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Role and functions of beneficial microorganisms in sustainable aquaculture

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ABSTRACT

This paper aims to review the development of scientific concepts of microecology and ecology of microbes and the role and functions of beneficial microorganisms in aquaculture and mariculture. Beneficial microorganisms play a great role in natural and man-made aquatic ecosystems based on the co-evolution theory in living biosphere on earth. Their functions are to adjust algal population in water bodies so as to avoid unwanted algal bloom; to speed up decomposition of organic matter and to reduce CODmn, NH_3–N and NO_2–N in water and sediments so as to improve water quality; to suppress fish/shrimp diseases and water-borne pathogens; to enhance immune system of cultured aquatic animals and to produce bioactive compounds such as vitamins, hormones and enzymes that stimulate growth, thus to decrease the FCR of feed.

1. Introduction

Since 1995 Lester Brown put forth his question "Who will feed China?", the grain security in China has been spotlighted in the world, especially the argument of "Grain Threat from China". China's fundamental policy is the basic self-sufficiency in grain requirements on internal resources. China will make her efforts to promote increment of grain production in country, and in general China will keep the grain self-sufficient rate not less than 95% and the net grain import less than 5% of the total consumption in country. It should be mentioned what we want to solve is food for the mankind, not only grain. We can supply enough food on the basis of Five Kingdoms of Nature. China has become the first producing country in the world since 2005 with the fish production of 51 million tons. China needs to develop aquaculture further to get more aquatic products as an important part of food security. But is it sustainable? Statistics shows that China has 17.47 million ha (hm^2) of inland water, among which 6.75 million ha could be used, but now the utilization rate is only 69%. China has 6.7 million ha rice fields, which could also be used to culture fish, but now the utilization rate is only 15%; China has 2.6 million ha brackish water areas, which could be used for mariculture, but now the utilization rate is only 28%. China could increase its productive capability in those areas so as to maintain the growth of aquatic products. Furthermore, we would raise the product quality from non-harmful food to organic food.

In the recent two decades, aquaculture and mariculture rapidly developed in scope and intensity. But the discharge of effluents from aquaculture or mariculture, industrial wastewater, and sewage without treatment seriously polluted water bodies nearby and damaged the ecology of culture areas, causing the pathogenic microorganisms to proliferate and to spread quickly. The diseases of cultured animals happened seriously causing heavy losses of aquaculture and mariculture. According to the incomplete statistics in China, the culture area of medium disease prevalence above accounted for 15–20% of the total culture areas with the loss of over 1 million tons a year.

Mariculture in the world suffered catastrophic losses caused by monodon baculovirus (MBV) in 1988 (Lightner et al., 1987; Chen et al., 1989) and white spot syndrome virus (WSSV) in 1993 (Lo and Kou, 1998). During that period 90% of the shrimp ponds were abandoned at 30–45 days after stocking post larvae from Ecuador and elsewhere. Most shrimp farmers in China switched to the newly introduced species *Penaeus vannarnei.* By early 1998, *P. vannarnei* became the most popular and highest-yield cultured shrimp in the world. However, from late 1998 to early 1999, due to high mortality, shrimp production dropped sharply to as little as 10% of the production in early 1998.

Antibiotics appeared ineffective due to overuse in aquaculture and mariculture. It not only increased disease resistance by bacteria, damaged or harassed normal microflora of culture environ-

Glossary: Microeubiosis, micro-eco equilibrium; Microdysbiosis, micro-eco dysfunction; Micro-eco-agents, micro ecological agents or preparation; Microecology, microecology in internal organs or epidermis of individual animal or plant; Ecology of microbes, ecology of microbes in certain medium (soil, air, water and sediments, even in hot springs).

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ment and caused microdysbiosis, as double pollution, but it also made antibiotic residue accumulated in aquatic products to be harmful for human consumption. Countermeasures have been taken including Integrated Disease and Pest Management (Li, 2008), especially by using beneficial microorganisms. Sustainable aquaculture or mariculture requires setting up of clean culture models by BM with no toxin, no side effects, no residue, and no resistance, while it is effective in improving environment and in raising immunity of cultured animals, in reducing diseases and in maintaining eco equilibrium (Bao and Shen, 2005).

2. Development of scientific concepts of microecology and ecology of microbes

Microbes were not known to mankind until Antonie Philips van Leeuwenhoek (1632–1723), a Dutch tradesman and scientist from Delft, The Netherlands, first found microbes in 1676. He is commonly known as the Father of Microbiology, and is considered to be the first microbiologist. Microorganisms outnumber all other organisms in both biomass and diversity. They are a major part of global ecosystem services and natural capital in the world. However, they are the least known species, and we know only 10% of their names, habits, structures, functions, and forms. They are the first occurring living organisms on earth. Their diversity plays an important role to maintain biosphere, and provides large amounts of resources through photosynthesis to release oxygen, and to change the O_2 content in the air. So we could say that microbes are the most primary productivity. Without microbes there will be no living creatures on the earth. However, our knowledge of them remains poor largely because approximately 99% of them are not readily culturable. Research on microorganisms in depth needs effective sophisticated apparatus and new methods. The Epifluorescence microscopy might be a powerful tool for counting the total numbers of bacteria, estimating their biomass and determining their distribution in land, water, and sediments. This technique has shown that bacteria are anywhere from 10 to 100,000 times more numerous than those shown by the classical microbiological techniques. Bacteria are now known to be far more active and to have a far more important role in lakes and seas than was thought 20 years ago (Moriarty David, 1997). The vast majority of microorganisms including fungi, algae, and even viruses around the globe will remain hidden, and need to be explored, identified, conserved and utilized for the benefit of humankind in particular, and the biota and environment (Dilip et al., 2005).

Ecologists often pay attention to the matter in scope of kilometers, but microbiologists in scope of 101–102 µm. Microbe communities and their physico-chemical environment are an interacted complex system. Without the above-mentioned great achievements, nature could not be summarized to have five kingdoms: the Animal Kingdom – Multi-cellular motile organisms, which feed heterotrophically; the Plant Kingdom – Multi-cellular organisms, which feed by photosynthesis; the Protista Kingdom – Protozoa and single-celled algae; the Fungus Kingdom – Fungi; and the Monera Kingdom – Bacteria and blue–green algae; parallel to these Kingdoms, but not included, are the Viruses, which should be called Kingdom Virus for they are becoming very important factors that they could affect the evolution of Animal and Plant Kingdoms (Lynn et al., 1988). Someone called it the 7th Kingdom when Archaebacteria is called the 6th Kingdom in nature (Carl et al., 1977).

In the history of life on earth, the co-evolution of microcosm and macrocosm is of particular importance. Conventional accounts of the origin of life usually describe the build-up of higher life forms in micro-evolution and neglect the macro-evolutionary aspects. But these two are the complementary aspects of the same evolutionary process, as Erich Jantsch has emphasized. From one perspective microscopic life creates the macroscopic conditions for its further evolution, and from the other perspective the macroscopic biosphere creates its own microscopic life. The unfolding of complexity arises not from the adaptation of organisms to a given environment but rather from the co-evolution of organisms and environment at all systems levels. Now we know that nature has a feature of co-evolution of all living organisms and environment on earth.

The concept of microecology was first put forth by German Dr Voeker Rusch in 1977. He Mingqing described that it is an ecological science to study interdependence and interaction between normal microbes and the inner environment of their hosts on cell and molecular level (He, 1994; Fu and Xu, 2005). Microecology as a science has only a history of less than 50 years. It was originally a theory to study microflora in animal stomach and digestive tracts under the concepts of microeubiosis, microdysbiosis, micro-eco-nutrition and micro-eco-prevention. Microecological agents can supplement normal microflora when the intestine of human being or animals is lack of normal microflora in order to regulate microflora and maintain microeubiosis in intestine, to strengthen immune functions, to promote digestibility and absorption of nutrients so as to reach prophylactic medicine if no diseases or to cure diseases if there is any and also to increase the FCR of food or feed. However, we must emphasize that microecology exists everywhere, not only in the gastrointestinal tracts in animals, but also in plants, and we could call it plant microecology. Chen Yanxi (1980) realized in his "Plant natural ecosystem" that "plant is not an entity. There are various microbes in its body and on its epidermis with specific and stable microbial communities. Those microbes and plant body both constitute a natural ecosystem on interaction and interdependence to some extent. This is the results of long-term evolution" (Cai, 2002). Plant microecology means that any plant is a compound of organelles and microbes within. Plant microecology is a branch of living science to study the composition, functions, evolution, their interrelationship and their interrelationship with their host (Cai, 2002).

It is the most important to differentiate ecology of microbes from microecology. There is ecology of microbes in soil, air, water and sediments. Ecology of microbes means microbial population, communities, diversity, biomass and their interrelationship with the impact of environment. Ecology of microbes occupies an important place in ecology in water and sediments. The sewage and industrial wastewater discharged from the anthropogenic activities pollute streams, rivers, lakes and seas, propagate harmful microbes, and finally jeopardize human health and aquatic animals, whereas BMs degrade pollutants, inhibit or kill harmful microbes by antagonism, purify water bodies and reach eco equilibrium in water. This is what past aquaculture and mariculture neglect (Xue and Xue 1997; Xue, 2006).

3. Classification of microorganisms

Microorganisms can be divided into three types: reviving, neutral and disintegrating. The reviving types are beneficial. They can enhance biological, chemical and physical properties of soil or water and sediments, while acting as synergists. The reviving types could also change the neutral types to beneficial types. These two types of microorganisms decompose organic matter to meet the growth requirements of cultured species and reduce pollution. In contrast, the disintegrating types cause diseases (Higa, 1999). Beneficial microorganisms are a professional term in ecology and ecology of microbes including micro algae, bacteria, fungi and virus. They could maintain the eco equilibrium, inhibit the proliferation of harmful organisms and disintegrate harmful chemical substances in ecological environment such as *Bacillus thuringiensis* in nature.

It is estimated that more than 70% of the antibiotics used in US are given to feed animals (e.g. chickens, pigs and cattle) in the absence of disease. Antibiotic use in food animal production has been associated with the emergence of antibiotic-resistant strains of bacteria including *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, and *Enterococcus* spp. Evidence from some US and European studies suggest that these resistant bacteria cause infections in humans that do not respond to commonly prescribed antibiotics. In response to these practices and attendant problems, several organizations (e.g. The American Society for Microbiology (ASM), American Public Health Association (APHA) and the American Medical Association (AMA)) have called for restrictions on antibiotic use in food animal production and for an end to all non-therapeutic uses (Antibiotic from Wikipedia, the free encyclopedia July 2008).

The positive role played by certain bacteria was first introduced by Russian scientist and Nobel laureate Eli Metchnikoff. He suggested in the beginning of the 20th century that it would be possible to modify the gut flora and to replace harmful microbes by useful ones. The term 'Probiotics' was first introduced in 1953 by Kollath, and probiotics are a professional term in microecology, namely, microorganisms and their metabolic products conducive to the equilibrium of microorganism populations in the intestine of animals. Probiotics seem to be the substitute for antibiotics, especially in preventive medicine. Probiotics generally include bacteria, cyanobacteria, micro algae and fungi, but these do not include micro algae in the English literature (Wang et al., 2008). Nowadays, the term "micro-eco-agent" is very popular in China for human health food and animal feed industries. Micro-eco-agents are of three kinds: probiotics, prebiotics, and synbiotics. Although there are a variety of micro-eco-agents, they are mainly composed of *Bacillus*, *Lactobacillus*, *Bifidobacteria*, *Bacteroides*, yeast and PSB (Fu and Xu, 2005). The present research is focused on probiotics and prebiotics. Probiotics are live microbial food additives that have been in use for some time, and are available in many food products, primarily in fermented milks (Cherie and Glenn, 1998; Collins and Gibson, 1999; Rolfe, 2000; Rastall and Vatsala, 2002).

Studies indicated that some oligosaccharides can selectively promote the proliferation of benign bacteria in animal intestine and prohibit the adhesion of pathogenic bacteria in intestine with the results similar to probiotics. Fructose oligosaccharides are able to modify the gut flora composition in favor of *bifidobacteria* (Cherie and Glenn, 1998). The substances found at the earliest are bifidus factor and oligosaccharides such as lactulose, oligo-sucrose, oligo-faffinose, oligo-maltose, corn oligosaccharides and soybean oligosaccharides. The saccharides can neither be digested nor be disintegrated by normal microflora, but can be utilized by *Lactobacillus* and *Bifidobacteria* to promote the propagation of beneficial microorganisms, to inhibit the growth of harmful bacteria up to eco balance of normal microflora. Some acids and salts such as glucose acid and glucolmin and some Chinese herbs such as the extract of ginseng, dangshen (root of hairy Asia-bell) and root of membranous milk vetch or Chinese tea have the same features (Bao and Shen, 2005). Synbiotics are micro-eco-agent compounds, which are composed of probiotics and prebiotics. Strictly speaking, only probiotics are beneficial microorganisms, for prebiotics are non-bioactive substances and synbiotics too because only part of synbiotics are beneficial bacteria. Probiotics actually are used to supplement normal microflora in the intestine of animals. So it is better to use the term "Beneficial microorganisms" for both microecology and ecology of microbes instead of the term "micro-eco-agents" for microecology only.

Apparently, harmless and beneficial bacteria far outnumber the harmful varieties. Microbes are vital to the environment because they participate in the Earth's element cycles such as the carbon and nitrogen cycles. Microorganisms are involved in the production of oxygen, biomass control and 'cleaning' the Earth of remnants of dead organisms. Beneficial microorganisms could be applied from a single strain such as PSB to multiple strains such as three strains: PNB, *Bacillus gemma* and *Pseudomonas*, to several dozen strains, not only bacteria but also fungi such as EM or Toyomoto™ products. Both are trade marks consisting of 80–90% beneficial bacteria (half anaerobic and half aerobic) and 10–20% beneficial fungi (personal communication with Dr. Li Yuanfeng, 2007).

Where do we isolate beneficial bacteria from? The best answer is from nature because there are lots of beneficial bacteria there; the more complicated the environment, the more the biomass and diversity of bacteria, from Chinese philosophy Ying and Yang. Based on the principle of natural ecosystems, the greater the diversity and number of the inhabitants, the higher the order of their interaction and the more stable the ecosystem. If we want to isolate BMs for aquaculture or mariculture, we have to analyze microbial diversity in freshwater and seawater, usually the dominant species in fresh water are *Aeromonas*, *Pseudomonas* and *Achromobacter*; the dominant species in seawater are *Pseudomonas*, *Vibrio*, *Achromobacter*, *Flavobacterium* and *Micrococcus.* There are other bacteria such as PSB (*Ectothiorhodospira*), *Bdellovibrio*, *Bacillus (Bacillus cereus, B. megatherium var. phosphaticum), Lactobacillus,* and *Desulphurobacterium, chitinase* in nature. Apart from bacteria, there are yeast, actinomycetes, bacteriophage and cyanobacteria virus.

As far as microflora in the intestine of fish are concerned, no matter in freshwater or saltwater, there are *E. coli* but are different from the common one with a feature of liquefying gelatin but not producing indole. A Japanese scientist found that the dominant species in tilapia intestine were *Vibrio* and *Aeromonas,* which accounted for 70% and above. The microflora depend on the feeding habits of their hosts: the dominant species in the intestine of carnivorous fish are *Enterococcus*, *Benecka vulnifica* and *Vibrio*, while the dominant species in the intestine of vegetative fish are *Lactobacillus acidophilus* and *Bifidobacteria* (Xue and Xue, 1997).

4. Role of beneficial microorganisms in aquatic ecosystem

Microbes include microalgae, bacteria, fungi and virus. Microalgae mainly photosynthesize to absorb CO_2 and to supply oxygen to aquatic animals. The main role of bacteria and fungi is to decompose organic matter in sediments so as to keep the water clean. However, due to the lack of oxygen there might be anaerobic decomposition or fermentation in sediments, producing harmful gases such as hydrogen sulphide and methane. There are two food chains in aquatic ecosystem: detritus food chain and prey–predatory food chain.

Microbes are the predominant photosynthetic organisms in most aquatic environments. In aerobic conditions (e.g. shallow water), algae, diatoms and Cyanobacteria predominate. In anaerobic conditions (polluted or eutrophic waters), other photosynthetic bacteria are dominant. There is a big difference between natural aquatic ecosystem and man-made aquatic ecosystem, especially in intensive culture with high-stocking density and high input.

The water body in a high-stocking density fishpond often in a small area has a limited self-purification capability. Therefore, there is a need to add BMs in stages to improve water quality to reduce the prevalence of fish diseases. When BM population in water body grows to a certain extent forming a specific population, BMs would hinder the growth of harmful ones during the competition for survival, thus the pond water is in a balanced and stable microbial ecological condition conducive to the growth of aquatic animals.

In a natural aquatic ecosystem, the factors on the abiotic aspect are water, heat, light, air, sediments and nutrients, while those on the biotic aspect are all the members of the five kingdoms in nature, which include autotrophs, not only aquatic plants but also algae and some autotrophic bacteria, such as PSB as producers and heterotrophs, and not only a variety of animals but also bacteria and fungi as decomposers. There are phytoplankton, zooplankton, worms, crustacean, fish, snails, and bivalves. Energy flows and material circulates in a balanced ecosystem. However, a fishpond, especially a high-density intensive fishpond, is not a balanced ecosystem. To a great extent, the good water of a fishpond depends on the large amount of BMs, just like a human body which contains 10–20 times more microbes than it does cells. When beneficial bacteria dominate, a human body is healthy.

There are national compulsory aquaculture water standards GB11607-89 we must adhere to if we want to get quality product. For example, dissolved oxygen (DO), 16 of 24 h, must be higher than 5mg/L and the rest must not be less than 3 mg/L; pH should be 6.5–8.5 in freshwater, while it should be 7.0–8.5 in brackish water; BOD_5 at 20 °C should be less than 5mg/L and less than 3 mg/L when frozen. NH_3 must be $\leqslant$0.02 mg/L. GB3838-88 indicates environmental quality standards for surface water, which are divided into five grades: Grade III for general fish species conservation area and Grade II for precious fish species conservation area and spawning grounds for fish and shrimp. In Grade III, Total Phosphorus (TP) should be $\leqslant$0.1 ($\leqslant$0.05 for lake and reservoirs) and Kjeldahl nitrogen should be $\leqslant$1 mg/L. As for most of the water bodies for aquaculture, it is difficult to reach these standards.

The health of aquatic animals depends upon both microeubiosis in the intestine of cultured animals and microbial ecological equilibrium. Both eco environments are interrelated closely (Peng and She, 2004). The general problems that fish farms have are due to over-stocking of fish or shrimp and creation of excess ammonium nitrogen in water. The concentration of wastes increases the microbial activity and decreases the dissolved oxygen in the ponds or tanks (http://www.emshop.co.nz/agriculture-and-farming/aquaculture...). In intensive aquaculture or mariculture, fish or other aquatic animals are under high-density stress, and fishponds with lots of humus (remnants of feeds and feces) are causing the fish or shrimp to be susceptible to diseases. Therefore, we must supplement beneficial microbes to speed up the decomposition of organic matter in water and sediments so as to maintain good water ambience.

5. Functions of beneficial microorganisms

Nowadays, beneficial microorganisms are applied in aquaculture and mariculture mainly in three forms: single strain, multiple strains and compound strains with some synergists. The BMs perform their functions on two aspects: one is to regulate microdysbiosis, to maintain microeubiosis, to raise the health level of hosts, and to promote the proliferation of BMs and their metabolic products in microecology. The other is to decompose organic matter (feces, wastes, remnants or leftovers of feed), to maintain dynamic ecological balance among organisms from the five kingdoms in nature and microbial ecological equilibrium in water and sediments, and to create a friendly environment for fish and shrimp growth.

1. The functions of BMs in microecology are as follows:

   (1) Beneficial microorganisms can produce lactic acid, acetic acid and propionic acid through metabolism. Those metabolic products can lower the pH in the intestine and enable beneficial bacteria to be the dominant ones.

   (2) Bacteria include beneficial, harmless, harmful and pathogenic bacteria. Healthy animals have normal bacterial population in their intestine, in which beneficial bacteria are dominant to maintain the balance in the intestinal microecosystem. Once the harmful and pathogenic bacteria dominate, the animal would be sick. In normal conditions the dominant species in intestine are anaerobic, which account for more than 99%, and they are *Bacillus, Bifidobacterium, Lactobacillus*, digestive *Bacillus*, *Saccharomycetes*, etc., aerobic and facultative bacteria only account for 1%. In Liaoning province they tried to add 0.1% compound microorganisms into formulated basic common carp diet, the results showed that average quality and gain weight were significantly higher than the control ($P < 0.05$), and the FCR in test pond was 1.78 significantly lower than the control (2.15) ($P < 0.05$), among which the FCR in test decreased 17.2% than the control (Fu et al., 2005).

   (3) Their metabolic products could decrease unionized ammonia NH_3 and ammonium in intestine.

   (4) BMs could secrete amylase, lipase, and proteinase, and increase the activity of digestive enzyme of aquatic animals. A test showed that ingestion of 200–300 mg *Bacillus Licheniformis* per kilogram could increase the activity of digestive enzyme and fish growth, and could be optimum for the growth of the fry of Allogygenetic Crucian Carp (Liu et al., 2006).

   (5) They could integrate B type Vitamin and antibiotic substances.

   (6) Beneficial microorganisms have a great impact on immune system of cultured aquatic animals. BMs as non-specific immune modulators would strengthen the antibody level and the activity of macrophage.

   (7) BMs can enhance diseases resistance of aquatic animals, e.g. PSB increased the survival rate of post larva of shrimp *Penaeus* orientalis on average to 58% in shrimp pond, and in control with 21% on average, and kinds and quantity of pathogens and their hazards in shrimp pond are greatly less than in the control (Zhang and Shi, 1999; Mao et al., 2006).

2. The functions of beneficial microorganisms in ecology of microbes in water and sediments are as follows:

6. Single strain

(1) PSB, especially PNB, can utilize sunlight as energy to separate hydrogen from hydrogen sulphide and hydrocarbon to raise dissolved oxygen, to remove nitrogen ion and metabolic substances so as to maintain the pond water clean, and to combine with CO_2 to integrate sugar, amino acids, vitamins and bioactive compounds in photosynthesis (Zhang and Shi, 1999).

(2) Acetobacter is a typical microbe that synthesizes nitrogen. It intakes saccharine from PSB and fixes nitrogen, part of which is left for plants and animals and part returned back to PSB to form a symbiotic structure with Acetobacter.

(3) *Bacillus gemma*, gram-positive bacteria, are non-toxic ubiquitous aerobic bacteria in nature. Kozasa (1986) isolated and used *Bacillus toyoi* to cure European eel infected by *Edwardsiella fujianensis.* It was the first application of BMs in aquaculture (Chou et al., 2002). Since then the research and application of BMs abruptly boomed due to their environment-friendly, health promotion and economic efficiency

enhancement features. Some scientists utilized *Bacillus subtilis* to have ammonia nitrogen reduced by 52.5% in nursery, reduced by 50% in grow-out period; nitrite content reduced by 50% in nursery, by 32.5% in grow-out period (Chou et al., 2002). There are two aspects of their function in microecology: one, as dominant species to inhibit pathogenic bacteria in intestinal and body surface of shrimp by competition; and two, some produce large amounts of proteinase, amylase and lipase, which can rapidly decompose protein, starch and lipid of remnant feeds and their excreta.

(4) Nitrobacteria belong to heterotrophic bacteria specifically aerobic, and they can be divided into two sub-groups: nitrite bacteria, which oxidize ammonia nitrogen into nitrite, and nitrate bacteria, which oxidize nitrite into nitrate. Thus, they reduce the toxicity of nitrite and ammonia nitrogen to aquatic animals in water and sediments.

(5) *Bdellovibrio* was first found by Stolp and Petold when they separated bacteriophage from the soil in 1962. It was a kind of parasite bacterium having aggressive, infectious and splitting action on other bacteria, similar to bacteriophage residing in water, which can split *Vibrio* and *Aeromonas hydrophila*.

(6) It belongs to *Bdellovibrio* and there are *Bdellovibrio bacteriovorus*, *Bdellovibrio Stolpii, Bdellovibrio Starrii* and others that are not defined. People are interested in their unique features. *Bdellovibrio* can prevent or reduce *Penaeus* diseases and its spread, and strengthen its immunity (Li et al., 2004).

(7) *Bifidobacterium* was successfully used in the breeding of *Penaeus* shrimp, and it raised the immunity of post larva of various stages of PL, promoted metamorphosis and increased the survival rate from nauplius to commercial juvenile shrimp by 55–60% (Zou and Kang, 1995).

(8) Actinomycetes are a diverse and a large group of gram-positive filamentous and/or branching bacilli. They can increase their number by using the nitrogen fixed by PSB as a substrate. They have a strong capability to degrade organic matter. Part of actinomycetes can endure high temperature of 55–65 °C. They can strengthen immunity of cultured animals and resistance to diseases. The most common organism in this group is *Nocardia*. Someone exploited *Actinomycetes* to treat pond water, reducing ammonia nitrogen by 32%. *Actinomycetes* are ubiquitous in nature, the correct identification of *Nocardia* species is essential to rule out *Streptomyces* species that is usually considered nonpathogenic.

(9) Lactobacteria such as *Lactobacillus acidophilus* can intake the substance produced by PSB, disintegrate lignin and cellulose, which are difficult to be decomposed at conventional temperature, and ferment organic matter that is not rotten to turn it into effective nutrients for plants and animals. Jiravanichpaisal et al. (1997) reported the use of *Lactobacillus sp.* as the probiotic bacteria in the giant tiger shrimp (*P. monodon* Fabricius). They designed to investigate an effective treatment of *Lactobacillus sp.* against vibriosis and white spot diseases in *P. monodon*.

(10) Yeast and *Aspergillus niger* belong to fungi. Fungi along with the bacteria are the primary decomposers of organic matter in almost all the terrestrial ecosystems worldwide and aquatic ecosystems as well. Many fungi are the producers of antibiotics, including β-lactam antibiotics such as penicillin and cephalosporin. Some fungi capable of competing with or infecting other organisms are considered beneficial for human use. For example in agriculture, some fungi may be used to restrict or eliminate the populations of harmful organisms such as pest insects. Yeast is a bioactive promoting bacterial division, and can fast decompose the saccharide dissolved in water and reduce oxygen consumption. Yeast is a mono-cell protein full of nutrients, Vitamin B, and amino acids in optimal proportion, so it can be used as additives in feeds too. The experiments indicated that the yeast can produce a high concentration of heat stress protein in the intestine of fish (Wang, 2006),

(11) Micro algae
Algal composition directly affects the growth of *Penaeus* shrimp. Diatoms are always dominant in ponds of good quality. As organic pollution increases, Pyrrophyta, Cyanophyta and Cryptophyta dominate instead of diatoms. Some diatoms can secrete some anti-bacterial substances and good food for post larvae of *Penaeus* shrimp. Some diatoms such as *Chaetoceros* can rapidly reduce ammonia nitrogen. In order to maintain the good quality of pond water, some scholars suggest that we inoculate diatoms and make them the dominant species. However, it is not easy to inoculate diatoms for they need strict growth conditions. The kind of ecological environment that is required to promote the growth of diatoms has not been reported so far (Xu, 2006). Austin et al. (1992) reported a kind of microalga (*Tetraselmis suecica*), which can inhibit pathogenic bacteria of fish. *Teraselmis suecica* was observed to inhibit *Aeromonos hydrophila*, *A. salmonicida*, *Serrstia liquefaciens*, *Vibrio anguillaram*, *V. salmonicida* and *Yersnia ruckeri* type I. When used as a food supplement, the algal cells inhibited laboratory-induced infection in Atlantic salmon. When used therapeutically, the algal cells and their extracts reduced mortalities caused by *A. salmonicida*, *Ser. liquefaciens*, *V. anguillaram*, *V. salmonicida* and *Yersnia ruckeri* type I. They suggested that there may be some bioactive compounds in the algal cells, and there appears to be a significant role for *Tetraselmis* in the control of fish diseases.

7. Multiple or compound strains

EMs is the abbreviation for effective microorganisms developed by Dr. Teruo Higa of the University of Ryukyus in Okinawa, Japan in the 1980s. EMs consist of more than 80 strains of beneficial microorganisms belonging to 10 Genera cultured in a brown solution with 100 million active microorganisms/ml, pH around 3.5. All these bacteria form a variety of microbial communities with complex composition, stable structure, and wide functions in interdependence, synergism and enhancement. They can quickly decompose organic matter, metabolize antioxidant substances and inhibit the proliferation of harmful microorganisms (Higa, 1997; Li and Liu, 2001).

EMs are not special set of microbes, nor are they genetically engineered organisms. EMs are only a combination of specially selected microorganism capable of producing multiple benefits, including predominant populations of lactic acid bacteria, yeasts, and smaller numbers of PSB, actinomycetes and other types of organisms. All these are mutually compatible with one another and can coexist in a liquid culture. All these microbes are present in nature. So EMs work in harmony with nature (Higa, 1997).

EM technology was introduced in 60 countries and applied in many sectors. It was introduced in China in 1991. The application of EM expanded from crop plantation, animal husbandry to environment protection such as waste deodorization, wastewater treatment and eutrophication control. The trial on rice, wheat, maize, soy bean, peanut, canola, vegetables, water melon, citrus, strawberry, seedling of trees and grassy sod indicated that EM technology had good effects. The yields of these crops obtained by using organic manure mixed with EMs were higher than those of crops obtained by using chemical fertilizers. The quality of produce was improved a lot. The application of EM technology in aquaculture began 10 years ago. The test showed that fish fed on

EM-mixed feeds grew quickly. In general, the weight gain increased by more than 30% for food eaters, and by 20–25% for filtering fish. The stocking density could be increased by 30%. The pond water treated with EMs appeared bloomy and clear, for EMs prohibit harmful bacteria from proliferating effectively, eliminating ammonia, raising DO level, and making plankton bloomy for fish to consume. EMs are more effective in intensive aquaculture factory, e.g. soft-shelled turtle, eel, and crab and prawn culture. At a higher temperature and heavy input, the pond water deteriorated easily. EMs can effectively control diseases such as enteritis, erythema, gill rot, and cholera caused by bacteria, and can promote the eco equilibrium of the water body. Mortality could be reduced almost to zero. Economic return would increase (Chen, 1997).

1. The functions of BMs in the ecology of microbes in water and sediments are as follows:

(1) To adjust the algal population in water body to avoid the deterioration of water quality
Used pond water usually appears black brown, blue–green or grey green, which indicate that blue–green algae such as Microcystis dominate with only a few species and less digestible algal species. The pond water is too bloomy with much turbidity, which is detrimental to the fish growth. The measure taken in the past is to pour new water to change the composition of algal population (Wang and Han, 2004). When the pond water becomes red with greater transparency, it indicates that the large amount of phytoplankton have died and dissolved after the outbreak of algal bloom, deteriorating physico-chemical factors. The application of BMs can regulate the composition of algae to a normal condition conducive to fish growth.
Another test was done in fishponds in Yongzhou Vocational College before the application of Lengshuibao™ living microbes with the concentration of 50mg/L at the temperature of 24 °C and illumination of 2800 l× in 3 repetitions. The algal composition before the test indicated that the dominant species are *Microcystis*, *Merismopedia*, *Dactylococcopsis*, *Chroococcus*, *Phormidium* and *Rhizosolenia*. The above except the last one (diatom) belong to Cyanophyta (blue–green algae), among which *Microcystis* and *Merismopedia* accounted for 70.05%, while *Chlorophyta* and *Bacillariophyta* accounted for 24.07%, the total amount was 150×10^4 individuals/L and the total biomass was 20.04 mg/L. six days after adding EMs with the concentration of 5000 mg/L, the dominant species were *Scenedesmus*, *Staurastrum*, *Coelastrum* and *Synedra* (diatom) genera among which *Chlorophyta* and *Bacillariophyta* accounted for 80.12%, whereas *Cynophyta* only accounted for 8.76% and the algae conducive to fish growth increased from 24.07% to 80.12%. The total biomass increased from 20.04 mg/L to 31.87mg/L. The maximum inhibiting rate of blue–green algae could be up to 75%. EMs can reduce the content of C, N and P, and cyanobacteria cannot reproduce owing to the lack of nutrients in water body. Thus, EMs can control eutrophication of fishponds (Jiang, 2005).

(2) Beneficial microorganisms composed of *Bacillus gemma*, *Lactobacillus*, yeast, denitrification bacteria in powder form, pH 7, the total content of bacteria 24×10^9/g, which are not pathogenic and not harmful to aquatic animals, were soaked in 100 times water for three hours, then were applied after resurrection with the optimal concentration of microorganisms, $25–50 \times 10^7$, to improve the water quality and sediment conditions (Mao et al., 2006).

(3) BMs can inhibit the prevalence of fish disease and the putrefaction of certain aquatic plants in summer.

(4) Beneficial microorganisms have a great impact on the immune system of aquatic animals. BMs can enhance the disease resistance of aquatic animals, e.g. PSB increased the survival rate of post larva of shrimp *Penaeus* orientalis on average to 58% in shrimp pond, and in control with 21% on average, and the kinds and quantity of pathogens and their hazards in shrimp pond are greatly less than in the control (Zhang and Shi, 1999; Mao et al., 2006).

(5) Antioxidant substances generated by effective microorganisms and the antioxidant emission of waves that accompany such processes have the strength to suppress the harmful effects of oxidation, and BMs can deactivate the free radicals that occur in materials and living organisms (Higa, 1999).

(6) EMs help overcome the problems of dioxin and PCBs (Higa, 1999).

There are two cases that indicate how to solve environmental problems: one is integrated crab culture in freshwater and another is ecological poly culture of shrimp, fish, shellfish and algae in mariculture.

In integrated crab culture in freshwater they applied three bioremediation techniques: (1) microbe remediation; (2) aquatic vascular plant remediation; and (3) aquatic animal remediation. Finally, the effluent from the crab pond reached National Surface Water Standard Grade III with total phosphorus 0.05mg/L and total nitrogen 1.0 mg/L, while NH_3 and NO_2 were only one-tenth of the input water. Like in nature there is a benign energy flow and nutrient cycles within crab ponds. Most of the feeds put into crab ponds are fed upon by the crabs and the remnants were fed upon by the shrimp and trash fish. Feces of aquatic animals are decomposed by bacteria into detritus and fed upon by the aquatic animals; inorganic elements or minerals are absorbed by the aquatic plants. Both aquatic plants and animals are good food for top omnivorous crabs (Liu et al., 2005a,b).

In ecological poly culture of shrimp, fish, shellfish and algae in mariculture, they used a water circulating system in a cluster of ponds. The system has integrated four different functionally subsystems: shrimp culture subsystem, fish subsystem, shellfish subsystem and large-scale marine algae cultivating subsystems. In addition, there are one water treatment area and one emergency drainage canal. The water quality of shrimp pond was bio- manipulated by different organisms in different ponds within the closed circulatory system. The results indicated that the contents of SS, COD and NH_3 were significantly lower than those in monoculture control ponds ($P < 0.01$). The effluent was no longer eutrophic ($E < 1$). The system produced 0.667 kg Penaeus type shrimp per kg of feed, besides harvesting 0.037 kg of tilapia, 0.738 kg of oyster, and 0.437 kg of *Gracilaria* as well. The food conversion rate decreased and economic benefits increased notably (Shen et al., 2004). The system has some properties: disease prevention, environment protection, and high efficiency. But if we add EMs or BMs we might phase out antibiotics in mariculture. In sum, sustainable aquaculture should be practiced on the basis of the co-evolution of a variety of organisms in an aquatic ecosystem in which microorganisms will play a great role.

8. Problems and prospect

There are several problems in the application of beneficial microorganisms in aquaculture and mariculture. One problem is that farmers do not distinguish microecology from the ecology of microbes as mentioned above. It is important to adopt different methods of application of BMs for two ecosystems. Although the utilization of BMs has a history of ten years in China, we still focus on a single strain of beneficial bacteria. The function of single strain might be known better than that of the multiple ones. If we adopt

EMs we should understand the interaction, interdependence and synergism among the many strains of the beneficial bacteria. Moreover, we have to know some synergists such as prebiotics, such as oligosaccharide, calcium carbonate and stable ranges of silica in chelate state and super phosphate at a 1:3 ratio, which need to be added. It seems to be a decoction of Chinese herbal medicine, which puts emphasis on the compatibility of various medicines with different properties as master and slaves. Another problem is how to apply EMs. The correct application of EMs depends on a suitable time and place to facilitate the establishment of laboratory-cultured beneficial microorganisms. It is not a symptomatic therapy but a preventive therapy. It would be better if we isolate BM strains from the local conditions from freshwater or salt water. But the most important problem is that we know very less about the microbes so that research on microbes should be deepened worldwide, e.g. to study how to use cyanophage, a cyanobacteria virus to control cyanobacteria (blue–green algae); Myxomycetes, *Bdellovibrio* and *Bacillus gemma* to control harmful red tide; PSB to treat organic pollutants such as dioxin and PCBs in water. Indeed, water quality is the key to a sustainable aquaculture and mariculture.

Prospects of BM research are encouraging. The present status of the utilization of beneficial microorganisms cannot meet the demand of cultured aquatic animals and water environment requirements. Harmful microorganisms become dominant if conditions develop that are favorable to their growth, activity and reproduction. Under such conditions, water-borne pathogens can rapidly increase their populations with devastating effects on cultured species. If these conditions change, the pathogen population declines just as rapidly to its original state (Higa and James, 1994). When the prevalence of fish/shrimp diseases happens in aquaculture and mariculture medications are needed, but they are only transitional measures, leaving safety problems in aquatic food products for humans. The best way is to adopt biological and ecological measures to deal with these problems. Therefore, we have to put the research of microorganisms under the guidance of the theories of co-evolution, ecology, ecology of microbes and microecology. Research is needed to identify and quantify reliable and predictable biological/ecological indicators of water quality. The optimization of ecological structure of cultured areas would enable the development of aquaculture and mariculture in a benign circulation to gain better economic, social and ecological benefits.

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References

Austin, B., Baudet, E., Stobie, M., 1992. Inhibition of bacteria fish pathogens by *Tetraselmis suecica*. J. Fish Dis. 15, 53–61.
Bao Xinghao, Shen Weimin, 2005. Manufacture and application of micro ecological agents. In: www.BIOX.CN 2005-4-16.
Cai, Yuancheng, 2002. Plant micro ecology and the application of plant micro-ecoagents. Chin. J. Eco-agric. 10 (2).
Carl Woese et al., 1977. Six Kingdoms from Wikipedia.
Chen, Daliang, 1997. The Application of EM in Aquaculture. J. Sci. Fish Farming 7, 34.
Chen, S.N., Chang, P.S., Kou, G.H., 1989. Observation on pathogenicity and epizootiology of *Penaeus monodon* baculovirus (MBV) in cultured shrimp in Taiwan. J. Fish Pathol. 24, 189–195.
Cherie J. Ziemer, Glenn R. Gibson, 1998. An Overview of Probiotics, Prebiotics and Synbiotics in the Functional Food Concept: Perspectives and Future Strategies Institute of Food Research, Earley Gate, White knights Road Reading RG6 6BZ, UK. Available online 31 December 1998.
Chou, Li et al., 2002. The application of *Bacillus subtilis* in aquaculture. J. Fishery Modernization 2, 26.
Collins, M.D., Gibson, G.R., 1999, Probiotics, prebiotics, and synbiotics: approaches for modulating the ecology of microbes of the gut. Am. J. Clin. Nutr. ISSN:0002-9165.
Dilip, K. Arora, Ratul, Saikia, Ram, Dwievdi, David, Smith, 2005. Current status, strategy and future prospects of microbial resources collections. Current Sci. 89 (3).
Dianguo, Fu, Ming, Xu, 2005. Microecology preparation. J. of Jilin (provincial) Farming & Veterinary 4, 13–15.
Fu, Dongyan, Yu, Xiaoting, Wang, Feng, 2005. The effects of compound microorganisms on the growth and water quality in carps. Feed Res. 8, 43–45.
He, Mingqing, 1994. Animal Micro-ecology. Agriculture Publisher, Beijing.
Higa, Teruo, James, F. Parr, 1994. Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment. International Nature Farming Research Center, Atami, Japan.
Higa, Teruo., 1997. Great revolution to save the earth in Chinese edition. China Agriculture Publisher University.
Higa Teruo, 1999, Lecture of Application of Effective Microorganism for Sustainable Crop Production. Sustainable Community Development Kansas City, USA. <http://www.SCDworld.com>.
Jiang, Aiqing, 2005. The chemical water factors change of different kinds of hill ponds using microorganism. Animals Breeding Feed 4, 35–37.
Jiravanichpaisal, P., Chuaychuwong, P., Menasveta, P. 1997. The use of *Lactobacillus* sp. as the probiotic bacteria in the giant tiger shrimp (*Penaeus monodon* Fabricius). Poster session of the second Asia-Pacific marine biotechnology conference and third Asia-pacific conference on algal biotechnology. 7–10 May 1997, Phuket Thailand, p. 16.
Li Kangmin, 2008. Extending Integrated Fish Farming Technology to Mariculture in China. In: UNESCO/EOLSS Certification No. 6-58-08-10.
Li, Nan, Ni, Xueqin, Pan, Kangcheng, 2004. The characteristic of bdello-vibrio and its application in animal production. Feed Prod. 8, 58–62.
Li, Yingjun, Liu, Zhonglin, 2001. Study on current status of EM biotech application in China. J. Environ. Dev. 16 (3), 12–13.
Lightner, D.V., Hedrick, R.P., Fryer, J.L., Chen, S.N., Lao, I.C., Kou, G.H., 1987. A survey of cultured penaeid shrimp in Taiwan for viral and other important diseases. Fish Pathol. 22, 127–140.
Liu, Bo, Jiang, Guochun, Wang, Huiping, Ye, Xiang, 2005a. Environment optimization technology and application in crab culture. J. Sci. Fish Farming 10, 30–31.
Liu, Bo, Liu, Wenbin, Wang, Tian, 2005b. Effects of *Bacillus licheniformis* on digestive performance and growth of Allogynogenetic crucian. Trans. Nanjing Agri. Univ. 28 (4), 80–84.
Liu, Bo, Xie, Jun, Liu, Wenbin, Wang, Tian, Wang, Huifen, Du, Wei, 2006. Effects of Bacillus licheniformis on digestive performance and growth of Allogynogenetic crucian carp. J. Dalian Fisheries Univ. 21, 336–340.
Lo, C.F., Kou, G.H., 1998. Virus-associated white spot syndrome of shrimp in Taiwan: a review. Fish Pathol. 33, 365–371.
Lynn Margulis, Karlene V. Schwartz, 1988. Five Kingdoms: an Illustrated Guide to the Phyla of Life on Earth Wikipedia, 181–195.
Mao, Jianqiang, Zhou, Guoqin, Chen, Bing, Du, Xuan, 2006. The effects of compound microorganisms on improving water quality. J. Aquaculture 27, 25–27.
Moriarty David, J.W., 1997. The role of microorganisms in aquaculture ponds. Aquaculture 151, 333–349.
Peng, Kaisong, She, Ruiping, 2004. Research and application of aquatic BM. World Agriculture 11 (307).
Rastall, Robert A., Vatsala Martin, 2002. Prebiotics and synbiotics: towards the next generation.
Rolfe, Rial D., 2000. The role of probiotic cultures in the control of gastrointestinal health. J Nutr. 130, 396S–402S.
Shen, Yu-chun, Ye, Fu-liang, Liang, Guo-pan, Xiang, Xian-fen, 2004. Study of ecological poly-culture system of shrimp, fish, shellfish and algae in water circular cluster ponds. J. Zhanjiang Ocean Univ. 24, 1069–1073.
Wang Xianghong, Li Jun, Ji Weishang, Xu Huaishu, 2008. Application of Probiotics in Aquaculture, published to the Internet with the permission of Dr. Xu Huaishu by Alken Murray Corp.
Wang, Yangyang, 2006. Different functions of probiotics and its application in animal production. J. Feed Nutr. (3), 2006.
Wang, Zhiguo, Han, Xuemei, 2004. How to adjust water quality by using microorganism. Ningxia J. Agri. Forestry Sci. Technol. 6, 60.
Xu, Sheng, 2006. Research development on biological environment remediation in prawn culture. J. Hebei Fisheries 12, 1–4.
Xue, Hengping, 2006. Analysis of BM in aquaculture and mariculture and ecological control. J Animal Husbandry Vet. Sci. 38 (2), 19.
Xue, Hengping, Xue, Yanqing, 1997. Preliminary study on the relationship between aquaculture and micro ecology and ecology of microbes. J. Feed Ind. 18 (2), 25.
Zhang, Ming, Shi, Jialiang, 1999. Application of photosynthetic bacteria in aquaculture. Chin. J. Appl. Environ. Biol. 5, 328–331.
Zou, Xiangyang, Kang, Bai, 1995. The application of Bifid bacterium preparation on the Fry production in China. Chin. J. Micro-ecol. 7, 67–70.