



## ROLE OF PROBIOTICS IN AQUACULTURE- A REVIEW

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### Abstract

Aquaculture is concerned with the propagation and rearing of aquatic organisms under complete human control involving manipulation of at least one stage of an aquatic organism's life before harvest in order to increase its production. Resistance mechanisms of bacteria can arise in one of two ways chromosomal mutation or acquisition R-plasmid. Forty percent of world aquatic product (including capture fisheries) derives from aquaculture being valued at US\$ 78 billion. Aquaculture produced molluscs account for 21% of total aquaculture product and make up 65 % of total mollusc product when capture fisheries are considered (FAO, 2007). Diseases caused by *Vibrio* spp and *Aeromonas* spp are commonly implicated in episodes of mortality. This review article focuses the 4 use of probiotics in aquaculture.

**Key Words:** Aquaculture, probiotics, probiotics, feed, growth, immunostimulation.

### INTRODUCTION

Probiotics that currently used in aquaculture industry include a wide range of taxa-from *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, *Lactococcus* and *Carnobacterium* spp. *Bacillus*, *Flavobacterium*, *Cytophage*, *Pseudomonas*, *Alteromonas*, *Aeromonas*, *Enterococcus*, *Nitrosomonas*, *Nitrobacter* and *Vibrio* spp., and Yeast *Saccharomyces*, *Debaryomyces* (Irianto and Austin 2002; Sahu *et al.*, 2008; Hemaiswarya *et al.*, 2013).

Aquaculture has grown tremendously during last years becoming an economically important industry (Subasinghe 2009). Today it is the fastest growing food- producing sector in the world with the greatest potential to meet the growing demand for aquatic food (FAO 2006). Although aquaculture activity in Nigeria is started about 50 years ago (Olagunju 2007), aquaculture production in Nigeria is currently about 40,000 metric tonnes contributing only 6% of domestic fish production (Adeogun 2007). Nigerians are high fish consumers and offer the largest market for fisheries production in Africa. Thus, Nigeria has become one of the largest fish importers in the developing world, importing about 600,000 metric tonnes annually (Olagunju 2007).

A significant issue affecting production is the loss of stock through disease. Diseases caused by *Vibrio* spp and *Aeromonas* spp are commonly implicated in episodes of mortality. Aquaculture is viewed as an important food security sector for a growing global human population and has rapidly developed due to intensified culture methods (Hai 2015). Although probiotics offer a promising alternative to chemicals and antibiotic in aquatic animals (Rekiel *et al.*, 2007) and as an aid in the protection of aquacultured species, the ways that probiotics are used in aquaculture need to be considered to avoid producing negative results.

As aquatic animals interact with a diverse range of micro-organisms within animals and their habitat, a screening probiotic process for particular fish species plays a vital role to make them species specific for obtaining desired results in which *in vitro* and *in vivo* tests need to be carried out carefully.

Probiotic administrations have been widely applied via water routine or feed additives (Moriarty 1998 ;Skjeremo and Vodstein 1999 ) with either single or a combination of probiotics or even a mixture with prebiotics or other immunostimulants ( Hai and Fotedar 2009).

There is a developing social attitude against unnecessary use of ADS and where possible it is the move away from non- essential AD use that the responsible farmer now seeks. Give the threat that both ADs and bacterial pathogens pose to farmers, as well as in human health, alternatives are being sought probiotics is one field commanding considerable attention.

### Probiotics Definition

The term "probiotic" comes from Greek *pro* and *bios* meaning "prolife" (Gismondo *et al.*, 1999). Elie Metchnikoff's work at the beginning of this century is regarded as the first research conducted on probiotics (Fuller 1989 ). As the intestine micro biota in aquatic animals constantly interacts with the environment and the host functions, a probiotic is defined as a live microbial adjunct which provides beneficial effects viz,

1. Modifying the host – associated or ambient microbial community,
2. Improving the use of feed or enhancing its nutritional value, enhancing the response of the host towards diseases or
3. Improving the quality of its ambient environment (Verchueren *et al.*, 2000).

In addition, probiotics have been widely used in human and veterinary medicine. They are mainly lactic acid bacteria, putative *Lactobacillus* spp. (Fullar 1989). He defined a probiotic as a "live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". Today probiotics are quite commonplace in health promoting "functional foods" for humans, as well as therapeutic, prophylactic and growth supplements in animal production and growth supplements in animal production and human health (Mombelli and Gismondo 2000; Ouweh and et al., 2002 Sullivan and Nord 2002; Senok et al., 2005). Other commonly studied probiotics include the spore forming *Bacillus* spp and Yeasts *Bacillus* spp. have been shown to possess adhesion abilities, produce bacteriocins (antimicrobial peptides) and provide immunostimulation (Chetif et al., 2001; Cladera Oliver et al., 2004; Due et al., 2004; Barbosa et al., 2005).

### Potential Probiotic Bacteria In Use

Probiotics are harmless bacteria that help the wellbeing of the host animal and contribute directly or indirectly to protect the host animal against harmful bacterial pathogens. The highly researched and most used probiotics according to Berger (2002) are *Lactobacillus acidophilus*, *L. bulgaricus*, *Bifidobacterium longum* and *Bifidobacterium infantis* some of the commercial probiotics currently available in use include Aqualact, probela; Lacto-SaccEpicin, Biogreen, Enviro, wunopu-15 and Epizyme (Abidi, 2003).

Desirable characteristics for the selection of potential probiotics include (i) no harm to the host; (ii) acceptance by the host through ingestion, and colonization and proliferation within the host; (iii) ability to reach target organs where they can work; and (iv) no virulent resistance or antibacterial resistance genes (Verschuere et al., 2000; Kesarcodi Watson et al., 2008).

### Aquatic probiotics are mainly of two types

1. Gut probiotics which can be blended with feed and administered orally to enhance the use full microbial flora of the gut and
2. Water probiotics which can proliferate in water medium and exclude the pathogenic bacteria by consuming all available nutrients. Thus the pathogenic bacteria are eliminated through starvation (Sahu et al., 2008).

### Application of Probiotics In Aquaculture

These organisms can be administered to the aquaculture organisms through feeding injection or immersion of the probiotic bacteria (Irianto and Ansiti 2002).

### Application In Feed

Probiotics are applied with the feed and a binder (egg or liver oil) and most commercial preparation contain either *Lactobacillus* spp or *Saccharomyces*

*cervisiae* (Abidi 2003). Regular use of probiotic in feed of fish in U.K and other European countries has been reported to have several health benefits.

Live feed are very important for shrimp larvae. Unicellular algae (*Chlorella* and *Skeletonema*), brine shrimp *Artemia*, and Rotifer are the two live feeds widely used in shrimp seed production (Naessene et al., 1997). Uhi cellular diatoms and algae are given as feed before the Nauplii moult into Zoea. According to FAO and WHO guidelines, probiotic organisms used in food must be capable of surviving passages through the gut i.e they must have the ability to resist gastric juices and exposure to bile (Senok et al., 2005).

Feed additives such as probiotics (*Lactobacillus rhamnosus*) improved the fecundity of zebra fish (*Danio rerio*) (Gioacchini et al., 2010). The immersion method is also useful (Sung et al., 1994; Itami et al., 1998).

### Direct To Culture Water/Pond

The water probiotics contain multiple strains of bacteria like *Bacillus acidophilus*, *B. Subtilis*, *B. leicheniformis*, *Nitrobacter* sp., *Aerobacter* and *Saccharomyces cerevisiae*. Application of probiotic through water of tanks and ponds may also have an effect on fish health by improving several qualities of water, since they modify the bacteria composition of the water and sediments (Ashraf 2000; Venkateswara, 2007). When probiotics are applied in culture water they multiply and overgrow the pathogenic organism present in the water. Beside this Venkateswara (2007) reported that probiotic bacteria are generally called bacteria which can improve the water quality of aquaculture and inhibit the pathogens in water thereby increasing production.

### Application Through Injection

Application of probiotics by injection is a possibility. Austin et al., (1995) suggested the possibility of freeze drying the probiotic like vaccine and applied either through bathing, or injection. Except for experimental purposes application of probiotic by injection has not been widely reported.

Yassir et al., (2002) has demonstrated the experimental administration of probiotic *Micrococcus luteus* to *Oreochromis niloticus* by injection through intra peritoneal route which had only 25% mortality as against 90% with *Pseudomonas* using the same route. According to Yassir et al., (Yassir 2002; Nikoskelainen 2003). The use of probiotics stimulate Rainbow trout immunity by stimulating phagocytes activity. Complement mediated bacterial killing and immunoglobulin production (Noh 1994).

### Developing Probiotics For Aquaculture

It has been widely published that a probiotic must possess certain properties (Verschuere et al., 2000a). These properties were proposed in order to aid correct establishment of new effective and safe products. The properties include:

1. The probiotic should not be harmful to the host it is desired for,
2. It should be accepted by the host, e.g. through ingestion and potential colonization and replication within the host,
3. It should reach the location where the effect is required to take place,
4. It should actually work *in vivo* as opposed to *in vitro* findings,
5. It should preferably not contain virulence resistance genes or AB resistance genes.

The future application for probiotics in aquaculture looks bright. There is an ever-increasing demand for aquaculture, Probiotics and a similar increase in the search for alternatives to antibiotics. The field of probiotics intended for aquacultured animals is now attracting considerable attention and a number of commercial products are available, particularly directed at shrimp larval cultures. However the advent of new probiotic screening techniques that incorporate an initial *in vivo* component will allow for a wider range of bacteria to be identified as probiotics. The successful acquisition of such novel probiotics might also depend on obtaining a better understanding of the microbial ecology of a cultured species as well as restricting the probiotic screens to the bacterial species that share the immediate environment with the cultured species. Probiotic strains that are already adapted, through natural processes, to the dynamics of an aquaculture, production system will probably lessen any farm management environmental manipulation practices required to achieve the desired probiotic effect in the final product. Introducing such specifically intended probiotics is bound to favour an increase in the application of probiotics, particularly in mollusk production.

### Benefits of Probiotics

Although some of the effects of probiotics have been documented clearly, research is still on going in the area with so many questions on the reality of some of the benefit remaining unanswered. However it is crucial to remember that different probiotic strains are associated with different health benefits (Senok et al., 2005).

### Improvement In Water Qualities

According to Venkateswara (2007), probiotics have reported to regulate micro flora, control pathogenic ones, enhances the decomposition of the undesirable organic substance, improve ecological environment by minimizing the toxic gasses like  $\text{NH}_3$ ,  $\text{N}_2\text{O}$ ,  $\text{H}_2$   $\text{O}_2$ ,

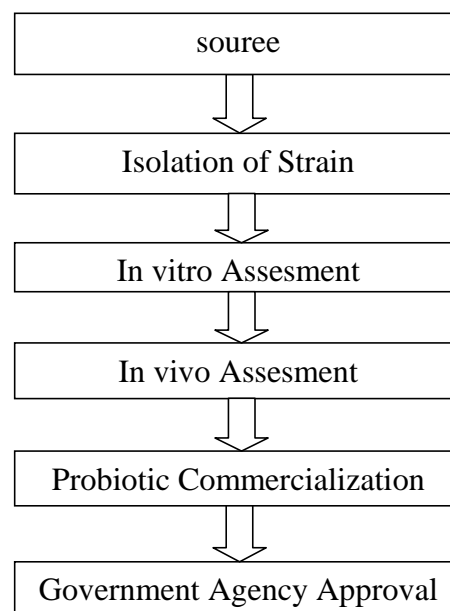
Methane etc, increases population of food organism in the water increases nutritional level of the aquatic host and improve their immunity in the culture water. In several studies, improved water quality has been recorded during the addition of the probiotics especially with *Bacillus* sp (Verschuere et al.,2000). The rationale is that Gram positive *Bacillus* sp. are generally more effective in converting organic matter back to  $\text{CO}_2$  than G negative bacteria which could convert a greater percent age of organic carbon to bacterial biomass or slime.

Probiotics have proven their effectiveness in improving water quality in different approaches. They enhanced decomposition of organic matter reduced nitrogen and phosphorus concentrations and controlled ammonia, nitrite and hydrogen sulphide(Boyd and Massaut1999 ; Ma et al 2009 ; Cha et al ., 2013). Probiotics reduced metabolic wastes during transportation of cardinal tetra (*Paracheirodon aexlorodi*) (Gomes et al.,2009). Probiotics improved water quality by reducing a number of pathogenic bacteria ( Park et al.,2000 ; Dalmin et al.,2001).

### Selection of Probiotics

The initial major purpose of using probiotics is to maintain or re-establish a favourable relationship between friendly and pathogenic Microorganism that constitute the flora of intestinal or skin mucus of fish. A successful probiotic is expected to have a few specific properties in order to certify a beneficial effect.

Guidelines recommend that in the cause of selecting probiotics; the probiont should be evaluated for a number of parameters such as, antibiotics susceptibility patterns, toxic production, metabolic and hemolytic activities infectivity in immune compromised host and side effects.



And in order to produce probiotics for commercialization, the following steps are to be put in to consideration as in Figure: 1

A healthy source of microorganisms from a digestive tract of healthy aquatic animals must be selected. The microorganisms with which the work is to be carried out are isolated and identified by means of selective culture.

A new culture with only colonies of interest for conducting in vitro evaluation such as inhibition of pathogens; pathogenicity to target species; resistance conditions of host; among others are performed.

In case of the absence of restrictions on the use of the target species, experiments with in vivo supplementation and small and large scale, are carried out to check if there are real benefits to the host. Finally the probiotic that presented significantly satisfactory result can be produced commercially and utilized.

### Characteristics of Good Probiotics

Filler (1989) listed the following as features of good probiotic bacteria;

1. It should be a strain, which is capable of exerting a beneficial effect on the host animal e.g increased growth or resistance to disease.
2. It should be non - pathogenic and non -toxic.
3. It should be present as viable cells preferable in large numbers.
4. It should be capable of surviving and metabolizing in the gut environment e.g. resistance to low pH and organic acid.
5. It should be stable and capable of remaining viable for periods under storage and field conditions.

A probiotic agent with all these features has considerable advantage over antibacterial supplements such as antibiotics currently in use. They do not induce resistance to antibiotics which will compromise therapy.

### Reference

- Abidi, R. Use of probiotics in larval rearing of new candidate species. *Aqua culture Asia*, April-June vol. VIII, no 2 (2003): pp 15-16.
- Adeogun, O.A., Ogunbadejo, H.K., Ayinla, O.A., Oresgun, A. Urban Aquaculture; producer perceptions and practices in Lagos State Nigeria. *Middle East Journal of Scientific Research* (2007): 2:21-27.
- Ashraf Ali. Probiotics in fish farming. Evaluation of a candidate bacterial mixture. Vattenbruksintitutionen. Report 19, Umea, ph-Licentiate thesis (2000): pp 1-18.
- Austin B., Stuke L.F., Robrtton P.A., Effendi R., and Griffith D.R.W. A probiotic strain of *Vibrio alginolyticus* effective in reducing disease caused by *Aeromonas salmonicida* *Vibrio anguillarum* and *Vibrio ordalii*. *Journal of fish diseases*, (1995): 18: 93-96.
- Barbosa, T.M., Serra, C.R., La Regione, R.M., Woodward, M.J., Henriques A.O.. Screening for bacillus isolates in the broiler gastrointestinal tract *Applied and Environmental Microbiology* (2005): 71(2), 968-978.
- Berger, A. Probiotics *British medical journal* (2002): 224 (7350): 1364 online.
- Boyd C.E., and Massaut I. Risks associated with the use of chemicals in pond aquaculture. *AquacEng* (1999): 20; 113-132.
- Cha J.H., Rahimnejad S., Jang S.Y., Kim K.W and Lee K.J. Evaluations of *Bacillus* spp. as dietary additives on growth performance of olive flounder (*Paralichthys olivaceus*). Against *Streptococcus iniae* and as water additives *Aquaculture* (2013): 402-403, 50-57.
- Cherif, A., Ouzari, Daffonchio, D., Cherifi, H., Ben Slama, K., Hassen, A., Jaoua, S., Boudabous, A. Thurichun7: a novel bacteriocin produced by *Bacillus thuringiensis* BGM 1-7 a new strain isolated from soil. *Letters in Applied Microbiology* (2001): 38, 243-247.
- Cladera - Olivera, Caron, G.R., Brandelli, A. Bacteriocin - like substance production by *Bacillus licheniformis* strain P40. *Letters in Applied Microbiology* (2004): 38, 251-256.
- Dalmin G., Kathiresan K., and Purusothaman A. Effect of probiotics of bacterial population and health status of shrimp in culture pond. *Ecosystem, Indian J. Exp Biol* (2001): 39; 939-942.
- Duc, L.H., Hong, H.A., Barbosa, T.M., Henriques, A.O., Cutting S.17., Characterization of *Bacillus* probiotics available for human use. *Applied and Environmental Microbiology* (2004): 70 (4), 2161-2171.
- FAO . The state of food insecurity in the world. Food and agriculture organization of the United Nations, Rome, Italy (2006): 235-240.
- FAO Fishery Information, Data and Statistics unit (FIDI) c., Fishery statistical Collections. FIGIS Data Collection FAO - Rome Updated March (2002, 2007) . Available via FIGIS From: <http://www.fao.org/figis/servlet/static?dom=Collection&xml=global-aquaculture-production.Xml>.
- Fuller, R. A review probiotics in man and animals *Journal of Applied Bacteriology* (1989): 66: 365-378.
- Fuller, R., Probiotics in man and animals. *J. Appl Bacteriology* (1989): 66: 365-378.
- Gioacchini, G., Maradonna, F., Lombardo, F., Bizzaro, D., Olivotta, I and Camevali, O. Increase of fecundity by probiotic administration in zebra fish (*Danio rerio*) *Reproduction* (2010): 140, 953-959
- Gismondo, M.R., Iroago, L., Lombardi, A. Review of probiotics available to modify gastrointestinal



- flora, *International Journal of Antimicrobial Agents* (1999):12:287-292.
- Gomes, L.C., Brinn, R.P., Marcon, J.L., Dantas, L.A., Brandao, F.R., De.Abreaj.S., Lemos P.M., mcombc D.M. Benefits of using the probioicfinol I during transportation of cardinal tetra, *paracheirodonexelrodi* (Schultz), in the Amazon. *Aqua Res bio*, (2009): 157-165.
- Hai,N.H. The use of probiotics in aquaculture. *Journal of Applied Microbiology* (2015) .
- Hai,N.V and Fotedar R. Comparison of the effects of the prebiotics (Bio Mos and [deta] -1,3- D-glucan) and the customized probiotics (*pseudomonas synxantha* and *P.aeruginosa*) on the culture of Junenile western king prawns (*penaeuslatisulcatuskishisouye*, 1896). *aquaculture* (2009): 289.
- Hemaiswarya, S., Raja, R.,Ravikumar, R., Carvalho Is. Mechanism of Action of probiotics Braz Arch *BiolTechnol* (2013): 56:113-119.
- Irianto A, Austin B. use of probiotics to control furunculosis in rainbow trout, *oncorhynchusmykiss*(walbaum). *J Fish Diseases* (2002): 25:333-342.
- Irianto, A and Austin B. Probiotics in aquaculture. *Journal of fish diseases* (2002): 25,633-642.
- Itami T., Asano H., Tokushige K., Kubono K., Wakagawa A., Takeno W., Nishimura H., Maeda M. Enhancement of disease oral administration of peptidoglycan derived from *Bifidobacteriumthermophilum*. *Aquaculture* (1998): 164, 277-288.
- Kesarcodi-Watson, A., Kaspar,H., Lategan, M.J. and Gibson, L. Probiotic in aquaculture; the need, principles and mechanisms of action and screening processes, *Aquaculture* (2008): 274-1-14.
- Mac W., Cho Y.S., and Oh K.H. Removel of pathogenic bacteria and nitrogens by *Lactobacillus* spp. JK -8 and JK -11. *Aquaculture* (2009): 287, 266-270.
- Mombelli ,B., Gismondo, M.R. The use of probiotics in medical practice.*IntemationalZoumal of Antimicrobial Agents* (2000):16, 531-536.
- Moriarty, D.J.W.. Control of luminous vibrio species in penaeid aquaculture ponds. *Aquaculture* (1998 ):164,351-358.
- Naessens E., Lavens P., Gomez I., BrowdyC.I., McGovem-Hopkins K., Spencer A.W. Maturation performance of *Peneaus&vannamei* co-fed *Artemia* biomass preparations. *Aquaculture* (1997): 155:87-10.
- Nikoskelainen S., Ouwehand A.C., Byland G., Salminess, lilus E. Immune enhancement in rainbow trout (*oncorhyndus my. kiss*) by potential probiotic bacteria (*Lactobacillus rharnnosus*). *Fish and fish immunology* (2003):15: 443-452.
- Noh S.H., Han K., Won T.H., Choi Y.Y. Effect of antibiotics, enzymes, yeast culture and probiotics on the growth performance of Israeli carp. *Korean journal of Animal Science* .(1994): 36; 480-486.
- Olagunju,F.I.,Adesiyan, I.O., Ezekiel, A.A. Economic viability of caifish production oyo state, Nigenia. *Journal of Human Ecology* (2007): 21:121-124.
- Ouwehand, A.C., Salminen, S., Isolauri, E. Probiotics; as overview of beneficial effects. *Antonic van leewenhock* ,(2002): 82,279-289.
- Park S.C., Shimamura L, Fukunaga M., Mori K., and Wakai T. Isolation of bacteriophages specific to a fish pathogen, *Pseudomonas plecogloss* *Environ Microbiol* .(2000): 66, 1416-1422.
- Rekiel,A., Wiecek,J., Bielecki, W., Gajewska, J., Cichowicz, M., Kulisiewicz, J., Batorska.M., Roszkowski, T, Effect of addition of feed antibiotic flavomycin or probiotic Bio Mos on production results of fatteners, blood biochemical parameters, morphometricindics of intestines and composition of microflora. *ArchirTierzuchtDummerstorf* (2007):50, 172-180.
- Sahu, M.K., Swanakumar, N.S., Sivakumar, K., Thangaradjou, T., Kannan, L. Probiotics in aquaculture importance and future respective. *India. J.Micribio* (2008): 48; 299-308.
- Sahu, M.K., Swarnakumar N.S., Sivakumar,K., Thangaradjou, T., Kannan,L., Probiotics in aquacture importance and future perspectives, *Indian J Microbio* (2008):48:299-308.
- Senok A.C., Ismed A.Y., Botta G.A. Probiotics facts and myths clinical microbiology and infection diseases . (2005):11; 958-960.
- Senok, A.C., Ismaeel,A.Y., Botta, G.A. Probiotics facts and myths clinical Microbiology and Infection (2005): 11(12) 958-966.
- Skjermo, J and Vadstein,O. Techniques for Microbial control in the intensive raering of marine larvae aquaculture (1999): 177,333-34 3.
- Subasinghe,R., Soto,D.,Jia, J. Global aquaculture and its role in sustainable development. *Reviews isaquacul* (2009): 1:2-9.
- Sullivan,A., Nord,C.E. The place of probiotics in human intestinal infections. *Intemational Journal of Antimicrobial Agents* .(2002): 20, 313- 319.
- Sung, H.H., Kou G.H and Sony Y.R Vibriosis resistance induced by glucan treatment in tiger shrimp (*Penaeusmonodon*). *Fish pathology* (1994): 29, 11-17.
- Venkateswara A.R.. Bioremediation to restore the health of aquaculture. *Pond ecosystem Hyderabad 500082 India* (2007): pp1-12.
- Verschere,L., Rombatu, G., Sorgeloos, P., Verstraete, W. Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Review* (2000a): 64, 655-671.

Verschuere, L., Rombaut, G., Songeloos, P and Verstraete, W.. Probiotic bacteria as biological control agents in aquaculture *Microbio/Mol.Biol Rev* (2000): 64,655-671.

Yassir A.L., Adel M.E., Azze A. Use of probiotic bacteria as growth promoters antibacterial and the effect on physiological parameters of *oreochromus niloticus*. *Journal of fish diseases* (2002): 25; 633-642.

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