Use of Probiotics for Improving Soil and Water Quality in Aquaculture Ponds

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ABSTRACT: A number of biological products including live bacterial inocula, enzyme preparations, and extracts of plant products are being promoted for use as water and soil quality conditioners in aquaculture ponds and particularly in shrimp ponds. Although there is much anecdotal information about these products and considerable promotional material from vendors, little independent research has been conducted. Several studies have shown no benefits of bacterial inocula, but one recent study at Auburn University demonstrated higher survival of fish and thus greater net production in ponds treated frequently with live *Bacillus* of three species than in control ponds. No improvements in water quality were noted, and the mechanism by which the bacteria improved survival is unknown. Laboratory studies of a bacterial inoculum demonstrated a greater rate of nitrogen loss. Liming of laboratory systems tended to improve bacterial activity. Pond studies also showed that applications of an enzyme preparation tended to enhance microbial mineralization of organic matter, but no effect on net fish production was observed. An extract of grapefruit seed caused greater survival of shrimp and higher production resulted. Again, the mode of action is unknown because water quality was not measurably improved. These few studies suggest that probiotics possibly can be beneficial in aquaculture ponds. Too little is known about their modes of action, the conditions under which they may be effective, and application rates and methods for general recommendation of their use. Nevertheless, the products are safe to humans and the environment, and their use poses no hazards. Thus, commercial producers are encouraged to conduct trials with these products, and researchers should conduct experiments with them.

KEYWORDS: probiotics, bacterial inocula, water conditioners

INTRODUCTION

There is considerable interest in use of probiotics to improve conditions for production in pond aquaculture. The most common probiotics are live bacterial inocula that sometimes are supplemented by yeast extracts with extracellular enzymes. Some companies sell extracellular enzyme preparations without live bacteria. Extracts of plant products also are used as probiotics. The most common are extracts of citrus seed and Yucca plants (*Yucca_schidigera*). Claims about the potential benefits of probiotics in aquaculture ponds include: enhanced decomposition of organic matter; reduction in nitrogen and phosphorus concentrations; better algal growth; greater availability of dissolved oxygen; less cyanobacteria (blue-green algae); control of ammonia, nitrite, and hydrogen sulfide; lower incidence of disease and greater survival; greater shrimp and fish production. Vendors usually sell probiotics for prophylactic treatments to protect against disease and to improve environmental conditions for culture. Few independent studies have been conducted on probiotics in aquaculture ponds. However, vendors have conducted trials on aquaculture farms and obtained testimonials from farmers. Results of trials and testimonials are used as the basis for exalted claims of the benefits of probiotics. Farmers usually do not have sufficient knowledge of microbiology and water quality to evaluate the claims of vendors. However, they are quite receptive to any new product that might enhance water and soil quality, reduce the incidence of disease, or increase production, and there is a brisk trade in probiotics for shrimp and fish farming.

BACTERIAL INOCULA

The argument for using bacterial inocula is that populations of beneficial bacteria in ponds can be increased by applying live bacteria or their propagules. Two types of inocula have been used. One type consists of the spores or other resting bodies of one or more species in a medium designed to prevent germination and retard growth. The media may be a liquid or granular material coated with bacterial propagules. Relatively small amounts of these inocula are placed in ponds with the assumption that propagules will quickly multiply and increase the abundance of the inoculated species. The second type of inoculum is a source of propagules that is inoculated into a nutrient solution and a culture of bacteria is produced for inoculating the pond. Initially, bacterial inocula were applied to ponds at 2- to 4-week intervals. Today, most vendors recommend that inocula be applied at more frequent intervals or even daily. Species of *Bacillus* are most commonly used, but species of

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Boyd CE, Gross A (1998) Use of probiotics for improving soil and water quality in aquaculture ponds. *In* Flegel TW (ed) Advances in shrimp biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok.

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Nitrobacter, *Pseudomonas*, *Enterobacter*, *Cellulomonas*, *Rhodopseudo-monas*, and photosynthetic sulfur bacteria have been used as bacterial inocula (Boyd 1990). The number of bacteria introduced per application varies greatly, but the initial increase in the pond water normally ranges from 10^2 to 10^4 colony forming units (CFU) ml^{-1}.

The mode of action of bacterial inocula is claimed to be the enhancement of natural processes such as organic matter degradation, nitrification, ammonia removal, denitrification, sulfide oxidation, and degradation of toxic pollutants. Some vendors state that by increasing the abundance of useful bacteria, competitive exclusion of undesirable species occurs. They even declare that the bacterial inocula can reduce the incidence of fish and shrimp disease in ponds.

Ponds have a natural microbial flora capable of conducting the entire range of organic matter and nutrient transformation that occur in natural ecosystems. Bacterial populations respond to the availability of substrate. For example, if a pond has ammonia in the water, natural nitrifying bacteria will oxidize the ammonia to nitrate. If ammonia concentration increases, the population of nitrifying bacteria will respond to the increase in substrate by rapidly increasing in abundance. When ammonia concentrations decline, the abundance of nitrifying bacteria will decline. Nitrification rates are sensitive to ammonia concentration, but they also are regulated by environmental variables such as temperature, dissolved oxygen concentration, and pH. If nitrification does not increase in response to increasing ammonia concentration, it is because some environmental variable is not satisfactory, and not because there is a shortage of nitrifying bacteria. The same argument may be made for organic matter decomposition and other microbial processes. It does not seem likely that applications of bacterial inocula to most ponds would influence the rate of bacterially-mediated processes. Although some vague explanations of how application of beneficial bacteria can reduce the incidence of undesirable bacteria have been advanced, no convincing explanation of how bacterial inocula can reduce the frequency of disease is available.

Boyd et al. (1984) treated four channel catfish ponds with a bacterial inoculum containing seven species of bacteria. Water quality and fish production were compared between treated and control ponds. The inoculum was claimed by the vendor to contain more than 10^9 CFU ml^{-1}, and 0.25 mg l^{-1} of inoculum was applied at monthly intervals between June and September. Concentrations of inorganic nitrogen, total phosphorus, chemical oxygen demand, 5-day biochemical oxygen demand, and chlorophyll a; numbers of bacteria and phytoplankters per milliliter; and percentages of blue-green algae did not differ significantly ($p > 0.05$) between treatments. On three sampling dates between mid July and mid August, there were significantly higher ($p < 0.05$) dissolved oxygen concentrations in ponds treated with the bacterial suspension. Fish production did not differ between treatments ($p > 0.1$). Tucker and Lloyd (1985) conducted trials with the same bacterial inoculum used by Boyd et al. (1984) with almost identical results.

Chiayvareesajja and Boyd (1993) treated laboratory microcosms with up to 40 mg l^{-1} of a granular microbial inoculum, and no changes in total ammonia nitrogen concentrations related to concentrations in controls were observed ($p > 0.05$). Pond waters were treated with the inoculum at 5 mg l^{-1} initially, 2.5 mg l^{-1} after week 1, and 0.5 mg l^{-1} for the next two weeks. No reduction in ammonia concentration relative to the control ponds was noted ($p > 0.05$).

A commercial bacterial inoculum consisting of *Bacillus* spp. cultured on site was applied to three channel catfish *Ictalurus punctatus* ponds at Auburn, Alabama, USA, three times per week from May until October 1996 (Queiroz & Boyd 1998). There were few significant differences ($p < 0.1$) in concentrations of water quality variables between ponds treated with bacteria and control ponds. In addition, bottom soil carbon and nitrogen did not differ between treated and control ponds. However, survival and net production of fish was significantly greater ($p < 0.1$) in ponds that received the bacterial inoculum than in controls (Table 1). The mechanism by which the bacterial treatment influenced fish survival cannot be explained from data collected in this study.

Because of the high variability encountered in water quality among aquaculture ponds treated alike and the difficulty of establishing a given level of water quality impairment in production ponds, a laboratory study of a bacteria inoculum was conducted at Auburn University. Pond soil-water mesocosms were established by placing 2 liters of pond soil enriched with 5 g of fish feed (32% crude protein) and 20 liters of pond water enriched with nutrients for BOD dilution water (Eaton et al. 1995). The following treatments were replicated four times in mesocosms: (1) control (soil and water with nutrients); (2) soil, water with nutrients, and bacterial inoculum; (3) soil, water and nutrients, calcium oxide, and bacterial inoculum; (4) autoclaved soil, water and nutrients, and bacterial inoculum. These mesocosms provided conditions similar to those found in intensive aquaculture ponds, i.e., high nutrient concentrations and or-

Table 1. Average fish production data (± SD) for channel catfish ponds treated and untreated (control) with bacterial inoculum.

Variable	Treated	Control
Stocking rate (per hectare)	15,000	15,000
Fish surviving until harvest (per hectare)	13,150 ± 2,007[a]	8,425 ±2,850[b]
Average harvest weight per fish (g)	311 ±63[a]	400 ±41[a]
Net production (kg ha^{-1})	4,020 ±438[a]	3,301 ±838[b]
Feed conversion ratio	1.5 ±0.07	1.82 ±0.36

[a]Means for the same variable were different at a probability level of 0.1

Table 2. Grand means for soil-water mesocosms in which a bacterial inoculum was tested. Means with the same letter did not differ at p = 0.05 (horizontal comparisons only).

		Bacterial inoculum added		
	Control	Initially-sterile soil	Un-sterile soil	Un-sterile soil and calcium oxide
Total phosphorus (mg l^{-1})	2.68^{a}	3.50^{a}	3.43^{a}	1.36^{b}
Soluble reactive phosphorus (mg l^{-1})	1.73^{a}	1.83^{a}	1.87^{a}	0.72^{b}
Total ammonia nitrogen (mg l^{-1})	4.50^{a}	5.45^{a}	3.32^{b}	2.72^{b}
Organic nitrogen (mg l^{-1})	2.70^{a}	3.50^{a}	2.04^{a}	2.31^{a}
Nitrate-nitrogen (mg l^{-1})	0.27^{a}	0.01^{a}	0.19^{a}	0.11^{a}
Nitrite-nitrogen (mg l^{-1})	0.37^{a}	0.03^{a}	0.60^{a}	0.17^{a}
Chemical oxygen demand (mg l^{-1})	31.7 b	42.2^{a}	42.7^{a}	34.9^{b}
Biochemical oxygen demand (mg l^{-1})	9.0^{a}	8.2^{a}	7.5^{a}	10.8^{a}
Dissolved oxygen (mg l^{-1})	8.2^{a}	7.8^{a}	7.1^{a}	7.9^{a}
pH (standard units)	7.7^{a}	7.6^{a}	7.4^{a}	8.3^{b}
Bacteria (cfu ml^{-1})				
Water	3.2×10^{5b}	2.9×10^{6a}	2.5×10^{5b}	2.3×10^{6a}
Soil	3.9×10^{6a}	4.0×10^{6a}	1.3×10^{6a}	7.0×10^{6a}

ganically-enriched bottom soil. The treatment with autoclaved soil provided mesocosms with few bacteria at the time of inoculation with bacterial product. The soil in microcosms had a pH of about 6, so a calcium oxide treatment was included to simulate liming.

The averages of water and soil analyses conducted over a 20-d period (Table 2) revealed bacteria abundance was greater in mesocosms treated with calcium oxide and in mesocosms with autoclaved soil than in the other treatments. Calcium oxide raised the pH to 8.34 and a pH of 8.0 to 8.5 is optimum for bacterial activity (Boyd & Pipoppinyo 1994). Sterilization of the soil eliminated the natural bacterial populations. This may have reduced competition of natural bacteria to provide a greater opportunity for growth for the inoculated bacteria.

Some differences (p< 0.05) were noted among treatments (Table 2). Total phosphorus and soluble reactive phosphorus concentrations in water were less in mesocosms treated with calcium oxide than in the others. This resulted from the precipitation of phosphorus by calcium oxide and was not related to bacterial activity. Total ammonia nitrogen concentrations were higher in waters of mesocosms containing autoclaved soil and control mesocosms than the other two treatments. Lower total ammonia nitrogen in the calcium oxide-treatment microcosm was related to the higher pH causing a greater loss of ammonia to the air by diffusion. The reason that application of bacteria to non-sterile soil resulted in lower total ammonia concentrations than found in control mesocosms or in mesocosms with initially-sterilized soils is not apparent. The chemical oxygen demand increased in the unlimed, microbial-treated mesocosms relative to the control. No differences in organic nitrogen, nitrate-N, nitrite-N, biochemical oxygen demand, or dissolved oxygen concentrations were noted among treatments.

This study failed to reveal a positive influence of the bacteria inoculum on water quality in the mesocosms. The only difference among treatments was the lower concentrations of total phosphorus, soluble reactive phosphorus, and total ammonia nitrogen in mesocosms treated with calcium oxide. Thus, liming to neutralize acidity in ponds with acidic soil and low alkalinity water may have a much greater effect on water quality than can be expected from bacterial augmentation. In ponds with higher pH and alkalinity, neither treatment is likely to enhance water quality.

ENZYMES

Reversible chemical reactions attain a state of equilibrium when the velocities of forward and reverse reactions become equal. The equilibrium state is influenced by temperature, pressure, concentrations of reactants, and catalysts. A catalyst can speed up both forward and reverse reactions to allow equilibrium to be reached quickly. For molecules to react, they must pass through a configuration known as the activated state in which they have the activation energy necessary to react. Catalysts reduce the activation energy to facilitate more rapid reaction of molecules. Catalysts are not used up in reactions, and they can be used over and over. In biochemical reactions, catalysts are specialized protein molecules called enzymes that are very specific in the reactions that they catalyze. They occur in living cells, and extracellular enzymes are produced and excreted by microorganisms. Enzymes are named for the reaction that they catalyze. For example, cellulase catalyzes the breakdown of cellulose into smaller molecules, and oxidases catalyze oxidations.

Bacteria excrete extracellular enzymes that degrade large molecules into smaller particles that can be absorbed for further degradation by enzyme-catalyzed reactions within their cells. It should be obvious that enzyme additions cannot speed up degradation of organic matter or toxic substances unless bacteria are present. Extracellular enzymes are only the first step in the degradation process. In cases where there is a high abundance of a substance, enzyme blocking may occur because of excess substrate.

A fermentation product that is rich in enzymes and contains stabilizers, nutrients, and minerals was applied to soil in laboratory respiration chambers (Boyd & Pippopinyo 1994). There were no significant differences ($p > 0.05$) in soil respiration between controls and soils treated at 50 and 200 mg kg^{-1} with the enzyme preparation. Queiroz et al. (1998) tested an enzyme preparation in channel catfish ponds. Although there were no significant differences ($p > 0.1$), ponds treated with the enzyme preparation tended to have higher concentrations of dissolved oxygen during summer months than control ponds. No differences in water quality or soil condition were noted between enzyme-treated and control ponds. However, there was a trend towards greater organic matter decomposition in soils treated with the enzyme product, but because of the high variation, the difference was not significant ($p > 0.1$). Fish survival was higher in the treated ponds ($p > 0.1$), but net fish production did not differ between treated and control ponds. Ponds used in this study were stocked at a moderate rate (15,000 fish ha^{-1}) and maximum daily feeding rate was only 75 kg ha^{-1}. Thus, water and soil quality were not severely impaired in the ponds. The enzyme product might produce greater benefits in ponds with higher stocking and feeding rates where the water and soil quality deteriorate greatly during the production period.

PLANT EXTRACTS

There has been preliminary research on the use of natural compounds extracted from plants for improving pond water quality or for controlling blue-green algae. Some of these products contain substances that are toxic to bacteria, while others contain compounds that are toxic to plants and especially to blue-green algae.

Yucca

Extracts of the Yucca plant contain glycocomponents which bind ammonia (Wacharonke 1994). Under laboratory conditions, 1 mg l^{-1} of a commercial Yucca extract reduced total ammonia nitrogen concentrations by 0.1-0.2 mg l^{-1}. Because pH data were not provided, the removal of unionized ammonia nitrogen cannot be computed. Pond treatments were made at 15-d intervals with 0.3 mg l^{-1} of the Yucca preparation per application. It was reported that ammonia concentrations were lower and shrimp survival better in ponds treated with Yucca extract than in control ponds (Wacharonke 1994). In a trial conducted at Auburn University, the commercial Yucca extract was applied at 0.3 mg l^{-1} at 2-week intervals to channel catfish ponds. Concentrations of total ammonia nitrogen in treated ponds averaged 0.1 mg l^{-1} lower than those of control ponds ($p > 0.1$). Further research on the use of Yucca extracts to reduce ammonia concentrations are needed to verify the benefits of this treatment.

Citrus seed extracts

There has been considerable use of citrus extracts for treating shrimp ponds in Ecuador to enhance soil and water quality. The most popular one, KILOL, is an extract of grapefruit seed. It is either applied directly to ponds or mixed with lime and applied to ponds. It also can be mixed into shrimp feed. KILOL has been approved by the United States Food and Drug Administration for use on foods and it does not cause environmental harm. One of the authors (CEB) designed and supervised an on-farm trial in Ecuador of KILOL and KILOMAR (KILOL powder mixed with agricultural limestone).

Five ponds were treated with KILOL and KILOMAR and KILOL was incorporated in feed at rates suggested by the manufacturer, and four ponds served as untreated control ponds. At the time of treatment, bottom soil conditions and water quality were similar among ponds. Ponds were stocked with an average of 15 postlarval *Penaeus vannamei*/m^2. Slightly more feed and fertilizer were applied to control ponds than to treated ponds. No large differences in soil and water quality were observed between treated ponds and control ponds, but on several dates, the color of water in treated ponds was yellowish brown, indicating large populations of diatoms, while waters of control ponds often were green in color. At harvest, shrimp in treated ponds had an average survival of 44.92%, a live weight production of 732 kg ha^{-1}, and a feed conversion ration (FCR) of 1.26 (Table 3). The control ponds had a survival of 35.86%, a production of 596 kg ha^{-1}, and a FCR of 1.83. Thus, treated ponds performed better than control ponds. Shrimp size at harvest was not different between control ponds and treated ponds. The greater production of shrimp in treated ponds was a result of higher shrimp survival in treated ponds than in control ponds. The farm manager reported fewer disease problems in treated ponds than in control ponds. However, it is not clear how KILOL and KILOMAR acted to reduce the incidence of disease and enhance shrimp survival.

Table 3. Summary from a shrimp farm in Ecuador of shrimp production data for control ponds (untreated) and ponds treated with KILOL plus KILOMAR.

Variable	Control	KILOL and KILOMAR
Stocking density (Postlarvae/m^2)	15.0	14.7
Feed applied (kg ha^{-1})	1,060	884
Average culture period (days)	141	143
Survival (%)	35.86	44.92
Average size of shrimp at harvest (g)	11.08	11.19
Production (kg ha^{-1})	596	732
Feed conversion ratio (kg feed/kg shrimp)	1.83	1.26

Potassium ricinoleate

Potassium ricinoleate, a natural compound derived from the saponification of castor oil and potassium hydroxide, was reported to be selectively toxic toward blue-green algae (van Aller & Pessoney 1982). Ricinoleate is structurally similar to other allelopathic compounds isolated from the aquatic angiosperm *Eleocharis microcarpa*. Additional field testing revealed that the percentage of blue-green algae in phytoplankton communities in ponds receiving potassium ricinoleate was not reduced (Tucker & Lloyd 1987, Scott et al. 1989).

Tannic acid

Tannic acid has also been reported to inhibit the growth of certain species of blue-green algae (Chung et al. 1995). However, Schrader et al. (1998) found that tannic acid was neither extremely toxic nor selectively toxic towards *Oscillatoria chalybea*, but they identified several other natural compounds that were selectively toxic towards *O. chalybea* in the laboratory. Also, decomposing barley straw has been identified as a method for controlling blue-green algae growth in reservoirs (Everall & Lees 1996), and Newman and Barrett (1993) found decomposing barley straw to be inhibitory towards the blue-green algae *Microcystis aeruginosa*. Phenolic compounds released during the decomposition of barley straw may undergo oxidation to form quinones that are toxic towards blue-green algae (Everall & Lees 1997).

DISCUSSION

The material reviewed above reveals that relatively little research has been done on probiotics in pond aquaculture. This is unfortunate because many companies are marketing these products, and they advertise that probiotics can enhance water and soil quality, improve production, and increase profits. The usual approach is to use low concentrations of probiotics for prevention and higher concentrations when a specific problem has been identified.

Results have shown very few positive benefits of probiotics to water or bottom soil quality. It may be that probiotics only produce improvements when specific problems occur, and possibly, these conditions never occurred in the ponds and laboratory trials reported above. Also, the advertising literature often is based on laboratory studies where high concentrations of the probiotics were used. In practice, it is too expensive to use such high concentrations, so the vendor recommends much lower doses for ponds. This is a common problem with the adoption of amendments in pond management. If there is an effect that is known to be produced by a certain agent, and if this effect would be good in ponds, the agent may be adopted for use with no idea of the appropriate concentration or of the conditions under which the effect is produced. There have been a few cases of improved survival and production of shrimp and fish through application of probiotics. However, the mechanism of action of probiotics are not known and the conditions under which improvements may be expected cannot be identified.

Obviously, considerable research should be conducted on probiotics in pond aquaculture by independent parties not interested in sales of the products. This research needs to elucidate the effects of probiotics on aquatic ecosystems, identify conditions under which probiotics can be beneficial, and develop appropriate doses and methods of application. In addition, the economic benefits that can accrue from probiotic use need to be determined. Until these findings are available, one should be cautious about probiotics. Although no damage to the fish or shrimp crop or to the environment should result from probiotics, one might spend considerable money on these products and receive little in return. Nevertheless, if using probiotics in your pond will make you feel better and you are willing to risk the investment, there is no reason not to use them.

LITERATURE CITED

Boyd CE (1990) Water quality in ponds for aquaculture. Alabama Agricultural Experiment Station, Auburn University, Alabama

Boyd CE, Pippopinyo S (1994) Factors affecting respiration in dry pond bottom soils. Aquaculture 120:283-294

Boyd CE, Hollerman WD, Plumb JA, Saeed M (1984) Effect of treatment with a commercial bacterial suspension on water quality in channel catfish ponds. Prog Fish-Cult 46:36-40

Chiayvareesajja S, Boyd CE (1993) Effects of zeolite, formalin, bacterial augmentation, and aeration on total ammonia nitrogen concentrations. Aquaculture 116:33-45

Chung K-T, Zhao G, Stevens, Jr. SE (1995) Inhibitory effects of tannic acid and its derivatives on growth of the cyanobacteria Nostoc sp. strain MAC and *Agmenellum quadruplicatum* PR-6. J Aquat Animal Health 7:341-344

Eaton AD, Clesceri LS, and Greenburg AE (1995) Standard methods for the examination of water and wastewater, 19th edition. American Public Health Association, Washington, DC

Everall NC, Lees DR (1996) The use of barley-straw to control general and blue-green algal growth in a Derbyshire reservoir. Water Res 30:269-276

Everall NC, Lees DR (1997) The identification and significance of chemicals released from decomposing barley straw during reservoir algal control. Water Res 31:614-620

Newman JR, Barrett PRF (1993) Control of *Microcystis aeruginosa* by decomposing barley straw. J Aquat Plant Man 31:203-206

Queiroz JF, Boyd CE (1998) Effects of a bacterial inoculum in channel catfish ponds. J World Aquacult Soc 29:67-73

Queiroz JF, Boyd CE, Gross, A (1998) Evaluation of a bio-organic catalyst in channel catfish, *Ictalurus punctatus*, ponds. J. Appl Aquacult: in press

Schrader KK, DeRegt MO, Tidwell PD, Tucker CS, Duke SO (1998) Selective growth inhibition of the musty-odor producing cyanobacterium *Oscillatoria chalybea* by natural compounds. Bull Environ Contam Toxicol 60:651-658

Scott JH, Bayne DR, Boyd CE (1989) Effects of potassium ricinoleate on water quality, phytoplankton, and off-flavor in channel catfish ponds. J. Aquat Plant Man 27:26-31

Tucker CS, Lloyd SW (1985) Evaluation of a commercial bacterial amendment for improving water quality in channel catfish ponds. Mississippi Agriculture and Forestry Experiment Station, Mississippi State University, Res Rpt 10:1-4

Tucker CS, Lloyd SW (1987) Evaluation of potassium ricinoleate as a selective blue-green algicide in channel catfish ponds. Aquaculture 65:141-148

van Aller RT, Pessoney GF (1982) U.S.M. algal research team makes major off-flavor/water quality discovery. Aquaculture Magazine 8:18-22