Effective Utilization of *Azotobacter chroococcum*, *Pseudomonas* and *Gluconacetobacter diazotrophicus* on Fish Growth Status in Freshwater and Inland Saline Water

Nalle Datta A., Deshmukh Abhaysinh R., and Shembekar Vishwas S.
Research Scholar, Department of Zoology and Fishery Science,
Rajarshi Shahu Mahavidyalaya, Latur
Research Scholar, Department of Zoology and Fishery Science,
Rajarshi Shahu Mahavidyalaya, Latur
Professor and Head, Department of Zoology and Fishery Science, Rajarshi Shahu Mahavidyalaya, Latur
Email**:** iprometheous007@gmail.com, d.abhaysinh@gmail.com

Abstract: *In present investigation we had been go through withTwo experiments to evaluate the effect of Azotobacter chroococcum (nitrogen fixer strain, Mac-27 and high temperature tolerant strain, HT-51), Pseudomonas (Phosphate solubliser strain PS-21) and Gluconacetobacterdiazotrophicus (high salinity tolerant strain 35-47) on nutrient status, plankton production and fish biomass in inland saline water fish ponds.Hydrobiological parameters of pond waters, net primary productivity (NPP) and fish growth were studied. Significantly (P<0.05) high values for alkalinity, kjeldahl's nitrogen, NO_3-N, turbidity, pigment concentration and fish growth were observed in ponds inoculated with co-culture, followed by ponds inoculated with Mac- 27 and PS-21. o-PO_4 concentration was significantly (P<0.05) higher both in PS-21 and in co- culture inoculated ponds. In Experiment 2, high temperature tolerant (HT- 51) strain of Azotobacterchroococcumand high salinity tolerant (Gluconacetobacterdiazotrophicus35-47) strainwere used. Alkalinity, hardness, TDS and chlorophyll a concentration were significantly (P<0.05) higher in ponds inoculated with high temperature tolerant mutant of A.chroococcum.Similar experiment was carried out in fresh water fishes on Catlacatla Two experiments were conducted to evaluate the effect of Azotobacter chroococcum (nitrogen fixer strain, Mac-27 and high temperature tolerant strain, HT-51), Pseudomonas (Phosphate solubliser strain PS-21) and Gluconacetobacterdiazotrophicus (high salinity tolerant strain 35-47) on nutrient status, plankton production and fish biomass in fresh water fish ponds.Ponds were stocked with Catlacatla at 100 fish per ponds. Irrespective of the treatment, ponds were fertilized using cow dung at 75 kg per annum. In Experiment 1, ponds were inoculated with nitrogen fixing Azotobacter chroococcum, Mac 27 (Treatment 1), phosphate solubilizing Pseudomonas, PS-21 (Treatment 2) and also with a co- culture of Mac-27 and PS-21 (Treatment 3).*

Hydrobiological parameters of pond waters, net primary productivity (NPP) and fish growth were studied. Significantly (P<0.05) high values for alkalinity, kjeldahl's nitrogen, NO_3-N, turbidity, pigment concentration and fish growth were observed in ponds inoculated with co-culture. In Experiment 2, high temperature tolerant (HT- 51) strain of Azotobacterchroococcumand high salinity tolerant (Gluconacetobacterdiazotrophicus35-47) strainwere used. Alkalinity, hardness, TDS and chlorophyll a concentration was significantly (P<0.05) higher in ponds inoculated with high temperature tolerant mutant of A. chroococcum.
Key Words: *Fish Growth, G. diazotrophicus, A. chroococcum*

Introduction:

To combat pollution and to reduce the excessive use of organic/inorganic fertilizers for sustaining the production system and also for assuring food security, it has become a necessity to apply newer techniques and technologies that can facilitate the culture of aquatic organisms without adversely affecting the pond ecology. The present-day global interest in biological nitrogen fixation is a direct consequence of this necessity to provide some economic assistance to the small and marginal fish farmers and also to reduce pollution. Therefore, attempts have been made to utilize microbial

bioinoculants such as *Azotobacter* which is a free living diazotroph and is reported to occur even in aquaticecosystem (Bhatnagar et al., 2004, Sayeda*et al.*, 2011).

Earlier studies of this laboratory (Garg et al., 1998; 2001; Garg and Bhatnagar, 1999a, b) have revealed that inoculation of *Azotobacter chroococcum* (Mac 27-nitrogen fixer and PS-21 Phosphate solubilizer) in freshwater fish culture ponds enhances pond productivity and fish production. Most of these studies have dealt with the fresh water ecosystem. Since the underground water in many of the Indian states such as Haryana, Punjab, Rajasthan and Gujarat is moderately to highly saline, therefore, there is a need to test the efficacy and suitability of these bioinoculants as biofertilizer in inland saline ground waters. Recently, Narula *et al*. (2005) have reported the development of a high temperature tolerant strain of *A. chroococcumand* high salinity tolerant*Gluconacetobacterdiazotrophicus*strain suitable fortropical and subtropical areas for agriculture crops where the soil is saline.

Keeping in view the importance of these biofertilizers in aquaculture system and increasing salinity of inland saline ground water of this part of India, attempts were made to utilize Nitogen fixing, Phosphate solubilising strains of *A. chroococcum* and high salinity and high temperature tolerant strains of *G. diazotrophicus* in saline water ponds in conjunction with fish culture. The impact of inoculation of *Azotobacter* strain, Mac 27- (Nitrogen fixer), phosphate solubilizer *Pseudomonas* strain PS-21; high salinity tolerant (*Gluconacetobacter* 35-47) strain and high temperature tolerant, HT-51 strain of *Azotobacter* were studied on the physico-chemical characteristics of inland saline water pond waters, their nutrient status, plankton production and fish biomass.

Materials and Methods

Effect of inoculation of Nitrogen fixer A. chroococcum Mac-27 and Phosphate solubilizer PS-21 strains on pond productivity and fish growth in Inland saline groundwater

In this experiment nitrogen fixer (Mac- 27) and phosphate solubilizer (PS-21) strains were used as biofertilizers. Four treatments were maintained, Mac- 27 strain of Azotobacter and PS-21 strain of Pseudomonas were inoculated in treatment 1 and 2 respectively (dosage 1.5 L per 375m^2. In treatment 3 co-culture containing 0.75 L of Mac-27 strain and 0.75 L of PS-21 strain was used. Treatment 4 served as control and no microbial biofertilizer was inoculated. Two weeks after the application of the first dose of fertilizer, milkfish, *Chanoschanos* fingerlings (mean body wt. 43.36±0.12g and mean body length 18.51±0.04 cm) were stocked during September. No supplementary feed was given to the fish during the experimental period. Two replicates of each treatment were maintained.

Analysis:

Water samples for physico-chemical and biological characteristics were analysed following APHA (1998). Dissolved oxygen (DO), pH, conductivity were measured with a portable kit (F- set 3, E-Merck Ltd., Germany). At the end of the experimental period (70 days for experiment 1 and 60 days for experiment 2), ponds were completely drained and fish were harvested and counted. Length (cm) and weight (g) of the individual fish was recorded. Carcass composition of fish (for experiment 1) on according to AOAC (1995).

Statistical Analysis:

The coefficient of correlation between different parameters and multiple regression between independent and dependent variables was determined by computer. One way ANOVA, followed by Tukey's studentized range (HSD) test was used to compare the group means (Gomez and Gomez, 1984).

Results:

Effect of inoculation of nitrogen fixer A. chroococcum- Mac-27 and phosphate solubilizer - PS-21 strains on pond productivity and fish growth in inland saline groundwater.

Viable Counts (Colony Forming Units)

High values of total viable counts were observed in ponds inoculated with nitrogen fixing strain (Mac-27), followed by ponds inoculated with phosphate solubilizer strain (PS -21). Bacterial counts remained low in ponds where a co-culture (Mac-27 and PS-21) was inoculated. Significantly ($P<0.05$) low bacterial counts were seen in control ponds. Observations have further shown that irrespective of the strain of *Azotobacter* inoculated, a significant ($P<0.05$) increase in viable counts was observed up to day 7 following inoculation and thereafter a significant ($P<0.05$) decline in viable counts on day 14 was observed (Figure 1).

Hydrobiological Characteristics

Electrical conductivity remained high in microbial inoculated ponds. pH was alkaline in

all the treatments and fluctuated between 8.27-8.38. Weekly variations in DO concentration (Figure 2) showed no significant differences with respect to microbial inoculation; however, the values remained higher in ponds inoculated with mixed culture. Total alkalinity, bicarbonates and turbidity remained high, while carbonates were low in ponds inoculated with mixed culture (Mac-27 + PS-21), followed by PS-21 and Mac-27 alone. No significant effect of microbial inoculation was observed on chlorides, calcium, magnesium, total hardness and sulphates and the values remained high in all the treatments (Table 1).

In general, release of nutrients viz. total Kjeldahl nitrogen (Figure 3), NO_3-N, and NH_4-were high in ponds inoculated with mixed culture (Mac-27 + PS-21), followed by ponds inoculated with Mac-27 and PS-21 alone (Table 1). On the other hand, o-PO_4 release was high in ponds inoculated with PS-21 (Figure 4). No significant variations in NO_2-N levels were observed among different treatments.

Primary productivity, pigment concentrations and biotic community: Followinginoculation with microbial culture, *a* significant ($P<0.05$) increase in NPP (Figure 5), GPP (mg C l^{-1} d^{-1}), chlorophyll *a*, pheophytin *a* (µg l^{-1}) and phytoplankton population was observed in all the treatments in comparison to controls (Table 1). High values in most of these parameters, however, were observed in ponds inoculated with co-culture. No significant ($P<0.05$) variations in plankton (phytoplankton and zooplankton) population (nos. l^{-1}) and species diversity (d) were observed in microbial inoculated ponds (Table 1).

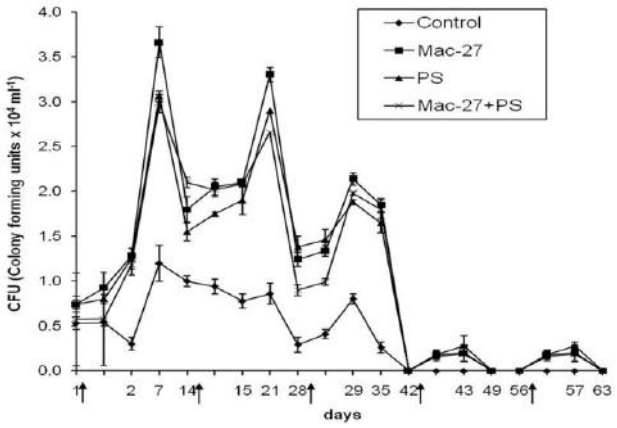

Figure1. Variations in viable counts (CFU) in fish culture ponds inoculated with *Azotobacterchroococcum*strains (Mac 27), *Pseudomonas* (PS-21) and in control ponds (Experiment 1). Inoculationday is indicated by arrows **(→)**.

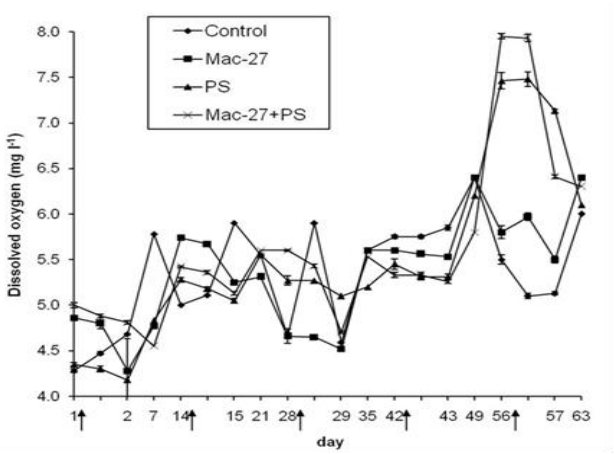

Figure 2. Effect of inoculation Mac 27, PS-21 and co-culture (Mac 27+ PS 21) on dissolved oxygen (DO) concentration in fish ponds. (Experiment 1).

Irrespective of the treatments, plankton communities principally consisted of two groups of phytoplankton (Bacillariophyceae and Chlorophyceae), and one group of zooplankton (copepoda). Phytoplankton were represented by 8 genera, 4 belonging to Bacillariophyceae and 4 to Chlorophyceae. While zooplankton was represented by copepoda (i.e. only *Cyclops*) alone. Among

Bacillariophyceae *Synedra* and *Navicula*, while among Chlorophyceae *Closterium* formed the stable community.

Table 1. Effect of inoculation of *Azotobacter* strain Mac-27 and PS-21 on physico-chemical characteristics of pond water stocked with *Chanoschanos* (70-day treatment) (Experiment 1)

Parameters	Treatments			
	Mac-27	PS	Mac-27+PS	Control
EC µ mhos cm^{-1}	16.48 ± 0.09^{A}	16.49 ± 0.09^{A}	16.73 ± 0.13^{A}	15.98 ± 0.05^{B}
pH	8.27 ± 0.01^{B}	8.32 ± 0.21^{B}	8.38 ± 0.01^{A}	8.29 ± 0.02^{B}
Dissolved oxygen mg l^{-1}	5.76 ± 0.09^{B}	5.89 ± 0.13^{B}	6.44 ± 0.12^{A}	5.34 ± 0.08^{C}
Carbonates mg l^{-1}	8.46 ± 0.38^{A}	8.32 ± 0.37^{A}	6.61 ± 0.28^{B}	7.86 ± 0.55^{A}
Bicarbonates mg l^{-1}	203.43 ± 2.24^{BC}	208.79 ± 2.30^{B}	217.41 ± 2.28^{A}	199.14 ± 2.14^{C}
Total alkalinity mg l^{-1}	211.93 ± 2.04^{C}	217.04 ± 2.15^{B}	223.94 ± 2.35^{A}	207.00 ± 1.80^{C}
Chlorides mg l^{-1}	6859.49 ± 66.60^{A}	6918.07 ± 61.11^{A}	6938.49 ± 50.38^{A}	6886.11 ± 76.20^{A}
Total hardness mg l^{-1}	3425.00 ± 40.15^{A}	3414.29 ± 45.11^{A}	3464.29 ± 37.23^{A}	3430.36 ± 34.28^{A}
Calcium mg l^{-1}	518.90 ± 12.28^{AB}	504.64 ± 13.25^{B}	521.16 ± 11.12^{AB}	542.18 ± 7.78^{A}
Magnesium mg l^{-1}	520.05 ± 9.04^{A}	527.40 ± 10.37^{A}	528.24 ± 9.33^{A}	507.71 ± 7.77^{A}
Total Kjeldahl nitrogen mg l^{-1}	7.13 ± 0.30^{A}	5.73 ± 0.27^{B}	7.80 ± 0.37^{A}	4.74 ± 0.24^{C}
NO_3-N mg l^{-1}	1.38 ± 0.04^{B}	1.13 ± 0.03^{C}	1.52 ± 0.03^{A}	1.05 ± 0.03^{C}
NO_2-N mg l^{-1}	0.75 ± 0.02^{B}	0.80 ± 0.02^{B}	0.68 ± 0.0^{C}	0.88 ± 0.02^{A}
NH_4-N mg l^{-1}	1.26 ± 0.03^{A}	1.08 ± 0.03^{B}	1.25 ± 0.03^{A}	0.94 ± 0.03^{C}
o-PO_4mg l^{-1}	0.19 ± 0.01^{B}	0.26 ± 0.01^{A}	0.24 ± 0.01^{A}	0.13 ± 0.01^{C}
SO_4 mg l^{-1}	68.68 ± 1.39^{B}	71.16 ± 1.54^{B}	71.44 ± 1.47^{B}	83.01 ± 1.17^{A}
Turbidity NTU	69.42 ± 1.86^{B}	68.61 ± 1.43^{B}	82.73 ± 1.49^{A}	55.61 ± 0.82^{C}
NPP mg C l^{-1} d^{-1}	0.70 ± 0.05^{BC}	0.79 ± 0.03^{B}	1.11 ± 0.06^{A}	0.61 ± 0.02^{C}
GPP mg C l^{-1} d^{-1}	2.20 ± 0.04^{C}	2.33 ± 0.04^{B}	2.86 ± 0.05^{A}	2.00 ± 0.03^{D}
Chlorophyll 'a' µg cm^{-2}	1.97 ± 0.04^{B}	1.88 ± 0.04^{B}	2.43 ± 0.05^{A}	1.72 ± 0.03^{C}
Pheophytin 'a' µg cm^{-2}	0.75 ± 0.06^{B}	0.73 ± 0.05^{B}	1.26 ± 0.06^{A}	0.64 ± 0.64^{B}
Phytoplankton nos l^{-1}	15975.00 ± 1172.00^{A}	15050.00 ± 944.00^{A}	16150.00 ± 838.00^{A}	11925.00 ± 752.00^{B}
Zooplankton Nos. l^{-1}	750.00 ± 153.00^{A}	425.00 ± 90.00^{AB}	625.00 ± 124.00^{AB}	325.00 ± 65.00^{B}
Phytoplankton (d)	2.22 ± 0.08^{A}	2.43 ± 0.07^{A}	2.37 ± 0.07^{A}	2.30 ± 0.07^{B}

All values are mean±SE of mean. Means with the same letter/s in the same row are not significantly (P<0.05) different. Water temperature during the experimental period dropped from 29.4 to 16. 3°C. Mac-27= Nitrogen fixing strain, PS-21 = Phosphate solubilizing strain,
Mac-27 + PS-21 = Co-culture of both strains. Control = No biofertilizer.

Fish Growth

No disease was detected during the entire period of investigations. Survival remained high in all the treatments. ANOVA revealed a significant (P<0.05) increase in growth performance of milkfish (in terms of mean live weight gain, SGR, growth per day and fish biomass) in all the treatments in comparison with controls (Table 2). High weight gain and biomass was observed in ponds inoculated with co-culture (Mac-27+PS-21), followed by ponds inoculated with Mac-27 and PS-21 strain alone. A study of length weight relationship (LWR) at the end of the experimental period of 70 days revealed that the values of exponential 'n' of LWR was high (2.77) for the fish stocked in ponds inoculated with co-culture (Mac-27+PS-21). In other treatments, the value of 'n' fluctuated between 2.00-2.6 (Table 2).

Carcass composition also appears to be significantly affected in different treatments as accumulation of protein, fat and energy were significantly higher in fish (P<0.05) grown in ponds inoculated with co-culture (Mac 27+PS-21) in comparison with other treatments (Table 2).

Discussion:

Since *Azotobacter* is a highly aerobic organism (Lakshminaryana, 1993), therefore inoculation of *Azotobacter* in freshwater fish ponds resulted a decrease in dissolved oxygen (DO) concentration (Garg *et al.*, 1998; Garg and Bhatnagar, 1999, 2002). No drastic reduction in DO concentration was observed in the present studies, which may be attributed to the comparatively large surface area (375 m^2) of the ponds used in the present studies. The pH values and alkalinity indicate that the pond waters were well buffered and thus remained suitable for the release of nutrients in optimum concentrations required for growth and survival of biotic communities.

Significantly high (P<0.05) values of alkalinity, total Kjedahl nitrogen, NO_3-N, NO_2-N, NH_4-N and o-PO_4 in microbial inoculated ponds indicate that ponds were in high trophic status. Statistically significant positive correlation of DO with fish weight gain (r=0.25, P<0.001; r=0.52, P<0.01), NPP (r=0.20, P<0.05; r=0.25, P<0.01) and GPP (r=0.33, P<0.01; r=0.41, P<0.001) was observed both in Experiment 1 and in Experiment 2, respectively. This may indicate that DO was at optimal levels which favoured high pond productivity/fish growth.

The rate of nitrogen fixation in terms of total Kjeldahl nitrogen, NO_3 -N and NH_4-N remained significantly (P<0.05) higher in ponds inoculated with co-culture in comparison with other treatments and control ponds in Experiment 1. Total Kjeldahl nitrogen showed a significant (P<0.05) and positive correlation with NO_3-N (r=0.60, P<0.001; r=0.40, P<0.001), NH_4-N (r=0.35, P<0.001; r=0.56, P<0.0001), phytoplankton population (r=0.82, P<0.001; r=0.61, P<0.0001), chlorophyll *a*

(r=0.34, P<0.001; r=0.27, P<0.01), NPP (r=0.18, P<0.05; r=0.21, P<0.05) and GPP (r=0.13, P<0.05; r=0.18, P<0.05) both in Experiment 1 and in Experiment 2.

Table 2. Effect of inoculation of *Azotobacter* strain Mac-27 and *Pseudomonas* PS-21 on growth performance and carcass composition of *Chanoschanos* (Experiment 1)

Treat-ments	INITIAL FISH STOCK			FINAL FISH STOCK (after 70 days)			Increase in mean fish wt. (g) (Mean length cm)	Growth d^{-1}	Cf (k)	LWR
	Stocking density/ 375m²	Total Biomass (kg)	Mean fish weight (g) (Length cm.)	Survival (%)	Total Biomass (kg)	Mean fish wt. (g) (Length cm.)				
Mac-27	200	8.62	43.12 ± 0.51^{A} (18.54 ± 0.11)	96.75	17.63^{B}	91.13 ± 2.15^{B} (23.31 ± 0.22)	48.01^{B} (4.77)	0.69^{B}	0.71^{B}	$W=0.000023L^{2.6}$ Log W= -4.63+ 2.6 Log L
PS	200	8.75	43.74 ± 0.54^{A} (18.60 ± 0.11)	95.00	13.41^{C}	70.56 ± 0.87^{C} (21.41 ± 0.10)	26.82^{C} (2.81)	0.38^{C}	0.72^{B}	$W=0.00005\times L^{2.33}$ Log W=-4.29+ 2.33 Log L
Mac-27+ PS-21	200	8.64	43.20 ± 0.54^{A} (18.49 ± 0.12)	94.0	20.25^{A}	107.13 ± 4.19^{A} (24.13 ± 0.35)	63.93^{A} (5.64)	0.91^{A}	0.74^{A}	$W=0.000021\times L^{2.77}$ Log W=-4.81+ 2.77 Log L
Control	200	8.67	43.39 ± 0.54^{A} (18.39 ± 0.09)	93.50	10.73^{D}	57.39 ± 0.94^{D} (20.64 ± 0.17)	14.00^{D} (2.25)	0.20^{D}	0.65^{C}	$W=0.0003\times L^{2}$ Log W=-3.88+

Carcass Composition

Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Phosphorus (%)	Energy (kJ g^{-1})
Initial	71.00 ± 0.17^{A}	17.00 ± 0.09^{D}	3.12 ± 0.12^{C}	3.84 ± 0.04^{A}	0.44 ± 0.02^{C}	6.12 ± 0.07^{C}
Mac-27	68.20 ± 0.18^{B}	18.71 ± 0.10^{B}	3.82 ± 0.11^{B}	3.34 ± 0.14^{A}	0.64 ± 0.01^{BC}	6.95 ± 0.09^{B}
PS	68.40 ± 0.13^{B}	18.44 ± 0.12^{B}	3.78 ± 0.11^{B}	3.47 ± 0.16^{B}	0.62 ± 0.01^{B}	6.87 ± 0.06^{BC}
Mac-27 + PS-21	67.65 ± 0.48^{C}	19.10 ± 0.10^{A}	4.15 ± 0.07^{A}	3.01 ± 0.01^{C}	0.67 ± 0.01^{A}	7.20 ± 0.02^{A}
Control	68.48 ± 0.24^{B}	17.94 ± 0.10^{C}	3.46 ± 0.19^{C}	3.51 ± 0.18^{B}	0.55 ± 0.01^{BC}	6.74 ± 0.07^{BC}

Means with the same letter/s in the same column are not significantly (P<0.05) different. Mac-27=Nitrogen fixing strain, PS-21 = Phosphate solubilizing strain, Mac-27 + PS-21 = Co-culture of both strains. Control = No biofertilizer. Condition factor (k) = Wt × $10^5/L^3$, where Wt is weight in grams and L=total length in milimeters. Length weight relationship (LWR): $W=cL^n$ or log W=log c+n log L, Where, W=Weight in kg, C=Constant, n=exponential value of length and L=length of fish in cm

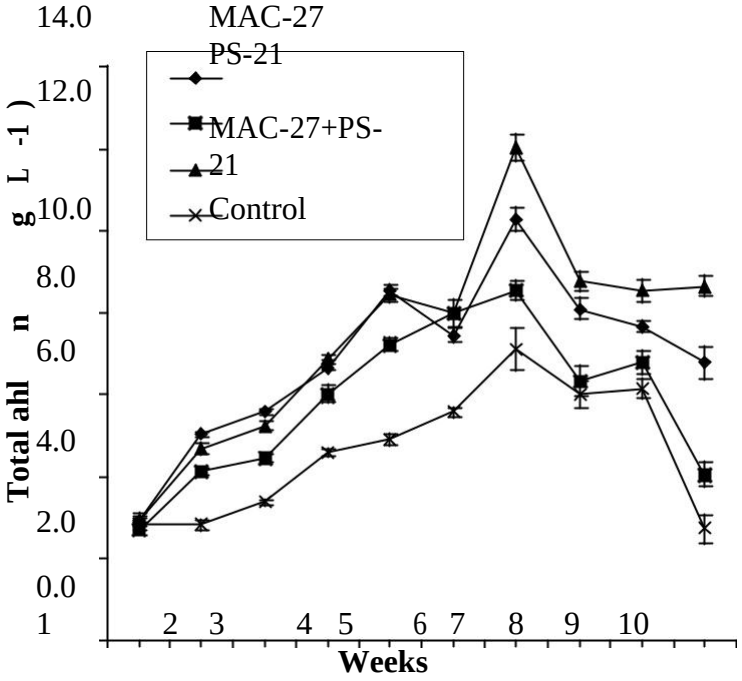

Figure 3. Weekly variations in total Kjeldahl nitrogen concentration in fish culture ponds inoculated with Mac 27, PS-21 and co-culture (Mac 27 + PS 21) and in control ponds (Experiment 1).

These results have clearly revealed that nitrogen fixation, its conversion and utilization is a continuous process. In experiment 2 rate of nitrogen fixation in terms of total Kjeldahl nitrogen, NH_4-N and NO_3 -N were higher in ponds inoculated with salinity tolerant strain indicating that this strain can also be used as biofertilizer in inland saline groundwater ponds. DO (Gnaf and Horne, 1975) and ammonia ($N\text{-}NH_3$, $N\text{-}NH_4$) (McFarland and Toetz, 1988) are considered to be the strong inhibitors of nitrogen fixation, however, biological nitrogen fixation process in different treatments was not affected. According to Pearl (1985), calm conditions in the water allow the creation of anoxic microzone around the cells, which may be considered conducive to nitrogen fixation. Even though NH_4-N levels were higher in different treatments, the rate of nitrogen fixation was not affected, which can be attributed to the lower effect of ammonia in water than in sediment (Howarth et al., 1988).

Nitrogenase activity appears to be closely related to phosphorus availability and phosphorus was not a limiting factor in any of the treatment thus allowing the biological nitrogen fixation process to proceed uninterrupted.

Bacterial viable counts showed an increase upto day 7 following inoculation and thereafter a significant ($P<0.05$) decline in viable counts on day 14 was observed in both the experiments. Viable counts varied with respect to the microbial strain used in the experiments (Figure 1, Table 2 and 6), however, the rate of nitrogen fixation was not affected in relation to multiplication rate of inoculated bacteria. These results suggest that growth rate and nitrogen fixing efficiency of *Azotobacter* need not to have a direct relationship. Ninawe and Paulraj (2003) have also reported that nitrogen fixing efficiency is independent of multiplication rate of bacterial strains. Even though, viable counts were higher, however, the rate of nitrogen fixation by high salinity/temperature tolerant strains remained low in comparison to the nitrogen fixing strains used in experiment 1. Low rate of nitrogen fixation may either be attributed to the high salinity of the medium or to the high-water temperature.

A significant and positive correlation of NO_3-N with phytoplankton ($r=0.62$, $P<0.01$ and 0.48, $P<0.005$) and o-PO_4 with chlorophyll *a* ($r=0.29$, $P<0.001$), NPP ($r=0.23$, $P<0.001$) and GPP ($r=0.29$, $P<0.001$) further indicate that nutrients increase natural fertility of the water body on which primary productivity depends (Delince, 1992).

High fish growth observed in the present studies also coincided with high plankton population and nutrients. Statistically also fish weight gain showed a positive correlation with alkalinity ($r=0.19$, $P<0.05$), NO_3–N ($r=0.46$, $P<0.001$, $r=0.92$ $P<0.0001$), total Kjeldahl nitrogen ($r=0.34$, $P<0.001$, $r=0.72$, $P<0.001$), o-PO_4 ($r=0.15$, $P<0.05$), phytoplankton population ($r=0.19$, $P<0.05$, $r=0.78$, $P<0.001$), zooplankton population ($r=0.17$, $P<0.05$), chlorophyll *a* ($r=0.38$, $P<0.001$), NPP ($r=0.26$, $P<0.001$) and GPP ($r=0.37$, $P<0.001$) clearly revealing that fish growth is positively correlated with the trophic status of the ponds also. Many other studies (Knud-Hansen and Batterson, 1994; Garg and Bhatnagar, 1999b; 2003; Garg et al., 1994, 1998, 2001; Sayeda et al., 2012) have also reported that fish growth is significantly correlated with the trophic status of pond waters.

Although values of constant 'n' (LWR) for *Chanoschanos* were higher in microbial inoculated ponds in comparison to controls, however, the values were <3, indicating that *Chanoschanos*grown in inland saline groundwater (13.0-13.5 ppt) do not follow the cubelaw. Thus, more studies are warranted to investigate the reasons for the deviation of growth patterns from the cube law.

Conclusion:

Use of microbial bioinoculants in inland saline groundwater ponds for the culture of euryhaline fish species such as milkfish, *Chanoschanos* and mullet, *Mugil cephalus* will help in the development of an eco-friendly, economically viable and sustainable aquaculture technology. Use of high temperature tolerant (HT-51) strain of *Azotobacter chroococcum* and high salinity tolerant, (*Gluconacetobacterdiazotrophicus* 35-47) strain along with nitrogen fixer and phosphate solubilizer strain of Azotobacter would be advantageous in inland saline groundwater ponds for reducing the use of inorganic fertilizers and thereby preventing the

References:

1. AOAC Association of Official Analytical Chemists (1995). Official methods of analysis. *Assoc. Off. Anal. Chem. Inc*. Arlington, USA, p 684.
2. APHA (1998). *Standard methods for the examination of water and waste water*. 20thedn. American Public Health Association New York.
3. Bhatnagar, A., Garg, S.K., Kumar, V. (2004). Total bacterial and free-living nitrogen fixing microflora in lentic and lotic habitat. *Proceeding of the National Workshop on rationaluse of water resources for aquaculture,* March 18-19, CCS Haryana AgriculturalUniversity, Hisar, India pp 86-94.
4. Cavalcante, V.A., Dobereiner, J. (1998). A new acid tolerant fixing bacterium associated with sugarcane. *Plant Soil*, 108, 23-31.
5. Delince, G. (1992). The ecology of the fish pond ecosystem with special reference to Africa. Kluwer Academic Publishers, p 230.
6. Edwards, P., Pacharaprakiti, C., Yomjinda, M. (1994). An assessment of the role of buffalo manure for pond culture of tilapia. I. On station experiment. *Aquaculture*, 21 , 261-279.
7. Ganf, G.G., Horne, A.J. (1975). Diurnal stratification, photosynthesis and nitrogen-fixation in a shallow equatorial Lake (Lake George, Uganda). *Freshwater Biology*, 5, 13-59.

8. Garg, S.K., Bhatnagar, A. (1996). Effect of varying doses of organic and inorganic fertilizers on plankton production and fish biomass in brackish water in fish ponds. *AquacultureResearch*, 27, 157-166.
9. Garg, S.K., Bhatnagar A. (1999a). Effect of Azosprillium and Azotobacter inoculation aon pond productivity and fish growth under fresh water conditions. *Indian Journal ofMicrobiology*, 39, 227-233.
10. Garg, S.K., Bhatnagar, A. (1999b). Azotobacter in aquatic system. In: Narula, N. (ed.). *Azotobacter in sustainable Agriculture*. CBS Publishers, New Delhi, pp 148-160.
11. Garg, S.K., Bhatnagar, A. (2002). Determination of dosage of *Azotobacter* and organic fertilizer for optimum nutrient release, net primary productivity and fish growth in freshwater fish ponds. *Aquaculture International*, 10, 87-107.
12. Garg, S.K., Bhatnagar, A. (2005). Use of microbial biofertilizers for sustainable aquaculture/fish culture. In: *Microbial Biotechnology in Agriculture and Aquaculture* (Vol. I). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. p 261-297.
13. Garg, S.K., Bhatnagar, A., Kalla, A., Narula N. (2001). *In vitro* nitrogen fixation, phosphate solubilization, survival and nutrient release by Azotobacter strains in an aquatic system. *Bioresource Technology*, 80, 101-109.
14. Garg, S.K., Bhatnagar, A., Narula, N. (1998). Application of Azotobacter enhances pond productivity and fish biomass in still water ponds. *Aquaculture International*, 6, 219-231.
15. Gomez, K.A., Gomez, A.A. (1984). *Statistical procedures for agricultural research*. Second ed. Willey, New York. Howarth, R.W., Marino R., Cole, J.J. (1998). Nitrogen fixation in freshwater, estuaries and marine ecosystems.2. Biogeochemical controls. *Limnology and Oceanography*, 33, 688-701.
16. Jensen, V. (1951). Notes on the biology of *Azotobacter*. *Proceedings of the Society of AppliedBacteriology*, 74, 89-93. King, E.O., Ward, M.K., Rancy, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine*, 44, 301-307.
17. Knud-Hansen, C.F., Batterson, T.R. (1994). Effect of fertilization frequency on the production of Nile Tilapia (Oreochromis niloticus). *Aquaculture*, 123, 271-280.
18. Lakshminarayana, K. (1993). Influence of Azotobacter on nitrogen nutrition of plants and crop productivity. *Proceedings of Indian National Science Academy*, 59, 303-308.
19. McFarland, M.A., Toetz, D.W. (1988). Nitrogen fixation (acetylene reduction) in Lake Hefner, Oklahoma. *Achives of Hydrobiology*, 114, 213-230.
20. Narula N, 2000. Azotobacter as an organism. In: *Azotobacter in sustainable agriculture* (Narula, N. ed.). CBS Publisher, New Delhi, pp 1-12.
21. Narula, N., Saharan, B.S., Kumar, V., Bhatia, R., Bishnoi, L.K., Lather, B.P.S., Lakshminarayan, G.K. (2005). Impact of the use of biofertilizers on cotton crop under irrigated agroecosystem. *Archives of Agronomy and Soil Science*, 51, 69-77.
22. Ninawe, A.S., Paul Raj, R. (2003). Effect of salinity on the growth and nitrogen fixation of *Azotobacter beijerinckii*. *J. Aquaculture*, 11, 7-17.
23. Pearl, H.W. (1985). Microzone formation: its role in the enhancement of aquatic N_2 fixation. *Limnology and Oceanography*, 30, 1246-1252.
24. Sayeda, M.A., Mohamed, I.A. Wafa and Abbas, W.T. (2011) Evaluation of *Azotobacter* and *Azospirillum*Biofertilizers as a Probiotics in *Oreochromis niloticus*aquaculture. *Journal of Fisheries and Aquatic Sciences.* Vol.6 (5), 534-544.
25. Van Rijn, J., Shilo, M. (1989). Environmental factors in fish culture system, in: M. Shilo and S. Sarig (Eds). *Fish culture in warm water system, problems and trends*. CRC Press: Boca Raton, FL, 163-177.
26. Wohlfarth, G.W., Schroeder, G.L. (1979). Use of manure in fish farming- a review. *Agricultural Wastes*, 1, 279-299.