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The use of Probiotics in Aquaculture

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Abstract
Disease outbreaks are being increasingly recognized as important constraints to aquaculture production and trade, affecting the economic development of the sector in many countries. An increase of productivity in aquaculture has been accompanied by ecological impacts including emergence of a large variety of pathogens and bacterial resistance. These impacts are in part due to the indiscriminate use of chemotherapeutic agents as a result of management practices in production cycles. There is an urgent need in aquaculture to develop microbial control strategies. One of the alternatives to antimicrobials in disease control could be the use of probiotic bacteria as microbial control agents. Most of the work reviewed in this article describes commercial hatchery experiments, probiotic research in the culture of fish, crustaceans and live food with an evaluation of results obtained so far. Directions for further research are discussed.

Key words: Probiotics, Chemotherapeutic, Fish Culture, Crustaceans, Water Quality,
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Introduction

Aquaculture is the fastest growing food production sector in the world. It provides a significant supplement to and substitutes for wild aquatic organisms and creates employment, generates income and provides opportunities for human development (Subasinghe, 2000). Disease is a primary constraint to the growth of many aquaculture species and is now responsible for severely impeding both economic and socio-economic development in many countries of the world (Bondad-Reantaso and Subasinghe 2009; Edun *et al*., 2007; Verschuere *et al*., 2000; Subasinghe *et al*., 2001).

Addressing health questions with both pro-active and reactive programmes has thus become primary requirement for sustaining aquaculture production and product trade. Conventional approaches, such as the use of antimicrobial drugs and disinfectants, have had limited success in the prevention or cure of aquatic diseases (Subasinghe, 1997). Furthermore, there is a growing concern about the use and particularly the abuse of antimicrobial drug not only in human medicine and agriculture but also in aquaculture (Verschere *et al*., 2000).

Several alternative strategies to the use of antimicrobials in disease control have been proposed and have already been applied very successfully in aquaculture. Yasuda and Taga (1980) anticipated that bacteria would be found to be useful both as food and as biological control agents of fish disease and activators of the rate of nutrient regeneration in aquaculture. The addition of probiotics is now also in common practice in commercial shrimp hatcheries (Rico-Mora *et al*., 1998). This review provides an overview on the use of probiotics in aquaculture with an evaluation of the results obtained so far and directions for further research.

Definition of Probiotics: Elie Metchnikoff's work is regarded as the first research conducted on probiotics (Fuller, 1992). He described them as 'microbes' ingested with the aim of promoting good health. This definition originally applied to farm animals (ruminants, poultry and pigs) or humans, since the first studies were carried out in these species.

Probiotics are now being used in aquaculture and therefore, the definition has been modified. Many researchers have already investigated the relationship of the intestinal microbial to the aquatic habitat or food (Gomez-Gil *et al*., 2000; Cahill, 1990). The authors summarized the result of these investigations on fishes, giving evidence that the bacteria present in the aquatic environment influence the composition of the gut microbial and vice versa.

Therefore, the following modified definition is proposed, which allows a broader application of the term "probiotic" and addresses the objections made earlier. A probiotic is defined as a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment (Verschuere *et al*., 2000).

Use of Probiotics

Probiotics Studies in the Larval Culture of Shrimp: Several bacteria have been used in the larval culture of aquatic organisms (Table 1), either delivered directly into the water freeze-dried, or via live carriers such as *Artemia nauplii* or rotifers. Griffith (1995) reported that following the introduction of probiotics in Ecuador in 1992, hatchery down-time batches was reduced from 7 days per month to 21 days annually, production volumes increased by 35% and overall antimicrobial use decreased by 94%.

Vibrio alginolyticus is a frequently tested bacterium with promising results. *V. alginolyticus* may have characteristics capable of conferring some degree of protection against disease (Austin *et al*., 1995; Garriques and Arevalo, 1995). The use of *V. alginolyticus* may require some caution since some strains could be pathogenic (Lightner, 1993) it could be a probiotic candidate for shrimp larviculture.

Bivalves: Vibrio spp*.* are important pathogens in the culture of the scallops *Pecten. maximus* (Nicholas *et al*., 1996) and oyster larvae (Elston *et al*., 1991) and probionts have been used in an attempt to control them. *Alteromonas* spp*.* was also used to in pacific oyster (*Crassostrea gigas*) larviculture (Douillet and Langdon, 1993, 1994). Oyster larvae fed with algae and this bacterium showed enhanced survival and growth compared with those fed on algae alone. They found that 10^5 cell ml^{-1} was optional for the enhancement of larval culture.

Finfish: The digestive tract of fish contains a much higher number of microorganisms than the surrounding water, as many as 10^8 cells g^{-1} (Ringo and Vadstein, 1995). Kennedy *et al*., (1998) showed that the addition of a gram-positive probiotic bacterium increased survival, size uniformity and growth rate of marine fish larvae (snook, red drum, spotted sea trout and stripped mullet). They also noted that the external and internal bacterial environments of the fish moved from the predominance of Vibrios to greater numbers of other gram-negative and gram-positive bacteria.

Improving Water Quality: Improvement in water quality has been recorded during the addition of probiotics, especially *Bacillus* spp. The rationale is that gram-positive *Bacillus* spp. are generally more efficient in converting organic matters back to CO_2 than are gram-negative bacteria which would convert a greater percentage of organic carbon bacteria biomass or slime (Stanier *et al*., 1963). It is reasoned that by maintaining higher levels of these gram-positive bacteria in the production pond, farmers can minimize the build up of dissolved and particulate organic carbon during the culture cycle while promoting more stable phytoplankton blooms through the increased production of carbon dioxide (Scura, 1995). Cultures containing nitrifying bacteria are being used to control the ammonia level in culture water and are aimed especially at aquarium lobbyists (Verschuere *et al*., 2000). Nitrifiers are responsible for the oxidation of ammonia to nitrite and subsequently to nitrate. Nitrifying cultures could also be added to the ponds or the tanks when an incidental increase of ammonia or nitrite levels is observed. Besides ammonia, nitrite toxicity is a common problem in fish culture (Lewis and Morris, 1986) for example in pond rearing of catfish (Tucker and Lloyd, 1985).

Rationale for selecting and developing probiotics in aquaculture: The development of probiotics applicable to commercial use in aquaculture is a multistep and multidisciplinary process requiring both empirical and fundamental research, full scale trials and an economic assessment of its use (Verschuere *et al*., 2000). This is schematically presented in Figure 1. Many of the failures in probiotic research can be attributed to the selection of inappropriate microorganisms (Gomez-Gil *et al*., 2000). Selection steps have been described, but they need to be adapted for different host species and environments.

Methods to select probiotic bacteria for use in the larviculture of aquatic animals might include the following steps (1) collection of background information (2) acquisition of potential probiont (PP) (3) evaluation of the ability of PP to out-compete pathogenic strains (4) assessment of the pathogenicity of the PP (5) evaluation of the effect of the PP in larvae, and (6) an economic cost benefit analysis (Gomez – Gil *et al*., 2000).

Table 1. Bacterial probionts employed in the larval culture of aquatic organisms

Species of bacteria	Target organism	Reference
Vibrio alginolyticus	Shrimp (*Penaeus vannamel*)	(Garriques and Arevalo, 1995: Zherdmant *et al*., 1997)
T. utilis (PM-4)	Shrimp (*Penaeus monodon)*	(Maeda and Liao, 1991)
V. harveyi, Pseudomonas sp., *Nitrobacter* sp. *Nitrosomonas* sp. and *Bacillus* sp	Shrimp (*P. monodon and* P. *penicillatus*)	(Anonymous, 1991)
T. utilis (PM-4)	Crab (*Portunus trituberculatus*)	(Nogami and Maeda, 1992; Maeda, 1994; Nogami *et al*., 1997)
V. Pelagius	Turbot *(Scophthalmus maximus*)	(Ringo and Vadstein, 1998)
Bacillus toyoi and *Bacillus* sp. Spores	Turbot via rotifers *(Brachionus plicatilis*)	(Gatesoupe, 1989, 1991b)
Lactic bacteria	Turbot via rotifers	(Gatesoupe, 1990)
Lactobacillus plantarum and *Lactobacillus helveticus*	Turbot via rotifers	(Gatesoupe, 1991a)
Lactobacillus bulgaricus and *Streptococcus lactis*	Turbot via *Artemia*	(Garcla-de-la-Banda et al., 1992)
Alteromonas sp.	Oyster *(Crassostrea gigas*)	(Doutilet and Langbon, 1993, 1994)
Aeromonas media	Oyster	(Gibson et al., 1998)
Roseeobacter sp. (BS107)	Scallop *(Pecten maximus)*	(Rulz-Ponte et al., 1999)
Vibrio sp.	Chilean scallop *(Argopecten purpuratus*)	(Riquelme et al., 1997)

Gomez-Gil *et al.,* 2000

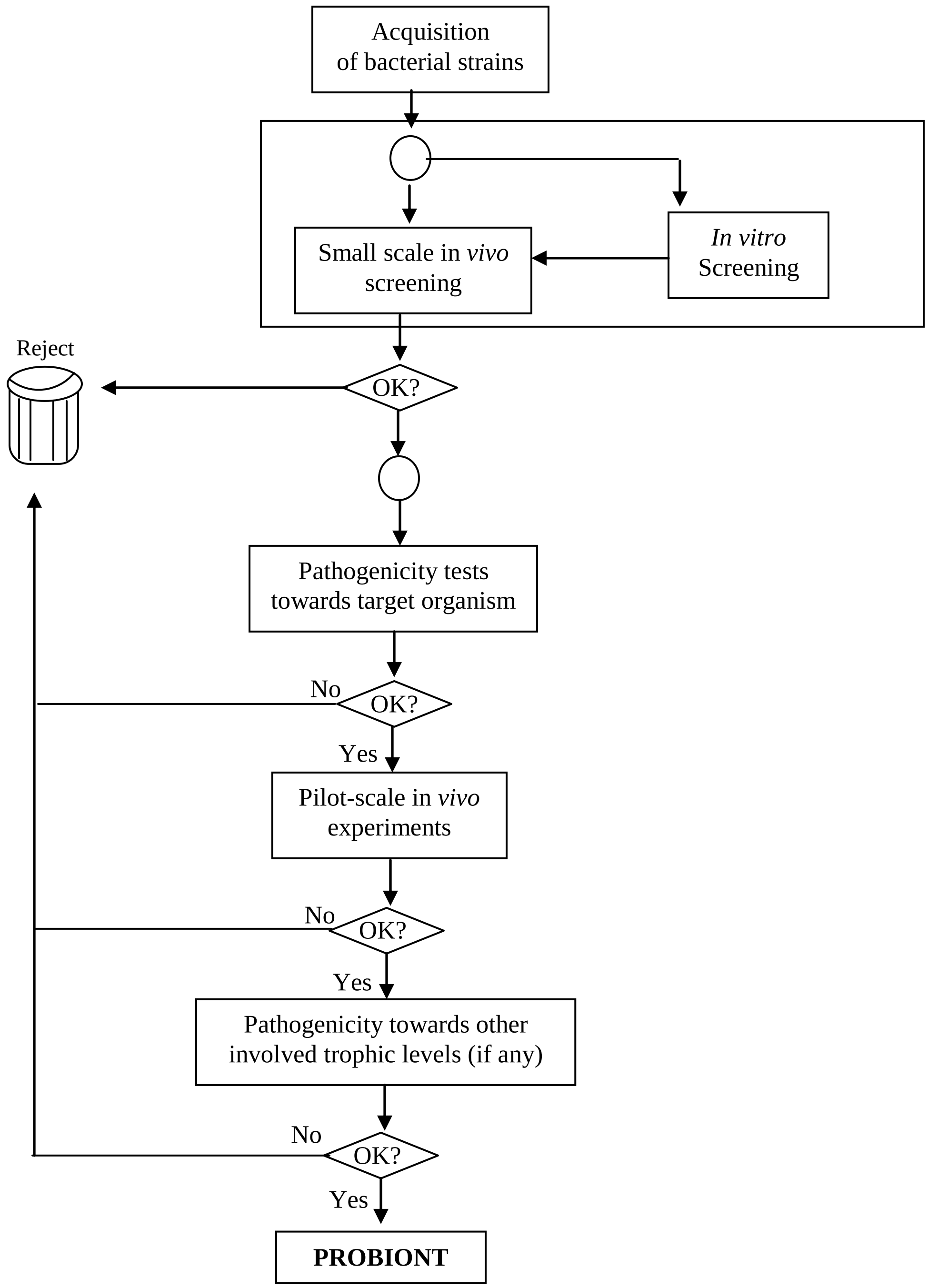

Fig. 1 Rationale for the research and development of probiotics as biological control agents in aquaculture (Verschuere *et al.,* 2000)

Conclusion

There are evidences to support the fact that prophylactic use of probiotics would improve the health and performance of cultured aquatic species. Since remarkable progress has been made in the culture of live food, crustacean, molluscs and fish, probionts have a great deal of potentials. The use of probiotics in aquaculture could be regarded as a kind of insurance since it may not produce any notable benefit but will be very helpful when there is a disease outbreak.

Many questions remain unanswered regarding the use of probiotics in aquaculture. Are they acting as food or are they competing with potentially harmful bacteria? How will probiotics perform when a stressful situation arises? Can they become pathogenic? Most of these questions are still unanswered. The selection of probiotics for the larval culture of aquatic organisms has been based on empirical observations and not on scientific data. There is still a lack of knowledge about the mode of action of a probiotic. The answer to these questions is to produce, formulate and preserve the probiotics applicable for use in aquaculture on a large scale and set out quality control guideline

References

Austin, B., Stuckey, L.F., Robertson, P.A., Efendi, I. Griffith, D.R.W., 1995. A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida, Vibrio anguillarum* and *Vibrio ordali*. *J. Fish Dis.* 18, 93-96.

Bondad-Reantaso, M.G., Subasinghe, R.P., Arthur, J.R., Ogawa, K., Chinabut, S., Adlard, R., Tan, Zilong and Shariff, M. (2005). Disease and health management in Asian aquaculture. *Veterinary Parasitology,* 132:249-272.

Cahill, M.M. (1990) Bacterial flora of fisheries: a review. *Microbial Ecology* 19:21-41.

Douillet, P.A. Langdon, C.J. (1993). Effects of Marine bacteria on the culture of axenic oyster (*Crassostrea gigas* Thunberg) larvae. *Biological Bulletin* 84: 36-51.

Douillet, P.A., Langdon, C.J. (1984) Use of a Probiotic for the culture of larvae of the pacific oyster (*Crassostrea gigas* Thunberg). Aquaculture 119: 25-40.

Edun, O.M., Akinrotimi, O. Opara, J.Y., Owhonda, K.N., Onunkwo, D.N. and Anyanwu, P.E. (2007). Public Health and economic implication of the microbial flora of cultivable freshwater fishes. *Journal of Fisheries International* 2(4):274-276.

Elston, R., Leibouvitz, L., Relyea, D., Zatila, J. (1981) Diagnosis of vibriosis in a commercial Oyster hatchery epizootic: diagnostic tools and management features. *Aquaculture* 24:53-62.

Fuller, R. (1992) History and development of probiotics. In: Fuller, R. (Ed). Probiotics: The Scientific Basis. Chapman and Hall, New York, pp.1-8.

Garriques, D., Arevalo, G. 1995. An evaluation of the production and use of a live bacterial isolate to manipulate the microbial flora in the commercial production of *Penaeus vannamei* post larvae in Ecuador. In: Browdy, C.L., Hopkins, J.S. (Eds), Swimming through Troubled Waters. Proceedings of the special session on shrimp farming. Aquaculture 95. World Aquaculture Society, Baton Rouge, pp.53-59.

Gomez-Gil, B., Roque, A. and Turnbull, J.F. (2000). The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture 191:259-270.

Griffith, D.R.W. (1995). Microbiology and the role of Probiotics in Ecuadorian shrimp hatcheries, In: Lavens, P., Jaspers, E., Roelands, I. (Eds, Larvi 91-fish and Crustacean Larviculture symposium. European Aquaculture Society, Gent, p.478; Special publication no.24.

Kennedy, S.B., Tucker, J.W., Thoresen, M. and Seonet, T. D.G. (1998). Current methodology for the use of probiotic bacteria in the culture of marine fish larvae. Aquaculture 98. World Aquaculture Society, Baton Rouge, p.286.

Lightner, D.V. (1993) Diseases of cultured penaed shrimps. In: McVey, J.P. (Ed.) CRC Handbook of Mariculture. CRC Press, Boca Raton, pp.393-486.

Nicholas, J.L., Corre, S., Gauthier, G., Robert, R., Ansquer, D. (1996). Bacterial problems associated with scallop. *Pecten maximus* larval culture. *Dts. Aquat. Org.* 27:67-76.

Rico-Mora, R., Voltolina, D., and Villaescusa-Celaya, J.A. (1998). Biological control of *Vibrio alginolyticus* in *Skeletonema costatum* (Bacillariophycae) cultures. *Aquaculture Engineering* 19:1-6.

Ringo, E. and Vadstein, O. (1995) Colonization of *Vibrio pelagius* and *Aeromonas caviae* in early developing turbot, *Scalphtalmus maximus* (L.) larvae. *Journal of Applied Microbiology* 84:227-233.

Subasinghe, R. (1997). Fish health and quarantine, P.45-49. In: Review of the state of the World Aquaculture. FAO fisheries circular no. 886. Food and Agriculture Organization of the United Nations, Rome, Italy.

Subasinghe, R.P., Bondad-Reantaso, M.G. and McGladdery, S.E. (2001). Aquaculture Development, Health and Wealth. In: R.P. Subasinghe, P. Bucno, M.J. Philips, C. Hugh, S.E. McGladdery and J.R. Arthur, (Eds). Aquaculture in the third Millennium Technical Proceedings of the conference on Aquaculture in the third millennium, Bangkok, Thailand, NACA, Bangkok and FAO, Rome, pp.167-191

Yasuda, K. and N. Taga, (1980). A mass culture method for *Artemia salina* using bacteria as food. *Microbology* 18:53-62.