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Glucans and disease resistance

Aquaculture Department, Southeast Asian Fisheries Development Center

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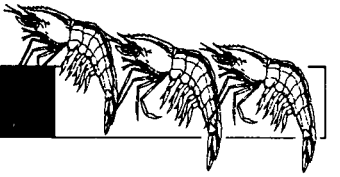


Glucans and disease resistance

Crustaceans have both cellular and humoral defense systems. Hemocytes, phagocytes, nodulation and encapsulation are part of the former. Phenoloxidase, pro-phenoloxidase activating system, bactericidin, and lectins are part of the latter.

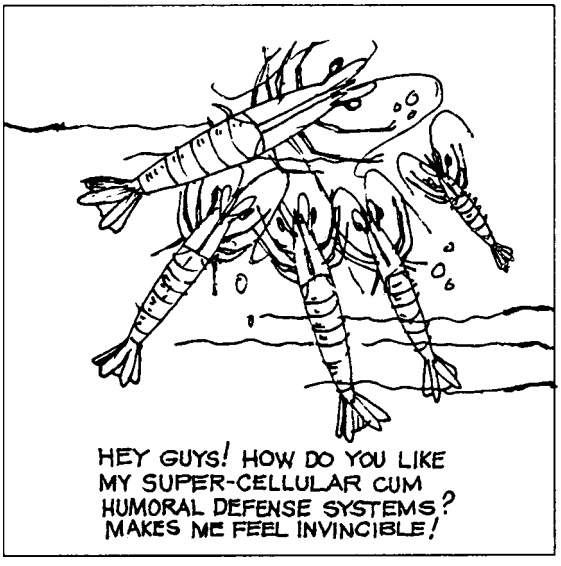
These defense factors "cooperate" together to provide a defense barrier against invading pathogens. They normally construct an elaborate network, and can be damaged when the organism is stressed under culture conditions (high stocking density, environmental pollution).

Beta-glucans (like insoluble beta-1,3 and 1,6-linkage polyglucose) have been successfully used as immunostimulants for strengthening non-specific defense systems of a wide range of animals. Beta-glucans are the most important structural elements of cell walls of fungi.

In mice, glucans enhance non-specific antimicrobial activity as well as anti-cancer mechanisms. In fish, beta-glucans activate cytotoxic macrophages, lymphocytes, natural killer cells, complement-mediated hemolytic activity, and the complement system through the alternative pathway. In addition, beta-glucans strengthen the non-specific disease resistance of salmon. Long-term administration of peptiglycan may enhance the disease resistance and growth of juvenile rainbow trout.

In crustaceans, glucans activate hemolymph clotting in the horseshoe crab and activate the prophenoloxidase system, causing increases in such activations as phagocytosis and encapsulation, both of which are associated and protective reactions in crayfish.

Based on previous studies involving crustaceans, the protection provided by glucans is possibly due, in part, to its activation of the prophenoloxidase (proPO) system. The proPO system represents the terminal component of a complex cascade of enzymes which function in non-self recognition and host defense; these mechanisms include bactericidal activity and phagocytosis. The proPO system is present in the blood of a wide range of marine invertebrates, especially crustaceans.



Testing a beta-glucan

Researchers from the National Taiwan University tested the effects of beta-glucans on the vibriosis resistance of tiger shrimp *Penaeus monodon*. They immersed 30-day old hatchery-produced shrimp postlarvae (at 100 shrimp per liter) in aerated beta-glucan suspensions for 3 hours. The beta-glucan used was insoluble beta-1,3 and 1,6-linkage polysaccharide that was extracted from the cell walls of the fungus *Saccharomyces cerevisiae*. The glucan concentrations tested were 0.25, 0.5, 1.0 and 2.0 mg per ml of pond water. Control shrimp were immersed in glucan-free pond water.

Following immersion, shrimp were kept in aerated pond water and fed commercial feed three times per day. Around 15-20 shrimp sam-

ples were collected and challenged with viable *Vibrio vulnificus* via water-borne infection at 10, 18, and 43 days following beta-glucan treatment. The shrimp were immersed for 12 hours in either 600 or 800 ml of bacterial suspension at a concentration of 5 x 10^7 colony-forming units per ml.

The researchers noted that shrimp immersed in 0.5, 1.0 and 2 mg per ml beta-glucan suspension grew faster than the 0.25 mg per ml suspension or the control. But gill tissue became noticeably shrunken immediately following immersion in 2 mg per ml; this concentration is hyperosmotic to shrimp.

The researchers also noted these results:

percent mortality of tiger shrimp exposed to *Vibrio* after immersion in beta-glucan

Beta-glucan	Days of challenge following treatment		
	10 (n=15)	18 (n=20)	43 (n=15)
2.0 mg/ml	55.6%	50	86.7
1.0	0*	30	80
0.5	0*	20*	93.3
0.25	41.7	50	86.7
control	54.5	60	73.3

n is number of shrimp used in each experiment.
*significantly different from the control group (α=0.05).

Glucan concentrations ranging 0.5-1.0 mg/ml were able to protect shrimp from *Vibrio* up to 18 days following immersion.

Better growth may have been the result of disease resistance of shrimp. This situation, the researchers noted, is in some degree similar to antibiotic-enhanced poultry feed. But higher levels of peptiglycans (one type of beta-glucans) are not feasible because of adverse effects.

After considering cost and labor, the researchers suggest that supplementation of beta-glucan at 0.5 mg per ml is sufficient in strengthening the non-specific defense mechanism of postlarval shrimp. Beta-glucan may have to be administered continuously. Hatchery-reared juvenile shrimp are particularly susceptible to microbial infections and consequently, high mortality rates. Thus, the addition of a single immunostimulant may provide added insurance against disease outbreaks by strengthening non-specific immunity.

Other than the challenge test, the researchers also examined *in vitro* the phenoloxidase (PO) activity of shrimp hemocytes to clarify the mechanism of the defense system enhanced by the beta-glucan. Their results indicated that the beta-glucan enhances pro-PO system in shrimp hemocytes.

Other researchers noted that beta-glucans can enhance bactericidal activity, oxygen production of macrophages, and serum lysozyme activity. Beta-glucans can also increase phagocytic activity of hemocytes. In general, beta-glucans may act as fundamental elicitor of host defense mechanisms in shrimp.

REFERENCES

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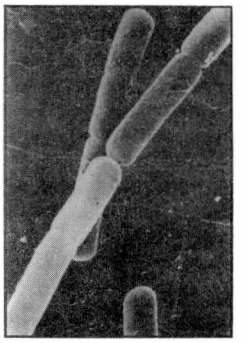

PROBIOTICS/*Bacillus* - FROM PAGE 13

under stressful conditions (or when nutrients are limited). Endospores allow *Bacillus* to reproduce when conditions are favorable.

- *Bacillus* produce antibiotics, of which bacitracin, polymyxin, tyrocodin, gramicidin, and circulin are examples
- *Bacillus* produce special compounds (enzymes) that can break down polysaccharides (sugars), nucleic acids, and lipids
- *Bacillus* cells measure around 0.8 µm in diameter
- *Bacillus* are easily transformable (free DNA is easily incorporated to change its genetic

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