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The power of postbiotics: Natural defenses for better shrimp yields

Postbiotics do not contain live microbes but still provide strong functional benefits for vannamei shrimp post larvae

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For decades, yeast and bacteria, either in their live form (probiotics) or as components (prebiotics), have been used to enhance performance and improve survival in both terrestrial and aquatic animals. Recently, there is growing attention on postbiotics - inactivated, fermented forms of yeast and bacteria - which have often demonstrated superior results compared to traditional pre- and probiotics.

Postbiotics explained

These are health-promoting compounds created during the controlled fermentation of beneficial microorganisms such as bacteria (*Lactobacillus* and *Bifidobacterium*) and yeast (*Saccharomyces cerevisiae*). Unlike probiotics, postbiotics do not contain live microbes but still provide strong functional benefits.

Postbiotics are produced in several steps, beginning with selecting specific strains of microorganisms with proven ability to produce desirable bioactive compounds during fermentation. These microbes are introduced into a nutrient-rich culture medium (usually containing carbohydrates, amino acids, vitamins, and minerals) and placed in bioreactors under carefully controlled conditions (temperature, pH, and oxygen levels etc). During fermentation, these microorganisms metabolise the nutrients in the medium, breaking down sugars and other substrates through enzymatic activity. This metabolic activity results in the production of a wide range of beneficial compounds, including short-chain fatty acids (e.g. butyrate and acetate), antimicrobial peptides, exopolysaccharides (EPS), enzymes, vitamins, and cell wall components such as peptidoglycans and lipoteichoic acids.

The above compounds are the key functional ingredients in postbiotics. Often during or after fermentation, microorganisms undergo additional induced stresses (oxygen and nutrition depletions, space competition, heat treatment) which will stimulate the microorganisms to produce and release their various defense molecules in large quantities (such as up to 5% EPS in the medium of fermented *Lactobacillus*).

At the optimal point of fermentation, microorganisms are deliberately inactivated, typically through heat treatment or pressure. This step ensures that no live microbes remain, which enhances product stability and eliminates the risks associated with live microbial supplementation.

Postbiotics from yeast versus bacteria

It is clear that the defense mechanisms between yeast and bacteria are very different and therefore the effects of postbiotics from yeast or bacteria when applied in feeds are also very different.

The first line of defense of yeast cells (particularly *S. cerevisiae*) is a robust cell wall comprising β-glucans, mannoproteins, and chitin which acts as a first protective barrier. Additionally, yeast can initiate autophagy or programmed cell death to limit viral spread within the population.

Another defense mechanism of yeast is through the production of heat shock proteins (HSPs) which help yeast survive stress from bacterial toxins or viral infections by refolding damaged proteins. A last defense mechanism is through the production of antioxidant enzymes (like superoxide dismutase and catalase) to neutralise reactive oxygen species (ROS) that might be produced during bacterial attacks.

In contrast, bacteria have developed different defense mechanisms which can be categorised into structural, chemical and genetic strategies.

Some defenses are similar to those of yeasts' competitive nutrition exclusion and space resource competition. Chemical defenses are antimicrobial compound production such as bacteriocins, antibiotics and acids (organic acids)/ hydrogen peroxide. Furthermore, they produce proteases, lysozymes and peptidoglycan hydrolases.

Another powerful strategy is the genetic defense strategy (CRISPR-Cas system) which bacteria use to recognise and destroy foreign genetic material from bacteriophages (viruses that infect bacteria). This system allows bacteria to develop immunity against past viral infections by storing viral DNA fragments and using them to target future invaders. Another genetic defense strategy is the use of restriction enzymes to cut foreign DNA, such as bacteriophage genomes, while protecting their own DNA with specific methylation patterns (restriction-modification systems). These defense strategies allow bacteria to out-compete rivals, and resist infections, and adapt to changing environments, ensuring their long-term survival in diverse ecosystems.

Biofilm protection

Bacteria have a strong defense mechanism through the production of long-chain sugar polymers (EPS) which are key components of biofilms. These biofilms create a protective environment that shields the bacteria from environmental stresses (like pH, salinity, antibiotics, or immune responses) and have antimicrobial effects, by physically blocking pathogens or by directly inhibiting them. Even after bacterial inactivation (as in postbiotics), EPS and biofilm fragments retain bioactivity. Both post and probiotics of *Lactobacillus farciminus* show a strong co-aggregation with various pathogens (Figure 1).

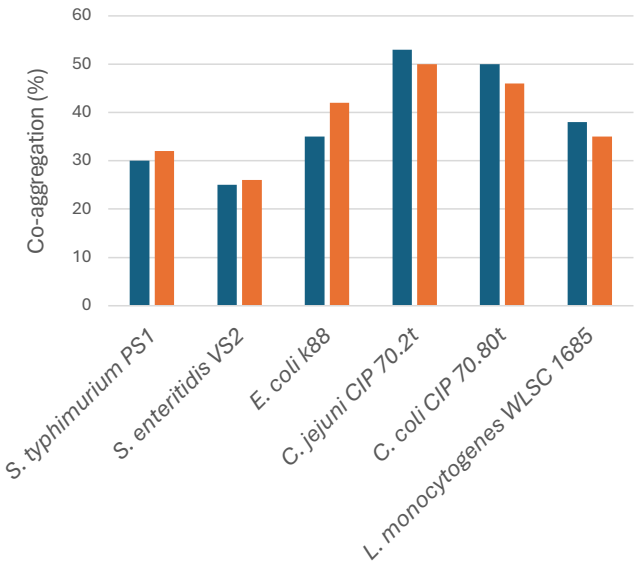

Figure 1. Co-aggregation capacities of the probiotic form (dark blue) and the postbiotic form (orange) of *Lactobacillus farciminis* CNCM-I-3699 with different pathogens.

Postbiotics in white shrimp

In a series of trials, we demonstrated the effectiveness of the postbiotics Metalac (STI Biotechnologie, France) in the white shrimp *Penaeus vannamei*. This postbiotic product is produced through the double fermentation of two strains of *Lactobacillus* (*Lactobacillus rhamnosus* and *L. farciminis*). It has been shown to significantly boost shrimp performance, enhance immune parameters, increase pathogen agglutination, and improve resilience to environmental stress.

Four trials in 2012 (Orapint, 2012) showed that this postbiotic strongly improved the resistance of shrimp against environmental stresses. This is important given that strong and sudden rains are often accompanied by rapid changes in temperatures, particularly within Southeast Asia. In these trials, two groups of *Penaeus vannamei* post larvae were fed from PL5 to PL30, a control diet or the control diet enriched with 1kg of Metalac per tonne of feed. Post larvae were subjected to brusque challenges - low and high temperature, low and high salinity, and high formalin (Figure 2). Average survival was higher by 20%.

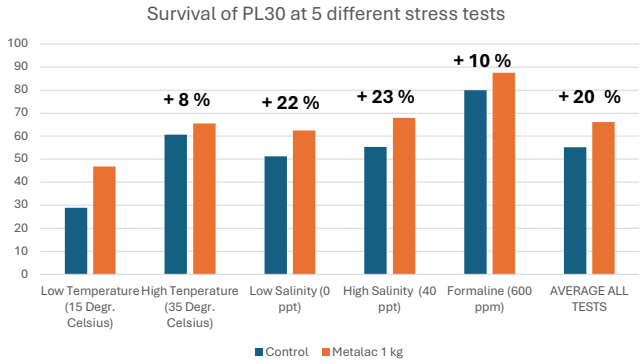

Figure 2. Survival of *Penaeus vannamei* post larvae PL30 in five different environmental challenge tests: Low temperature, dropping from 29°C to 15°C; high temperature, increasing from 29°C to 35°C; low salinity, changing from 25 to 0ppt; high salinity, changing from 25 to 40ppt and formalin at 600ppm (Orapint, 2012).

In a recent trial, post larvae were fed Metalac (0.5kg/tonne feed) over 4 weeks and the effect of the postbiotic supplemented feed on growth, gut microbiome and shrimp immunity were evaluated. Shrimp immune parameters included total haemocyte count (THC), differential haemocyte count (DHC), bacterial agglutination, phagocytosis, and mRNA expression of prophenoloxidase enzyme.

The results showed that post larvae fed diets supplemented with the postbiotic were 14% larger (4.16±0.6g) than for the control group (3.64±0.6g). Although there were no significant differences in THC and DHC, (Figure 3), shrimp fed with this postbiotic demonstrated the significant induction of haemocyte phagocytic activity and haemocyte phagocytic index ($p<0.01$) (Figure 4 and 5).

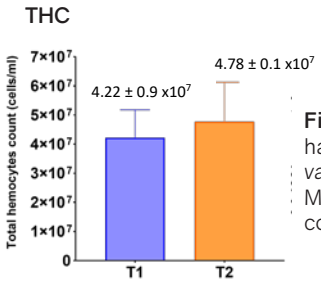

Figure 3. Changes in total haemocyte count (THC) in *Penaeus vannamei* post larvae fed with Metalac-supplemented feed (T2), compared to control group (T1).

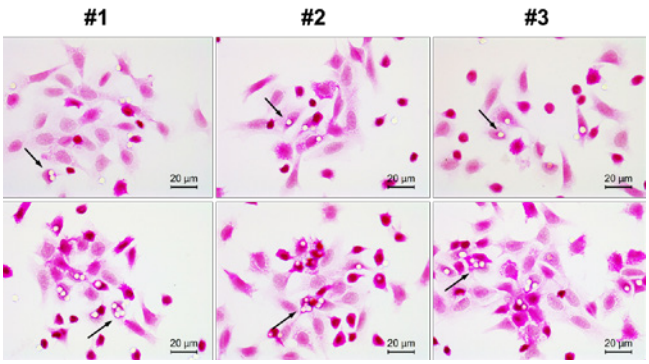

Figure 4. Images represent haemocyte phagocytic activity in shrimp fed with different feeds. High numbers of both bead-ingested haemocytes and number of bead ingesting (arrows) were mainly observed in T2 group. Phagocytic activity and index in white shrimp fed without (T1) and with Metalac (T2).

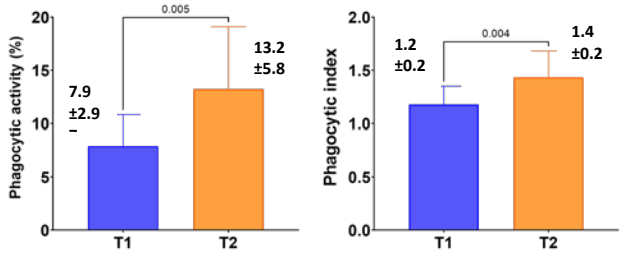

Figure 5. Phagocytic activity and phagocytic index in two groups of shrimp (T1; control; T2 Metalac).

The bacterial agglutination activity was tested both *in vivo* (in haemocyte lysate supernatant, HLS) of Metalac-fed shrimp and *in vitro* (direct incubation of Metalac with the bacterial cells). The bacterium used in the study was *Vibrio parahaemolyticus* 5HP (VP-AHPND strain, Joshi et al., 2014). Agglutination was observed with a light microscope. The postbiotic supplemented group (T2) with visible microbial agglutination compared with the non-supplemented group (T1), is shown in Figure 6.

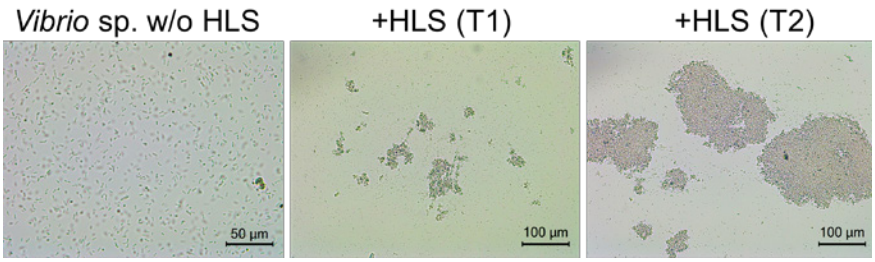

Figure 6. Bacterial agglutination in the haemocyte lysate supernatant (HLS) of the white shrimp in non-Metalac (T1) and Metalac-supplemented group (T2). At the same HLS dilution, a high degree of bacterial clumping was observed in HLS of T2.

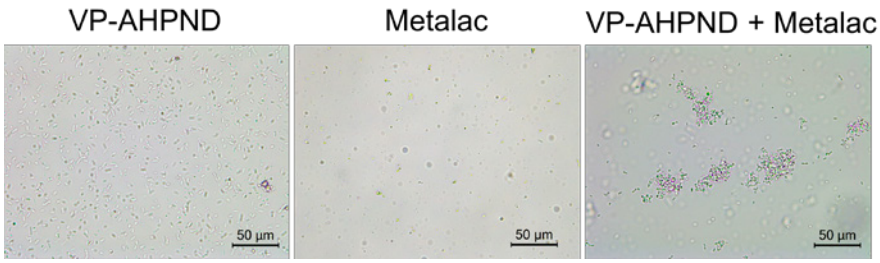

Figure 7. Bacterial agglutination. The bacterial suspension was incubated with Metalac and the agglutination was observed under light microscopy. VP-AHPND agglutination could be observed when incubated with Metalac.

The *in vitro* effect of postbiotic inclusion on VP-AHPND agglutination was demonstrated as shown in Figure 7. Agglutination activity was not observed for VP-AHPND suspension without the postbiotic.

The prophenoloxidase (proPO) system is crucial for innate immunity, particularly against pathogens, working in concert with cellular mechanisms like phagocytosis, and it plays a vital role in melanisation and pathogen clearance (Amparyup et al., 2013). Shrimp fed with postbiotic added feed showed high expression of proPO transcript suggesting the elevation of the proPO system (Figure 8).

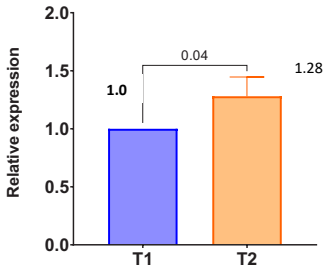

Figure 8. Quantitative PCR analysis showed significant up-regulation of prophenoloxidase (proPO) in shrimp haemocytes fed with non-Metalac group (T1) and Metalac group (T2). Vertical bars represent the mean ± SE (N=3).

In this study, shrimp fed with T2 with 0.5kg/tonne of feed of the postbiotic exhibited significant effects on agglutination of VP-AHPND cells. In addition, they showed high phagocytic activity and phagocytic index.

We conclude that postbiotics from *Lactobacillus* show interesting potential in shrimp farming by reducing bacterial challenges and environmental stress.

References

Orapint Jinatsataporn et al., 2012. Effects of Heat-Inactivated *Lactobacillus* on growth performance and stress resistance in white shrimp (*Penaeus vannamei*) post larvae. Study 1,2,3,4. Internal report.

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