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## Cellulose decomposing bacteria in fish ponds of West Bengal

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The study has been made to find out the population density, seasonal variation and spatial distribution of cellulose decomposing bacteria in water and sediments of fish ponds used for polyculture, monoculture and traditional systems of fish farming during a period of two and a half years. It has been found that a number of environmental factors of both water and sediments were responsible for different microbial population size and seasonal changes of cellulose decomposing bacteria in these ponds.

**Key words :** Cellulose decomposing bacteria, population density, fish culture ponds, seasonal variation, environmental factors

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### INTRODUCTION

Microorganisms play a decisive role in nutrient cycle both in water and sediments. They are able to breakdown the natural organic compounds into the simple components. Heterotrophic microorganisms have a significant role in decomposition of organic matter. Cellulose decomposing bacteria are also heterotrophic ones and they are involved in decomposition in aquatic ecosystem. Cellulose is an organic substance embedded in plant cell wall. During decomposition of plant parts in water cellulose is broken down into simple organic matter. Organisms like cellulose decomposing bacteria are predominantly active in decomposition of cellulose. Ostertag (1950) stated that the bacteria are primarily responsible for break down of cellulose in the River Elbe and fungi are not so of importance (Von Brandt, 1953). Depending on the quality and concentration of the organic materials, various environmental factors, different management practices, the density of the microbial population were found to be different (Patel, 1982; Roy, 1984; De, 1993). The present paper describes the occurrence of cellulose decomposing bacteria in water and bottom sediments of fish ponds of West Bengal.

### MATERIALS AND METHODS

Six fish ponds farmed under polyculture, monoculture and traditional systems were selected for present investigation. These ponds are distributed in Kalyani and Naihati fish farm. Two fish farms were separated by a distance of 12 km. The water area and mean depth of these ponds are 0.04 ha to 6.4 ha and 1.0 to 3.6 m respectively.

The polyculture ponds were stocked with three species of Indian major carps (*Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*) and three exotic carps (*Hypophthalmichthys molitrix*, *Ctenopharyngodon idella* and *Cyprinus carpio*) on a ratio of 6:2:3:4:2:3 with a total stocking density of 6000/ha. In monoculture system fingerlings of *Cirrhinus mrigala* were introduced following the same stocking rate as in the polyculture ones. The traditional fish ponds did not follow the modern pisciculture technique. It is rather a conventional system of fish cultivation being practised in majority of rural areas. The traditional fish ponds were stocked largely with three species of Indian major carps without maintaining any specific ratio. A number of stray fishes such as *Puntius ticto*, *Heteropneustes fossilis*, *Clarias batrachus*, *Channa marulius* etc. were also present in these ponds. All the fish ponds with the exception of those managed in the traditional way, where fertilised at monthly intervals with the application of 50 kg ammonium sulphate or 10.5 kg N/ha. or 8 kg P/ha and manured with 2000/ha cattle dung (wef).

The water samples were collected from surface of the pond for microbiological and physicochemical analysis. Water samples were obtained from test pond twice a month at regular intervals for the period of 28 months (from July 1978 to October 1980). The collection procedures were followed according to methods described by Rodina (1972) and Antipchuk (1979).

Samples of surface water from each of six ponds were collected in sterilized glass bottles (125 ml capacity) for enumeration of cellulose decomposing bacterial population and the other in a 500 ml plastic sampling bottles were used for the examination of physicochemical factors of water. The density of bacteria occurring in the water samples was estimated by spread plate technique in triplicates, using nutrient agar (Difco, Bacto) medium. The methods adopted by Antipchuk and Jana (1978a,b) were followed for enumeration of bacteria.

## RESULTS

### *Cellulose Decomposing Bacteria: Water*

The cellulose decomposing bacterial population of water of these experimental ponds regardless of culture were found to be very high and monthly counts exhibited 0.50 to  $25.5 \times 10^3$  cells  $\text{ml}^{-1}$ . The cellulose decomposing bacteria did not differ in density between two farms sites. The statistical treatment of water data (Table 1) was not significant ( $F_{1,2} = 7.10; P > 0.05$ ). On the other hand, the farming management was found to have a strong influence on the distribution pattern of cellulose decomposing bacteria in these ponds ( $F_{2,2} = 28.9; P < 0.05$ ). Thus the bacterial density found in monoculture pond ( $291.1 \times 10^3$  cells  $\text{ml}^{-1}$ ) was significantly higher ( $P < 0.05$ ) than the counts in either traditional ( $235 \times 10^3$  cells  $\text{ml}^{-1}$ ) or polyculture ( $173.47 \times 10^3$  cells  $\text{ml}^{-1}$ ) ponds.

A distinct seasonal periodicity of cellulose decomposing bacteria was found to occur in these fish farming ponds ( $F_{27,54} = 10.27; P < 0.001$ ). Although two peaks were observed in majority of fish ponds, they were widely separated from each other in mono and polyculture ponds of Kalyani while the peaks were closely distributed in the ponds of Naihati. None the less, in Monoculture-2 observed in February ( $13.8 \times 10^3$  cells  $\text{ml}^{-1}$ ) was as large as twice the magnitude of the peak observed in November ( $6.6 \times 10^3$  cells  $\text{ml}^{-1}$ ).

**Table 1.** Analysis of Variance : Cellulose Decomposing Bacteria

| Sources             | df  | MS    |          | Variance Ratio (F) |          | Significance |          |
|---------------------|-----|-------|----------|--------------------|----------|--------------|----------|
|                     |     | Water | Sediment | Water              | Sediment | Water        | Sediment |
| Farm Site(R)        | 1   | 15.20 | 4946.50  | 7.10               | 33.400   | P>0.05       | p<0.05   |
| Culture (C)         | 2   | 61.00 | 46.83    | 29.90              | 0.316    | p<0.05       | P>0.05   |
| Error - A           | 2   | 2.05  | 148.06   |                    |          |              |          |
| Season (T)          | 27  | 34.1  | 794.93   | 9.56               | 17.470   | P<0.001      | P<0.001  |
| Interaction (C X T) | 54  | 4.82  | 52.15    | 1.35               | 1.146    | P>0.05       | P>0.05   |
| Interaction (R X T) | 27  | 18.86 | 897.71   | 5.32               | 19.72    | P<0.001      | P<0.001  |
| Error - B           | 54  | 3.57  | 45.50    |                    |          |              |          |
| Total               | 167 |       |          |                    |          |              |          |

The mean annual curve found in three ponds of Naihati (P-2, M-2 and T-2) and polyculture-1 (Kalyani) were fitted well with sine and cosine model. The test statistics (Table 2) was highly significant ( $r_s > 0.671$ ;  $P < 0.01$ ) in each case.

**Table 2.** Coefficients of Harmonic functions Cellulose Decomposing Bacteria : Water

| Months            | Coefficients   | FISH PONDS |        |        |         |
|-------------------|----------------|------------|--------|--------|---------|
|                   |                | Poly-1     | Poly-2 | Mono-2 | Tradi-1 |
| July              | a <sup>0</sup> | 2.646      | 3.754  | 6.050  | 4.558   |
| August            | a <sup>1</sup> | 1.067      | -1.897 | -3.550 | -1.861  |
| September         | a <sup>2</sup> | 0.106      | 0.006  | 0.708  | -0.073  |
| October           | a <sup>3</sup> | 0.370      | 0.952  | 0.733  | 1.122   |
| November          | a <sup>4</sup> | 0.107      | -0.870 | -1.008 | -1.162  |
| December          | a <sup>5</sup> | -0.637     | -0.405 | -0.182 | 0.014   |
| January           | a <sup>6</sup> | -0.160     | 0.259  | 0.050  | 0.402   |
| February          | b <sup>1</sup> | -0.353     | -0.603 | -1.999 | -1.608  |
| March             | b <sup>2</sup> | 0.078      | 0.045  | 1.159  | 0.825   |
| April             | b <sup>3</sup> | 0.098      | -0.700 | -1.838 | -0.972  |
| May               | b <sup>4</sup> | -0.071     | -0.007 | 0.004  | 0.098   |
| June              | b <sup>5</sup> | -0.289     | -0.079 | -0.556 | 0.003   |
| Spearman's rank   |                | 0.825      | 0.671  | 0.713  | 0.671   |
| Correlation       |                |            |        |        |         |
| Coefficients (rs) |                | P<0.01     | P<0.05 | P<0.01 | P       |

**Moving points of moving averages cellulose Decomposing Bacteria : Water**

|               | FISH PONDS |         |
|---------------|------------|---------|
|               | Mono-1     | Tradi-2 |
| Moving points | 4          | 4       |

The distribution pattern of cellulose decomposing bacteria in these fish ponds was found to be less affected by interactions between fish farming and season ( $F_{54,54} = 1.35$ ;  $P < 0.05$ ), but strongly affected by the interactions between farm sites and season ( $F_{27,54} = 5.32$ ;  $P < 0.001$ ).

#### *Cellulose Decomposing Bacteria : Sediment*

The cellulose decomposing bacteria of these ponds was much higher in numbers ( $0.8$  to  $118 \times 10^3$  cells  $g^{-1}$ ) in sediments than in surface water. The density of cellulose decomposing bacteria differed significantly ( $F_{1,2} = 33.4$ ;  $P < 0.05$ ) from one farm to another with distinctly higher population in Naihati ponds as compared to Kalyani ponds. However, differences in monthly mean of cellulose decomposing bacteria among three cultural ponds were not significantly different from Zoro ( $F_{2,2} = 0.316$ ;  $P > 0.05$ ). The density of bacteria ranged from  $10.8 \times 10^3$  cells  $g^{-1}$  in polyculture system to  $9.0 \times 10^3$  cells  $g^{-1}$  in monoculture type. The seasonal periodicity of cellulose decomposing bacteria was well pronounced ( $F_{27,54} = 17.47$ ;  $P < 0.001$ ) in these ponds (Table 1). The number of peaks in these ponds ranged from one to two. A well defined single peak was found in March in Traditional-2 (Naihati), while the peak in Traditional-1 (Kalyani) was not well defined.

The three point running average (Table 3) was the best model for predicting the seasonal trend of cellulose decomposing bacteria in all these fish ponds with the exception of Monoculture-1 where the seasonal data were predicted by means of polynomial equations of second degree.

**Table 3.** Coefficients of Polynomial Equation Cellulose Decomposing Bacteria : Sediments

| Pond                                                                            | Equation                          | Confidence level |        |         |         |
|---------------------------------------------------------------------------------|-----------------------------------|------------------|--------|---------|---------|
| Mono-1<br>(Kalyani)                                                             | $Y = 6.714 - 1.040 X + 0.097 X^2$ | 95 %             |        |         |         |
| Moving points of the moving averages Cellulose Decomposing Bacteria : Sediments |                                   |                  |        |         |         |
| FISH PONDS                                                                      |                                   |                  |        |         |         |
|                                                                                 | Poly-1                            | Poly-2           | Mono-2 | Tradi-1 | Tradi-2 |
| Moving<br>Points                                                                | 3                                 | 3                | 3      | 3       | 3       |

#### *Physico-chemical examination of water*

The temperature of water in these fish ponds ranged from  $18^{\circ}\text{C}$  in winter to  $34^{\circ}\text{C}$  in summer. The water of these ponds remained always within alkaline range and pH varying between 7.3 and 8.6.

- \* There was not well marked seasonal changes of pH found in these ponds. The values of  $\text{CO}_2$  were relatively higher in the ponds of Naihati ( $14.6 \text{ mg l}^{-1}$ ) as compared to the ponds of Kalyani ( $7.4 \text{ mg l}^{-1}$ ). Monoculture pond ( $12.36 \text{ mg l}^{-1}$ ) showed relatively higher values of  $\text{CO}_2$  as compared to either polyculture ( $8.1 \text{ mg l}^{-1}$ ) or traditional ponds ( $9.5 \text{ mg l}^{-1}$ ). The concentration of bicarbonate alkalinity ranged between  $24 \text{ mg l}^{-1}$  and  $285 \text{ mg l}^{-1}$ . The values of bicarbonate remained higher during the period of July through January but markedly declined in the rest of the year. The total hardness of water ranged from 34 to

266 mg  $\Gamma^{-1}$  in these ponds. The highest concentration of total hardness (266 mg  $\Gamma^{-1}$ ) was found in summer and lower values found in monsoon (July to August). The dissolved oxygen concentration in these ponds ranged from 1.0 mg  $\Gamma^{-1}$  to 19.8 mg  $\Gamma^{-1}$ . On a cultural basis, the polyculture ponds showed higher concentration of oxygen (9.7 mg  $\Gamma^{-1}$ ) than the traditional (8.0 mg  $\Gamma^{-1}$ ) and monoculture (9.1 mg  $\Gamma^{-1}$ ) ponds. There was definite seasonal trend in the values of dissolved oxygen of water in all these fish ponds observed.

The ammonia-nitrogen concentration showed maximum mean values (1.50 mg  $\Gamma^{-1}$ ) in monoculture ponds and this followed by polyculture system (1.25 mg  $\Gamma^{-1}$ ) followed by the traditional (0.54 mg  $\Gamma^{-1}$ ) ones. The values of ammonia-N showed a peak in winter months and when a steady decline was noticed. The nitrite-N concentration of water ranged from 0.19 to 3.80 mg  $\Gamma^{-1}$  in these ponds. The highest value of nitrite-N was found in monoculture (2.50 mg  $\Gamma^{-1}$ ) and lowest one was found in traditional (0.77 mg  $\Gamma^{-1}$ ) ponds. The seasonal trend of nitrite level of nitrogen showed a rising trend in July and reached the peak in winter months (Nov. and Dec.) followed by decline so that lowest values were found in summer.

There was a marked difference in concentration of nitrate-N observed. The value of nitrate-N found in monoculture pond (1.98 mg  $\Gamma^{-1}$ ) was 1.2 and 1.5 times higher than the values found in polyculture (1.61 mg  $\Gamma^{-1}$ ) and traditional ponds (1.3 mg  $\Gamma^{-1}$ ) respectively.

The monthly mean values of phosphate concentration obtained in polyculture ponds (0.15 mg  $\Gamma^{-1}$ ) was more than two times higher than the mean concentration found in monoculture (0.085 mg  $\Gamma^{-1}$ ). The maximum concentration of phosphate was found in winter months and lowest values in April or May.

Amount of sulphate varied between 12 and 49.7 mg  $\Gamma^{-1}$  in these six ponds. The sulphate concentration of polyculture ponds (28.4 mg  $\Gamma^{-1}$ ) showed the highest values as compared to monoculture and traditional type of fish ponds. The highest and lowest values of sulphate were observed in winter and summer months respectively.

The concentration of dissolved organic matter of water ranged from 1.0 to 30 mg  $\Gamma^{-1}$  in these ponds. Among three types of fish farming ponds the highest concentration of organic matter was observed in polyculture pond and the lowest in traditional system. Values of dissolved organic matter were found to be maximum in April and the minimum values were observed in winter.

The organic carbon content of water ranged from 1.6 to 13.5 mg  $\Gamma^{-1}$  in these experimental fish ponds. The highest value of organic carbon concentration was found in polyculture (8.15 mg  $\Gamma^{-1}$ ) and this followed by monoculture (7.5 mg  $\Gamma^{-1}$ ) and traditional (5.25 mg  $\Gamma^{-1}$ ) ponds respectively. In July the highest peak of organic carbon content was observed and lowest value was observed in winter months.

#### *Physico-chemical analysis of sediments*

The hydrogen-ion-concentration of bottom sediments of these six ponds ranged from 6.0 (Traditional-1) to 7.8 (Polyculture-2 and Monoculture-I). There was negligible difference in the mean values of pH between three types of fish farming ponds, ranging from 7.0 in traditional system to 7.3 in polyculture type. Similarly the mean values of pH did not differ between the ponds of two farm sites.

Seasonal fluctuations of pH in sediments of these fish farming ponds were very minor. However, relatively lower values were observed in winter in all the fish ponds.

Free carbonate of bottom sediments varied between 0.8 and 9.6% in six ponds investigated. The monthly mean values were found to differ considerably between two ponds of either polyculture (P-1; 2.9 % and P-2; 4.5%) or monoculture systems (M-1; 3.2% and M-2; 5.6%). The differences between two traditional ponds were minor ranging from 3.9 % in Traditional-2 to 4.9 % in Traditional-1, and on a cultural basis, no difference was found between monoculture (4.4%) and traditional (4.4%) ones. These values differed from the mean values observed in polyculture system (3.7).

The organic carbon of mud samples ranged between 0.5 and 3.9% on a cultural basis. The monthly mean was lowest in traditional system (1.1%), while the highest value (2.05%) was recorded in monoculture pond. The organic carbon content of Kalyani ponds (1.86%) was higher than the Naihati ponds (1.46%).

The total nitrogen content of bottom sediments of these ponds ranged from 0.014% to 0.99%. The monthly mean was distinctly higher in monoculture system (0.07%) as compared to polyculture (0.059%) and traditional (0.03%) ones. The mean values of total nitrogen did not differ much between Kalyani (0.056%) and Naihati (0.052%) farms.

The percentage of available nitrogen of mud sediments of these ponds was much lower than the amount of total nitrogen. The amount of available nitrogen observed in the traditional ponds (0.013%) was more than two times lower than the values observed either in polyculture (0.027%) or monoculture (0.035%) ponds. There was no difference between ponds of Kalyani (0.026%) and Naihati (0.024%).

Carbon:nitrogen ratio was markedly higher in traditional ponds (37.6) as compared to either monoculture (29.3) or polyculture (31.3) ones. The mean value was relatively lower in Naihati farm (31.4) as compared to Kalyani (34.1).

The concentration of phosphate in these fish ponds ranged from  $3.9 \text{ mg l}^{-1}$  to  $30 \text{ mg l}^{-1}$ . Maximum value observed in monoculture system ( $17.3 \text{ mg l}^{-1}$ ) while the minimal in traditional ponds ( $9.6 \text{ mg l}^{-1}$ ).

Nitrogen : phosphate ratio varied from 0.2 to 3.7 on cultural basis and maximum value (2.05) was recorded in monoculture ponds and lowest value (1.45) was found in traditional system.

#### *Regression analysis*

With a view to identify the principal environmental factors responsible for seasonal changes of cellulose decomposing bacteria in the fish ponds regardless of culture, a stepwise multiple regression analysis (STEP R multivariate statistics package) was run in the computer (Burroughs B 6700) using the linear concept between the dependent (Y) and independent variables (X). The analysis was performed using a reduced set of independent variables; the logarithmic transformations ( $\log_{10}$ ) of monthly mean counts of bacteria occurring in these six ponds regardless of culture were used as dependent variables (Y) and 14 possible predictor variables of pond environment (physico-chemical factors) as independent parameters (X). Variables were entered into or removed from the regression

equation by computing a partial F-statistics for each variable at each step and comparing it to a preselected F-value based on the number of observations. The results of the multiple regression analysis (Table 4) of cellulose decomposing bacteria in water was highly significant ( $P < 0.001$ ).

**Table 4** Step wise multiple regression analysis of cellulose decomposing bacteria in water and bottom sediments and significance environmental covariates.  
Total sum of squared based on 166 degrees of freedom.

A. In surface Water

| Step | Variable        | Multiple R | R <sup>2</sup> | $\Delta R^2$ | Over all F |
|------|-----------------|------------|----------------|--------------|------------|
| 1    | DOM             | 0.349      | 0.121          | 0.121        | 22.95      |
| 2    | CON             | 0.426      | 0.181          | 0.060        | 11.97      |
| 3    | NH <sub>4</sub> | 0.472      | 0.222          | 0.162        | 8.57       |
| 4    | DOC             | 0.510      | 0.260          | 0.098        | 8.39       |
| 5    | SO <sub>4</sub> | 0.537      | 0.288          | 0.190        | 6.25       |

$$\text{Equation : Log Cellulose Decomposing Bacteria} = 0.035 (\text{DOM}) + 0.357 (\text{CON}) + 0.192 (\text{NH}_4) - 0.027 (\text{DOC}) - 0.008(\text{SO}_4) + 0.232$$

B. In Bottom sediments

| Step | Variable | Multiple R | R <sup>2</sup> | $\Delta R^2$ | Over all F |
|------|----------|------------|----------------|--------------|------------|
| 1    | CON (BW) | 0.290      | 0.078          | 0.078        | 14.21      |
| 2    | DOM (BW) | 0.398      | 0.158          | 0.080        | 15.59      |

$$\text{Equation : Log Cellulose Decomposing Bacteria} = 0.726 (\text{CON}) + 0.026 (\text{DOM}) + 0.208.$$

## DISCUSSIONS

The growth of microorganisms is affected by different physico-chemical factors as well as other biological factors of the water. Microbial population in the fish farming ponds depend upon several factors such as organic loading, geochemical properties of the ponds and also the fish stocking. The present observation showed the density difference of cellulose decomposing bacteria among both the waters and sediments of these fish farming ponds. The different population structure of cellulose decomposing bacteria in these fish ponds might be explained due to the effect of qualitative and quantitative differences of organic matter resulting from differences in the fish farming management. Rheinheimer (1980) stated that the composition of organic material had a strong influence on the growth of particular bacterial population.

Large number of cellulose decomposing bacteria found in the surface water of polyculture and monoculture ponds were perhaps due to prevalence of specific nutrient condition mediated through the ratio of various combinations of nutrient load for the growth of definitive group of bacteria. It has been observed in this study that large numbers of cellulose decomposing bacteria occurred in bottom sediments than the surface water. Kuznetsov (1968) and Rheinheimer (1980) also found the similar results. This showed that

amount of nutrients was much higher in sediments because it was a principal site of decomposition and this increased nutrients level was conducive to the massive microbial growth. Lack of significant difference in the density of cellulose decomposing bacteria among sediments of three cultural ponds implied that sediments of all fish ponds regardless of culture system perhaps contained adequate amount of nutrients necessary for the massive growth of various groups of bacteria including cellulose decomposers.

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