BIOTEC

ANNUAL REPORT 2017

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National Science and Technology Development Agency (NSTDA)
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# Annual Report 2017

National Center for Genetic Engineering and Biotechnology (BIOTEC)

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# MESSAGE FROM THE BIOTEC EXECUTIVE DIRECTOR

I have a great pleasure to reflect on the past exciting year of BIOTEC in this 2017 BIOTEC Annual Report. We started our 2017 fiscal year with some re-arrangement of our research units located at the Thailand Science Park in order to accommodate the growing number of researchers, sharpen the focus of each research unit and better address new research challenges. Agricultural Biotechnology Research Unit has been split into three new units, namely Plant Biotechnology Research Unit, Animal Biotechnology Research Unit and Virology and Antibody Technology Research Unit. Similarly, Bioresource Technology Research Unit has been dissolved and two new units have been established: Biodiversity and Biotechnological Resource Research Unit and Microbial Biotechnology and Biochemicals Research Unit. We now have a total of nine research units based at the Thailand Science Park to support the growing demand for technological development of our local industries.

In this past year, we were able to turn a number of our research results into well-adopted products and technologies. Derived from our long-term rice breeding program in collaboration with Kasetsart University, Riceberry and Sinlek were the two rice varieties that have been registered as new plant varieties under the Plant Varieties Protection Act B.E. 2542. In addition, another two varieties — salt-tolerant and blast-resistant RD73 and blast-resistant RD75 — have been certified by the Rice Department and thus become recommended varieties for cultivation. Other impressive news is the launch of cassava flour under the brandname "Sava" by Chorchaiwat Industry Company Limited. The company obtained a license of our technology to produce low-cyanide cassava flour. Sava flour is marketed as gluten-free and GMO-free flour for cooking and baking, an alternative product for people suffering coeliac disease and discerning consumers. These are just some highlights and more cases can be found in this report. The process of getting new plant varieties registered or certified, or the technologies and products to the market takes months if not years of refining and perfecting the technologies and products by our scientists and technology transfer team with active participation of technology users, customers and clients. I would like to express my appreciation and congratulations to BIOTEC team and our collaborators for all their hard work and accomplishments made in the past year.

On the international front, our scientists have been taking a prominent role in regional and global projects. Dr. Lily Eurwilaichitr, our Deputy Executive Director, has been elected to the Executive Board of the World Federation for Culture Collections (WFCC). Three of our young scientists — Dr. Panit Kitsubun, Dr. Anan Jongkaewwattana and Dr. Peera Jaruampornpan — will be working with a team of UK experts to establish biopharmaceutical and animal vaccine production capacity in Thailand and neighboring Southeast Asian countries with the grant awarded by the Global Challenges Research Fund. With the funding from Newton Institutional Links, Dr. Kallaya Sritunyalucksana will establish an International Network for Shrimp Health (INSH) aiming to link leading research laboratories in the UK and Thailand with government and industry stakeholders in order to meet the needs for rapid access to pathogen detection and surveillance services, an important component to our lucrative shrimp farming industry. These examples demonstrate our commitment to work with international partners not only for the betterment of Thailand, but our region and the world.

NSTDA started to implement its 6$^{th}$ Strategic Plan in 2017. NSTDA's 6$^{th}$ Strategic Plan constitutes the 5-year framework for the operation of NSTDA and its affiliated centers, BIOTEC included. Introduced in this new Strategic Plan is Targeted Research which focuses on the translation of research to real-world application in order to realize tangible socio-economic impact by 2021. Target Research, which is in line with Target Industries declared by the Thai Government, consists of five topics: Modern Agriculture, Biofuels and Biochemicals, Food for the Future, Enhancement of Public Health and Quality of Life and Next-Generation Automotive and Logistics. Our Center clearly has much to contribute to this Target Research and I am extremely proud that three of our senior scientists, Dr. Theerayut Toojinda, Dr. Kuakoon Piyachomkwan and Dr. Wonnop Visessanguan have been appointed by NSTDA to spearhead three of the five topics, i.e. Modern Agriculture, Biofuels and Biochemicals and Food for the Future, respectively.

In the coming years, BIOTEC will consolidate our effort to support the development of bio-economy. Chief among our projects is the establishment of “Bio-bank,” the national biological database. This is an extension of an already accomplished Thailand Bioresource Research Center (TBRC) launched in 2015. Bio-bank will collect and digitize bio-information including genome sequences of organisms, ranging from plant (seed, cell and tissue cultures), animals, microorganisms and human, as well as develop tools to mine useful data for supporting industrial research and biotechnology development. In addition, we will set up a new initiative on “plant factory” to develop an industrial-scale herb cultivation under controlled environment, in order to improve the quality of herbs and increase their functional ingredients. This project will greatly enhance the capacity of our medical, nutraceutical and cosmetic industries.

A number of exciting projects have been rolled out to underpin the Thailand 4.0 policy announced by the Thai Government to transform the country to a more creative and innovative economy. These include the launch of the Eastern Economic Corridor of Innovation (EECi) to serve as a new growth hub, offering a complete ecosystem for innovation and a new economic estate specializing in research, innovation, and National Quality Infrastructure (NQI). NSTDA has been tasked to lead the development of EECi and we could not be more thrilled to contribute to this project.

With these new projects and initiatives, we will have a busy year ahead of us.  But I am certain that with the brilliant team that we have and excellent partnerships that we have formed, we will be able to employ biotechnology to solve national and global challenges, and continue to make a significant contribution to the economy and society.

Somvong Tragoonrung, Ph.D.
Executive Director, BIOTEC

# FACTS AND FIGURES

BIOTEC was first set up under the Ministry of 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), Agricultural Technology and Innovation Management Institute (AGRITECH) and Technology Management Center (TMC).

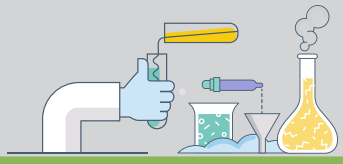
As a premier research institute in Thailand and Asia, BIOTEC operates research units located at Thailand Science Park and specialized laboratories hosted by various universities, covering a wide spectrum of research topics from agricultural science to biomedical science and environmental science. In addition to research units, a number of translational research facilities have been established with special emphasis to promote translational research in collaboration with industry, as well as provide services to industry.

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

# Organization

## BIOTEC Research Units at Thailand Science Park

- Animal Biotechnology Research Unit
- Plant Biotechnology Research Unit
- Food Biotechnology Research Unit
- Virology and Antibody Technology Research Unit
- Medical Molecular Biology Research Unit
- Genome Technology Research Unit
- Biosensing Technology Research Unit
- Biodiversity and Biotechnological Resource Research Unit
- Microbial Biotechnology and Biochemicals Research Unit



## Translational Research Facilities and Multi-disciplinary Laboratory

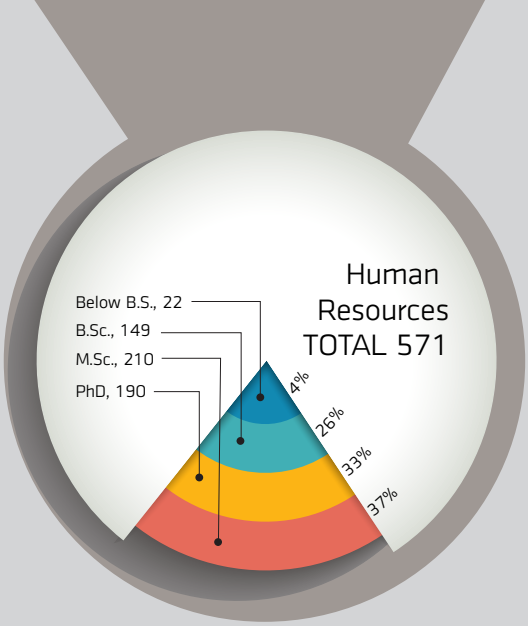
- Shrimp Genetic Improvement Center
- Nuclear Polyhedrosis Virus Pilot Plant for Insect Pest Control
- Thailand Bioresource Research Center
- Food and Feed Innovation Center
- Integrative Biorefinery Laboratory
- National Biopharmaceutical Facility (in collaboration with King Mongkut's University of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

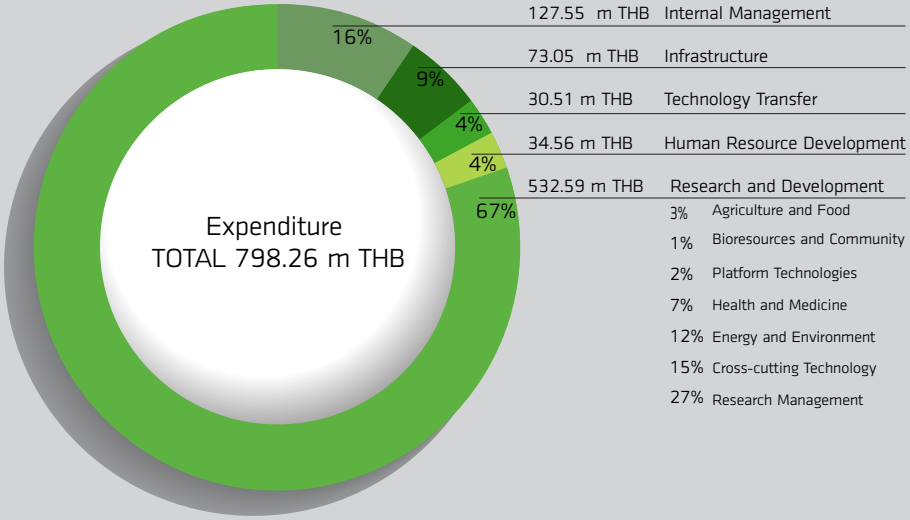
## Collaborative Research Laboratories at universities and government organization

- Biochemical Engineering and Pilot Plant Research and Development Laboratory - at King Mongkut's University of Technology Thonburi (KMUTT)
- Biopharmaceutical 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
- 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
- Shrimp Molecular Biology and Biotechnology Laboratory - at Mahidol University
- Peat Swamp and Rainforest Research Station - jointly established with the National Park, Wildlife and Plant Conservation Department and located in Narathiwat Province

## Administration

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



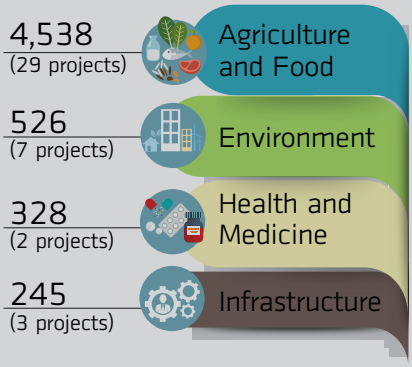




## Socio-economic Impact of Completed Projects

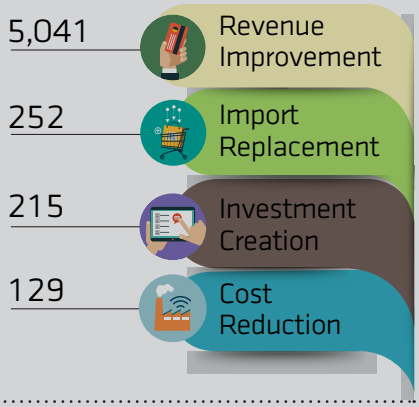
TOTAL **5,637** m THB

from **41** selected projects

### By Project Themes

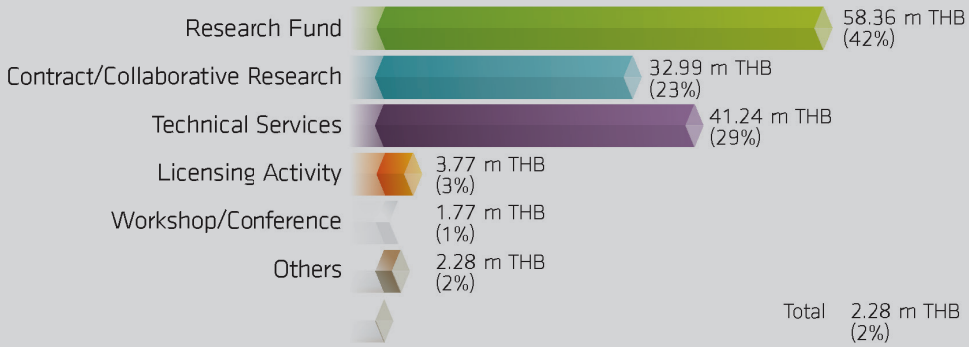



### By Impact Categories

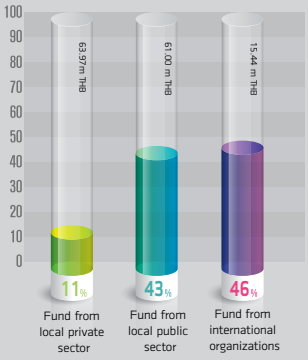



## Income from sources outside NSTDA TOTAL **140.41** m THB

**By Type of Income**




**By Souree of Income**




## Major Outputs

**Publications**

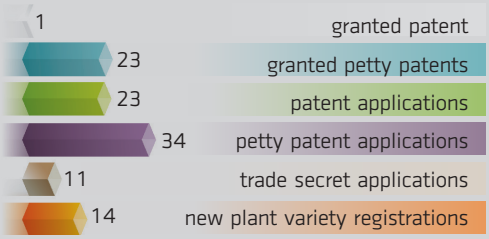
221 publications papers, including papers published in journals with impact factors greater than 4

**Awards**

10 international awards

17 domestic awards

**Intellectual Properties**



# 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

## Rice varieties granted protection and certification

BIOTEC has long collaborated with Kasetsart University employing biotechnology as a tool to make improvements in Thai rice through an establishment of a joint laboratory called the Rice Gene Discovery Unit. Target traits for improvement include cooking quality, nutrition and resilience to biotic and biotic stresses.

Two rice varieties developed with a marker-assisted selection namely, salt-tolerant and blast-resistant RD73 and blast-resistant RD75, have been certified by the Rice Department in 2017. RD73 is derived from a cross between Khao Dawk Mali 105 (jasmine rice) and IR66946-196-3R-1-1, aiming for salt-tolerance and blast-resistance as well as fragrance. It is suitable for the area with saline soil and rice blast disease, especially in the northeastern part of Thailand. RD75 is derived from a cross between IR77955-24-75-284 and DHL279 as a resistance donor. It is blast resistant and can be grown in rainfed highland area in the northeast of Thailand.

In 2017, a 12-year protection was granted to two new rice varieties under the Plant Varieties Protection Act B.E. 2542. These two varieties are Riceberry and Sinlek. Riceberry is derived from a cross between Jao Hom Nin, a local non-glutinous purple rice and Khao Dawk Mali 105. It is enriched with both water soluble, mainly anthocyanin, and lipid soluble antioxidants, such as carotenoid, gamma oryzanol and vitamin E. Sinlek is also derived from the same cross. Its distinct properties are low glycemic index and high iron content.

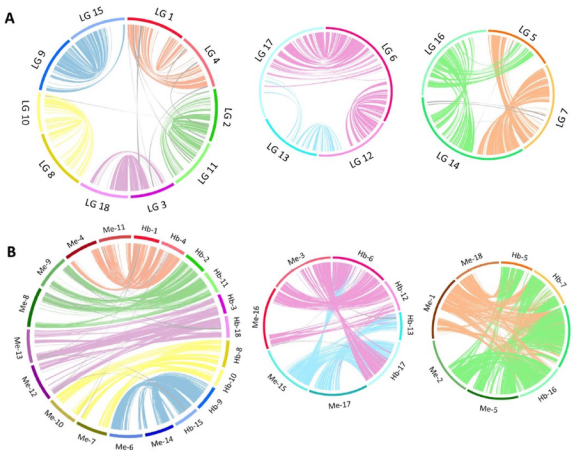
## *De novo* hybrid assembly of the rubber tree genome

Para rubber tree (*Hevea brasiliensis*) is an important economic species as it is the sole commercial producer of high-quality natural rubber. The genome assembly is a valuable resource for studying marker-trait association at a whole genome level and for developing desirable high-yielding cultivars to meet growing demands for natural rubber.

Researchers reported a *de novo* hybrid assembly of the *H. brasiliensis* genome from BPM24 (Bank Pertanian Malaysia 24) clone, which exhibits resistance to major fungal pathogens in Southeast Asia. Deep-coverage 454/Illumina short-read and Pacific Biosciences (PacBio) long-read sequence data were acquired to generate a preliminary draft, which was subsequently scaffolded using a long-range "Chicago" technique to obtain a final assembly of 1.26 Gb. The assembled genome contains 69.2% repetitive sequences and has a GC content of 34.31%. Using a high-density SNP-based genetic map, they were able to anchor 28.9% of the genome assembly (363 Mb) associated with over two thirds of the predicted protein-coding genes into rubber tree's 18 linkage groups. These genetically anchored sequences allowed comparative analyses of the intragenomic homeologous synteny, providing the first concrete evidence to demonstrate the presence of paleotetraploidy in *Hevea species*. Additionally, the degree of macrosynteny conservation observed between rubber tree and cassava strongly supports the hypothesis that the paleotetraploidization event took place prior to the divergence of the *Hevea* and *Manihot species*.

This study was performed by the Genome Technology Research Unit in collaboration with the Rubber Authority of Thailand.

**Ref:** Pootakham W, Sonthirod C, Naktang C, Ruang-Areerate P, Yoocha T, Sangsrakru D, Theerawattanasuk K, Rattanawong R, Lekawipat N, Tangphatsornruang S. (2017) *De novo* hybrid assembly of the rubber tree genome reveals evidence of paleotetraploidy in *Hevea species*. *Scientific Reports*, 7, 41457. doi: 10.1038/srep41457.




*Syntenic analysis of 18 linkage groups suggests paleotetraploidy in H. brasiliensis.*

## Potential probiotic *Lactobacillus* with inhibitory effect on a foodborne pathogen

Antibiotics have been commonly used for prevention and treatment of pathogenic diseases in farm animals. However, the extensive use of antibiotics in livestock has been linked to the emergence of antibiotic-resistant bacteria found in humans and animals. As a result, the use of several antibiotics in farm animals has been banned in several countries. Probiotics are preferred alternatives to antibiotics.

Researchers attempted to search for probiotic *Lactobacillus* with protective effects against Salmonella infection in pigs. To do this, *Lactobacillus* isolated from pigs of four ages, pregnant sows, newborn, suckling, and weaned piglets, were preliminarily screened for anti-*Salmonella* activity. The strains exhibiting anti-*Salmonella* activity were selected and further characterized for probiotic properties such as acid and bile salt tolerance, adhesion to Caco-2 cells, and production of antimicrobial substances. The results of probiotic characterization and co-culture studies suggest that LJ202 is a good probiotic which exhibits inhibitory activity against *Salmonella* spp. and fecal coliform bacteria. Therefore, LJ202 is a suitable candidate for further study of its protective effects against pathogen infections in pigs.

This investigation was undertaken by researchers from the Food Biotechnology Research Unit.

**Ref:** Abhisingha M, Dumnil J, Pitaksutheepong C. (2017) Selection of Potential Probiotic *Lactobacillus* with Inhibitory Activity Against *Salmonella* and Fecal Coliform Bacteria. *Probiotics and Antimicrobial Proteins*. doi: 10.1007/s12602-017-9304-8.

## Climatic factors and prevalence of *Campylobacter* in Thai poultry farms

*Campylobacter* are bacteria associated with human foodborne disease worldwide. Poultry and poultry products are generally considered as a main source of these organisms. Since information on *Campylobacter* in Thai broiler flocks is still insufficient, more intensive surveillance is necessary.

In this study, the prevalence of *Campylobacter* and the influence of climatic factors (i.e., rainfall, ambient temperature and relative humidity) on *Campylobacter* colonization in broiler flocks in the central and northeastern regions of Thailand was studied. A total of 442 commercial broiler flocks reared in the central and northeastern regions of Thailand during 2012 to 2014 were investigated. *Campylobacter* positive status was identified in 252 examined flocks. Prevalence of *Campylobacter* in the northeastern region was slightly lower than that of the central region. Rainfall and relative humidity were identified as climatic conditions correlated with *Campylobacter* colonization in Thai broiler flocks, while no association between ambient temperature and colonization was observed.

This study was a collaborative effort between the Food Biotechnology Research Unit, Chulalongkorn University, Khon Kaen University, King Mongkut's University of Technology Thonburi, the University of Liverpool (UK) and Swansea University (UK).

**Ref:** Prachantasena S, Charununtakorn P, Muangnoicharoen S, Hankla L, Techawal N, Chaveerach P, Tuitemwong P, Chokesajjawatee N, Williams N, Humphrey T, Luang-tongkum T. (2017) Climatic factors and prevalence of *Campylobacter* in commercial broiler flocks in Thailand. *Poultry Science*, 96, 980–985.

## Starter cultures for reduced-salt soy sauce fermentation

Soy sauce fermentation involves microorganisms, some of which produce compounds related to flavor development. The use of suitable microbial starter cultures in the fermentation can lead to consistent quality of soy sauce with production of desirable flavor compounds. With the trend of healthy diet, reduced-salt soy sauce is gaining popularity among health conscious consumers. Starter cultures for the fermentation of reduced-salt soy sauce is therefore under development to meet this market demand.

Researchers investigated the use of starter cultures in reduced-salt moromi fermentation. These starter cultures, consisting of *Tetragenococcus halophilus* TS71, *Zygosaccharomyces rouxii* A22, and *Meyerozyma (Pichia) guilliermondii* EM1Y52, were previously isolated during the fermentation of Thai soy sauce. The trial salt-reduced fermentation (12%NaCl) was conducted and changes of microbial, physicochemical and biochemical properties were monitored in pilot scale of 3-month fermentation with industrial practice. The results demonstrated the feasibility of starter cultures in reduced-salt moromi fermentation. Salt reduction and application of starter cultures could affect microbial population and physico-chemical properties during moromi fermentation. Low salt concentration was found to be more supportive to the growth of halotolerant microorganisms and thus enhance the formation of volatile flavor compounds of yeasts. For food safety concern, biogenic amines content in the reduced-salt soy sauce obtained in this work were within safety margins and the fermentation was under salt concentration and acidic pH that restricts the growth of pathogenic bacteria. The knowledge obtained from this study would be beneficial to the production of reduced-salt soy sauce and the development of rapid fermentation process.

This study was a combined effort of researchers from the Food Biotechnology Research Unit and Mahidol University.

**Ref:** Singracha P, Niamsiri N, Visessanguan W, Lertsiri W, Assavanig A. (2017) Application of lactic acid bacteria and yeasts as starter cultures for reduced-salt soy sauce (moromi) fermentation. *LWT - Food Science and Technology*, 78, 181-188.

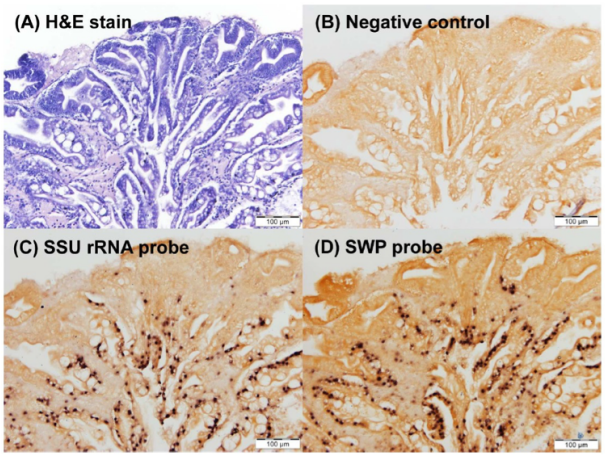
## Superior detection method for *Enterocytozoon hepatopenaei* infection in shrimp

Hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP) is an important disease of cultivated shrimp. Heavy infections may lead to retarded growth and unprofitable harvests. Existing PCR detection methods target the EHP small subunit ribosomal RNA (SSU rRNA) gene (SSU-PCR). However, these methods can give false positive test results due to cross reactivity of the SSU-PCR primers with DNA from closely related microsporidia that infect other aquatic organisms. This is problematic for investigating and monitoring EHP infection pathways.

To overcome this problem, a sensitive and specific nested PCR method was developed for detection of the spore wall protein (SWP) gene of EHP (SWP-PCR). The new SWP-PCR method did not produce false positive results from closely related microsporidia. The first PCR step of the SWP-PCR method was 100 times more sensitive than that of the existing SSU-PCR method but sensitivity was equal for both in the nested step. Since the hepatopancreas of cultivated shrimp is not currently known to be infected with microsporidia other than EHP, the SSU-PCR methods are still valid for analyzing hepatopancreatic samples despite the lower sensitivity than the SWP-PCR method. However, due to its greater specificity and sensitivity, the SWP-PCR method is recommended for screening for EHP in feces, feed and environmental samples for potential EHP carriers.

This work was a collaborative study by researchers from the Shrimp Molecular Biology and Biotechnology Laboratory, the Animal Biotechnology Research Unit, Mahidol University, the University of Exeter (UK) and the Center for Environment, Fisheries and Aquaculture Science (UK).

**Ref:** Jaroenlak P, Sanguanrut P, Williams BAP, Stentiford GD, Flegel TW, Sritunyalucksana K, Itsathitphaisarn O. (2016) A Nested PCR Assay to Avoid False Positive Detection of the Microsporidian *Enterocytozoon hepatopenaei* (EHP) in Environmental Samples in Shrimp Farms. *PLoS ONE,* 11(11): e0166320. doi:10.1371/journal.pone.0166320.




*DIG-labeled SWP and SSU probes give comparable in situ hybridization results in EHP-infected shrimp.*

## Double-stranded RNA treatment for SPF shrimp stocks of newly discovered viral infections

Using post-larvae derived from specific pathogen free (SPF) stocks in penaeid shrimp farming has led to a dramatic increase in production. At the same time, new pathogens of farmed shrimp are continually being discovered. Once a new shrimp viral pathogen is discovered, the stock must be checked for freedom of that pathogen even if it has never shown any gross signs of that disease. If the SPF stock is found to be positive for the newly discovered pathogen, serious problems can arise. The stock developers are faced with destroying their existing stock which has been developed over a long period of time at considerable cost, and starting the whole stock development process anew with the newly discovered pathogen added to the SPF list. An alternative option to save time and cost is in need.

Researchers hypothesized that injection of complementary double-stranded RNA (dsRNA) into viral-infected broodstock prior to mating might inhibit replication of the target virus sufficiently to reduce or eliminate its transmission to their offspring. Subsequent selection of uninfected offspring would allow for post-clearing of the new virus from the existing stock and for conversion of the stock free of the new virus. This hypothesis was tested using the stock infected with Laem Singh virus (LSNV). The transmission was substantially reduced in several treated broodstock compared to much higher transmission in buffer-injected broodstock. Based on these results, the model is proposed for post-clearing of SPF stocks using dsRNA treatment. The model may also be applicable to post-clearing of exceptional, individual performers from grow-out ponds for return to a nucleus breeding center.

This study was a collaborative effort between the Shrimp Molecular Biology and Biotechnology Laboratory and Mahidol University.

**Ref:** Saksmerprome V, Charoonnart P, Flegel TW. (2017) Feasibility of dsRNA treatment for post-clearing SPF shrimp stocks of newly discovered viral infections using Laem Singh virus (LSNV) as a model. *Virus Research*, 235, 73–76.

## Causative agent of scale drop and muscle necrosis disease in barramundi

Symptoms of scale drop and muscle necrosis have been considered as an emerging problem in farmed barramundi (*Lates calcarifer*) in Vietnam since 2013. Diseased fish exhibited remarkable external clinical signs of scale loss, muscle degradation and eventually died. The causative agents of this scale drop and muscle necrosis disease are still unknown.

Researchers conducted a study to determine the infectious causative agent of the clinically diseased fish collected from barramundi caged culture in central Vietnam in 2015. Histological examination from naturally sick fish revealed signs of severe necrotic muscles with infiltration of massive immune-related cells, severe hemorrhage and blood congestion in the brain, collapsed kidney tubules and epithelial cells sloughing into the lumen. Five different bacterial species were recovered from diseased fish and identified as *Vibrio harveyi*, *Vibrio tubiashii*, *Tenacibaculum litopenaei*, *Tenacibaculum* sp. and *Cytophaga* sp. based on homology

of 16S rDNA sequences and biochemical characteristics. Experimental infection revealed that only *V. harveyi* killed the fish with similar clinical signs and histological changes compared to naturally diseased fish. In addition, several unculturable bacteria including *T. maritimum* were also uncovered from DNA extracted from necrotic muscles by species-specific PCR and 16S rDNA clone library sequencing, but their roles in disease manifestation need further investigation.

This investigation was undertaken by researchers from the Shrimp Molecular Biology and Biotechnology Laboratory, the Animal Biotechnology Research Unit, Mahidol University and King Mongkut's University of Technology Thonburi.

**Ref:** Dong HT, Taengphu S, Sangsuriya P, Charoensapsri W, Phiwsaiya K, Sornwatana T, Khunrae P, Rattanarojpong T, Senapin S. (2017) Recovery of Vibrio harveyi from scale drop and muscle necrosis disease in farmed barramundi, Lates calcarifer in Vietnam. *Aquaculture*, 473, 89–96.

## Characterization of the nucleocapsid protein of porcine epidemic diarrhea virus

Recurrent porcine epidemic diarrhea virus (PEDV) outbreaks have resulted in enormous economic losses to swine industries worldwide. To combat this disease, it is necessary to understand how this virus replicates and evades host immunity. Characterization of viral proteins provides important clues to mechanisms by which viruses survive and spread.

Researchers characterized an intriguing phenomenon in which the nucleocapsids of some PEDV strains are proteolytically processed by the virally encoded main protease. Growth retardation in recombinant PEDV carrying uncleavable N suggests a replication advantage provided by the cleavage event, at least in the cell culture system. These findings can lead to a more complete understanding of PEDV replication and pathogenicity.

This study was performed by the Virology and Antibody Technology Research Unit in collaboration with the University of East Anglia.

**Ref:** Jaru-Ampornpan P, Jengarn J, Wanitchang A, Jongkaewwattana A. (2017) Porcine Epidemic Diarrhea Virus 3C-Like Protease-Mediated Nucleocapsid Processing: Possible Link to Viral Cell Culture Adaptability. *Journal of Virology*, 91:e01660-16. doi: 10.1128/ JVI.01660-16.

## The role of ORF3 accessory protein in the replication of porcine epidemic diarrhea virus

The ORF3 accessory protein has been shown to impede reverse genetics of cell-culture-adapted porcine epidemic diarrhea virus (PEDV). Its absence or truncated variants are also associated with viral attenuation *in vivo*.

In this study, three ORF3 variants ($ORF3_{NP12}$, $ORF3_{NP14}$ and $ORF3_{RB14}$) and their truncated counterparts were investigated for their regulatory role in recovery of cell-adapted PEDV *in vitro*. It was shown that the $ORF3_{NP12}$, but not the truncated form, can inhibit recovery of reverse-genetics-derived PEDV when expressed in trans. When testing with other RNA viruses, ORF3 was found to inhibit rescue of porcine respiratory and reproductive syndrome virus (PRRSV), but not of influenza virus. Results from mutagenesis of $ORF3_{NP12}$ suggest that F81 and M167 are responsible for impairing PEDV rescue *in vitro*. By changing specific residues of ORF3, the recombinant PEDV bearing the modified $ORF3_{NP12}$ can be productively propagated in VeroE6-APN cells. These results may provide mechanistic insights into ORF3-mediated inhibition of PEDV replication in new host cells.

This investigation was undertaken by the Virology and Antibody Technology Research Unit.

**Ref:** Wongthida P, Liwnaree B, Wanasen N, Narkpuk J, Jongkaewwattana A. (2017) The role of ORF3 accessory protein in replication of cell-adapted porcine epidemic diarrhea virus (PEDV). *Archives of Virology*, 162: 2553. doi: 10.1007/s00705-017-3390-5.

## Highlights from Health and Medicine

### Development of a novel anti-malaria compound, P218

Following successful discovery, a compound targeting malarial enzyme dihydrofolate reductase (DHFR) that effectively kills both sensitive and resistant parasites, P218 has been selected by Medicines for Malaria Ventures, a Swiss-based not-for-profit organization, for further development. P218 is currently undergoing the first-in-human study in the UK with MMV's financial support. Phase Ib study is expected to commence in 2018. In addition, the Thai Government Pharmaceutical Organization and MMV have signed a Non-Disclosure Agreement (NDA) in order to enter into discussions regarding P218 development in Thailand, focusing on synthetic route optimization, scale-up and formulation, as well as an assessment of Thailand's capability in manufacturing drugs that meet international standards.

### A simple detection platform for drug-resistant malaria

Drug resistance is a major threat to global efforts to control and eliminate malaria. Mechanisms of resistance to several classes of antimalarial drugs have been linked to key mutations in the *Plasmodium falciparum* genes. Pyrimethamine targets the dihydrofolate reductase of the bifunctional dihydrofolate reductase thymidylate synthase (DHFR-TS), and specific point mutations in the *dhfr-ts* gene have been assigned to resistant phenotypes. Several molecular methods are available to detect the mutant genotypes including DNA sequencing and PCR-based methods, but some of these methods can be tedious and not practical for routine surveillance.

A team of researchers has developed a *Pf*SNP-LAMP platform to detect nucleotide polymorphism in the *dhfr* gene associated with N51I mutation and antifolate resistance. The *Pf*SNP-LAMP method was validated with genomic DNA samples and parasite lysates prepared from sensitive and pyrimethamine resistant strains of *P. falciparum*. The method showed 100% specificity, while further optimization may improve the detection limit of the assay and make the method more useful as surveillance or diagnostic tools.

This study was a combined effort of researchers from the Medical Molecular Biology Research Unit and the Biosensing Technology Research Unit.

**Ref:** Yongkiettrakul S, Kampeera J, Chareanchim W, Rattanajak R, Pornthanakasem W, Kiatpathomchai W, Kongkasuriyachai D. (2017) Simple detection of single nucleotide polymorphism in *Plasmodium falciparum* by SNP-LAMP assay combined with lateral flow dipstick. *Parasitology International*, 66, 964-971.

### Physical and functional properties of nonstructural protein 1 secreted from dengue virus-infected insect cells

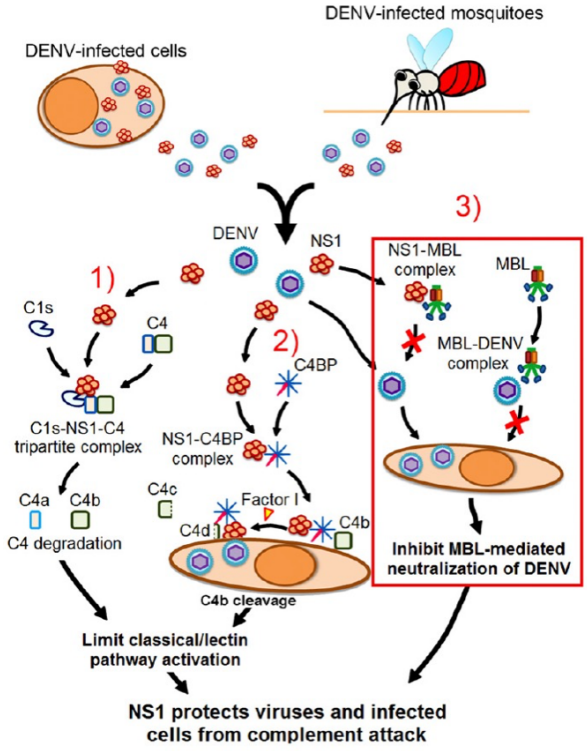
Flavivirus nonstructural protein 1 (NS1) is a unique secreted nonstructural glycoprotein. Although it is absent from the flavivirus virion, intracellular and extracellular forms of NS1 have essential roles in viral replication and the pathogenesis of infection. The fate of NS1 in insect cells has been more controversial, with some reports suggesting it is exclusively cell associated.

A team of researchers has confirmed NS1 secretion from cells of insect origin and characterized its physical, biochemical, and functional properties in the context of dengue virus (DENV) infection. Unlike mammalian cell–derived NS1, which displays both high mannose and complex type N-linked glycans, soluble NS1 secreted from DENV-infected insect cells contains only high mannose glycans. Insect cell–derived secreted NS1 also has different physical properties, including smaller and more heterogeneous sizes and the formation of less stable NS1 hexamers. Both mammalian and insect cell–derived NS1 bind to complement proteins C1s, C4, and C4-binding protein, as well as to a novel partner, mannose-binding lectin. Binding of NS1 to MBL protects DENV against mannose-binding lectin–mediated neutralization by the lectin pathway of complement activation. As secreted NS1 and DENV were detected in the saliva of infected mosquitoes, these findings suggest a mechanism of viral immune evasion at the very earliest phase of infection.

This study was jointly conducted by researchers from the Medical Biotechnology Research Laboratory;

Faculty of Medicine, Siriraj Hospital, Mahidol University; the Armed Forces Research Institute of Medical Sciences and Washington University School of Medicine (USA).

**Ref:** Thiemmeca S, Tamdet C, Punyadee N, Prommool T, Songjaeng A, Noisakran S, Puttikhunt C, Atkinson JP, Diamond MS, Ponlawat A, Avirutnan P. (2016) Secreted NS1 Protects Dengue Virus from Mannose-Binding Lectin–Mediated Neutralization. *Journal of Immunology*, 197(10), 4053–4065.




*Model of complement antagonism by DENV NS1. Together with DENV virions, soluble NS1 is released from DENV-infected insect or mammalian cells. Attenuation of the classical and lectin pathways of complement activation in tissue is promoted by three mechanisms. Overall, NS1 possesses complement evasion functions and protects DENV and DENV-infected cells from complement activation and inhibition.*

## The role of mitogen-activated protein kinase JNK1/2 in dengue virus-induced liver injury

High viral load with liver injury is exhibited in severe dengue virus (DENV) infection. Mitogen activated protein kinases (MAPKs) including ERK1/2 and p38 MAPK were previously found to be involved in the animal models of DENV-induced liver injury. However, the role of JNK1/2 signaling in DENV-induced liver injury has never been investigated.

JNK1/2 inhibitor, SP600125, was used to investigate the role of JNK1/2 signaling in the BALB/c mouse model of DENV-induced liver injury. SP600125-treated DENV-infected mice ameliorated leucopenia, thrombocytopenia, hemoconcentration, liver transaminases and liver histopathology. DENV-induced liver injury exhibited induced phosphorylation of JNK1/2, whereas SP600125 reduced this phosphorylation. An apoptotic real-time PCR array profiler was used to screen how SP600125 affects the expression of 84 cell death-associated genes to minimize DENV-induced liver injury. Modulation of caspase-3, caspase-8 and caspase-9 expressions by SP600125 in DENV-infected mice suggests its efficiency in restricting apoptosis via both extrinsic and intrinsic pathways. Reduced expressions of TNF-$\alpha$ and TRAIL are suggestive to modulate the extrinsic apoptotic signals, where reduced p53 phosphorylation and induced anti-apoptotic Bcl-2 expression indicate the involvement of the intrinsic apoptotic pathway. This study thus demonstrates the pivotal role of JNK1/2 signaling in DENV-induced liver injury and how SP600125 modulates this pathogenesis.

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

**Ref:** Sreekanth GP, Chuncharunee A, Cheunsuchon B, Noisakran S, Yenchitsomanus P, Limjindaporn T. (2017) JNK1/2 inhibitor reduces dengue virus-induced liver injury. *Antiviral Research*, 1417, 7-8.

## Association of human leukocyte antigen (HLA) class II and susceptibility to TB

Tuberculosis (TB) occurs as a result of complex interactions between the host immune system and pathogen virulence factors. Human leukocyte antigen (HLA) class II molecules play an important role in the host immune system. However, no study has assessed the association between HLA class II genes and susceptibility to TB caused by specific strains.

Researchers investigated the possible association of HLA class II genes with TB caused by modern and ancient *Mycobacterium tuberculosis* (MTB). The study included 682 patients with TB and 836 control subjects who were typed for HLA-DRB1 and HLA-DQB1 alleles. MTB strains were classified using a large sequence polymorphism typing method. Association analysis was performed using common HLA alleles and haplotypes in different MTB strains. HLA association analysis of patients infected with modern MTB strains showed significant association for *HLA-DRB1*09:01* and *HLA-DQB1*03:03* alleles with susceptibility to TB. Haplotype analysis confirmed that these alleles were in strong linkage disequilibrium and did not exert an interactive effect. The results of this study showed an association between HLA class II genes and susceptibility to TB caused by modern MTB strains, suggesting the importance of strain-specific analysis to determine susceptibility genes associated with TB.

This work was a collaborative study by researchers from the Medical Molecular Biology Research Unit, Mahidol University, Ministry of Public Health, the University of Tokyo (Japan), Japan Anti-Tuberculosis Association and RIKEN Center for Integrative Medical Sciences (Japan).

**Ref:** Toyo-Oka L, Mahasirimongkol S, Yanai H, Mushiroda T, Wattanapokayakit S, Wichukchinda N, Yamada N, Smittipat N, Juthayothin T, Palittapongarnpim P, Nedsuwan S, Kantipong P, Takahashi A, Kubo M, Sawanpanyalert P, Tokunaga K. (2017) Strain-based HLA association analysis identified *HLA- DRB1*09:01* associated with modern strain tuberculosis. *HLA, 90(3)*,149-156.

## Highlights from Energy and Environment

### An active synergistic enzyme system for lignocellulose saccharification

Efficient hydrolysis of lignocellulosic materials to sugars for conversion to biofuels and chemicals is a key step in biorefinery. Designing an active saccharifying enzyme system with synergy among their components is considered a promising approach.

In this study, a lignocellulose-degrading enzyme system of *Chaetomium globosum* BCC5776 (CG-Cel) was characterized for its activity and proteomic profiles, and synergism with accessory enzymes. The highest cellulase productivity of 0.40 FPU/mL was found for CG-Cel under the optimized submerged fermentation conditions on 1% (w/v) empty palm fruit bunch, 2% microcrystalline cellulose (Avicel®) and 1% soybean meal at 30 °C, pH 5.8 for 6 days. CG-Cel worked optimally at 50–60 °C in an acidic pH range. Proteomics analysis by LC/MS/MS revealed a complex enzyme system composed of core cellulases and accessory hydrolytic/non-hydrolytic enzymes attacking plant biopolymers. A synergistic enzyme system comprising the CG-Cel, a β-glucosidase (Novozyme® 188) and a hemicellulase Accellerase® XY was optimized on saccharification of alkaline-pretreated rice straw by a mixture design approach. Applying a full cubic model, the optimal ratio of ternary enzyme mixture containing CG-Cel: Novozyme® 188: Accellerase® XY of 44.4:20.6:35.0 showed synergistic enhancement on reducing sugar yield with a glucose releasing efficiency of 256.4 mg/FPU, equivalent to a 2.9 times compared with that from CG-Cel alone. This work provides an alternative lignocellulose-degrading enzyme system potent for on-site enzyme production for the development of a feasible biorefinery industry.

This study was a combined effort of researchers from BIOTEC and King Mongkut's University of Technology Thonburi.

**Ref:** Wanmolee W, Sornlake W, Rattanaphan N, Suwannarangsee S, Laosiripojana N, Champreda V. (2016) Biochemical characterization and synergism of cellulolytic enzyme system from *Chaetomium globosum* on rice straw saccharification. *BMC Biotechnology*, 16:82. doi: 10.1186/s12896-016-0312-7.

### Techno-economic assessment of high-solid simultaneous saccharification and fermentation

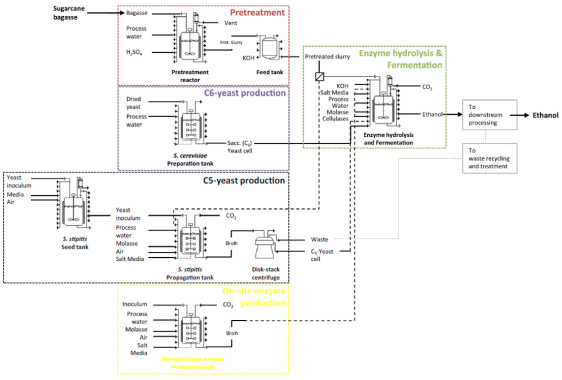
The use of lignocellulosic biomass for the production of biofuels and biochemicals has received great attention due to its low cost, renewability and abundance. Sugarcane bagasse is one of the major lignocellulosic feedstocks

that could potentially be used for biorefinery process. The development of an integrated simultaneous saccharification and fermentation (SSF) process at high solid loading of bagasse for high-titer ethanol production is thus of great interest. Due to the interdependency of each processing step, turning lignocellulosic ethanol production towards a successful industrial scale is only possible by optimizing and defining optimal integrated process options meeting the techno-economic feasibility of cellulosic biomass conversion technologies.

In this study, technological and economical potential of high-solid SSF for sugarcane bagasse-to-ethanol conversion process previously developed by BIOTEC research team was analyzed based on process flowsheet simulation for an estimation of the minimal ethanol selling price (MESP). Based on techno-economic assessment, a high-solid SSF process platform for low-cost lignocellulosic ethanol production was designed, comprising (1) yeast consortium for C5 and C6 sugars co-fermentation and (2) cellulase/on-site hemicellulase enzyme mixtures acting synergistically for efficient saccharification. Implementing the integrated SSF process with on-site enzymes and yeast consortium, the MESP could be reduced to as low as 15.7 Baht/L equivalent to 1.66 US$/gal which is a 6% lower than the current market selling price of 1.76 US$/gal. Thus, the on-site enzymes together with cellulase-hemicellulase synergism to lower enzyme demand as well as the yeast consortium technology to increase ethanol titer from C5/C6 co-fermentation would provide economic feasibility for the future cellulosic ethanol production in an industrial scale. Such process platform is also an important strategy for the development of low-cost biorefinery industry that can outperform the current sugar-based process for the production of biofuels.

This investigation was undertaken by researchers from the Microbial Biotechnology and Biochemicals Research Unit.

**Ref:** Khajeeram S, Unrean P. (2017) Techno-economic assessment of high-solid simultaneous saccharification and fermentation and economic impacts of yeast consortium and on-site enzyme production technologies. *Energy*, 122, 194-203.




*Proposed process diagram of fed-batch, high-solid SSF by S. stipitis/S. cerevisiae cell consortium and on-site hemicellulose enzyme production for low-cost ethanol production from sugarcane bagasse.*

## Highlights from Bioresources

### Antitubercular activity of *Ganoderma* lanostanoids

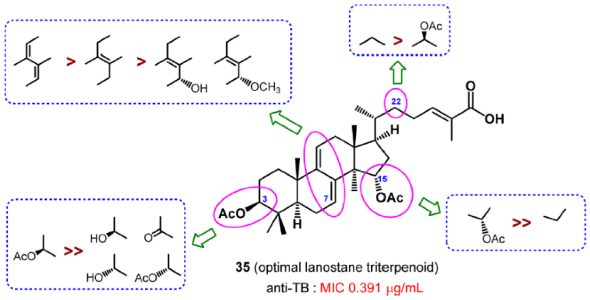
*Ganoderma* has long been regarded as one of the most significant medicinal mushrooms worldwide. More than 250 lanostane-type triterpenoids have been isolated from basidiocarps (natural or cultivated mushroom specimens) and/or mycelial cultures of *Ganoderma*. These lanostanoids have been shown to possess various biological activities. Most importantly, some of the lanostanoids have been considered as potent anticancer agents, showing cytotoxic activities against tumor cell lines. As part of the exploration of fungal resource utilization in Thailand, a team of researchers previously reported the isolation of lanostanoids from mycelial cultures of the relatively rare species *Ganoderma orbiforme*, strain BCC 22324, and their antituberculosis (anti-TB) activity and relatively weaker cytotoxicity. It was the first report of the significant anti-TB activity of *Ganoderma* lanostanoids.

In a continuation of the research into antitubercular lanostane triterpenoids from submerged cultures of *Ganoderma* species, three strains, *Ganoderma orbiforme* BCC 22325, *Ganoderma* sp. BCC 60695, and *Ganoderma australe* BCC 22314, have been investigated. Fourteen new lanostane triterpenoids, together with 35 known compounds, were isolated. Antitubercular activities of these mycelium-associated *Ganoderma* lanostanoids

against *Mycobacterium tuberculosis* H37Ra were evaluated. Taken together with the assay data of previously isolated compounds, structure–activity relationships of the antitubercular activity are proposed. Most importantly, 3β- and 15α-acetoxy groups were shown to be critical for antimycobacterial activity. The most potent compound was (24E)-3β,15α-diacetoxylanosta-7,9(11), 24-trien-26-oic acid.

This study was performed jointly by researchers from BIOTEC, Mae Fah Luang University and the Biodiversity-Based Economy Development Office.

**Ref:** Isaka M, Chinthanom P, Sappan M, Supothina S, Vichai V, Danwisetkanjana K, Boonpratuang T, Hyde KD, Choeyklin R. (2017) Antitubercular Activity of Mycelium-Associated *Ganoderma* Lanostanoids. *Journal of Natural Products*, 80, 5,1361-1369




*Structure-activity relationship for the anti-TB activities of mycelium-associated Ganoderma lanostanoids.*

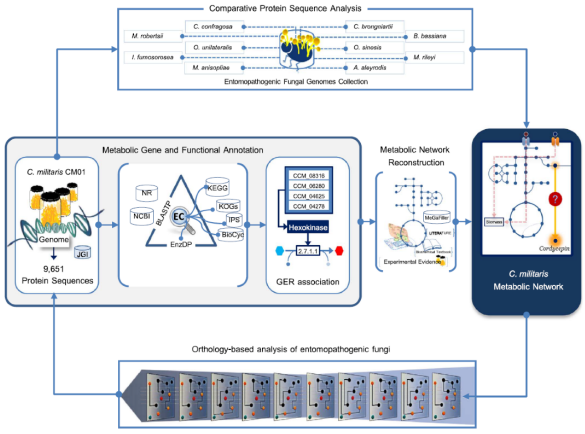
## Construction of genome-scale metabolic network of *Cordyceps militaris*

*Cordyceps militaris* is an entomopathogenic fungus belonging to *Cordyceps*, one of the largest genus of fungi, with over 500 species. *C. militaris* is a target source of natural products with various pharmaceutical functions and thus is being used as an herbal medicine for multiple clinical purposes. One of the bioactive compounds being extensively investigated is cordycepin. It exhibits a broad spectrum of biological activities, including anti-bacterial, anti-fungal, anti-tumor, anti-leukemia, anti-viral and immuno-regulative activities. The genes and relevant metabolic functions underlying cordycepin biosynthesis in this fungus and related entomopathogenic fungi would have an impact on industrial biotechnology as pertaining to the rational development of platform fungal cell factories for efficient production of high-value bioactive compounds with pharmaceutical interest.

The first genome-scale metabolic network of *C. militaris* (iWV1170) was constructed representing its whole metabolisms, which consisted of 894 metabolites and 1,267 metabolic reactions across five compartments, including the plasma membrane, cytoplasm, mitochondria, peroxisome and extracellular space. The *i*WV1170 could be exploited to explain its phenotypes of growth ability, cordycepin and other metabolites production on various substrates. A high number of genes encoding extracellular enzymes for degradation of complex carbohydrates, lipids and proteins existed in *C. militaris* genome. By comparative genome-scale analysis, the adenine metabolic pathway towards putative cordycepin biosynthesis was reconstructed, indicating their evolutionary relationships across eleven species of entomopathogenic fungi. The overall metabolic routes involved in the putative cordycepin biosynthesis were also identified in *C. militaris*, including central carbon metabolism, amino acid metabolism (glycine, L-glutamine and L-aspartate) and nucleotide metabolism (adenosine and adenine). Interestingly, a lack of the sequence coding for ribonucleotide reductase inhibitor was observed in *C. militaris* that might contribute to its over-production of cordycepin.

This study was a collaborative effort between BIOTEC, Kasetsart University and the National University of Singapore.

**Ref:** Vongsangna W, Raethong N, Mujchariyakul W, Nguyen NN, Leong HW, Laoteng K. (2017) Genome-scale metabolic network of *Cordyceps militaris* useful for comparative analysis of entomopathogenic fungi. *Gene*, 626, 132-139.




*Overview of genome annotation and construction process of the metabolic network of C. militaris.*

## Microbial communities in the reef water in the lower Gulf of Thailand

Coral reefs are among the most diverse habitats on Earth, but their knowledge on microbes living in such habitats (marine microbiome) remains limited. To gain more insights into coral reef ecosystem in the lower Gulf of Thailand, researchers utilized 16S and 18S rRNA gene-based amplicon-based metagenomics to identify the prokaryotic and eukaryotic microbiota present in the reef water at Kham Island, Trat province, Thailand. The resulting microbiome data was then compared with the published microbiota from different coral reef water and marine sites.

Kham Island's coral reefs are of the fringing type and remain preserved and abundant. The community similarity indices indicated that the prokaryotic composition from Kham was closely related to that of Kra, another fringing reef site in the lower Gulf of Thailand, followed by the coral reef water microbiota at GS048b (Cooks Bay, Fr. Polynesia), Palmyra (Northern Line Islands, United States) and GS108b (Coccos Keeling, Australia), respectively. Additionally, the microbial eukaryotic populations at Kham was analyzed and compared with the available database at Kra. Both eukaryotic microbiota, in summer and winter seasons, were analyzed. An abundance of *Dinophysis acuminata* was notable in the summer season which is similar to other reports causing by diarrhoeatic shellfish outbreak in the summer season. The slightly lower biodiversity in Kham than that of Kra might reflect the partly habitat difference due to coastal anthropogenic activities and minor water circulation. Note that Kham locates close to the mainland and is surrounded by islands. The global marine microbiota comparison suggested relatively similar microbial structures among coral sites irrespective of geographical location, supporting the importance of coral-associated marine microbiomes, and Spearman's correlation analysis between community membership and factors of shore distance and seawater temperature indicated potential correlation of these factors (p-values < 0.05) with Kham, Kra, and some other coral and coastal sites. Together, this study provides the second marine microbial database for the coral reef of the lower Gulf of Thailand, and a comparison of the coral-associated marine microbial diversity among global ocean sites.

This study was conducted by BIOTEC research team from the Genome Technology Research Unit working in collaboration with researchers from Chulalongkorn University and the Marine and Coastal Resources Research Center, Lower Gulf of Thailand.

**Ref:** Somboonna N, Wilantho A, Monanunsap S, Chavanich S, Tangphatsornruang S, Tongsima S. (2017) Microbial communities in the reef water at Kham Island, lower Gulf of Thailand. *PeerJ*, 5:e3625. doi: 10.7717/peerj.3625.

## Effect of pH on the biological function of Vip3Aa toxin

Vip3Aa insecticidal protein is produced from *Bacillus thuringiensis* and exerts a broad spectrum of toxicity against lepidopteran insect species. Although Vip3Aa has been effectively used as part of integrated pest management strategies, the mechanism of the toxin remains unclear. The study of environmental factors influencing Vip3A function will provide a better understanding of the molecular basis of toxicity. One of the factors that may affect the biological function of Vip3Aa toxin is the environmental pH.

Researchers investigated the effect of pH on the pore-forming activity of the trypsin activated Vip3Aa (actVip3Aa) via *in vitro* pore-forming assays. The results revealed a direct correlation between the pore-forming

properties of Vip3Aa toxin and the pH conditions. The actVip3Aa was able to permeabilize membrane and formed pores at acidic and neutral conditions, while the alkaline condition induced the conformational change resulting in the loss of pore-forming activity. Overall, pH regulates the pore-forming properties of actVip3Aa, supporting the existence of a pore-forming step in the mechanism of Vip3Aa toxicity. The study thus provides a better understanding of the molecular basis of Vip3Aa toxicity and opens the possibility to improve pest control management in the future.

This study was a collaborative effort between BIOTEC, Mahidol University, Tokyo University of Agriculture and Technology (Japan), Hokkaido University (Japan), Tohoku University (Japan) and Japan Science and Technology Agency.

**Ref:** Kunthic T, Watanabe H, Kawano R, Tanaka Y, Promdonkoy B, Yao M, Boonserm P. (2017) pH regulates pore formation of a protease activated Vip3Aa from *Bacillus thuringiensis*. *BBA - Biomembranes*, 1859, 2234-2241.

### Plant diversity increases with the strength of negative density dependence

Theory predicts that higher biodiversity in the tropics is maintained by specialized interactions among plants and their natural enemies that result in conspecific negative density dependence (CNDD). By using more than 3000 species and nearly 2.4 million trees across 24 forest plots worldwide, scientists demonstrate that global patterns in tree species diversity reflect not only stronger CNDD at tropical versus temperate latitudes but also a latitudinal shift in the relationship between CNDD and species abundance. CNDD was stronger for rare species at tropical versus temperate latitudes, potentially causing the persistence of greater numbers of rare species in the tropics. This study reveals fundamental differences in the nature of local-scale biotic interactions that contribute to the maintenance of species diversity across temperate and tropical communities.

This investigation was a global effort of researchers from Asia, Europe, Africa and North America led by the Tyson Research Center, Washington University in St. Louis, USA. As part of the Smithsonian Center for Tropical Forest Science–Forest Global Earth Observatory (CTFS-ForestGEO) network, Mo Singto Plot in Thailand was among 24 forest plots examined in this study. Located in the center of Khao Yai National Park, the 30.5-ha plot was established as a forest dynamics plot under the collaboration between BIOTEC and the Department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment.

**Ref:** LaManna JA, Mangan SA, Alonso A, Bourg NA, Brockelman WY, Bunyavejchewin S, Chang LW, Chiang JM, Chuyong GB, Clay K, Condit R, Cordell S, Davies SJ, Furniss TJ, Giardina CP, Gunatilleke IAUN, Gunatilleke CVS, He F, Howe RW, Hubbell SP, Hsieh CF, Inman-Narahari FM, Janík D, Johnson DJ2, Kenfack D, Korte L, Král K, Larson AJ, Lutz JA, McMahon SM, McShea WJ, Memiaghe HR, Nathalang A, Novotny V, Ong PS, Orwig DA, Ostertag R, Parker GG, Phillips RP, Sack L, Sun IF, Tello JS, Thomas DW, Turner BL, Vela Díaz DM, Vrška T, Weiblen GD, Wolf A, Yap S, Myers JA. (2017) Plant diversity increases with the strength of negative density dependence at the global scale. *Science*, 356, 1389–1392.

## Highlights from Cross-cutting Technology

### Triple antibody sandwich enzyme-linked immunosorbent assays for begomovirus detection

*Tomato yellow leaf curl Thailand virus*, TYLCTHV, is a begomovirus that causes severe damages to tomato crops in Thailand and other countries across Southeast and East Asia. The development of monoclonal antibodies (MAbs) and serological methods for detecting TYLCTHV is essential for epidemiological studies and screening for virus-resistant cultivars for breeding program.

Researchers developed triple antibody sandwich enzyme-linked immunosorbent assays (TAS-ELISAs) for begomovirus detection using two MAbs (M1 and D2) generated against recombinant TYLCTHV coat protein expressed in *E. coli* cells. The efficiency of these newly established TAS- ELISAs in detecting TYLCTHV and other begomoviruses was evaluated with tomato, pepper,

eggplant, okra and cucurbit plants collected from different provinces of Thailand. TAS-ELISA using the narrow-specificity MAb M1 proved highly efficient for the detection of TYLCTHV and Tobacco leaf curl Yunnan virus (TbLCYnV), whereas TAS-ELISA using the broad-specificity MAb D2 was highly efficient for the detection of TYLCTHV, TbLCYnV, Tomato leaf curl New Delhi virus (ToLCNDV) and Squash leaf curl China virus (SLCCNV). Both newly developed assays allow for sensitive, inexpensive, high-throughput detection of begomoviruses in field plant samples, as well as screening for virus-resistant cultivars.

This work was a collaborative study by researchers from the Virology and Antibody Technology Research Unit and Kasetsart University.

**Ref:** Seepiban C, Charoenvilaisiri S, Warin N, Bhunchoth A, Phironrit N, Phuangrat B, Chatchawankanphanich O, Attathom S, Gajanandana O. (2017) Development and application of triple antibody sandwich enzyme-linked immunosorbent assays for begomovirus detection using monoclonal antibodies against *Tomato yellow leaf curl Thailand virus*. *Virology Journal*, 14, 99.

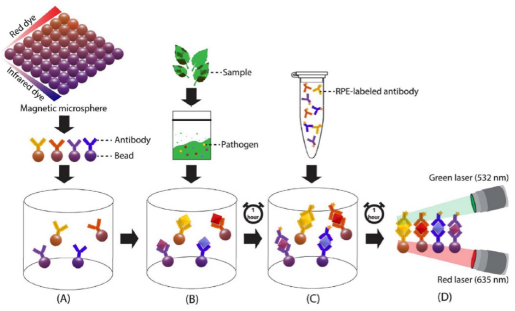
## A microsphere immunoassay for multiplex detection of four diseases in cucurbits

In plant disease testing that deals with a large number of samples, multiplex immunoassay methods are favorable as they minimize the sample preparation step allowing for rapid and accurate results to be obtained without specialized workers.

Researchers have developed and optimized a microsphere immunoassay (MIA) to simultaneously detect multiple plant pathogens (potyviruses, *Watermelon silver mottle virus*, Melon yellow spot virus, and *Acidovorax avenae* subsp. *citrulli*) in cucurbits. A simple extraction method using a single extraction buffer was selected to detect the four pathogens in various cucurbit samples (cucumber, cantaloupe, melon, and watermelon). The extraction method and assay performance were validated with inoculated and field cucurbit samples. The MIA showed 98–99% relative accuracy, 97–100% relative specificity and 92–100% relative sensitivity when compared to commercial ELISA kits and reverse transcription PCR. In addition, the MIA was also able to accurately detect multiple-infected field samples. The results demonstrate that one common extraction method for all tested cucurbit samples could be applied to detect multiple pathogens; avoiding the need for multiple protocols to be employed. This multiplex method can therefore be instrumental for high-throughput screening of multiple plant pathogens with many advantages such as a shorter assay time (2.5 h) with single assay format, a lower cost of detection ($5 vs $19.7 for 4 pathogens/sample) and less labor requirement. Its multiplex capacity can also be expanded to detect up to 50 different pathogens upon the availability of specific antibodies.

This study was a combined effort of researchers from the Virology and Antibody Technology Research Unit and the Biosensing Technology Research Unit.

**Ref:** Charlermroj R, Makornwattana M, Himananto O, Seepiban C, Phuengwas S, Warin N, Gajanandana O, Karoonuthaisiri N. (2017) An accurate, specific, sensitive, high-throughput method based on a microsphere immunoassay for multiplex detection of three viruses and bacterial fruit blotch bacterium in cucurbits. *Journal of Virological Methods*, 247, 6–14.




*Scheme of a microsphere immunoassay (MIA).*

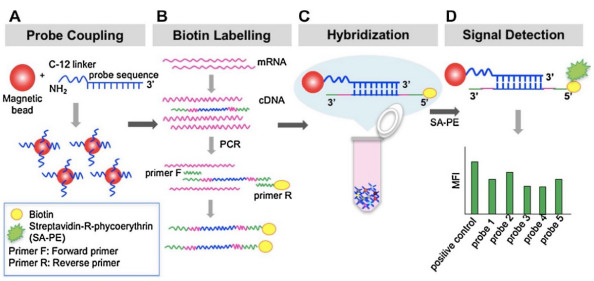
## A multiplex assay for immune gene expression analysis in shrimp

The application of immunostimulants is recognized as a safe and effective strategy to promote shrimp immunity to prevent losses caused by infectious diseases. To efficiently evaluate immunostimulatory effects of candidate compounds, it is crucial to identify their mode of action at a molecular level to shrimp immune response system.

Researchers developed a 9-plex bead-based array as a tool to evaluate molecular effects on transcription levels of immune-related genes in the black tiger shrimp (*Penaeus monodon*). The bead array technology allows simultaneous detection of multiple target genes in a single sample, reducing time, labor and cost. The oligonucleotide probes were designed to target eight immune-related genes that involve in antimicrobial activity, melanization, pathogen pattern recognition proteins, lysozyme and one housekeeping gene as an internal control. The nine probes were coupled to carboxylated-magnetic bead sets. The 9-plex PCR primers were designed and optimized for conditions to allow multiplex detection. The specificity of the assay was validated and the sensitivity was determined. The 9-plex immune gene expression assay was applied to determine transcript levels in gills of *P. monodon* under exposure to a shrimp pathogen, *Vibrio harveyi*, and gene expression patterns were consistent to patterns observed under a traditional realtime PCR method. While realtime PCR method gave a better sensitivity but limited multiplexity, this 9-plex immune gene expression assay was able to simultaneously measure expression of multiple target genes, providing useful alternative assay in the need of higher-throughput gene expression analysis such as evaluation of immune stimulatory effects in different feed additives under various dosages and time points in shrimp.

This study was performed by the Biosensing Technology Research Unit.

**Ref:** Arayamethakorn S, Karoonuthaisiri N, Rungrassamee W. (2017) A multiplex bead-based assay for immune gene expression analysis in shrimp. *Journal of Biotechnology*, 260, 74-78.




*Schematic representation of the bead array assay for multiplex gene expression analysis.*

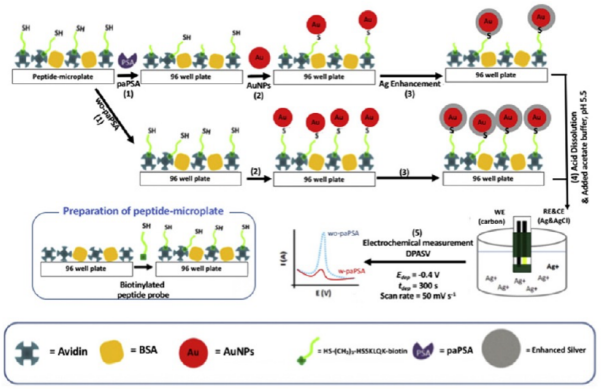
## Electrochemical assay for prostate cancer diagnosis

Total prostate specific antigen (PSA), a serine protease, in blood has been used as a screening test for prostate cancer. Because specificity of the test is poor at the cutoff PSA value and PSA levels can be elevated with benign conditions, alternative methods are being sought.

Researchers demonstrated the determination of proteolytically active prostate specific antigen (paPSA), a potential biomarker for prostate cancer diagnosis, in human serum using a sensitive and low-cost electrochemical sensor. A specifically designed peptide probe was immobilized on the surface of a 96-well plate. The probe could be recognized by paPSA causing cleavage of the peptide, resulting in a decrease in the thiols group remaining on the probe. Gold nanoparticles (AuNPs) were attached to the peptide thiol groups by self-assembly. Hence the amount of AuNPs relates to the length of peptide probe. After cleavage and binding of AuNPs, an amplification step was performed using a silver enhancer solution. The quantity of deposited silver was then measured by differential pulse anodic stripping voltammetry (DPASV) using a disposable screen-printed carbon electrode (SPCE). The signal for paPSA detection was the linear range from 0.1 to 100 ng/mL, with a detection limit of 27 pg/mL. The assay was shown to be reliable and has potential for clinical applications.

This study was a collaborative effort between the Biochemical Engineering and Pilot Plant Research and Development Laboratory, King Mongkut's University of Technology Thonburi and the University of PGRI Banyuwangi (Indonesia).

**Ref:** Parnsubsakul A, Safitri R E, Rijiravanich P, Surareungchai W. (2017) Electrochemical assay of proteolytically active prostate specific antigen based on anodic stripping voltammetry of silver enhanced gold nanoparticle labels. *Journal of Electroanalytical Chemistry*, 785, 125–130.




*Schematic illustration of preparation and detection strategy for paPSA catalyzed peptide cleavage in 96-well plate, measured by differential pulse anodic stripping voltammetric (DPASV) analysis of Ag catalytically deposited on the Au nanolabel surface in 96-well plate.*

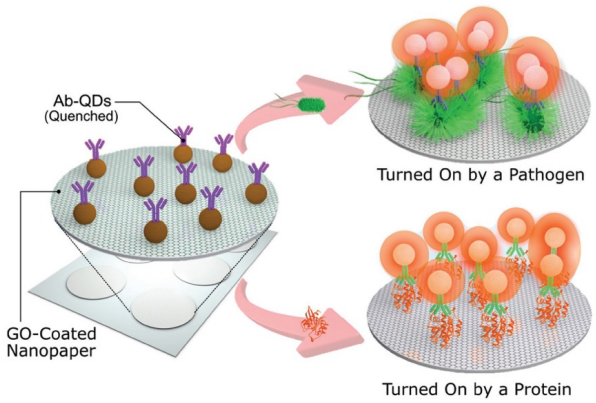
### Immunosensing platform based on graphene oxide-decorated nanopaper

Immunoassays are nowadays a crucial tool for diagnostics and drug development. However, they often involve time-consuming procedures and need at least two antibodies in charge of the capture and detection processes, respectively. Cost-efficient and simple approaches are of interest to facilitate innovative immunosensing approaches.

Researchers developed an advantageous and highly transformative immunosensing platform based on graphene oxide-coated nanopaper (GONAP) that requires no-washing steps and exploits a single antibody. The hydrophilic, porous, and photoluminescence-quenching character of GONAP allows for the adsorption and quenching of photoluminescent quantum dots nanocrystals complexed with antibodies (Ab-QDs), enabling a ready-to-use immunosensing platform. The photoluminescence is recovered upon immuno-complex (antibody-antigen) formation which embraces a series of interactions that trigger desorption of the antigen-Ab-QD complex from GONAP surface. However, the antigen is then attached onto the GONAP surface by electrostatic interactions leading to a spacer between Ab-QDs and GONAP and thus hindering non-radiative energy transfer. It is demonstrated that this simple—yet highly sensitive—platform represents a virtually universal immunosensing approach by using small-sized and big-sized targets as model analytes. In addition, the assay is proved effective in real matrices analysis, including human serum, poultry meat, and river water. GONAP opens the way to conceptually new paper-based devices for immunosensing, which are amenable to point of care applications and automated diagnostics.

This study was jointly conducted by researchers from the Biochemical Engineering and Pilot Plant Research and Development Laboratory, King Mongkut's University of Technology Thonburi, Catalan Institute of Nanoscience and Nanotechnology (Spain), Centro de Investigaciones en Óptica A.C. (Mexico) and Beni-Suef University (Egypt).

**Ref:** Cheeveewattanagul N, Morales-Narváez E, Hassan A-RHA, Bergua JF, Surareungchai W, Somasundrum M, and Merkoçi A. (2017) Straightforward Immunosensing Platform Based on Graphene Oxide-Decorated Nanopaper: A Highly Sensitive and Fast Biosensing Approach. *Advanced Functional Materials*, 27, 1702741. doi: 10.1002/adfm.201702741.




*Operational concept of the immunosensing approach (schematic representation, not to scale).*

## Highlights from Platform Technology

### A tool for identifying nucleotide enrichment signals in feature-enriched RNA-seq data

Biochemical methods are available for enriching 5' ends of RNAs in prokaryotes, which are employed in the differential RNA-seq (dRNA-seq) and the more

recent Cappable-seq protocols. Computational methods are needed to locate RNA 5' ends from these data by statistical analysis of the enrichment. Although statistical-based analysis methods have been developed for dRNA-seq, they may not be suitable for Cappable-seq data. The more efficient enrichment method employed in Cappable-seq compared with dRNA-seq could affect data distribution and thus algorithm performance.

Researchers developed a tool called ToNER (Transformation of Nucleotide Enrichment Ratios). ToNER uses a simple yet robust statistical modeling approach, which can be used for detecting RNA 5' ends from Cappable-seq data, in particular when combining information from experimental replicates. The ToNER tool could potentially be applied for analyzing other RNA-seq datasets in which enrichment for other structural features of RNA is employed. The program is freely available for download at ToNER webpage (http://www4a.biotec.or.th/GI/tools/toner) and GitHub repository (https://github.com/PavitaKae/ToNER).

This work was a collaborative study by researchers from the Genome Technology Research Unit and the National Electronics and Computer Technology Center (NECTEC).

**Ref:** Promworn Y, Kaewprommal P, Shaw PJ, Intarapanich A, Tongsima S, Piriyapongsa J. (2017) ToNER: A tool for identifying nucleotide enrichment signals in feature-enriched RNA-seq data. *PLoS ONE*, 12(5): e0178483. doi: 10.1371/ journal.pone.0178483.

### Effects of enzymes on genome complexity reduction and enrichment of genic SNPs in a GBS approach

Advances in next generation sequencing have facilitated a large-scale single nucleotide polymorphism (SNP) discovery in many crop species. Genotyping-by-sequencing (GBS) approach couples next generation sequencing with genome complexity reduction techniques to simultaneously identify and genotype SNPs. Choice of enzymes used in GBS library preparation depends on several factors including the number of markers required, the desired level of multiplexing, and whether the enrichment of genic SNP is preferred.

In this study, researchers evaluated various combinations of methylation-sensitive (*Aat*II, *Pst*I, *Msp*I) and methylation-insensitive (*Sph*I, *Mse*I) enzymes for their effectiveness in genome complexity reduction and enrichment of genic SNPs. It was found that the use of two methylation-sensitive enzymes effectively reduced genome complexity and did not require a size selection step. On the contrary, the genome coverage of libraries constructed with methylation-insensitive enzymes was quite high, and the additional size selection step may be required to increase the overall read depth. Furthermore, the effectiveness of methylation-sensitive enzymes in enriching for SNPs located in genic regions was demonstrated. When two methylation-insensitive enzymes were used, only 16% of SNPs identified were located in genes and 18% in the vicinity of the genic regions, while most SNPs resided in the intergenic regions. In contrast, a remarkable degree of enrichment was observed when two methylation-sensitive enzymes were employed. Almost two thirds of the SNPs were located either inside or in the vicinity of the genic regions. These results provide useful information to help researchers choose appropriate GBS enzymes in oil palm and other crop species.

This study was performed by the Genome Technology Research Unit.

**Ref:** Pootakham W., Sonthirod C, Naktang C,, Jomchai N, Sangsrakru D, Tangphatsornruang S. (2016) Effects of methylation-sensitive enzymes on the enrichment of genic SNPs and the degree of genome complexity reduction in a two-enzyme genotyping-by-sequencing (GBS) approach: a case study in oil palm (*Elaeis guineensis*). *Molecular Breeding*, 36,154. doi: 10.1007/ s11032-016-0572-x.

## Highlights from Policy Research

### Strategic plan to promote an animal vaccine industry in Thailand: A case study of Porcine Epidemic Diarrhea Virus and Porcine Reproductive and Respiratory Syndrome Virus

Pigs are recognized as an important livestock species in Thailand and their economic impact is growing

steadily. However, disease outbreaks have been a constant threat to the industry and are impacting pig production. Porcine Epidemic Diarrhea Virus (PED) and Porcine Reproductive and Respiratory Syndrome Virus (PRRS) are two of the most economically significant diseases to affect the swine industry today. While vaccination is one of the most effective solutions, imported vaccines have been proven rather ineffective in Thailand as they were developed from different viral strains from the ones found in Thailand. This calls for the development of animal vaccine industry in Thailand.

BIOTEC has successfully developed prototype vaccines for PED and PRRS at lab-scale with high specificity and efficacy. The National Biopharmaceutical Facility (NBF) located at King Mongkut's University of Technology Thonburi (KMUTT) is well-established and can serve as an important infrastructure for industrial-scale vaccine production facility. Market value of PRRS and PED vaccines in Thailand is sizable and is expected to grow steadily into the next decade. To effectively promote an animal vaccine industry and maximize the impact of the industry in Thailand, short- and long-term strategies have been formulated. Short-term strategy involves positioning NBF as the main production facility for PED and PRRS vaccines and simultaneously implementing financial measures such as an innovation coupon program in order to entice local businesses to commission NBF to produce vaccines at a relatively competitive price to imported products. To sustain this industry in the long run, Thailand would need to 1) enhance the national assessment of applications for registration of domestically-produced animal vaccines which can be achieved through the public-private collaboration in developing guidelines for registration of animal vaccines derived from domestic research projects; 2) develop R&D excellence in animal vaccine technology by committing to long-term research funds for large-scale projects in this discipline; 3) attract world-class vaccine companies to set up a base in Thailand; 4) accelerate the development of manpower to perform toxicity, safety and efficacy assessment of vaccines through the collaboration with international research agencies and foreign companies.

### Status and future prospect of agricultural microbiome research in Thailand

Microbiome is defined as the totality of microorganisms and their collective genetic material present in a particular environment, including inside and on the skin of living organisms. Research into microbiome has recently taken center stage and rapid progress has been made in this area to gain basic understanding that will lead to novel applications of microorganisms. Agricultural microbiomes can significantly contribute to the development of probiotics to combat pathogenic microbes in animal guts or the development of microbial products to be used in place of chemical pesticides which has adverse effects on human and animal health as well as the environment. Upon reviewing the global trend in microbiome research, it was found that the research priority is placed on work that would result in application as reflected by the high number of patent applications in animal and plant microbiomes in the US and China. Microbiome research in Thailand is still in its early stage, most of the work aiming at knowledge creation and not much on product or process development. Therefore, to develop agricultural microbiome research to enhance Thailand's competitiveness, the following are recommended research priorities: 1) diversity and types of microbiome for application, and for the long term focusing on in-depth research into the characterization of microbiome-host or microbiome-environment systems; 2) study of hosts that are economically important plants and animals aiming to produce organic plants and animals with the use of probiotics and prebiotics to boost plant and animal health; 3) investment in technology development and scientific equipment that will support research priorities and establishment of research networks and databases with proper mechanisms to encourage information sharing and collaboration.

### Criteria and minimum requirements for application of food biosafety assessment of genetically-modified animals

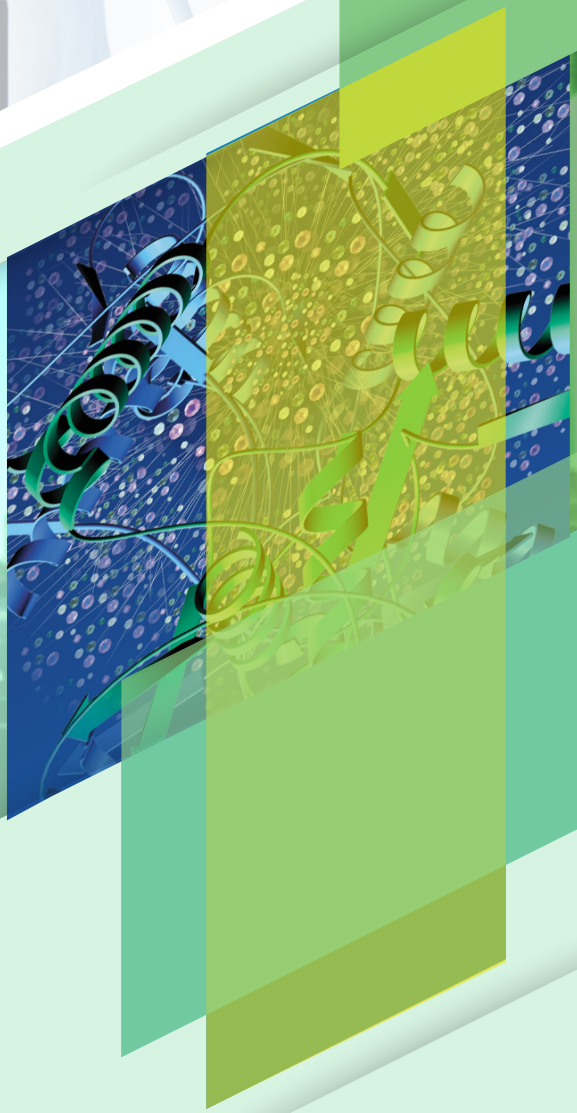
Recognizing the contribution of modern biotechnology to sustainable agriculture and food industry, BIOTEC and

the Thai Food and Drug Administration (Thai FDA) work cooperatively to ensure the safety of products derived from modern biotechnology and strengthen the nation's capacity in food biosafety assessment. In 2015, genetically modified salmon, engineered to grow faster than its non-engineered counterpart, was approved by the US FDA for consumption and became the first genetically engineered animal to be cleared for safe release as food. In view of this recent development, BIOTEC, as the secretariat of Thailand's Technical Biosafety Committee (TBC), established criteria and minimum requirements for application of food biosafety assessment of genetically-modified animals, referencing the Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA animals developed by Codex Alimentarius Commission. These criteria and minimum requirements cover the scope of using engineered animals for both direct consumption and food processing. Safety assessment is evaluated in various aspects including molecular biology, nutrition, toxicity, allergenicity, and aquaculture or animal husbandry. The Thai FDA will incorporate these newly developed criteria in the regulations for foods derived from genetically modified animals.

### Biosafety guidelines for modern biotechnology (2016 Edition)

BIOTEC, in its capacity as the TBC secretariat, developed and released the Biosafety Guidelines for Modern Biotechnology in 1993. The guidelines provided relevant basic biosafety information, general principles and framework for work related to modern biotechnology to safeguard personnel, the community and the environment. The guidelines are reviewed and updated every two years to keep abreast of the technological advancement and international biosafety protocols.

The 2016 edition includes the following changes: 1) the scope is expanded to include emerging technology such as genome editing technology and synthetic biology; 2) notification and approval process for conducting research is amended and the list of microorganisms and their corresponding risk level is revised according to the Pathogens and Animal Toxins Act, B.E. 2558 (2015); and 3) requirements of laboratories for each biosafety level are revised to correspond to the current international practice.



# 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 2017:

**ENZbleach: Alkaline–tolerant Enzyme for Pulp Bleaching Process.** ENZbleach contains xylanase which has been developed and optimized for pulp biobleaching without the need of pH adjustment of the pulp, making the large-scale operations more simple and cost effective. This new biobleaching process using enzymes leads to a significant reduction of chlorine and chlorine-based compounds used in the conventional chemical bleaching process. The technology has been licensed to GeneFerm Biotechnology Company Limited, a leading fermentation company in Taiwan specializing in microbial fermentation, strain screening, recovery and purification technology. Currently, GeneFerm undertakes a manufacturing development and marketing of fermented fungi, microbial and probiotic products.


*EZbleach: Alkaline–tolerant Enzyme for Pulp Bleaching Process*

**One-step production process of mangosteen vinegar with "MV-F1" starter culture**. This technology has an advantage over the traditional fermentation using naturally-present microorganisms. It takes only 12-15 days to achieve 5% acetic acid, which is shorter than the traditional fermentation. The technology has been licensed to A&P Orchard 1959 Company Limited. The company operates the largest organic mangosteen plantation in Thailand and manufactures products and beverages from mangosteen.


*One-step production process of mangosteen vinegar with "MV-F1" starter culture*

**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 fungal spores. *B. bassiana* BCC 2660 is a proven biocontrol agent against brown planthopper, cassava mealybug and aphids based on the field trials in rice, cassava and yardlong bean plantations. The fermentation technology developed by BIOTEC includes the use of rice grains as the main substrate. This specially-designed medium produces 78-fold increased spore yields over conventional media. This technology has been licensed to TAB Innovation Company Limited, a company with the main business in import, export and distribution of agrochemical, biopesticide, fertilizer, seeds, and irrigation systems.

**Production technology for low-cyanide cassava flour**. The technology allows bitter cassava, containing high level of toxic cyanide, to be processed into high quality cassava flour with less than 10 ppm cyanide content, the safety limit for cyanide content in cassava food enforced by Codex Alimentarius Commission of WHO/FAO. This technology applies equipment and machines that can be manufactured locally. This high quality cassava flour can replace wheat flour in various products and provides relatively similar texture and taste, and therefore this production technology can reduce wheat flour volume imported from other countries. The technology has been licensed to Chorchaiwat Industry Company Limited, a starch-processing company.

**Commercial production of cane silage with *Lactobacillus plantarum* BCC 65951**. The technology application includes the lab-scale production of *L. plantarum* BCC 65951 and the fermentation technology of forage cane using *L. plantarum* BCC 65951 as a starter culture. The use of starter culture speeds up the fermentation process and yields a high quality silage, over the natural fermentation. Silage produced with this technology has an extended shelf life of more than 6 months, without diminishing its quality. The technology has been licensed to Micro Innovate Company Limited, the first company to establish an industrial-scale microbial production facility for the production of probiotics and animal feeds in Thailand.

**LAMP-dipstick diagnostic device for detecting *Mycobacterium tuberculosis***. The detection kit, a combination of loop-mediated isothermal amplification (LAMP) technique and lateral flow dipsticks, is designed for rapid diagnosis of *M. tuberculosis* with high sensitivity and specificity. This kit takes 75 minutes to obtain the result which is faster than PCR-Gel electrophoresis and LAMP-Gel electrophoresis methods. A set of four primers was designed for LAMP to target six distinct regions of *M. tuberculosis*, making the test highly specific. The quick result means that patients can receive treatment in a timely manner. This technology has been licensed to Strategic Wisdom and Research Institute, Srinakharinwirot University.

## Gluten free cassava flour

A team of researchers from BIOTEC and Kasetsart University successfully developed a production technology for cassava flour. It is a mechanization process that can turn bitter cassava, containing high level of toxic cyanide, into high quality cassava flour with less than 10 ppm cyanide content. This technology can be implemented in the cassava starch factory with some process modifications, enabling the factory to diversify its products and keep the production cost competitive. As cassava flour contains no gluten, it is suitable for people suffering from celiac disease and health conscious consumers. Therefore, it has potential to be commercialized as a premium product. The conversion to premium cassava flour significantly adds value to cheap bitter cassava.

BIOTEC has been working in collaboration with Chorchaiwat Industry Company Limited to implement a pilot-scale machine to demonstrate the feasibility of the technology and produce cassava flour for market testing. Chorchaiwat Industry has been in the business of manufacturing and selling cassava starch for over 60 years. As the bakery market in Thailand has progressively expanded, the company recognized an opportunity to introduce a gluten-free product derived from a local cash crop to the market. Chorchaiwat Industry therefore obtained the license of cassava flour manufacturing technology from NSTDA and became the first Thai company to launch the gluten free all-purpose cassava flour under the brand name “SAVA” in Thailand in 2017.

## 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 2017, BIOTEC performed 80 collaborative/commissioned research projects, of which 61 projects with clients/collaborators in the private sector and 19 projects with private companies. Of these, 37 projects were initiated in 2017. These are categorized into 24 projects in food and agriculture theme, 5 projects in energy and environment theme, 5 projects in bioresources theme and 3 projects in healthcare and medicine theme. In addition, 8 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 the deposit and provision of microbial cultures and biological materials; fungal isolation and identification; bioactive compound screening; compound extraction and analysis; monoclonal antibody production; animal cell deposit; cattle reproductive system management; cassava starch quality analysis; design and performance experiments in shrimp aquaria; shrimp disease diagnostics and solutions; and DNA analysis for aquaculture. In 2017, as many as 17,968 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 innovating industrial processes and products. Open Lab often leads to collaborative research, commissioned research, or the provision of analytical services. In 2017, an activity was co-organized with the Asia and Pacific Seed Association (APSA) to introduce an efficient inoculation protocol for tomato necrotic ringspot virus (TNRV) and capsicum chlorosis virus (CaCV) resistance screening in tomato to seed companies. Seventeen representatives from local and international seed companies attended the event.



*An introduction on efficient inoculation protocol for tomato necrotic ringspot virus (TNRV) and capsicum chlorosis virus (CaCV) resistance screening in tomato to seed companies.*


# HUMAN RESOURCES DEVELOPMENT AND PUBLIC AWARENESS

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.

## Human Resource Development in Biotechnology

**Postdoctoral Fellowship Program** BIOTEC Postdoctoral Fellowship Program offers opportunities to recent PhD graduates from all over the world 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 2017, one new fellowship was granted, adding to ten 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 interests. 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 6 doctoral students, 15 master's students and 9 YSTP scholars. In addition, BIOTEC offers opportunity to undergraduate students to experience a research laboratory and gain insight into various disciplines of biotechnology through an internship program. In 2017, 94 students took part in this internship program.

**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 2017, BIOTEC organized 54 events, including conferences and workshops on 27 topics, drawing in 3,078 participants in total (8,189 man-days). Eleven events were open to international participants. Examples were Thailand Metabolic Workshop, Workshop on Fungal Contamination & Its Mycotoxin in the Food Products and Food Processing, Seminar on Genome Editing Technology, the Thai-Israeli Tomato Conference, the International Curator Lecture and Workshop on Emerging Technologies and Management of Microbial Resources and Capacity Building Program on Biosafety Assessment.

*Participant from International Curator Lecture and Workshop on Emerging Technologies and Management of Microbial Resources.*

## Public Awareness in Science and Technology

**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: ENZease: an enzyme for textile industry, a production technology for cassava flour, Amp-Gold: a detection device for acute hepatopancreatic necrosis disease in shrimp, and a testing device for melon diseases based on magnetic beads.


*Exhibition of BIOTEC/NSTDA at Thai Tech Expo 2017.*


*ENZease: an enzyme for textile industry on Modern 9 TV showed on September 2018.*


*BIOTEC activity for students at National Science and Technology Fair in 2017.*

**Exhibitions.** BIOTEC participated in a number of exhibitions and displays at major events, disseminating results and ideas to several demographics and industrial sectors. In 2017, BIOTEC exhibited its work in fourteen events ranging from scientific events to trade fairs; for example, Thai Tech EXPO 2017, NSTDA Annual Conference 2017, National Science and Technology Fair 2017 and SETA 2017 (Sustainable Technology Energy Asia 2017).

**Lab Tour.** 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 2017, 155 groups toured BIOTEC laboratories, 50 of which were from overseas.

## AGBIO2017: International Conference on Sustainable Agriculture and Bioeconomy 2017

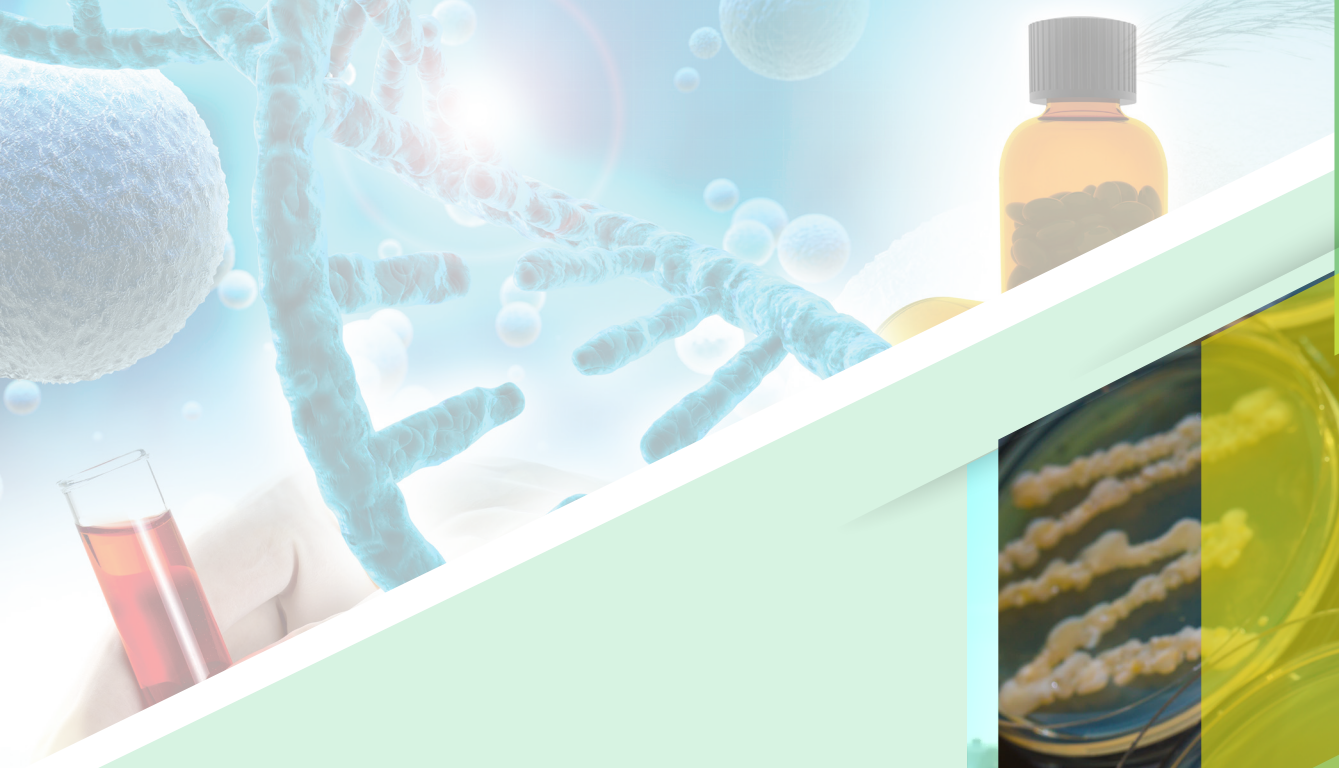
BIOTEC, NSTDA in collaboration with local and international partners organized the International Conference on Sustainable Agriculture and Bioeconomy 2017 or AGBIO2017 during 27 February – 2 March 2017 at Bangkok International Trade & Exhibition Centre (BITEC), Bangkok, Thailand. The opening ceremony was auspiciously presided over by Her Royal Highness Princess Maha Chakri Sirindhorn.

AGBIO2017 offered a platform where international researchers, policy makers and industry leaders can exchange ideas, build partnerships, and update on latest innovations and technologies relevant to key topics of the conference. The conference program consisted of special lectures by international renowned speakers, oral and poster presentation, exhibition, and business matching. The conference addressed key topics of sustainable agriculture and the bioeconomy such as seed technology; plant diseases; bioresource collection and biobased industry; biorefinery products, chemicals and biomaterials; and enzyme technology. The event was attended by approximately 1,000 participants from Thailand and overseas.

## Starch Update 2017: The 9th International Conference on Starch Technology


BIOTEC-NSTDA in collaboration with the Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), Kasetsart University has been organizing a biennial Starch Update, an international conference on starch technology to serve as a platform to connect scientists, technologists and industrialists to enhance development of starch technology and innovation. In 2017, Starch Update was convened on 27-28 February 2017, concurrently with AGBIO2017, offering participants a comprehensive experience on agricultural technology.

The program of Starch Update 2017 consisted of keynote and plenary speakers as well as oral and poster presentation sessions, covering the topics of starch properties and analysis, starch processing, starch modification and starch application.



# 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 establishing the BIOTEC International Advisory Board.

## The 2017 Meeting of BIOTEC International Advisory Board

BIOTEC International Advisory Board (IAB) was established in 2005 to enable the Center to better meet the constant challenges posed by globalization and the emergence of new technologies. The IAB Meeting is held annually with the aim to review the performance and progress of BIOTEC, as well as to provide comments and feedback on particular research topics presented each year.

The 2017 Meeting of BIOTEC International Advisory Board was held during 3-4 March 2017. Current BIOTEC International Advisory Board, serving from November 2017 to November 2019, comprises eleven eminent experts from reputable organizations, with diverse backgrounds in biotechnology. In addition to overall performance of the Center, research topics discussed in this year's meeting were shrimp, plant and biorefinery. The board members also actively participated in a forum on "How to survive in current scientific research environment" specially organized for BIOTEC young researchers.

*BIOTEC International Advisory Board Members and Executives*

*The 2017 Meeting of BIOTEC International Advisory Board*

## International Exchange Program

A number of programs have been established to host foreign researchers and students in BIOTEC laboratories as a strategy to initiate and promote research collaboration with international partners. 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; two from Myanmar, two from Vietnam, three from Indonesia, three from the Philippines and two from Laos.
- **International Exchange 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 eighty-five students participating in this program in the past year from eighteen countries, namely Indonesia, Vietnam, Malaysia, Laos, Singapore, Hong Kong, Japan, Taiwan, India, Nepal, China, Australia, the UK, France, Ireland, Germany, the US and Mexico.

*11 Fellowships under the Human Resource Development Program in Biotechnology.*

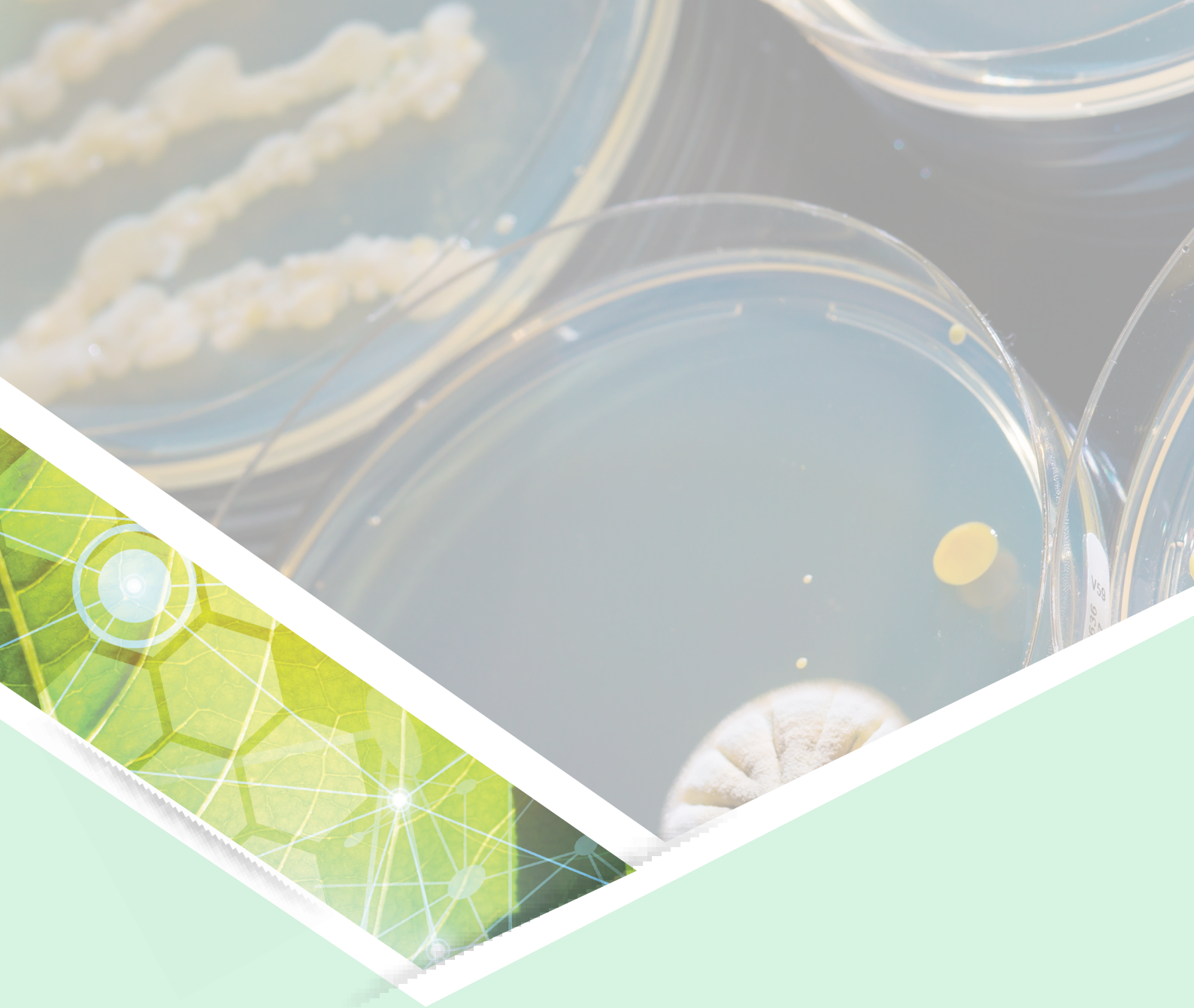
*Student internship from Ireland and Indonesia.*

## Fostering Collaboration

In fiscal year 2017, BIOTEC signed/renewed eight Memorandam of Understanding (MOUs)/Agreements for academic and research collaboration covering eight different countries.


*An MOU Signing Ceremony between BIOTEC and Asahi Industries Co., Ltd., Japan to promote research collaboration on organic fertilizers.*

| Organization | Detail |
|---|---|
| Institut Teknologi Bandung (ITB), Indonesia | To organize a study visit on biotechnology in Thailand. |
| Institute of Biotechnology, Vietnam Academy of Science and Technology, Vietnam | To foster cooperation in the fields of bioresource utilization and microbial management. |
| Vietnam National University Ho Chi Minh City - University of Science, Vietnam | To foster cooperation in the fields of bioresource utilization and microbial management. |
| Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, China | To support research collaboration on disease prevention and control of aquaculture shrimp. |
| WFCC-MIRCEN World Data Center for Microorganisms, China | To promote cooperation in the field of data management of microorganisms. |
| National Agriculture and Forestry Research Institute, Lao PDR | To promote academic, research and capacity building cooperation and technology transfer in the field of agriculture. |
| Asahi Industries Co., Ltd., Japan | To promote research collaboration on organic fertilizers. |
| Institute for Macromolecular Studies (ISMAC), National Research Council, Italy | To promote research collaboration on textiles. |



# 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 of 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 2017, 41 projects were selected for impact assessment. An estimated total of 5.64 billion baht was generated from these projects. This amount was categorized as investment generation (215 million baht), revenue generation to licensees or users (5.04 billion baht), cost reduction (129 million baht) and import replacement (252 million baht).

| Sector | Number of Evaluated Projects | Impact (million baht) | | | | |
|---|---|---|---|---|---|---|
| | | Investment Creation | Revenue Improvement | Cost Reduction | Import Substitute | Total |
| Agriculture and Food | 29 | 13 | 4,509 | 8 | 8 | 4,538 |
| Health and Medicine | 2 | 202 | 126 | - | - | 328 |
| Environment | 7 | - | 406 | 96 | 24 | 526 |
| Infrastructure | 3 | - | - | 25 | 220 | 245 |
| **Total** | **41** | **215** | **5,041** | **129** | **252** | **5,637** |

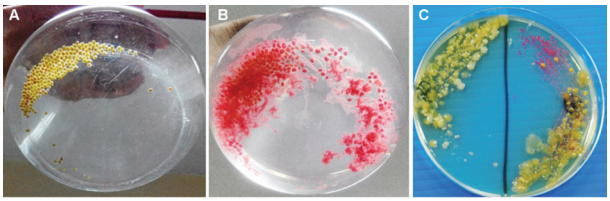
## Agriculture and Food

A total of 29 projects were evaluated, generating 4.54 billion baht impact.

**New rice varieties.** This includes varieties that have been developed by using marker-assisted selection technology, namely flood-tolerant rice (Homcholasit), fragrant rice (Homwarin), blast-resistant glutinous rice (Thanyasirin), dwarf glutinous RD6 and antioxidant-rich Riceberry. These new varieties were introduced to farmers, along with the training in seed production in all regions of Thailand. A total of 88 million baht in income increment was estimated.

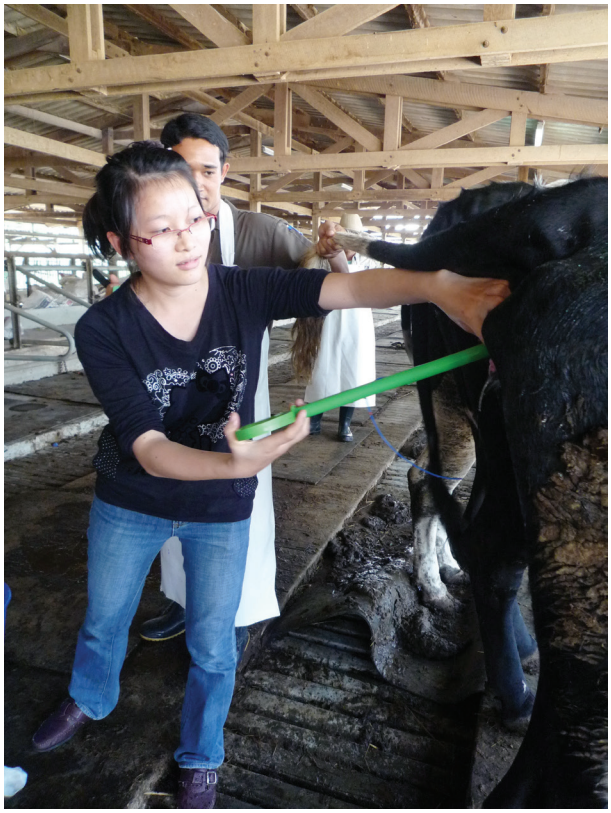
**Technologies for crop production.** BIOTEC engaged in various projects on plant improvement and technologies for crop production, such as improvement on the production capacity of disease-free sugarcane, screening for salt-tolerant crops, ecologically-friendly cultivation system for eucalyptus trees, plant improvement services, development of agroforestry farming with community participation, plant multiplication for land rehabilitation, diagnostic devices and reagents for plant disease detection and biocontrol production. These technologies have generated additional income to farmers and industries, as well as an investment to the economy at the value of 510 million baht in total.

**Aquaculture production.** The impact assessment was made on selected completed projects and inventions, including research on shrimp diseases, research on tilapia diseases, shrimp breeding, LAMP-based shrimp disease detection kits and an aquaculture system. These technologies have generated an impact of 1.14 billion baht.




*Tilapia eggs and isolated bacteria.*

**Animal production.** BIOTEC has developed a steroid-based synchronization of ovulation protocol to be used in combination with artificial insemination of cattle. The protocol improves the efficiency of the insemination. Farmers' earning due to this technology has been estimated to be 419 million baht.



*A steroid-based synchronization of ovulation protocol.*

**Food and agro-industry.** Technologies that have been used at the commercial scale include starter culture for animal feed production, production of animal feed enzymes and identification of physical and chemical functional properties of egg and egg products. These technologies have generated income to the companies involved, substitute for imported products and provided an increase in return from the investment at the value of 241 million baht.


*Pasteurized liquid egg products.*

**Development of testing protocols.** BIOTEC founded DNA Technology Laboratory and now serves as its consultant. DNA Technology Laboratory developed the protocol for Thai jasmine rice identification, enabling an authentication of Thai jasmine rice and commanding a premium price for the rice. This has generated an additional 2.14 billion baht to the economy.

## Health and Medicine

Two projects were evaluated and created an impact of 328 million baht.

**Process improvement for traditional medicine.** BIOTEC was commissioned by a manufacturer of traditional medicine to make improvements to its production process. The project has resulted in an investment made by the company to upgrade the production process. The improvement has enhanced the productivity of the manufacturer and consequently increased the profit. Economic impact was estimated at 223 million baht.

**Establishment of a biopharmaceutical facility.** BIOTEC and King Mongkut's University of Technology Thonburi (KMUTT) founded the National Biopharmaceutical Facility (NBF) at the KMUTT Bangkhuntien campus. This facility has generated investment to the economy of 105 million baht.

## Environment

Seven projects were evaluated, resulting in an impact of 526 million baht.

**Efficiency improvement in cassava starch processing.** Technology has been developed to improve the production efficiency with near zero waste concept in the cassava starch production process. The technology was introduced to cassava starch factories through practical training of managers and staff working in the factories. Cost reduction and improved production yields have been estimated to 406 million baht.

**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 installed in agro-industry factories, including cassava starch, palm oil mill and food processing. The benefit of this technology includes wastewater compliance and energy cost savings of 87 million baht.


*Biogas production at Cassava Factory*

**Environmentally-friendly products.** BIOTEC and a private company conducted a joint research project to develop a commercial bioremediation product based on oil-degrading microbes. The company later acquired the license to commercialize the technology and since then has released a wide range of products. The company's revenue and the value of import substitution were estimated to 33 million baht.

## Infrastructure Establishment

Three projects were evaluated, resulting in an impact of 245 billion baht.

**Manpower development.** BIOTEC developed a training module on biosafety assessment in order to build up capacity of Institutional Biosafety Committees (IBCs). The training module has been used by various academic and research institutes. From 2011-2016, a total of 2,652 individuals have been trained. The impact was quantified to 20 million baht.

*Training on biosafety assessment on May 2017.*

**Analytical and testing services.** With well-equipped research facilities and qualified laboratory staff, BIOTEC provides analytical and testing services, as well as research partnership opportunities to the public and private sector. Services that were assessed for their impact include proteomic analysis and bioactive compound screening. By eliminating the need to seek such services from overseas, the savings were estimated to 225 million baht.

07



# IAPPENDICES

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

# List of Publications


1. Abdel-Wahab, M.A., Dayarathne, M.C., Suetrong, S., Guo, S.Y., Alias, S.A., Bahkali, A.H., Nagahama, T., Elgorban, A.M., Abdel-Aziz, F.A., Hodhod, M.S., Al-Hebshi, M.O., Hyde, K.D., Nor, N.A.B.M., Pang, K.L. and Jones, E.B.G. (2017). New saprobic marine fungi and a new combination. *Botanica Marina*, 60(4), 469-488.
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# List of Intellectual Properties

## List of Issued Intellectual Properties

| Title | Granted Date | Ref. No. | Country |
|---|---|---|---|
| **Patent** | | | |
| Interactive learning materials on GMOs and biosafety | 10 February 2017 | 53705 | Thailand |
| **Petty Patent** | | | |
| Primer pair specific for *Vibrio harveyi* detection | 7 October 2016 | 12009 | Thailand |
| Enzyme-additive formulation for desizing and scouring of fabric and the use of such formulation in desizing and scouring of fabric | 7 October 2016 | 12012 | Thailand |
| Method for enhancing germination rate of hard seeds by soaking seeds in high-temperature water and high-acidic solution | 13 October 2016 | 12030 | Thailand |
| Detection method for Infectious Spleen and Kidney Necrosis Virus in fish | 28 October 2016 | 12061 | Thailand |
| Detection method for *Streptococcus iniae* in fish | 28 October 2016 | 12062 | Thailand |
| The solid-state fungal fermentation process for gamma-linolenic acid production using vinasses | 4 November 2016 | 12098 | Thailand |
| Bacterial cellulose/hydroxyapatite/BMP-2 composite | 13 January 2017 | 12299 | Thailand |
| Cultivation process of *Bacillus thuringensis* for the production of Vip3A protein for controlling insect pests | 13 January 2017 | 12300 | Thailand |
| Primers and PCR protocols for simultaneous detection of yellow head virus genotypes YHV and GAV | 13 January 2017 | 12302 | Thailand |
| A method for fosmid library construction | 10 February 2017 | 12409 | Thailand |
| Recombinant yeast *Pichia stipitis* directly produces ethanol from cellulose and hemicellulose and its applications thereof | 10 February 2017 | 12410 | Thailand |
| Recombinant yeast *Pichia stipitis* directly produces ethanol from cellulose and its applications thereof | 10 February 2017 | 12411 | Thailand |
| Reporter plasmid and method of using this plasmid in anti-intracellular *M. tuberculosis* agent assay | 3 March 2017 | 12462 | Thailand |
| A method to improve plants tolerated to drought stress using polyethylene glycol, chemical mutagen and ultrasonic wave | 28 April 2017 | 12621 | Thailand |
| Animal feed formulated with insect fungal biopolymer | 7 July 2017 | 12839 | Thailand |
| Genetically Engineered Beauveria bassiana for insect pest control | 7 July 2017 | 12840 | Thailand |
| Detection method for *Francisella noatuensis subsp. orientalis* in fish | 7 July 2017 | 12841 | Thailand |
| Species-specific DNA probes for nucleocapsid gene of CaCV, MYSV, TNRV and WSMoV | 14 July 2017 | 12855 | Thailand |
| Method for multiplex detection and identification of CaCV, MYSV, TNRV and WSMoV using RT-PCR-ELISA | 14 July 2017 | 12856 | Thailand |
| The solid medium formula of fungal cultivation for gamma-linolenic acid production | 15 August 2017 | 12984 | Thailand |
| Plasmid vectors for extracellular production of heterologous proteins from *Lactobacillus plantarum* and *Bacillus subtilis* | 15 August 2017 | 12985 | Thailand |
| Plasmid vectors for extracellular production of heterologous proteins from *Bacillus subtilis* | 15 August 2017 | 12986 | Thailand |
| Development and synthesis of flexible 2,4-diamino-6-phenyl-5-(3-(2-(2-carbonylethyl) phenoxy) propoxy pyrimidine derivatives for antimalarial drugs | 22 September 2017 | 13134 | Thailand |
| **Plant Registered** | | | |
| Tomato line FD1-2 (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line FD4 (A4) (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line FD6 (A5) (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line FD7 (A6) (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |

| Title | Granted Date | Ref. No. | Country |
|---|---|---|---|
| Tomato line FD10 (A7) (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line FD12-3 (A8-3) (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line FD12-4 (A8-4) (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line FD12-LK (A8-LK) (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line PC3 (A9) (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line PC5 (A10) (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line PC6 (A11) (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line PC11 (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line PC12-20 (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |
| Tomato line PC8 (A12) (Resistant to Tomato Yellow Leaf Curl Geminivirus) | 8 June 2017 | N/A | Thailand |

## List of Applied Intellectual Properties

| Title | Filling Date | Ref. No. | Country |
|---|---|---|---|
| **Patent** | | | |
| Plasmid for extracellular production of halophilic protease from *Bacillus subtilis* | 7 October 2016 | 1601006067 | Thailand |
| Primers specific for DNA markers in sugar metabolism genes and a method for using such primers to select for sugarcane variety with high sugar content | 25 November 2016 | 1601007041 | Thailand |
| [5]HELICENE DERIVATIVES AS MOLECULAR REPORTERS FOR DIAGNOSTIC APPLICATIONS AND METHODS OF SYNTHESIS THEREFOR | 1 February 2017 | PCT/TH2017/000072 | N/A |
| A *gshA* deficient surrogate model for testing glutathione-related molecules and compounds | 9 March 2017 | 1701001258 | Thailand |
| DNA primers and probes specific for detecting unique SNP loci for cultivar identification and seed purity test in chilli pepper | 29 March 2017 | 1701001737 | Thailand |
| DNA primers and probes specific for detecting unique SNP loci for cultivar identification and seed purity test in watermelon | 29 March 2017 | 1701001738 | Thailand |
| DNA primers and probes specific for detecting unique SNP loci for cultivar identification and seed purity test in cucumber | 29 March 2017 | 1701001739 | Thailand |
| Monoclonal antibodies to the NSs protein of Capsicum chlorosis virus and its application in detection of Capsicum chlorosis virus | 7 April 2017 | 1701001950 | Thailand |
| A mutant strain of *Aspergillus aculeatus* for production of cellulases and xylanase and methods for production of enzyme thereof | 7 June 2017 | 1701003166 | Thailand |
| Screening process for selection of additive mixture formulations that enhance activity and stability of enzyme in liquid form during operational and storage conditions | 30 June 2017 | 1701003739 | Thailand |
| Enzyme-additive cocktail formulation for enhancing of activity and stability of enzyme in liquid form during operational and storage conditions | 30 June 2017 | 1701003740 | Thailand |
| Xylanase-containing additive formulations for enhancing catalytic activity of enzyme | 30 June 2017 | 1701003741 | Thailand |
| Primer set, peptide nucleic acid sequence and a method for detection of Isoniazid-resistant mycobacterium tuberculosis | 11 August 2017 | 1701004546 | Thailand |

| Title | Filling Date | Ref. No. | Country |
|---|---|---|---|
| Endotoxin removal process from fibronectin protein | 11 August 2017 | 1701004549 | Thailand |
| Culture medium of insect fungi and its preparation process | 18 August 2017 | 1701004656 | Thailand |
| Manufacture of antimicrobial textile from agricultural by-product | 5 September 2017 | 1701005062 | Thailand |
| Dry scaffolds of bacteria cellulose and bone morphogenetic 2 and their process of preparation | 8 September 2017 | 1701005130 | Thailand |
| High temperature stable bone morphogenetic 2 using recombinant statherin-fibronectin fusion protein and their process of preparation | 8 September 2017 | 1701005131 | Thailand |
| An episomal plasmid system for high-level protein expression | 22 September 2017 | 1701005536 | Thailand |
| Organic dyes based on derivatives of [5]helicene isoindoledione as a reporter molecule for diagnostic applications and methods of synthesis therefor | 22 September 2017 | 1701005538 | Thailand |
| Organic dyed from derivatives of 13-((n-oxoalkyl)oxy)-1,2,5,6-tetrahydrodibenzo[c,g]phenanthrene-3,4-dicarbonitrile) as a reporter molecule for diagnostic applications and method of synthesis therefor | 25 September 2017 | 1701005608 | Thailand |
| Organic dyed from derivatives of 13-((n-oxoalkyl)oxy)dibenzo[c,g]phenanthrene-3,4-dicarbonitrile) as a reporter molecule for diagnostic applications and method of synthesis therefor | 25 September 2017 | 1701005612 | Thailand |
| Organic dyed from derivatives of n-((3,4-dicyanodibenzo[c,g]phenanthren-13-yl)oxy)alkanoic acid as a reporter molecule for diagnostic applications and method of synthesis therefor | 25 September 2017 | 1701005613 | Thailand |
| Multi-color fluorescent reporter system for flaviviruses | 25 September 2017 | 1701005614 | Thailand |
| **Petty Patent** | | | |
| Production of odorless antioxidative hydrolyzed collagen from seabass (*Lates calcarifer*) skin without descaling | 8 December 2016 | 1603002520 | Thailand |
| A CRISPR-dCas9 based system for fine-tuning carotenoid production in yeast | 28 December 2016 | 1603002635 | Thailand |
| A CRISPR-dCas9 based system for fine-tuning gene expression in microorganisms | 28 December 2016 | 1603002634 | Thailand |
| crRNA specific for *pyrG* gene disruption in fungi by CRISPR-Cas9 system | 27 January 2017 | 1703000136 | Thailand |
| Loop-mediated isothermal amplification coupled fluorescent dye for semi-quantitative detection of staphylococcal enterotoxin A in *Staphylococcus aureus* | 27 January 2017 | 1703000138 | Thailand |
| Real-time reading system for multi-fluorescent color | 28 February 2017 | 1703000334 | Thailand |
| The solid medium formula for fungal cultivation of the genus *Metarhizium* | 3 March 2017 | 1703000360 | Thailand |
| Portable Breath Biomarker Analyser | 9 March 2017 | 1703000403 | Thailand |
| *Ogataea* spp. Recombinant plasmid for extracellular expression of heterologous protein from sucrose in yeast *Ogataea* spp. | 17 March 2017 | 1703000456 | Thailand |
| Gluten-free bread containing Job's tears flour and protease hydrolyzed egg white and its process | 12 April 2017 | 1703000628 | Thailand |
| The process of Chilli thrips control by Biocontrol agent | 28 April 2017 | 1703000720 | Thailand |
| Hydrocyclone for Separation of Cassava Starch Granules from Impurities | 4 May 2017 | 1703000753 | Thailand |
| Transgenic *Plasmodium falciparum* for discovery of Gamma-glutamylcysteine synthetase enzyme inhibitors that have inhibitory effects in the parasite. | 4 May 2017 | 1703000754 | Thailand |
| A method to improve rice plants tolerated to blast disease using induced mutation | 4 May 2017 | 1703000755 | Thailand |
| Method for lignocellulose degradation using cell wall degrading enzymes with recombinant lytic polysaccharide monooxygenase | 19 May 2017 | 1703000855 | Thailand |

| Title | Filling Date | Ref. No. | Country |
|---|---|---|---|
| Products for constitutive gene expression to produce target proteins or biological compounds from thermotolerant yeast and the process of using such products | 23 June 2017 | 1703001116 | Thailand |
| Products for methanol-inducible gene expression to produce target proteins or biological compounds from thermotolerant yeast and the process of using such products | 23 June 2017 | 1703001117 | Thailand |
| Simultaneous saccharification and fermentation process with recycling enzymes and cells for lignocellulosic ethanol production | 30 June 2017 | 1703001160 | Thailand |
| High cell density fermentation of recombinant bacterial cells by fed-batch fermentation without supplement of oxygen | 30 June 2017 | 1703001161 | Thailand |
| A method to inhibit growth of bacterial in plant tissue culture process using povidone-iodine and ultrasonic wave | 4 August 2017 | 1703001416 | Thailand |
| A thyA folA tolC knockout bacteria with *Toxoplasma gondii* dihydrofolate reductase-thymidylate synthase expression | 4 August 2017 | 1703001417 | Thailand |
| Specific oligonucleotide primer for detection of *Lactobacillus brevis* | 4 August 2017 | 1703001418 | Thailand |
| The method for detection of mycorrhizal *Astraeus* mushroom | 18 August 2017 | 1703001523 | Thailand |
| The Process of Preparation of Double Layered Microencapsulation from Biodegradable Polymeric Pectin and Riceberry Rice Protein Extracts for Bitter Melon Extracts | 25 August 2017 | 1703001621 | Thailand |
| Specific primer set and a method for detection of foodborne *Vibrio parahaemolyticus* in food | 1 September 2017 | 1703001682 | Thailand |
| Diagnostic tool using a small set of single nucleotide polymorphisms (SNP markers) for classification of Bay-91 pig breed | 15 September 2017 | 1703001793 | Thailand |
| Diagnostic tool using a small set of singe nucleotide polymorphisms (SNP markers) for predicting a risk of carrying mummified piglets | 15 September 2017 | 1703001794 | Thailand |
| Recombinant *Pichia pastoris* for the production of esters of branched-chain alcohols | 22 September 2017 | 1703001861 | Thailand |
| Recombinant *Pichia pastoris* for the production of isobutanol | 22 September 2017 | 1703001862 | Thailand |
| Recombinant plasmid for production of bacteriocin Bac7293A | 22 September 2017 | 1703001863 | Thailand |
| Design species-specific primers and probes for identification of lactic acid bacteria | 22 September 2017 | 1703001864 | Thailand |
| Water treatment and recycle system for aquaculture | 28 September 2017 | 1703001922 | Thailand |
| **Trade Secret** | | | |
| Enzyme and additive formulations for degradation of plant biomass | 2 February 2017 | N/A | Thailand |
| Enzyme and additive formulations for degradation of plant biomass by energy-conserving process | 2 February 2017 | N/A | Thailand |
| Medium formulation for production of biomass degrading enzyme | 2 February 2017 | N/A | Thailand |
| Medium formula (mYPD) for inoculum preparation for production of yeast probiotic cells | 12 April 2017 | N/A | Thailand |
| Medium formula (AS-1) for fed-batch fermentation for production of yeast probiotic cells | 12 April 2017 | N/A | Thailand |
| Feeding method for fed-batch fermentation for production of yeast probiotic cells | 12 April 2017 | N/A | Thailand |
| Heat resistant and highly stable formulated enzyme | 8 May 2017 | N/A | Thailand |
| MV-F1 Mixed cultures for mangosteen vinegar fermentation | 5 July 2017 | N/A | Thailand |
| MV-F3 Mixed cultures for mangosteen vinegar fermentation | 5 July 2017 | N/A | Thailand |
| Single-step fermentation of mangosteen for vinegar production | 5 July 2017 | N/A | Thailand |
| Production of antibacterial agent from hen egg white protein | 1 August 2017 | N/A | Thailand |

# Honors and Awards

## International Awards

**Dr. Umaporn Uawisetwathana**

**Biosensing Technology Research Unit**

>> Carolina MacGillavry Award for an IFS-SEARCA-funded project on "Climate change adaptation strategy through application of biofloc technology for the improvement of productivity and environmental sustainability of white shrimp *Litopenaeus vannamei* production in South East Asia," awarded by the International Foundation for Science (IFS) in partnership with the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA).

**Dr. Pakkakul Sangsuriya**

**Animal Biotechnology Research Unit**

>> Carolina MacGillavry Award for an IFS-SEARCA-funded project on "Impact of high temperature on shrimp immune response associated with acute hepatopancreatic necrosis disease (AHPND)," awarded by the International Foundation for Science (IFS) in partnership with the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA).

**Dr. Walaiporn Charoensapsri**

**Shrimp Molecular Biology and Biotechnology Laboratory**

>> Carolina MacGillavry Award for an IFS-SEARCA-funded project on "Climate change impact on the pathogenic *Vibrio parahaemolyticus* isolates causing acute hepatopancreatic necrosis disease (AHPND)," awarded by the International Foundation for Science (IFS) in partnership with the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA).

**Dr. Chunya Puttikhunt and Ms. Tanapan Pruksamas**

**Medical Biotechnology Research Laboratory**

>> Silver Medal for "DEN-STEP hemorrhagic fever diagnostic kit," presented at the 45th International Exhibition of Inventions of Geneva, 13-17 April 2016, Switzerland.

**Ms. Wansika Kiatpathomchai and Mr. Narong Arunrut**

**Biosensing Technology Research Unit**

**Dr. Rungkarn Suebsing**

**Microbial Biotechnology and Biochemicals Research Unit**

>> Platinum Award for "VIP-Safe Plus: LAMP-electrochemical sensor for detection of foodborne pathogens," presented at the 13th Taipei International Invention Show & Technomart (INST 2017), 28-30 September 2017, Taiwan.

**Dr. Kunruedee Sangseethong**

**Cassava and Starch Technology Research Laboratory**

>> Gold Medal for "Cassava starch-based hydrogel as a superdisintegrant in drug tablets," presented at the 13th Taipei International Invention Show & Technomart (INST 2017), 28-30 September 2017, Taiwan.

## National Awards

**Dr. Saengchan Senapin**

**Shrimp Molecular Biology and Biotechnology Laboratory (Centex Shrimp)**

>> Taguchi Prize for Outstanding Researcher 2016 for her achievement in research work entitled "Current and emerging infectious diseases in fish and shrimp: Emphasis on molecular characterization, pathogenesis, and detection," awarded by the Thai Society for Biotechnology.

**Dr. Ubolsree Leartsakulpanich, Dr. Penchit Chitnumsub, Dr. Wichai Pornthanakasem, Ms. Aritsara Jaruwat and Ms. Pinpunya Riangrungroj**

**Biosensing Technology Research Unit**

**Dr. Chairat Uthaipibull and Dr. Darin Kongkasuriyachai**

**Medical Molecular Biology Research Unit**

**Ms. Wanwipa Itarat**

**Animal Biotechnology Research Unit**

>> The 2016 NRCT Research Award (Chemistry and Pharmaceutical Science) for "Serine Hydroxymethylatransferase as a Novel Drug Target for Malaria," awarded by the National Research Council of Thailand (NRCT).

**Dr. Vanvimon Saksmerprome, Dr. Patai Charoonnart, Ms. Sarocha Jitrakorn and Ms. Thitiporn Thammasorn**

**Shrimp Molecular Biology and Biotechnology Laboratory**

>> The 2016 NRCT Research Award (Agricultural Science and Biology) for "RNA interference technology for practical management of a slow growth virus (Laem-Singh virus, LSNV) in black tiger shrimp cultivation," awarded by the National Research Council of Thailand (NRCT).

**Dr. Chumpol Ngamphiw**

**Genome Technology Research Unit**

>> The 2016 NRCT PhD Dissertation Award (Agricultural Science and Biology) for "Genomic Characteristics of Argonaute Proteins Mediate Genes Containing LINE-1 Expression," awarded by the National Research Council of Thailand (NRCT).

**Dr. Walrati Limapichat**

**Biosensing Technology Research Unit**

>> The 2016 NRCT PhD Dissertation Award (Chemistry and Pharmaceutical Science) for "Probing the Roles of Receptor Structure, Drug-Receptor Interactions, and Receptor Crosstalk in Ligand-Gated Ion Channel Function," awarded by the National Research Council of Thailand (NRCT).

**Dr. Weerawat Runguphan**

**Biodiversity and Biotechnological Resource Research Unit**

>> The 2016 NRCT PhD Dissertation Award (Chemistry and Pharmaceutical Science) for "Reprogramming Alkaloid Biosynthesis in *Catharanthus roseus*: Synthetic Biology in Plants," awarded by the National Research Council of Thailand (NRCT).

**Ms. Wansika Kiatpathomchai, Mr. Narong Arunrut, Ms. Jantana Kampeera and Mr. Sarawut Sirithammajak**

**Biosensing Technology Research Unit**

>> The 2017 NRCT Invention Award (Agricultural Science and Biology) for "Amp-Gold: Sensitive Visual Detection Kit of AHPND Bacteria," awarded by the National Research Council of Thailand (NRCT).

**Dr. Orawan Chatchawankanphanich, Ms. Anjana Bhunchoth and Ms. Bencharong Phuangrat**

**Virology and Antibody Technology Research Unit**

>> The 2017 NRCT Invention Award (Agricultural Science and Biology) for "Cherry Tomato Resistant to Yellow Leaf Curl Disease," awarded by the National Research Council of Thailand (NRCT).

**Dr. Philip J. Shaw, Dr. Chairat Uthaipibull, and Dr. Aiyada Aroonsri**

**Medical Molecular Biology Research Unit**

>> DMSc Award (Medical Science Research and Development) for "A ribozyme tool for study of malaria parasite genes," awarded by the Department of Medical Sciences, Ministry of Public Health.

**Dr. Satinee Suetrong**

**Biodiversity and Biotechnological Resource Research Unit**

>> Oral Presentation Award for "Diversity of mangrove fungi in Thailand, area-based collaborative research for conservation," presented at the Center of Excellence in Fungal Research International Conference (COEIC 2017), 11-13 January 2017, Chiang Rai, Thailand.

**Dr. Nattawut Boonyuen**

**Biodiversity and Biotechnological Resource Research Unit**

>> Oral Presentation Award for "*Parafuscospora garethii* sp. nov. and notes on four new fungal taxa from freshwater habitats, Thailand," presented at the Center of Excellence in Fungal Research International Conference (COEIC 2017), 11-13 January 2017, Chiang Rai, Thailand.

**Dr. Umpava Pinruan**

**Biodiversity and Biotechnological Resource Research Unit it**

>> Poster Presentation Award for "Productivity of edible *Amanita* at Phusing Agricultural Development Center, Sisaket, Thailand," presented at the Center of Excellence in Fungal Research International Conference (COEIC 2017), 11-13 January 2017, Chiang Rai, Thailand.

**Ms. Charuwan Chuaseeharonnachai**

**Biodiversity and Biotechnological Resource Research Unit**

>> Poster Presentation Award for "Two novel species and two new records of *Endophragmiella* from Thailand," presented at the Center of Excellence in Fungal Research International Conference (COEIC 2017), 11-13 January 2017, Chiang Rai, Thailand.

**Ms. Jureerat Ueapattanakit**

**Biodiversity and Biotechnological Resource Research Unit**

>> Poster Presentation Award for "Biodiversity of geothermal soil-borne fungi in Thailand," presented at the Center of Excellence in Fungal Research International Conference (COEIC 2017), 11-13 January 2017, Chiang Rai, Thailand.

**Ms. Paveena Tapaneeyaworawong**

**Marine Biotechnology Laboratory**

>> Oral Presentation Award for "Growth of a small-sized diatom *Amphora coffeaeformis* BUUC1601

isolated from estuary, Chanthaburi Province, under batch and continuous cultivation," presented at the 16th Conference on Agriculture, 24-25 January 2017, Khon Kaen, Thailand.

**Dr. Sithichoke Tangphatsornruang**

**Genome Technology Research Unit**

>> Biotechnologist Award 2016 presented by Biogenomed Co., Ltd.

**Dr. Thidarat Nimchua and Mr. Phitsanu Pinmanee**

**Microbial Biotechnology and Biochemicals Research Unit**

>> Silver level of achievement of PTT Innovative Idea Award for the innovation entitled "PTT Yeast Technology Platform," awarded by PTT Public Company Limited.

# Executives and Management Team

**Chairman**

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| Sakarindr Bhumiratana | President, King Mongkut's University of Technology Thonburi |

**Vice Chairman**

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| Narong Sirilertworakul | President, National Science and Technology Development Agency (NSTDA) |

**Members**

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| Porametee Vimolsiri | Secretary General, Office of the National Economic and Social Development Board (NESDB) |
| Anan Suwannarat | Director, Rice Department, Ministry of Agricultural and Cooperation |
| Dechapiwat Na Songkhla | Deputy Director, Bureau of the Budget |
| Amaret Bhumiratana | Associate Fellow of the Royal Society of Thailand<br>Professor Emeritus, Faculty of Science, Mahidol University |
| Pimchai Chaiyen | Professor, Faculty of Science, Mahidol University |
| Wichar Thitiprasert | Advisor, National Bureau of Agricultural Commodity and Food Standards |
| Pornsilp Patcharintanakul | Advisor, Thai Chamber of Commerce<br>President, Thai Feed Mill Association<br>President, Thai AEO Importer & Exporter Association |
| Pachok Pongpanich | Executive Committee, Thai Seed Trade Association -THASTA<br>Managing Director, PacThai (Pacific Seeds Co., Ltd.) |
| Rutjawate Taharnklaew | Assistant Vice President, R&D Center, Betagro Group |
| Kittiphong Limsuwannarot | Managing Director, Solution Creation Co., Ltd. |
| Somvong Tragoonrung | Executive Director, BIOTEC |
| Dussadee Siamhan | Deputy Executive Director, BIOTEC |

## International Advisory Board

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| Jean-Marcel Ribaut | Director, Integrated Breeding Platform (IBP), MEXICO |

**Members**

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| Philippe Desmeth | President, World Federation for Culture Collections, BELGIUM |
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| Ikuo Hirono | Professor, Laboratory of Genome Science, Tokyo University of Marine Science and Technology, JAPAN |
| Martin Keller | Director and Alliance President, National Renewable Energy Laboratory, USA |
| Lene Lange | Professor, Department of Chemical and Biochemical Engineering, Technical University of Denmark, DENMARK |
| Vítor Martins dos Santos | Chair for Systems and Synthetic Biology, Wageningen University, THE NETHERLANDS |
| David Mathews | Expert Scientific Advisory Committee, Medicines for Malaria Venture (MMV) and Member of Earlham College Board of Trustees, USA |
| Fabien De Meenster | Owner of DMF Co., Ltd., POLAND |
| Ray Ming | Professor of Plant Biology, University of Illinois at Urbana-Champaign, USA |
| Frédéric Tangy | Director of Research, French National Centre for Scientific Research (CNRS) and Head of the Viral Genomics and Vaccination Unit, Institut Pasteur, FRANCE |

## Management Team

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| Somvong Tragoonrung | Executive Director |
| Dussadee Siamhan | Deputy Executive Director |
| Kuakoon Piyachomkwan | Deputy Executive Director |
| Lily Eurwilaichitr | Deputy Executive Director |
| Sirawut Klinbunga | Director, Animal Biotechnology Research Unit |
| 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 Ingsriswang | Director, Biodiversity ad Biotechnological Resource Research Unit |
| Verawat Champreda | Director, Microbial Biotechnology and Biochemicals Research Unit |

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