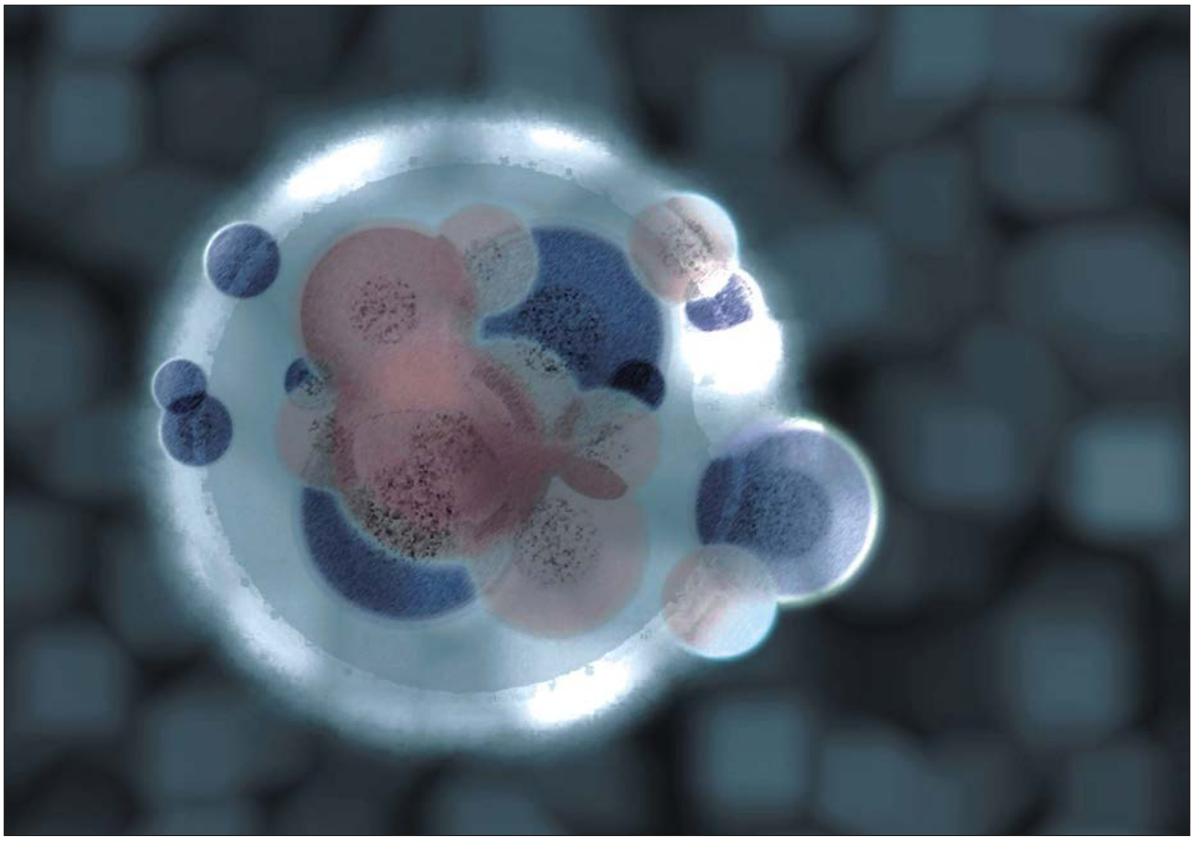

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 7 (6): 931-936, 2007
ISSN 1727-3048

Characterization of a Soil Cyanobacterium *Fischerella* sp. FS 18 under NaCl Stress

[1]N. Soltani, [2]G. Zarrini, [3]Y. Ghasemi, [4]Sh. Shokravi and [1]L. Baftehchi
[1]Department of Biology, ACECR, Institute of Applied Science, Tehran, Iran
[2]Department of Biology, Faculty of Science, Tabriz University, Tabriz, Iran
[3]Department of Pharmacognosy and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Shiraz University, Shiraz, Iran
[4]Department of Biology, Faculty of Science, Islamic Azad University, Gorgan Branch, Gorgan, Iran

Abstract: In this research we showed the effect of salinity (NaCl-free, 0.5 and 1%) on growth, photosynthesis, nitrogenase activity and antimicrobial effects in cyanobacterium *Fischerella* sp. FS18. In this way *Fischerella* sp. FS18 was treated with different concentrations of NaCl (0, 0.5 and 1%). Aqueous, petroleum ether and methanol extracts of the strain were examined against four bacteria and two fungi for antimicrobial purposes. The results indicated that the growth of *Staphylococcus epidermidis* PTCC 1114 was inhibited by all treatments. Aqueous extract did not have any inhibitory effect on *Bacillus subtilis* PTCC 1204. None of the treatments had decreasing effect on gram negative bacteria *Entrococcus feacalis* ATCC 8043 and *Escherichia coli* PTCC 1047. Among the fungi, *Candida kefyr* ATCC 1140 was more sensitive. Regarding physiological responses, the growth rate was higher in NaCl-free and salinity did not inhibit growth of other treatments. Similar results were seen in the case of other physiological processes. The light-saturated photosynthetic rate was higher in NaCl-free and it decreased with increasing salinity. With respect to nitrogenase activity, the highest rate belonged to NaCl-free and the cultures with higher growth rates reached to the maximum level of activity sooner. There was no significant difference between 0.5 and 1% NaCl treatments in relation to nitrogenase activity.

Key words: Antimicrobial activity, cyanobacteria, *Fischerella* sp., nitrogenase, photosynthesis, salinity

INTRODUCTION

The cosmopolitan community of cyanobacteria has a significant role in N_2 fixing improving the fertility of wetlands such as rice fields (Anand *et al*., 1990). They are common inhabitants of aquatic and terrestrial surfaces, including extreme environments (Noaman *et al*., 2004; Mazur and Pliñski, 2001; Quesada *et al*., 1998). Also the ability of producing biologically active compounds makes them a rich source of potentially useful natural products and the targets of screening programs (Soltani *et al*., 2005; Ghasemi *et al*., 2003; Jaki *et al*., 1999; Moore, 1996). *Fischerella* spp. have been reported to produce antimicrobial substances (Asthana *et al*., 2006; Tabatabaei Yazdi *et al*., 2005; Ghasemi *et al*., 2004; Hagmann and Jüttner, 1996; Falch *et al*., 1995) and some of these substances identified include Fischerindole L, Parsiguine and Nostocyclyne (Ghasemi *et al*., 2004; Plounto and Cameli, 2000).

On the other hand, salinity of soil is an important ecological variable and a serious problem in agriculture. The widespread distribution of cyanobacteria indicates that they can cope with a wide spectrum of global environmental stresses such as temperature, pH, desiccation, etc. Salt stress is one the limiting factors on the growth and productivity of microorganisms. They have developed a number of mechanisms by which cyanobacteria defend themselves against environmental stressors (Rajendran *et al*., 2007; Allakhverdiev *et al*., 2001). The physiological basis for the adaptation to high salinities in several cyanobabacterial species includes three main subprocesses: active extrusion of inorganic ions, leading to relatively unchanged internal salt concentrations; accumulation of large internal amounts of organic osmoprotective compounds; and expression of a set of salt stress proteins (Hagemann and Erdmann, 1997).

Iranian paddy fields are a source of enormous biological diversity, which is scarcely studied.

Corresponding Author: Neda Soltani, Department of Biology, ACECR, Research Institute of Applied Science, Tehran, P.O. Box 19835-169, Iran Tel/Fax: 0098 21 29903037

Particularly, few reports on microbial life are described. More recently, the characterization of microorganisms with biotechnological interest has been reported by Soltani *et al*. (2006, 2005) and Ghasemi *et al*. (2004). However, the production of antimicrobials by cyanobacteria isolated in this region is poorly described. Interest in antimicrobial and physiological activity of cyanobacterium *Fischerella* sp. FS18 isolated from paddy fields of Iran under salinity stress has been the goal of this work.

MATERIALS AND METHODS

Cyanobacterial cultures: *Fischerella* sp. FS18 was isolated from paddy fields of Gillan province near Roodsar city (2005), Iran. The samples transferred to lab (Department of Biology, ACECR, Univ. Shahid Beheshti). Pure culture of selected cyanobacterium was prepared by repeated subculture on solified medium,. Stock cultures were grown in the BG110. Temperature was maintained at 30°C and cultures were bubbled with air under a constant light intensity of 60 μmol photon m^{-2} sec^{-1} supplied by three fluorescent tubes. Cells in logarithmic phase of growth were collected from stock cultures and used as inoculate for experiments.

BG110 medium of different salinity was made for inoculation of *Fischerella* sp. FS18. The required salinity was obtained by adding sodium chloride. The flasks were maintained for 21 days at 30±1°C under constant illumination of about 60 μ E m^{-2} sec^{-1}.

Antimicrobial bioassay: Different treated, soil cyanobacterium *Fischerella* sp. FS18 has been tested for the release of substances that inhibit the growth of four bacteria and two fungi. Cyanobacterial cells were harvested during the stationary phase by centrifugation. The cells were extracted in petroleum ether and methanol for 20 min and the solvents were removed by rotary evaporation up to 10 mL at 40°C. *Bacillus subtilis* PTCC 1204, *Staphylococcus epidermidis* PTCC 1114, *Candida albicans* ATCC 14053, *Candida Kefyr* ATCC 1140, *Entrococcus feacalis* ATCC 8043, *Escherichia coli* PTCC 1047, were used as test organisms. Antimicrobial activity was determined by the disc diffusion method (Lorain, 1996). Filter paper discs (6.4 mm) were saturated with 40 μL of the test solution, dried and placed on the Muller-Hinton agar plate for the bacterium and saubouraud's dextrose agar plate for the fungus, which had been inoculated with a lawn of the test microorganisms. Plates were incubated at an appropriate temperature for fungi (25°C) and bacteria (37°C), for a period of 18-24 h. Antibiogram test was run for three times.

Physiological analysis: Growth determination was estimated as previously described (Soltani *et al*., 2006). For chlorophyll estimation, cells were extracted with pure methanol for 24 h at 4°C and the chlorophyll content was determined spectrophotometrically at 665 nm. Phycobiliproteins were extracted after osmotic shock and measured spectrophotometrically at 652, 615 and 562 nm.

Nitrogenase activity was determined by acetylene reduction in 15 mL aliquots of cell suspensions placed in stoppered 25 mL vials. Prior to incubation 10 % of the air inside the vial was replaced with the same volume of acetylene. Cells were incubated for 1 h under the same conditions as they were cultured. After incubation 0.5 mL of gas samples were taken and ethylene concentration was determined in a Shimadzu GC-8 gas chromatograph.

O_2 evolution was measured with a Clark-type O_2 electrode (Hach Chemical Company). Two milliliter aliquots of cell suspensions were placed in a temperature controlled cuvette (30°C) and illuminated at desired quantum flux density.

All the experiments were repeated three times. Data are the means of triplicate tests±SD. Statistical differences were examined using the ANOVA test.

RESULTS

To show the microenvironment of sample locality, the mentioned soil was analyzed. Results of physicochemical analysis of the soil of collection site are demonstrated in Table 1.

Neutral pH and high EC is a common feature in Gillan paddy fields and *Fischerella* sp. had wide distribution in that region. Since antimicrobial screening was first step of our research, three extracts with polar and nonpolar behavior were prepared from *Fischerella* sp. FS18. Results showed that all treatments had antibacterial activity against *S. epidermidis* (Table 2). Salinity did not have any inhibitory effect on this behavior significantly. Diameters of inhibition zone were 19.1 mm in NaCl-free medium and 17 and 15.6 mm in 0.5 and 1% NaCl respectively. Same results were seen in methanol and petroleum extracts.

The data of antibiogram test did not show any activity against *B. subtilis* in aqueous extracts, but it was seen in methanol and petroleum extract. The highest effect was indicated in NaCl 0.5%. Same results were demonstrated in the case of *C. albicans* ATCC 14033.

Table 1: Physicochemical analysis of the soil of collection site (Roodsar, Gillan)

pH	EC (ds m^{-1})	O.C (%)	P (%)	K (%)	N (%)	C/N
7.4	13.4	10.4	0.37	1.0	1.45	10.4/1.45

Table 2: Antimicrobial activity of extracts from different treatments (diameter of inhibition zone, mm)

NaCl treatment (%)	*Bacillus subtilis* PTCC 1204	*Staphylococcus epidermidis* PTCC 1114	*Candida albicans* ATCC 14033	*Candida kefyr* ATCC 1140	*Entrococcus feacalis* ATCC 8043	*Escherichia coli* PTCC 1047
A-0	-	19.1	-	-	-	-
A-0.5	-	17.0	-	-	-	-
A-1	-	15.6	-	9.9	-	-
M-0	9.8	22.3	12.2	14.5	-	-
M-05	10.1	22.7	11.6	14.2	-	-
M-1	10.0	22.0	13.0	14.1	-	-
P-0	4.8	15.2	8.7	10.4	-	-
P-0.5	9.6	17.3	10.9	12.1	-	-
P-1	6.1	17.0	9.8	12.2	-	-

A: Aqueous extract; M: Methanol extract; P: Petroleum ether extracts

Table 3: Growth rate and Chlorophyll contents in different NaCl concentrations in *Fischerella* sp. FS18

Salinity (%)	Growth rate (day^{-1})	Chlorophyll content (μg chl mg dw^{-1})
0	0.34±0.02	11.78±2.5
0.5	0.42±0.06	11.57±4.5
1	0.13±0.01	8.50±2

Salinity increased the antimicrobial activity. Diameter of inhibition zone in NaCl-free, 0.5 and 1% were 8.7, 10.9 and 9.8 mm, respectively in petroleum ether extract. The results were similar to *C. kefyr* ATCC 1140, another selected fungus for antimicrobial screening with wider spectrum of activity in the case of the latter. Two Gram negative bacteria, *E. coli* and *E. feacalis* were resistance to all treatments (Table 2). Totally, methanol and petroleum ether extracts were more active than aqueous section. According to physiological responses of *Fischerella* sp. FS18 to salt stress, it was concluded that the growth rate decreased with increasing salinity. The biomass in 0.5% NaCl was higher than that of the control but the difference was not significant.

In next step, the effect of salinity on chlorophyll content was measured. The results are shown in Table 3. Table 3 reveals the lower Chlorophyll content by higher salinity, so the minimum content belonged to 1% NaCl. Salinity 0 and 0.5% were in decreasing order (11.78 and 11.57 μg Chl mg dw^{-1}, respectively).

According to photosynthesis rate we had planned short time and long time photosynthesis experiments. As it is shown in Fig. 1 the highest rate was seen in third day and NaCl-free medium. This is in agreement with chlorophyll content. 0.5 and 1% NaCl were in decreasing order.

Regarding short time experiments of photosynthesis, oxygen evolution of the treated cyanobacteria in the exposure of different light intensities was measured (Fig. 2). As it is shown, maximum photosynthesis rate (P_{max}) belongs to the control. Also, photosynthesis efficiency is seen in 0.5% NaCl. The light in which photosynthesis would be saturated (I_k) is higher in the control. These are 0.86 and 0.67 $\mu E\ m^{-2}\ sec^{-1}$ in 0.5 and 1% NaCl, respectively.

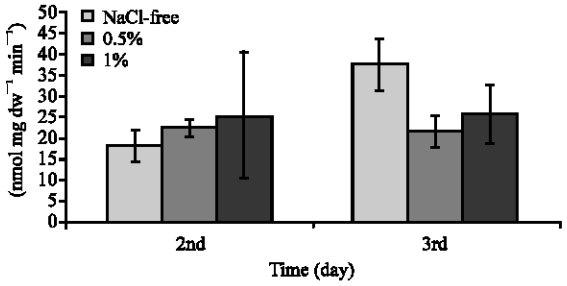

Fig. 1: Photosynthetic rate in 2nd and 3rd days after inoculation in *Fischerella* sp. FS18

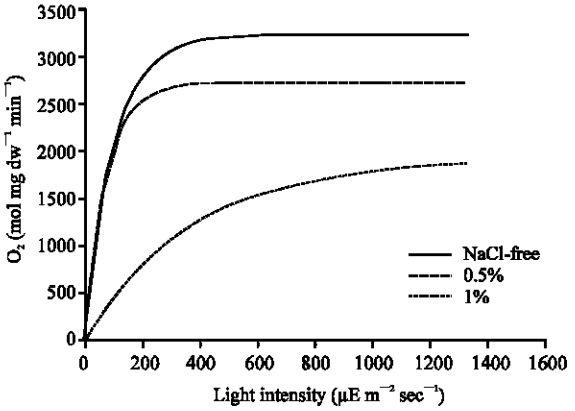

Fig. 2: Photosynthetic rate in different light intensities and NaCl concentrations

Taking the nitrogenase activity into account, the maximum rate was seen in the 3rd day after inoculation in all treatments (Data not shown). The highest amount belonged to the controls and decreased with increasing salinity. This effect was significant (ANOVA, $p<0.001$).

DISCUSSION

A few studies have been done to apply cyanobacteria isolated from local habitats for organic antimicrobial active compounds with respect to their

metabolism in salinity stress especially in the Middle East. Many species of cyanobacteria, including those normally living in brackish and fresh waters, are capable of growth and bloom formation over a wide range of salinity.

According to antimicrobial activity, this study is in agreement with other researches that confirmed the ability of cyanobacteria to produce of antimicrobial substances (Asthana *et al*., 2006; Tabatabaei Yazdi *et al*., 2005). Regarding antimicrobial screening, our results indicated that *S. epidermidis* was the most sensitive bacteria among selected strains, as activities were seen in all treatments. It means that NaCl 1% increased antibacterial activity. These data are similar to the results of growth and photosynthesis. Among the fungi, *C. kefyr* ATCC 1140 was more sensitive than *C. albicans* ATCC 14033. These data are in agreement with Soltani *et al.* (2005) and Ghasemi *et al.* (2004). *Fischerella* sp. FS18 did not affect the two Gram negative bacteria *E. coli* PTCC 1047 and *E. feacalis* ATCC 8043. These results are in agreement with Ghasemi *et al.* (2004) and also Safonova and Resser (2005) that revealed a slightly inhibiting effect of *C. turgidis* on *E. coli*, but no significant inhibition on either *E. coli* or *M. luteus* by *Nostoc* sp. and *Synechocystis* sp.

Most of antibacterial the activity could be seen in methanol and petroleum ether extracts. It indicated the possible locality of active metabolite in the biomass of cyanobacterium.

Production of active metabolites was not absolutely similar by same the cyanobacteria because several factors could affect it, for instance the presence or absence of other bacteria, as has been shown by Mazure and Plinski (2001). Temperature, pH, incubation period or different nitrogen and carbon sources in some media have different effects on both growth and antimicrobial activity (Noaman *et al*., 2004). Moreover, in the case of some species of cyanobacteria, salinity was found to be one of the most important factors influencing metabolism.

Regarding physiological responses of *Fischerella* sp. FS 18 to NaCl, as shown in Fig. 1 and 2, the growth rate decreased with increase in salinity though it continued in NaCl 1%. These data were also seen in *Nostoc* sp. (Sekar and Subramanian, 1999) and *Nostoc moscorum* (Bhargava and Singh, 2006). On the other hand, Moisander *et al.* (2002) showed that *Anabaenopsis* sp. had similar growth in the NaCl ranged 2-20 g L^{-1} whilst *Anabaena aphanizomenoide* grew in NaCl up to 15 g L^{-1} but 20 g L^{-1} had inhibiting affect on it. However, the limiting point in *Cylinderospermopsis* was 4 g L^{-1} NaCl (Moisander *et al*., 2002). These results confirm the variation of chlorophyll content in different salinity. Figure 2 can demonstrate a similar growth rate pattern which confirms the role of chlorophyll in cyanobacterial growth and its change with varied environmental factors. The tolerance of this strain against NaCl 1% is also concluded. Rosales *et al.* (2005) indicated that *Synechococcus* isolated from hypersaline habitats had maximum dry weight, chlorophyll a, betacarotene and zeaxeanthin in 100 ppt. This strain is halotolerant (Rosales *et al*., 2005). Of course Na^+ requirement in cyanobacteria should be considered. Elimination of this essential nutrient, reduced capacity for photosynthesis and nitrogenase activity.

Maximum photosynthesis rate (P_{max}) is seen in the control and confirms the results of short time experiments of photosynthesis. P_{max} decreased in NaCl 0.5 and 1%. The efficiency of photosynthesis reached its maximum in salinity 0.5%, but the difference with the control was not significant. Taking the results into account, it is concluded that salinity has a significant effect on photosynthesis and affect the usage of minimum light for photosynthesis. This cyanobacterium needs more light to survive in saline environment. This result is in agreement with that of growth.

Cyanobacteria are not only able to fix atmospheric nitrogen, but also have an important role in rice fields by their frequency (Roger and Kulasooriya, 1980). Maximum nitrogenase activity was seen in the 3rd day and NaCl-free medium (18.5 nmol C_2H_4 mg dw^{-1} h^{-1}). It decreased with increasing NaCl with a delay in reaching the maximum activity. These results are in the same line with Rai *et al.* (2001) but are in contrast with Sekar and Subramanian (1999). Comparison of photosynthesis and nitrogenase activity indicates overlapping of their maximum activity. It can be explained by preparing the carbon skeleton and required energy via photosynthesis for nitrogen fixation. Nitrogenase activity seems to be different, depending on the strain. On this basis *Anabeana* showed higher nitrogen fixation than *Nostoc* in saline water (Hashem, 1998).

According to antimicrobial effects, the results indicated that *Fischerella* sp. FS18 has more antimicrobial activity in saline environments. It could be explained not only by the role of NaCl in the life of bacteria but also by decreasing the growth rate of cyanobacterium. Regarding physiological responses, *Fischerella* sp. FS18 demonstrated tolerance in NaCl 1%. Its growth was not inhibited completely but it decreased. This decrease has followed in other metabolic process like photosynthesis or nitrogen fixation. Researches have shown that halotolerant cyanobacteria synthesis accumulates different osmolites for modulation hypersalinity caused by increasing NaCl out of cells. In this way, saline stress proteins has also an important role. Research on the kind of osmolite produced should be followed in future.

ACKNOWLEDGMENTS

This research was supported by a grant from the Research Council of Shiraz University of Medical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran.

REFERENCES

Allakhverdiev, S.I., M. Kinoshita, M. Inaba, I. Suzuki and N. Murata, 2001. Unsaturated fatty acids in membrane lipids protect the photosynthetic machinery against salt-induced damage in *Synechococcus*. Plant Physiol., 125: 1842-1853.

Anand, N., L. Radha, R.S. Shanthakumar Hopper, G. Revathi and T.D. Subramanian, 1990. Blue-green Algae as Biofertilizers: Certain View Points on the Choice of Suitable Isolates. In: Perspective in Phycology. Rajarao, V.N. (Ed.), Today and Tomorrow's Printer and Publisher, New Delhi, pp: 383-391.

Asthana, R., A. Srivatava, A.P. Singh, Deepali, S.P. Singh, G. Nath, R. Srivastava and B.S. Srivastava, 2006. Identification of an antimicrobial entity from the cyanobacterium *Fischerella* sp. isolated from bark of *Azadirachta indica* (Neem) tree. J. Applied Phycol., 18: 33-39.

Bhargava, S. and K. Singh, 2006. Differential response of NaCl-resistant mutants of the cyanobacterium *Nostoc muscorum* to salinity and osmotic stresses. World J. Microb. Biotech., 22: 783-789.

Falch, B.S., G.M. König, A.D. Wright, O. Sticher, C.K. Angerhofer, J.M. Pezzuto and H. Bachmann, 1995. Biological activities of cyanobacteria: Evaluation of extracts and pure compounds. Planta Med., 61: 321-328.

Ghasemi, Y., M. Tabatabaei Yazdi, A. Shafiee, M. Amini, Sh. Shokravi and G. Zarrini, 2004. Parsiguine, a novel antimicrobial substance from *Fischerella ambigua*. Pharm. Biol., 42: 318-322.

Ghasemi, Y., M. Tabatabaei Yazdi, Sh. Shokravi, N. Soltani and G. Zarrini, 2003. Antifungal and antibacterial activity of paddy-fields cyanobacteria from the north of Iran. J. Sci. Islamic Republic of Iran, 14: 203-209.

Hagemann, M. and N. Erdmann, 1997. Environmental Stresses. In: Cyanobacterial Nitrogen Metabolism and Environmental Biotechnology. Rai, A.K. (Ed.), Springer, Heidelberg, pp: 156-221.

Hagmann, L. and F. Jüttner, 1996. Fischerellin A, a novel photosystem-II-inhibiting alleolochemical of the cyanobacterium *Fischerella muscicola* with antifungal and herbicide activity. Tetrahedron Lett., 37: 6539-6542.

Hashem, M.A., 1998. Ecophysiological Studies of Cyanobacteria in Paddy Soils. In: Nitrogen Fixation with Non-legumes. Malik, K.A., M.S. Mirza and J.K. Kluwer (Eds.), Academic Publishers, Ladha, pp: 333-344.

Jaki, B., J. Orjala, H.R. Burgi and O. Sticher, 1999. Biological screening of cyanobacteria for antimicrobial and molluscicidal activity, brine shrimp lethality and cytotoxicity. Pharm. Biol., 37: 138-143.

Lorain, V., 1996. Antibiotic in Laboratory Medicine. Baltimore, William and Wilkins, pp: 1-51.

Mazur, H. and M. Pliński, 2001. Stability of cyanotoxins, microcystin-LR, microcystin-RR and nodularin in seawater and BG-11 medium of different salinity. Oceanologia, 43: 329-339.

Moisander, P.H., E. McClinton and H.W. Pearl, 2002. Salinity effects on growth, photosynthetic parameters and nitrogenase activity in estuarine planktonic cyanobacteria. Microb. Ecol., 43: 432-442.

Moore, R.E., 1996. Cyclic peptides and depsipeptides from cyanobacteria: A review. J. Ind. Microbiol., 16: 134-143.

Noaman, N.H., A. Fattah, M. Khaleafa and Sh. Zaky, 2004. Factors affecting antimicrobial activity of *Synechococcus leopoliensis*. Microbiol. Res., 159: 395-402.

Plounto, A. and Sh. Cameli, 2000. Nostocyclyne A, a novel antimicrobial cyclophane from the cyanobacterium *Nostoc* sp., J. Nat. Prod., 63: 1524-1526.

Quesada, A., M. Nieva, F. Leganés, A. Ucha, M. Martín, C. Prosperi and E. Fernández-Valiente, 1998. Acclimiation of cyanobacterial communities in rice fields and response of nitrogenase activity to light regime. Microb. Ecol., 35: 147-155.

Rai, V., S.P. Tiwari and A.K. Rai, 2001. Effect of NaCl on nitrogen fixation of unadapted and NaCl-adapted *Azolla pinnata-Anabaena azollae*. Aqua. Bot., 71: 109-117.

Rajendran, U., E. Kathirvel and A. Narayanaswamy, 2007. Desiccation-induced changes in antioxidant enzymes, fatty acids and amino acids in the cyanobacterium *Tolypothrix scytonemoides*. World J. Microb. Biotech., 23: 251-257.

Roger, P.A. and S.A. Kulasooriya, 1980. Blue-green algae and rice. The International Rice Res. Institute, pp: 3-9.

Rosales, N., J. Ortega, R. Mora and E. Morales, 2005. Influence of salinity on growth and biochemical composition of the cyanobacterium *Synechococcus* sp., Cienc. Mar., 31: 349-355.

Safonova, E. and W. Resser, 2005. Growth promoting and inhibiting effects of extracellular substances of soil microalgae and cyanobacteria on *Escheichia coli* and *Micrococcus luteus*. Phycol. Res., 53: 189-193.

Sekar, S. and S. Subramanian, 1999. Influence of low levels of salinity on the primary metabolism of the fresh water cyanobacteria *Phormidium* and *Nostoc*. Rev. Bras. Fisiol. Veg., 11: 83-89.

Soltani, N., R. Khavari-Nejad, M. Tabatabaie, Sh. Shokravi and E.F. Valiente, 2005. Screening of soil cyanobacteria for antimicrobial activity. Pharm. Biol., 43: 455-459.

Soltani, N., R.A. Khavari-Nejad, M. Tabatabaie, Sh. Shokravi and E.F. Valiente, 2006. Variation of nitrogenase activity, photosynthesis and pigmentation of cyanobacterium *Fischerella ambigua* strain FS18 under different irradiance and pH. World J. Microb. Biotech., 22: 571-576.

Tabatabaei Yazdi, M., Y. Ghasemi, A. Ghasemian, Sh. Shokravi, H. Niknahad, M. Amini, A. Dehshahri and M.A. Faramarzi, 2005. Bioconversion of hydrocortisone by cyanobacterium *Fischerella ambigua* PTCC 1635. World J. Microb. Biotech., 21: 811-814.