



# SYNSEA Report

## The Development and Selection of Probiotics for Aquaculture

SYNBIO TECH INC.



*“Probiotic solutions for antibiotic free farming era”*





**W**ith the rapid development of countries, the global demand for sources of protein is continuously increasing. According to the statistics from FAO 2010, fish and other aquatic animals have become one of the most important sources of protein, accounting for 29% of animal protein consumed. A total of 90 million tons of fish and other aquatic organisms were captured, and an additional 56 million tons were produced by aquaculture. In addition, the feed conversion ratio of aquatic animals is better than that of terrestrial animals. Thus, FAO 2010 estimated that aquaculture would become the third leading source of protein by 2012. Furthermore, the aquaculture industry is developing by leaps and bounds, in part because of the stagnated growth of the wild capture fisheries worldwide, which is as an important economic industry. However, rapid growth and high demand for aquaculture fishery products have led to high-density farming and high morbidity of aquacultured organisms, which has consequently led to many issues, such as drug residue in organisms, bacterial resistance, and safety concerns regarding human ingestion of aquatic products. These problems not only undermine consumer confidence, but also obstruct the development of the aquaculture industry. Modern fisheries focus on sustainable development, and green farming will become essential. The aquaculture industry must continuously search for farming approaches to reduce medication and ensure animal health and

farmer profits. For example, probiotic feed additives that promote aquatic animal growth and increase immunity against diseases were estimated to have had a market value of approximately 7.49 million US dollars in 2015, and consequently has become the most sought after product. Nevertheless, an in-depth understanding on how to select an efficient probiotic product in the market is lacking. The following content will detail the requirements of aquatic probiotics for aquatic animals and explain the application of AquaProbiotics, which use SYNTEK® and were developed by SYN BIO TECH, INC., in the feeding of white shrimp.

### **The importance of probiotics in aquaculture**

Lactic acid bacteria as probiotics constitute a large group of beneficial bacteria in healthy aquatic animals. Probiotics contribute to gastrointestinal functions and the development of immunity. The intestine is the organ that is most significantly involved the support of immunity. The lack of the healthy microflora causes the host to die rapidly because of the ease of infection with diseases. Therefore, probiotics and the host organism represent a symbiotic relationship. Aquatic animals are mostly oviparous or ovoviviparous species. Their eggs are sterile, unlike newborn terrestrial animals in which there is a rapid colonization of bacteria obtained from their mother during birth. In recently hatched fish and shrimp, there are no microorgan-

isms in their intestines, gills, or skin because of the undeveloped intestinal system and immunity. This is the main reason the mortality of newly hatched aquatic larvae is high. Therefore, it is necessary to quickly build a healthy microflora for newly hatched aquatic larvae. The direct method is to apply probiotics via the environment and food to rapidly promote the development of the intestinal and immune systems. Consequently, establishing microbiota in both the water environment and the intestine of aquatic animals is the most important key point for successful aquaculture farming.

Probiotics are beneficial live microorganisms that improve and balance intestinal microflora when consumed, including lactic acid bacteria (e.g., *Bifidobacterium* spp.), yeast, and other bacteria (e.g., *Bacillus* spp.). The choice of aquatic probiotics focuses on their ability to exert antagonistic effects against pathogenic bacteria, enhance growth of aquatic animals, enzymatically hydrolyze organic substances for the host to absorb, degrade harmful

substances in residues and feces, improve water quality, increase and promote the immunity response of the host, and reduce farming loss. The desired capabilities of aquatic probiotic strains are different from the probiotic strains for terrestrial species, such as poultry and swine. Therefore, probiotics for aquaculture must comply with requirements of aquatic physiology and be adaptable to the aquatic environment.

### Fish-origin and patented lactic acid bacteria

For probiotics serving aquatic animals, the challenges are stricter than for probiotics in terrestrial animals; hence, not every probiotic strain can be used in aquaculture. As aquatic probiotics, they



Fig. 1. PP4012 improved body weight and growth rate of cobia infected by *Pdp*.

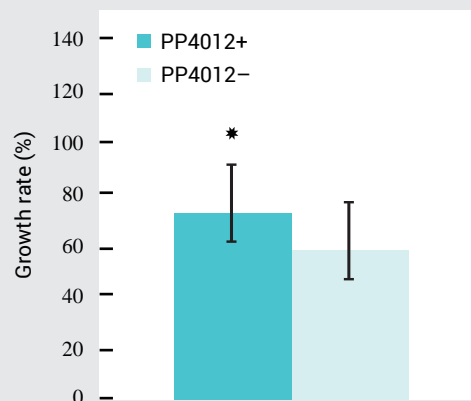
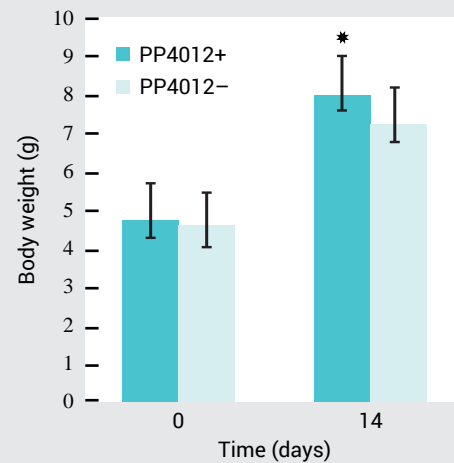
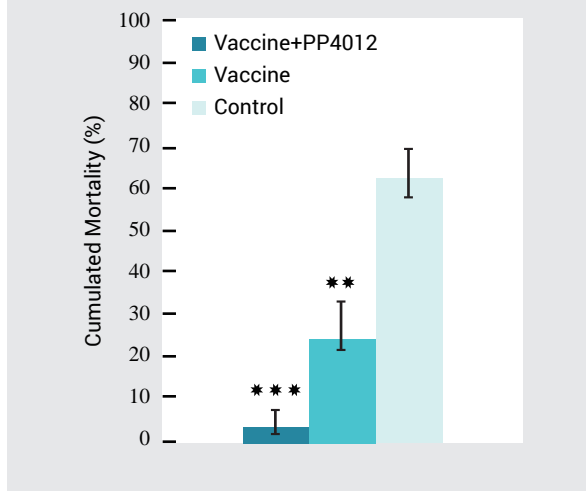


Fig. 2. PP4012 significantly decreased mortality rate of vaccinated cobia against Pdp challenge.



must be able to survive in high salinity, and tolerate large variation in temperature and the aerobic environment. In addition, they must tolerate the acid and bile salts in the gastrointestinal tracts of aquatic organisms, as well as adhere to the intestinal epithelium. Furthermore, they must be approved by field trials and challenge tests to ensure probiotic capabilities. Such a complicated screening and selection process has resulted in a few probiotic products of questionable origin available on the market. The qualified fish-origin probiotic strains are more adaptable for aquaculture. According to studies, *Lactobacillus plantarum* is an aquatic probiotic, which when applied in the water increased the survival rate of flatfish after hatching for two weeks thanks to its immunomodulation activity in the host. In addition, SYN BIO TECH, INC. cooperated with the National Penghu University of Science and Technology and National Taiwan University to develop a specific strain of *Pediococcus pentosaceus* PP4012 isolated from the intestine of cobia, which can inhibit the growth of *Photobacterium damsela* subsp. *piscicida* (*Pdp*) and *Vibrio anguillarum* that cause Early Mortality Syndrome (EMS). In a *Pdp* immersion challenge test, 2 weeks of feeding of PP4012 was adequate to significantly elevate average body weight by 10% and the growth rate by 12% (Fig. 1). When cobia were fed PP4012 with a *Pdp* vaccine, the antagonistic effect against pathogens was enhanced and resulted in remarkably reduced morbidity and mortality in

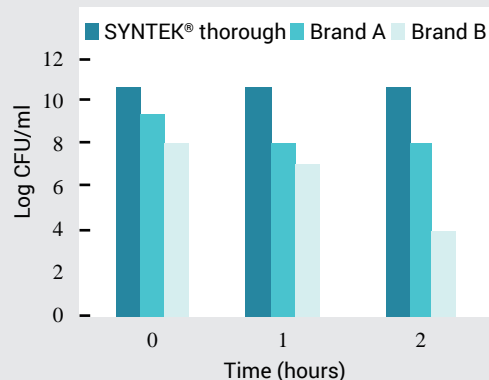
cobia (Fig. 2). The relevant studies of PP4012 have been published in international journals and protected by an invention patent.

### The SYNTEK® thorough probiotic optimizing development system

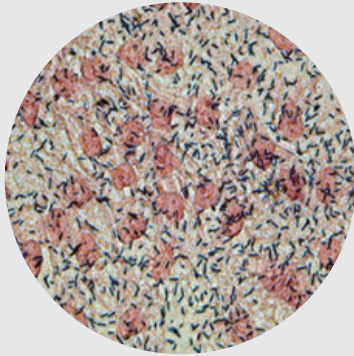
To produce products with the best performance, effective probiotic strains must be formulated using state-of-the-art production technology. SYN BIO TECH, INC. is well known as a leading biotech company specializing in research and manufacture of probiotics in Taiwan. We have our own culture collection and databank with outstanding microbiological experts and a research team that developed our core competency SYNTEK® thorough system, a probiotics optimizing development system, which selects the effective strain via an analytical platform of functionality, formulation of growth medium, and optimization of fermentation, cryoprotectant, and lyophilization conditions that systematically improves the properties of probiotics regarding acid/bile salt tolerance, adhesive ability on intestinal mucosal cells, immunomodulation, and storage stability (Fig. 3). Using the globally known strain *Lactobacillus rhamnosus* LGG as an example, the adhesive ability in the intestinal epithelium was enhanced from 30 CFU/cell to 73 CFU/cell after being optimized by SYNTEK® thorough (Fig. 3b). In addition, the stability of the strain is a substantial

Fig. 3. SYNTEK® thorough: analytical platform and tests

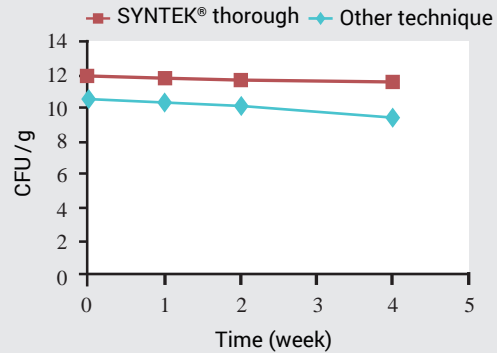
#### A. Comparison of acid tolerance of strain at pH 3.0 by different companies and techniques



B. SYNTEK® thorough promoted the adhesive ability on intestinal epithelial cells



C. Comparison of storage stability of strain at 37°C produced by different companies and techniques



concern of customers. It not only indicates the survival rate of the strain, but also represents the efficacy of product. Fig. 3 illustrates that in an accelerated test at 37°C for 4 weeks, which is equivalent to a stability test at room temperature for 12 months, the strain was more stable when it was produced by SYNTEK® thorough. The minimum cell count of 100 billion CFU/g provides cost savings benefits to the customer, as well as new product development. Using the SYNTEK® thorough system, SYNBIOTECH, INC. selected specific strains with various functions, including PP4012 from cobia, *Lactobacillus plantarum* LP28 for immunomodulatory benefits, three strains of kefir origin produced by symbiotic fermentation for enhancement of the absorption of nutrients, and *Bacillus subtilis* for degradation of organic substances. These strains were formulated into the SYNSEA™ series of products for aquatic animals, including SYNSEA™ FeedAd applied as a feed additive, and SYNSEA™ Clean for the improvement of water quality. Thus, the products are applicable for different stages of the farming process.

### Field trials with white shrimp

In a pond with mixed farming of milkfish (*Chanos chanos*) and white shrimp, the shrimp diet was mixed with SYNSEA™ FeedAd, and water was fermented for one day and fed to 500,000 shrimps. In the trial, the researcher used an umbrella fishing net to observe shrimp intake for 48 days, after which

the shrimps were captured. All data were compared with data collected over the last two years. Fig. 4 shows that feeding shrimp SYNSEA™ FeedAd significantly increased profits for the fisheries in terms average weight of the first capture of shrimp, which increased from 9% to 28%, growth cycle, which decreased by 29 days, as well as survival rate and body weight, which were more than double that of the previous years.

To summarize the discussion above, when aquatic animals are newly hatched or in the larval stage, the intestine, which is the largest immunity organ, is undeveloped. This is the period of highest loss during the entire farming process because the young aquatic animals are highly sensitive to the environ-

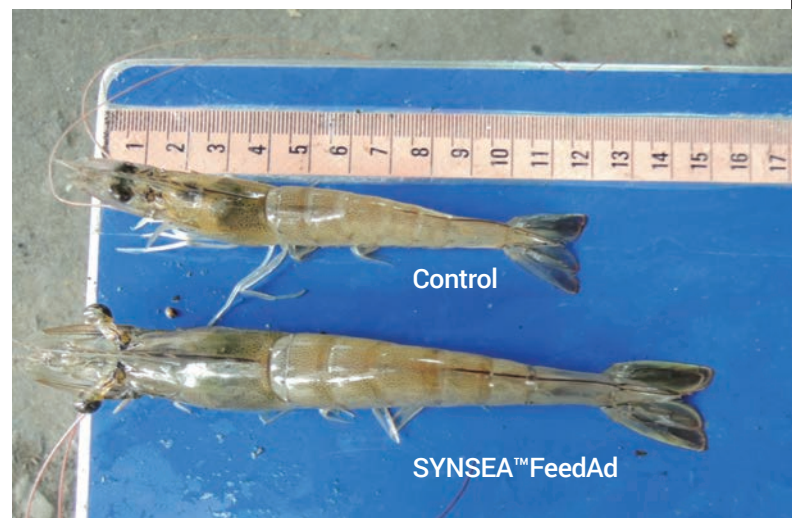
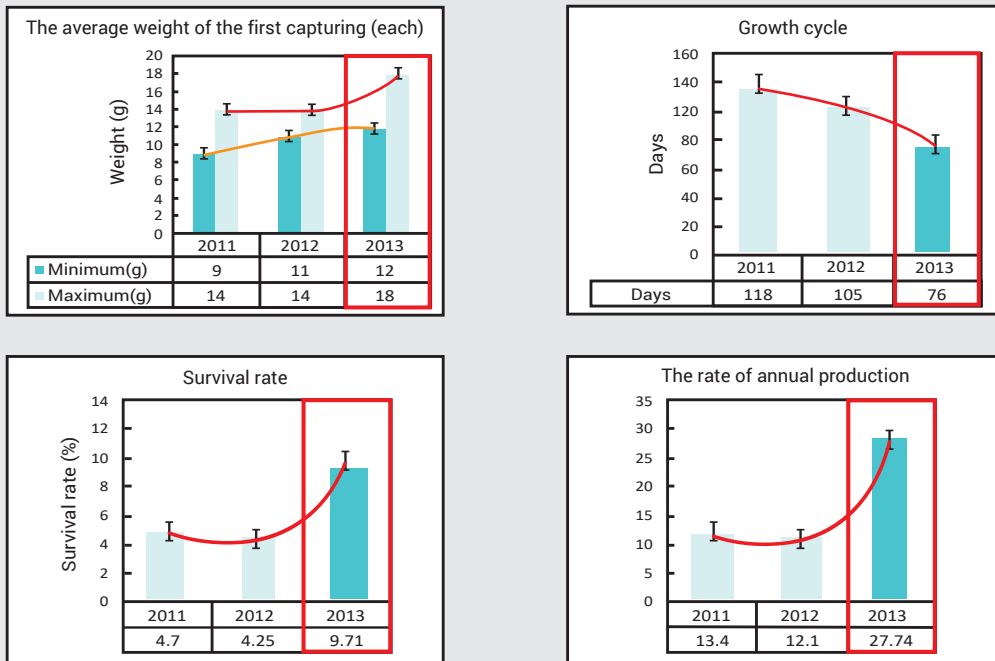


Fig. 4. Comparison of farming performance over the few years on white shrimp farming with SYNSEA™ FeedAd; □ feeding with SYNSEA™ FeedAd



ment and are not protected by a comprehensive immune system. A suggestion to solve this problem is to build resistance to diseases in the host and promote growth via probiotics. Probiotics can modulate immunity, establish dominant microflora, and improve the farming environment. The selected probiotics for aquaculture should be validated by strict selection and evaluation criteria, including identification of specific strains, product quality, and functionality. SYN BIO TECH, INC., is devoted to

research, development, and manufacture of aquatic probiotics and with the unique SYNTEK® thorough can perform screening for effective strains, functionality analysis, and production processes to enhance probiotic value, and meet the demand for low-cost farming. SYN BIO TECH, INC., established this remarkable model in the market to provide the aquaculture industry the best choice of probiotic products.



## SYN BIO TECH INC.

No. 66, Beiling 6<sup>th</sup> Rd., Luzhu Dist., Kaohsiung City 821, Taiwan  
[www.synbiotech.com](http://www.synbiotech.com) | **Email:** [service@synbiotech.com.tw](mailto:service@synbiotech.com.tw)  
**Tel:** +886-7-6955680 | **Fax:** +886-7-6955713

