ALLIANCE™
(https://www.globalseafood.org)

Responsible Seafood ADVOCATE

Health & Welfare

Effect of a microencapsulated probiotic on the intestinal microbiome of Pacific white shrimp

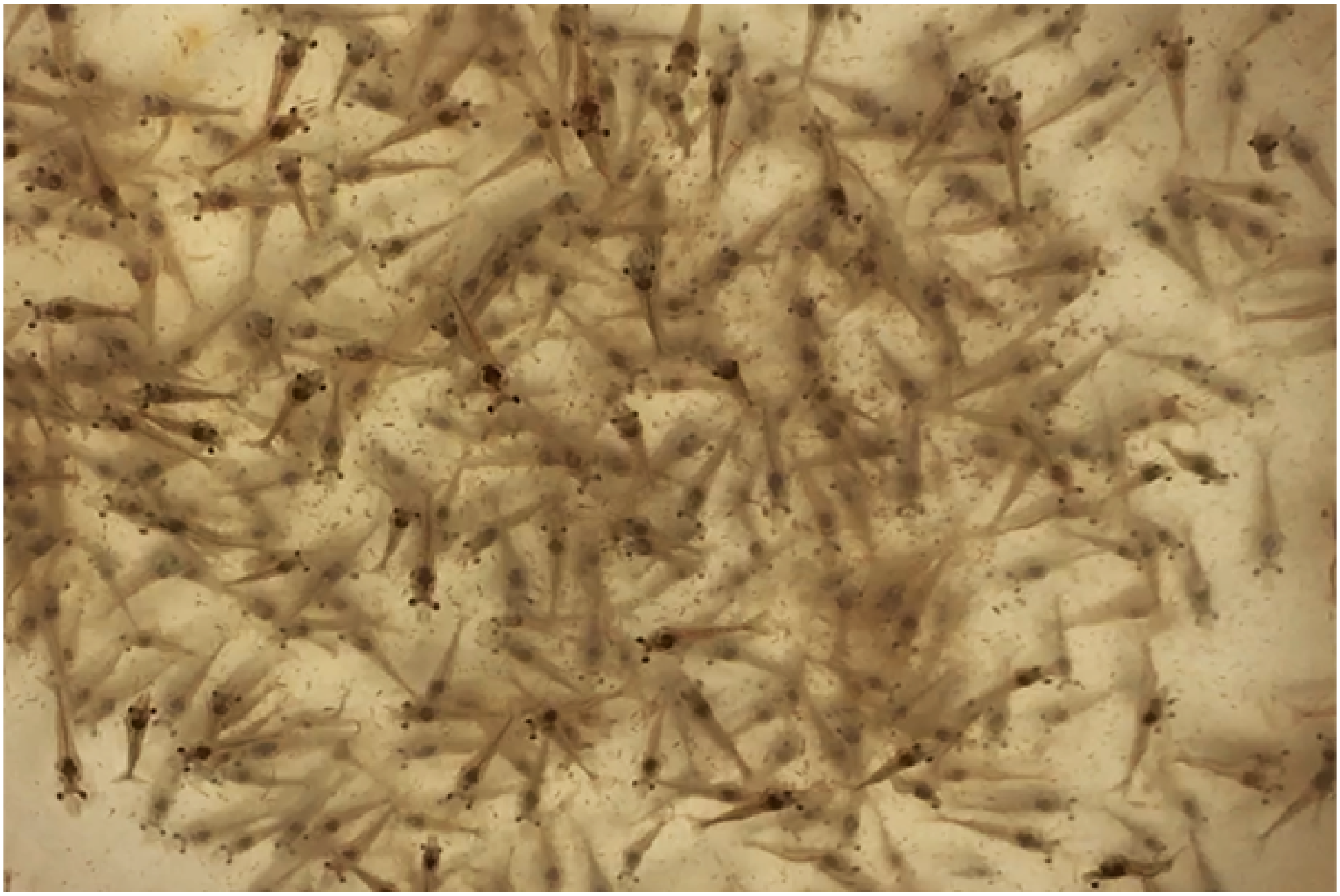
21 August 2023
By Chung-Hung Liu

Microencapsulation of *Bacillus subtilis* E20 proved effective in addressing probiotic issues related to inclusion during feed manufacturing

Several studies have reported the benefits of including probiotics in shrimp diets and modifying the bacterial profile of the shrimp intestine. Despite the benefits reported, it is believed that the direct administration of live probiotics reduces their cell viability, undermining the full potential of the probiotic. In particular, the sensitivity of probiotic bacteria to heat limits its application in the shrimp feed manufacturing processes, which use high temperatures.

Encapsulation techniques such as spray drying, freeze drying, and electrodynamics are deemed effective strategies to permit high viability and provide a high degree of protection against processing, storage and gastrointestinal conditions. These techniques control the release of probiotics in the intestine to exert modulatory effects on gut microbiota.

The application of next-generation sequencing (NGS; a technology for determining the sequence of



Results of this study showed that microencapsulation was effective in addressing probiotic issues related to inclusion during feed manufacturing, that encapsulated B*. subtilis* E20 administration increased beneficial strains of bacteria such as *Bacillus* and reduced the harmful bacteria belonging to the *Vibrio* species, and that encapsulation of *B. subtilis* E20 has the potential to modulate gut microbiota and control *Vibrio* species in shrimp. Photo by Darryl Jory.

DNA or RNA to study genetic variations associated with various biological phenomena; it allows the sequencing of many DNA strands at the same time, instead of one at a time as with traditional sequencing) techniques for shrimp can help elucidate shrimp-bacteria interaction. To date, no studies have specifically addressed the effects of encapsulated probiotics on the composition, diversity and function of microbiota in shrimp.

This article – summarized from the **original publication** (https://doi.org/10.3390/fishes8050264) (Cheng, A-C. et al. 2023. Microencapsulation of *Bacillus subtilis* E20 Probiotic, a Promising Approach for the Enrichment of Intestinal Microbiome in White Shrimp, *Penaeus vannamei. Fishes* 2023, 8(5), 264) – reports on a study that analyzed the microbiota associated with the intestine of encapsulated probiotic-fed Pacific white shrimp (*Litopenaeus vannamei*) and unencapsulated-fed shrimp using Next-Generation Sequencing (NGS) of 16S ribosomal RNA (16S rRNA; it is the most widely used marker gene in microbial ecology).

Study setup

L. vannamei shrimp were obtained from the Department of Aquaculture at the National Pingtung University of Science and Technology, in Pingtung, Taiwan. Before the study, shrimp at intermolt stage were acclimated for seven days in 10-cubic-meter cement tanks holding 5 tons of seawater at 20 ppt salinity and air stones for aeration. Then, 200 juvenile shrimp (1.89 ± 0.06, mean ± SE), with all appendages in good condition, were distributed into two cement tanks (6 × 2 × 1 m).

Shrimp were allocated to two dietary treatments (n = 100 each), one being a control and the other being the encapsulated probiotic, *B. subtilis* E20 (EP). The probiotic *B. subtilis* E20 was encapsulated in alginate-chitosan bilayer microparticles following a **published procedure** (https://doi.org/10.1016/j.foodres.2007.11.001). Diet formulations using the encapsulated probiotic at 10^7 CFU/kg (EP), and a basal control diet was prepared and fed to shrimp for 60 days. The experimental diets were prepared based on our previous study’s diet with the highest growth performance and improved health status.

For detailed information on the experimental design, animal husbandry, data collection, intestinal microbiota analysis using Next-Generation Sequencing, and biodiversity and abundances of intestinal microbiota determinations, refer to the original publication.

Results and discussion

The shrimp intestinal microbiome consists of several microbes and genes critical for health, metabolism, as well as disease pathogenesis. As shrimp are intimately connected to the aquatic environment, much of their intestinal microbes are influenced by the microbes present in the surrounding environment. Consequently, culture systems that are either intensive or unfavorable adversely affect the microbial interaction between the shrimp and the environment, resulting in the proliferation of opportunistic pathogens that cause disease outbreaks.

Live probiotic bacteria – which are generally regarded as safe due to their immunomodulatory, antimicrobial, and antioxidant beneficial effects – are often incorporated into feeds as dietary supplements to maintain the microbial balance in shrimp gut. However, the viability of probiotics is greatly affected by numerous factors, especially during production, storage, feeding and passage through the gastrointestinal system. Several techniques for microencapsulation have been reported to preserve and protect the viability of probiotic cells

While most of these studies focused on the immune response and growth performance as well as intestinal microbiota upon live probiotic administration without encapsulation, studies on the intestinal microbiome upon administering encapsulated probiotics are limited.

In this study, the *B. subtilis* E20 strain was encapsulated with alginate-chitosan to protect cell viability and determine the bacterial communities generated. Data from NGS analysis revealed a dominant presence of Proteobacteria in all shrimp microbiota. Other researchers reported similar results when *B. subtilis* E20-fermented soybean meal (FSBM) was provided to shrimp.

These results suggest that microencapsulation of *B. subtilis* E20 (EP) can induce the proliferation and diversification of bacteria in shrimp microbiota.

The taxonomical analysis revealed that the majority of the bacterial genera were distributed among different families (193), with shrimp fed the control diet indicating a higher number of genera (275) than the shrimp fed the EP diet (236) (Fig. 1). Among the intestinal samples in the control and EP group, the shared bacterial genera were 89 and 67, respectively.

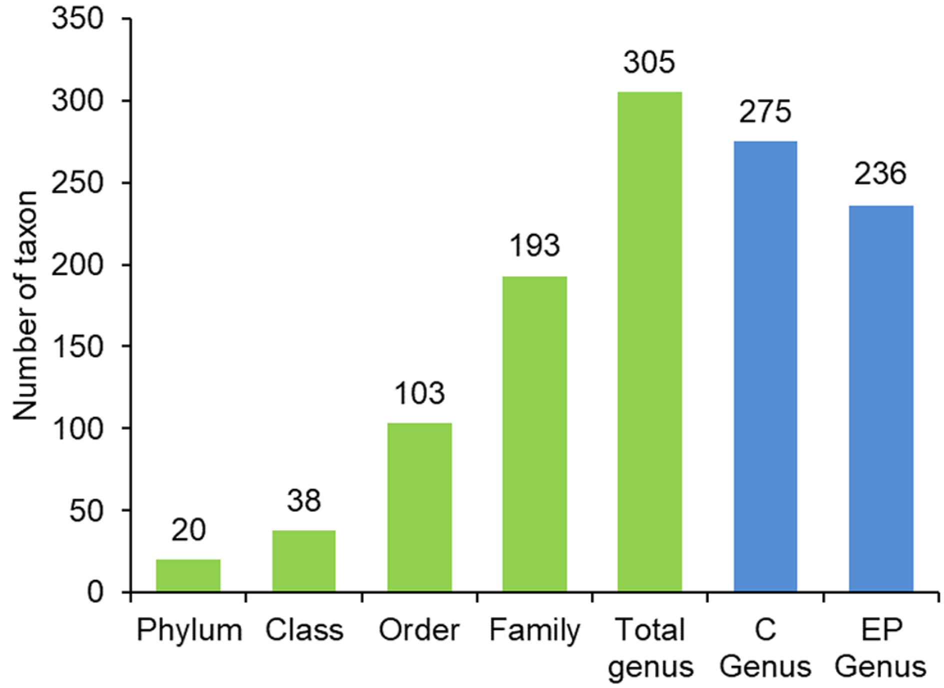


Fig. 1: Taxonomic identification of the intestinal microflora of white shrimp fed with the control (C) and the encapsulated-probiotic B. subtilis E20 (EP) diet.

At the phylum level, the relative abundance of bacterial groups in the intestinal microbiota of shrimp fed the control and EP diet was predominantly *Proteobacteria* at 85.24 percent and 63.13 percent, respectively. The EP diet was further influenced by the phylum *Tenericutes* (12.96 percent), *Bacteroidetes* (10.80 percent) and *Firmicutes* (10.68 percent), all of which were minimally expressed in the control group. In shrimp fed the control diet, the most abundant at a generic level were *Vibrio* (70.74 percent), compared to the EP group, which had a lower *Vibrio* abundance of 30.25 percent.

Generally, the health condition of shrimp and fish can be reflected by the relative abundance of *Proteobacteria*, which is a microbial sign of dysbiosis (an imbalance of microbial species and a reduction in microbial diversity within certain body microbiomes) and disease in gut microbiota. *Tenericutes* are free-living organisms and exhibit metabolic and adaptivity flexibility commensal to the host. *Firmicutes* help ferment carbon sources and control energy balance within the host.

Similarly, *Bacteroidetes* ferment plant-derived substrates in the intestines by producing short-chain fatty acids (SCFAs) that allow the host to obtain excess energy; SCFAs also play major roles in the homeostasis of immune cells in several organisms. Therefore, the interaction between *Firmicutes* and

Bacteroidetes likely promoted more efficient fermentation of carbohydrates in the diet and increased the energy absorption in the intestine of shrimp fed with encapsulated *B. subtilis* E20.

Shrimp feeds and management: Golden rules for success

With decreasing prices and increasing raw-material costs, efforts to reduce shrimp feed costs and improve management top the agenda for most farmers.

Global Seafood Alliance

In addition, *Vibrio* species are among the dominant members of the *L. vannamei* shrimp microbiota and are considered the most important bacterial pathogens responsible for several diseases and mass mortalities. Several studies have reported the importance of *Vibrio* during the different developmental stages of shrimp. *Vibrio* populations in the shrimp gut microbiota are typically higher during the nursery stage than in the adult stage, indicating that the microbiota in the latter stage is more diverse than in the nursery stage.

Vibrio species are considered opportunistic pathogens that have detrimental effects on shrimp's growth, metabolic activity, microbial balance and immune response. High expression in the gut is an indicator of disease in shrimp. For example, in infected shrimp, *V. parahaemolyticus* increases intestinal permeability and impairs the ability to absorb the amino acids and glucose that are necessary for maintaining physiological activities.

The supplementation of probiotics is a beneficial biocontrol agent for reducing *Vibrio* counts and preventing vibriosis. In this study, by analyzing the microbial community we determined that the *Vibrio* count and abundance levels of *Vibrio* species were suppressed in the intestine of shrimp fed the encapsulated *B. subtilis* E20 compared to shrimp fed the control diet.

The microencapsulated probiotic in our study also increased the abundance of *Candidatus Bacilloplasma* in shrimp. *Candidatus Bacilloplasma* are the dominant bacteria in the intestines of healthy shrimp, and a change in its abundance can contribute to shifting the intestinal microbial communities in shrimp suffering pathogens. *Candidatus Bacilloplasma* are recognized as symbionts (two organisms of different species that have a close and long-term biological interaction) and can be used as potential taxonomic indicators for assessing the health status of shrimp.

In previous studies, the detection of *Candidatus Bacilloplasma* showed commensal activities which inhibited the proliferation of *Vibrio* bacterial strains and infection. The greater expression of *Candidatus Bacilloplasma* in this study suggests that the encapsulation of probiotics can preserve their viability to such an extent that it stimulates the growth of various beneficial bacteria that might be lost when the probiotic is unencapsulated.

Perspectives

This study concludes that microencapsulation of *B. subtilis* E20 can be helpful in tackling the sensitivity problems associated with probiotics during processing and application. Our results indicate that encapsulated *B. subtilis* E20 administration increased beneficial strains of bacteria such as *Bacillus* and reduced the harmful bacteria belonging to the *Vibrio* species. Thus, the encapsulation of *B. subtilis* E20 has the potential to modulate gut microbiota and control *Vibrio* species in shrimp.

Author

CHUNG-HUNG LIU

Corresponding author
Department of Aquaculture, National Pingtung University of Science and Technology, Pingtung 912301, Taiwan

chliu@mail.npust.edu.tw (mailto:chliu@mail.npust.edu.tw)