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The Study of Cyanobacterial Flora from Lamazan Hot Springs of West Hormozgan, Iran

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ABSTRACT: Cyanobacteria, known as blue-green algae, blue-green bacteria, cyanoprokaryots, and cyanophytes, are oxygenic photosynthetic prokaryotes Cyanobacteria show considerable morphological diversity. They are morphologically, physiologically and metabolically very diverse group and have been the thrust area for investigation since long. The blue-green algae are considered to be thermophilic when part or all of their optimal growth temperature range is above 40°C. Only a few species of eucaryotic protists or animals tolerate higher temperatures. Hot springs and their drain ways provide the most abundant aquatic habitats for thermophilic blue-green algae. Therefore, The present investigation involves study of mat forming cyanobacterial flora from Lamazan hot springs located in west part of Hormozgan province. This thermal spring are inhabited by characteristic thermophilic organisms including cyanophyta. Cyanophyta are among the few organisms that can occupy high temperature aquatic environments including hot springs. In alkaline and neutral hot springs and streams flowing from them cyanophyta can form thick colourful mats that exhibit banding patterns. Sampling was done based on temperature gradients; physicochemical analysis of the water was performed due to a dramatic effect on species distribution, Samples collected at different temperature ranges and were divided into two parts, One part was fixed in 4% formaldehyde to minimize quantitative and qualitative changes in phytoplankton and the other part were kept in a refrigerator for provide the pure isolates. Taxonomic determination was carried out by morphometric study of the isolates with light microscopy, based on Desikachary, Prescott, Whitford and Schumacher, John et al. and Wehr et al. by prepared semi-permanent slides. The vegetative and reproductive characters used in the taxonomic determination were: shape, colour and size of the cell, colony, thallus and trichome; as well as their width and length; shape, size and colour of vegetative cells. As result of this study, Total of 23 thermophilic cyanobacterial species representing 2 orders, 6 families and 10 genera were recorded.
Key words: Blue Green Algae, Hot Springs , Thermophilic, Hormozgan Province

INTRODUCTION

Cyanobacteria, also known as blue-green algae, blue-green bacteria, cyanoprokaryots, and cyanophytes, are oxygenic photosynthetic prokaryotes that possess features familiar to both bacteria (prokaryota) and algae (eukaryota). Their cell structure and composition are similar to those of prokaryotic cell in that they lack the cell nucleus and distinctive organelles of eukaryotic cell, and their special structure and chemical composition of the cell wall are basically the same as those of gram-negative bacteria [1 and 2]. However, in contrast to typical prokaryotes, they contain chlorophyll a and several accessory pigments providing them with oxygenic photosynthetic ability like other algae, and their bluegreen color. They have two photosystems (PSII and PSI) and use water as an electron donor during photosynthesis, leading to the production of oxygen. Several cyanobacteria can also perform anoxygenic photosynthesis using only photosystem I if electron donors such as hydrogen sulphide are present. All the known cyanobacteria are photoautotrophic, using primarily CO2 as carbon source [2]. The environmental condition prevailing during the origin of cyanophyta in Precambrian included high temperature, poor amount of free oxygen, high concentration of sulfur and lots of reducing gases like methane, ammonia, carbondioxide. These environmental conditions are similar to those of present day thermal springs [3].Few organisms such as unicellular prokaryotes known as thermophiles from the domain of Archaea and Bacteria can withstand these hostile environments. In microbial mats of geothermal springs, highly developed cyanobacterial mats are common at temperature less than 74°C and pH level above 5.0. Synechococcus, Phormidium, Calothrix, Mastigocladus are some of the cyanobacterial taxa which are found in thermal springs. However their distribution pattern varies with temperature range.The cyanobacteria living in thermal springs of the world have been studied by many workers [3, 4 and 5]. Blue-green algae (BGA) are the largest group of photosynthetic prokaryotes identified by a variety of names, including cyanobacteria, blue-greens, blue-green algae, myxophyceans, cyanophytes, cyanophyceans, and cyanoprokaryotes that exist in large diversity and distribution in the world [1].Geographic position of Iran determines high diversity of its nature. Mountains and

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plains, wet subtropics with annual precipitation up to 2000 mm and dry areas of rocky and sandy deserts and salt marshes (a.p. 100 mm), coasts of the Caspian and Oman seas – such conditions result in high variety of water bodies types with wide range of salinities, pH values and temperatures [7]. Cyanobacteria are known to inhabit the various extreme environments with high and low temperatures, in very dry climate, and in high concentration of salts [8]. Therefore, for Iran with its original nature, it is interesting to reveal specific composition and distribution of blue-green algae division that has adapted to extreme conditions of the environment. Information about the blue-green algae of Iran's different water bodies are limited and presented by small groups in publications such as Woronichin [9], Löffler [10,11], Hirano [12], Wasylik [13], and Compere [14]. Cyanobacteria in hot springs are largely unexplored, Hot springs are well-isolated habitats occurring as clusters in globally distant regions, and the microorganisms that inhabit them are extremophiles adapted to conditions quite different from the ambient milieu (e.g. air, water) through which they would have to disperse. Cyanobacteria are well represented in this hardy group, which owe their survival to unusual genetic adaptations that protect the organism. The hot spring mat communities in terrestrial habitats have long attracted the attention of microbial ecologists because of the unique adaptations of the microbial flora to these harsh environments [15]. During the survey of literature it was observed that most of the screening efforts have been done using mesophilic cyanobacteria [16] and no reports exist so far on thermotolerant or thermophilic genera except one Therefore, present study was aimed to screen and survey distribution of thermophilic cyanobacterial cultures isolated from Lamazan thermal springs in Hormozgan.

MATERIALS AND METHODS

Collection site

Lamazan hot spring chosen for the present study that are usually covered with cyanobacterial mats all year round (fig.1), this spring located in eastern part of Hormozgan province which is characterized by highly alkaline water and high temperature

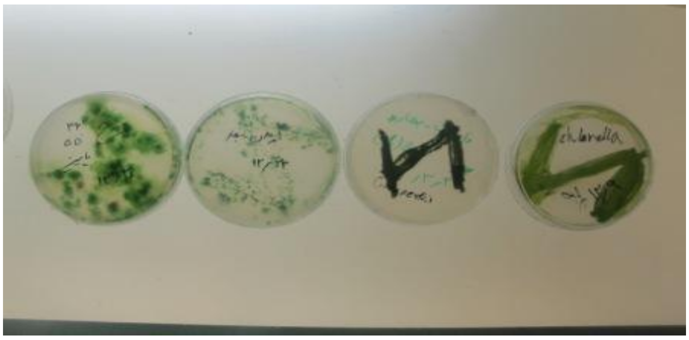
Physico-chemical analysis of hot spring water

Due to the influence of physic- chemical properties of water in the species distribution, Water samples were collected to be analyzed for chemical factors, including, total hardness (TH), Total Dissolved Solid(TDS), alkalinity, Potassium (K^+), Calcium (Ca^{++}), Sodium (Na^+) , Ammonium (NH_4^+) ,; nitrite (NO_2^-), sulphate (SO_4^{-2}), dissolved oxygen (DO), chloride (Cl^-) and inorganic phosphate (PO_4^{-3}) Spring water temperature was measured in the field by thermometer. Analyses were performed in the laboratory by standard methods according to Iran Standard (1053).

Sampling and morphological study

The mass was divided into two parts after transferring the samples to the laboratory, in order to obtain pure culture and isolation was kept in refrigerator one sample and other part was fixed using 4% formalin for taxonomically determination. For microscopic study, semi-permanent preparations in glycerine were made and sealed with enamel paint. Sketches were prepared with the help of camera lucida and Olympus binocular microscope and various dimensions were measured with the help of calibrated ocular micrometer, Taxonomic determination was carried out based on Komrek and Anagnostidis [17] and latest morphological article up to species level. The vegetative and reproductive characters used in the taxonomic determination were: shape, color and size of the cells and thallus; width and length of trichomes; shape, size and color of vegetative cells, apical cell shape, presence or of mucilage sheath .Cell length and width measurements were made for 10 individuals of each species. Species constrbuting hot spring cyanobacterial mats were isolated from water samples by an agar plate method using BG11 medium [1]. for purifying blue-green algae, alternative culture methods were used, Algal samples were transferred onto sterile solid medium, The medium used in this study was the BG-11 medium, After sufficient growth of algae on solid media ,pure algae were obtain through several re-culturing (fig 2).

Fig 1. Lamazan hot spring



A B C D

Fig 2. A and B impure culture of algae 3, pure culture (single cell) 4, pure culture (Filamentous)

Table1. Physicochemical analysis of Lamazan hot spring

Order	Family	Subfamily	Genus	56 °c	53°c	4°c	43°c	41°c
Oscilatoriales	Pseudanabaenaceae	Pseudanabaenaoideae	Jaaginema	Angustissimum	Angustissimum	Angustissimum	Angustissimum	Angustissimum
				Metaphyticum	Metaphyticum	Metaphyticum	Metaphyticum	Metaphyticum
				Pseudogeminatum	Pseudogeminatum	Pseudogeminatum	Pseudogeminatum	Pseudogeminatum
				Quadripunctuata				
			Limnothrix		Redeki	Redeki		Redeki
								Obliqueacuminatum
			Pseudanabeana		Catantea			Catantea
								Mucicola
			Geitlerinema		Amphibium	Amphibium	Amphibium	
		Spirulinoideae	Spirulina				Tenerrima	
							Subtlissima	
	Broziaceae		Komvophoron				Bourrellyi	Bourrellyi
								Minutum
	Phormidiaceae	Phormidioideae	Phormidium				Acuminatum	Acuminatum
	Oscillatoriacea	Oscillatorioideae	Lyngbya				Thermalis	Martensiana
								Nigra
								Auesteri
Chroococales	Merismopediaceae	Merismopedioideae	Synechocystis			Aquatilis		Aquatilis
						Minuscola		Minuscola
						Thermalis	Thermalis	Thermalis
	Chroococcaceae		Chroococcus	Thermalis			Minutus	Minutus
							Turgidus	Turgidus
							Membraninus	Membraninus

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Table 2. Physicochemical analysis of Lamazan hot spring

Parameters	Standard no.	Scale	Result
Temperature (during a		°C	25.3
color	2120C	Pt-Co	----
Electrical conductivity(EC)	2510B	µS/cm	27110
Turbidity	2130B	NTU	0.369
PH	HB 4500		6.86
TDS (in180 °c)	2540 C	mg/L TDS	17800
OH^- (mg L^{-1})/ compare with phenol fetalei	2220 .B	mg/L $Caco_3$	0
OH^- (mg L^{-1}) compare with metyle orange	2220. B	mg/L $Caco_3$	165.2
Total hardness (TH)	2240 .B	mg/L $Caco_3$	25.5
Ca^{++}(mg L^{-1})	3500 .B	mg/L Ca	633.26
Mg^+(mg L^{-1})	3500-Mg. B	mg/L Mg	224.77
Na^+(mg L^{-1})	3500-Na. B	mg/L Na	6555
K^+(mg L^{-1})	3500-K .B	mg/L K	84
Fe^{2+}(mg L^{-1})	3500-Fe .B	mg/L Fe	-
Mn^+(mg L^{-1})	3500-Mn. B	mg/L Mn	-
NH_4^+(mg L^{-1})	4500-NH_2.G	mg/L NH3-N	-
Cl^- (mg L^{-1})	4500-Cl.B	mg/L Cl^-	10860
F^- (mg L^{-1}	4500-F.B	mg/L F	5.55
$SO_4{}^{-2}$ (mg L^{-1})	4500-$So_4{}^{2-}$.E	mg/L $So_4{}^{2-}$	1945
PO $_4{}^{-3}$ (mg L^{-1})	4500-P.D	mg/L $Po_4{}^-$	-
$NO_3{}^-$ (mg L^{-1})	4500-No_3.B	mg/L No_3	1.9
$NO_2{}^-$ (mg L^{-1})	4500-No_2.B	mg/L No_2	0.01<

Species identification: Species determination: Cyanobacterial species were observed under microscope within 24 hours of collection. The mat samples temporary preparations were made using 10% glycerol. Microphotographs were taken using Olympus-CH 40 microscope (Olympus, Australia). Identification of cyanobacterial samples were done following Desikachary [18] Komarek and Anagnostidis [17, 19]. Voucher specimens have been preserved in 4% formalin and deposited in department of Botany, Shahid Beheshti University, Tehran, Iran

Isolation from mat: The mat samples taken from spring substrates were washed by double distilled water repeatedly and placed in Petri plates and flask (for enrichment culture) containing BG-11 medium. Homogenized mat samples were also used for plating. All cultures incubated at 35 ± 2°C under 25-30 µmol photons m-2 s-1 light (12 h: 12 h L/D cycle) at incubator. Pure cultures were obtained under aseptic condition by streaking the cells repeatedly on BG- 11 medium agar plate followed by growth in liquid culture medium.

RESULTS

Physico-chemical parameters of four hot springs: Chemical analysis of surface water chemistry (Table 2) indicate that all the springs studied. because of high density of Ca++, Mg+ , Na+ ,nitrate, ammonium, phosphate, silicate and sulfate these springs are part of the brackish and very hard water (TDS >5000 mg L -1,TH > 300 mg L -1) type of hot springs, spring water is transparent and colorless, and Smelling strongly of sulfur and slight alkaline waters (pH > 6).

Mat morphology and cyanobacterial species composition: Results show that microbial mats in geothermal springs are predominated by cyanobacterial flora, The identification of the most representative cyanobacteria of the communities revealed the presence of 23 species, distributed in 10 genera, 6 families and 2 orders (Table 1) A at a temperature range of 41-56°C,total of 17 species belonging to Oscillatoriales (73.91%), 6 species to chroococales (26.08%) and Nostocalean genera were absent in all three thermal springs, Jaaginema , chroococcus and Lyngbya are the most abundant genera with 4 species followed by Spirulina and Synechocystis each with 3 species. The only genus that is present in all the mat communities is Jaaginema, adapted to a thermal gradient of 41-56 °C. as result show One of the most interesting ecological features of most hot springs is the great constancy of temperature and chemistry of the water at the source, species distribution when temperature increase from 41 to 56 species distribution notably decrease as in 56 C $_o$, there are a few species (6) in compare to 41 $_o$ C with 19 species (Fig 1).

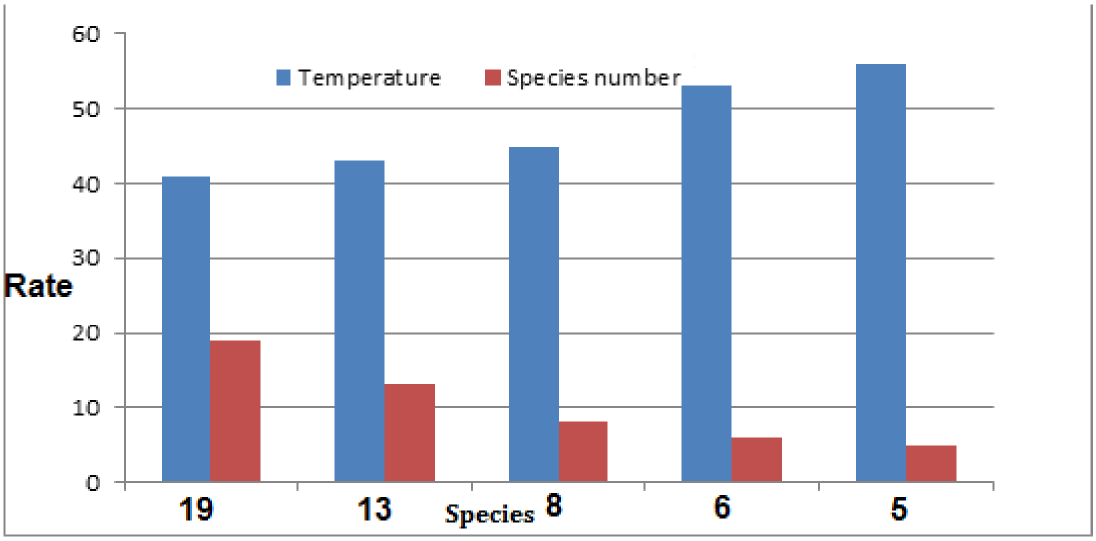

Fig1. Species distribution in temperature gradient

DISCUSSION

Several authors have commented on the dominance of cyanobacteria in different thermophilic mats [6]. Studies on cyanobacterial diversity in low temperature thermal springs have been carried out earlier. Temperature is one of the most important parameter for cyanobacterial species diversity in microbial mat of hot springs. The studies revealed that cyanobacterial diversity and complexity decreased with increasing temperature ((Ferris 1996) , Results of this study also support the previous studies as if there is 19 species in 41°C in contrast only 5 species in 56°C. Some authors reported the dominance of unicellular forms of cyanophyta like *Synechococcus* in thermal gradients from 50 °C to 75 °C but in lower temperature between 40 °C to 50 °C filamentous form like *phormidium* , *Oscillatoria*, etc are dominant. Present study show the filamentous cyanophyceae, jaaginema can live in all temperature gradients between 41°C to 56°C and other unicellular and filamentous form present in 41°C to 56°C . Diazotrophic cyanobacteria are able to colonize in the springs where nitrogen levels are lower than proper condition for the other taxa. Conversely, they may be out-competed by non diazotrophic cyanobacteria in spring with sufficient combined nitrogen [6]. In this study in hot springs with high levels of combined nitrogen, diazotrophic cyanobacteria are absent.

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