Probiotics in aquaculture

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Aquaculture, especially prawn (shrimp) culture, is a major and rapidly increasing source of income in the agribusiness sector of tropical countries. Although probiotic bacteria are being used in aquaculture, there is scope, and indeed a need, for them to be used much more widely as replacements for antibiotics.

Antibiotics, such as oxytetracycline, chloramphenicol, florfenicol and fluoroquinolones are used in aquaculture industries throughout the world [1] and may persist for many months in marine sediments around aquaculture facilities. Pathogens are increasing in resistance and virulence as a result, and bacteria with multiple antibiotic resistance traits are found not only in the cultured species, but in other animals and sediments in the surrounding water [2-4]. Antibiotics are therefore actually creating worse problems for the aquaculture industry than would be the case if they were not used at all, or if other methods for disease control were used.

The microflora of the sediment and water in which the cultured prawns, fish and other aquaculture species live is influenced by the microbes released from faeces of all the animals in their environment. If a pathogen is present, its population density can be magnified through interactions in the intestinal tracts of the animals and in the faeces. When food for aquatic animals is added to the water, it adsorbs or absorbs bacteria from the water before ingestion. Thus it is a vehicle for introducing microbes to the gut and so any pathogens present can enter via the feed. As it is possible to alter the bacterial composition of pond or tank water by adding beneficial bacteria, which compete with potential pathogens and displace them, they can be considered to be probiotics, especially when the probiotic bacteria adsorb to feed and enter the gut of cultured animals [5].

Two of the reasons why it is beneficial for aquaculture producers to use probiotics rather than antibiotics are:

- From the human health perspective, the risk of multiple antibiotic resistance (MAR) and virulence gene transfer to human gut microflora and other bacteria that interact within humans is minimised. Many importing countries restrict entry of shrimp and other seafood products with antibiotic residues.
- From the aquaculturists' perspective, because the spread of virulence and pathogenicity in aquatic bacteria has increased with antibiotic usage, productivity and profits are increased when probiotics are substituted for antibiotics.

Penaeus monodon **(tiger prawns) from a London supermarket, with signs of vibriosis and white spot virus.**

In Thailand and India, two major prawn-producing countries, probiotic usage is increasing, and there are indications that antibiotic use is decreasing due to intervention by Governments and by farmers themselves. They are concerned about the negative impact on markets of antibiotic residues such as nitrofurans and chloramphenicol in shrimp exports; indeed Europe has banned imports with such residues.

Another reason why some prawn producers in Asia and Latin America are now avoiding antibiotics is because they have found that they are losing money due to crop losses resulting from increased virulence linked to the increase in antibiotic resistance. Not only are they finding that the antibiotics are ineffective or very costly when used at high doses, but the disease is worse. This provides circumstantial evidence that horizontal gene transfer of virulence genes together with antibiotic resistance genes in *Vibrio* populations is increasing. Therefore, if appropriate probiotics can be provided at a cost-effective price, and productivity increases, then the abuse of antibiotics in the aquaculture industry will be curtailed.

The microbial flora of fish and invertebrates is not as well studied as that of higher animals. The flora becomes established when the gut opens after the larvae hatch, and is influenced initially by the composition of the bacterial flora in the surrounding water. It changes with

time as diet changes and thus becomes influenced more by the nature of the flora attached to, or inside, ingested food. In marine fish and invertebrates, the gut flora is usually dominated by *Vibrio species.* Other marine gut bacteria include species of *Flavobacterium, Alcaligenes, Acinetobacter, Photobacterium, Staphylococcus, Enterococcus, Micrococcus* and *Pseudomonas.* Strictly anaerobic flora of fish includes *Bacteroides, Eubacteria* and *Fusobacterium* which are found mainly in herbivorous and omnivorous fish [6].

In freshwater animals, enterobacters, aeromonads and plesiomonads are commonly found. Lactic acid bacteria, in particular species of *Carnobacterium* and *Lactobacillus,* generally constitute only a small proportion of the gut flora of fish. Several reports of effective probiotics among these genera have been published. In some fish culture sectors, such as salmon in Norway and Canada, vaccination is proving effective and antibiotic use has declined in the last 10 years. In Asia where 80% of the world's cultured fish are produced, vaccines are not used.

In prawns, *Vibrio* is the dominant gut genus; the species composition is variable and depends on season, location, phytoplankton species composition and food. Many of the bacteria that are opportunistic fish and prawn pathogens may also be pathogenic to humans e.g. *Vibrio cholerae, Vibrio vulnificus, Vibrio parahaemolyticus, Aeromonas hydrophila, Pseudomonas aeruginosa, Pseudomonas putida.*

Successful harvest shrimp *(Litopenaeus vannamei)* in Ecuador using probiotics to control vibriosis and white spot virus.

A large prawn farm in Ecuador. Production is on a semi-intensive scale with about 10-12 prawns/m^2 and large ponds (10-50 hectares each).

Fish, like higher vertebrates, have a relatively stable gut flora and an adaptive immune system. Young fish can be protected from pathogens by exposure to non-virulent strains of pathogenic species in the gut [7]. More research by microbiologists on microbial interactions of aquatic animals will provide a much better understanding of how we can manipulate the natural flora to minimise disease and maximise production without recourse to the use of antibiotics or other antimicrobial compounds.

Crustaceans have a much less developed gut microflora than homeotherms or fish. It is mostly transient in the foregut and hind gut, which have a chitinous epithelium that is lost with each moult. We do not know much about the colonisation of the crustacean gut by bacteria and whether there are specific attachment sites. Although bacterial abundance and composition varies with feeding and defaecation, there is a persistent community in the midgut and mid gut gland (hepatopancreas) of prawns that is dominated by *Vibrio* spp. When these vibrios include pathogens, severe losses can occur in ponds or hatcheries.

There are some reports of the use of non-pathogenic strains of *Vibrio, Aeromonas, Pseudomonas* and *Alteromonas* that have antagonistic properties to pathogenic strains. They are added to tank water by some hatchery operators to colonise the surfaces and guts of larvae and successfully inhibit growth of pathogens. However, the antagonistic capacity can be lost during storage or subculture. These strains are susceptible to change such as loss of inhibitory activity or even the acquisition of virulence genes during repeated subculture. As these gram negative bacteria do not produce resistant spores, they are not suitable for large scale commercial production and distribution.

Bacillus species, being spore formers and producing a wide range of antagonistic compounds are, however, valuable as probiotics on a commercial scale in aquaculture. Several strains of species such as *Bacillus subtilis* and *Bacillus licheniformis* occur naturally in fresh and sea water environments and are found naturally in the intestinal tracts of prawns. They are used commercially as probiotics in aquaculture, mostly for prawn (shrimp) culture [8, 9]. Probiotics for commercial fish culture are not used as extensively as they are in prawn culture, perhaps because in tropical countries the profit margins are low for fish and there has not been much research and development in this field. However, there is potential for such use [8].

Some of the chemicals on sale to prawn farmers at the wharf in Machala, Ecuador.

Aquaculturists generally think of probiotics in the same terms as a medicine or antibiotic: they expect a quick and decisive effect, and are discouraged from using probiotics when the results are not immediate or dramatic. The presence of ineffective products that are sold as probiotics has caused many potential users to question the probiotic concept, rather than the nature or mode of action of the probiotic bacteria. The farmers need to understand that the probiotics are not therapeutic agents, but are designed to modify the microbial communities in the alimentary tract of animals and in their aquatic environment.

Unfortunately, there are many products being sold to aquaculture producers as probiotics that contain inappropriate species or strains of bacteria, or population densities that are too low to be effective. Many products sold for crustaceans contain *Lactobacillus* species that were produced for human or land animals and are not appropriate for marine animals. Some products in Asia have labels indicating species of *Clostridium, P. putida, P. aeruginosa* etc. One product even advertised 96 different species present! As such products are usually ineffective or may even contain potential fish or shrimp pathogens, they give aquaculture producers a misleading impression of the probiotic concepts and benefits of correct modification of the microbial flora of animals and ponds or tanks.

Where appropriate and effective products are used, results are very good. For example in Ecuador, the industry was devastated by a combination of vibriosis and White Spot Virus Syndrome disease in 1999-2000. Producers in southern Ecuador who are using a commercial product based on *Bacillus* species have now raised production from a loss-making 0-25% survival in 2000 to a profitable 60-90% survival in the presence of the pathogens in 2002 [10]. This was achieved by incorporating the *Bacillus* at a high density (>1 x 10^8/g) in all feed, so that the gut of the animals was always colonised by *Bacillus* species that competed successfully with the pathogenic *Vibrio* species.

References

1. Moriarty DJW. Microbial ecology: the key to controlling virus and bacterial disease in aquaculture. *Microbiology Australia* 1998; 9:22-27.
2. Thyssen A & Ollevier F. In vitro antimicrobial susceptibility of *Photobacterium damselae* subsp. *piscicida* to 15 different antimicrobial agents. *Aquaculture* 2001; 200:259-269.
3. Hektoen H, Berge JA, Hormazabal V & Yndestad M. Persistence of antibacterial agents in marine sediments. *Aquaculture* 1995; 133:175-184.
4. Tendencia EA & de la Pena LD. Antibiotic resistance of bacteria from shrimp ponds. *Aquaculture* 2001; 195:193-204.
5. Moriarty DJW. Disease control in shrimp aquaculture with probiotic bacteria. In: Bell CR, Brylinsky M & Johnson-Green P (Eds). *Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology.* Halifax, Canada: Atlantic Canada Society for Microbial Ecology, 1999.
6. Sakata T. Microflora in the digestive tract of fish and shellfish. In: Lesel R (Ed). *Microbiology in poecilotherms.* Amsterdam: Elsevier, 1990, p.171 -176.
7. Olafsen JA. Interactions between fish larvae and bacteria in marine aquaculture. *Aquaculture* 2001; 200:223-247.
8. Gatesoupe FJ. The use of probiotics in aquaculture. *Aquaculture* 1999; 180:147-165.
9. Moriarty DJW. Control of luminous *Vibrio* species in aquaculture ponds. *Aquaculture* 1998; 164:351-358.
10. Harris, L, de Wind A, Serrano W, Moriarty DJW & Villamar DF. Use of microbial technology to improve farm results in shrimp farms in Ecuador. *Diseases in Aquatic Organisms* (in press).