1996

Using bacteria to fight bacteria

Aquaculture Department, Southeast Asian Fisheries Development Center

---

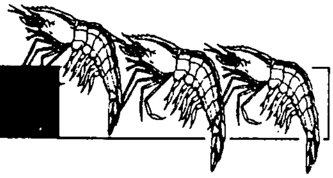
Southeast Asian Fisheries Development Center, Aquaculture Department (1996). Using bacteria to fight bacteria. Aqua Farm News, 14(4-5), 12-13, 17, 27.

---

http://hdl.handle.net/10862/2431

---

option 1 PROBIOTICS

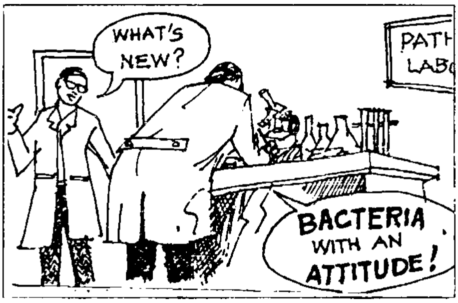


Using bacteria to fight bacteria

Experience has shown that intensive shrimp aquaculture often results in good yields for a few years, but is usually followed by a collapse due to viral and bacterial diseases (especially caused by the *Vibrio* species).

Following medical and veterinary practices, farmers initially respond by using antibiotics or antimicrobial chemicals in large doses. But antibiotics exacerbate the disease problem, and its use in aquaculture is doomed to failure (see related article, pages 14-15).

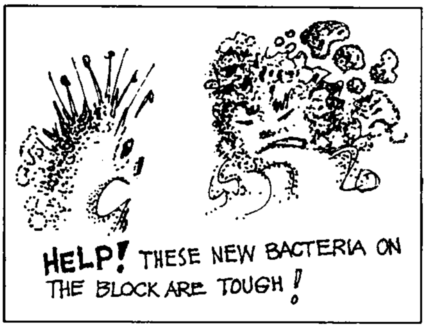
Instead, the use of beneficial bacteria to "fight" pathogenic bacteria (by competitive exclusion, see box next page) is an accepted practice in agriculture, being seen as a better remedy than antibiotics. It is slowly gaining acceptance in aquaculture.



What are probiotics?

Probiotics are single or mixed culture of live microorganisms (selected strains of bacteria, among others) that when applied to culture systems benefit the farmed stock by improving the indigenous microflora. The probiotic bacteria modify the microbial composition of the water and sediment; increase species diversity; and minimize the effect of, if not eliminate, pathogens directly.

Probiotic bacteria also enter the gut (ingested with food) or attach to external surfaces of farmed stock. In aquaculture, these are used as feed supplements and water conditioners.



How do probiotics work?

In natural aquatic ecosystems, bacterial growth and biomass are controlled mainly by the rate at which large compounds or polymers (such as protein or starch) are broken into small units. These particles are present in ponds as dead algae, uneaten food, and feces. Bacteria that are efficient at breaking down polymers would have a selective advantage and hence, dominate, provided that other conditions are not limiting (dissolved oxygen, pH, and essential nutrients like phosphate). Gram-positive bacteria (the *Bacillus* in particular) are among the most efficient in breaking down polymers. However, these are not normally present in high proportions in the water column; their natural habitat is the sediment.

When certain *Bacillus* strains are added to the water sufficiently, they can make an impact. They compete with the bacterial flora naturally present for the available organic matter (leached or excess feeds and shrimp feces). The result is less accumulation of slime or organic matter on the pond bottom, better penetration of oxygen into the sediment, and a better environment for farmed stock in general. And because shrimp are not stressed, its natural resistance can fight off diseases. Pathogens can not invade and can not proliferate.

Competitive exclusion is one of the ecological process that can be manipulated to modify the species composition of a soil or water body or other microbial environment. Species composition of a microbial community in a pond is determined by chance and by factors that allow a particular species to grow and divide more rapidly than others. Chance favors those organisms that happen to be in the right place at the right time to respond to a sudden increase in nutrients (e.g., from the decomposition of feed pellets that fall around them).

Shrimp farmers can manipulate species composition by seeding large numbers of desirable strains of bacteria or algae. In intensive culture, farmers often can not afford to wait for natural processes to readjust and deal with new conditions. Bioremediation and the use of probiotics are significant management tools, but their efficacy depends on the correct number of bacterial strains, viability of bacterial strains, and correct application procedures.

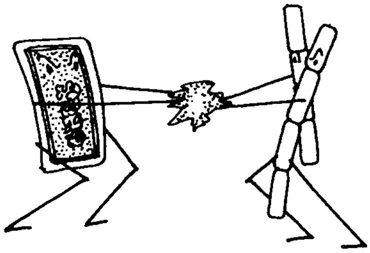
Probiotic field tests

AQD FIELD TEST. AQD fish health consultant Rogelio Gacutan is spearheading the field-test of three types of commercially available probiotics in eight ponds in west Negros. These probiotics are made up of selected strains of bacteria, fungi and yeasts which can rapidly convert solid sediments into simpler and utilizable substances.

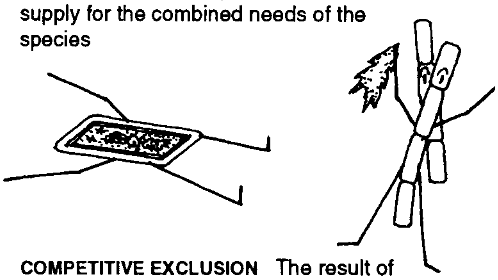
The jury is still out, so-to-speak. The results are not yet conclusive. The researchers suspect that the actual composition of these probiotics must be known first before they can understand what is happening in the ponds and advise farmers.

BIOPOND SYSTEMS FIELD TEST. Dr. DJW Moriarty, an honorary associate professor of the University of Queensland (Australia) discussed the value of adding selected strains of *Bacillus* as probiotic bacteria to control luminescent vibriosis in ponds. He compared farms in Indonesia that use the same water source which contained luminous vibrio strains.

The farm that did not use the *Bacillus* cultures experienced almost complete failure in all ponds, with luminescent vibriosis killing shrimp before 80 days of culture was reached. In contrast, a farm using the probiotics was able to grow shrimp for over 160 days without problems. Around $1x10^4$ to $1x10^5$ cells per ml of *Bacillus* was used.



COMPETITION In ecology, the use of the same resource by two or more species, when the resource is present in insufficient supply for the combined needs of the species



COMPETITIVE EXCLUSION The result of competition between species for a limiting resource in which one species completely eliminates the other

The bacterial species composition was different in the pond water on the two farms, demonstrating that it is possible to change bacterial species composition and improve shrimp production in large water bodies. The number of vibrios, especially *Vibrio harveyi*, was low in ponds where a large number of specially selected *Bacillus* species was maintained in the water column. Vibrio count was also low in sediment and no luminous vibriosis outbreak occurred in sediments where the probiotic *Bacillus* was used.

What are *Bacillus*?

Knowing more about *Bacillus*, possibly the main component of effective probiotics, might be helpful in understanding probiotics. There are many *Bacillus*, around 15 species have been identified. Microbiologists have long catalogued their general characteristics:

- *Bacillus* can easily move around (motile) because they have a whip-like "tail" (flagella)
- *Bacillus* form endospores which are useful

☞ *page 17, 2nd column*

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 5×10^7 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.

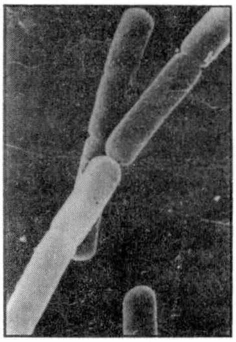
Other researchers noted that beta-glucans can enhance bactericidal activity, oxygen production of macrophages, and serum lysozyme activity. Beta-glucans can also increase phagocytic activity of hemocytes. In general, beta-glucans may act as fundamental elicitor of host defense mechanisms in shrimp.

REFERENCES

Y Takahashi, T Itami and M Kondo. 1995. *Immunodefense system of crustacea*. **Fish Pathology** 30 (2): 141-150.

HH Sung, GH Kou and YL Song. 1994. *Vibriosis resistance induced by glucan treatment in tiger shrimp (Penaeus monodon)*. **Fish Pathology** 29 (1): 11-17.

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

☞ page 27

Discounted 5-year cash flow of semi-intensive milkfish production in newly renovated ponds at 15% discount rate; per hectare (estimated by R. Agbayani)

Year	Revenue	Cost	Net cash flow	Discount factor	Discounted revenue	Discounted cost	Discounted net cash flow
0	0	50,000	(50,000)	1.00	0	50,000	(50,000)
1	144,875	122,651	22,224	0.87	125,983	106,657	19,326
2	156,465	132,463	24,002	0.76	118,303	100,155	18,148
3	168,982	143,060	25,922	0.66	111,106	94,062	17,044
4	182,501	154,505	27,996	0.57	104,354	88,346	16,008
5	197,101	166,865	30,236	0.50	97,999	82,965	15,033
Net present value							**₱ 35,560**
Benefit-cost ratio							**1.07**
Internal rate of return							**40.53%**

"But the profit taking was short-lived because of risks associated with environmental deterioration. This was not considered in the computations then." The high discount rate used in Rene's analysis is intended as fair warning for investors to look into the associated risks in high-density aquaculture systems to avoid similar losses and to prevent environmental deterioration which caused many intensive shrimp farms to go under.

REFERENCE

R. Agbayani. 1996. *Economic analysis of various milkfish culture systems.* Paper presented at the **National Conference-Exhibit on Technical Considerations for the Management and Operation of High-Density Milkfish Culture Systems**; 24-25 October 1996; Quezon City; U.P. Aquaculture Society, Inc. Miag-ao, Iloilo.

PROBIOTICS/*BACILLUS* - FROM PAGE 17

make-up). This is very useful in making "designer" bacteria.

- *Bacillus* are thermophilic, growing at high temperatures (50-70°C) in areas like hot springs and heated industrial wastes
- *Bacillus* are easy to isolate from soil or air. They grow well on synthetic media containing sugars, organic acids, alcohols, among others, as sole carbon sources. Ammonium can be its sole nitrogen source. Few isolates have vitamin requirements.

REFERENCES

DJW Moriarty. ***Asian Shrimp News,*** 2nd Quarter 1996. p. 3.

DJW Moriarty. Control of luminous *Vibrio* species in aquaculture ponds. Abstract of seminar given at SEAFDEC / AQD. Undated.

RQ Gacutan, MY Tabbu and RT Dal. 1996. The beneficial effects of probiotics on selected chemical and growth parameters in *Penaeus monodon* pond waters. Abstract of seminar given at SEAFDEC / AQD. Undated.

TD Brock and MT Madigan. 1991. **Biology of Microorganisms**. Prentice-Hall Intematl Ed. p. 44, 249, 327, 693, 775-777.