Role of Probiotics in Aquaculture with Special Reference to Water Quality, Disease Control and Feed Digestibility

Nisha Manish
Associate Professor, Zoology Dept.,
Meerut College, Meerut
Email: drnishamanish@gmail.com

Reference to this paper should be made as follows:

Nisha Manish

"***Role of Probiotics in Aquaculture with Special Reference to Water Quality, Disease Control and Feed Digestibility***",

Voyager: Vol. VIII, No. 1, June 2017, pp. 78-87

Abstract

To increase the feed efficiency and disease resistance among the fish species and other culture organisms, use of probiotics proven to be instrumental for aquaculture. Probiotics affect the water quality and disease causing microorganisms by improving the quality of water and decreasing the rate of infection in an aquatic system. Further, the effect of probiotics on the gut microbiota has also been discussed. In the present review article ,an attempt has been made to gather the data pertaining to the effect of probiotics on the water quality, infection and digestibility in aquatic organisms with special reference to aquaculture.

Keywords : *Probiotics, water quality, aquaculture, disease resistance, Infection*

Introduction

It goes without saying that aquaculture plays a pivotal role in the food requirements of a country. Aquaculture is the fastest growing food producing industry with an average annual growth rate of 8.9 % . A large number of the world population depends upon this to fulfil the nutritional requirements.it is practiced in various agro-climatic zones including tropical to temperate regions.(Subasinghe, 2005).In the present scenario, due to several anthropological activities, excessive use of chemotherapeutic substances and pesticides for over production, the aquaculture is drastically affected in terms of water quality. Further, due to high stocking density and disease caused by various fish pathogens, the production of aquaculture has decreased remarkably. (Bondad-Reantaso, 2005; Gaddi Pati *et a*l., 2015). This directly affects the economy of the country. Over stocking and over production, accumulation of excessive organic matter formed by feces and feed of aquatic organisms and phytoplanktons in aquaculture leads to the growth of a number of pathogens. Moreover, degradation of organic matters leads to the oxygen consumption and formation of a number of nitrogen wastes such as ammonia, nitrites and nitrates . Removal of these substances becomes mandatory for a sustainable aquaculture system. Therefore, to maintain the water quality and reduce the pathogens in an aquaculture system ,use of probiotics is of great importance. (Sahu *et al.*,2008)

What are Probiotics?

In Greek , the meaning of "pro" and "bios" is "profile"((Schrezenmeier *et al.*,2001). According to Parker (1974) ,probiotics may be defined as "organisms and substances which contribute to intestinal microbial balance'. Fuller (1989) described probiotics as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance." Probiotics may also be defined as "a live microbial adjunct which has a beneficial effect on the host by modifying the host- associated or 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). As defined, a probiotic should benefit the host either nutritionally or by changing its immediate environment (Kesarcodi-Watson *et al.* 2008). According to FAO/WHO definition of a probiotic "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (as reviewed by Colin Hill *et al*., 2014) Probiotics may also be defined as "monocultures or mixed cultures of microorganisms applied to animals or humans that benefit the host by improving properties of indigenous microflora" (Havenaar, R. Huis, I, 1992). Gatesoupe in 1999, defined probiotics as "microbial cells administered in a certain way, which reaches the gastrointestinal tract and remain alive with the aim of improving health". Lazado *et al.* (2014a,) defined probiotics as 'live or dead, or even a component of the microorganisms that act under different modes of action in conferring beneficial effects to the host or to its environment'

Use of Probiotics in Aquaculture

In 1905, Dr. Elie Metchnikoff described the positive role of some bacteria among farmers who consumed pathogen-containing milk and that "reliance on gut microbes for food makes it possible to take steps to change the flora of our bodies and to replace harmful microbes by beneficial microbes" .However, a formal application of probiotics in aquaculture was implemented first by Kozasa (1986) by using spores of *Bacillus toyoi* as feed additive to increase the growth rate of yellow tail, *Seriola quinqueradiata.* (As described by Cruz *et al.*, 2012)

Probiotics Improve the Water Quality

It has been investigated that probiotics affect the water quality in an aquaculture system (Boyd, *et al.*,1998; Nimrat*et al., 2012*).

In 1991, Porubcan stated that *Bacillus* sp have an ability to increase productivity of *Penaeus monodon* farming and they also help to increase water quality by decreasing the concentrations of ammonia and nitrite. A number of studies have shown that besides playing a significant role in maintaining an optimum level of ammonia in cultured ponds, probiotic bacteria are also responsible for the decomposition of organic matter and production of food material from dissolved organic matter. (Jana ,B.B.and. De U.K, 1990)

Nimrat *et al* (2012) during their study, compared the water quality of treated and control ponds and showed that administration of mixed probiotics (*Bacillus s*p) affects the growth of beneficial bacteria. They act as enhancer to improve the water quality as level of ammonia, pH and Nitrite were noticeably decreased.

Sunitha *et al.* (2013) during their experimentation, stocked three fish species in three different earthen ponds. The three species were catfish (*Pangasius sutchi)* and Indian major carps, catla (*Catla catla)* and rohu (*Labeo rohita*). In two ponds , the probiotics *Nitrosomonas* and *Nitrobacter* species were used and the remaining pond was used as control. The results showed that the concentrations of ammonia, nitrite and orthophosphates were higher in control ponds than in the treated ponds. In treated ponds, zooplankton and beneficial bacterial loads were observed to be increased whereas pathogenic Pseudomonas loads decreased. They concluded that use of probiotics maintains an optimum level of water parameters (dissolved oxygen, ammonia, nitrite ,nitrate and phosphate, bacterial loads and zooplanktons) thus acting as an instrument for growth, survival and prevention from disease in aquaculture (Sunitha *et al.* ,2013).

Probiotics and Feed Digestibility

Use of probiotics in aquaculture has shown nutritional benefits such as improving feed digestibility and feed utilization (Fuller 1989; Kesarcodi-Watson *et al.* 2008; Das *et al.,* 2008).

It is stated that metabolism of the microorganisms plays an important role in biogeochemical cycles/processes in the aquatic environment through the processes of aerobic and anaerobic decomposition. Probiotics play an important role in recycling of organic matter. They also help to dissolve insoluble inorganic salt and regeneration of nutrients. (Das *et al*., 2008). Thus, having a positive effect on the utilization of nutrients, digestibility and growth. (Ravi *et al*., 1998)

Probiotics decompose the organic matter, producing a variety of exoenzymes like proteases, lipases. These exo-enzymes play an important role in improvement of feed digestibility and help in digesting the undigested feed by degrading the undigested feed and feces in a pond (Das *et al*., 2008). They are a good stimulator of appetite ,help in absorption ofnutrients and play an important role to modulate the immunity. (Irianto, A, Austin B., 2002)

Probiotics in Disease Control

Studies have revealed that probiotic bacteria are proven to be beneficial for the health of the host (Guarner and Schaafsma, 1998). They have an inhibitory action on pathogens. The widely used probiotic species are *Bacillus sp*. (Banerji *et al*., 2007), *Lactobacillus* (Rollo *et al*., 2006), *Saccharomyces sp.* (Ahilan *et al*., 2004), *Enterococcus s*p. and *Bacillus subtilis* (Ghosh *et al*., 1999). These species are now used for oral bacteriotherapy in aquaculture. *Bacillus* sp. have been shown to produce bacteriocins- the antimicrobial peptides produced by the bacteria that inhibit the growth of closely related pathogenic strains. Yeast *Saccharomyces cerevisiae* reported to show immunostimulatory activity and the production of inhibitory substances. (Das *et al.*, 2008) thus helping in disease control. Moriarty (1998) showed that *Bacillus* spp. decrease the proportion of *Vibrio* spp. in shrimp ponds.

Further,Probiotic bacteria act as competitors for attachment sites in the gut and nutrients in the aquatic environment. They alter enzymatic activity of pathogens. They have shown immunostimulatory functions. (Balcazar *et al*. 2006).

Marine *Actinobacteria a*re proven to be magnificent probiotics for aquaculture. As reviewed by Das *et al*.(2006a) *Actinobacteria* isolated from the marine environment include *Aeromicrobium,, Marinophilus, Salinispora, Marinispora, Solwaraspora, Salinibacterium, Kocuria, Williamsia, and Verrucosispora, in addition to Actinomyces, Actinopolyspora, Micromonospora, Micro- polyspora, Nocardia, Rhodococcus, Streptomyces, Strepto sporangium, and Streptoverticillium.*

You *et al.* (2005) stated the effect of probiotic actinomycetes on pathogenic

Vibrio sp. in shrimp culture .He was of opined that marine actinomycetes are remarkable probiotic strains due to their ability to degrade macromolecules, such as starch and protein in culture pond water; the production of antimicrobial agents and the formation of heat- and desiccation-resistant spores.Studies have shown use of marine actinomycetes to control the aquatic pathogens. In aquaculture practices formation of biofilm by a number of pathogenic microorganisms leads to disease outbreak.You *et al.* (2007) reported that marine actinomycetes work against biofilms produced by *Vibrio sp*, thus helping in prevention of infection caused by *Vibrio sp.* Das *et al.* (2006b) studied the use of Streptomyces on the growth of black tiger shrimp and found a positive effect on the growth and survival of black tiger shrimp.

Studies have revealed that a number of compounds are produced by these probiotics having bactericidal effect like antibiotics, siderophores and exoenzymes (proteases and lipases) An antibiotic product was extracted from marine actinomycetes and after mixing with feed , in vivo studies have shown that this product was quite effective on white spot syndrome virus in black tiger shrimp (Kumar *et al*., 2006). Streptomyces release antibiotics that act upon the competing microorganisms in an aquatic environment. These antibiotics are small molecules and interfere with gyrase protein that help in DNA replication. As a result, growth of the bacterial population was hampered due to abnormal division. However, as reported by Das *et al.*(2008) Streptomyces protects itself from its own antibiotics by the production of efflux pumps (used against the influx of antibiotics), ribosomal protection proteins (protect ribosome and prevents interfering with protein synthesis), and modifying enzymes (neutralize antibiotics by the production of acetyl or phosphate groups).

Actinobacteria are saprophytic microorganisms . They are capable of being involved in the process of mineralization and nutrient cycles in the aquaculture ponds. Being colonized in the host intestine, they produce exoenzymes helpful in facilitating feed utilization and digestion. Moreover, they play an important role in the resistance to infectious diseases by producing antibacterial substances. According to Wang *et al*. (2008), these probiotics adhere and colonize on the mucosal surfaces of the intestine, thus exhibit protective mechanisms against pathogens.

Number of species of *Pseudomonas* and *Aeromonas* have been reported pathogenic and reported to cause skin ulcerations like Epizootic ulcerative syndrome (EUS), albinoderma, erythroderma, tail and fin rot and hemorrhagic septicemia (Das, 2004). Padmavathi *et al*. (2012) and Sunitha *et al.,* (2013) showed that with the introduction of probiotic bacteria the disease causing species were suppressed by the probiotics as they have a stronger appetite thus eliminating the bad bacteria.

Gaddipati *et al.* (2015) in their study demonstrated that Ecotech is a mixture of probiotic microorganisms and gave an effective zone of inhibition.They further stated that a minimum effective dose (µl) of Ecotech was sufficient to inhibit the growth of pathogenic *Vibrio sp* in the shrimp industry. The probiotic preparation includes a combination of bacterial strains viz. *Lactobacillus bulgaricus, Lactobacillus plantarum, Streptococcus thermophilus, Enterococcus faecium, Enterococcus faecalis, Bifidobacterium sp. and Escherichia coli.*

Probiotics and Gut Microbiota of Cultured Organisms

It is important to have an optimum population of gut microbiota for maintaining gut health.Occurrence of any infection causes disturbance in naturally inhabiting microorganisms in the gut which leads to several health-related issues. . Supplementation of dietary probiotics improves fish health (Han *et al.,*2015). Further, for maintaining the gut health of cultured organisms a proper selection of probiotic supplement is essential as it varies greatly from one species to another. Some important bacterial species with probiotic potential are *Bacillus* sp., *Micrococcus* sp., *Enterococcus*sp., *Phaeobacter* sp., *Shewanella* sp., lactic acid bacteria, and *Pseudomonas* sp that play an important part in maintaining the gut flora in fish (Lobo *et al*., 2014; Merrifield *et al.*, 2010a, b).. Newaj-Fyzul *et al.*(2007) reported that probiotic dietary supplement enhanced the beneficial population of bacterium *Bacillus subtilis* in rainbow trout and colonization of *B. subtilis* on the gut epithelial surface protects against pathogenic strain of *Aeromonas* sp.Similar results were shown by Bagheri *et al.* (2008), who used commercial probiotic product (Bioplus) containing a mixture of *B. subtilis* and *Bacillus licheniformis*. Similarly, an enhancement in the population of *B. subtilis* on the intestinal mucosal surface has been observed in four fish species (*Poecilia sphenops*, *Xiphophorus maculatus*, *Poecilia reticulata*, and *Xiphophorus helleri*) fed with *B. subtilis* containing diet and (Ghosh, Sinha, & Sahu, 2008).. Among several probiotic strains, *lactobacillus* groups as probiotics in aquaculture have been studied extensively. Studies have shown that due to their property to colonize on the epithelial surface of the gut,lactobacilli has beneficial effects on gut microbiota (Merrifield &Carnevali, 2014) Modulation of gut microbiota by probiotics is independent of age and maturation ranging from larvae to adult (Merrifield & Carnevali, 2014) . However,a number of factors like water quality, temperature and pH,timing of probiotic diet and age of fish may influence the properties of probiotics.

A number of factors such as strain, species, temperature, water activity, hydrogen-ion concentration (pH),oxygen and .osmotic pressure influence the functioning of probiotics So, special attention must be paid during the process of selection of a probiotic strain, as in probiotic research, screening experiments involve a large number of tests to obtain a promising strain (Kesarcodi-Watson *et al.* 2008).

Conclusion

It may be concluded that probiotics are the appropriate alternative to maintain the sustainability of an aquatic environment. They are proven to be effective as far as water quality, disease control and feed digestibility is concerned. However, further research should be carried out in estimation of appropriate doses and quantity incorporated with feeds and in consortium of bacteria and various physical and chemical factors those affect the probiotics must be studied

Acknowledgments

Acknowledgments are due to all eminent contributions quoted by the author in this short review article.

References

1. Ahilan, D.H., Salmine, S. & Lilius, E.M (2004). Innate immune responses in rainbow trout (*Oncorhynchus mykiss,* Walbaum) induced by probiotics. *Fish Shellfish Immunol*. 21: 513-24.
2. Bagheri, T., Hedayati, S. A., Yavari, V., Alizade, M., & Farzanfar, A. (2008). Growth, survival and gut microbial load of rainbow trout (*Oncorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. *Turkish Journal of Fisheries and Aquatic Sciences*, *8*, 43–48.Google Scholar
3. Balcazar JL, Decamp O, Vendrell D, De BlasI, Ruiz-Zarzuela I (2006) Health and nutritional properties of probiotics in fish and shellfish. *Microb Ecol Health Nutri* 18:65–70
4. Banerjee, R., Misra, C.K., Das, B.K., Mukherjee, S.C. & Meher, P.K.(2007). The immunomodulatory effects of tuftsin on the nonspecific immune system of Indian major carp, *Labeo rohita. Fish Shellfish Immunol*. 20: 728–738. [4].
5. Bondad-Reantaso, M. G., Subasinghe, R. P., Arthur, J. R., Ogawa, K., Chinabut, S., Adlard, R., Tan, Z. & Shariff, M.(2005). Disease and health management in Asian aquaculture. *Vet. Parasitol.* 132: 249-272
6. Boyd,C.E.and Gross,A.(1998). Use of Probiotics for Improving Soil and Water Quality in Aquaculture Ponds. In Flegel ,T.W.(Ed).Adv.Shrimp Biotech. National Centre for Genetic Engineering and Biotechnology, Bangkok .
7. Colin Hill, Francisco Guarner ,Gregor Reid,Glenn R. Gibson, Daniel J. Merenstein, Bruno Pot,Lorenzo Morelli, Roberto Berni Canani, Harry J. Flint, Seppo Salminen, Philip C. Calder&Mary Ellen Sanders, (2014)The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic *Nature Reviews Gastroenterology & Hepatology 11*: 506–514

8. Cruz, P. M., Ibáñez,A.L., Hermosillo, O.A.M.and Saad,H.C.R.(2012).Use of Probiotics in Aquaculture.*ISRN Microbiol.*: 916845.Published online. doi: 10.5402/2012/916845
9. Das.B.K.(2004). Bacterial diseases of fish and their control, In: P. Swain, P.K. Sahoo and B.K. Das (Ed.), Disease Diagnosis and Health Management of Freshwater Fish and ShellFish, Bhubaneswar, Central Institute of Freshwater Aquaculture, 45-52.
10. Das ,S., Ward, L. R. & Burke ,C. (2008).Prospects of using marine actinobacteria as probiotics in aquaculture.*Appl Microbiol Biotechnol* 81:419–429
11. Fuller R., Turvey, A. (1971). Bacteria associated with the intestinal wall of the fowl *(Gallus domesticus)*. *J. Appl. Bacteriol* . 34: 617-622 [7].
12. Fuller R. (1978). Epithelial attachment and other factors controlling the colonization of the intestine of the gnotobiotic chicken by *lactobacilli*. *J. Appl. Bacteriol*, 46: 335-342
13. Fuller, R. (1989). Probiotics in man and animals – A review. *J. Appl. Bacteriol*. 66: 365–378.
14. Gaddipati, G .Rahul, Gunturu,C.,,R.and R Rajkumar and Raghavaiah ,G. (2015).Ecotech : A probiotic for maintaining water quality and control of *Vibrio sp in Vitro*.*J. Res.Agricul. Ani. Sci.*3(6):1-4
15. Gatesoupe FJ.(1999;) The use of probiotics in aquaculture. *Aquaculture.*180(1-2):147–165.Google Scholar
16. Ghosh, S., Kumar, R., Mukherjee, S.C., Prasad, K. ,Pal ,A.K.(1999). Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp *Labeo rohita* (Ham.). *Aqua. Res*. 37: 1215-21.
17. Ghosh, A., Sinha, A., & Sahu, C. (2008). Dietary probiotic supplementation in growth and health of live-bearing ornamental fishes. *Aquaculture Nutrition*, *14*(4), 289–299.CASGoogle Scholar
18. Guarner F, Schaafsma GJ. Probiotics.(1998)*Intern.J.Food Microbiol.* ;39(3):237–238. [PubMed] [Google Scholar]
19. Han, B., Long, W. Q., He, J. Y., Liu, Y. J., Si, Y. Q., & Tian, L. X. (2015). Effects of dietary *Bacillus licheniformis* growth performance, immunological parameters, intestinal morphology and resistance of juvenile Nile tilapia (*Oreochromis niloticus*) to challenge infections. *Fish and Shellfish Immunology*, *46*(2), 225–231.CASPubMedGoogle Scholar
20. Havenaar R, Huis I.(1992). The Lactic Acid Bacteria in Health and Disease, *Volume 1.* New York, NY, USA: *Elsevier*; (Edited by B. J. B. Wood).Google Scholar
21. Irianto A, Austin B. *(*2002); Probiotics in aquaculture. *J. Fish Diseases.*25(11):633–642.
22. Jana B.B.and. De U.K, (1990)Spatial and seasonal distribution of heterotrophic bacteria in pond water and sediments under different management practices, *Internationale Revue der Gesamien Hydrobiologie*, 75, 639-648
23. Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L (2008) Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquacult* 274:1–1

24. Kozasa M. (1986)Toyocerin (*Bacillus toyoi*) as growth promoter for animal feeding. *Microbiologie Aliments Nutrition.*;4(2):121–135. [Google Scholar]

25. Lazado, C. C.,Christopher,M A., Caipang.(2014a). Mucosal immunity and probiotics in fish. *Fish Shellfish Immunol.* 39:78–89. [Crossref], [PubMed], [Web of Science ®], [Google Scholar]

26. Lobo, C., Moreno-Ventas, X., Tapia-Paniagua, S., Rodríguez, C., Morínigo, M. A., & de La Banda, I. G. (2014). Dietary probiotic supplementation (*Shewanella putrefaciens* Pdp11) modulates gut microbiota and promotes growth and condition in *Senegalese sole larviculture*. *Fish Physiology and Biochemistry*, *40*(1), 295–309. CASPubMedGoogle Scholar

27. Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. T. M., Bøgwald, J., Castex, M., & Ringø, E. (2010a). The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture*, *302*(1-2), 1–18.

28. Merrifield, D. L., Harper, G. M., Dimitroglou, A., Ringø, E., & Davies, S. J. (2010b). Possible influence of probiotic adhesion to intestinal mucosa on the activity and morphology of rainbow trout (*Oncorhynchus mykiss*) enterocytes. *Aquaculture Research*, *41*(8), 1268–1272.

29. Merrifield, D. L., & Carnevali, O. (2014). Probiotic modulation of the gut microbiota of fish. In D. L. Merrifield, & E. Ringø (Eds.), *Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics*, .85–2221 Oxford, UK: Wiley-Blackwell Publishing.Google Scholar

30. Metchnikoff ,E.(1907). The Prolongation of Life, Optimistic Studies.Lactic acid as inhibiting intestinal putrefaction; UK:London,Mitchell Heinemann;. 161–183.Google Scholar

31. Moriarty, D.J.W., 1998. Control of luminous Vibrio species in penaeid aquaculture ponds. *Aquacult.*, 164: 351-358.

32. Nimrat, S. Suksawat, S., Boonthai,T andVuthiphandchai, V. (2012) Potential *Bacillus* probiotics enhance bacterial numbers, water quality and growth during early development of white shrimp (*Litopenaeus vannamei*).*Vet.Microbio*l.159(2-4):443-450

33. Newaj-Fyzul, A., Adesiyunz, A. A., Mutani, A., Ramsudhag, A., Brunt, J., & Austin, B. (2007). *Bacillus subtilis* AB1 controls Aeromonas infection in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of Applied Microbiology*, *103*(5), 1699–1706.CASPubMedGoogle Scholar

34. Padmavathi, P ,Sunitha, K. and Veeraiah, K.(2012).Efficacy of probiotics in improving water quality and bacterial flora in fish ponds .*Afri. J. Microbiol. Res.* 6 (49): 7471-7478

35.Parker R B (1974). Probiotics the other half of the antibiotic story. *Animal Nutrition and Health*, 29:4-8

36. Porubcan RS. (1991). Reduction of ammonia nitrogen and nitrite in tanks of *Penaeus monodon* using floating biofilters containing processed diatomaceous earth media pre-inoculated with nitrifying bacteria. Proceedings of the Program and Abstracts of the 22nd Annual Conference and Exposition; Puerto Rico, Spain. *World Aqua. Soci.;* Google Scholar

37. Porubcan RS.(1991). Reduction in chemical oxygen demand and improvement in *Penaeus monodon* yield in ponds inoculated with aerobic *Bacillus* bacteria. Proceedings of the Program and Abstracts of the 22nd Annual Conference and Exposition; Puerto Rico, Spain. *World Aqua. Soci.*; Google Scholar
38. Ravi, V., S. Ajmalkhan & S. Rajgopal, (1998). Influence of probiotics on growth of Indian white prawn Penaeus indicus. *J. Sci. Id. Res.*, 57(10-11): 752-756
39. Rollo, G.K., Ei-Sery, N.A., Abdel-Razek, F.A. & Taha, S.M.(2006). Evaluation of various probiotic bacteria for the survival of Penaeus japonicus larvae. *Fresenius Environ. Bull.* 15: 1506-1511.
40. Sahu, M. K., Swarna Kumar, N. S., Sivakumar, K. Thangaradjou, T. and Kannan,.L.(2008).Probiotics in aquaculture: importance and future perspectives.I*ndian J. Microbiol.*48:299–308 299 DOI: 10.1007/s12088-008-0024-3
41. Schrezenmeir J, De Vrese M.(2001). Probiotics, prebiotics, and synbiotics—approaching a definition. *American Journal of Clinical Nutrition.*;73:361S–364S. PubMedGoogle Scholar
42. Subasinghe, R.P.,(2005). Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. *Prev. Vet. Med*. 67: 117– 124.
43. Sunitha k., and Padmavathi.(2013) Influence of Probiotics on Water Quality and Fish Yield in Fish Ponds *Int. J. Pure Appl. Sci. Technol.*, 19(2): 48-60
44. Verschuere L, Rombaut G, Sorgeloos P, Verstraete W (2000) Probiotic bacteria as biological control agents in aquaculture. *Microbiol Mol Biol Rev* 64:655–671
45. Wang Y-B, Jian-Rong L, Junda L (2008) Probiotics in aquaculture: challenges and outlook. Aquaculture doi:10.1016/j.*Aquacult*. 2008.06.002
46. You J, Cao LX, Liu GF, Zhou SN, Tan HM, Lin YC (2005) Isolation and characterization of actinomycetes antagonistic to pathogenic *Vibrio sp.* from nearshore marine sediments. *World J Microbiol Biotechnol* 21:679–682
47. You J, Xue X, Cao L, Lu X, Wang J, Zhang L, Zhou S (2007) Inhibition of Vibrio biofilm formation by a marine actinomycete strain A66. Appl *Microbiol Biotechno*l 76:1137–1144