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Beneficial and destructive effects of probiotics in aquaculture systems-A review

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Abstract
Aquaculture is one of the fastest on rising sectors in the world today. An aquaculture system is diversified as fresh, brackish and marine water culture systems. However, the occurrence of the disease is a major constraint for its sustainability. Probiotics are often employed to control bacterial pathogens in the aquaculture systems. Beneficial probiotics such as *Lactobacillus, Bifidobacterium, Streptococcus thermophilus, Pediococcus, Streptococcus and Carnobacterium* spp. *Bacillus, Flavobacterium, Cytophaga, Pseudomonas, Alteromonas, Aeromonas, Enterococcus, Nitrosomonas, Nitrobacter, and Vibrio* species are used commercially. But with very limited studies have been conducted on destructive effects of probiotics in aquaculture systems and environment. This paper is a review of both the effects of probiotics in relation to the environment and aquaculture sustainability.

Keywords: Aquaculture, Probiotics, Livefeed, Hatchery, Water quality

1. Introduction
Aquaculture globally has undergone tremendous growth during the last fifty years from a production of less than a million tonnes in the early 1950s to over 50 million tonnes in the year 2008 (FAO,2009) [22]. Aquaculture is expanding into new directions, intensifying and diversifying. With increasing demand for environment friendly aquaculture, the use of alternatives to antibiotic growth promoters in fish nutrition is now widely accepted. Science-based knowledge on probiotics and prebiotics has increased in recent years (Subasinghe *et al*., 2009; FAO, 2006) [62, 20]. Aquaculture of finfish, crustaceans, molluscs, and algal plants is one of the fastest-growing food-producing sectors today. While probiotic research in aquaculture focused, in the beginning, on fish juveniles, more attention has recently been given to larvae of fish and shellfish and live food organisms in aquaculture as they are important causes of diseases in organisms and also provide health benefits to the organisms in several ways (Verschuere, 2000) [74]. They are important sources for C, N, S cycles and any imbalance in the micro flora of systems leads to pathogenesis (Rengpipat, 1996) [59]. The probiotics in aquaculture, one must be concerned with their indirect effects on ecosystem cycles and food chains. Although the use of antibiotics and chemotherapy remains the method of choice as disease control strategy, the abuse of chemotherapeutics, especially antibiotics has resulted in development of multiple antibiotic resistant bacteria (Kim *et al*., 2004; Cabello 2006; Bestha Lakshmi *et al*., 2013) [40, 15, 11]. There is an increasing interest within the industry in the control or elimination of antimicrobial use. Therefore, alternative methods need to be developed to maintain a healthy microbial environment in the larval rearing tanks. Biological control has been described as the utilization of natural enemies to reduce the damage caused by noxious organisms to tolerable levels. Many years, studies focused on microorganism's characteristic from intestinal microbiota, and the term "probiotic" was mainly restricted to gram-positive lactic acid bacteria, particularly representative of the genera *Bifidobacterium, Lactobacillus,* and *Streptococcus.*
The researchers have been attempting to isolate beneficial bacteria from various sources like soil, water and animal gut to control disease causing pathogens in aquaculture systems (Austin and Day 1990; Munro *et al*., 1995; Gomez-Gil *et al*., 2000; Ahmed *et al*., 2005; Skrodenyte-Arbaciauskiene *et al*., 2006; Kim *et al*., 2007; Bestha Lakshmi *et al*., 2013) [7, 48, 30, 5, 68, 39, 11]. Probiotics that currently used in aquaculture industry include a wide range of taxa – from *Lactobacillus, Bifidobacterium, Pediococcus,*

Lake *Streptococcus and Carnobacterium* spp. *Bacillus, Flavobacterium, Cytophaga, Pseudomonas, Alteromonas, Aeromonas, Enterococcus, Nitrosomonas, Nitrobacter, and Vibrio* spp., and *yeast Saccharomyces, Debaryomyc* (Irianto and Austin 2002; Sahu *et al*., 2008; Hemaiswarya *et al*., 2013) [35, 63, 34]. While using some beneficial probiotic bacteria for fish, some might be highly pathogenic e.g. *Vibrio alginolyticus.* It will lead to destructive effect in the aquaculture systems. So, it is necessary to take care of the choice of probiotic before administration. The best known probiotic strains such as *Bifidobacteria*, *Lactobacilli*, and *Streptococcus thermophilus* are employed as the dietary supplementation with probiotic bacteria in the aquaculture industry and it will increase the efficiency and sustainability of aquaculture production (Kim *et al*., 2007) [39].

2. Definition – Probiotics

The term “probiotic” comes from Greek *pro* and *bios* meaning “prolife” (Gismondo *et al*., 1999) [28]. Elie Metchnikoff’s work at the beginning of this century is regarded as the first research conducted on probiotics (Fuller, 1989) [23]. He defined a probiotic as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance”. This definition is still widely referred to. However, the term probiotic was introduced until 1965 by Lilly and Stillwell [41], as a modification of the original word “probiotika.” It was used to describe substances produced by a microorganism that prolong the logarithmic growth phase in other species. It was described as an agent which has the opposite function of antibiotics. Later, Sperti (2006) [67] modified the concept of “tissue extracts that stimulate microbial growth”.

Probiotics are now also being used in aquaculture. As not only the digestive tract is important, but also the surrounding water, the definition may therefore have to be modified. Gatesoupe (1999) [27] defines probiotics as microbial cells that are administered in such a way as to enter the gastrointestinal tract and to be kept alive, with the aim of improving health. A live microbial supplement can beneficially affect the host animal by improving its microbial balance. Moriarty (1999) [47] proposed an extending definition as “living microbial additives that benefit the health of hydrobionts and increase productivity”.

3. Effects of probiotics use in Fish/shrimp hatchery

Aquatic probiotics are mainly of two types: 1) gut probiotics which can be blended with feed and administrated orally to enhance the useful 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) [63]. Moreover, it will act as antagonistic against pathogens; enhancement of the immune response and disease resistance, improving enzyme activity, feed digestibility and feed utilization, fish health and performance (Verschuere *et al*., 2000; Balcazar *et al*., 2007) [74, 10]. The first type probiotics are used mainly in finfish aquaculture and the second type in shrimp aquaculture. Commercially available probiotics include pure strains, defined mixture of specific strains, and also consortia of strains and undefined mixtures. Generally, probiotics proposed as biological control agents in fish aquaculture are applied in the feed or as a water additive supplement.

The introduction of microbial control practices by means of probiotics may have a beneficial effect on the cultures in hatcheries. A relatively dense, nonpathogenic and diverse adherent microbiota present on the eggs would probably be an effective barrier against colony formation by pathogens on fish eggs (Hansen and Olafsen, 1989) [33]. This rationale has been tried with cod eggs. He attempted to manipulate the egg microbiota of cod (*Gadus morhua*) by incubating gnotobiotic eggs in cultures of defined inhibitory bacterial strains; however, these strains failed to prevent colonization of the eggs by the microbiota naturally present in the incubator. Nevertheless, it should be noted that the choice of strains is very important.

The screening and preselection of potential or putative probiotics should be based on extensive experimental work performed *in vivo*. Therefore, it would be better if the experimental setup described by Hansen and Olafsen (1989) [33] was used as a preselection tool, not to verify the effect of selected bacteria, as is often done now. The experimental evidence suggests that the bacteria may have provided essential nutrients not present in the algae, or improved larval digestion by contributing enzymes. A clear distinction has to be made between the probiotic function of a bacterium and that of a feed. Probiotic organisms colonize the digestive tracts of their hosts and out compete potential disease-causing organisms. There is evidence that probiotics inhibit a wide range of fish pathogens, with a common added benefit of appetite stimulation, which can last longer than the probiotic treatment period (Brunt *et al*., 2007) [14]. Hence, successful application of the principle of competition to natural situation is not easy and this remains as a major task for microbial ecologists (Sahu *et al*., 2008) [63].

4. Beneficial use of probiotics in fish and shrimp culture systems

Beneficial effects	Species	Reference
Reduction of nitrogen compounds, Improved water quality, Increase oxygenation, and growth rates	Nitrifies, Sulphur bacteria, *Bacillus* spp, *Pseudomonas* spp. *Bacillus* spp., *Bacillus toyoi, Streptomyces*	Das *et al*. (2006) [17]
Production of fingerlings in females	*L. acidophilus, L. casei, E. faecium,* and *B. thermophilum*	Abasali and Mohamad, (2010) [1]
Reduced chemical oxygen demand; Better digestive enzyme activities; Better growth performance and feed efficiency	*Bacillus* sp.	Porubcan, 1991; Bagheri *et al.* (2008); Yanbo and Zirong (2006) [55, 9, 79].
To enhance the immune responses	*Lactobacillus plantarum*	Mohapatra, *et al*. (2014) [45]
Antagonistic activity towards shrimp pathogen vibriosis	*V. alginolyticus* (NCIMB 1339) and *V. gazogenes*	Sahu *et al*. (2008) [63]

	(NCIMB 2250)	
Antioxidant defences and oxidative stress of *Litopenaeus Vannamei*	*Pediococcus acidilactici*	Bestha Lakshmi *et al*. (2013) [11]
Compete with other bacteria in pond and clearing the organic matter	*Bacillus Licheniformis*	Moriarty, (2005) [45]
To overcome the temperature-associated stress, also substantially improved the fish growth.	*B. subtilis, L. lactis* and *S. Cerevisiae*	Mohapatra, *et al*. (2014) [45]
Increased production of erythrocytes and leukocytes	*Lactobacillus sporogens*	Mohapatra, *et al*. (2014) [45]
Increase the digestibility in fish and shrimp	*B. subtilis, B. licheniformis,* and *B. Cereus*	Raida *et al*. (2003); Merrifield *et al*. (2010) and Vine *et al*. (2006) [56, 44, 75]
Enhance the cellular and humoral immune responses in fish	*Carnobacterium maltaromaticum* B26 *Carnobacterium divergens*	Kim and Austin (2006a) [38]
Increased resistance to *Aeromonas* spp. and *Salmonicida* sp.	*Lactobacillus rhamnosus*	Nikoskelainen *et al*. (2001) [52]
Positive effects on growth and increased in body weight in European sea bass	*Lactobacillus delbrueckii*	Carnevali *et al*. (2006) [16]
Enhance the growth performance and immunity in *Oreochromis niloticus*	*Lactobacillus rhamnosus* GG	Pirarat *et al*. (2008) [54]
Enhanced the non-specific immune parameters; Improved resistance against *Edwards iella tarda* infection; Higher growth performance, survival rate and feed utilization; Enhanced fish resistance against *Aeromonas hydrophila* infection in *Oreochromis niloticus*	*Micrococcus luteus*	Taoka *et al*. (2006a) [70] Abd El-Rhman *et al*. (2009) [2]

5. Water quality management using Probiotic microbes

Fish or shrimp farmers are using chlorine in water to eliminate pathogens between crop cycles, and also add chemicals detrimental to bacterial survival throughout the grow-out periods (Boyd, 1998)[13]. Later on probiotics are used, where they directly uptake or decompose the organic matter or toxic material and improve the quality of water (Hemaiswarya, *et al*., 2013) [34] by reducing the disease (including *Vibrio* sp. and *Aeromonas* sp.) incidences, enhancing zooplankton numbers, reducing odours and ultimately enhancing aquaculture production. The addition of a probiotic solution should ensure that sufficient bacteria are present to decompose the organic matter of the sludge and release nutrients for oxygen-producing phytoplankton, thereby improving the overall quality of water in the pond (Moriarty, 1999; Visscher and Duerr, 1991) [47, 76].The microbial cultures produce a variety of enzymes such as amylase, protease, lipase, xylanase and cellulase in high concentrations than the native bacteria, which help in degrading the waste. These bacteria have a wide range of tolerance for salinity, temperature and pH (Hemaiswarya *et al*., 2013) [34]. The philosophy of this type of biotechnology recognizes that the root of the water quality problem is not the water or the shrimp, but the bacterial populations.

Probiotics are now widely used for reducing ammonia, nitrite and nitrate in the rearing water, which are harmful for fish larvae. Some of the probiotic strains are more efficient in converting organic matter or large polymers into smaller units, reducing the organic load in the aquatic environment. By speeding up the rate of organic matter breakdown, free amino acids and glucose are also released, providing food sources for the beneficial microorganisms. Inorganic forms of nitrogen, such as ammonia, nitrate and nitrite are also reduced. Gram-positive genus *Bacillus* group is more efficient than gram-negative in transforming organic matter to CO_2. It is suggested that maintaining high levels of probiotics in production ponds, fish farmers can minimize the accumulation of dissolved and particulate organic carbon during the growing season. In addition, this can balance the production of phytoplankton (Balcázaret *et al*., 2006) [8]. However, published evidence for improving water quality is limited; except for the nitrification (Verschuere, 2000) [74].

Methane-reducing bacteria use carbon dioxide as a source of molecular oxygen. Methane diffuses into the air and thereby improves the water quality (sahu *et al.*, 2008) [63] as methane diffuse into the air it causes environmental damage. Meanwhile, Wang *et al*. (2000) [78] showed that a commercial product made from *Bacillus* sp., *Saccharomyces cerevisiae*, *Nitrosomonas* sp., and *Nitrobacter* sp., had the ability to increase the beneficial bacterial micro-biota of *Penaeus vannamei* shrimp, (Jiqiu *et al*., 2007) [36]. Probiotic strains are a completely natural and ecologically harmless method of maintaining proper pond chemistry and environments for shrimp and fish aquaculture operations, but aquaculturists have the trend to change the water regularly from the hatchery (Raja, 2011)[57] and so organic matter is washed out of the system and microbes are lost in that way.

6. Use of Probiotics in Live feed

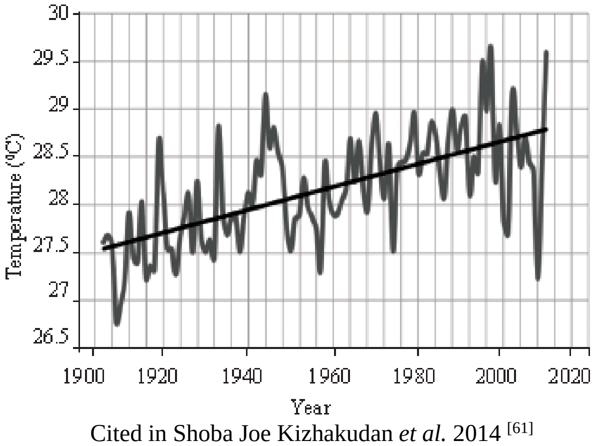
Live feeds are very important for shrimp larvae. Unicellular algae (*Cheatocerous* and *Skeletonema*), brine shrimp *Artemia*, and Rotifer are the two live feeds widely used in shrimp seed production (Naessens *et al.,* 1997) [50]. Uni-cellular diatoms and algae are given as feed before the nauplii moults into zoea. Devi *et al.* (2004) [19] reported that micro algae species, *Artemia*, and rotifers play a vital role in larval growth and survival of shrimp and fishes (Munro *et al*., 1995; Fukami, 1997; Suminto, 1997) [48, 24, 66]. Both algal culture and *Artemia* hatching should be done in exclusive sections using disinfected sea water and screened for pathogen presence before giving to the larvae (Verschuere, 2000) [74], because more *Vibrio* pathogen population are present in live feed (Raja, 2011) [57]. Vibriosis has been reported as a severe barrier to further development of shrimp aquaculture in India (Karunasagar *et al.,* 1994) [37]. However, many authors reported that *Artemia* nauplii represent as a vector for introducing potentially pathogenic bacteria into the hatchery systems (Austin and Allen, 1982; Gomez-Gil, *et al.,* 1994; Lopez-Torres and Lizarraga-Partida, 2001; Vandenberghe *et al.,* 2003; Kennedy *et al.,* 2006) [6, 29, 42, 73, 12]. *Artemia* nauplii harboured and introduced considerable population of luminous bacteria (log 2.44 cfu.42/g) into the post-larval rearing tanks (Abraham *et al.,* 2004; Villamil *et al.,* 2003; Thomson *et al.,* 2005) [3, 77, 72]. Careless use of *Artemia* nauplii has been found to be responsible for the development of disease and mortality of *P. monodon* larvae in hatcheries (Hameed *et al.,* 2002) [64]. However, the shrimp hatchery operator has not used the effective pathogen screening methods for their larval rearing systems (Raja *et al*., 2013) [58], hence, to avoid pathogen entry into post-larval tanks, the *Artemia* nauplii need to be enriched with probiotics (FAO, 2007) [21]. Widely fish and shrimp hatchery operators has applied mass population of *Artemia* for external disinfection by using probiotics, but still the methodology is not clear to identify and screening of microbes in infected *Artemia*.

Rotifers are indispensable as the first live feed for larvae of most cultured aquatic species due to their small size and they are more accessible to larvae. Planas *et al*., (2004) [53] reported that the lactic acid bacteria to increase the growth of the rotifer *Brachionus plicatilis* and obtained best results with the addition of *Lactococcus casei, Pediococcus acidilactici,* and *Lactobacillus lactis*. Thus, rotifers are a live feed employed mainly in the rearing of fish larvae (Gatesoupe *et al*., 1989) [25]. Even though, the rotifer is carrier of pathogenic microbes in to the fish larval rearing systems, contaminated rotifer will be a risk for fish hatcheries. Hence, the enriched or treated probiotic is essential for healthy fish larvae production. (Gatesoupe, 1990; Harzevili *et al*., 1998) [26, 32]. It has also been reported that the inoculation of a probiotic bacterium, B. *toyoi* used for rotifer disinfection and enhanced growth rate of turbot against *Vibrio* spp. in fish rearing hatchery.

7. Use of Probiotics in Fish/ Shrimp health management

Fish fed with probiotics showed lower blood glucose levels; this might be due to the capability of probiotics to reduce the effects of stressors (Mohapatra *et al*., 2014) [45]. Probiotics for bacterial diseases like vibriosis is well reported but for viral diseases the authentic strains still need research. According to Bestha Lakshmi *et al*. (2013) [11] probiotics acts as anti-viral agents that was a novel approach. Probiotics have the capability of enhancing the immune response in fish and shrimp. For the protection of shrimp from viral diseases, no specific drug was designed; besides the use of antibiotic gives rise to a new type of resistant strains. Enhancement of the disease resistance in animal and the development of the immune power are the best option to prevent and resist the viral infections. For this purpose, a proper understanding of the immune system and the type of immune response in the animal is necessary. The nutritional effect of probiotic bacteria on the growth and survival of fish and shellfish larvae have been studied (for review see Tinh *et al*., 2008) [71]. Hence, successful application of the principle of competition to the natural situation remains as a major task for microbial ecologists (Sahu *et al*., 2008) [63].

8. Impacts of Probiotics on temperature



Cited in Shoba Joe Kizhakudan *et al.* 2014 [61]

Fish are poikilothermic vertebrates that inhabit aquatic ecosystem and are most susceptible to seasonal and diurnal variations in water temperature. Reported that Shoba Joe Kizhakudan *et al.* (2014) [61] the Sea Surface Temperature (SST) has increased from 1906 to 2010, it's indicated that increasing water temperature in upcoming years. The rising temperatures have an adverse effect in aquaculture when there is change of negative average rainfall (Muralidhar *et al*., 2012) [49]. The aquaculture has distributed around global. However, the rainfall level is not equal in all the geographical regions. Increase in temperature will affect all chemical and biological processes in fish and it will have a direct effect on food requirements and food conversion efficiency (Boyd, 1998) [13]. Therefore, the food conversion efficiency can be increased using probiotic species such as *bacillus licheniformis, bacillus cereus and bacillus subtilis* (Vine *et al*., 2006; Merrifield *et al*., 2010) [44, 75]. Elevated water temperature alters several hemato-immunological parameters, enhances the synthesis of stress related proteins production and induces apoptosis; ultimately resulting in an overall reduction of fish growth. Fluctuation of temperature increases the vulnerability of fish to Vibriosis in aquaculture system; high fish mortality has been noticed to occur during high water temperature and *Vibrio* species to be the most abundant bacteria exist in diseased fish and shrimp (Raja, 2011; Albert and Ransagan, 2013) [57, 4]. The fluctuation of temperature is not only increase bacterial pathogens but also will influence viral virulence for mortality in the aquaculture species (Rodriguez *et al*., 2003; Albert and Ransagan, 2013) [60, 4]. Temperature is known to regulate the growth and other physiological and biochemical functions of fish. A three species combination of probiotics (*B. subtilis, L. lactis* and *S. Cerevisiae*) has been used to overcome the temperature-associated stress, which has also substantially has improved the fish growth (Mohapatra *et al*., 2014) [45].

9. Destructive effects of probiotics in Aquaculture system

The use of probiotics is receiving considerable attention as an alternative approach to controlling microbiota in aquaculture, especially in hatching facilities. However, application with consistent results is hampered by insufficient information on their modes of action (Marques *et al*., 2004) [43]. In natural populations of aquatic animals, the microflora of the gut might reflect that of the aquatic environment. However, in massive artificial larval cultures, the balance can be altered by the use of disinfected water. As a result, a protective microbial community may not develop either in the environment or the digestive system of the larvae (Gomez-Gil *et al*., 2000) [30]. Aquaculturists have the trend to change the water regularly from the hatchery (Raja, 2011) [57] and grow-out farms, the outlet of organic matter will lead to the environmental degradation and aquatic pollution.

The effect of probiotics in aquatic environment, showing the causal link between the beneficial effects of the probiont and *in vivo* suppression of a pathogen is rarely noticed. According to Verschuere *et al.* (2000) [74] the use of probiotics as biological control agents should be considered to be a kind of risk insurance that may not provide any notable benefit when the culture is performed under optimal conditions and in the absence of (opportunistic) pathogens. The mode of mechanism is still not clear because nutrients and other microbes also involved; more in-depth studies of the competitive processes between bacteria must be carried out, and the ecological relevance of the different processes *in-situ* remains to be elucidated. Also, the interaction between the cultured aquatic species and its associated microbiota deserves further research into possible immunostimulative effects (Verschuere *et al*., 2000) [74]. Therefore, the need for in-depth research on both beneficial and destructive effects of probiotics with reference to a specific type of water, species and geospace of aquatic culture systems is suggested.

When culturing the aquatic species, microbial populations in the intestinal contents are much higher than those in the surrounding water. This indicates that the intestines provide favorable conditions for these organisms (Stefan Denev, 2009) [69]. So, there is a chance of risk associated with the transmission of resistant bacteria from aquaculture environments to humans.

The beneficial probiotic bacteria are used as oral administration along with feed to improve the fish/ shrimp digestibility (Nageswara and Babu 2006; Sahu *et al*., 2008) [51, 63]. However, the role of individual microbes play in the health and nutrition of fish is still poorly understood. There are no reports of any harmful effect of probiotics but it is found that the biological oxygen demand level may temporarily be increased with its application. Therefore, it is advisable to provide subsurface aeration to expedite the establishment of probiotics organisms. A minimum dissolved oxygen level of 3% is recommended during probiotics treatment (Stefan Denev *et al*., 2009) [69].

Development of suitable probiotics for aquaculture requires empirical and fundamental research, full-scale trials as well as the development of appropriate monitoring tools and production under stringent quality control. A performing mixture of probiotic strains can be designed after evaluating the ability of individual strains to grow in low/high salinity under micro-aerophilic or anaerobic conditions, produce various enzymes and more importantly, produce a range of inhibitory compounds (Decamp, 2004) [18].

The hatchery operators need to strengthen the implement of the recirculatory systems in larval rearing aquaculture (Raja *et al*., 2013) [58]. It can be established well in static or low water exchange systems (re-circulatory system) and are effective if only applied as soon as the water medium is sterilized before contamination with other microbes (Sahu *et al*., 2008) [63]. There is evidence that probiotics are effective at inhibiting a wide range of fish pathogens, but the reason for the inhibitions is often unstated (Irianto and Austin 2002) [35]. The balance between the phytoplankton, zooplankton and beneficial bacteria during the culture process play a crucial role in the maintenance of pond health (Irianto and Austin 2002) [35]. Hence, the pond probiotics also have a special blend of denitrifying bacteria that remove the algae’s primary source of food namely nitrogen from the pond water. This drastic reduction in nitrogen concentration makes it difficult for the algae to bloom (Hallegraeff, 1993) [31]. According to Verschuere (2000) [74] probiotic microorganisms have the ability to release chemical substances with bactericidal or bacteriostatic effect on pathogenic bacteria that are in the intestine of the host, thus constituting a barrier against the proliferation of opportunistic pathogens. The probiotics can easily be destroyed by any other chemical or drug which generally interferes with the establishment of useful microbes. When the temperature is raised, fast digestibility occurs in aquatic organisms and so the organic matter is passed out in excess to reduce this effect. Probiotics should be used as biofilters but the proper mechanism is not known for biopurifications.

10. Conclusion

Probiosis is based on the principle of competitive exclusion and involves the use of living bacteria in the diet or culture water to ensure that the gut of the cultured species is initially colonised with beneficial micro-organisms to improve digestion. This approach is being actively investigated, but work is still at an early stage. Moreover, many private industries have promoted their probiotic product without understanding the environmental impacts. In addition, the end users also may not be aware of the proper method of administration. Hence, effective probiotic species in relation with the environment research studies and farmer awareness are required for sustainability.

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