Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 11	No. 1	pp. 177-180	2012

Original Research Paper

In Vitro Molecular Assessment of Cyanobacteria for Salt Tolerance

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com
Received: 24/8/2011
Accepted: 18/10/2011

Key Words:
Cyanobacteria
Salt tolerance
Molecular assessment
Pigments

ABSTRACT

Blue green algae (Cyanobacteria) constitute the largest, most diverse and most widely distributed group of photosynthetic prokaryotes. These are known to be primary colonizers of inhospitable ecosystems in which they colonizeed almost all kinds of aquatic ecosystems. Salt tolerance is a good taxonomic character too because of their diverse morphological organization and different biochemical machineries. Four species of cyanobacteria, namely *Oscillatoria tenue, Lyngbya estruarii, Scytonema major* and *Calothrix juliana* were collected from the salt pans of Huma and cultured in medium containing varying concentrations from 0 to 25 ppt of NaCl for 20 days to asses the tolerance level of them. The growth and various macromolecular contents were determined along with the pigments on 20th day of culture. These organisms are able to tolerate the salt concentration of 5 ppt to 15 ppt.

INTRODUCTION

The cyanobacteria are ubiquitous in their occurrence and are well adapted to various ecological conditions. The cell structure of the cyanobacteria is well suited for adaptation to diverse ecological conditions (Strainer & Van Neil 1962). The cyanobacteria also occur in salt affected soils of Haryana (Kausik 1983). Presence of excess sodium salt affects the agricultural soils leading to the development of poor physical soil structure by dispersing the soil aggregates. This in turn results in blocking of the soil porosity. The cyanobacteria have the tendency to grow and cover the available surface soil for growth and proliferation. During their growth phase some of the fixed carbonaceous material is released in the form of extracellular substances (Roy Chaudhury et al. 1985). The soil carbon status increases growing cyanobacteria on such salt affected soils leading to enrichment of soils with nitrogen (Subasini & Kaushik1981, 1984, Kaushik 1983, 1984, 1985). The present study is aimed at assessing the salt tolerance of *Oscillatoria, Lyngbya, Scytonema* and *Calothrix* sps. cultured in the medium with varying concentrations of salt. Consistent with this objective, experiment was carried out to determine the growth, macromolecules and pigments like chlorophyll, carotenoids and phycocyanin. Cyanobacteria are seen as a coherent group, which characteristically lack membrane bound sub-cellular organelles such as mitochondria and chloroplast, which do not have their genetic material located on more than one chromosome in a membrane bound nucleus (Stainer & Van Niel 1962). The histomorphological and physiochemical peculiarities of cyanobacteria tend to their different ecological distribution. Cyanobacteria are ubiquitous in water of a great range of salinity having low state of cell differentiation. Singh (1961) reported the occurrence of species of microflora, *Scytonema* and *Porphyrosiphon* on the loose as well as compact salty soils. In succession these genera were followed with species of *Camphylonema*, *Cylindrospermum* and *Nostoc*. Kaushik (1983) reported the occurrence of species of *Lyngbya*, *Microcoleus, Oscillatoria* and *Aphanocapsa* on the saline and sodic salts of Haryana. Presence of excess sodium results in clogging of soil pores through which air and water move. Cyanobacetria have the tendency to grow and cover the available soil crust for their proliferation and establishment.

Although cyanobacteria have featured in a number of water monitoring studies in which the entire algal community has been considered, the effectiveness of monitoring using the cyanobacetria has not been assessed. Thus, the aim of the study was to determine whether or not the cyanobacetria constitute a well developed and suitable community that could be used to measure the environmental quality of saline ecosystems. The results clearly indicate the potential of cyanobacetria to enrich salt affected soils with carbon, nitrogen and increase in availability of phosphorus (Subhashini & Kaushik 1981), which is helpful in amelioration of sodic soils with blue green algae.

MATERIALS AND METHODS

Algal samples were collected from different sub-habitats of Huma salt pans and then identified under the microscope by staining with aniline blue and further allowed to grow on both solid as well as liquid media in aseptic laboratory conditions for the pure algal culture. To make bacterial free cul-

ture, benzyl-penicillin was added. Test cultures were incubated at 24±2°C temperature and at 2200 lux light intensity for a period of three weeks. Stock cultures were grown from this unialgal bacteria free material, and tested regularly for their purity under microscope. The experimental strains *Oscillatoria tenue, Lyngbya estuarii, Scytonema major and Calothrix juliana* were selected for their wide spectrum of adaptation to different ecological niches.

Growth measurement: Growth was estimated by dry weight measurements in triplicate. Aliquot samples were taken from uniformly suspended culture. The cells were separated from medium by centrifuging at 3,000 rpm for 20 minutes and dried in oven at 105°C. The growth was recorded by measuring the optical density (OD) of homogenized suspension of the culture at 766 nm. The dry weight values were determined from the alga by taking the dry weight in mg, versus optical density at 760 nm. It expressed a linear relationship between dry weight and optical density up to an optical density value of 1.0. In each case, it indicates the reliability of direct optical density measurements in estimating the growth of the algae. The results were represented as the mean of three closely concordant determinations.

Estimation of pigments: The cultures were homogenized in homogenizer by using acetone. The homogenized cultures were centrifuged for 20 minutes at 3,000 rpm. The clean extract was separated from the cell residue by re-extractions until all the lipid soluble pigments were removed. The concentration of chlorophyll and the carotenoid was measured by standard formulae.

Estimation of nitrogen: Total nitrogen was determined by the Kjeldahl distillation and Nesselerization method of Folin et al. (1916) at various time intervals.

Estimation of total carbohydrate: The total carbohydrates (free and bound) in samples were estimated by the anthrone method (Hewitt 1958).

Estimation of total protein: Water soluble fractions of protein in the algae were estimated by Folin phenol reagent method of Lowry et al. (1951).

RESULTS AND DISCUSSION

The effect of salinity on the growth of cyanobacterial strains is shown in Fig. 1, effect on pigments in Fig. 2 (a-d), and effect on macromolecular contents in Fig. 3 (a-d).

The chemical composition of micro-algae varies greatly from species to species resulted by a variety of environmental factors (Piorreck & Pohl 1984). A variety of useful compounds such as pigments, proteins, carbohydrates, lipids and hydrocarbons are synthesized and accumulated by the algae and can be exploited on commercial scale by increasing their production through experimental and greater manipulation. Based on their chemical composition, particularly lipid, cyanobacteria may represent transitional position between bacteria and photosynthetic eukaryotes (Piorreck & Pohl 1984).

The above experimented strains of the cyanobacteria should be tested for their applied aspects as food, industry, therapeutic and in the field of biotechnology. All the above four cyanobacterial strains do not show equal growth dynamics, pigment and macromolecular contents. Their diversifying nature provides the greater knowledge in various fields of application. Factor such as size, toxicity, digestivity and biochemical composition of these microalgae can be accounted for the difference in their food values (Webb & Chu 1983). With respect to the biochemical composition, the total concentration of protein, lipid and carbohydrate in microalgae can vary substantially with the species and culture conduct (Parsons et al. 1961, Dortch 1982, Redalije & laws 1983). A precise knowledge of microalgae composition could theoretically make it possible to tailor specific algal diets to the requirements of animal species. Taking into account of the above factors, the present study was designed to compare the food value of *Oscillatoria tenue, Lyngbya estruarii, Scytonema major* and *Calothrix juliana,* which are very common in salt pans of Huma. A detailed and complete analysis of different species, grown under the same experimental condition was investigated, so that composition differences between the species can be properly identified. All cultures were grown under standard set of conditions and harvested at a definite stage of growth.

The present study was the preliminary phase of programme to determine the qualitative value of some cyanobacterial strains. The effect of salt pans on the four selected blue green algae, was studied with respect to the growth and biological composition of the species.

Analysis of the findings of chemical composition of all algal isolates at different salinity showed that the growth rate is maximum at 5‰ to 10‰ as compared to the control, which gradually decreases with increasing salinity. In case of *Oscillatoria tenue* after 20th day of observation the same growth pattern was observed in *Lyngbya estruarii*. Both forms shows halotolerance capacity. In case of *Scytonema major* and *Calothrix juliana,* the growth rate is maximum at 10 ‰ than that of the control value and gradually the growth decreases with increasing the saline value. These two strains have more potential towards the salinity tolerance than that of the *Oscillatoria tenue* and *Lyngbya estruarii.*

Effect of salinity on different macromolecular contents: Analysis of the chemical composition of all the algal isolates at different saline concentrations showed that in case

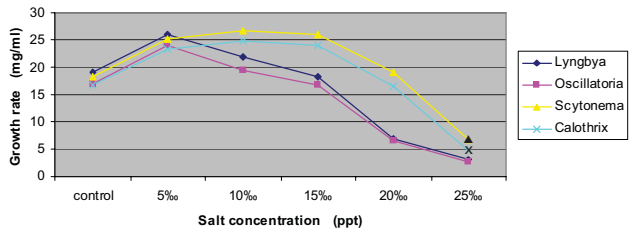

Fig. 1: Effect of salinity on growth of cyanobacterial strains after 20th day of observation.

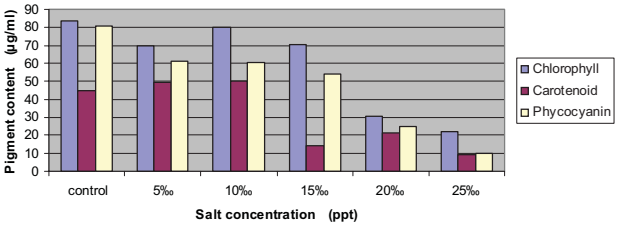

Fig. 2 (a): Effect of different salinities on pigments of *Oscillatoria tenue* after 20th day of observation.

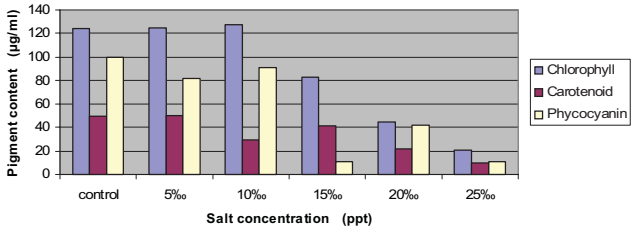

Fig. 2 (b): Effect of different salinities on pigments of *Lyngbya estuarii* after 20th day of observation.

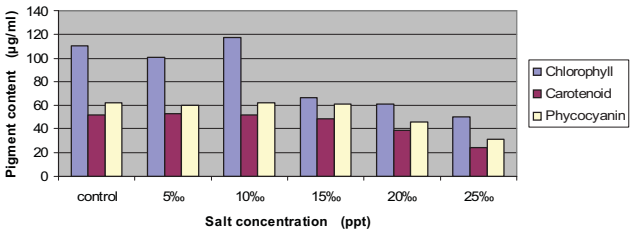

Fig. 2 (c): Effect of different salinities on pigments of *Calothrix juliana* after 20th day of observation.

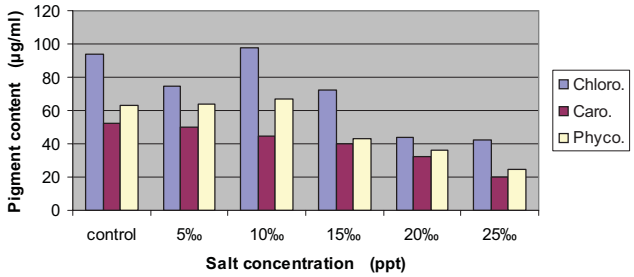

Fig. 2 (d): Effect of different salinities on pigments of *Scytonema major* after 20th day of observation.

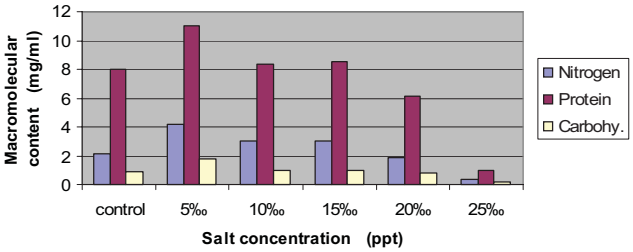

Fig. 3 (a): Effect of different salinities on macromolecular content of *Oscillatoria tenue* after 20th day of observation.

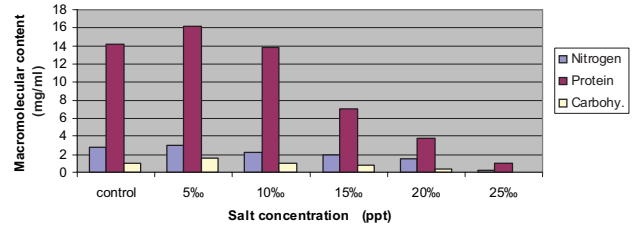

Fig. 3 (b): Effect of different salinities on macromolecular content of *Lingbya estuarii* after 20th day of observation.

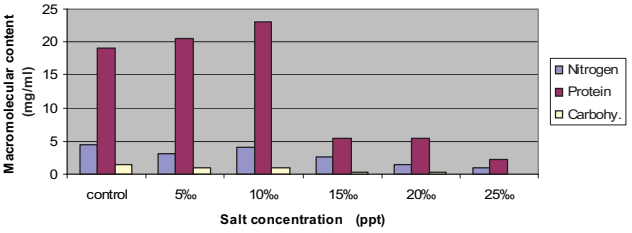

Fig. 3 (c): Effect of different salinities on macromolecular content of *Calothrix juliana* after 20th day of observation.

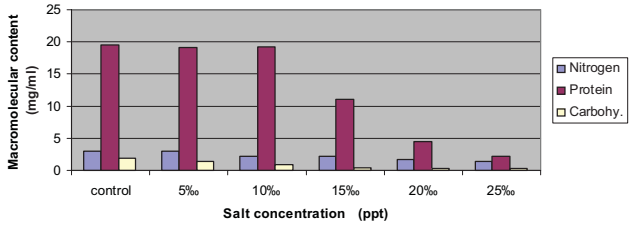

Fig. 3 (d): Effect of different salinities on macromolecular content of *Scytonema major* after 20th day of observation.

of *Oscillatoria tenue,* the protein, nitrogen and carbohydrate content is more at 5‰, which gradually decreases with increasing the salinity. Carbohydrate content is very less at 25‰ as compared to protein and nitrogen. In case of *Lyngbya estruarii*, protein, carbohydrate and nitrogen are maximum at 5‰ than that of the control and gradually decrease with increasing the salinity and very less at 25‰.

In case of *Scytonema major* the protein, nitrogen and carbohydrate content is more in control and gradually

decreases with increasing the salinity. The macromolecular content is greatly affected at the higher saline concentration. In case of *Calothrix juliana* the protein, nitrogen and carbohydrate contents are more at 10‰ than that of the control, and gradually decrease with increasing the salinity.

Pigment composition after 28th day of observation showed that in case of *Oscillatoria tenue*, the chlorophyll, carotenoid and phycobillin are maximum at 10‰ as compared to the control, and then gradually decrease with increasing the salinity concentration. In case of *Lyngbya estruarii* and *Scytonema major* also, the chlorophyll, carotenoid and phycocyanin are maximum at 10‰ as compared to the control and 5‰, and gradually decrease with increasing the salinity. In case of *Calothrix juliana* the chlorophyll, carotenoid and phycocyanin are maximum at control, which gradually decrease with increasing the salinity value at 25‰ but the pigment content is more as compared to the other strains. Hence, *Calothrix juliana* shows more sensivity to pigment contents at higher salinity conditions. Exploring research for assessing the food value of the local algal flora should be initiated to eliminate the protein shortage.

ACKNOWLADGEMENT

The authors are thankful to the Principal, HOD, Biotechnology and Management, GIET, Gunupur for providing the facilities for this research. They are also thankful to the farmers and local people of different study sites for their help and cooperation during the sample collection.

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