *Lophiostoma bipolare* (BCC25910), along with the known compounds, (–)-scleroderolide, (–)-sclerodin, and oxasetin. Chemical structures were elucidated based on NMR spectroscopic data and HRESIMS analysis. In addition, the absolute configurations of the phenalenones were resolved using specific rotations and chemical means, while the relative configuration of bipolarenic acid was confirmed by X-ray crystallographic analysis. The compounds were evaluated for biological activity against the *Plasmodium falciparum* K-1 strain, *Candida albicans* and *Bacillus cereus*, and for cytotoxicity against both cancerous and non-cancerous cells.
 - Several known compounds, including tricycloalternarenes 2b (also known as ACTG-toxin D), 3a, 3b, A, H, and 11a, as well as infectopyrone, were isolated from the marine fungus *Paradendryphiella arenariae* BCC 17999. Tricycloalternarenes 2b and 3b displayed weak cytotoxic activity against the human small-cell lung cancer cell line NCI-H187.
 - The novel compounds, astronypyrone, astronyquinone, and astronyurea, together with three known compounds, xestodecalactone A, ent-coryoctalactone B, and dimethoxy-O-methylpulvinone were isolated from the marine-derived fungus *Astrospheariella nypae* BCC 5335. Their structures were elucidated on the basis of NMR spectroscopy and mass spectrometry data. Antimalarial activity against *Plasmodium falciparum* K1 was observed for dimethoxy-O-methylpulvinone. Astronyquinone showed weak antituberculosis activity and exhibited cytotoxic activity against Vero cell lines.
 - Three new oxygen-bridged cyclooctadiene containing compounds, phomoxydienes A-C, and two new dothiorelones I and J, together with 10 known compounds were isolated from an endophytic fungus, *Phomopsis* sp. BCC 45011. Structures were determined by NMR spectroscopic and MS spectrometric analyses. Mycoepoxydiene, deacetylmycoepoxydiene, phomoxydienes A and C, and cytosporone E displayed antimalarial and cytotoxic activities while 1893 A and cytosporone P showed only cytotoxic and antimalarial activity, respectively.
 - Two new compounds, allahabadolactones A and B, along with 10 known compounds including 16- amino-isopimar-7-en-19-oic acid, 16- α -D-glucopyranosyloxyisopimar-7-en-19-oic acid, 16- α -D- mannopyranosyloxyisopimar-7-en-19-oic acid, ergosterol, (22E)-5 α ,8 α -epidioxyergosta-6,22-dien-3 β -ol, cerevisterol, (R)-(–)-methoxycarbonylmellein, (–)-piliiformic acid, 7-dechlorogriseofulvin, and cytochalasin D, were isolated from the endophytic fungus, *Aspergillus allahabadii* BCC45335. Their chemical structures were determined based on NMR spectroscopic and mass spectrometric analyses. Antibacterial activity against *Bacillus cereus* and cytotoxicity against MCF-7, KB, NCI-H187, and Vero cells of the isolated compounds were evaluated.
 - Five new compounds including three dimeric chromanones, one cyclohexenone and one benzamide, together with four known metabolites, were isolated from the endophytic fungus *Xylaria* sp. PSU-H182. Some of the isolated compounds were evaluated for antimalarial, antimycobacterial and cytotoxic activities.
 - Chaetone G, a new dibenzo[b,e]oxepinone, along with the known derivative, 5 α ,8 α -epidioxy-(22E,24R)-ergosta-6,22-dien-3 β -ol, and zeorin were isolated from the insect pathogenic fungus, *Aschersonia luteola* BCC 31749. The structure of chaetone G was elucidated based on the analysis of NMR spectroscopy and mass spectrometry. 5 α ,8 α -Epidioxy-(22E,24R)-ergosta-6,22-dien-3 β -ol exhibited antibacterial activity against *Bacillus cereus* and also showed cytotoxicity toward NCI-H187 and Vero cells.
 - Six new compounds, including three acremonidins FeH, two acremoxanthonones FeG, and acremonoxide, along with eight known compounds, were isolated from *Verticillium* sp.

BCC33181. Their structures were determined based on extensive 1D and 2D NMR spectral analyses, mass spectrometric data and by comparison with the known compounds reported previously. The relative configurations were identified based upon NOESY spectroscopic information as well as comparison of CD spectrum with the known analogs. Both acremonidins and acremoxanthones exhibited anti-*Bacillus cereus* activity, while acremonidins F, A, G, and C also displayed anti-*Enterococcus faecium* activity. In addition, acremoxanthones F and G showed antimalarial activity against *Plasmodium falciparum* K1 strain, multidrug-resistant strain, while others were inactive.

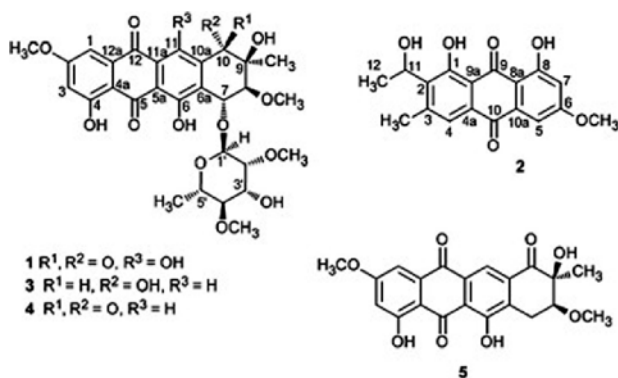
- Three new lovastatin analogues together with four known lovastatin derivatives, namely, lovastatin, α,β -dehydrolovastatin, α,β -dehydrodihydromonacolin K, and α,β -dehydro-4a,5-dihydromonacolin L, were isolated from the soil-derived fungus *Aspergillus sclerotiorum* PSU-RSPG178. Their structures were established using spectroscopic evidence. A lovastatin analogue exhibited the most potent activity against HMG-CoA reductase. This lovastatin analogue was considered to be noncytotoxic against noncancerous Vero cells, whereas lovastatin displayed much stronger activity.
- Two new compounds including one phthalide (asperlide) and one depsidone (aspersidone) together with twelve known compounds were isolated from the soil-derived fungus *Aspergillus unguis* PSU-RSPG199. Known emeguisin A exhibited potent antibacterial activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus* as well as strong antifungal activity against *Cryptococcus neoformans*. Additionally, known pilobolusate was strongly active against the human oral carcinoma (KB) cell line. Emeguisin A and pilobolusate were noncytotoxic against noncancerous cell lines.
- Five new metabolites including four β -resorcylic macrolides and one octahydronaphthalene derivative were isolated from a seagrass-derived fungus *Fusarium* sp. PSU-ES123 together with seven known compounds. Their structures were characterized by analysis of the spectroscopic data. The absolute configuration was determined by Mosher's method and circular dichroism spectroscopy. One β -resorcylic macrolide displayed weak antifungal activity against *Cryptococcus neoformans* and no cytotoxic activity against noncancerous Vero cell lines. In addition, known zearalenone displayed a protective activity against INS-1 832/13 pancreatic β -cells and was noncytotoxic to Vero cells.
- Two new trichothecenes and a new chloroderivative of a trichothecene analogue together with four known trichothecenes, crotochin, trichothecin, 8-deoxytrichothecinol B, and a trichothecene analogue, were isolated from the fungus *Acremonium crotochinigenum* BCC 20012. The structures of these compounds were elucidated by extensive spectroscopic analysis. Among the tested metabolites, trichothecin itself showed strongest antimalarial activity against *Plasmodium falciparum* K1, and cytotoxic activity against Vero cell lines.
- Sixteen new lanostane triterpenoids, together with 26 known compounds, were isolated from cultures of the basidiomycete *Ganoderma* sp. BCC 16642. Antitubercular activities of these *Ganoderma* lanostanoids against *Mycobacterium tuberculosis* H37Ra were evaluated, and structure-activity relationships are proposed.
- A new seco-tremulane, 11,12-epoxy-5,6-secotremula-1,6(13)-dien-5,12-olide, was isolated together with the known compounds, conocanolides A and B tremulenediol A, tremulenolide A, and two lanostane triterpenoids, trametenolic acid B, and pinicolic acid A, from cultures of the basidiomycete *Flavodon flavus* BCC 17421. Interconversion of conocanolides A/B was demonstrated. The new compound exhibited weak cytotoxic activities, whereas tremulenediol A showed antiplasmodial activity. Pinicolic acid A exhibited activity against herpes simplex virus type-1 as well as cytotoxic activities.
- Twelve new lanostane triterpenoids, astraeusins A-L, together with six known compounds, were isolated from the food mushroom *Astraeus odoratus*. Their structures were elucidated by spectroscopic analysis and chemical correlations.

Antibacterial and cytotoxic activities of these lanostanoids were evaluated.

- Two new hirsutane sesquiterpenes, marasmiellins A and B, were isolated from cultures of the basidiomycete *Marasmiellus* sp. BCC 22389. The structures were elucidated on the basis of NMR spectroscopic and mass spectrometry data. The absolute configuration of marasmiellin B was determined by application of the modified Mosher's method.



Lovastatin Analogues from the Soil-Derived Fungus *Aspergillus sclerotiorum* PSU-RSPG178



Chemical structures of compounds from *Streptomyces* sp. BCC27095

Highlights from Cross-cutting Technology Program

Sensitive visual detection of AHPND bacteria

Acute hepatopancreatic necrosis disease (AHPND) is a component cause of early mortality syndrome (EMS) of shrimp. In 2013, the causative agent was found to be unique isolates of *Vibrio parahaemolyticus* (VP_{AHPND}) that contained a 69 kbp plasmid (pAP1) carrying binary Pir-like toxin genes *Pir^{VP}A* and *Pir^{VP}B*. After VP_{AHPND} was characterized, a major focus of the AHPND control strategy was to monitor broodstock shrimp and post larvae for freedom from

VP_{AHPND} by nucleic acid amplification methods, most of which required use of expensive and sophisticated equipment not readily available in a shrimp farm setting.

Scientists developed a simpler but equally sensitive approach for detection of VP_{AHPND} based on loop-mediated isothermal amplification (LAMP) combined with unaided visual reading of positive amplification products using a DNA-functionalized, ssDNA-labeled nanogold probe (AuNP). The target for the special set of six LAMP primers used was the VP_{AHPND} *Pir^{VP}A* gene. The LAMP reaction was carried out at 65°C for 45 min followed by addition of the red AuNP solution and further incubation at 65°C for 5 min, allowing any *Pir^{VP}A* gene amplicons present to hybridize with the probe. Hybridization protected the AuNP against aggregation, so that the solution color remained red upon subsequent salt addition (positive test result) while unprotected AuNP aggregated and underwent a color change from red to blue and eventually precipitated (negative result). The total assay time was approximately 50 min. The detection limit of 100 CFU was comparable to that of other commonly-used methods for nested PCR detection of VP_{AHPND} and 100-times more sensitive than 1-step PCR detection methods that used amplicon detection by electrophoresis or spectrophotometry. There was no cross reaction with DNA templates derived from non-AHPND bacteria commonly found in shrimp ponds, including other *Vibrio* species. The new method significantly reduced the time, difficulty and cost for molecular detection of VP_{AHPND} in shrimp hatchery and farm settings.

This study was a collaborative effort between BIOTEC, Mahidol University and Charoen Pokphand Foods PLC.

Ref: Arunrut, N., Kampeera, J., Sirithammajak, S., Sanguanrut, P., Proespraiwong, P., Suebsing, R. and Kiatpathomchai, W. (2016). Sensitive Visual Detection of AHPND Bacteria Using Loop-Mediated Isothermal Amplification Combined with DNA-Functionalized Gold Nanoparticles as Probes. *PLoS ONE*, 11(3): e0151769. doi:10.1371/journal.pone.0151769.

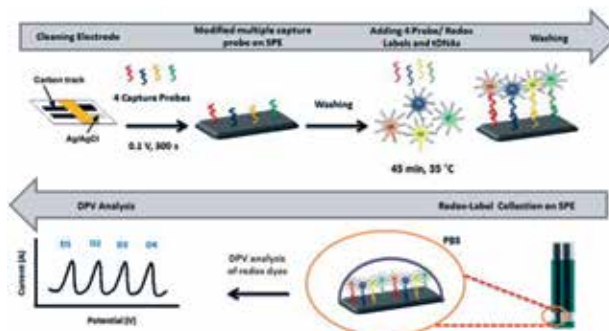
Loading of silicon nanoparticle labels with redox mediators for detection of multiple DNA targets within a single voltammetric sweep

The detection of specific sequences of DNA has many applications including the sensing or diagnosis of viruses, pathogenic microorganisms, human genetic diseases and biological warfare agents. Electrochemical techniques have been intensively researched for these purposes. One analytical application is the identification of different but related DNA sequences, where an accurate concentration value is not required, but more than one sequence needs to be identified. In this case a technique which can provide simultaneous, semi-quantitative detection for greater than one analyte and can be repeated for each target sequence is desired. For a detection system involving voltammetric measurement, one route to this goal is to use different redox species possessing well separated standard potentials. This then gives the possibility of detecting different target sequences within one voltammetric sweep.

Researchers have constructed electrochemical labels by loading four different redox mediators into silica nanoparticles (SiNPs) through electrostatic attraction. The mediators were methylene blue, ferrocinium tetrafluoroborate, tris (2,2-bipyridyl) dichlororuthenium (II) hexahydrate and acridine orange. The SiNPs were coated with avidin, which enabled attachment to biotin-tagged DNA sequences, forming signal probes. The immobilisation of four different capture probes onto screen printed electrodes enabled a simple qualitative and semi-quantitative detection of four different target probes within a single voltammetric sweep using differential pulse voltammetry. This was illustrated by detecting sequences found in four forms of the influenza virus, the influenza A sub-types H1N1, H3N2, H5N1 and influenza B. The limits of detection were 1.6 pM, 1.6 pM, 4.7 pM and 2.9 pM, respectively.

This study was jointly conducted by researchers from Biochemical Engineering and Pilot Plant Research and Development Institute at King Mongkut's University of Technology Thonburi.

Ref: Cheeveewattanagul, N., Rijiravanich, P., Surareungchai, W. and Somasundrum M. (2016). Loading of silicon nanoparticle labels with redox mediators for detection of multiple DNA targets within a single voltammetric sweep. *Journal of Electroanalytical Chemistry*, 779, 61–66.



Schematic representation of simultaneous DNA sandwich assay.

Highlights from Platform Technology Program

A novel salt-inducible vector for efficient expression and secretion of heterologous proteins

Bacillus subtilis is commonly used as a host for heterologous protein production via plasmid-based expression system. Expression vectors used for heterologous gene expression in *B. subtilis* have been developed by using various types of promoters. Many inducible promoters have some drawbacks in terms of food safety, carbon catabolite repression problem or high large-scale production cost.

In order to improve product safety, avoid carbon catabolite repression and lower production cost, a novel salt-inducible vector, pSaltExSePR5, was developed based on a natural plasmid of *Lactobacillus plantarum* BCC9546. Salt-inducible promoter *opuAA* and a DNA fragment encoding a signal peptide of subtilisin E (*SubE*) were sequentially added to the core shuttle vector to facilitate expression and secretion of a target protein in *B. subtilis*. To evaluate the effectiveness of this system under salt induction, a protease gene from *Halobacillus* sp. without its native signal sequence was inserted in the pSaltExSePR5 plasmid downstream of *SubE* signal sequence and transformed into *B. subtilis* WB800. The results demonstrated that pSaltExSePR5 provides

an alternative vector for efficient and simple production of heterologous proteins in *B. subtilis* with a safer and more economic inducer.

This study was jointly conducted by researchers from Bioresources Technology Research Unit and Food Biotechnology Research Unit.

Ref: Promchaia, R., Promdonkoyb, B., Tanapongpipata, S., Visessanguanc, W., Eurwilaichitrd, L. and Luxananil, P. (2016). A novel salt-inducible vector for efficient expression and secretion of heterologous proteins in *Bacillus subtilis*. *Journal of Biotechnology*, 222, 86–93.

Genetic analysis of Thai cattle

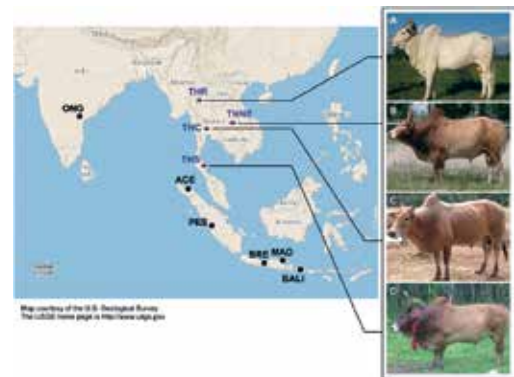
Cattle commonly raised in Thailand have characteristics of *Bos indicus* (zebu), but there have been no formal evidences of genetic testing to support the claim. There are four native breeds officially recognized by the Department of Livestock Development, Ministry of Agriculture, Thailand, namely Kho-Khaolumpoon (northern Thailand origin), Kho-Isaan (northeastern Thailand), Kho-Lan (central Thailand) and Kho-Chon (southern Thailand). Genetic variation of native Thai bovines has been studied using different marker types, i.e., STR, mitochondrial DNA and SNPs, but none of these studies provide any information on the origins and ancestries of these cattle, since the markers used are insufficiently informative.

Researchers performed a population genetics study of 28 individuals sampled from the four Thai native breeds: (Kho-Khaolampoon, Kho-Isaan, Kho-Lan and Kho-Chon). Genotyping data were obtained using the Illumina BovineSNP50K chip array platform. These data were analyzed together with 1,369 worldwide cattle from 88 breeds previously published. Data has confirmed that all four Thai varieties are *Bos indicus* and that they are distinct from other indicine breeds. Among these Thai cattle, a distinctive ancestry pattern is apparent, which is the purest within Kho-Chon individuals. This ancestral component is only present outside of Thailand among other indicine breeds in Southeast Asia. From this pattern, it was concluded that a unique *Bos indicus* ancestor originated in Southeast Asia, and native Kho-Chon Thai cattle retain the

signal of this ancestry with limited admixture of other bovine ancestors.

This study was conducted by researchers from BIOTEC, Khon Kaen University, Mahidol University, CIRAD (France) and INRA (France).

Ref: Wangkumhang, P., Wilantho, A., Shaw, P.J., Flori, L., Moazami-Goudarzi, K., Gautier, M., Duangjinda, M., Assawamakin, A. and Tongsima, S. (2015). Genetic analysis of Thai cattle reveals a Southeast Asian indicine ancestry. *PeerJ*, 3:e1318; DOI 10.7717/peerj.1318.



Four native Thai cattle varieties

Actin interacts with dengue virus 2 and 4 envelope proteins

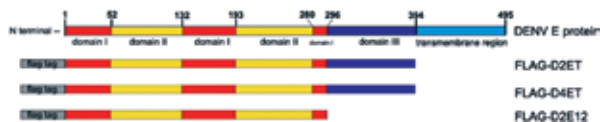
Dengue virus (DENV) remains a significant public health problem in many tropical and sub-tropical countries worldwide. The DENV envelope (E) protein is the major antigenic determinant and the protein that mediates receptor binding and endosomal fusion. In contrast to some other DENV proteins, relatively few cellular interacting proteins have been identified.

To provide a better understanding of the mechanism of viral replication and pathogenesis of dengue virus, researchers performed a study to identify interactions between DENV E and host cellular proteins, as well as cellular pathways that might be used as targets for anti-dengue therapy development by employing a co-immunoprecipitation strategy. The predominant co-immunoprecipitating proteins identified were actin and actin related proteins, however the results suggested that actin was the only bona fide interacting partner. Actin was shown to interact with the E protein of DENV 2 and 4, and

the interaction between actin and DENV E protein was shown to occur in a truncated DENV consisting of only domains I and II. Actin was shown to decrease during infection, but this was not associated with a decrease in gene transcription. Actin-related proteins also showed a decrease in expression during infection that was not transcriptionally regulated. Cytoskeletal reorganization was not observed during infection, suggesting that the interaction between actin and E protein has a cell type specific component.

This study was performed by researchers from BIOTEC Proteomics Research Laboratory and Mahidol University.

Ref: Jitboam, K., Phaonakrop, N., Libsittikul, S., Thepparit, C., Roytrakul, S. and Smith, D.R. (2016) Actin Interacts with Dengue Virus 2 and 4 Envelope Proteins. *PLoS ONE*, 11(3):e0151951. doi:10.1371/journal.pone.0151951.



Diagrammatic representation of dengue virus E protein showing the three domains of this protein (domains I, II and III) and representation of the three constructs generated.

Highlights from Policy Research

10-Year technology roadmap for biotechnology development (2017-2026)

A study has been conducted to formulate a 10-year Technology Roadmap for Biotechnology Development covering the period from 2017 to 2026. The roadmap serves as a plan to develop platform technology that will support the long-term goals of Thailand's biotechnology development in each sector.

1. Agricultural biotechnology focuses on technologies that will enable sustainable agriculture, increase yield, reduce inputs while maintaining maximum productivity with less effects on the environment, minimize greenhouse gas emission and deal with the impacts of a climate change. Topics for research and development includes photosynthesis, plant breeding and precision farming.

2. Food biotechnology aims at improving nutrition and safety of food and feed, as well as developing food to individual needs (personalized nutrition) and food to prevent or treat diseases (nutrigenomics). Technologies to be exploited are intestinal microbiome, nutrigenomics and diagnostic testing for food safety.
3. Environmental biotechnology promotes the sustainable development principle. This includes improving resource efficiency in the production process, utilizing waste, bioremediation and biorefinery.
4. Medical biotechnology focuses on personalized medicine, molecular diagnostics, genetic testing, regenerative medicine, toxicity and drug testing with stem cells and disease modeling.

Precautionary approach to managing biosafety under the upcoming ASEAN Economic Community

BIOTEC in collaboration with the Office of Natural Resources and Environmental Policy and Planning carried out a study on the impact of ASEAN Economic Community (AEC) on the cross-border movements of transgenic crops in and out of Thailand. The study classified the transgenic crops with likelihood of movement into three categories:

Low Risk: Cotton, papaya, eggplant, tomato, bell pepper, soybean, carnation and potato

Medium Risk: corn, sugarcane and rice (excluding high-vitamin rice)

High Risk: Golden rice (high-vitamin A rice)

High-vitamin A rice has a high likelihood of movement because rice is a staple food of this region and does not normally contain vitamin A. Also, vitamin A is one of essential nutrients with recommended daily intake.

The Cartagena Protocol on Biosafety outlines a precautionary approach for the transboundary movement of transgenic crops into three stages as follows:

Stage 1 Pre-importation: Risk assessment must be conducted before permission is granted. High-risk crop is prohibited, whereas medium- and low-risk crops will proceed to socio-economic impact assessment. The crop causing high impact to the

society and economy is prohibited from importation.

Stage 2 Post-importation: Transgenic crops with medium risk must undergo biosafety assessment in the greenhouse and the field and an assessment on socio-economic impact. In addition, management and separation plans must be submitted.

Stage 3 Post-commercialization: Should the transgenic crop cause damage to the environment and human health, the responsible organization must stop the commercialization of the crop and appropriate actions must be taken. Fines can be imposed on the party responsible for the damages.

As Thailand currently has no specific law for this matter, modification of existing law is suggested. Preparation in other aspects at the national and regional levels are called for in order to have efficient mechanisms.

Biosafety guidelines for contained use of genetically modified microorganisms at pilot and industrial scales (2016 Edition)

Genetically Modified Microorganisms (GMMs) have been utilized to produce specially designed products mostly in the industrial scale from pharmaceutical and medical equipment industry, food industry, to biotechnology industry. To ensure the safety of operators as well as humans and the environment from using GMMs, “Biosafety Guidelines for Contained Use of Genetically Modified Microorganisms at Pilot and Industrial Scales” was first released in 2004 by the Technical Biosafety Committee (TBC). The guidelines have been regularly updated with the most recent information and technology.

The 2016 edition provides an additional topic on waste management, a revised GMM list. GMM practices at pilot and industrial scales are classified into 4 classes as:

- **GILSP:** Work in this category involves the use of GMMs that do not cause any harm and adopts good industrial large scale practice. GMMs used must be non-pathogenic, must not involve any viral DNA, bacteriophage or plasmid that may cause disease and must be derived from microorganisms that have a long history of safe

use in industry or have limited survival in the natural environment.

- **Class 1:** Work in this category involves the use of GMMs that do not cause any harm but do not fulfill the GILSP conditions. It requires the minimum of large-scale containment level 1 (LS1).
- **Class 2:** Work in this category involves the use of GMMs that have low potential to cause harm to operators, the community or the environment. It requires a minimum of large-scale containment level 2 (LS2).
- **Class 3:** Work in this category involves the use of GMMs that are potentially harmful to human health, the community or the environment. GMMs that fall into this class may cause disease but not disease epidemics and such diseases can be prevented and treated. This class also includes work with an unidentified level of risk. It requires a minimum of large-scale containment level 3 (LS3).



Biosafety Guideline edition 2016

Guideline for food safety assessment of food derived from recombinant-DNA microorganisms

Currently, there are countless substances used in food production that are produced with the help of genetically modified microorganisms. Food derived from recombinant-DNA microorganisms need to undergo a thorough safety assessment before they are allowed into the food supply. Currently, “Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA microorganisms” developed by FAO/WHO (Codex Alimentarius Commission) is widely adopted. However, this Codex guideline does not address food additives, processing aids and enzymes used in the production process. Therefore, BIOTEC, in its capacity

as Secretariat of the Food Biosafety Subcommittee, has developed “Guideline for food safety assessment of food derived from recombinant-DNA microorganisms” for Thailand. The Guideline classifies food derived from recombinant-DNA microorganisms into three categories:

- **Category 1:** Food and food ingredients containing viable or non-viable recombinant-DNA microorganisms as addressed in Codex guideline. Full food safety assessment according to the Codex guideline is required.
- **Category 2:** Food and food ingredients produced by fermentation using recombinant-DNA microorganisms from which the recombinant-DNA microorganisms or antibiotic resistance marker genes may have been removed as addressed in Codex guideline. Full food safety assessment according to the Codex guideline is required.
- **Category 3:** Food derived from recombinant-DNA microorganisms from which the recombinant-DNA microorganisms may have been removed and are not addressed in the Codex guideline. This includes food additives, processing aids and enzymes used in the production process. The following assessments are required: safety assessment of the recombinant-DNA microorganisms and residue of recombinant-DNA microorganisms or genetic materials in the final product.

TECHNOLOGY TRANSFER



BIOTEC places strong emphasis on exploiting our research through the transfer of technologies to public or private sectors. The main mechanisms used to implement the technology transfer include technology and product licensing, capacity building, along with collaborative and commissioned research, as well as consultancy services. As technologies and knowledge can offer benefit to community enterprises, educators and youth, our technology transfer mission extends to include these groups with the ultimate goal to enhance the nation's competitiveness.

Licensing Agreements

The following technologies were licensed during fiscal year 2016:

Production technology for *Beauveria bassiana*. This technology includes the use of *Beauveria bassiana* BCC 2660 and 20 kg solid-state fermentation technology for the production of *Beauveria*. *B. bassiana* BCC 2660 is a proven biocontrol agent against rice and cassava pests and the fermentation technology developed by BIOTEC has been demonstrated to produce high quality *Beauveria* with long shelf life at an economical cost. The technology has been licensed to Ladda Company Limited, a leading agro-chemical company in Thailand, for commercial production of *B. bassiana* BCC 2660.

Large-scale production technology for micro-rhizome of ginger. This technology employs tissue culture techniques to produce disease-free micro-rhizomes of ginger with a capacity of 300,000 - 1,000,000 micro-rhizomes per year. Micro-rhizomes produced from this technology cost 1.27 times less than tissue-culture ginger plantlets. The technology was licensed to Blue Ocean Food and Drinks Company Limited, a leading manufacturer of ginger tea.

On-site microbial reactor 1. The 10L reactor is equipped with an automated system controlling aeration and influent/effluent for the production of oil-degrading microorganisms within 24-48 hours. It comes with starter (specially formulated oil-degrading microbes) and concentrated culture medium. With this reactor, microorganisms can be produced on-site for the treatment of oil-contaminated wastewater. It will eliminate the cost of packaging and transportation of bioremediation agents, making it suitable for industrial sites that need to regularly manage oil-contaminated wastewater. KEEEN Limited obtained the license to manufacture this reactor for commercial purposes.

On-site microbial reactor 2. This reactor, with the capacity of less than 100L, is equipped with an automated system controlling aeration and influent/effluent for the production of oil-degrading microorganisms within 24-48 hours. The technology has been licensed to Thai Bio Oxzine Company Limited for commercial use.

NPV for pest control. Nucleopolyhedrosis virus (NPV) is an entomopathogenic virus which can destroy larval stage of insects of the order Lepidoptera. Due to its highly specific effect on the insect pest targets and safety for humans, beneficial insects and the environment, NPV has been used for insect pest control as a microbial insecticide. NPV is effective against beet armyworm, cotton worm and common cutworm. This technology has been licensed to Bright Organic Company Limited for commercial production of NPV.

Improving extraction yield in cassava starch factory by recovery of starch in pulp at high solid loading with enzyme cocktails as a continuous integrated process. This process is able to extract more than 40% of starch in cassava pulp and increase overall productivity by 6-10%. Starch from this process is of high quality. The technology has been licensed to Siam Victory Chemicals Company Limited.

IC strip test for detection of bacterial fruit blotch disease in cucurbits. The immunochromatographic strip test based on mouse monoclonal antibodies against *Acidovorax avenae* subsp. *citrulli* – the bacterium which causes fruit blotch disease in cucurbits (watermelon, melon, squash, cantaloupe, cucumber and pumpkin) – has been developed to provide seed exporters and governmental sector a tool to cost-effectively, conveniently, accurately and rapidly detect the disease in 5 minutes. The technology has been licensed to Parwel International Company Limited.

On-site Microbial Reactor

A microbial reactor was developed to allow for on-site production of microorganisms used in the treatment of oil-contaminated wastewater. It will eliminate the cost of packaging and transportation of bioremediation agents, making it suitable for industrial sites that need to regularly manage oil-contaminated wastewater. The 10L reactor is equipped with an automated system controlling aeration and influent/effluent for the production of oil-degrading microorganisms within 24-48 hours. It comes with starter (specially formulated oil-degrading microbes) and concentrated culture mediums. Field tests have demonstrated that the effluent from the factories installed with this reactor has met the discharge requirements. Petty patent (No. 1503001926) has been applied on 15 November 2016.



Press Conference Launching
On-site Microbial Reactor.

Collaborative and Commissioned Research

To promote the application of biotechnology in finding solutions to problems faced by industries and other government agencies, BIOTEC employs its expertise to work with partners in the form of collaborative and commissioned research. The thorough study of the problems and repeated tests of solutions are critical to ensuring that solutions developed by BIOTEC will perform effectively and meet the requirements of clients and partners.

In fiscal year 2016, BIOTEC carried out 65 collaborative/commissioned research projects. Of these, 26 projects were initiated in 2016. These are categorized into 13 projects in food and agriculture theme, 7 projects in energy and environment theme, 4 projects in bioresources theme and 2 projects in manufacturing and service theme. In addition, 6 consultancy projects were carried out.

Analytical and Technical Services

With the skill and expertise of BIOTEC staff and well-equipped facilities, BIOTEC offers analytical and technical services to clients from the public and private sectors. Available services include microbial cultures, biological materials, fungal isolation and identification, enzyme assay, bioactive compound screening, compound extraction and analysis, monoclonal antibody production, culture and cell deposit, cattle reproductive system management, cassava starch quality analysis, design and performance experiments in shrimp aquaria, shrimp disease diagnostics and DNA analysis for aquaculture. In 2016, 19,824 services were performed.

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 2016, an activity was organized under the diagnostic theme with a visit to Microarray Laboratory and Monoclonal Antibody Production Laboratory. A total of 26 participants attended the Open Lab, twenty-two of which came from the private sector.



BIOTEC provides services and consultancies on hormone-based technology for ovulation synchronization and artificial insemination for dairy farm cooperatives, veterinary clinics, large and small-scale dairy farmers. The technology helps induce pregnancy in cows with reproductive system difficulties.

Production Technology for High-quality *Beauveria* for Thai Farmers

BIOTEC developed a solid-state fermentation process using rice grain as a substrate for the effective production of *Beauveria bassiana* BCC2660 at low cost. The process and conditions have been optimized to yield maximum spore production of 10^{12} spores/kg. The process includes quality assurance and control steps, thus ensuring high quality of *Beauveria* produced. *B. bassiana* BCC2260 is an effective strain of biocontrol agent, which has exhibited efficacy against green peach aphid (*Myzus persicae*), mealybug (*Pseudococcus cryptus*) and brown planthopper (*Nilaparvata lugens*).

BIOTEC-NSTDA launched a joint project with the

Department of Agricultural Extension (DOAE) in 2015 aiming to promote the use of high quality *Beauveria* among farmers. Under this project, BIOTEC transfers the production technology and the effective strain (*B. bassiana* BCC2660) to regional centers of DOAE responsible for inoculum production and distribution, as well as the training of farmers on *Beauveria* production from inoculum.

In 2016, the training was implemented in regional centers in Chiang Mai and Phitsanuloke provinces. The training included production process, quality control, storage, formulation and field application. DOAE staff was also trained on product testing, to keep the product at highest quality possible. In addition, the BIOTEC team also assisted DOAE in training 100 farmers from Phrae and Nakhon Sawan provinces.

Distribution of New Rice Varieties and the Transfer of Rice Seed Production Technology to Farmers

BIOTEC has successfully developed a number of new rice varieties through its marker-assisted rice breeding program. Since 2008, the new varieties have been distributed to farmers, along with training on rice seed production to enable farmers to produce high quality seed for long-term new rice variety production.

Locations at which new rice varieties and seed production technology have been transferred (data from 2008-2016).

Blast-resistant Glutinous Thanyasirin Rice

Number of provinces	22 Kamphaeng Phet, Phrae, Lamphun, Chiang Mai, Lampang, Chiang Rai, Phayao, Nan, Loei, Nong Bua Lam Phu, Nong Khai, Udon Thani, Bueng Kan, Sakon Nakhon, Nakhon Phanom, Chaiyaphum, Mukdaharn, Maha Sarakham, Yasothon, Ubon Ratchathani, Buriram and Nakhon Ratchasima
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Number of farmers	1,900
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Land area	6,800 rai (1,088 hectares)
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Tonnage of rice production	46,000
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RD6 Glutinous Rice with Blast and Bacterial Blight Resistance

Number of provinces	15 Chiang Mai, Lampang, Chiang Rai, Nan, Loei, Nong Khai, Bueng Kan, Sakon Nakhon, Nakhon Phanom, Mukdahan, Maha Sarakham, Amnat Charoen, Ubon Ratchathani, Buriram and Khon Kaen
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Number of farmers	600
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Land area	800 rai (128 hectares)
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Tonnage of rice production	490
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Flash-flooding Tolerant Homcholasit Rice

Number of provinces	14 Kamphaeng Phet, Uthai Thani, Chainat, Phichit, Phetchabun, Nong Khai, Ubon Ratchathani, Phra Nakhon Si Ayutthaya, Chachoengsao, Prachuap Khiri Khan, Nakhon Si Thammarat, Phatthalung, Songkhla and Pattani
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Number of farmers	200
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Land area	4,000 rai (640 hectares)
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Tonnage of rice production	3,200
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Development of Organic Rice Community Enterprise

In 2014, BIOTEC launched a project to develop a thriving and sustainable organic rice community enterprise with a project site set in Yasothon province which could later serve as a model for other provinces. The strategy is to improve organic rice cultivation and raise production standards through science and technology in order for farmers to increase productivity and obtain organic certification. This is achieved through the identification of appropriate technologies, implementation of technology transfer program and facilitation of technology adoptions. The technology introduced in 2014 was a software application, called TAMIS (Thailand Agriculture Mobility Information System), specially designed by NECTEC-NSTDA to facilitate the inspection, by enabling farmers and inspectors to input, store and retrieve all the farming data using computer tablets, thus eliminating labor-intensive paperwork. The software helps to expedite the organic certification process. By the end of 2015, 1,949 farmers successfully obtained organic certification.

In 2015, the project concentrated on the improvement of rice productivity and the promotion of the rice processing, as more income could be earned from selling milled or processed rice, rather than un-milled rice. Three approaches were taken. At an individual farmer level, the work focused on improving productivity and reducing costs. For farmer groups, the focus was on capacity building. The concept of fair trade was propagated at the network level. A total of 4,565 farmers participated in the project.

In 2016, farmers were introduced to alternative crops after rice harvesting. Legume crops such as jack bean, sunn hemp and cowpea can be grown for making organic fertilizer and for seed which can be used for future planting seasons, saving on seed expenses. Other economic crops such as watermelon, corn, sesame, turmeric, lemon grass, mungbean, soybean and peanut can be cultivated for additional income. This training resulted in 314 farmers growing crops after rice harvesting. More than 10 tons of

additional farm produce were harvested, on the area of 978 rai (150 hectares).

Upgrading Manufacturing Standards in Food Processing Industry

BIOTEC, in collaboration with KMUTT, has implemented a capacity building program in Good Hygiene Practice (GHP) and Good Manufacturing Practice (GMP) for the food processing industry, focusing on food processing facilities/factories under the Royal Project and community enterprises. Training and consultancy on GMP and GHP are provided to ensure the safety of produce and products, whereas the 5S Process is applied to ensure a safer, more efficient, and more productive operation. The activities include facility inspection, training sessions and regular follow-up.

The program has been implemented in 38 facilities, such as Royal Agricultural Stations in Chiang Mai, Chiang Rai and Mae Hong Son. In addition, 28 training workshops have been organized for staff working in community enterprises, with 1,466 participants in total (1,913 man-days).



GMP and GHP consultancy to members of Royal Project in Chiang Mai.

Promotion of Science Education in Rural Schools

BIOTEC set up a program dedicated to the improvement of science education in rural schools, through activities such as curriculum development, training programs for science teachers, developing teaching and learning materials and media in science, organizing activities such as science project contests, science camps and science fairs.

In 2016, 113 schools from six provinces (Nan, Mae Hong Son, Chiang Mai, Sakon Nakhon, Narathiwat and Pangnga) partook in the program and 748 teachers were trained on the use of learning media and techniques to engage students. Workshops and follow-up meetings were organized with the schools to monitor the improvement in student learning abilities. “Science Project Clinic” was set up by the program to mentor students on science projects and facilitate students to enter regional and national competitions. Five science projects of students under this program won awards at the 2016 National Science and Technology Fair.

Support Science Education in the Royal Initiative Projects

BIOTEC has been implementing activities to enhance science education in monastic schools under an initiative of HRH Princess Maha Chakri Sirindhorn since 2004. In 2016, a total of 69 monastic schools situated in Nan, Phrae, Chiang Rai, Phayao, Lampang and Sisaket provinces took part in the program which tackles the science education system of the schools by identifying competencies and skills that students need to develop and set up STEM curriculum (STEM: Science Technology Engineering and Mathematics Education). Major tasks carried out in 2016 included 1) enhance capability of school administrators to be able to develop quality assurance system; 2) enhance capability of science teachers to develop and implement STEM curriculum, encourage students to enter science project contests and integrate the use of e-learning contents freely available through internet and satellite television into the classroom; 3) enhance capability of students through activities such as science project contests and 4) enhance the quality of education through follow-up meetings. Improvement in quality is measured from student performance in the standardized test.



Students of the monastic schools that took part in science education improvement under the Royal Initiative Projects.

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 scientific workforce. Several activities were designed to assist different segments of the workforce, as well as address a variety of objectives, ranging from providing fellowships, to training post-graduate students, to organizing scientific conferences and training workshops for academics and industry. The Center also recognizes that the public at large needs to understand the benefit of science and technology in order to create a knowledge-based society, therefore public outreach is part of our mandate.

Postdoctoral Fellowship Program

BIOTEC Postdoctoral Fellowship Program offers opportunities to recent PhD graduates to carry out exciting research projects available in BIOTEC laboratories. The fellowship helps enrich research skills, build long-term research careers and networking connections for young researchers. In 2016, seven new fellowships were granted, adding to five on-going fellowships from the previous year.

BIOTEC Academic Program

To groom the future generation of biotechnology professionals, BIOTEC offers the expertise of its researchers and well-equipped facilities for university students to carry out research of their interest. This is conducted under the Thailand Graduate Institute of Science and Technology (TGIST) and Young Scientist and Technologist Program (YSTP). Both TGIST and YSTP are sponsored by NSTDA with the concept of having students co-supervised and mentored by NSTDA researchers. While TGIST offers scholarships for university students to study in the master's and doctoral program, YSTP is designed to provide research funds to undergraduate students to conduct senior projects under the co-supervision of NSTDA researchers and university faculty members. In the past year, BIOTEC researchers co-supervised 5 doctoral students, 11 master's students and 7 YSTP scholars. Additionally, over 100 undergraduate students partook of an internship at BIOTEC offering opportunity to experience a research laboratory and gain insight into various disciplines of biotechnology that the Center has to offer.

Conference and Training Courses

BIOTEC regularly organizes conferences, seminars and training workshops as part of its commitment to enhance research professionals, industry workforce and university students in the field of bioscience and biotechnology. The training topics on offer ranged from advanced technology for academia to staying abreast of cutting-edge technologies, to technologies that will improve efficiency in manufacturing or farming sectors.

In fiscal year 2016, BIOTEC organized 41 events, including conferences and workshops on 36 topics, drawing in 2,068 participants in total (3,857 man-days). Examples of the workshops were Breeding and Cultivation Techniques of Seedless Watermelon, Variant Discovery from Next Generation Sequencing (NGS) Post-processing Data, Intensive Training Workshop on Ethanol Production from Cassava and the Establishment of a Cassava Industry Cluster.



Workshop on Multiplex Detection of Plant Pathogen in July 2016.



Workshop on Microbial Preservation Techniques: Liquid Drying in May 2016.



Collecting and Preservation of Fungi and Mushroom Samples for Scientific Research in September 2016.



Since 2014, BIOTEC has been serving as a Regional Hub of the Integrated Breeding Platform (IBP), developed by the CGIAR Generation Challenge Programme. IBP provides resources that breeders need to design and manage plant breeding projects. At the heart of the Platform is the Breeding Management System (BMS) – a package composed of software applications for breeders to plan, conduct, analyze and assess the outcomes of their work. BMS helps breeders manage breeding activities in a very user-friendly and efficient way.

As a Regional Hub of IBP, BIOTEC organizes training workshops and operates a help-desk to provide technical support to interested crop breeders. In the past year, training workshops on database management and statistics have been organized for 72 breeders from Rayong Field Crops Research Center (Thailand), Nakhon Sawan Field Crops Research Center (Thailand) and National Agriculture and Forestry Research Institute (Laos). Nakhon Sawan Field Crops Research Center is now using BMS in its corn breeding program to improve drought tolerance; whereas Rayong Field Crops Research Center is applying BMS to its cassava germplasm management. BMS is used in maintaining BIOTEC's marker data for its rice breeding program and QTL mapping for drought tolerant traits in rice population.

Public Outreach

Television Programs. BIOTEC created content for two NSTDA TV shows for regular broadcast on public channels as part of NSTDA TV. The content is based on BIOTEC's work that has, or will, create social or economic impact. Examples of content that has been made into NSTDA TV shows over the past year are: production of *Beauveria bassiana* for controlling brown planthopper and synthesis of hydrogels from cassava starch for tablet disintegrants.

Press Activities. Events have been organized regularly for local journalists to be informed of our scientific research and the use of our technologies in actual industries. In 2016, the following activities were organized:

- » A visit to farmer group in Ayuthaya province that received the transfer of *Beauveria* production technology;
- » A visit to Shrimp Genetic Improvement Center which serves as a production facility for the selective breeding of black tiger shrimp;
- » An interview with BIOTEC research team on the application of monoclonal antibody technology in developing diagnostic tests for food and agricultural purposes;
- » An interview with BIOTEC team on the development of malaria parasite detection assay;
- » An interview with BIOTEC team on water management as preparedness measure for climate change; and
- » Press conference on on-site microbial reactor innovation

News and articles on these stories were subsequently published reaching out to the general public.



Press Interview to Minister of Science and Technology, Dr. Pichet Durongkaveroj, on a transfer of *Beauveria* production technology to the farmer.



Journalist visiting investigating black tiger shrimp at Shrimp Genetic Improvement Center.

Open Lab. BIOTEC has an open-door policy and welcomes the public to tour laboratory facilities on a regular basis as a means to create awareness and appreciation of science, as well as to explore future collaboration in the case of academia and industry. In 2016, 146 groups toured BIOTEC laboratories, 51 of which were from overseas.



BIOTEC Exhibition at Thailand Tech Show 2016 in September 2016.



Students visited BIOTEC counter at National Science and Technology Fair 2016 in August 2016.

Exhibitions. BIOTEC participated in a number of exhibitions and displays at major events, disseminating results and ideas to several demographics and industrial sectors. In 2016, BIOTEC exhibited its work in fourteen events; for example, Thailand Tech Show 2016, NSTDA Annual Conference 2016, National Science and Technology Fair 2016 and Thailand Lab International 2016.

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.

The 2016 Meeting of BIOTEC International Advisory Board

BIOTEC International Advisory Board was established in 2005 to enable the Center to better meet the constant challenges posed by globalization and the emergence of new technologies. BIOTEC appreciates that active international participation and exchange in science and technology is essential for the future growth of biotechnology in Thailand.

The 2016 Meeting of BIOTEC International Advisory Board was held on 22-23 February 2016. The advisory board, serving from October 2014 - September 2016, consists of 8 international experts in various areas of biotechnology. In addition to the Board Meeting, a workshop was organized for board members to share their expertise on technology trends and their research experience with BIOTEC young researchers. The workshop aimed to motivate young staff and provide tools for their career development.



BIOTEC International Board Members, Advisor to NSTDA President, and BIOTEC Executives.



The 2016 Meeting of BIOTEC International Advisory Board.

Fostering Collaboration

In fiscal year 2016, BIOTEC signed/renewed ten Memorandum of Understanding (MOUs)/agreements covering eight different countries.

Organization	Detail
University of Zurich, Switzerland	To investigate cyanide content in cassava.
Center for Environment, Fisheries and Aquaculture Sciences, UK	To foster cooperation in the field of shrimp disease.
Cebu Technological University, The Philippines	To foster cooperation in the fields of microbial biotechnology and agricultural biotechnology.
Nonglam University, Vietnam	To support the placement of students from Nonglam University in BIOTEC laboratories and promote research collaboration on microbial biotechnology.
Airlangga University, Indonesia	To promote cooperation in the field of enzyme technology.
College of Life Science, National Taiwan University, Taiwan	To support the placement of students from College of Life Science in BIOTEC laboratories.
Bioprocessing Technology Institute, Singapore	To promote collaboration in the field of bioprocessing technology.
Osaka University, Japan	To foster staff/student exchange and joint research in the field of microbial biotechnology.
Meiji University, Japan	To support research collaboration on agricultural biotechnology.
National Institute of Technology and Evaluation, Japan	To promote collaboration on microbial biotechnology.



An MOU between BIOTEC and National Institute of Technology and Evaluation, Japan to continue collaboration on microbial biotechnology.



Opening Ceremony of 2016 Human Resource Development Program in Biotechnology.



Student internship from UK, Ireland, Taiwan, Vietnam, Indonesia.

International Exchange Programs

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

- » **Human Resource Development Program in Biotechnology.** This program annually provides fellowships to young researchers from ASEAN to undergo research-based training in BIOTEC laboratories for a period of 3-6 months. This year, BIOTEC welcomed a total of twelve young researchers under this program; five from Myanmar, three from Vietnam, three from Indonesia and one from Laos.
- » **International Student Internship Program.** BIOTEC offers internship placements in laboratories for undergraduate and graduate students from overseas to enrich their research skills and serve as a vehicle for long-term collaboration between researchers and faculty members. There was a total of ninety-six students participating in this program in the past year from twenty countries, namely the UK, France, Ireland, Germany, the Netherlands, USA, Mexico, Singapore, Laos, Myanmar, Indonesia, Vietnam, Malaysia, India, Nepal, China, Korea, Hong Kong, Japan and Taiwan.

International Seminars and Workshops

BIOTEC regularly collaborates with its international partners to organize seminars and workshops as a platform for researchers from Thailand and overseas to exchange information and explore research collaboration.

Thai-German Bioeconomy Conference: Cooperation Opportunities for a Sustainable Bioeconomy. This Conference was organized in partnership with the German Federal Ministry of Education and Research Forschungszentrum Jülich, Gemeinschaft zur Förderung von Pflanzeninnovation e.V. (GFPI) and Bioeconomy Science Center (BioSC). The conference was held in Bangkok on 28 January 2016 and in Chiang Mai on 29 January 2016. The program covered topics such as national bioeconomy policy of Thailand and Germany, biomass research and bioproduct development.

Microbial Utilization and Management: Sustainable Utilization of Bioresources for a Growing Bioeconomy. The workshop was co-organized by the National Institute of Technology and Evaluation (NITE), Japan; Institute of Microbiology, Chinese Academy of Sciences (IMCAS); World Data Centre for Microorganisms (WDCM); World Federation for Culture Collections (WFCC) and ASEAN Network on Microbial Utilization (AnMicro). The 5-day workshop consisted of lecture and lab sessions, with experts from Thailand, Japan, France, the Netherlands and Belgium. A total of 78 participants from 12 countries attended this workshop.

and discussed their research with peers and workshop mentors, as well as broadening their industrial perspective through lectures from representatives of industry, academia, government and inter-governmental bodies.



Participants from Thai-German Bioeconomy Conference: Cooperation Opportunities for a Sustainable Bioeconomy.

Mycotoxins in ASEAN Countries and Post-Harvest Control Measures. This international workshop was co-organized by SEA-EU-NET project, the French Agricultural Research Centre for International Development (CIRAD), the Italian Institute of Sciences of Food Production (ISPA-CNR) and Thammasat University. Held from 14-16 March 2016, the workshop offered insight into the current situation of mycotoxin contamination in Southeast Asia and provided knowledge on various strategies to prevent and treat mycotoxin contamination in order to assist food companies to ensure safer products. Speakers at this Workshop consisted of local and international experts as well as representatives from seven ASEAN countries. The Workshop attracted over 100 participants.



Participants from International Workshop on “Microbial Utilization and Management: Sustainable Utilization of Bioresources for a Growing Bioeconomy”.

Scientific, Technological and Social Solutions for Sustainable Aquaculture in Thailand: a Key Player in Global Aquatic Food Supply. This Workshop was supported by Newton Fund Researcher Links Workshop Programme, funded by The British Council and the Thailand Research Fund to encourage international research collaboration between ambitious young researchers from the UK and Thailand. Coordinated by Dr. Kallaya Sritunyalucksana (BIOTEC, Thailand) and Prof. Grant Stentiford (Centre for Environment, Fisheries and Aquaculture Science, the UK), this workshop focused on finding the solutions to aquaculture diseases faced by countries in Asia, the world’s top aquaculture supplier. The Workshop was attended by 33 early-career researchers, 16 from Thailand and 17 from the UK. During the 5-day workshop, participants made presentations



A visit of participants from International Workshop on “Mycotoxins in ASEAN Countries and Post-Harvest Control Measures” to Food and Feed Innovation Center, BIOTEC.



Participants from Workshop on “Scientific, technological and social solutions for sustainable aquaculture in Thailand: a key player in global aquatic food supply” at Shrimp Genetic Improvement Center.

IMPACT OF BIOTEC'S OUTPUT



Every year, a number of projects are selected for detailed impact study. These are projects that have created technologies or products that have actually reached the end users, through various forms of technology transfer such as technology licensing, provision of consultancy and training and establishing core infrastructure that would advance research in academic and industrial communities. Impact is measured in terms income generated by our clients from products and technologies and, where appropriate, assessed for the impact to the nation's socio-economy in the forms of import substitution, investment and employment generation.

In 2016, 45 projects were selected for impact assessment. An estimated total of 2.50 billion Baht was generated from these projects. This amount was categorized as investment generation (688 million Baht), revenue generation to licensees or users (1.67 billion Baht), cost reductions (21 million Baht) and import replacement (122 million Baht).

Agriculture and Food

A total of 33 projects were evaluated, generating 1.02 billion Baht impact.

New rice varieties. This includes varieties that have been developed by the Rice Gene Discovery Laboratory, in collaboration with the Rice Department, namely blast-resistant glutinous rice (Thanyasirin), flood-tolerant rice (Homcholasit) and antioxidant-rich Riceberry. These new varieties were introduced to farmers with the training in seed production in four regions of Thailand. A total of 174 million Baht in income increment was estimated.



Homcholasit, flood-tolerant rice.



Thanyasirin, blast-resistant glutinous rice.

Technologies for crop production. BIOTEC engaged in various projects on plant improvement and technologies for crop production, such as improvement on the production capacity of disease-free sugarcane, screening for salt-tolerant crops, design of plant cultivation system and reagents for plant disease detection. These technologies have generated additional income to farmers and industries at the value of 166 million Baht.

Animal and aquaculture production. Technologies and products under assessment include steroid-based synchronization of ovulation protocol for artificial insemination of cattle, shrimp breeding, a closed recirculating aquaculture system integrated with hybrid nitrification biofilter tanks and patented Tubular Denitrification Reactor (TDNR) and shrimp disease detection kits. These technologies have generated an impact of 334 million Baht.

Food and agro-industry. Technologies that have been used at the commercial scale include starter culture for traditional fermented pork sausage, production technology of fermented soybean meal for animal feed and production of animal feed enzymes. These technologies have created income to the companies and investment to the economy at the value of 131 million Baht.

Community development and rehabilitation of saline land. BIOTEC works with partners and local authorities in applying science and technology to support community development. Appropriate technologies, mainly related to farming and food processing are made accessible to villagers with proper training. Examples of projects include agroforestry farming in Phrae province, science village in Phatthalung and Phra Nakhon Si Ayutthaya provinces, and area-based community development in Nan and Sakon Nakhon provinces. These projects have improved the farming practice and food products, resulting in better quality produce. The impact from investment and additional income is estimated at 46 million Baht. In addition, a project on saline land remediation has been carried out by BIOTEC in collaboration with Siam Cement Group (SCG) and several public agencies. The project was able to turn saline land into arable land for rice and other crops in four provinces -- Sakon Nakhon, Udon Thani, Nakhon Ratchasima and Khon Kaen. Economic impact, based on the value of produce cultivated, totaled 168 million Baht.

Health and Medicine

Two projects were evaluated and created an impact of 763 million Baht.

Drug and vaccine development. BIOTEC, in partnership with King Mongkut's University of Technology Thonburi (KMUTT), carried out process development, optimization and scale up for the production of biopharmaceuticals. This project has generated investment to the economy of 582 million Baht.

Anti-cough pill production process. BIOTEC was commissioned by a manufacturer of a traditional anti-cough pill to make improvements to its production process. The project has resulted in an investment made by the company and the improvement has enhanced the productivity of the manufacturer. Economic impact was estimated to 181 million Baht.



Traditional anti-cough pill.

Energy and Environment

Eight projects were evaluated, resulting in an impact of 628 million Baht.

Efficiency improvement in cassava starch processing. Technology has been developed to improve the production efficiency in terms of energy and water consumption and raw material utilization in the cassava starch production process. The technology was introduced to cassava starch factories through practical training and advice to practitioners working in the factories. A total of 524 million Baht in savings was estimated from cost reduction and improved production yields.

Biogas production from agro-industrial waste. Wastewater treatment and biogas production technology was developed by EcoWaste, a joint laboratory between BIOTEC and KMUTT. The technology has been implemented in agro-industry factories (cassava starch, palm oil mill, food processing), enabling wastewater compliance and energy cost savings of 71 million Baht.



Wastewater from cassava cleaning process to be treat and generate biogas.

Environmentally-friendly products. BIOTEC and a private company collaborated on a joint research project to develop a commercial bioremediation product based on oil-degrading microbes. The technology was subsequently licensed to the company for commercialization. Impact assessment was made on the revenue and the value of import substitution, totaling 33 million Baht.

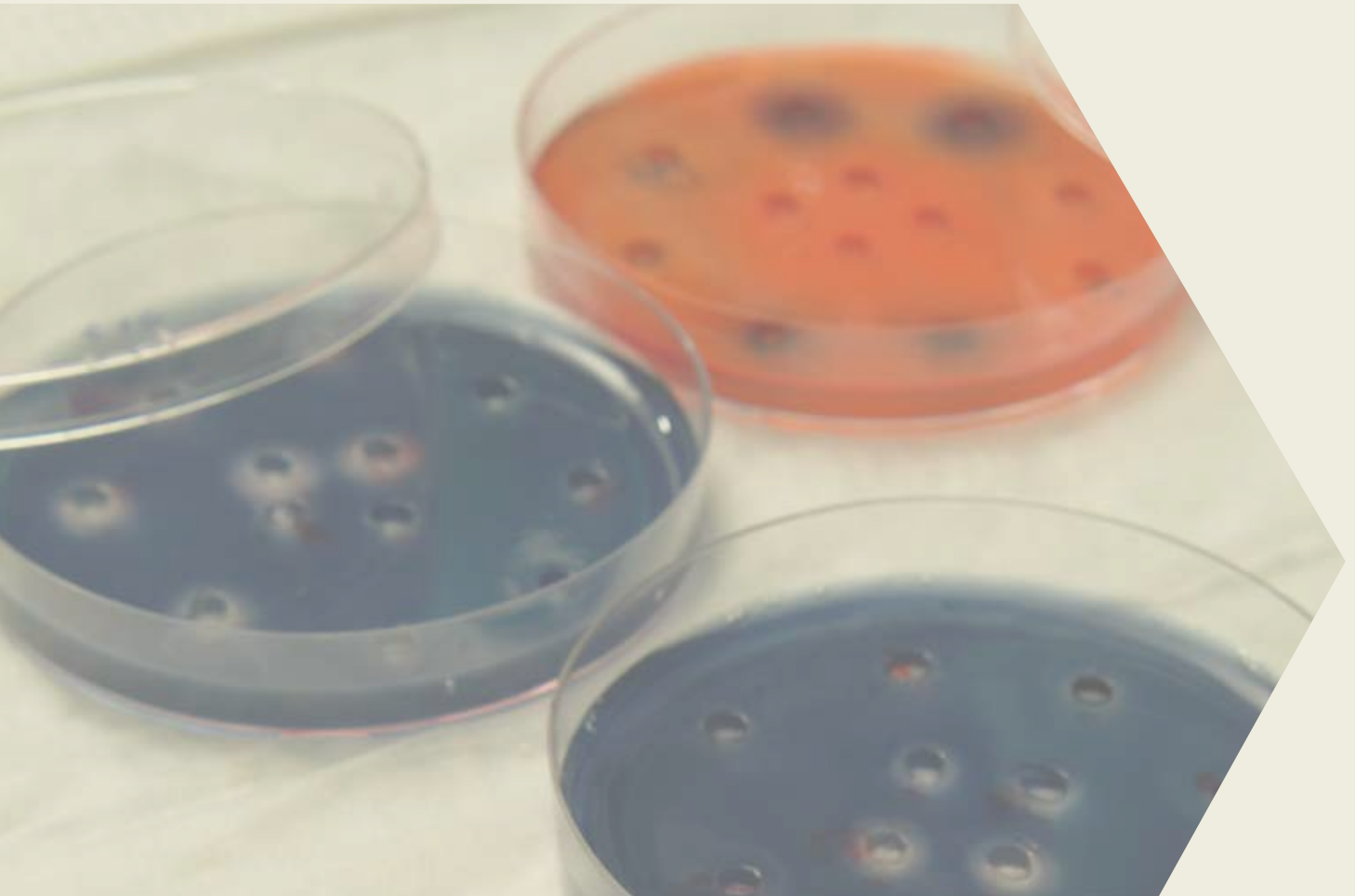


A variety of bioremediation product based on oil-degrading microbes.

Infrastructure Establishment

Two projects were evaluated resulting in an impact of 91 billion Baht.

Analytical and testing services. With well-equipped research facilities and qualified laboratory staff, BIOTEC offers analytical and testing services, as well as research partnership opportunities to the public and private sector. Services and expertise available include proteomic analysis and bioactive compound screening. The savings, by obviating the need to seek such services from overseas, were estimated to 91 million Baht.



APPENDICES



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

List of Publications

1. Akekawatchai, C., Roytrakul, S., Kittisenachai, S., Isarankura-Na-Ayudhya, P. and Jitrapakdee, S. (2016). Protein Profiles Associated with Anoikis Resistance of Metastatic MDA-MB-231 Breast Cancer Cells. *Asian Pacific Journal of Cancer Prevention*, 17(2), 581-590.
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LIST OF INTELLECTUAL PROPERTIES

List of Intellectual Properties

Title	Granted Date	Ref. No.	Country
Patent			
Anti-folate antimalarials with dual-binding modes and their preparation	7 April 2015	US 9,000,003	USA
Petty Patent			
Degradation of plant cell wall polysaccharides using enzymes and expansin	9 October 2015	10514	Thailand
Preparation method of cassava starch-based hydrogel for use as disintegrant in drug tablet	9 October 2015	10517	Thailand
Preparation of soluble recombinant bone morphogenetic protein-2	9 October 2015	10518	Thailand
Method to improve salinity and drought tolerance in soybean using the combination of plant growth regulator and mutagen	9 October 2015	10523	Thailand
Method to improve crop yield using the combination of high carbon dioxide treatment and mutagen	9 October 2015	10524	Thailand
Screening method for latent infection in sugarcane stalk	9 October 2015	10525	Thailand
Recombinant plasmid for extracellular expression of heterologous protein in yeast <i>Ogataea</i> spp.	30 October 2015	10661	Thailand
Production of bolete spawn (<i>Thaeogyroporus porentosus</i> (berk. ET. Broome)) on semi-solid culture media	30 October 2015	10662	Thailand
Culture media for lignocellulolytic enzyme production from fungi	30 October 2015	10663	Thailand
Method to improve crop yield in rice using the combination of high concentration sugar syrup, mutagen and ultrasonic energy	30 October 2015	10665	Thailand
Culture media and cultivation process to enhance mycelium growth of <i>Boletus</i> mushroom	13 November 2015	10731	Thailand
A marker recycling DNA tool for genome modification of yeast strain <i>Ogataea thermomethanolica</i> and its application	4 December 2015	10852	Thailand
Simultaneously serotyping of dengue virus by using anti-dengue NS1 monoclonal antibodies	8 January 2016	10980	Thailand
Primers specific to lysogenic bacteriophages of <i>Bacillus</i> spp. and their applications	28 January 2016	11103	Thailand
Gene expression system for construction of recombinant yeasts directly produce ethanol from cellulose and hemicellulose	28 January 2016	11104	Thailand
Gene expression system for the production of target protein at high temperature without induction using thermotolerant <i>Pichia thermosmethanolica</i>	4 February 2016	11117	Thailand
Specific primers for identification of thrips species found in pepper, tomato and cucurbits fields in Thailand	4 February 2016	11121	Thailand
Primers specific to lytic bacteriophages of <i>Bacillus</i> spp. and their applications	4 February 2016	11122	Thailand
Methanol-inducible expression system for the production of target protein	11 February 2016	11158	Thailand
Starter Culture Technology for ensiling forage sugarcane	11 February 2016	11159	Thailand

Title	Granted Date	Ref. No.	Country
Sucrose measuring device based on pulse amperometry for sugar mill factory	1 April 2016	6127	Thailand
Method of preparing nanocomposite material from magnetic nanoparticles and cationic starch for chromium (VI) absorption	1 April 2016	11333	Thailand
Preservation technique for fungal spores used as biocontrol agent, its preparation and usage	19 May 2016	11508	Thailand
Detection method for <i>Shewanella</i> spp. in Nile tilapia and red tilapia	19 May 2016	11509	Thailand
Detection method for <i>Flavobacterium columnare</i> in Nile tilapia and red tilapia	19 May 2016	11510	Thailand
Immunomagnetic beads for specific isolation of <i>Acidovorax avenae</i> subsp. <i>citrulli</i>	5 August 2016	11810	Thailand
A marker recycling DNA tool for genome modification of yeast strain <i>Ogataea thermomethanolica</i> and its application	5 August 2016	11811	Thailand
Fungal fermentation process for omega-6 fatty acid production	26 August 2016	11882	Thailand
Detection method for <i>Streptococcus iniae</i> in fish	23 September 2016	11955	Thailand
Detection method for <i>Streptococcus agalactiae</i> in fish	23 September 2016	11956	Thailand

List of Applied Intellectual Properties

Title	Filing Date	Ref. No.	Country
Patent			
Microarray-based method to screen a phage display library for phage clone specific to target bacteria	29 December 2015	1501007937	Thailand
In situ loop-mediated isothermal amplification for highly sensitive detection of infectious hypodermal and hematopoietic necrosis virus (IHHNV) in shrimp tissues	22 January 2016	1601000350	Thailand
Alkaliphilic xylanase mutants for hydrolysis of plant biomass	29 July 2016	1601004382	Thailand
Plasmid vectors for secreted protein expression in <i>Lactobacillus plantarum</i> , <i>Bacillus subtilis</i> and <i>Escherichia coli</i>	26 August 2016	1601004946	Thailand
Electrochemical cell with an interdigitated electrode arrays (IDEA) and process of fabrication	9 September 2016	1601005225	Thailand
Monoclonal antibodies to the nonstructural protein (NSs protein) of Watermelon silver mottle virus and its application in immunodiagnosis	16 September 2016	1601005378	Thailand
Cytotoxic tropolone compounds	23 September 2016	1601005547	Thailand
Cytotoxic bicyclic tropolone compounds	23 September 2016	1601005548	Thailand
Fendleral - an active ingredient composition	23 September 2016	1601005549	Thailand
Automated workflow for band detection and band classification from gel electrophoresis	23 September 2016	1601005587	Thailand
n,n'-((3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g]phenanthrene-8,13-diyl)bis(oxy))di alkanolic acid as a reporter molecule for diagnostic applications and methods of synthesis	30 September 2016	1601005887	Thailand

Title	Filing Date	Ref. No.	Country
n,n'-((2-(n-carboxyalkyl)-1,3-dioxo-2,3,4,5,14,15-hexahydro-1H-dinaphtho[2,1-e:1',2'-g]isoindole-7,12-diyl) bis (oxy))bis(alkane-1-sulfonate) as a reporter molecule for diagnostic applications and methods of synthesis	30 September 2016	1601005888	Thailand
n-((13-((n-Carboxyalkyl)oxy)-3,4-dicyano-1,2,5,6-tetrahydrodibenzo[c,g]phenanthren-8-yl)oxy)alkane-1-sulfonate as a reporter molecule for diagnostic applications and methods of synthesis	30 September 2016	1601005889	Thailand
n-((3,4-Dicyano-13-hydroxy-1,2,5,6-tetrahydrodibenzo[c,g]phenanthren-8-yl)oxy)alkanoic acid) as a reporter molecule for diagnostic applications and methods of synthesis	30 September 2016	1601005890	Thailand
Genetically modified bone morphogenetic protein 2	30 September 2016	1601005894	Thailand
A surface coating composition for cell culturing and spreading	30 September 2016	1601005900	Thailand
Petty Patent			
Bioprocess for constitutive production of a heterologous protein in recombinant thermotolerant yeast Ogataea thermomethanolica	6 November 2015	1503001894	Thailand
Onsite Microbial Reactor (OMR)	13 November 2015	1503001926	Thailand
The solid-state fungal fermentation process for gamma-linolenic acid production using vinasses	29 December 2015	1503002225	Thailand
The solid medium formula of fungal cultivation for gamma-linolenic acid production	29 December 2015	1503002226	Thailand
Detection method for shrimp acute hepatopancreatic necrosis disease (AHPND) strain of Vibrio parahaemolyticus	26 February 2016	1603000321	Thailand
Bead array method for multiplex gene expression analysis in the black tiger shrimp	11 August 2016	1603001428	Thailand
Plasmid vectors for secreted protein expression in <i>Lactobacillus plantarum</i> and <i>Bacillus subtilis</i>	26 August 2016	1603001559	Thailand
Plasmid vectors for secreted protein expression in <i>Bacillus subtilis</i>	26 August 2016	1603001560	Thailand
A thyA -, folA -, and tolC knockout E. coli with no antibiotic resistance gene and its application	2 September 2016	1603001633	Thailand
Development and synthesis of flexible 2,4-diamino-6-phenyl-5-(3-(2-(2-carbonylethyl) phenoxy) propoxy pyrimidine derivatives for antimalarial drugs	2 September 2016	1603001634	Thailand
Enzyme mixtures for rheology modification of cassava in very high gravity fermentation for production of biofuels and chemicals	2 September 2016	1603001635	Thailand
The process of plant breeding for drought tolerance using polyethylene glycol together with mutagenic and ultrasonic waves	16 September 2016	1603001816	Thailand
The process of plant breeding for resistance to soil acidity (acid soil) using acid together with mutagenic and ultrasonic waves	16 September 2016	1603001817	Thailand
Trade Secret			
The formulation of reagent for All in one TSV Nested PCR test kit	13 January 2016	N/A	Thailand

Title	Filing Date	Ref. No.	Country
The formulation of reagent for All in one YHV Nested PCR test kit	13 January 2016	N/A	Thailand
The formulation of reagent for All in one IMNV Nested PCR test kit	13 January 2016	N/A	Thailand
The formulation of reagent for All in one MrNV Nested PCR test kit	13 January 2016	N/A	Thailand
The formulation of reagent for All in one HPV Nested PCR test kit	13 January 2016	N/A	Thailand
The formulation of reagent for All in one MBV Nested PCR test kit	13 January 2016	N/A	Thailand
The formulation of reagent for WSSV LAMP COLOR test kit	13 January 2016	N/A	Thailand
The formulation of reagent for TSV LAMP COLOR test kit	13 January 2016	N/A	Thailand
The formulation of reagent for IHNV LAMP COLOR test kit	13 January 2016	N/A	Thailand
The formulation of reagent for All in one WSSV Nested PCR test kit	13 January 2016	N/A	Thailand
The formulation of reagent for All in one IHNV Nested PCR test kit	13 January 2016	N/A	Thailand
The formulation of reagent for All in one LSNV Nested PCR test kit	13 January 2016	N/A	Thailand
The formulation of reagent for YHV type1 LAMP COLOR test kit	13 January 2016	N/A	Thailand
Medium formulation and culture conditions for multienzyme production by fungi used in starch and wax removal of natural fiber	24 May 2016	N/A	Thailand
Extraction and downstream processes of multienzyme produced by fungi for removal of starch and wax from natural fiber	24 May 2016	N/A	Thailand
Additive compositions and formulation processes of multienzyme for removal of starch and wax from natural fiber	24 May 2016	N/A	Thailand

Honors and Awards

Dr. Chunya Puttikhunt, Dr. Sansanee Noisakran and Dr. Thaneeya Roytrakul

Medical Biotechnology Research Laboratory

- » Research Grant for a project on “The dynamic of anti-dengue antibodies over time”, awarded by UK-Thailand: Joint Health Research Call, a joint program between NSTDA of Thailand and the Medical Research Council of the UK.

Dr. Phanramphoei Namprachan Frantz

Animal Biotechnology Research Unit

- » Marie Skłodowska-Curie Individual Fellowship on a project entitled “Making a yeast-based candidate vaccine against Porcine Epidemic Diarrhea Virus (PEDV)”, awarded by Horizon 2020, the EU Framework Programme for Research and Innovation.

Dr. Thidarat Nimchua

Bioresources Technology Research Unit

- » Leader in Innovation Fellowship for “ENZease: two-in-one enzyme for one-step desizing and scouring process of cotton fabric in textile industry” to participate in a training program on entrepreneurship and commercialization of research in the UK, awarded by the Royal Academy of Engineering, UK.

Ms. Wansika Kiatpathomchai and Ms. Jantana Kampeera

Biosensing Technology Research Unit

- » Gold Medal and Special Prize of the Korea Invention Promotion Association for “AflaSense Plus, a portable aflatoxin sensor for agricultural products and animal feed”, presented at the 44th International Exhibition of Inventions of Geneva, 13-17 April 2016, Switzerland.

Dr. Oraprapai Gajanandana, Dr. Orawan Himananto and Ms. Mallika Kumposiri

Animal Biotechnology Research Unit

Dr. Plearnpis Luxananil

Bioresources Technology Research Unit

- » Silver Medal and Special Prize of the Taiwan Invention Association for “Fruit Blotch Easy kits”, presented at the 44th International Exhibition of Inventions of Geneva, 13-17 April 2016, Switzerland.

Prof. Prida Malasit, MD., Dr. Chunya Puttikhunt, Dr. Sansanee Noisakran, Dr. Thaneeya Roytrakul and Dr. Dumrong Mairiang

Medical Biotechnology Research Laboratory

Medical Biotechnology Research Laboratory

Dr. Sittiruk Roytrakul

Genome Technology Research Unit

- » The 2015 NSTDA Research Chair Grant for a project on “Integrative researches for a set of novel biomarkers applicable to diagnosis, prognosis and treatments of patients with dengue hemorrhagic fever”, awarded by the National Science and Technology Development Agency (NSTDA).

Dr. Weerawat Runguphan

Bioresources Technology Research Unit

- » The 22nd TTSF Science and Technology Research Grant for “Development of MultiCRISPR, a Simple and High-Throughput Platform for Multi-Gene Pathway Optimization based on CRISPR/Cas”, awarded by Thailand Toray Science Foundation (TTSF).

Dr. Sissades Tongsim

Genome Technology Research Unit

- » The 2015 TRF-OHEC-Scopus Researcher Award in the field of Health Sciences for his work on “Genome Wide Association Study Computational Workflow for Identification of Genetic Markers Associated with Common Complex Diseases”, awarded by the Thailand Research Fund (TRF), Office of the Higher Education Commission (OHEC) and Elsevier.

Dr. Jittima Piriyaongsa

Genome Technology Research Unit

- » The 2015 TRF-OHEC-Scopus Young Researcher Award in the field of Health Sciences for her work on “Application of Bioinformatics for Studying MicroRNA Gene Regulation and Human Diseases”, awarded by the Thailand Research Fund (TRF), Office of the Higher Education Commission (OHEC) and Elsevier.

Dr. Jittima Piriyaongsa and Ms. Pavita Kaewprommal

Genome Technology Research Unit

- » The 2015 NRCT Research Award (Agricultural Science and Biology) for “Genetic evaluation of ORF5 and Nsp2 genes of PRRSV in a swine herd following an acute outbreak with highly pathogenic PRRSV”, awarded by the National Research Council of Thailand (NRCT).

Dr. Thidarat Nimchua

Bioresources Technology Research Unit

- » The 2016 NRCT Invention Award (Engineering and Industrial Research) for “ENZease: two-in-one enzyme for one-step desizing and scouring process of cotton fabric in textile industry”, awarded by the National Research Council of Thailand (NRCT).

Dr. Wirulda Pootakham

Genome Technology Research Unit

- » The 2015 NRCT PhD Dissertation Award (Agricultural Science and Biology) for “Elucidation of Functional and Regulatory Aspects of Sulfate Transport in *Chlamydomonas reinhardtii*”, awarded by the National Research Council of Thailand (NRCT).

Dr. Suganya Yongkiettrakul

Biosensing Technology Research Unit

- » Winner of DMSc Award (Development of Healthcare Service) for her work on “LAMP-LFD assay for malaria parasite detection”, awarded by the Department of Medical Sciences, Ministry of Public Health.

Dr. Ubolsree Leartsakulpanich

Biosensing Technology Research Unit

- » Second Runner-up of DMSc Award (Research and Development in Medical Science) for her work on “*Plasmodium* serine hydroxymethyltransferase for antimalarial drug development”, awarded by the Department of Medical Sciences, Ministry of Public Health.

Dr. Piti Amparyup

Animal Biotechnology Research Unit

- » The 2016 Thailand Frontier Researcher Award for his work on “Prophenoloxidase system and its role in shrimp immune response against major pathogens”, awarded by Thomson Reuters.

Dr. Atikorn Panya

Food Biotechnology Research Unit

- » Poster Award for “Apolar Radical Initiated Conjugated Autoxidizable Triene (ApoCAT) Assay: Effects of Oxidant Locations on Antioxidant Capacities and Interactions”, presented at TRF-OHEC Annual Congress 2016, 6-8 January 2016, Phetchaburi, Thailand.

Mr. Narong Arunrut, Ms. Jantana Kampeera and Ms. Wansika Kiatpathomchai

Biosensing Technology Research Unit

- » Poster Award (Molecular Biology and Bioinformatics) for “Development of loop-mediated isothermal amplification combined with lateral flow dipstick for sensitive and rapid detection of EMS/AHPND”, presented at the 27th Annual Meeting of The Thai Society for Biotechnology and International Conference: TSB 2015, 17-20 November 2015, Bangkok, Thailand.

Ms. Manlika Makornwattana, Dr. Rattaphol Charlermroj and Dr. Nitsara Karoonuthaisiri

Biosensing Technology Research Unit

Dr. Orawan Himananto, Dr. Orapapai Gajanandana, Dr. Channarong Seepiban

Animal Biotechnology Research Unit

- » Poster Award (Molecular Biology and Bioinformatics) for “A practical application of a microsphere immunoassay for multiplex detection of plant diseases”, presented at the 27th Annual Meeting of The Thai Society for Biotechnology and International Conference: TSB 2015, 17-20 November 2015, Bangkok, Thailand.

Ms. Mallika Kumposiri, Dr. Orawan Himananto, Dr. Orapapai Gajanandana and Dr. Siwat Sangsritavong
Animal Biotechnology Research Unit

- » Research Award (Animal Science) for “Development of Competitive Enzyme-Linked Immunosorbent Assay for Quantification of Progesterone Levels in Milk of Dairy Cattle”, presented at the 54th Kasetsart University Annual Conference, 2-5 February 2015, Bangkok, Thailand.

Ms. Chayaphat Wongsombat
Medical Molecular Biology Research Unit

- » Poster Presentation Award for “Bacterial model for identification of new drug resistance mutations in *P. falciparum* dihydrofolate reductase”, presented at the 5th International Biochemistry and Molecular Biology Conference 2016: BMB 2016, 26-27 May 2016, Songkhla, Thailand.

Mr. Sarunyou Wongkanoun
Bioresources Technology Research Unit

- » Poster Award for “*Durotheca macrostroma* sp. nov, a new species from Thailand”, presented at the 6th Conference on Taxonomy and Systematics in Thailand: TST2016, 25-27 May 2016, Khon Kaen, Thailand.

Ms. Wasana Noisripoom
Bioresources Technology Research Unit

- » Oral Presentation Award for “Two new *Shimizuomyces* species discovered in a community forest in Phitsanulok Province”, presented at the 6th Conference on Taxonomy and Systematics in Thailand: TST2016, 25-27 May 2016, Khon Kaen, Thailand.

Dr. Khaow Tansomboon
Biosensing Technology Research Unit

- » Winner and People’s Choice Award Winner of FameLab Thailand 2016, a science communication competition organized by the British Council Thailand, for his presentation on “I Remember Nothing”

Dr. Wanarit Wimuttisuk
Bioresources Technology Research Unit

- » Second Runner-up of FameLab Thailand 2016, a science communication competition organized by the British Council Thailand, for her presentation on “How Bacteria Becomes Drug Resistant”

Executives and Management Team

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Ikuo Hirono	Professor, Laboratory of Genome Science, Tokyo University of Marine Science and Technology, JAPAN
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Martin Keller	Director and Alliance President, National Renewable Energy Laboratory, USA
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Lene Lange	Professor, Department of Chemical and Biochemical Engineering, Technical University of Denmark, DENMARK
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Vítor Martins dos Santos	Chair for Systems and Synthetic Biology, Wageningen University, THE NETHERLANDS
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David Mathews	Expert Scientific Advisory Committee, Medicines for Malaria Venture (MMV) and Member of Earlham College Board of Trustees, USA
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Fabien De Meenster	Owner of DMF Co., Ltd., POLAND
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Ray Ming	Professor of Plant Biology, University of Illinois at Urbana-Champaign, USA
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Frédéric Tangy	Director of Research, French National Centre for Scientific Research (CNRS) and Head of the Viral Genomics and Vaccination Unit, Institut Pasteur, FRANCE
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Somvong Tragoonrung	Executive Director
Dussadee Siamhan	Deputy Executive Director
Kuakoon Piyachomkwan	Deputy Executive Director
Lily Eurwilaichitr	Deputy Executive Director
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Wonnop Visessanguan	Director, Food Biotechnology Research Unit
Theerayut Toojinda	Director, Plant Biotechnology Research Unit
Anan Jongkaewwattana	Director, Virology and Antibody Technology Research Unit
Sumalee Kamchonwongpaisan	Director, Medical Molecular Biology Research Unit
Sithichoke Tangphatsornruang	Director, Genome Technology Research Unit
Nitsara Karoonuthaisiri	Director, Biosensing Technology Research Unit
Supawadee Ingsruswang	Director, Biodiversity and Biotechnological Resource Research Unit
Verawat Champreda	Director, Microbial Biotechnology and Biochemicals Research Unit

BIOTEC¹¹
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National Center for Genetic Engineering and Biotechnology (BIOTEC)

National Science and Technology Development Agency (NSTDA)

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