

## Bacterial diversity in organic manured fish ponds

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Indian freshwater aquaculture is mainly based on organic fertilization with the use of raw cow manure along with inorganic fertilizers for pond fertilization (Ayyappan *et al.*, 1990). In integrated fish farming recycled animal excreta, poultry droppings and agricultural byproducts are used as fertilizers. Processing of organic matter prior to application to fish ponds would provide ready substrate to decomposing bacterial communities which otherwise would have caused a lag period before the requisite bacterial activity (Zhu *et al.*, 1990). Organic manuring results in composite fertilization providing nutrients on a sustained basis to the fish culture system (Wohlfarth and Schroeder, 1979). Significance of bacterial communities in the recycling of organic material in aquatic ecosystems is important as they maintain ecological balance through various microbial processes and these microbial processes increases the fertility of the ecosystems. Bacterial communities in fish pond ecosystems serve as reliable indices of the efficiencies of manurial practices, based on the concept of substrate utilization (Jha *et al.*, 2008; Das *et al.*, 2013). The objective of the present study was to evaluate the different bacterial genera involved in nutrient cycling of both raw and processed organic manured carp culture fish ponds.

The water and sediment samples were collected aseptically from the organic manured fish ponds with cow manure and biogas slurry and transferred to laboratory

under sterile conditions for the assessment of bacterial diversity. Standard dilution plate count method was employed for enumeration of aerobic heterotrophic bacterial populations once in a month both in water and sediment for a period of one year using nutrient glucose agar (Norris and Ribbons, 1970; Collins and Lyne, 1976) at the farm of Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Orissa (Lat.20<sup>0</sup>11'06"-20<sup>0</sup>11'45" N; Long. 85<sup>0</sup>50'52"-85<sup>0</sup>51'35"E). The isolation of bacterial cultures was carried out using serial dilution technique. While 10<sup>-3</sup> dilutions with sterile physiological saline (0.85% NaCl) were used for water samples, 10<sup>-5</sup> dilutions were employed for sediment samples. Samples in sterile petridishes (15 Psi, 121°C for 20 minutes) were mixed in nutrient agar medium by the pour plate method and the plates were incubated at room temperature (30° ± 2°C) in a bacteriological incubator (BEC model, 1989) for 48 hours. Hundred numbers of different bacterial isolates were selected and were sub cultured in nutrient agar slants and subjected to staining procedures. For generic identification, physiological and biochemical tests (motility, catalase production, nitrate reduction, starch hydrolysis, gelatin liquefaction, H<sub>2</sub>S production, utilization of carbohydrate sources like glucose, sucrose, lactose, mannitol, indole production, Methyl Red-Voges Proskaver test, urea hydrolysis, arginine hydrolysis, casein hydrolysis) were carried out following standard methods of

Bergey's Manual of Systematic Bacteriology (Krieg, 1984; Sneath, 1986).

(Wolen and Miller, 1983; Guo Xianshen *et al.*, 1984; Gijzen *et al.*, 1987).

The bacterial communities isolated were identified up to generic level (Table.1). The generic representations among the heterotrophic bacterial isolates comprised *Methylococcus*, *Pseudomonas*, *Aquaspirillum*, *Bacillus*, *Planococcus*, *Micrococcus*, *Streptococcus*, *Listeria*, *Lactobacillus*, *Sporosarcina* and *Sporolactobacillus*. The dominance of *Bacillus* sp. (Fig.1) along with other gram positive forms like *Planococcus* (Fig.2) and *Micrococcus* in the isolates was conspicuous. The bacterial strains isolated from pond water and sediment indicated diverse occurrence of different bacterial genera. This is typical of the shallow freshwater fish ponds with allochthonous organic enrichment and active sediment-water nutrient interactions (Ram *et al.*, 1982; Qin *et al.*, 1995; Zhang *et al.*, 2002). The occurrence of other genera among the isolates may be attributed to the sources of application of different organic manures

## CONCLUSION

In the present investigation, an attempt was made to identify the bacterial genera associated with the nutrient cycling of both raw and processed organic manured carp culture fish ponds. Eleven bacterial species isolated from water and sediment media from organic manured carp cultured ponds with cow manure and biogas slurry comprised diverse bacterial strains of *Methylococcus*, *Pseudomonas*, *Aquaspirillum*, *Bacillus*, *Planococcus*, *Micrococcus*, *Streptococcus*, *Lactobacillus*, *Listeria*, *Sporosarcina* and *Sporolactobacillus*. *Bacillus* sp. (44.1%) along with other gram-positive forms like *Planococcus* sp. (14.3%) and *Micrococcus* sp. (10.7%) were the important microbial groups and their occurrence may be attributed to allochthonous organic enrichment and active sediment-water nutrient interactions.

**Table 1: Generic representation among bacterial isolates from organic manured fish ponds**

Sl.No.	Genus	Occurrence,%
1	<i>Methylococcus</i>	1.2
2	<i>Pseudomonas</i>	7.1
3	<i>Aquaspirillum</i>	1.2
4	<i>Bacillus</i>	44.1
5	<i>Planococcus</i>	14.3
6	<i>Micrococcus</i>	10.7
7	<i>Streptococcus</i>	7.1
8	<i>Lactobacillus</i>	5.9
9	<i>Listeria</i>	2.4
10	<i>Sporosarcina</i>	3.6
11	<i>Sporolactobacillus</i>	2.4

## REFERENCES

- Ayyappan, S., Rao NGS, Rao GRM, JankiRam K, Purushothaman CS, Saha PK, Pani KC, Muduli HK, Sinha VRP and Tripathi SD. 1990. Production efficiencies of carp culture ponds under different management practices. *J. Aqua. Trop.*, **5**: 67-73.
- Bergey's Manual of Systematic Bacteriology*, 1984. Krieg (Ed.), Vol.1, The Williams and Wilkins Co. Baltimore, USA, 1268pp.
- Bergey's Manual of Systematic Bacteriology*, 1986. Sneath (Ed.), Vol.11, The Williams and Wilkins Co. Baltimore, USA, 1599pp.
- Collins CH and Lyne PM. 1976. *Microbiological Methods*. Butterworth & Co. (Publ.) Ltd., London, 521pp.
- Das Madhumita, Ayyappan S. and Dash Biswajit. 2013. Heterotrophic bacterial populations and dehydrogenase activity in fish ponds under different fertilization practices. *Indian J. of fisheries*, Vol., **60**(3), pp. 87-90.
- Gijzen HJ Lubberding HJ Verhagen FJ Jwart KB and Vogels GD. 1987. Application of rumen microorganisms for an enhanced anaerobic degradation of solid organic waste materials. *Biol. Wastes*, **22**:81.
- Guo Xianshen *et al.* 1984. The Preliminary Studies on the Analysis of Bacterial Types in the Fish Ponds Applied with Animal Manure and their Law of Growth and Death. *Freshwater Fisheries of China*, **1**:31-35.
- Jha PS Barat K and Nayak CR. 2008. Fish production, water quality and bacteriological parameters of Koi carp ponds under live-food and manure based management regimes. *Zoological Research*, **2**:165-173.
- Norris JR and Ribbons DW. 1970. *Methods in Microbiology*, Volume 3A, Academic Press Inc., London, 505pp.
- Qin J Culver DA and Yu N. 1995. Effect of organic fertilizer on heterotrophs and autotrophs, implications for water quality management. *Aquaculture Research*, **26**: 911-920.
- Ram NM Zur O and Avnimelech Y. 1982. Microbial changes occurring at the mud water interface in an experimental fish aquarium. *Aquaculture*, **27**: 63-72.
- Wohlfarth GW and Schroeder GL. 1979. Use of manure in fish farming - a review, *Agricult. Wastes*, **1**: 279-299.
- Wolen MJ and Miller TL. 1983. Interaction of microbial population in cellulose fermentation. *Fed. Proc.*, **42**: 109-113.
- Zhang YC Ronimus RS Turner N Zhang Y and Morgan HW. 2002. Enumeration of thermophilic *Bacillus* species in composts and identification with a random amplification polymorphic DNA (RAPD) protocol. *Syst. Appl. Microbiol.*, **25**: 618-626.
- Zhu Y Yang Y Wan Z Hua D and Mathias JA. 1990. The effect of manure application rate and frequency upon yield in integrated fish ponds. *Aquaculture*, **91**: 233-251. *Indian J. Fish.*, **46**(1): 79-85.

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