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Isolation, characterization and evaluation of growth of salt tolerant mangrove microbes grown under NaCl stress

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An attempt Was made to evaluate the salt tolerance behaviour of *Aspergillus* sp. and *Bacillus* sp. isolated on Brain heart infusion agar from muddy water of Bhitarkanika mangrove ecosystem by growing them under different salt concentration in broth medium . It was observed that fungus could tolerate salt stress upto 16% where as bacteria could be able to grow upto 11 %. The *Aspergillus* sp. showed increase in growth with the increasing concentration of NaCl where as bacteria declined its growth in this condition. It was observed that acidic metabolites was produced by fungus, therefore, its pH was decreased with the increasing level of NaCl . Simultaneously, bacteria did not show any significant changes in pH of filtrate of different saline treated cultures. The protein content in the culture filtrate of fungus increased in higher concentration of salt . However, bacteria did not behave in the same manner rather showed negative effect of salinity on growth. The present attempt made to analyze the salt stress in fungus and bacteria was of primary nature that provided us various clues to work further.

Key words : Mangrove, Saline, Fungi, Bacteria, Salt tolerant , NaCl, Halotolerant

INTRODUCTION

It is well known that many bacteria and other microorganisms can proliferate in the presence of high concentrations of sodium chloride, provided that conditions are other wise favorable (Su and Lee, 2001; Turcoe *et al*., 1999) . For many microorganisms sodium chloride is an indispensable nutrient; some grow best in a medium that is almost saturated with sodium chloride. It has long been known that various types of bacteria occur in the great salt lake the dead sea , and other natural waters of extremely high salinity (Goyal and Kaushik ,2002). In habitats of lesser salinity as, for instance, in the sea and in marine mud, a rich and varied population of microbes can be found (Yadav and Yadav , 2003, Sengupta and Chadhury, 1995 and Purkaystha and Pal, 1998, Schmit and Shearer, 2004)). Many of the microbes indigenous to such habitats are halophilic and the degree of their halophilism directly related to the salt concentration of their environment. Mangrove ecosystems are one of the important saline habitat accommodating various groups of organisms. Thus study is based on the salt tolerant isolates obtained from such habitat.

MATERIALS AND METHODS

Source of Organisms :

The fungus and Bacteria were isolated from the water samples collected from different locations of creek and vegetations of Khola region of Bhitarkanika mangrove ecosystem, Orissa.

Isolation and characterization :

The test organisms were isolated on brain heart infusion agar (Hi.Media) being used for the isolation of salt tolerant organisms. The bacteria was characterized for its morphology and biochemical behaviour. The fungus was identified according to morphological norms.

Experimental set up for salt tolerance :

Twenty one different concentrations of sodium chloride i.e. 0 to 20 % was taken into consideration along with the other factors remaining constant. Potato dextrose broth medium added with different concentration of NaCl was prepared and final pH 6.0 was maintained (Narsen and Patel, 2000). The fungus was inoculated by 5 mm disc cut into the previous fresh culture into the individual sets of NaCl added medium in triplicate. Finally after 15 days of incubation at 30°C, fresh weight biomass and pH of the culture filtrate were measured.

The bacteria was inoculated in the same experimental set separately at the rate of $1x10^2$ CFU in 25 ml potato dextrose broth and incubated at 37 C. The 10^{-3} diluted inoculum of 96 hrs. old bacterial cultures were used for analysis of population count on potato dextrose agar medium .The total protein was estimated by Lowry Method (1951).

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RESULTS AND DISCUSSION

The salt tolerant fungi obtained from mangrove area was identified as *Aspergillus* sp. The salt tolerant bacteria studied was gram positive rods and fermentative. The isolate was producing acid from sucrose, dextrose and manitol while it was not utilizing lactose and glycerol. It was catalase positive and gave growth under medium of 9.6 pH. This bacteria was found to be VP positive. The effect of NaCl on growth of fungi and bacteria was studied in batch culture under different concentration of salt. The results obtained are as follows.

Effect of NaCl on fungus :

Effect on growth :

The fungus showed growth upto 16% of NaCl concentration (Fig.1). The maximum growth in terms of biomass (g) was recorded in 7 and 8 % NaCl treatment. However, there was reduction in growth above 9% and fungus could not grow on more than 16% NaCl treatment. This observation of increase in growth with the increasing amount of NaCl into the medium is corroborated with studies done by Narisen and Patel (1997) where adverse

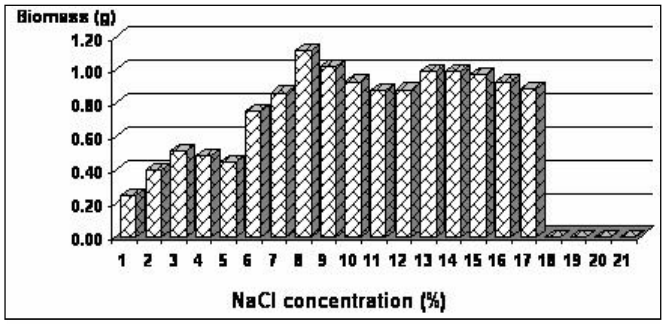

Fig. 1 Pattern of growth of fungi grown in potato dextrose broth under NaCl concentrations

effects of high concentration of NaCl was mentioned. Similar observations have been made by Betty *et al.* (1971) where they had concluded the NaCl and glycerol as one of the important factor for the growth of salt tolerant microbes. There was morphological changes also occurred due to high salinity. Spore shrinkage was observed after 6% of NaCl treatment where as in higher concentration from 12 to 16 % spores were found to be highly irregular and accumulative nature.

Effect on pH :

In connection with this study, pH of the culture filtrate was also measured (Fig2). It was noteworthy that from 0 to 5 % NaCl treatment, pH was of alkaline range and then after started declining upto 4-5. Exceptionally, at 6% NaCl treatment, the culture filtrate showed 3.55 pH. Overall, in higher concentration of NaCl, pH was declining gradually. The production of acidic metabolites in

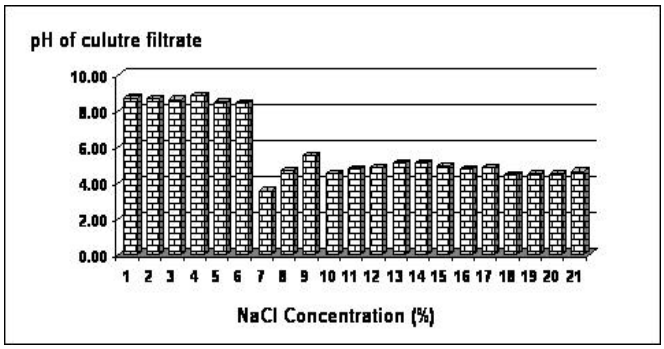

Fig. 2 Pattern of pH of culutre filtrate of fungi grown in potato dextrose broth under NaCl concentrations

extracellular condition may be the one of the important factor used by this fungus for cope up with the salt stress at high level.

Effect on protein content :

The analysis of extracellular protein in the culture filtrate of fungus treated with different concentrations of salt have shown gradual increase in protein content (Fig. 3). It was increased upto 11% and then started decline. At this concentration the total protein content was 1.94 mg/ml/mg culture. The enhancement in protein synthesis at higher concentration in fungus was indicative of synthesis of salt specific proteins and their absence at more high salt stress may be due to inhibition of particular group at higher salinity levels (Apte and Bhagwat, 1989). Similarly, Su and Lee (2001) studied the saline tolerant extracellular protease that was produced by *Aspergillus* sp. FC 10 upto 18% NaCl concentration. Our results are very contradictory with the findings of Goyal and Kaushik (2002) who stated the decrease in protein synthesis with the increase in salt concentration. Therefore, our findings may require further investigations to clarify the differences and deviations.

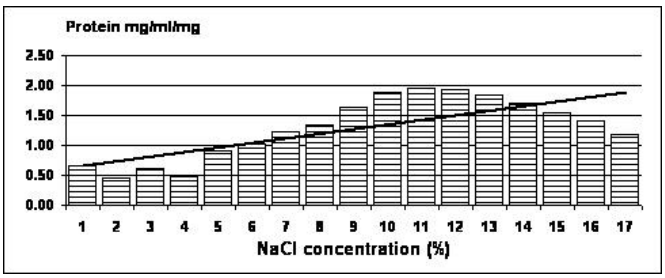

Fig. 3 Pattern of protein concnetration in culutre filtrate of fungi grown in potato dextrose broth under NaCl concentrations

Effect of NaCl on Bacteria :

Effect on growth :

The growth recorded in case bacteria grown under different NaCl treatment have shown in Fig. 4. It was observed that total population count was decreased with the increase of NaCl concentration. Somehow, the bacteria tolerated salt stress upto 10 % and later no growth of any colony could be observed on plates of higher salt concentration.

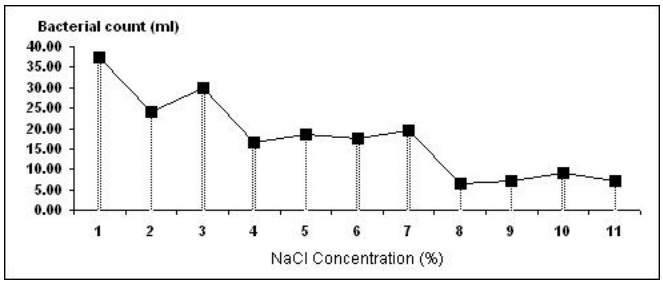

Fig. 4 Pattern of growth of bacteria grown in potato dextrose broth under NaCl concentrations

Effect on pH :

The data recorded on pH of the culture filtrate have shown in Fig. 5. It is quite interesting observation that very minor changes in the pH of bacterial culture filtrate was observed. As mentioned, acidic metabolites might have not been produced in the case of bacteria as it was produced in the fungal culture of same kind of experiment.

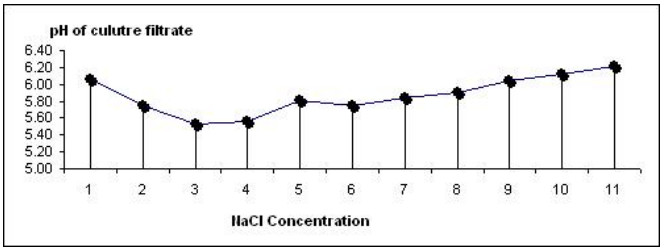

Fig. 5 Pattern of pH of culutre filtrate of bacteria grown in potato dextrose broth under NaCl concentrations

Effect on protein content :

The quantitative estimation of extracellular protein present in the bacterial culture filtrate ispresented in Fig.6. The decline in the protein content was observed with the increase in concentration of NaCl in the culture medium. However, very minor differences could be observed in this regard when samples from 4 % to 9 % NaCl treatments were analyzed. This finding was very well corroborated with the Goel and Kaushik (2002) who observed the decrease in protein synthesis with the increasing concentration of NaCl.

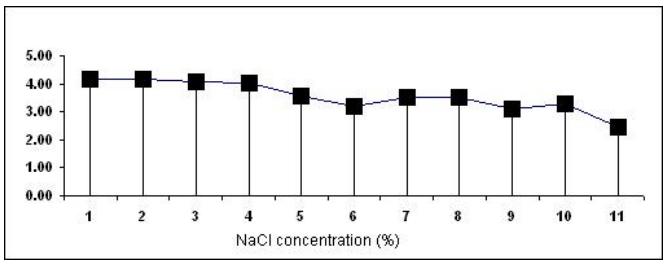

Fig. 6 Pattern of protein concnetration in culutre filtrate of bacteria grown in potato dextrose broth under NaCl concertration

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