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Note

Microcosm evaluation of indigenous microflora of traditional shrimp farming system as bioremediators

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ABSTRACT

The ability of indigenous microflora of traditional shrimp farming system to remove ammonia was evaluated under laboratory conditions. The indigenous microflora in combination were capable of removal of total ammoniacal nitrogen (TAN) to the tune of 85 - 99% within a week. The activity of nitrifying bacteria was observed to be substrate dependent. The results revealed that the TAN removal rate was affected by high initial TAN concentration and varied microbial activity. The results of the present study would release new avenues for future research and refinement of techniques on bioremediation in shrimp aquaculture.

Keywords: Ammonia oxidizing bacteria, *Bacillus* sp. P7, Bioremediation, Microcosm assay, Nitrite oxidizing bacteria.

The organic nitrogen content in aquaculture environment will be high compared to natural environment due to extraneous input like feed, excreta, fertilizer, *etc*. The increase in intensification of culture system leads to increased metabolite production which in turn supports higher population of protein mineralizing and ammonifying bacteria (Barat and Jana, 1987). The protein mineralizing bacteria and the ammonifying bacteria in ponds carry out active decomposition of this organic nitrogen into inorganic ammonia through mineralization (Moriarty, 1997). In aerobic sediments and in the water column, the nitrifying bacteria oxidize ammonia and nitrites for their energy needs and fix inorganic carbon dioxide (CO_2) to fulfill their carbon requirements. The capability of *Bacillus* spp. to utilize ammonia as nitrogen source for its growth is also assuming much importance. Gram-positive *Bacillus* spp. are generally more efficient in converting organic matter back to CO_2 than Gram-negative bacteria, which would convert a greater percentage of organic carbon to bacterial biomass or slime.

A number of methods using submerged flow biofilters (Abeysinghe *et al*., 1996), high rate linear-path trickling nitrification filters (Twarowska *et al*., 1997), bench scale fluidized bed bioreactors (Ng *et al*., 1996), continuous bioreactors with immobilized alginate beads (Kim *et al*., 2000), pellet immobilization of nitrifying bacteria (Shan and Obbard, 2001) and other total ammoniacal nitrogen (TAN) removal approaches have been developed for the control of TAN concentration in aquaculture systems. Achieving a high rate of TAN removal using microbes exhibiting specific functions under conditions of continuous TAN production is a major challenge in aquaculture systems. The present study was taken up to evaluate the efficacy of indigenous microflora of traditional shrimp farming system in TAN removal through microcosm assays.

The water and sediment samples collected from traditional shrimp farms (Bheries) in Malancha (Lat. 22° N; Long. 88°45'E), South 24 Parganas District, West Bengal were inoculated into modified Winogradsky medium and Winogradsky medium for the isolation of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), respectively (Rodina, 1972) and incubated for 2 - 3 weeks at 30±2°C. Presence of AOB was tested using Greiss reagent. The positive samples were further purified by subculturing fortnightly. The purified AOB was, then grown in enrichment medium (Rodina, 1972**)** for 7 days and used. Presence of NOB was tested using diphenylamine reagent. The NOB was further purified by subculturing fortnightly in Winogradsky medium and used for experimental purposes.

An aerobic Gram-positive, spore forming rod, hereafter referred to as *Bacillus* sp. P7, was isolated from traditional shrimp farm sediment sample on to tryptic soy agar supplemented with 1% (w/v) sodium chloride (NaCl) (TSAS) at 30±2°C. The biochemical tests were performed as per Collins *et al.* (1989) and the strain was identified

following the scheme for Gram-positive bacteria (LeChevallier *et al.,* 1980). For experimental purpose, *Bacillus* sp. P7 was grown in tryptic soy broth supplemented with 1% NaCl (TSBS) for 24 h at 30±2°C.

A mixture of pond water (100 ml) and sediment (50 g) from traditional shrimp farm was inoculated into minimal medium containing 1 g glucose and 0.1 g ammonium chloride in 1000 ml seawater (salinity 15 ppt) and incubated at 30±2°C for 24 h. Subculturing of this microbial consortium (Microcon) was done in minimal medium fortnightly.

Forty glass aquaria of 50 l capacity were first rinsed in water, treated with 10 ppm chlorine and then thoroughly washed in clean water to remove traces of chlorine and then dried. Pond sediment collected from shrimp farms (bheries) was spread uniformly to get a 5 cm thick layer in all the aquaria. Saline water (10 ppt) was added into these tanks slowly without disturbing the sediment layer. All the tanks were kept undisturbed for three days. The tanks were divided into 4 sets of ten tanks each. In each of the tanks in sets 1 to 4, the TAN concentration was adjusted to 1 ppm, 2 ppm, 3 ppm and 4 ppm, respectively by the addition of liquid ammonia solution (20%). Each set contained eight treatment tanks (A, B, C and D in duplicate) and two control tanks (E in duplicate). The bacterial cells were obtained by growing them in appropriate medium followed by centrifugation at 5000 rpm for 15 min. The washed cells of AOB, NOB and Microcon were resuspended separately in physiological saline (0.85% NaCl w/v) and enumerated in respective medium by Most Probable Number (MPN) technique. *Bacillus* sp. P7 was enumerated on to TSAS by spread plating. The treatment tanks were seeded with indigenous microflora as described below:

A - *Bacillus* sp. P7 at 10^5 ml^{-1} tank water

B - AOB at 10^5 ml^{-1} tank water and NOB at 10^5 ml^{-1} tank water

C - AOB at 10^5 ml^{-1} tank water and *Bacillus* sp. P7 at 10^5 ml^{-1} tank water

D - AOB at 10^5 ml^{-1} tank water, *Bacillus* sp. P7 at 10^5 ml^{-1} tank water and Microcon at 10^5 ml^{-1} tank water.

E - Control tanks, which received no bacterial inoculum.

The surface water temperature of each experimental tank was recorded daily by a mercury centigrade thermometer and the pH was measured using a digital pH meter (Systronics: model No. MK-VI). The total alkalinity was estimated following the acid base titration method (APHA/AWWA/WEF, 1998). The levels of TAN, nitrite (NO_2) and nitrate (NO_3) were monitored spectrophotometrically following APHA/AWWA/WEF (1998) methods daily for 8 days. One-way ANOVA was followed to test the level of significance among treatments and critical difference was calculated to examine which of the treatments varied significantly (Snedecor and Cochran, 1962).

Ammonia oxidizing bacteria and nitrite oxidizing bacteria are highly sensitive to sudden changes in temperature and pH (Abraham *et al*., 2004). There were no substantial differences in water temperature, pH and total alkalinity among the experimental tanks. The water temperatures were nearly the same in all the tanks and varied between 30 ^{0}C and 32 ^{0}C. The levels of pH exhibited a decrease with increasing days in all the experimental tanks from 8.08 ± 0.04 to 7.83 ± 0.06. The same occurred in the total alkalinity, which decreased from 136.48 ± 4.16 mg l^{-1} to 123.46 ± 3.92 mg l^{-1}. The reduction in pH and alkalinity of tank water was perhaps because of the reduction of basic TAN to acidic nitrate by AOB and NOB.

The traditional shrimp farm sediment samples were selected for the isolation of nitrogen cycle bacteria to be used in the microcosm assay, as they harboured good number of these bacteria (Abraham *et al*., 2004). As shown in Fig. 1a-d, the indigenous microflora in combination were capable of removal of TAN to the tune of >85 - 99% within a week. There existed significant differences ($p<0.05$) in the reduction of TAN at different initial TAN levels and among treatments. Higher initial TAN concentration and varied microbial population affected the TAN removal rate in both treatment and control tanks. The differential rates of TAN removal was probably due to the varying degrees of oxidation of ammonia. The reduction of TAN in tanks with 1 and 2 ppm initial TAN varied markedly ($p<0.05$) when compared to 3 and 4 ppm initial TAN. The mixture of AOB and NOB worked well ($p<0.05$) when the initial TAN levels were low, *i.e*., 1 ppm and 2 ppm, removing more than 91-95% of TAN. While at higher ammonia levels, the removal of TAN was about 86%. This could be due to the fact that the activity of nitrifying bacteria is substrate dependent.

Bacillus sp. P7 also exhibited similar results removing TAN to the tune of 92-95% at initial TAN levels of 1 and 2 ppm but only about 88-89% at high initial TAN levels. This could probably be attributed to the varying degrees of oxidation of ammonia by *Bacillus* sp. P7 at varying initial TAN levels. On the other hand, the initial TAN levels did not have any significant effect on the TAN removal rate of AOB, *Bacillus* sp. P7 and Microcon mixture, and was effective in removing more than 96% of TAN (Figs. 1a-d) at all the initial TAN levels. This could be due to the synergistic effect of all the three groups to oxidize ammonia and / or to utilize it as nitrogen source for growth. It has been reported that coculture with a heterotroph is able to

increase the nitrifying activity of an autotrophic strain *Nitrosomonas* sp., or to reduce its lag phase (Kuenen and Gottschall, 1982). The combination of AOB and *Bacillus* sp. P7 was also equally effective, removing 97% of TAN (Figs. 1a-d).

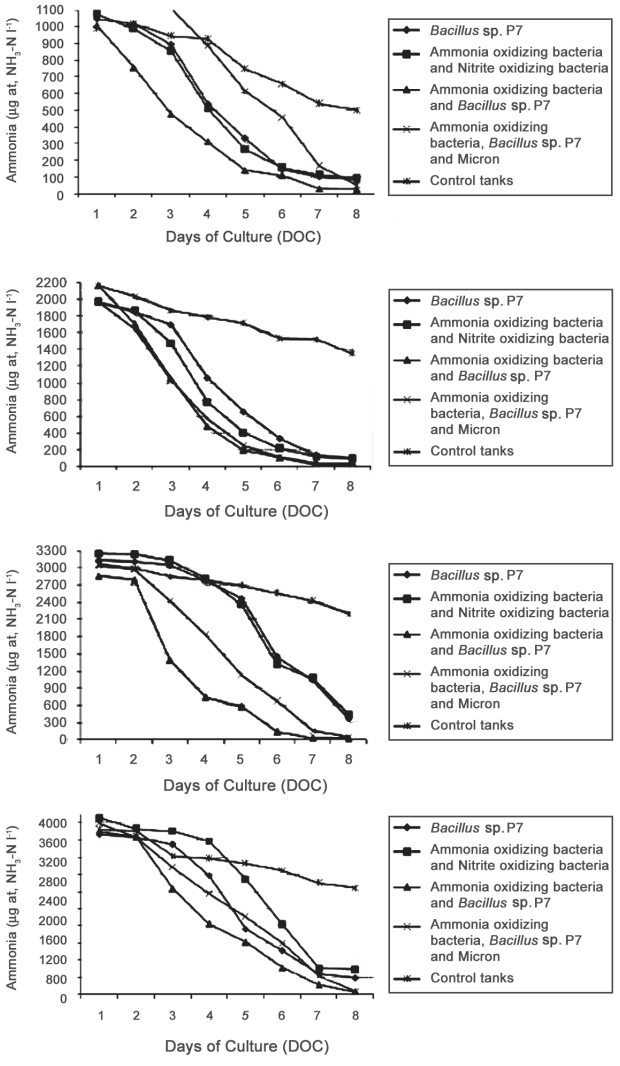

Fig. 1a-d. Efficacy of indigenous microflora of traditional shrimp farm in removing ammonia at various initial ammonia levels – a: 1 ppm; b: 2 ppm; c: 3 ppm; d: 4 ppm.

The NO_2 levels increased in all the experimental tanks except the tanks seeded with a combination of AOB and *Bacillus* sp. P7 at 1 and 3 ppm levels of TAN indicating that the ammonia is getting oxidized to NO_2 by nitrifying bacteria (Figs. 2a-d). Significant differences ($p<0.05$) existed among treatments and also among initial TAN concentrations. The increase in NO_2 was generally high at 3 and 4 ppm levels of TAN in majority of the cases than at low initial TAN levels.

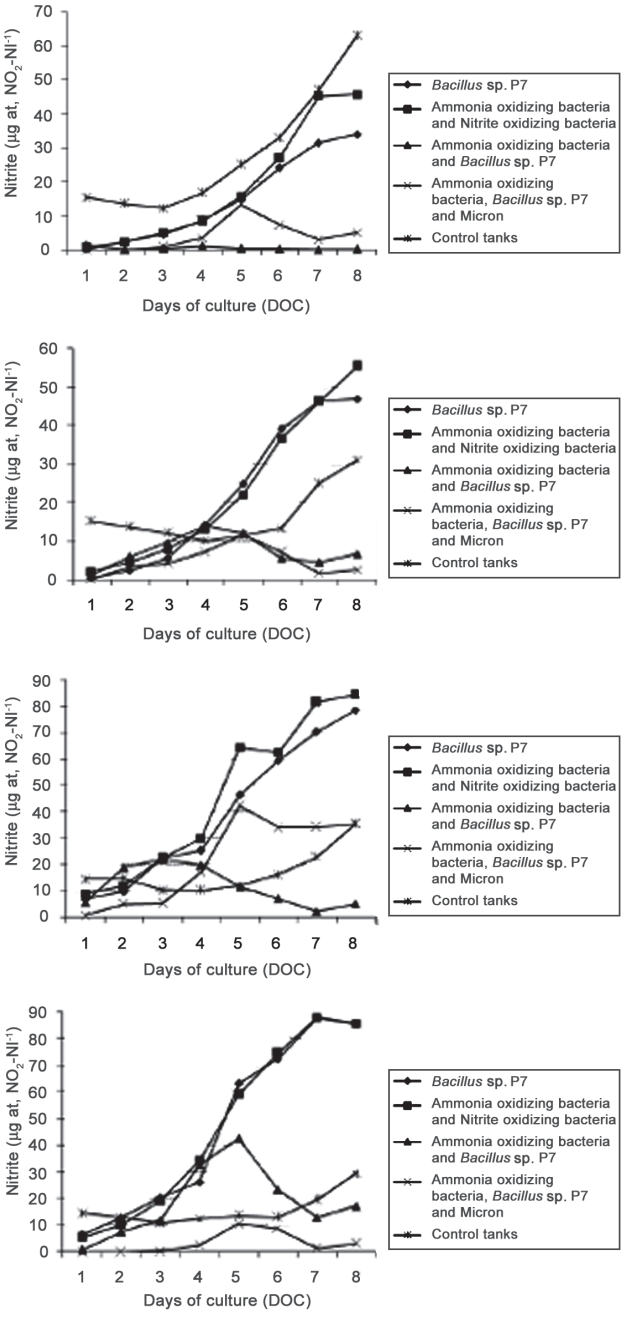

Fig. 2a-d. Effect of addition of indigenous microflora on nitrite in microcosm experiments at different initial ammonia concentrations – a: 1 ppm; b: 2 ppm; c: 3 ppm; d: 4 ppm.

The NO_3 levels varied in all treatment tanks seeded with indigenous flora and decreased in control tanks (Figs. 3a-d). The NO_3 levels in control tanks were significantly different ($p<0.05$) from those of seeded tanks at all the four different initial TAN levels. At 1 ppm and 2 ppm levels of TAN, the observed levels of NO_3 in tanks seeded with a mixture of *Bacillus* sp. P7 and AOB and *Bacillus* sp. P7,

AOB and Microcon were significantly different ($p<0.05$) from those of tanks seeded with a mixture of AOB and NOB and those seeded with *Bacillus* sp. P7 alone (Figs. 3a-d). The differences in the mechanism of NH_3 utilization by nitrifying bacteria and/or different rate of substrate utilization by indigenous flora (Koops and Muller, 1992) could explain the observed differences in the NO_2 and NO_3 levels.

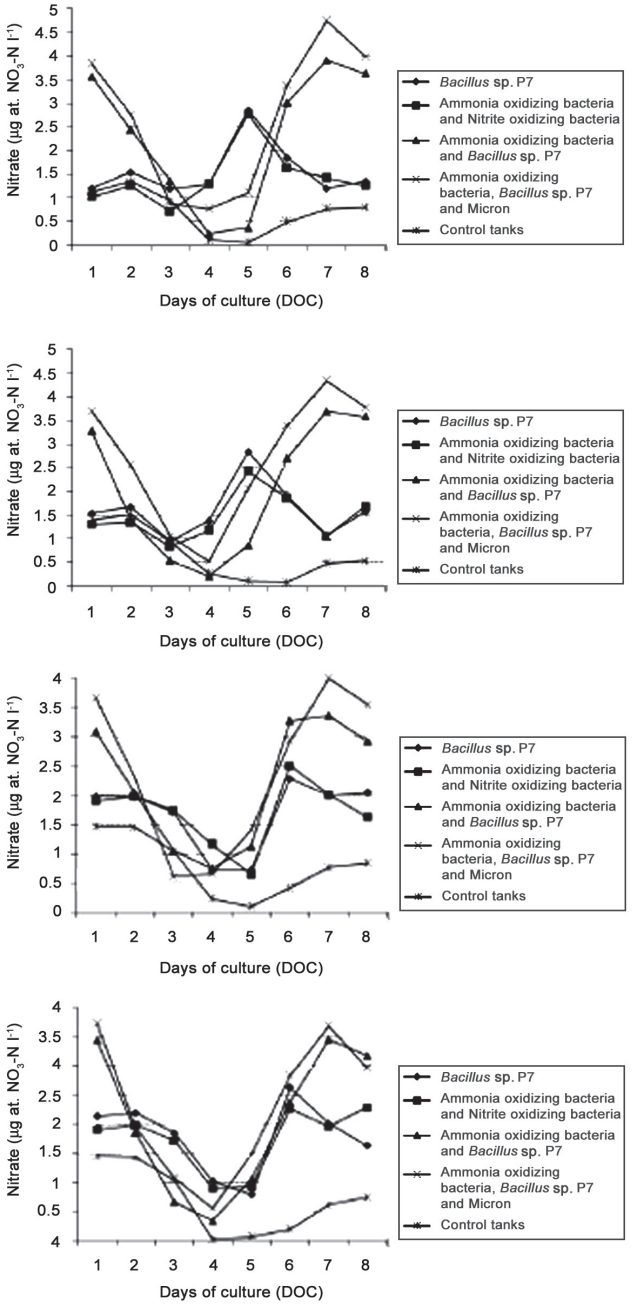

Fig. 3a-d. Effect of addition of indigenous microflora on nitrate in microcosm experiments at different initial ammonia levels - a: 1 ppm; b: 2 ppm; c: 3 ppm; d: 4 ppm.

The results of the present study are in full agreement with earlier published report (Shan and Obbard, 2001) on the proficiency of immobilized nitrifying bacterial cultures in removing TAN when TAN was continuously supplied at concentrations similar to that produced in aquaculture as a result of shrimp excretion and food addition to pond water. The indigenous bacterial flora can induce immediate TAN removal without the constraint of acclimatization and inter-specific competition associated with the use of proprietary and non-indigenous cultures (Shan and Obbard, 2001). The challenge in maintaining a viable culture of indigenous bacteria at high cell density in active growth phase is a key factor in providing an effective treatment for shrimp culture pond water. The results of the present study would provide a basis for the future research and refinement of techniques on bioremediation in shrimp aquaculture.

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References

Abeysinghe, D. H., Shanableh, A. and Rigden, B. 1996. Biofilter for water reuse in aquaculture. *Water Sci. Technol.,* 34: 253-260.

Abraham, T. J., Ghosh, S., Nagesh, T. S. and Sasmal, D. 2004. Distribution of bacteria involved in nitrogen and sulphur cycles in shrimp culture systems of West Bengal, India. *Aquaculture,* 239: 275 - 288.

APHA/AWWA/WEF, 1998. *Standard Methods for the examination of water and wastewater*, 20th edn., American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, p. 4-125.

Barat, S. and Jana, B. B. 1987. Effect of farming management on the distribution pattern of ammonification rates, protein mineralizing and ammonifying bacterial population in experimental culture tanks. *Bamidgeh,* 39(4): 120-132.

Collins, C. H., Lyne, P. M. and Grange, J. M. 1989. *Microbiological methods*, 6th edn., Butterworth, London, UK, 409 pp.

Kim, S., Kong, I., Lee, B., Kang, I., Lee, M. and Suh, K. H. 2000. Removal of ammonia–N from a recirculation aquaculture system using an immobilized nitrifier. *Aquacult. Engg.*, 21: 139-150.

Koops, H. P. and Muller, U. C. 1992. The lithotrophic ammonia oxidizing bacteria. In: Balows, A., Truper, H. G., Dworkin, M., Harbder, W. and Schleifer, K. H. (Eds.), *The Prokaryotes*. Vol. III, Springer-Verlag, Berlin, Germany, p. 2625-2637.

Kuenen, R. D. and Gottschall, J. C. 1982. Competition among chemolithotrophs and methylotrophs and their interactions with heterotrophic bacteria. In: Bull, A. T. and Slater, J. H. (Eds.), *Microbial Interactions and Communities*. Vol. 1, Academic Press, London, UK, p. 153-188.

LeChevallier, M. W., Seidler, J. and Evans, T. M. 1980. Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. *Appl. Environ. Microbiol.,* 40 (5): 922-930.

Moriarty, D. J. W. 1997. The role of microorganisms in aquaculture ponds. *Aquaculture,* 151: 333-349.

Ng, W. J., Kho, K., Ong, S. L., Sim, T. S. and Ho, J. M. 1996. Ammonia removal from aquaculture water by means of fluidized technology. *Aquaculture*, 139: 55-62.

Rodina, A. G. 1972. *Methods in Aquatic Microbiology*, University Park Press, Baltimore, USA, 461 pp.

Shan, H. and Obbard, J. P. 2001. Ammonia removal from prawn aquaculture water using immobilized nitrifying bacteria. *Appl. Microbiol. Biotechnol.,* 57: 791-798.

Snedecor, G. W. and Cochran, W. C. 1967. *Statistical Methods*. Oxford and IBH Publishing Co., Calcutta, India, 593 pp.

Twarowska, J. G., Westerman, P. W. and Losordo, T. M. 1997. Water treatment and waste characterization evaluation of an intensive recirculating fish production system. *Aquacult. Engg.,* 16: 133-147.

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