Shrimp Biotechnology in Thailand

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Biotechnology is important for the rational exploitation of national resources in an environmentally friendly way. It has the potential for a wide range of applications in activities ranging from agriculture to medicine and thus has the potential to impact on the economy and the quality of life of everyone.

The shrimp industry serves as a good example of how biotechnology has helped Thailand to become the world's leader in cultivated shrimp production for the past decade. This is in spite of the fact that there have been serious problems with shrimp loss due to disease, environmental problems, a shortage of broodstock and insufficient knowledge on shrimp biology. Biotechnology has been useful in helping to solve all of these problems and the National Center for Genetic Engineering and Biotechnology (BIOTEC) has taken a leading role in this by setting up a program in shrimp biotechnology. This program aims to promote research and development (R&D) in shrimp towards the goal of sustainable shrimp production by aquaculture with a minimum negative affect on the environment. By improving the efficiency and quality of shrimp production through R&D activities, BIOTEC believes that Thailand can continue to lead the world in high quality, healthy shrimp production.

This book entitled, Shrimp Biotechnology in Thailand presents the BIOTEC projects relevant to achieving the goal of environmentally friendly and sustainable production of high quality shrimp. Readers will understand the importance of the industry for Thailand's economy and they will see how biotechnology can help the country maintain its position of leadership in the highly competitive international shrimp market.

Shrimp Biotechnology in Thailand

Since the beginning of the 1990's Thailand has led the world in the export of farmed shrimp. In 2000, there were 25,000 shrimp farms producing 350,000 tons of exported black tiger shrimp at a value of 100,000 million baht and employing approximately 150,000 people.

Thus, cultivated shrimp is one of the top 10 exports from Thailand, generating approximately 1.5 billion dollars in yearly export earnings. These earnings are especially important because they are based very largely on inputs of local origin. Although the shrimp farming industry has not developed without production and environmental difficulties, both government and industry are unified in efforts to reach the goal of a sustainable enterprise with minimal negative impact on the environment. The National Center for Genetic Engineering and Biotechnology (BIOTEC) has been particularly active in supporting research and in stimulating cooperation to solve production and environmental problems in the shrimp industry (Flegel, et al. 1997a).

Shrimp Biotechnology Program in BIOTEC

As Thailand is now one of the largest shrimp producers and exporters in the world, BIOTEC pays special attention to this field. It lends itself very well to modern biotechnology through breeding, feed development and supplementation, disease control, quality assurance and waste treatment and recovery. As worldwide shrimp stocks diminish, the country is turning more to farming the sea and biotechnology has a major role to play in this transition. Strategically speaking, Thailand is well located for year-round shrimp culture and it has the potential to become a long-term sustainable industry based on renewable resources and with high competitive export potential. Researchers and all concerned sectors feel the urgent need to take necessary action and carry out research to support shrimp farmers and the strategic government plan.

The Shrimp Biotechnology Program general objectives are to make the shrimp industry in Thailand more competitive, to sustain its leading position in world shrimp production, to maintain or increase production, to lower the production cost and to reduce the negative environmental impact to a minimum. This can be achieved by:

- Promoting better communication and coordination amongst shrimp industry players in academia and business
- Providing funding support for R&D and cooperation amongst all industry sectors
- Increasing technology transfer from academia to industry
- Supporting training of scientists, students and technology users at the post-graduate and post-doctoral levels in shrimp biotechnology
- Arranging seminars, training courses and round table discussions among concerned agencies to assess current status, to present research results, know-how and new techniques, to develop strategies and to solve technical problems for the shrimp industry
- Preparing publications (both Thai and international) and providing rapid and convenient access to information

All of the activities are coordinated through a BIOTEC shrimp biotechnology sub-committee and sub-research groups that consist of experts from both the private and public sectors. The groups function to formulate research and development strategy, set research priorities and to act as a task force to react to emergency problems. Altogether, there are 5 sub-research groups, one each in the following areas:

- Broodstock and Genetics

This is an important area of research and development for the long-term future of the shrimp industry in Thailand as continued reliance on wild broodstock represents a significant disease risk and prevents the development of genetically improved strains with better production characteristics. Although the Shrimp Culture Research and Development Co. Ltd. (SCRD), of which BIOTEC is a major shareholder, is already involved in the development of a selection programme, there remains much to be done to demonstrate the advantages and improve the efficiency of maturation techniques.

- Nutrition

Nutrition is another key area for research and development activities to improve the overall performance and cost-effectiveness of shrimp farming. In farms, for example, feed can represent up to 60 percent of variable costs of production. However, given the highly competitive nature of the Thai shrimp feed industry, BIOTEC's role is limited to areas of common benefit or common interest to feed producers and the industry.

- Health Management

This is probably the major research area identified by most farmers and industry sectors given the high cost of disease to the Thai (and international) industry. Estimates of direct losses due to shrimp diseases, and particularly the virus diseases white spot syndrome virus and yellow head virus, have been estimated to be several billion baht. Some examples of achievements in this program are presented in this brochure.

- **Environment**

Environmental issues have been increasingly prominent within the shrimp industry over the past few years. National and international NGO's have highlighted problems and increased awareness of the environmental impact of shrimp farming and their claims have impacted on the international marketing of shrimp. Environmental problems have also been recognized within the industry as a problem which impacts on the efficiency of production. Pollution from shrimp farms and other sources for example, may play a significant role in the occurrence and severity of disease outbreaks. The development of methods to improve control of the environment in shrimp production facilities will reduce environmental impacts and pollution, and increase the efficiency of production. BIOTEC believes that biochemical engineers can make significant contributions in this area.

- **Pond and Farm Management**

The effectiveness of research programs to improve the production and efficiency of shrimp farming is frequently reduced by the impact of individual farm (and hatchery) management on production. The development of better, standardized management techniques (such as "Best Management Practice" or "BMP" programs) will increase the consistency of production. In addition, the practical implementation of research results by the private sector must become an integral part of the research and development program. Again, BIOTEC believes that careful research by shrimp biologists and biochemical engineers on hatchery and pond rearing systems will help to improve efficiency and take the guesswork out of production.

In this brochure, some examples will be given of projects in the area of shrimp health management, particularly with respect to viral diseases. There include discovery of new infectious disease agents, DNA probe technology for rapid diseases detection, training courses, workshops and seminars, and the establishment of the Shrimp Culture Research and Development Company (SCRD) consortium for production of domesticated shrimp stocks. BIOTEC also supports in-house research on shrimp at **the Marine Biotechnology Research Unit at Chulalongkorn University and the Center of Excellence for Shrimp Molecular Biology and Biotechnology at Mahidol University** In addition **the Shrimp Biotechnology Service Laboratory (SBSL)**, provides standardization services and reagents for DNA diagnostic work.

The BIOTEC projects have already shown considerable benefit for the Thai shrimp farming industry. WSSV was discovered in Thailand through a BIOTEC supported project before it became a problem in farms. When difficulties at the farm level arose, rapid diagnostic reagents were quickly developed by a BIOTEC project and the technology was rapidly transferred to industry. As a result of this research, technology transfer and training, the Thai industry did not suffer the same level of catastrophic fall in shrimp production faced by other countries in the region. Indeed, Thailand still leads in cultured shrimp production.

Molecular Diagnostic Tools for Shrimp Viruses

DNA diagnostic reagents for shrimp viral diseases. These are produced by the Shrimp Biotechnology Service Laboratory (SBSL).

Other important milestones in the BIOTEC program have been the development of DNA probes for the rapid detection of all major shrimp pathogens in thailand (Flegel, 1997). These probes are essential for the development and monitoring of certified shrimp broodstock and fry. They are also critical tools for the prevention of shrimp diseases which may cause disastrous production losses, even though they present no health risk to shrimp consumers. Indeed, BIOTEC's support for research on the development of a DNA probe for one of these pathogens, white spot syndrome virus (WSSV), has yielded a benefit that can be valued at approximately 1 billion US$ per year since 1996. This example serves to illustrate clearly how research can be vitally important to the Thai economy.

Work on WSSV in Thailand (Wongteerasupaya, et al. 1995a) began unpredictably in 1994 as the result of a chance infection in laboratory reared shrimp. These shrimp were under observation for a different major shrimp viral pathogen (yellow head virus or YHV) originally discovered in Thailand in 1992 (Chantanachookin, et al. 1993; Flegel, et al. 1995; Wongteerasupaya, et al. 1995b). In retrospect, we now know that WSSV probably originated in China in 1993 and gradually spread from there to the rest of Asia in succeeding years. In China, it resulted in a drop of shrimp production from approximately 155,000 metric tons in 1992 to 35,000 metric tons (77% decrease) by 1994 (Rosenberry,

1994). When the chance discovery of WSSV occurred in the laboratory in Thailand, the virus had not yet caused any known farm losses, so the Thai production for 1994 was 225,000 metric tons (Rosenberry, 1994), and that for 1995 was 220,000 metric tons (Rosenberry, 1995). In the meantime, work was done to investigate the nature of the virus and to develop diagnostic probes for it (Wongteerasupaya, 1996). A major discovery with the probe was that shrimp fry used to stock shrimp ponds could be the source of the virus. By the time the virus began to affect farms in Thailand, a good amount of research information had already been accumulated and this helped considerably in mitigating its negative impacts (Withyachumnarnkul, 2000).

WSSV began to cause Thai farm losses in late 1995, but its occurrence was too late to have a very significant effect on 1995 production figures. However, farms stocked in late 1995 (i.e., to be harvested in early 1996) and those stocked in 1996 were hit heavily such that export of farmed shrimp dropped to 160,000 metric tons in that year (Rosenberry, 1996). Although this was a massive loss (60,000 metric tons or 27% of production from the preceding year), we believe that, like China, the losses would have reached 170,000 metric tons (i.e., 77% of the previous year's production) without Biotec's research results. The research allowed for the development of effective prevention programs (Flegel, et al. 1997b) which included the use of DNA technology to screen stocking fry so that WSSV positive batches could be rejected. The difference between a loss of 60,000 metric tons and 170,000 metric tons (110,000 tons) had an export value of approximately 1 billion US$ and should be considered as a return on a WSSV research investment of less than 200,000 dollars. That constitutes a return on investment of 5,000 times in a single year! It is also important to note that a substantial part of the knowledge gained was paid for by funding provided to work on a different virus. In other words, it was not known at the time of its discovery that work on WSSV would eventually be useful to the shrimp industry.

Appearance of shrimp affected by WSSV.

The research benefit is international. For example, Chinese shrimp production subsequently recovered to approximately 80,000 tons by 1996 (Rosenberry 1996), largely due to the result of better knowledge of WSSV derived from research (Flegel, 1997; Flegel, et al. 1997b). Taken over the whole Asian shrimp production region, the gains must be very large indeed. BIOTEC continues with its strong commitment to research programs in shrimp biotechnology and to integration of all relevant work in the private and public sectors. The few examples given here serve to illustrate that the time and money invested can yield substantial returns.

Yellow Head Virus Detection and Control

Appearance of shrimp affected by YHV.

Another serious problem for Thai shrimp farmers is yellow head disease. It has also been the subject of intensive research in Thailand and much of this has been supported by BIOTEC (Chantanachookin, et al. 1993; Wongteerasupaya 1995b). It was named after gross signs of yellowing of the shrimp "head" or cephalothorax region and generally pale overall body color. The disease is caused by yellow head virus (YHV) and the first outbreaks of this disease were reported in Thailand in 1991, long before the advent of white spot disease. We now know that yellow head virus is an RNA virus related to the *Coronavirus* family, the virus family that includes some human cold viruses. At the early stages of infection, shrimp may sometimes have a very large appetite and their feeding rate may increase to 2 times that of normal shrimp. The reason for this is still unknown. After 1-2 days of high feed intake, they stop eating, swim to the surface and then die at the pond dike or pond bottom. If not harvested, most of the shrimp in an affected pond will die within 1–2 days after they stop eating.

Yellow head disease is widespread in every area of shrimp culture in Thailand, including Samut Songkram and Samut Sakorn in central Thailand, Rayong, Chantaburi and Trad in easthern Thailand, and provinces in southern Thailand, especially on the gulf of Thailand. Although losses due to yellow head disease are less than those for white spot disease, they have continued sporadically since 1991 and they constitute a serious problem for Thai shrimp farmers.

The molecular diagnostic probes developed for detection of YHV by RT-PCR were quickly developed both in Thailand (Wongteerasupaya, et al. 1995b) and in Australia through cooperation between Mahidol University and CSIRO (Cowley, et al. 1999) with the support of the BIOTEC and the Australian Centre for International

Agricultural Research (ACIAR). These tools are now being used to study the molecular epidemiology of this virus so that sources of entry into the cultivation system can be more rationally controlled. As with the WSV probes, these will also be important for screening the domesticated broodstock in the SCRD program.

Stunted or Dwarf Shrimp Disease Detection and Control

Normal shrimp compared to shrimp stunted by HPV or MBV.

Hepatopancreatic parvovirus (HPV) is associated with stunted shrimp or dwarf shrimp that Thai farmers call "kung jikko". Infected shrimp exhibit very slow growth such that they may weigh only 2–5 g/pc body weight at the end of a 4-5 month cultivation period. In some cases 30-40% of the shrimp in a pond may constitute such stunted shrimp. Although HPV may not cause shrimp to die and although it is not a human health problem, it is still a problem because the small size shrimp have a very low price. In some cases they may constitute such a large proportion of a crop that a farmer cannot cover his rearing costs.

Thai scientists first became interested in this virus after farmers complained about losses due to large numbers of overly small shrimp in their rearing ponds. As a result of work sponsored by BIOTEC, it was found that HPV was associated with the slow growing shrimp (Flegel, et al. 1999). Subsequently, the virus was isolated, purified and characterized and a rapid PCR detection method was devised (Sukhumsirichart et al. 1999). This technique is now available for the screening of post larvae, so that they can be discarded rather than stocked in ponds, if infected with HPV. The technique is also being used to search for the reservoir of HPV in the rearing system. Once it is identified, more rational control measures will be possible.

Monoclonal Antibodies for Viral Detection

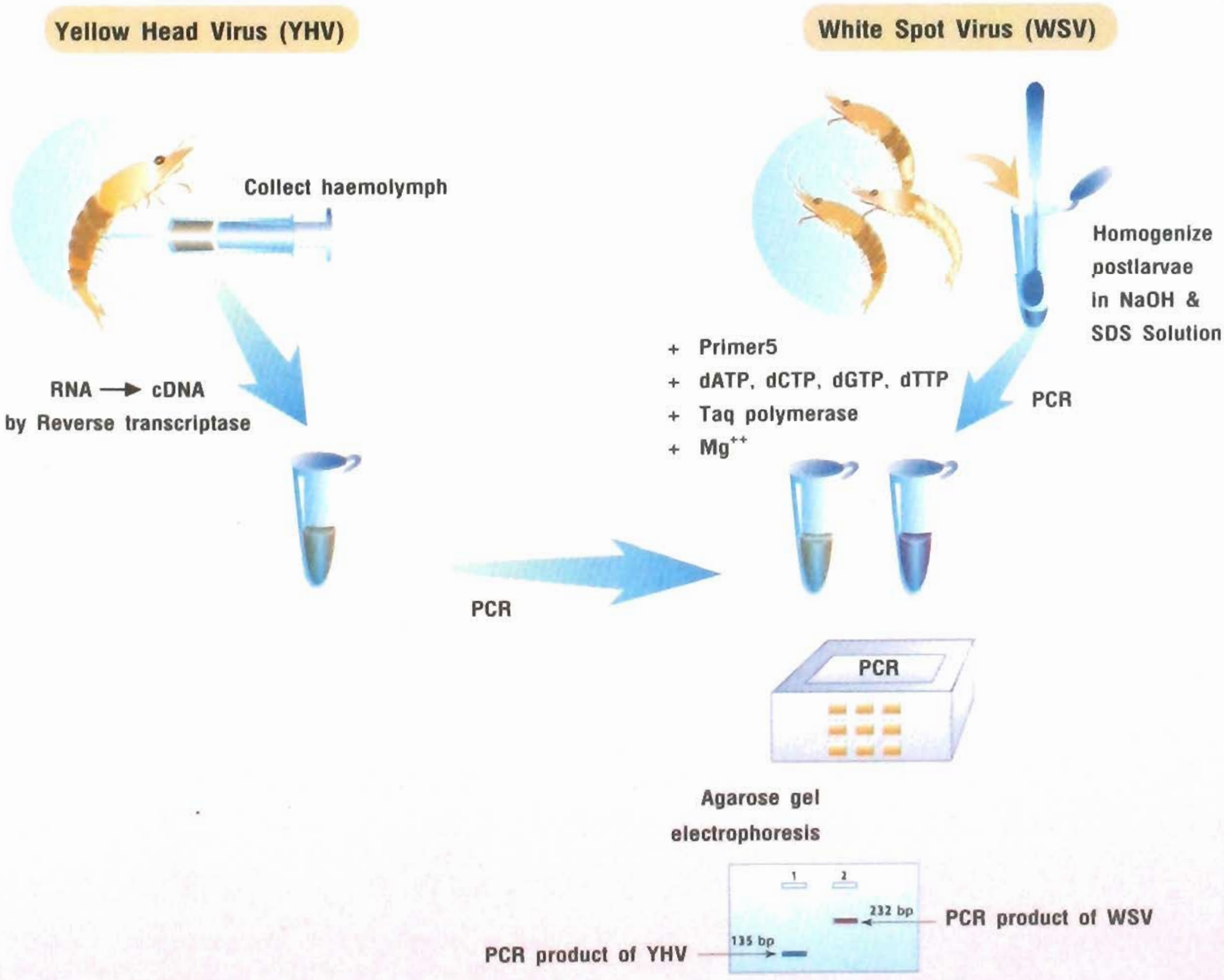
BIOTEC is also interested in the development of monoclonal antibody (Mab) techniques for shrimp disease detection and monitoring, because they may eventually be produced in simple kit formats that could be used by shrimp farmers. These would be

useful for rapid verification of disease outbreaks, but they would not serve as replacements for the very sensitive PCR techniques developed to screen carriers of the virus with very light infections. A successful Mab technique has recently been developed for YHV (Sithigorngul, 2000) and BIOTEC is supporting efforts to make it available and to realize the development of simple diagnostic kits. It is also supporting work for the development of similar reagents and kits for WSSV.

Onward Development of Techniques

The preceding descriptions are only examples of BIOTEC efforts in the realm of shrimp disease detection. Nor is the work final. Further studies are underway to refine the techniques to make them more rapid, more convenient and cheaper. For example, the normal PCR process is followed by detection procedures requiring the use of gel electrophoresis, staining with ethidium bromide and reading of results using UV-light. These steps are not convenient and are somewhat complicated, especially for the testing of large numbers of samples. Therefore, the research team has developed new methods for reading the result using a color reaction called PCR-ELISA that allows the results to be read by the naked eye.

Steps for Detection of Viruses by PCR



Additional work has also been done to improve the sensitivity of the PCR and RT-PCR detection techniques by a process called nested PCR amplification, all in a single PCR reaction vial. This can increase the sensitivity of the PCR process by 100 to 1000 times. Detection formats have also been developed for grading the severity of infections (e.g., light, moderate, heavy) by PCR. For example, the test developed for WSSV gives products of 1,100 bp, 526 bp, 250 bp and 143 bp in the following way. With heavy infections (+3), 4 distinct PCR products are obtained (1,100 bp, 526 bp, 250 bp and 143 bp), with moderate infections (+2), 3 different PCR products are obtained (526 bp, 250 bp and 143 bp), with light infections (+1), two products are obtained (250 bp and 143 bp) and with no infection (0) a single control product of 143 bp is obtained.

Yet another effort of BIOTEC supported scientists is to develop what are called "multiplex PCR" tests for the shrimp diseases important in Thailand. These techniques allow for simultaneous PCR detection of 2 or more targets in a single PCR reaction vial. By this new method, a lot of time and cost can be saved. For example, one multiplex PCR test has been developed for the simultaneous detection of WSSV (232 bp) and HPV (362 bp) by PCR and one for simultaneous detection of WSSV, HPV and monodon baculovirus (MBV) is being tested.

Technology Transfer and Support

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Diagnostic kit for detection of viruses in shrimp.

Realizing the need for implementation of research results, BIOTEC has an active program for publication or results and for training in the form of seminars and workshops. The molecular techniques described above have been widely applied in the Thai shrimp industry, largely as a result of these activities. In addition, BIOTEC has recently established a Shrimp Biotechnology Service Laboratory to further support the industry by making techniques and materials more easily and quickly available and by offering techni-

cal backup when necessary. This unit will take an active role in the commercialization of research results and the development of simple disease detection kits.

In 1998 and again in 1999, researchers working with BIOTEC on shrimp biotechnology applications were given the Golden Shrimp Award from the Suratthani Shrimp Farmer's Association in appreciation for their contributions to the development of the shrimp industry. These awards confirm the practical relevance of the research done and attest to the closed relationship between the scientists and the industry.

The Shrimp Culture Research and Development Company

View of a typical Thai shrimp farm.

An important milestone was achieved in 1996 when a five year effort of BIOTEC culminated with the establishment of the Shrimp Culture Research and Company Limited (SCRD), a government-industry consortium dedicated to the development of sustainable shrimp aquaculture. Currently, the most important program of SCRD involves the domestication and genetic improvement of shrimp stocks (Withyachumnarnkul, et al. 1998). The first domesticated stocks from this program were ready for pond production tests in 1999. For the genetic improvement part of the SCRD program, BIOTEC is also supporting advanced studies on DNA characterisation and DNA tagging of the shrimp stocks (Klinbunga, et al. 1998; Jarayabhand, et al. 1998; Vanavichit, et al. 1998). These studies are providing the tools that will be important for rapid genetic improvement strategies.

References

1. Chantanachookin C, Boonyaratpalin S, Kasornchandra J, Direkbusarakom S, Ekpanithanpong U, Supamataya K, Siurairatana S and Flegel TW (1993) Histology and ultrastructure reveal a new granulosis-like virus in *Penaeus monodon* affected by "yellow-head" disease. *Diseases of Aquatic Organisms* **17**, 145-57.
2. Cowley JA, Dimmock CM, Wongteerasupaya C, Boonsaeng V, Panyim S and Walker PJ (1999) Yellow head virus from Thailand and gill-associated virus from Australia are closely related but distinct prawn viruses. *Diseases of Aquatic Organisms* **36**, 159-61.
3. Flegel TW (1997) Special topic review: major viral diseases of the black tiger prawn (*Penaeus monodon)* in Thailand. *World J Microbiol Biotech* **13**, 433-42.
4. Flegel TW, Menasveta P and Paisarnrat S (eds) 1997a. Shrimp biotechnology in Thailand. National Center for Genetic Engineering and Biotechnology, Thailand, 244 pp.
5. Flegel TW, Boonyaratpalin S and Withyachumnarnkul B (1997b) Current status of research on yellow-head virus and white-spot virus in Thailand. *In* Flegel TW and McRae I (eds) Diseases in Asian Aquaculture III. Fish Health Section, Asian Fisheries Soc, Manila, pp 285-96.
6. Flegel TW, Sriurairatana S, Wongteerasupaya C, Boonsaeng V, Panyim S and Withyachumnarnkul B (1995) Progress in characterization and control of yellow-head virus of *Penaeus monodon*. *In* Browdy C and Hopkins S (eds) Swimming through Troubled Water; Proceedings of the special session on shrimp farming, Aquaculture '95, San Diego, February 1995, World Aquaculture Society, Baton Rouge, LA, pp 76-83.
7. Flegel TW, Thamavit V, Pasharawipas T and Alday-Sanz V(1999) Statistical correlation between severity of hepatopancreatic parvovirus (HPV) infection and stunting of farmed black tiger shrimp (*Penaeus monodon*). *Aquaculture* **174**, 197-206
8. Sukhumsirichart W, Wongteerasupaya C, Sriurairatana S, Boonsaeng V, Panyim s, Withyachumnarnkul B, Thamavit V and Flegel TW (1999) Characterization and PCR detection of hepatopancreatic parvovirus (HPV) from *Penaeus monodon* in Thailand. *Diseases of Aquatic Organism* 38, 1-10
9. Jarayabhand P, Uraiwan S, Klinbunga S, Tassanakajon A, Srimukda P, Pattanachan P, Panakulchaiwit R and Menasveta P (1998) Estimated heritabilities for early growth rate of the black tiger prawn, *Penaeus monodon*, Fabricius. *In* Flegel TW(eds) Advances in shrimp biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok, pp 67-70.
10. Klinbunga S, Penman DJ, McAndrew DJ, Tassanakajon A and Jarayabhand P (1998) Genetic variation, population differentiation and gene flow of the giant tiger shrimp (*Penaeus monodon*) inferred from mtDNA-RFLP data. *In* Flegel TW(eds) Advances in Shrimp Biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok, pp 51-59.
11. Rosenberry Bob (1996) World shrimp farming 1996. Shrimp News International, San Diego.
12. Rosenberry Bob (1995) World shrimp farming 1994. Shrimp News International, San Diego.

13. Rosenberry Bob (1994) World shrimp farming 1994. Shrimp News International, San Diego.
14. Vanavichit A, Wuthimaethavee S, Taechayinkphibool D, Kruthoo PS, Lumubol P and Tragoonrung S (1998) Development of simple sequence length polymorphisms in the black tiger shrimp (*Penaeus monodon*). *In* Flegel TW (eds) Advances in Shrimp Biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok, p 49.
15. Wongteerasupaya C, Vickers JE, Sriuraiwatana S, Nash GL, Akarajamorn A, Boonsaeng V, Panyim S, Tassanakajorn A, Withyachumnarnkul S and Flegel TW (1995a) A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in *Penaeus monodon*. *Diseases of Aquatic Organisms* **21**, 69-77.
16. Wongteerasupaya C, Sriurairatana S, Vickers JE, Anutara A, Boonsaeng V, Panyim S, Tassanakajon A, Withyachumnarnkul B and Flegel TW (1995b) Yellow-head virus of *Penaeus monodon* is an RNA virus. *Diseases of Aquatic Organisms*. **22**, 45-50.
17. Withyachumnarnkul B (2000) Results from black tiger shrimp *Penaeus monodon* culture ponds stocked with postlarvae PCR-positive or -negative for white-spot syndrome virus (WSSV). *Diseases of Aquatic Organism* **39**, 21-27.
18. Withyachumnarnkul B, Boonsaeng V, Flegel TW, Panyim S and Wongteerasupaya C (1998) Domestication and selective breeding of *Penaeus monodon* in Thailand. *In* Flegel TW(eds) Advances in Shrimp Biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok, pp 73-77.
19. Wongteerasupaya C , Wongwisansri S, Boonsaeng V, Panyim S, Pratanpipat P, Nash GL, Withyachumnarnkul B and Flegel TW (1996) DNA fragment of *Penaeus monodon* baculovirus PmNOBII gives positive *in situ* hybridization with viral infections in 6 penaeid shrimp species. *Aquaculture* **143**, 23-32.

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