MICROBIAL ECOLOGICAL STUDIES IN CHINESE FISH POND ECOSYSTEMS

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The paper presents an overview of research work on microbial ecology of freshwater aquaculture systems that has been conducted in the Republic of China since 1982. The aspects considered are bacterial populations in fish ponds under different management practices, bacteria-environment interactions, sediment-water interactions, carbon flow in fish pond ecosystems, heterotrophic bacterial activity and trophic significance of bacterial communities in aquaculture.

Fish pond is a semi-artificial ecosystem with four basic components of producers, consumers, decomposers and abiotic environment. A host of interactions take place from the stage of manure application to the pond till its utilisation by the fish food organisms and further the fish, where microbial activity is of great importance. The pond dynamics encompasses these interactions both with regard to matter in terms of nutrients and energy (Fenchel and Jorgensen, 1977). Studies on microbial ecology of pond ecosystem provide information on the extent to which the system can process the organic inputs and energy transfer processes as also the means for improving the production efficiencies (Brock, 1971; Moriarty, 1981, 1987). A number of autotrophic and heterotrophic bacteria constitute the coenoses which act on different types of substrates rendering them into simpler nutrients that would be utilised by the primary producers, and bacterial-algal interactions are of great importance at this stage. Heterotrophic pathway has been shown to be as important as the autotrophic, accounting for half of the resultant productivity in many systems (Schroeder, 1978, 1983; Ayyappan et al., 1991). The trophic significance of the bacterial communities in carp culture has also been demonstrated, suggesting that they are not only involved in the process of organic decomposition, but also contribute to the food spectrum of fish species (Zhang et al., 1989; Zhu, 1989; Ayyappan et al., 1990, 1991).

Considering the importance of aquatic microbiological studies in monitoring and maintaining the productivity status of fish pond ecosystems, and total lack of information in Chinese waters, relevant studies were initiated in the Republic of China in the early eighties, particularly at the Freshwater Fisheries Research Centre at Wuxi, China. The present paper reviews the work that has been done during the last ten years and its impact on fish pond environmental monitoring and management in China.
1. Studies on bacterial populations in fish ponds under different management practices

a. The heterotrophic bacterial populations were studied in fish ponds applied with four kinds of animal manure. In pond applied with chicken manure, the mean number of heterotrophic bacteria was $0.93 \times 10^3$/ml followed by duck manure applied pond (73% of the counts of chicken manure applied pond), cattle manure (53%), pig manure (49%) and the control (12%). The mean bacterioplankton population in the chicken manure pond was $11.05 \times 10^6$/ml followed by duck manure (57%), cattle manure (47%), pig manure (38%) and control (15%).

The total bacterial population reached a peak after 3.5 days of applications of animal manure and then decreased. They varied with the type of manure and the physicochemical conditions of the water medium.

In the bacterial populations, cocci were the most dominant, followed by bacilli and then filamentous forms. There were altogether 34 genera among which four dominating genera were Micrococcus, Achromobacter, Cytophaga and Bacillus (Guo et al., 1984, 1988; Fang et al., 1986).

b. In the feed-applied intensive fish culture pond, the range in the number of heterotrophic bacteria was 1.56-14.50 x $10^4$/ml, and the total bacteria 1.30-15.90 x $10^5$/ml. In the pond silt, the corresponding ranges were 1.94-19.90 x $10^5$/g wet weight and 1.20-68.00 x $10^6$/g wet weight.

The variations in bacterial number were closely associated with the variations of factors such as water temperature, feed application and feed consumption by fish. The heterotrophic bacteria in pond silt comprised 11 genera, including Pseudomonas, Micrococcus, Bacillus and Corynebacterium (Din et al., 1988; Fang et al., 1989).

2. Bacteria-environment interactions

Studies on relations between the bacterial populations and environmental parameters were made in natural ponds. The data were processed by computer and correlations between aquatic bacteria and pond ecosystem were further elucidated.

a. Aquatic bacteria were closely related to seven non-biological factors. Positive correlations were recorded with factors such as BOD, suspended matter, organic detritus, total nitrogen and total phosphate ($r=0.9$), while negative correlations were observed with factors such as dissolved oxygen and transparency ($r=0.6-0.7$).

b. Aquatic bacterial counts were positively correlated to phytoplankton and zooplankton ($r=0.9$) as also fish production rates ($r=0.8$) (Guo et al., 1992).
3. Studies on Sediment-water Interactions

Studies on the water-sediment interactions laid emphasis on chemical and bacteriological aspects associated with nitrogen cycle.

a. A vertical categorisation of the sediment layers could be made into three layers: oxygenated layer (0-1.5 cm), low-oxygenated layer (1.5-9.0 cm) and non-oxygenated layer (more than 9.0 cm). Due to different oxygen and organic matter contents in each layer, bacterial populations involved in nitrogen cycle were also different with highest activity in the oxygenated layer. The chemoautotrophic and heterotrophic bacteria were involved in different steps of nitrogen cycle like ammonification, nitrification and denitrification.

b. In oxygenated layer of the pond silt, dominating bacterial genera were *Pseudomonas*, *Flavobacterium*, *Alcaligenes* and *Bacillus* with anaerobic bacterial populations. In the low-oxygenated layer, dominating genus was *Pseudomonas* with the number decreasing with increasing silt depth. The number of anaerobic bacteria increased with increase in silt depth. In the non-oxygenated layer, almost all the bacteria were anaerobic (*Enterobacteraceae*).

c. The oxygenated layer and the low-oxygenated layer which were active in nitrogen cycle were categorised as active silt layers and non-oxygenated layer, inactive in nitrogen cycle, was defined as non-active silt layer. In practice, the nitrogen dynamics in the active silt layer should be exploited, and nitrogen in organic matter in inactive silt layer should be released and transferred to fish food organisms for increasing the fish yield. Maintenance of adequate depth of silt is required for sustained fish production levels. These provided new information on the benthic bacterial activity relevant to pond dynamics.

4. Carbon flow in fish pond ecosystems

a. Carbon isotope spectrographic analyses were conducted for tracking the flow of carbon in feed and manure in the integrated fish pond. The relationship between food chain and food web was explored. Spectrographic analysis using stable carbon isotope (13C/12C) were made to understand the flow of matter and energy between food and fish. The research results showed that in pond applied with organic manure, the energy source of filter-feeding fish comes largely from autotrophic algal production system. As regards omnivorous fish, about 1/3 of crucian carp yield comes from this autotrophic production system and 2/3 from aquatic bacterial heterotrophic production system, whereas in common carp, each of the food chains produced 1/2 of the energy source.

b. Studies made on the transformation and flow of carbon between feed, manure, grass, natural feed and the six polycultured carp fish species showed that for filter-feeding fish like silver carp and bighead carp, 2/3 of carbon is provided by algae in autotrophic
food chain and 1/3 of carbon is supplied by aquatic bacteria through heterotrophic food chain. For omnivorous fish like crucian carp and common carp, 2/3 of carbon comes from fish feed (barley powder and rapeseed cake) and the rest 1/3 from aquatic bacteria and organic detritus. For herbivorous fish such as grass carp and bream, 2/3 of carbon comes from feed (barley powder and rapeseed cake), and the rest 1/3 from rye grass and cabbage (Schroeder, 1978, 1983; Guo et al., 1987).

5. Heterotrophic bacterial activity

For the first time in China, aquatic bacterial heterotrophic productivity of different water layers with respect to time in the high-yielding fish ponds were measured using (3H) TdR liquid scintillation analysis. The autotrophic productivity of phytoplankton was also measured. Comparisons were made with regard to energy supply and fish yield in different production systems. The results showed that:

a. On an average, daily production of phytoplankton is 12.30 mg/l, P/B coefficient is 0.47, daily production of aquatic bacteria is 23.54 which is 1.91-fold higher than that of phytoplankton and P/B coefficient is 2.25, which is 4.79-fold higher than that of phytoplankton.

b. The productivity of phytoplankton is 63.87 g/m² per day and the productivity of aquatic bacteria is 161.37 g/m² per day which is 2.53-fold higher than phytoplankton.

c. Phytoplankton provides 2.90 g C/m² of energy per day, i.e., 30.74 kcal/m² per day; aquatic bacteria provides 16.1 g C/m² per day of energy, i.e., 171.08 kcal/m² per day, which is 5.57 times higher than that of phytoplankton.

d. As regards energy transfer to higher trophic levels, phytoplankton provides 0.73 g C/m² per day and aquatic bacteria provide 4.04 g C/m² per day, which is 5.53 times that by phytoplankton.

The radioactive tritium analysis quantified the heterotrophic productivity of aquatic bacteria and provided an insight into the quantified energy flow patterns.

6. Trophic significance of bacterial communities

Studies on the aquatic bacterial coenoses with regard to their feed value, nutritional composition, digestion and effects on fish farming using stable nuclide 15N conducted for the first time in China provided the following information:

a. With four kinds of different carbon/nitrogen sources, i.e., pig manure, feeds (barley, bran and soybean residues), rye grass and beef-extract-peptone as the culture medium, the heterotrophic bacteria were cultured in order to examine their nutritional composition and contents. With beef-extract-peptone as the routine control culture medium, the bacteria cultured on the pig manure culture medium showed the highest
nutritional contents (protein 55.85%, essential amino acids 23.69% and total content of 17 amino acids 46.42%), followed by those cultured on different feeds. Though the bacteria cultured on the rye grass culture medium showed the lowest nutritive value (protein 43.55%, essential amino acids 17.89% and total content of 17 amino acids 35.23%), they were better than those of phytoplankton or zooplankton. It was thus shown that the aquatic heterotrophic bacteria are fine natural food with rich nutritional contents.

b. Experiments on feeding aquatic bacteria to fish were also conducted. The $^{15}$N-labelled aquatic heterotrophic bacterial cultures were fed to silver carp and bighead carp for ten days and the digestion and absorption rates were 65.11% for silver carp and 64.82% for bighead carp. The increments in length and weight were 6.83% and 17.78% for silver carp and 6.10% and 16.34% for bighead carp. Their food coefficients are only 0.61 and 0.64 and their survival rates are 100%. With survival rates of 0.61 and 0.64 for silver carp and the bighead.

c. The studies clearly demonstrated the trophic significance of microbial communities for carp. They also brought out the utility of the stable isotope $^{15}$N as a useful tracer in studying the trophic utility of aquatic bacteria for filter-feeding fish. This proved to be an efficient and economical method as compared to other methods like dissection, chromic oxide tagging, use of radio isotopes, etc.

Following conclusions could be drawn from the preceding discussion:

(i) Aquatic bacterial flora regulate the nutrient status and further the pond ecological conditions.

(ii) Playing important roles in decomposition and trophic functions, aquatic bacteria form the basis of the trophic structure.

(iii) With considerable influence on the trophic structure, aquatic bacteria influence and even determine the fish yields.

(iv) Processes involved in pond dynamics are influenced by heterotrophic and autotrophic food chains.

(v) The studies will greatly aid in maximising the input of energy as fish yield on scientific basis and to optimise the production efficiencies for sustained fish production rates.

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