Journal of Agricultural Technology 2011 Vol. 7(3): 649-663
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Physiological and antimicrobial characterizations of some cyanobacteria isolated from the rice fields in Iran

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Asadi, A., Khavari-Nejad, R., Soltani, N., Najafi, F., Molaie-Rad, A. (2011). Physiological and antimicrobial characterizations of some cyanobacteria isolated from the rice fields in Iran. Journal of Agricultural Technology 7(3): 469-663.

In this study, physiological and antimicrobial characterizations of *Microchaete tenera* var._major (Thuret) K-Möbious ISC13 (Nostocales); *Anabaena* sp. Bory ISC55 (Nostocales); *Phormidium* sp. Kützing (Oscillatoriales) ISC31; *Chroococcus pallidus* Nägeli ISC35 (Chroococcales) isolated from the paddy fields in Iran were evaluated. Herterocystous strains, like *Microchaete tenera* var._major and *Anabaena* sp., showed maximum dry weights, growth rates and optical densities in comparison to non-heterocystous strain, *Phormidium* sp., and unicellular strain, *Chroococcus pallidus*. *Phormidium* sp. showed maximum chlorophyll a, carotenoid and protein accumulation in comparison to other strains. *Anabaena* sp. showed highest photosynthesis activity and nitrogenase activity. Antimicrobial activity using agar well diffusion and minimum inhibitory concentrations (MIC) methods indicated that the methanol extracts showed more potent activity than other organic and aqueous extracts. Results indicated that *Microchaete tenera* var._major had the highest antibacterial activity towards the tested bacteria. Also, the broth microdilution assay gave minimum inhibitory concentrations (MIC) values ranging from 0.052 to 27 µg/mL. The MICs of methanol extracts of *Microchaete tenera* var._major *Anabaena* sp. and *Chroococcus pallidus* appeared widespread spectrum of antimicrobial activities. This study revealed that basic research is needed to identify and develop biofertilizers and pharmaceuticals from local strains of cyanobacteria in rice fields.

Key words: Physiological characterization, antimicrobial activity, cyanobacteria, paddy fields, Iran

Introduction

Cyanobacteria or blue-green algae are a fascinating group of primitive phototrophic prokaryotic organisms whose long evolutionary history dates back to the Proterozoic era. These organisms, endowed with tremendous genome plasticity, are distributed in all possible biotypes of the world. These organisms have tremendous potential in environmental, management as soil conditioners,

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biofertilizers, biomonitors of soil fertility and water quality, amelioratory agents in reclamation of degraded ecosystems through biosorption of metals, feed for animals and protein supplement (Rai *et al., 1998;* Whitton and Potts, 2000). Cyanobacteria can photosynthesize and fix nitrogen, and these abilities, together with great adaptability to various soil types, make them ubiquitous. Cyanobacteria have been reported from a wide range of soils, thriving both in and below the surface. They are often also characteristic features of other types of sub-aerial environment and many intermittently wet ones such as rice fields. Most paddy soils have a natural population of cyanobacteria which provides a potential source of nitrogen fixation at no cost (Haroun and Hussein, 2003; Song *et al.,* 2005; Zaccaro *et al*., 1999).

Due to their occurrence in diverse habitats, these organisms are the excellent material for investigation by ecologists, physiologists, biochemists, pharmacists and molecular biologists. Accordingly, looking for cyanobacteria with antimicrobial activity has gained importance in recent years. Biologically active substances were proved to be extracted by cyanobacteria (Borowitzka, 1995; Kreitlow *et al.,* 1999; Mundt *et al.,* 2001; Volk and Furkert, 2006). Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal (John *et al.,* 2003), antibacterial and antifungal (Ghasemi *et al.,* 2003, 2007; Isnansetyo *et al.,* 2003; Jaki *et al.,* 1999; Kundim *et al*., 2003; Soltani *et al.,* 2005) and antiviral activity (Moore *et al.,* 1989). A few studies have been done to screen paddy fields cyanobacteria for production of antimicrobial substances.

Due to the pivotal role played by these organisms, it was considered worthwhile to examine growth parameters, physiological attributes and antimicrobial activity for possible biotechnological applications.

Materials and methods

Isolation and culture conditions

Four soil cyanobacterial strains used in this research, *Microchaete tenera* var._major (Thuret) K-Möbious ISC13 (Nostocales); *Anabaena* sp. Bory ISC55 (Nostocales); *Phormidium* sp. Kützing (Oscillatoriales) ISC31; *Chroococcus pallidus* Nägeli ISC35 (Chroococcales), obtained from microalgal culture collection of ACECR, RIAS. These species were isolated from paddy fields of different provinces in Iran. Soil samples were cultured by conventional methods (Andersen, 2005). For measurement of growth and certain physiological and antimicrobial activity, cultures were grown in nitrogen deficient medium for heterocystous, nitrogen fixing forms and nitrogen enriched medium for non-heterocystous and unicellular forms under controlled conditions. The cultures

were illuminated continuously (50 $\mu Em^{-2}s^{-1}$) supplied by six fluorescent lamps and following incubation at 30 ± 1ºC. Samples were daily homogenized with the help of incubator shaker (Labteck® model) and homogenizer (JENCONS-PLS model) during exponential phase of growth (15 days of incubation). Identification of cyanobacteria was carried out according to Desikachary, (1959) and John *et al*. (2003).

Analytical methods

Growth rate was estimated by both the optical density at 750 nm and by the cell dry weight as described by Leganés *et al*. (1987). Chlorophyll content was extracted using 90% aqueous methanol and measured at 665 nm, and concentration was calculated using the extinction coefficient of Marker (1972). The total carotenoids were extracted and measured in 80% acetone according to Chamovitz *et al*. (1993). Proteins were determined by the method of Bradford using bovine serum albumin as the standard (1976). Nitrogenase activity was determined by acetylene reduction technique. Ethylene concentration was determined in a Shimadzu GC-15A gas chromatograph as nmol ethylene/mg dry weight hour. Oxygen evolution was measured with a Clark-type O_2 electrode in a Chlorolab oxymeter (Hansatech Instruments, Norfolk, UK). Photosynthetic activity was estimated by measuring O_2 evolution for 2 minutes and expressed as nmol O_2 evolved/ µg chlorophyll a hour.

Antimicrobial methods

After incubation, cyanobacterial biomass was separated after 15 days from supernatant by centrifugation at 5000 rpm for 15 min. The pellets were collected, weighed and used for extraction of antimicrobial agents. All of the extracts were preserved at +4ºC (Val *et al.,* 2001). Antibacterial activities of cyanobacteria extracts were tested by agar-well diffusion method (Lorain, 1996). The following formula was used for comparison of the antimicrobial activity of the sample with that of the standard

$$\text{antimicrobial index} = \frac{\text{Inhibition zone of sample} \times 100}{\text{Inhibition zone of the standard}}$$

The samples with activity against the test organisms in the agar-well diffusion method were assayed for more evaluation of results obtained by agar-well diffusion method about the quantity of the compound with antimicrobial activity by minimum inhibitory concentration (MIC) of µg/mL, according to the standard reference method (NCCLS, 2008).

Statistical analysis

All the experiments were replicated three times. Data are means of triplicate tests ± SE. Statistical differences were examined using SPSS software.

Results

A wide variation of physiological activities was observed among different cyanobacterial.

Our results showed two heterocystous strains had the maximal optical density at 750 nm and cell dry weight in comparison to non-heterocystous and unicellular strains during seven days incubation. *Anabaena* sp. had an optical density and cell dry weight higher than that of *Microchaete tenera* var._major, *Phormidium* sp. and *Chroococcus pallidus* (Figs. 1, 2).

The specific growth rates of four strains were between 0.133 and 0.0188 d^{-1} during 2 to 7 days incubation. *Microchaete tenera* var._major had the maximal cell biomass and appropriate specific growth rate during 2 to 4 days cultivation (0.892 to 1.356 d^{-1}) (Fig. 3).

Chlorophyll a content of *Phormidium* sp. significantly ($P<0.05$) increased in comparison to *Chroococcus pallidus*, *Anabaena* sp. and *Microchaete tenera* var._major (Fig. 4). Chlorophyll a content of *Microchaete tenera* var._major increased between 0.317 and 0.593 μg/mg dw and chlorophyll a accumulation of *Anabaena* sp. decreased (0.748 to 0.661 μg/mg dw) during 2 to 5 days (Fig. 4). *Phormidium* sp. produced high chlorophyll a content during 2 to 4 days (0.896 to 1.282 μg/mg dw) and at the fifth day, *Phormidium* sp. produced low chlorophyll a (Fig. 4). *Chroococcus pallidus* had the maximal chlorophyll accumulation in 2 to 4 days and then its content decreased (Fig. 4).

Total carotenoid accumulation of *Phormidium* sp. significantly ($P<0.05$) increased in comparison to *Chroococcus pallidus*, *Anabaena* sp. and *Microchaete tenera* var._major (Fig. 5). Carotenoid content of *Microchaete tenera* var._major increased between 0.123 and 0.127 μg/mg dw during 2 to 3 days and that of *Anabaena* sp. increased (0.041 to 0.079 μg/mg dw) during 2 to 5 days (Fig. 5). *Phormidium* sp. produced high chlorophyll a content during 1 to 2 days (0.171 to 0.204 μg/mg dw) and at the third day, *Phormidium* sp. produced low carotenoid (Fig. 5). *Chroococcus pallidus* had the maximal carotenoid accumulation in 2 to 6 days and then its content decreased (Fig. 5). Therefore, Total carotenoid contents significantly ($P<0.05$) increased in *Phormidium* sp. *in comparison to Microchaete tenera* var._major *Anabaena* sp., and *Chroococcus pallidus* strains (Fig. 5). The lowest carotenoid contents belonged to *Anabaena* sp. (Fig. 5).

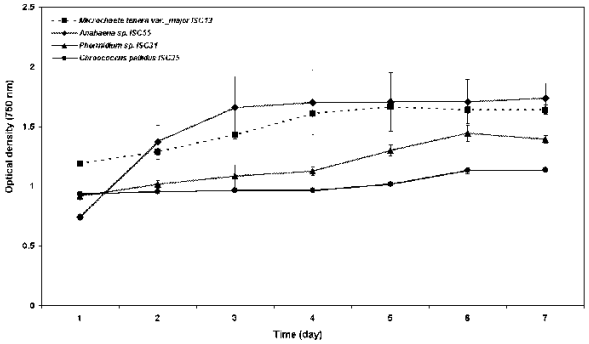

Fig. 1. Optical density of four cyanobacterial strains from paddy-field soil (Data are mean values of three experiments±SE).

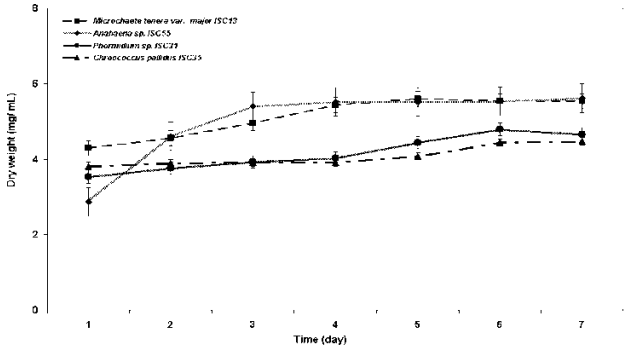

Fig. 2. Dry weight of four cyanobacterial strains from paddy- field soil (Data are mean values of three experiments±SE).

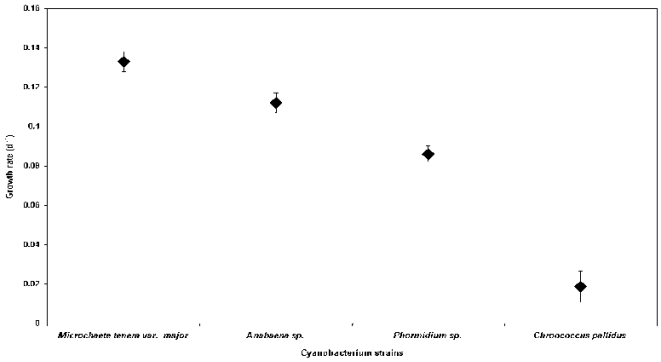

Fig. 3. Growth rate of four cyanobacterial strains from paddy- field soil (Data are mean values of three experiments±SE).

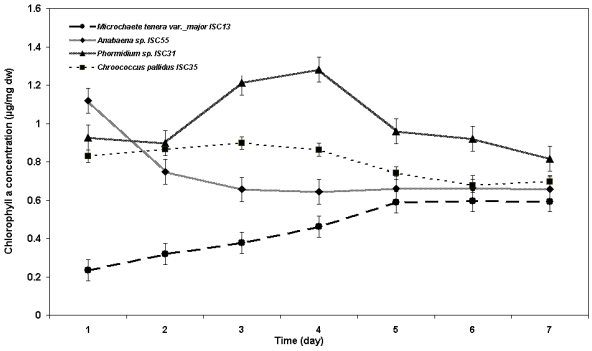

Fig. 4. Chlorophyll a contents of four cyanobacterial strains from paddy- field soil (Data are mean values of three experiments±SE).

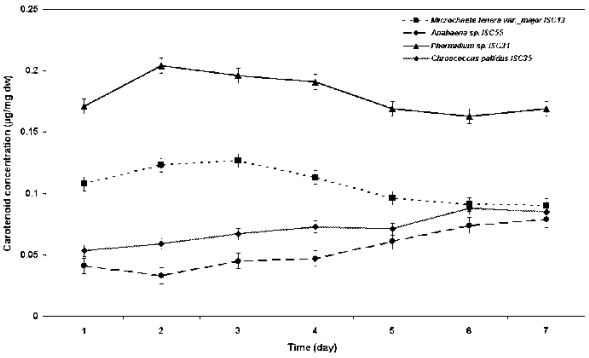

Fig. 5. Carotenoid contents of four cyanobacterial strains from paddy- field soil (Data are mean values of three experiments±SE).

Phormidium sp. and *Chroococcus pallidus* produced the highest (0.404 µg/mg dw) and lowest (0.127 µg/mg dw) soluble proteins, respectively (Fig. 6). The heterocystous strains, *Microchaete tenera* var._major exhibited maximum soluble protein content (0.356 µg/mg dw) in comparison to *Anabaena* sp. (0.24 µg/mg dw) (Fig. 6). In general, the ability to synthesize soluble proteins by the isolates of unicellular was markedly less than the ability observed by the heterocystous and non-heterocyctous forms.

We also evaluated nitrogen-fixing rates, as cyanobacteria are capable of fixing the atmospheric nitrogen.

Anabaena sp. (16.963 nmol ethylene/mg dw h) was the most efficient in terms of nitrogenase activity and *Chroococcus pallidus* (4.696 nmol ethylene/mg dw h) was the least efficient. Interestingly, *Anabaena* sp. showed the highest growth and *Chroococcus pallidus* poor growth in terms of optical density and dry weight. Nitrogenase activity of *Anabaena* sp. and *Microchaete*

tenera var._major significantly (P<0.05) increased in comparison to that of other two strains (Fig. 7).

Photosynthetic oxygen evolution of *Anabaena* sp. and *Chroococcus pallidus* significantly (P<0.05) increased in comparison to *Microchaete tenera* var._major ISC13 and *Phormidium* sp. (Fig. 8).

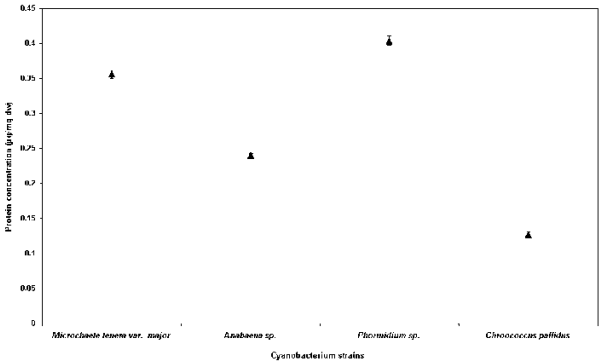

Fig. 6. Protein contents of four cyanobacterial strains from paddy- field soil (Data are mean values of three experiments±SE).

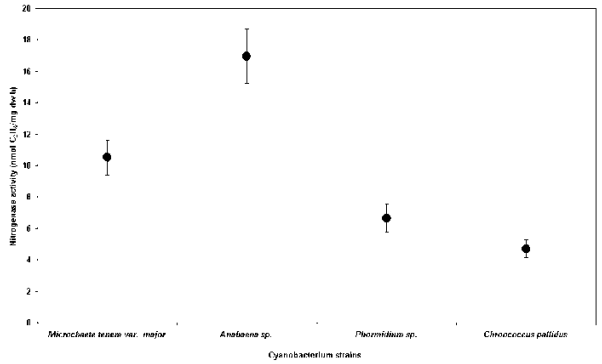

Fig. 7. Nitrogenas activity of four cyanobacterial strains from paddy- field soil (Data are mean values of three experiments±SE).

The results obtained from the biological activity of antimicrobial agents produced by selected cyanobacteria against different species of bacteria (Gram-positive bacteria: *Bacillus cereus* PTCC 1015 and *Enterococcus faecalis* PTCC 1237 and Gram-negative bacteria: *Pseudomonas aeruginosa* PTCC 1074, *Escherichia coli* PTCC 1047, *Klebsiella punemonia* PTCC 1053 and *Salmonella typhi* PTCC 1108) were recorded in Table 1.

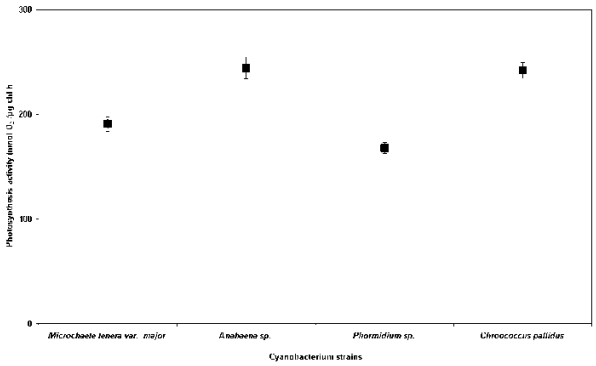

Fig. 8. Photosynthesis activity of four cyanobacterial strains from paddy- field soil (Data are mean values of three experiments±SE).

According to Table 1, the diameter of the inhibition zone depends mainly on the type of the species, the solvent used and the tested bacterial organisms. Antibacterial effects revealed that methanol extracts of *Microchaete tenera* var._major had the maximum biological activity against *Bacillus cereus* PTCC 1015*, Enterococcus faecalis* PTCC 1237, *Escherichia coli* PTCC 1047 *and Pseudomonas aeruginosa* PTCC 1074 similar results obtained from methanol extracts of *Chroococcus pallidus* against *Klebsiella punemonia* PTCC 1053 and *Salmonella typhi* PTCC 1108. No inhibition was observed by petroleum ether and aqueous extracts of all studied cyanobacteria. But, the results indicated that petroleum ether extracts of *Chroococcus pallidus* and *Phormidium* sp. gave biological activities against *Salmonella typhi* PTCC 1108 and *Bacillus cereus* PTCC 1015.

Among the studied cyanobacteria, *Microchaete tenera* var._major, *Anabaena* sp. and *Chroococcus pallidus* showed a widespread spectrum of antibacterial activities.

Antimicrobial index of species with activity against *Bacillus cereus* PTCC 1015, varied between 16 to 60. It varied in other cases; for instance 71-92 against *Enterococcus faecalis* PTCC 1237, 31-62 against *Pseudomonas aeruginosa* PTCC 1074 ,18-40 against *Klebsiella punemonia* PTCC 1053, 60-70 against *Escherichia coli* PTCC 1047 and 30-60 against *Salmonella typhi* PTCC 1108.

On the other hand, *Microchaete tenera* var._major exhibits the maximum antimicrobial index in the methanol extract against *Bacillus cereus* PTCC 1015 (60), *Enterococcus faecalis* PTCC 1237 (92), *Escherichia coli* PTCC 1047 (70) and *Pseudomonas aeruginosa* PTCC 1074 (62).

The effects of extracts obtained from cyanobacteria were lower than those of standard antibiotic used in this study.

Table 1. Antimicrobial activity of different cyanobacterial extracts as presented by inhibition zone diameter (in mm) and antimicrobial index (in parentheses).

Cyanobacterial strains	organisms	*B.c*	*E.f*	*P.a*	*E.coli*	*K.p*	*S.t*
Microchaete tenera var._major ISC 13	A	15 (60)	13 (92)	10 (62)	7 (70)	6 (27)	4 (40)
	B	-	-	-	-	-	-
	C	-	-	-	-	-	-
Anabaena sp. ISC55	A	12 (48)	12 (85)	5 (31)	6 (60)	4 (18)	5 (50)
	B	-	-	-	-	-	-
	C	-	-	-	-	-	-
Phormidium sp. ISC31	A	10 (40)	10 (71)	-	-	7 (31)	4 (40)
	B	-	-	-	-	-	-
	C	-	-	-	-	-	3 (30)
Chroococcus pallidus ISC35	A	14 (56)	11(78)	7 (43)	-	9 (40)	6 (60)
	B	-	-	-	-	-	-
	C	4 (16)	-	-	-	-	4 (40)
Control (Gentamicin)		25 (100)	14 (100)	16 (100)	10 (100)	22 (100)	10 (100)

A- Methanol extract**; B-** Aqueous extract**; C-** Petroleum ether extract
B.c- Bacillus cereus PTCC 1015*; E.f- Enterococcus faecalis* PTCC 1237*; P.a- Pseudomonas aeruginosa* PTCC 1074*; E.coli- Escherichia coli* PTCC 1047*; K.p- Klebsiella punemonia* PTCC 1053*; S.t- Salmonella typhi* PTCC 1108

As mentioned, the quantity of the compound produced antimicrobial activity measured by minimum inhibitory concentration of active crude extracts and results have been shown in Table 2.

Table 2. Minimum Inhibitory Concentration (MIC) of cyanobacterial active extracts.

Sample	*Microorganisms*	*27*	*13.5*	*6.75*	*3.37*	*1.68*	*0.843*	*0.421*	*0.210*	*0.105*	*MIC*
Microchaete	*P.a*	-	-	+	+	+	+	+	+	+	13.5
tenera	*E. coli*	-	+	+	+	+	+	+	+	+	27
var._major	*B. c*	-	-	-	-	-	+	+	+	+	1.68
ISC 13	*E.f*	-	-	-	+	+	+	+	+	+	6.75
	K.p	-	+	+	+	+	+	+	+	+	27
	S.t	-	+	+	+	+	+	+	+	+	27
Anabaena	*P.a*	-	-	-	+	+	+	+	+	+	6.75
sp. ISC55	*E.coli*	-	+	+	+	+	+	+	+	+	27
	B.c	-	+	+	+	+	+	+	+	+	27
	E.f	-	-	+	+	+	+	+	+	+	13.5
	K.p	-	+	+	+	+	+	+	+	+	27
	S.t	-	+	+	+	+	+	+	+	+	27
Phormidium	*B.c*	-	+	+	+	+	+	+	+	+	27
sp. ISC31	*E.f*	-	+	+	+	+	+	+	+	+	27
	K.p	-	+	+	+	+	+	+	+	+	27
	S.t	-	+	+	+	+	+	+	+	+	27
Chroococcus	*P.a*	-	-	+	+	+	+	+	+	+	13.5
pallidus	*B.c*	-	+	+	+	+	+	+	+	+	27
ISC35	*E.f*	-	-	-	+	+	+	+	+	+	6.75
	K.p	-	+	+	+	+	+	+	+	+	27
	S.t	-	+	+	+	+	+	+	+	+	27

(-): No growth observed; (+): Growth observed; Concentration of extracts in µg/mL
B.c- Bacillus cereus PTCC 1015*; E.f- Enterococcus faecalis* PTCC 1237*; P.a- Pseudomonas aeruginosa* PTCC 1074*; E.coli- Escherichia coli* PTCC 1047*; K.p- Klebsiella punemonia* PTCC 1053*; S.t- Salmonella typhi* PTCC 1108

The results indicated that The MICs of active crude extracts of *Microchaete tenera* var._major, *Anabaena* sp. and *Chroococcus pallidus* against *Pseudomonas aeruginosa* PTCC 1074 were 13.5, 6.75 and 13.5 µg/mL, respectively. Also, The MICs of active extracts of *Microchaete tenera* var._major and *Anabaena* sp. against *Escherichia coli* PTCC 1047, *Klebsiella punemonia* PTCC 1053 and *Salmonella typhi* PTCC 1108 were 27µg/mL. Whereas the active substances of *Microchaete tenera* var._major, *Anabaena* sp., *Phormidium* sp. and *Chroococcus pallidus* presented MIC values of 6.75, 13.5, 27 and 6.75 µg/mL against *Enterococcus faecalis* PTCC 1237 and so 1.68, 27, 27, 27 µg/mL against *Bacillus cereus* PTCC 1015. The results indicated that The MICs of active extracts of both *Phormidium* sp. and *Chroococcus pallidus* against *Klebsiella punemonia* PTCC 1053 and *Salmonella typhi* PTCC 1108 were 27 µg/mL.

Therefore, it appears that *Microchaete tenera* var._major had the highest antimicrobial activity range in both agar- well diffusion and MIC methods. It seems that this species may have potent antimicrobial activity and can be the subject of future investigations.

Discussion

Four of the genera (*Michrochaete, Anabaena, Phormidium,* and *Chroococcus*) have been described as common cyanobacteria in rice field soils, and have been isolated from various rice fields located in Iran. In those studies, which are based on culture-dependent methods, the highest growth rate, dry weight and optical density were recorded by the heterocystous cyanobacteria, *Microchaete tenera* var._major and *Anabaena* sp. while the lowest once was recorded by *Phormidium* sp. and *Chroococcus pallidus*. Meanwhile, *Phormidium* sp. showed the highest value of chlorophyll a, carotenoids and protein. The lowest values of chlorophyll a, carotenoids and protein contents were recorded by *Microchaete tenera* var._major, *Anabaena* sp. and *Chroococcus pallidus*, respectively. The highest values of nitrogenase and photosynthetic activity were recorded by *Anabaena* sp. Results obtained were interesting indicating a clear differential response exhibited by the various isolates.

Tiwari *et al*., (2005) reported seven genera of cyanobacteria isolated from arid zones of Rajasthan. In this study, *Phormidium* species showed maximum chilorophyll accumulation in comparison to heterocystous forms. Also, non-heterocystous forms, like *Phormidium* and *Oscillatoria*, also accumulated high levels of soluble proteins (Tiwari *et al*., 2005). Therefore, these results are similar to the concluded data of present study. Differential mechanisms of

tolerance might be responsible for the variability observed in the parameters examined amongst the isolates (Potts, 1994; Potts and Bowman, 1985).

In a survey of 102 rice soils from Philippines, India, Malaysia and Portugal, found that heterocystous cyanobacteria were present in all samples (Roger *et al.,* 1987). Under natural conditions in rice fields, cyanobacteria are exposed to the combined influences of several factors such as pH and irradiance, which vary both daily and over the crop cycle (Quesada *et al.,* 1995, 1998). Growth, biochemical and physiological characteristics of cyanobacteria are influenced by environmental factors (Grossman *et al.,* 1993; Soltani *et al.,* 2006).

Similar studies on ecophysiology of desert community particularly on non-heterocystous cyanobacteria have carried out by Potts and Friedmann (1981) who have observed variability in various parameters examined in the strains predominantly harbouring deserts. The physiological effects of environmental parameters on cyanobacteria are similar to those in prokaryotic organisms because water stress in arid areas and other areas in known to affect cells turgidity, the active sites and structural integrity of membranes, proteins and other vital biomolecules (Potts, 1994). In arid areas, the critical effect of dehydration is to increase osmotic stress so that the concentration of cytoplasmic solutes must increase as a protective response to the decrease in external water potential. From the studies undertaken, it can be concluded their colonization potential as indicated by their isolation from these areas can be attributed to the tolerance mechanisms and avoidance strategies developed by these organisms under stress situations (Kempf and Bremer, 1998).

The above findings which indicate the discovery of novel chemicals, can lead us to the development of pharmaceuticals by cyanobacteria. Cyanobacteria are potential sources of new active substances for medicine and pharmacy, from which numerous active compounds have been isolated (Falch *et al.,* 1995; Jaki *et al.,* 1999; Moore *et al.,* 1989). Possibly, the synthesis of highly active toxin can be a defense option of cyanobacteria in these environments against other organisms like bacteria, fungi, viruses, and eukaryotic microalgae (Mundt *et al.,* 2001). The results obtained from the present work are in harmony with those obtained by Volk and Furkert (2006). They found that certain cyanobacteria had high biological activity against *Bacillus subtilis, Bacillus thuringiensis, Bacillus megaterium, Escherichia coli, Pseudomonas aeruginosa, Candida tropicalis* and *Saccharomyces cerevisiae*.

Ghasemi *et al.* (2003) have surveyed antibacterial activities of paddy-fields cyanobactria of northern Iran. Following this research, a novel antimicrobial substance named parsiguine has been identified (Ghasemi *et al.*, 2004). According to a study of antimicrobial activity of strains belonging to rice

fields, culture media of cyanobacteria belonging to Nostocaceae, Microchaetaceae and Scytonemaceae isolated from the Argentine paddy fields were found to be active against *Staphylococcus aureus* and *Candida albicans* (De Caire *et al.,* 1993). In another study it was shown that cyanobacteria from the paddy fields of Northern Thailand produce bioactive substances with antibiotic activity against *Bacillus subtilis* (Chetsumon *et al.,* 1993).

Ozdemir *et al.* (2001) found that extracts of *Spirulina* obtained by different solvents exhibited antimicrobial activity on both Gram-positive and Gram-negative organisms. Many investigators mentioned that the methanol extracts of *Nostoc muscorum* revealed antibacterial activity on *Sclerotiorum* (De Mule *et al.,* 1991; Ishida *et al.,* 1997).

Also, the methanol extract of cyanobacteria has been investigated by Prashantkumar *et al*. (2006) for *in vitro* antimicrobial activity against *Proteus vulgaris, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus* and *Phizopus nigricans* using agar cup-plate method. Although it was found in the present study that antibacterial activity of the methanol extracts was higher against Gram positive bacteria than that of Gram negative bacteria, the same results have been observed in other studies (Ghasemi *et al.,* 2003, 2004; Soltani *et al.,* 2005). Generally, antibiotics are less effective against Gram negative bacteria because of their more complex multilayered cell wall structure, which makes it more difficult for the active compound to penetrate (Ördög *et al.,* 2004).

Among the studied cyanobacteria, *Phormidium sp*. exhibited the minimum activity against the test microorganisms. This is in agreement with the results of Ghasemi *et al*., (2007).

The MICs of active crude extracts of *Microchaete tenera* var._major, *Anabaena* sp. and *Chroococcus pallidus*, respectively, had a wide range of inhibitory activity against those bacteria that has already been described in other studies (Ghasemi *et al.,* 2003, 2004; Soltani *et al.,* 2005).

Among all of the cyanobacteria species studied in the present work for monitoring antibacterial activity, it seems that *Microchaete tenera* var._major is being reported for the first time as the producer of antibacterial substances. This strain showed a good activity against the test microorganisms.

The antimicrobial activity of cyanobacteria can be explained by the presence of cyclic peptides, alkaloids and lipopolysaccharides (Abedin and Taha, 2008). In particular, the identities and the chemical origins of bioactive compounds of *Microchaete tenera* var._major need to be elucidated and their beneficial effects as growth inhibitions on some pathogenic bacteria and fungi must be examined. In conclusion, cyanobacteria could be considered as a potent

biological source of antimicrobial activity and are of special interest in the development of a harmless environment.

This study revealed that this kind of investigation creates very general view towards cyanobacteria for their possible use in agricultural productivity (biofertilizer) and production of various secondary metabolites including pharmaceuticals. Basic research is needed to identify new cyanobacterial strains of high value products.

Acknowledgments

This research was supported by grant from Department of Biology, ACECR, Research Institute of Applied Science, Tehran, Iran.

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(Received 4 November 2010; accepted 20 March 2011)