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The analysis of morphological acclimation of cyanobacterium *Leptolyngbya* sp. Isc25 to combined treatment of mixotrophe-red light rays

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ABSTRACT

There are few surveys about morphological adaptation on cyanobacteria to monochromatic light. Also very limited studies were done on the evaluation of their behavior. In this research, the effect of monochromatic red light rays at treatment of liquid and solid of mixotrophe including 0.05 percent of soluble sugars (glucose, fructose and sucrose) on morphological responses, communities, structural changes, biometry and situation of trichome of Leptolyngbya sp. were studied. This investigation was carried out in weekly and daily periods of time. Also, molecular identification of partial sequence of 16S rRNA was determined. Morphological and biometrical study indicates that change in nutritional conditions and the quality of light could be the causes of this type of cellular changes. Due to the morphological variability of cyanobacteria in different conditions, molecular and genetics studies should be performed for identify, taxonomy and systematic of them.

Key words: *Leptolyngbya* sp., Cyanobacteria, Mixotrophe, Red light, Masjed Soleiman.

INTRODUCTION

Cyanobacteria are one of the prokaryotic organisms groups that growth forms range from uni-to multicellular. These various growth forms have enabled cyanobacteria to inhabit almost every terrestrial and aquatic habitat on Earth [30]. Based on fossil records, Cyanobacteria have occurred as long ago as 2,600 to 3,500 million years (Myr), [5, 15, 32]. These earliest cyanobacteria are believed to have been played an important role in producing an oxygen-rich atmosphere on earth about 2,300 Myr ago [4]. These organisms are identified according to their morphological characters such as morphology of vegetative cells, akinetes and heterocytes [27, 28]. Due to existence of changeable morphology in these organisms, other techniques such as molecular techniques are used to improve cyanobacterial taxonomy [21, 35]. Several properties of the 16S rRNA gene, such as evolutionary properties and ubiquity, have allowed it to become the most commonly used molecular marker to distinguish and establish relationships between cyanobacterial genera and species [6]. The environmental factors such as light are playing an important role in the production and combination of the photosynthetic pigments [3, 12, 34]. Light intensity and quality are the most important environmental factors influencing the photosynthetic pigments synthesis in cyanobacteria [36]. Mixotrophic culture is a potential mode for mass production of cyanobacteria by using heterotrophic capability of them [7, 20]. This type of culture medium can achieve high cell densities and synthesize light-induced products such as photosynthetic pigments and was especially suitable for the production of high value bioactive compounds [10]. The aim of this investigation was to study the morphological responses and structural changes *Leptolyngbya* sp. Isc25 to monochromatic red ray and mixotrophe - red monochromatic ray combined treatment.

MATERIALS AND METHODS

Isolation of strain

The strain *Leptolyngbya* sp. Isc25 was isolated from soils in the Masjed Soleiman region (x=0339545 y=3535959) of Iran. Isolation and purification was made by ordinary methods [2]. Following achievement of axenic culture, cyanobacterium was cultivated in liquid and solid BG-11 medium. Preliminary identification of this species was performed by [1, 8, 9, 16, 26].

Culture conditions

Stock cultures were grown in the solid BG-11 medium. Temperature was maintained at 30±1°C and cultures were bubbled with air under a constant light intensity of 60 µmol photon m^{-2} s^{-1} supplied by three white fluorescent tubes. Cells in logarithmic phase of growth were collected from stock cultures and used as inoculate for experiments. Three sets of solid and liquid BG-11 medium were prepared depending on 0.05 percent of soluble sugars (glucose, fructose and sucrose) and then solid and liquid of mixotrophe mediums containing *Leptolyngbya* sp. Isc25 were placed against monochromatic rays of red light and then morphological changes with the control sample (solid and liquid BG-11 medium containing *Leptolyngbya* sp. Isc25 against white rays) were compared.

Morphological studies

For morphological studies, semi permanent slides were prepared every day (for a month). These studies were performed by light (Labomed, X400) and florescence microscopes. Factors such as type meetings, fluidity filament, biometry, status and color of trichome, the size of the vegetative cells, morphology of the terminal cells and presence or absence of sheath were evaluated [27, 28]. More detailed morphological studies were carried out by scanning electron microscope (SEM).

PCR amplification, cloning, and sequence analysis of 16S rRNA

To extract DNA from the *Leptolyngbya* sp. a fresh biomass was prepared by centrifuging at 12000 rpm and using Fermentas kit (k0512). The applied PCR condition has been described by Nübel et al. [23]. PCR amplification, cloning and sequence analysis of 16S DNA content was first extracted from the cyanobactrium and then PCR was applied with using two set of primers [23]. Sequences were amplified using the primers PA (5´- AGA GTT TGA TCC TGG CTC AG -3´) as forward and PH (5´- AAG GAG GTG ATC CAG CCG CA)-3´) as reverse PCR products were obtained by electrophoresis in a 1% (w/v) agarose gel using TBE buffer containing DNA set stain. The sequence was determined by the Cina Gene Company. The sequence data was analyzed using a similarity search by using the BLAST through the website of the NCBI.

Statistical analysis

Analysis of the results was performed using SPSS ver. 18 software, and analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The classification of cyanobacteria has normally relied on morphological specifications which are not always reliable, as they may show variation on form depending on culturing and environmental conditions [22]. These problems of usual morphological classification, together with the deficiency of molecular data, pose serious hindrances for taxonomy of cyanobacteria [14, 17]. In this study morphological observations showed that in *Leptolyngbya* sp. Isc25. is scattered communities and verdant. Right trichome and sometimes curved at the end. Cells oblong to oblong - oval, granules are very vague, the walls have little or no compression, the dimensions of the cells 1/5mµ and 3/04 mµ; apical cell domical or clear rand sometimes has a bent, There are around the cell mucilage sheath is very thin amorphous that hardly visible. Morphological studies of *Leptolyngbya* sp. Isc25 at BG11 liquid medium (control) was performed by photos provided with optical and SEM and fluorescence microscopes that life cycle of *Leptolyngbya* sp. Isc25 and results were seen in figures 1, 2 and 3.

Morphological studies of *Leptolyngbya* sp. Isc25 at combined treatment of liquid mixotrophe - red light show only at medium containing sucrose until the fifth day vegetative cells have survived and at the other treatments were degenerated. Biometrical studies of *Leptolyngbya* sp. Isc25 at liquid BG11 medium (white light) and liquid mixotrophe - red light were shown in figures 5 and 6.

Biometrical studies of Leptolyngbya sp. Isc25 at liquid BG11 medium (white light) and combined treatment of liquid mixotrophe - red light were shown in figures 5 and 6. The pattern of changes in average length and diameter of vegetative cells at liquid BG11 medium (control) and combined treatment of liquid mixotrophe (sucrose) - red light (Fig. 5 and 6) have significant difference (ANOVA, $p<0.05$).

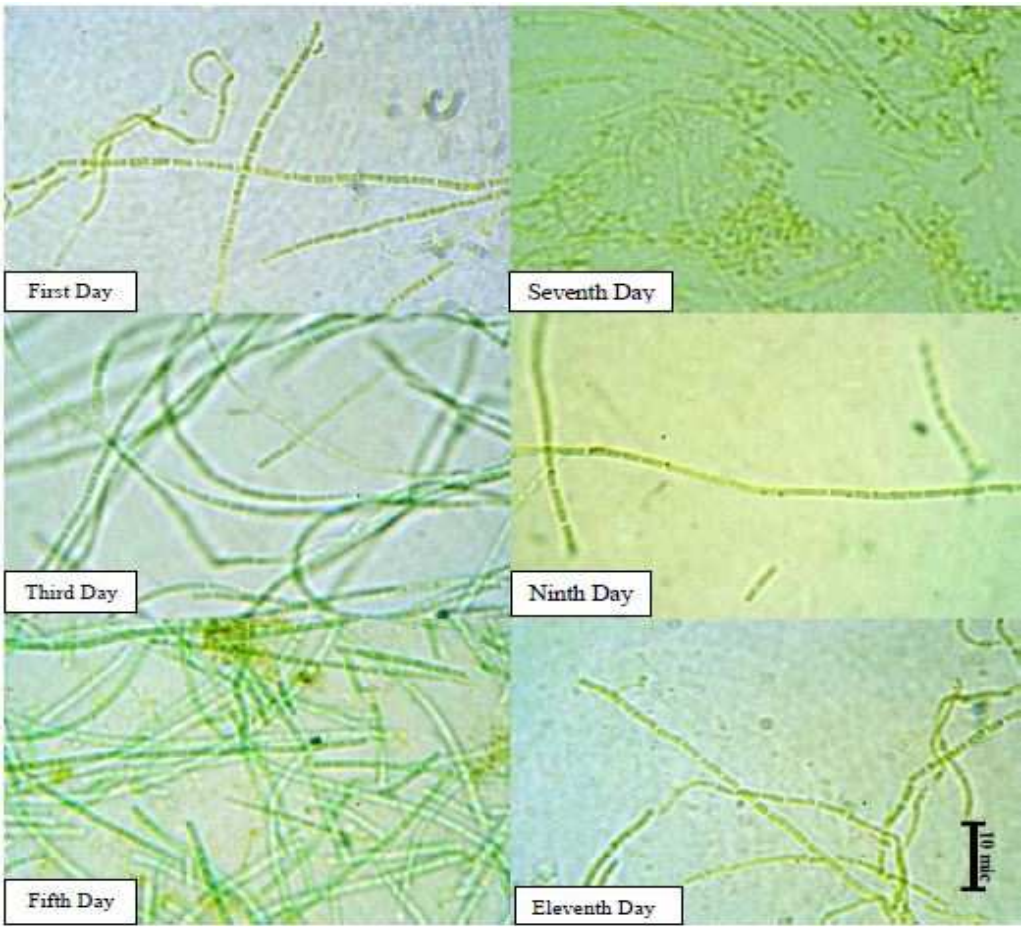

Fig 1.life cycle of *Leptolyngbya* sp. Isc25 at liquid BG11 medium (control) by light microscope(100x)

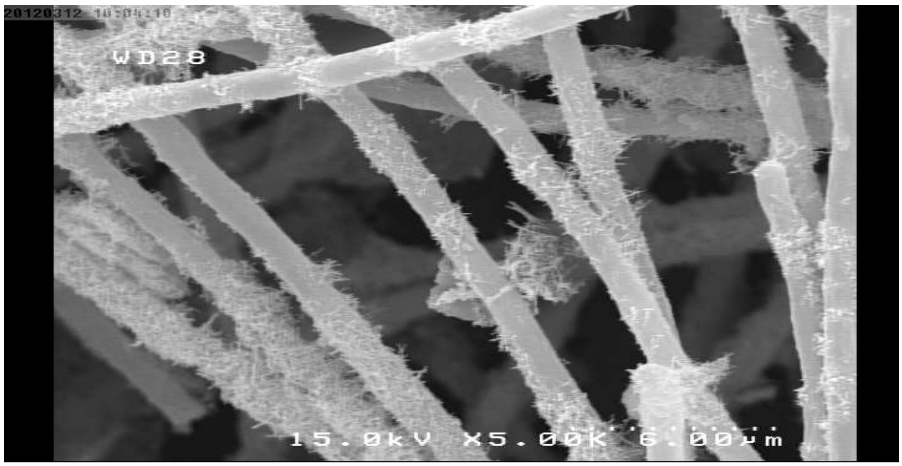

Fig 2. *Leptolyngbya* sp. Isc25 at liquid BG11 medium (control) by SEM microscope

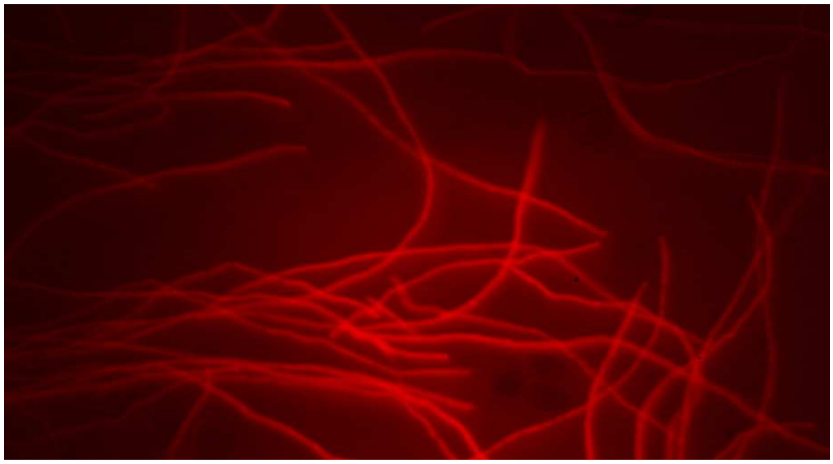

Fig 3. Leptolyngbya sp. Isc25 at liquid BG11 medium (controle) by fluorescence microscope (X40)

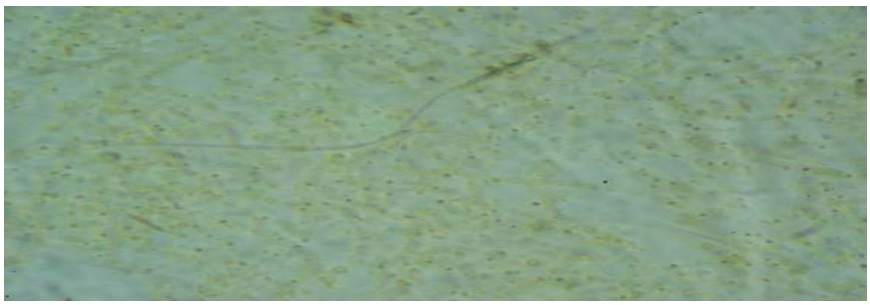

Fig 4. Degenerated cells and brown granules in *Leptolyngbya* sp. Isc25 at liquid mixotrophe (sucrose) - red light on the fifth day by light microscope(X100)

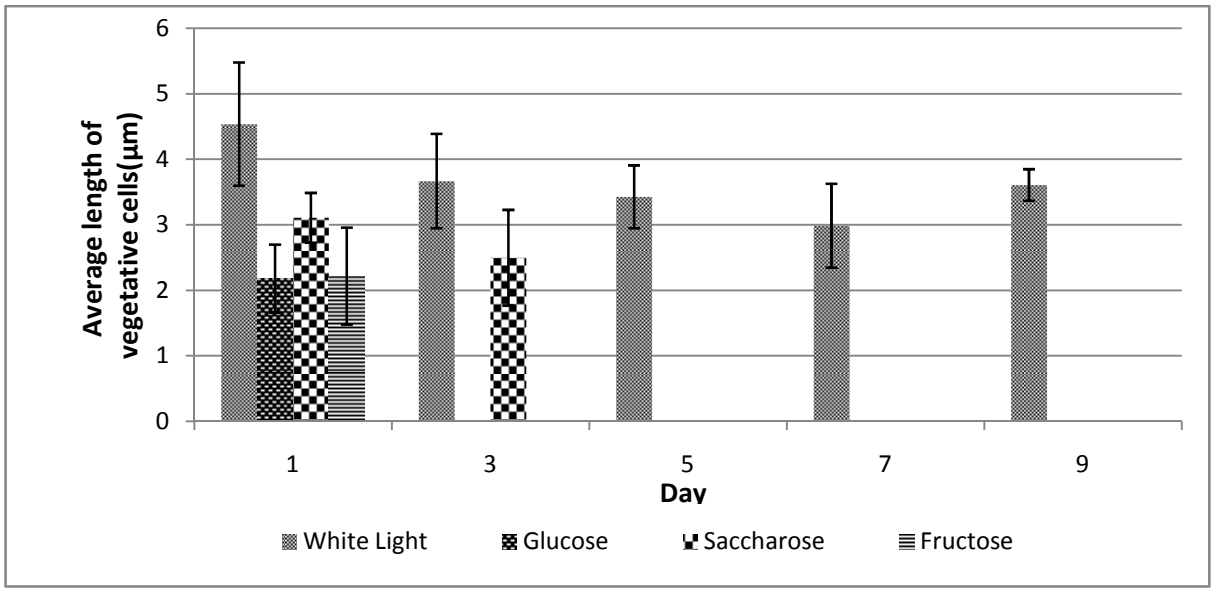

Fig 5. Comparison of the average length of vegetative cells liquid BG11 medium (white light) and liquid mixotrophe - red light

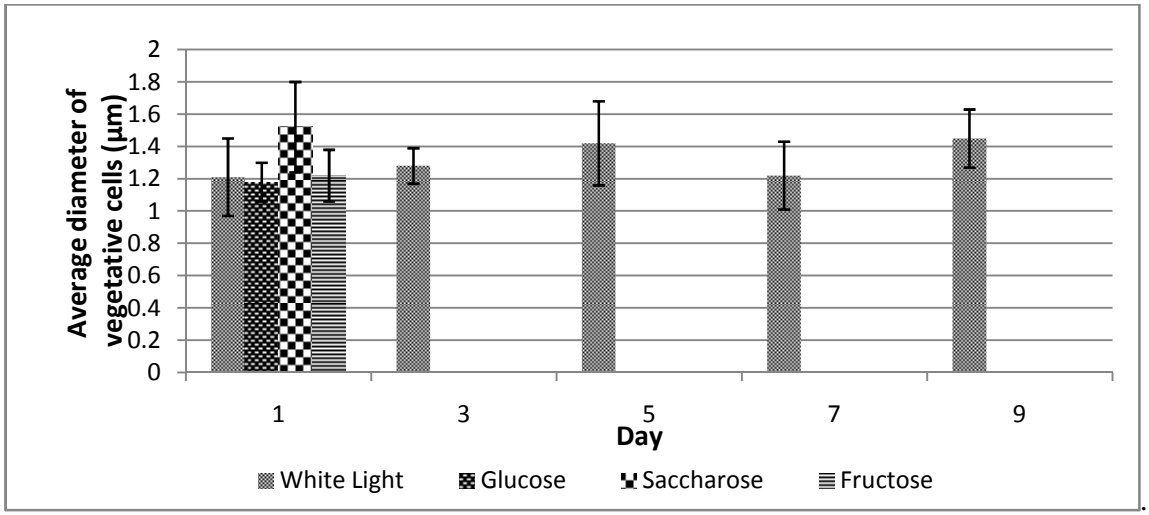

Fig 6. Comparison of the average diameter of vegetative cells at liquid BG11 medium (white light) and liquid mixotrophe - red light

Morphological and biometrical studies of *Leptolyngbya* sp. Isc25 at solid BG11 medium (control) and combined treatment of solid mixotrophe - red light were shown in table 1.

Table 1. Morphological characteristics of *Leptolyngbya* sp. Isc25 at solid BG11 medium (control) and solid mixotrophe - red light

Factor Conditions	Day	Average length of vegetative cells (µm)	Average diameter of vegetative cells (µm)	Communities form	Communities color	Terminal cell form	vegetative cells form	Granules	Mucilage sheath
Control (white light)	1	4.91±0.94	1.35±0.26	Expanded	Dark-green	Domed or flat	Rectangular cube	+	Very thin
	3	8.01±0.87	1.28±0.28	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
	5	6.38±0.65	1.53±0.47	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
	7	3.58±0.89	1.36±0.27	Expanded	Dark-green	Domed or flat	Rectangular cube	+	Very thin
	9	3.42±0.76	1.41±0.18	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
Mixotroph containing glucose and red light	1	2.38±0.79	1.11±0.28	Expanded	Dark-green	Domed or flat	Rectangular cube	+	Very thin
	3	2.15±0.68	1.01±0.62	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
	5	3.11±0.95	1.50±0.56	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
	7	2.72±0.79	1.76±0.45	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
	9	2.36±0.83	1.38±0.31	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
Mixotroph containing saccharose and red light	1	2.43±0.44	1.53±0.47	Expanded	Dark-green	Domed or flat	Rectangular cube	+	Very thin
	3	2.83±0.56	1.55±0.47	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
	5	3.38±0.62	1.54±0.61	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
	7	2.58±0.22	1.55±0.52	Expanded	Dark-green	Domed or flat	Rectangular cube	+	Very thin
	9	2.04±0.49	1.21±0.14	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
Mixotroph containing fructose and red light	1	2.28±0.72	1.42±0.16	Expanded	Dark-green	Domed or flat	Rectangular cube	+	Very thin
	3	2.87±0.33	1.45±0.58	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
	5	2.39±0.57	1.37±0.58	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
	7	3.13±0.55	1.50±0.40	Expanded	Dark-green	Domed or flat	Rectangular cube	-	Very thin
	9	3.13±0.33	1.41±0.55	Expanded	Dark-green	Domed or flat	Rectangular cube	-	-

Morphological studies of *Leptolyngbya* sp. Isc25 at combined treatment of solid mixotrophe - red light were shown in figure 7 and 8. At treatment of solid mixotrophe - red light (fructose and saccharose**),** filaments indicated particular spatial arrangement on the fifth day that in other treatments were not indicated Fig 7(A, B).

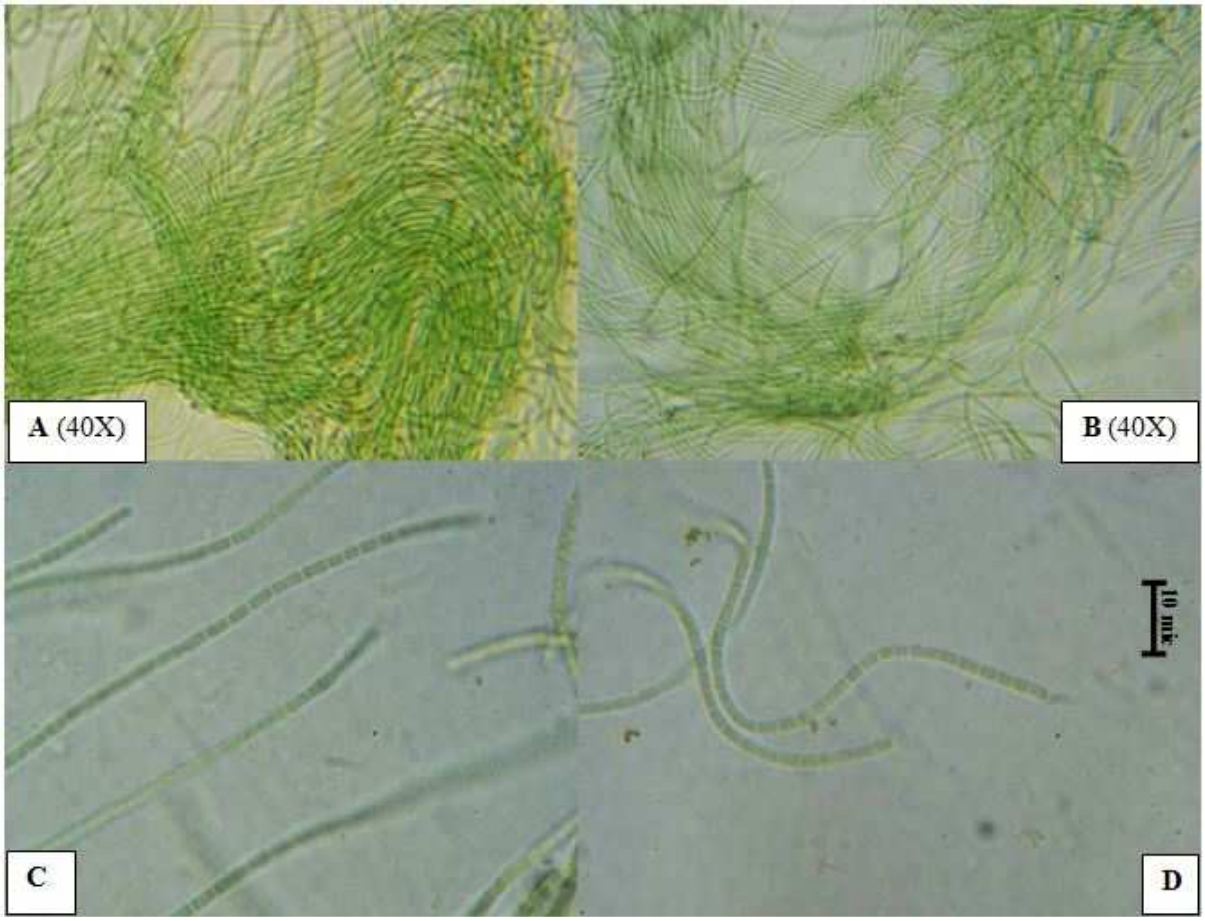

Fig 7. *Leptolyngbya* sp. Isc25 at solid mixotrophe - red light by light microscope on the fifth day

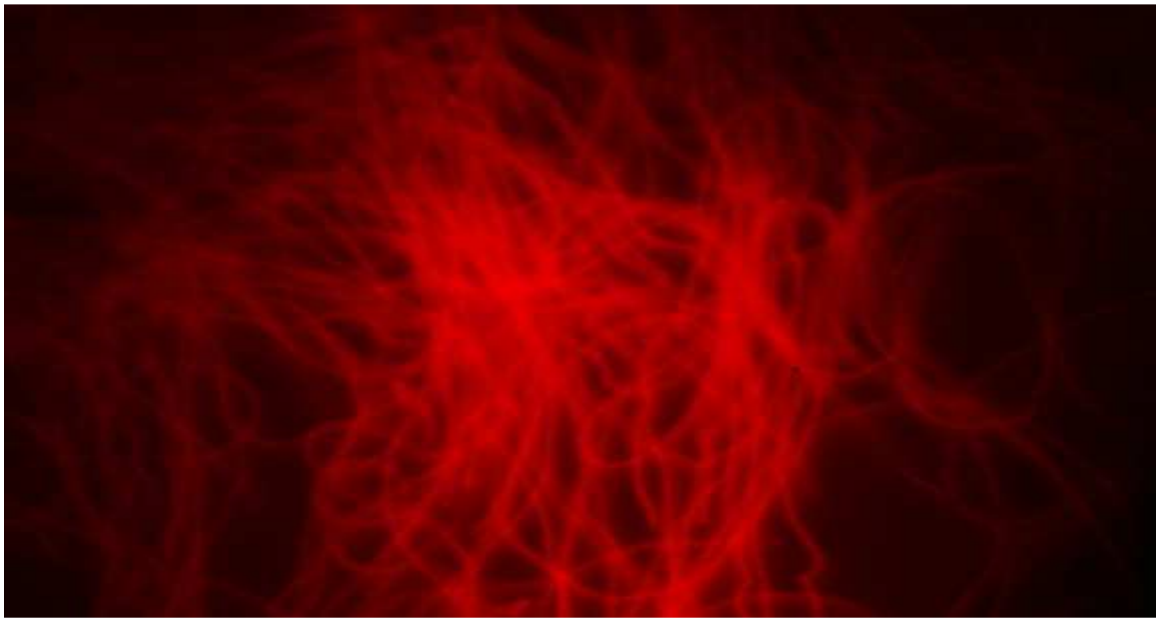

Fig 8. *Leptolyngbya* sp. Isc25 at solid mixotrophe (sucrose) - red light on the fifth day by fluorescence microscope (x40)

Biometrical studies of *Leptolyngbya* sp. Isc25 at solid BG11 medium (white light) and solid mixotrophe - red light were shown in figures 9 and 10.

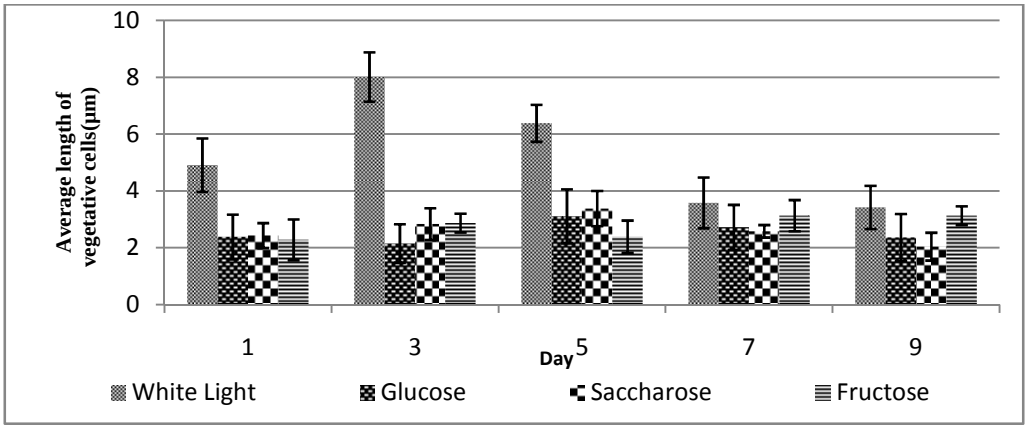

Fig 9. Comparison of the average length of vegetative cells at solid BG11 medium (white light) and solid mixotrophe - red light

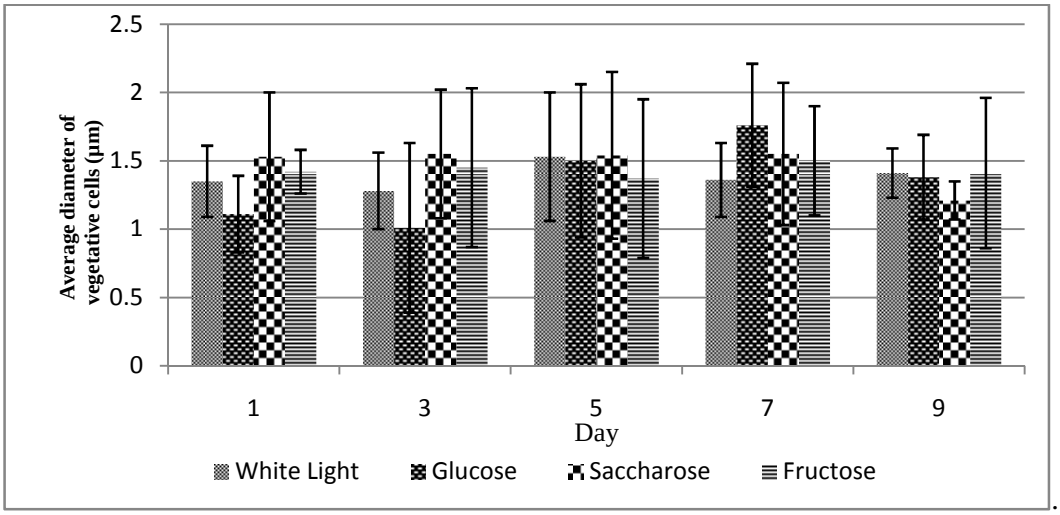

Fig 10. Comparison of the average diameter of vegetative cells at solid medium BG11 (white light) and solid mixotrophe - red light

The pattern of changes in the average of length and diameter of vegetative cells in control and the treatments nearly (Figure 9 and 10) is identical. This indicate that control and the treatments have not significant difference [ANOVA, $p<0.05$].

Algal cultures are influenced by a variety of environmental factors and they play a significant role in the production and composition of the photosynthetic pigments [3, 12, 34]. In both treatment and especially liquid mixotrophe - red light medium, in *Leptolyngbya* sp. Isc25. existence of 0.05 percent of soluble sugars (glucose, fructose and sucrose) at liquid and solid BG-11 medium the changes have created in morphology, size and survival of cells. That cause of it can be changes in photosynthetic products. Light intensity and quality are the most significant environmental factors influencing the photosynthetic pigments in cyanobacteria [36]. In this study was seen difference at the form and size of cells at red and white monochromatic light. Changes in cell pigmentation in response to spectral quality of light were resulted from modifications of the relative amounts of phycoerythrin (PE) and phycocyanin (PC). Phycobiliproteins are the major light-harvesting pigments used to stimulate photosynthesis. Only cyanobacteria which are able to synthesize PE can undergo complementary chromatic adaptation [33]. In addition, intensity, quality and the time of light impact affect photosynthesis, which is responsible for producing organic matter, cell division and the growth rate of organism [18, 24]. Morphological and biometrical studies in different environmental conditions indicate that there are morphological diversity and high adaptability in *Leptolyngbya* sp. Isc25. Therefore molecular studies are essential for accurate identification of this species.

Besides morphological studies, it is currently accepted that characterization and taxonomy of cyanobacteria must combine multidisciplinary approaches [11, 14, 17]. This so-called polyphasic methodology (including phenotypic, chemotaxonomic and genotypic data) has been increasingly followed by many cyanobacteriologists worldwide, e.g., Nayak *et al*. [22], Li *et al*. [19], Saker *et al*. [29] and Schleifer [31]. Among the molecular methods, the analysis of the 16S rRNA gene sequences has proved to be a useful tool for exploring phylogenetic relationships among cyanobacteria [13, 25, 35, 37]. Molecular studies in *Leptolyngbya* sp. Isc25. on the conserved region of 16S rRNA

was performed using specific primers for cyanobacteria. In this region there is a highly variable region that enables precise molecular diagnosis of genus. The results of 16S rRNA sequencing results confirm the morphological identification. Comparison of gene sequences of sample studied in Gene Bank (http://www.nbci.nlm.nih.gov/BLAST) NBCI due to blasting operations (BLAST) showed the highest similarity with *Nostoc* as much as 98% and the number JX972170 the NBCI database is accessible.

CONCLUSION

The results show that *Leptolyngbya* sp. Isc25. has fluidity morphological and in different situations in indicates high adaptability. Unmistakable, global expansion and survival of cyanobacteria on earth is related to the variability of their morphological and metabolic at millions of years.

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