Disease Control in Shrimp Aquaculture with Probiotic Bacteria

David J. W. Moriarty

Biomanagement Systems Pty. Ltd., 315 Main Road, Wellington Point. Queensland 4160 Australia, and Department of Chemical Engineering, The University of Queensland. Qld. 4072 Australia.

ABSTRACT

Shrimp aquaculture production in much of the world is depressed by disease, particularly caused by luminous *Vibrio* and/or viruses. Antibiotics, which have been used in large quantities, are in many cases ineffective, or result in increases in virulence of pathogens and, furthermore, are cause for concern in promoting transfer of antibiotic resistance to human pathogens. Probiotic technology provides a solution to these problems. The microbial species composition in hatchery tanks or large aquaculture ponds can be changed by adding selected bacterial species to displace deleterious normal bacteria. Virulence of luminous *Vibrio* species can be controlled in this manner. Abundance of luminous *Vibrio* strains decreased in ponds and tanks where specially selected, probiotic strains of *Bacillus* species were added. A farm on Negros, in the Philippines, which had been devastated by luminous *Vibrio* disease while using heavy doses of antibiotics in feed, achieved survival of 80-100% of shrimp in all ponds treated with probiotics.

Introduction

The UN FAO estimates that half of the world's seafood demand will be met by aquaculture in 2020, as wild capture fisheries are overexploited and are in decline. Shrimp (or prawn) culture is widespread throughout the tropical world. It is in an industry set for a period of strongly growing demand, and is currently worth around US$10 billion. *Penaeus monodon*, the black tiger shrimp, is the most widely cultured species.

In much of the world, however, the shrimp aquaculture industry is beset by disease, mostly due to bacteria (especially the luminous *Vibrio harveyi*) and viruses. The high density of animals in hatchery tanks and ponds is conducive to the spread of pathogens, and the aquatic environment, with regular applications of protein-rich feed, is ideal for culturing bacteria.

I feel these problems have been exacerbated because the interactions of microbes, animals and their environment at intensive production scales have been considered primarily from a clinical pathology perspective. That is, the disease was treated rather than the underlying cause. When pathogenic bacteria or viruses are detected, farmers apply antimicrobial compounds to the feed and water. Many farmers also use antibiotics as prophylactics in large quantities, even when pathogens are not evident. This has lead to an increase in vibrios, and presumably other bacteria, having multiple antibiotic resistance and to an increase in more virulent pathogens. Many of the pathogens appear to have mutated to more virulent forms than were present a decade ago, and thus even when the shrimps are not stressed by poor water quality they succumb to attack. Thus I feel that the incidence of disease has been exacerbated by the actions of the shrimp farmers.

Microbial Biosystems: New Frontiers
Proceedings of the 8th International Symposium on Microbial Ecology
Bell CR, Brylinsky M, Johnson-Green P (eds)
Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.

The solution lies in the field of microbial ecology, not in the field of pharmacology, i.e. in developing new antibiotics or vaccines [5]. Shrimp farmers have to learn to live with a complex community of microbes and manage them. The use of beneficial bacteria (probiotics) to displace pathogenic bacteria by competitive processes is a better remedy than administering antibiotics. And it works!

The microbial species composition in aquaculture ponds can be changed by adding selected species to displace deleterious common bacteria. Success depends upon defining the ecological process or processes to be changed, the types of deleterious species that are dominant and the desirable alternative species or strains of bacteria that could be added. Competitive exclusion is one of the ecological processes that allows manipulation of the bacterial species composition in the water, sediment and animal guts.

Antimicrobials and Pathogens

Vibrio spp., especially the luminous *V. harveyi*, have been implicated as the main bacterial pathogens of shrimps [2]. Antibiotics have been used in attempts to control these bacteria, but their efficacy is now, in general, very poor. In the Philippines, luminous *Vibrio* disease caused a major loss in shrimp production in 1996, and many farms have ceased to produce shrimps because survival was so poor. The *Vibrio* species were resistant to every antibiotic used, including chloramphenicol, furazolidone, oxytetracycline, and streptomycin, and were more virulent than in previous years.

In Thailand this year, a farmer who was using colloidal silver in all feeds experienced a large increase in mortality from vibriosis. This was managed for a while with large doses of norfloxacin in all feeds. However, when it was stopped all shrimps died within 2 days. Clearly, a highly virulent strain of luminous *Vibrio* had developed in response to the use of the silver and antibiotics.

Chlorine is widely used in hatcheries and ponds, but its use stimulates the development of multiple antibiotic resistance genes in bacteria [8]. Some farmers in Thailand have reported that when chlorine is used in ponds to kill zooplankton before stocking shrimp, there is a rapid increase in *Vibrio harveyi* numbers after the chlorine is removed. This is to be expected as marine vibrios have very fast growth rates, and the chlorine treatment will lower the numbers of competitors for nutrients and kill algae, thus increasing food resources. It is likely, therefore, that the vibrios surviving after chlorine treatment are not only more resistant to antibiotics, but are also pathogenic. Thus the problems have been exacerbated by the use of antimicrobial compounds.

If antibiotics or disinfectants are used to kill bacteria, some bacteria will survive, either strains of the pathogen or others, because they carry genes for resistance. These will then grow rapidly because their competitors are removed. Any virulent pathogens that re-enter the pond or hatchery tank, perhaps from within biofilms in water pipes or in the guts of animals, can then exchange genes with the resistant bacteria and survive further doses of antibiotic. Thus, antibiotic-resistant strains of pathogens evolve rapidly.

The transfer of resistance to human pathogens and gut bacteria is of major concern. Such transfers probably happen easily and often, as discussed by Salyers [10]. Resistance plasmids encoding for many antibiotic resistance genes were transferred between pathogenic and non-pathogenic Gram negative bacteria in several environments including sea water. In the presence of tetracycline concentrations that were not high enough to kill the bacteria, the rate of gene transfer between *Vibrio cholerae* and *Aeromonas salmonicida* increased 100 times [4].

This work raises questions not only about the use of antibiotics in aquaculture, but about the use of bacteria closely related to pathogenic species as probiotics. Not only antimicrobial resistance genes, but also genes for virulence can be transferred by R plasmids and transposons. As the R plasmids can transfer genes between widely different bacteria in the Gram negative group, it would be potentially dangerous to use *Vibrio* or *Pseudomonas*, for example, as probiotics.

Throughout Asia, shrimp farmers use antibiotics in large quantities. Warehouses supplying the industry in all the major centres sell a range of antibiotics in containers of 500 g or more in size. The antibiotics in current use include fluoroquinolones especially norfloxacin and enrofloxacin, furazolidone, oxolinic acid, oxytetracycline, trimethoprim and sulphadiazine. It is difficult to find out just how much antibiotic use there is in the industry, but it is possible to make an estimate from feed usage and production. In 1994 Thailand produced about 250,000 tonnes (a quarter of the world production) of farmed shrimps, which consumed about 500,000 - 600,000 tonnes of feed. With the disease problems, shrimp production last year was down to 150,000 tonnes. For each crop at semi-intensive to intensive scales of production, farmers use 5 - 10 g antibiotics per kg feed at least once per day at weekly intervals; some use them for more extensive periods. Thus antibiotics would be used in about 10% of feed. It is possible, therefore, that the antibiotic usage in shrimp farm production in Thailand in 1994 was as much as 500 - 600 tonnes, assuming all farmers used them — and this does not include that used in hatcheries for fry production.

As much of this will end up producing bacteria with multiple antibiotic resistance in farm effluents that then contaminate coastal waters, the potential impact on human health is significant. This problem was discussed by Austin in 1983 [1] with reference to fish farming, but it has become far worse with the major increase in shrimp farming that has occurred since then.

Probiotic Bacteria

The use of beneficial bacteria (probiotics) to displace pathogens by competitive processes is being used in the animal industry as a better remedy than administering antibiotics and is now gaining acceptance for the control of pathogens in aquaculture [3]. The term "probiotic" has been defined as: "a probiotic is a mono- or mixed culture of live microorganisms that, applied to animal or man, affect beneficially the host by improving the properties of the indigenous microflora" [3]. In this discussion, the authors considered only human and land farm animals. In extending their definition to aquaculture, I suggest that it also applies to the addition of live, naturally-occurring bacteria to tanks and ponds in which the animals live, because these bacteria modify the bacterial composition of the water and sediment. The health of animals is thus improved by the removal, or decrease in population density, of pathogens and by improving water quality through the more rapid degradation of waste organic matter.

Unlike land animals, aquatic farmed animals are surrounded by a milieu that supports opportunistic pathogens independently of the host animal, and so the pathogens can reach high abundance around the animal. *Vibrio* grow attached to algae, and may reach high population densities after being ingested with the algae and then excreted with lysed algae in faecal pellets by zooplankton; they are gut bacteria in fish and shrimps as well as zooplankton [7]. In aquaculture ponds, where animal and algal population densities are very high, *Vibrio* numbers are also high compared to the open sea. The onset of shrimp disease due to exposure to high numbers of *Vibrio*, especially when pathogenicity has increased by overuse of antimicrobial compounds indicates that a defense is needed.

The species composition of a microbial community, such as that in a pond, will be determined partly by stochastic phenomena, that is, chance, and partly by deterministic and predictable factors that allow one species to grow and divide more rapidly than others, and thus dominate numerically. Chance favours 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 lysis of algal cells or the decomposition of feed pellets that fall around them. The farmer can manipulate the species composition by seeding large numbers of desirable strains of bacteria or algae; in other words, by giving chance a helping hand.

Competitive exclusion is one of the ecological processes that can be manipulated to modify the species composition of a soil or water body or other microbial environment. Small changes in factors that affect growth or mortality rates will lead to changes in species dominance. We are still a long way from knowing all the factors that control growth rates of particular species. The complete species composition in natural environments is largely unknown, but enough is known to argue that it is possible to change species composition by making use of competitive exclusion principles [11]. Thus bacteria can compete by secreting antimicrobial compounds that do not necessarily kill all their competitors, but increase mortality rates just enough to tip the balance in resource utilization. For example, if a *Bacillus* strain were to produce an antibiotic that inhibited a *Vibrio*, then the *Vibrio*'s mortality rate would increase, shifting the dominance to the *Bacillus*, even if the antibiotic were not produced at high enough concentration to kill all or most *Vibrio* cells directly.

Microbial ecology and biotechnologies have advanced in the last decade, to the point that commercial products and technologies are available for treating large areas of water and land to enhance population densities of particular microbial species or biochemical activities. The practice of bioremediation (or bioaugmentation) is applied in many areas, but success varies greatly, depending on the nature of the products used and the technical information available to the end user. The bacteria that are added must be selected for specific functions that are amenable to bioremediation, and be added at a high enough population density, and under the right environmental conditions, to achieve the desired outcomes. Bioaugmentation and the use of probiotics are significant management tools for aquaculture, but their efficacy depends on understanding the nature of competition between particular species or strains of bacteria. They rely on the same concepts that are used successfully for soil bioremediation and probiotic usage in the animal industry.

Probiotics such as the Gram positive *Bacillus* offer an alternative to antibiotic therapy for sustainable aquaculture. *Bacillus* species are commonly found in marine sediments and therefore are naturally ingested by animals such as shrimps that feed in or on the sediment. An advantage of using *Bacillus* species is that they are unlikely to use genes for antibiotic resistance or virulence from the vibrios or related Gram negative bacteria. There are barriers at the transcriptional and translational levels to the expression of genes from plasmid, phage and chromosomal DNA of *E. coli* in *B. subtilis* [9].

Probiotic Applications in Aquaculture

Bacterial species composition in shrimp ponds, which are large water bodies up to a hectare or more in size, hatchery tanks and shrimp guts can easily be changed and thus result in an improvement in shrimp production. In particular, luminous *Vibrio* can be controlled in this manner. To my knowledge, there has not been any rigorous study made of *Vibrio* populations in shrimp on farms, in relation to antibiotic or probiotic usage. Thus the data referred to here are given as examples of what has been observed, but the conclusions need to be substantiated.

An example is the Viveros farm in Negros, where losses from luminous *Vibrio* had been catastrophic, even though antibiotics were used in the feed and gave protection some of the time. Luminous *Vibrio* abundance in the pond waters of that region were often as high as 10^3 to 10^4 per ml within 2 - 3 weeks of filling and fertilizing ponds. However, when the probiotic bacteria were used, no disease was experienced and indeed survival was very high (80-100%), even in the presence of luminous *Vibrio* species (Tables 1, 2) [6].

Table 1. Shrimp production on a farm in Negros, The Philippines, with Biomanagement Systems' probiotic bacterial technology, compared to controls with antibiotics [data from many other control ponds that collapsed after 30-60 days are not shown]. Ponds were harvested in December 1996/January 1997. All shrimps died overnight in pond 6 from vibriosis, which was a considerable loss at 120 days of culture.

	BMS Ponds			Control Ponds		
Pond #	1	2	3	4	5	6
Stocking: N/m^2	30	15	30	32	32	32
Days of Culture	195	189	196	182	190	120
Survival (%)	88	100	100	67	73	0
Harvest (tonne/ha)	7.2	5.4	8.6	6.5	6.8	0

Table 2. Second crop from a farm in Negros (stocked April 1997, harvested September) in 5 of the ponds referred to in Table 1 above.

Pond #	1	2	3	4	5
Pond area (m^2)	6300	8200	7200	7200	6000
Stocking: N/m^2	20	20	19	18	18
Days of Culture	158	159	158	147	146
Feed conversion ratio	1.54	1.62	1.54	1.47	1.46
Survival (%)	100	73	92	81	91
Harvest (tonne/ha)	6.1	4.9	5.3	5.0	5.1

In several ponds in the Philippines, luminous *Vibrio* numbers in the hepatopancreas of shrimps fell from around 1 x 10^4 per gut when antibiotics were used in the feed, to zero when probiotics were applied to the pond (Figure 1). The shrimp gut flora was often dominated by *Vibrio* species that were sucrose negative (green on TCBS agar) and luminous in the presence of antibiotic treatment, whereas sucrose positive strains (yellow colonies) were usually more abundant or equally abundant when probiotics were used (Fig. 1). Many farmers now realize that they cannot solve the vibriosis disease problem by using antibiotics.

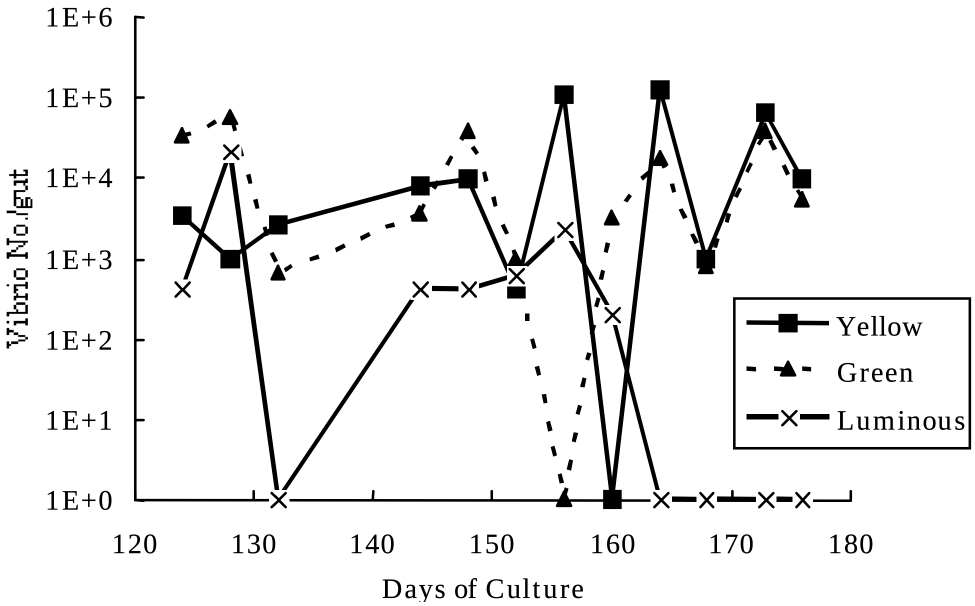

Fig. 1. Total and luminous *Vibrio* in midgut (hepatopancreas) of shrimps in Pond 25 , Roxas, Philippines. Luminous *Vibrio* were often abundant in the presence of antibiotics: i.e. they were resistant and shrimp mortality was high, although sometimes they were sensitive, e.g. at 132 Days of Culture, when the antibiotics were changed. After Day 160, when the probiotic Pondpro-VC was added daily to the pond, luminous *Vibrio* were eliminated from the gut. (Data were kindly supplied by AA Exico, Roxas City.)

Luminous *Vibrio* were completely eliminated from the water column and from the sediment of ponds in Indonesia when probiotic strains selected for their direct inhibitory effect were used [11]. In contrast, *Vibrio* numbers increased markedly from around 20 to over 200 cfu/ml in shrimp ponds where antibiotics were used in the feed. Survival, and thus production, was high in all ponds where probiotics were used.

These data show that the disease problems can be overcome by applying probiotic biotechnology, which is an application of microbial ecology. It makes use of the natural mechanisms by which bacteria compete against each other. In other words, shrimp farmers who learn to farm microorganisms will be far more likely to achieve successful harvests.

With the right combination of bacteria and aeration, water exchange can be minimized and water can be recycled between crops, thus lessening environmental impacts and the likelihood of introducing pathogens. The transfer of antibiotic resistance to human pathogenic bacteria, which is exacerbated by the abuse of antibiotics in the aquaculture industry, will decrease.

References

1. Austin, B (1993) Environmental issues in the control of bacterial diseases of farmed fish. In: Environment and Aquaculture in Developing Countries. Pullin, RSV. Rosenthal, H, Maclean, JL (eds). ICLARM Conference Proceedings 31. ICLARM, Manila. pp 237-251
2. Baticados, MCL, Lavilla-Pitogo, CR, Cruz-Lacierda, ER, de la Pena, LD, Sunaz, NA (1990) Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. Dis Aquat Org 9: 133-139

3. Havenaar, R, Ten Brink, B, Huis in't Veld, J H J (1992) Selection of strains for probiotic use. In: R. Fuller (ed), Probiotics: the scientific basis, Chapman and Hall, London. pp 209-224.
4. Kruse, H, Sørum, H (1994) Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural environments. Appl Environ Microbiol 60: 4015-4021
5. Moriarty, DJW (1997) The role of microorganisms in aquaculture ponds. Aquaculture 151: 333-349.
6. Moriarty, DJW (1998) Control of luminous *Vibrio* species in penaeid aquaculture ponds. Aquaculture 164: 351-358
7. Moriarty, D.J.W. 1990. Interactions of microorganisms and aquatic animals, particularly the nutritional role of the gut flora. In: R. Lésel (ed.), Microbiology in Poecilotherms: Proceedings of the International Symposium on Microbiology in Poecilotherms. Elsevier Science Publishers, B.V. pp. 217- 222.
8. Murray, G. E., Tobin, R. S., Junkins, B., Kushner, D. J. (1984) Effect of chlorination on antibiotic resistance profiles of sewage related bacteria. Appl Environ Microbiol. 48: 73-77
9. Rabinowitz JC, Roberts, M (1986) Translational barriers limiting expression of *E. coli* genes in *Bacillus* and other Gram-positive organisms. In: Levy, S.B, Novick, R.P (eds) Banbury Report 24: Antibiotic Resistance Genes: Ecology, Transfer and Expression. Cold Spring Harbour Laboratory pp297-312
10. Salyers, A A (1995) Antibiotic resistance transfer in the mammalian intestinal tract: implications for human health, food safety and biotechnology. Springer-Verlag, New York. pp 109-136.
11. Smith, V H (1993) Implications of resource-ratio theory for microbial ecology. Limnol Oceanogr 38:239-249.