

Studies of freshwater cyanobacteria strains from an ecological perspective in the western region of Saudi Arabia

Hassan Ibrahim Al Masaabi¹, Hatim Matouq Al-Yasi¹, Zakria Mohammad² Ali Hassan Abo Amer²

¹*Department of Biology, College of Science, Taif University, Saudi*

²*Department of Botany and Microbiology, Faculty of Science, Sohag University, Egypt*

Hb24.5.1437@gmail.com

Abstract. A class of photo-oxygenic bacteria known as cyanobacteria, or blue green algae, can be found from pole to pole in aquatic, aerophytic, and terrestrial environments. They are some of the most common species on Earth and belong to one of the oldest prokaryotic lineages. Only a little amount of research on cyanobacterial strains was conducted in the central and southern regions. This cyanobacteria ecological study is thought to be the first in the Kingdom of Saudi Arabia's western region. From this vantage point, the identification of cyanobacteria strains, their physical and chemical characteristics, and the impact of salinity in this area will take precedence. There are two primary components to the current research. The isolated strains were defined and isolated in the first section. The physical and chemical properties are examined in the second section.

Keywords: Cyanobacteria, Fresh water, Isolation of strains, Chemical and physical properties.

1. Introduction

The cyanobacteria are photosynthetic prokaryotes found in most, though not all, types of illuminated environment. They are also quantitatively among the most important organisms on Earth (Adams et al., 1990). A conservative estimate of their global biomass is 3×10^{14} g C or a thousand million tons (1015 g) wet biomass (Adams et al., 1990). They all synthesize chlorophyll a and typically water is the electron donor during photosynthesis, leading to the evolution of oxygen. Most produce the phycobilin pigment, phycocyanin, which gives the cells a bluish color when present in sufficiently high concentration, and is responsible for the popular name, blue-green algae; in some cases, the red accessory pigment, phycoerythrin, is formed as well (Whitton and Potts, 2012). The cyanobacteria are incredible ecosystem engineers, accounting for ca. 20–30 % of global oxygen production (Pisciotta et al., 2010) and have been credited with elevating the atmospheric oxygen levels ca. 2.5-2.2 bya (Schopf, 2000). Cyanobacteria are also known to fix atmospheric nitrogen, contributing greatly to the global nitrogen budget (Karl et al., 2002) serve as the centerpiece of aquatic food webs (Scott and Marcarelli, 2012) help to stabilize substrates, and are common photobionts.

This event is known as endosymbiosis, and it is also the origin of eukaryotic mitochondrion (Issa et al., 2014). Majority of cyanobacteria are aerobic photoautotrophs. Their life processes require only water, carbon dioxide, inorganic substances and light. Photosynthesis is their principal mode of energy metabolism. In the natural environment, however, it is known that some species are able to survive long periods in complete darkness. Furthermore, certain cyanobacteria show a distinct ability for heterotrophic nutrition (Fay, 1965). Cyanobacteria is often the first plants to colonize bare areas of rock and soil.

Adaptations, such as ultraviolet absorbing sheath pigments, increase their fitness in the relatively exposed land environment. Many species are capable of living in soil and other terrestrial habitats, where they are important in the functional processes of ecosystems and cycling of nutrient elements (Whitton,1992). Cyanobacteria have long been observed by phycologists in classic monographs (Agardh 1824; Kützing, 1849; Nägeli, 1849), but were first extensively documented by Bornet and Flahault (1886–1888) and later by Gomont (1892-1893). Their starting taxonomy was expanded by later researchers, most notably Geitler (1932), who helped revise many of the currently recognized genera familiar to most scientists. Recognizing the prokaryotic nature of the cyanobacteria, Stanier et al. (1978) advocated the transfer of the cyanobacteria from the International Code of Botanical Nomenclature (ICBN) to the International Code of Nomenclature of Bacteria (ICNB). While this appeal had much merit (after all, they are bacteria), it also meant that both codes could be utilized to name novel taxa, leading to an explosion of new names. The inclusion into the ICNB code allowed the cyanobacteria to be catalogued in the Bergey's Manual of Systemic Bacteriology, where Castenholz (2001) broke them into five major lineages based, in part, on type of cell division and the presence of differentiated cells. While this was a major revision, many felt that this approach missed a tremendous amount of the actual biodiversity of this lineage. All of this began to change in the 1980s–1990s when Komárek and Anagnostidis began their revisionary work on the cyanobacteria as a whole (Anagnostidis and Komárek,1985). Eschewing the simplified version of systematics proposed by Castenholz (2001), Komárek and Anagnostidis set about erecting smaller, monophyletic genera employing a polyphasic approach using a number of different characters including morphology, ecology, and genetic characters (most D.A. Casamatta and P. Hašler notably the 16S rDNA sequence as proposed by the ICNB). Their work, along with that of colleagues, has greatly increased our knowledge of cyanobacterial diversity and evolutionary relationships (Dvořák, et al., 2017).

Few attempts related to ecological investigation on fresh water Cyanobacteria located in the western region of Saudi Arabia are achieved (Zakaria and Al-Shehri, 2015). Therefore, our main target of the present study is to isolate the available strains of the cyanobacteria from the western region of Saudi Arabia and investigate the physical and chemical properties of the located area.

2. Materials and Methods

Sampling

The study sites were spread across the western region and included Al-Khalidea Al- Wesam, AL Sail, Moawya Dam (Ekrema), Wadi Wegg, Lyah, Lyah and Al-Qummria (table 1).

(Table 1). Tenth sites were selected along the study area at Taif governorate.

Site No.	Site	Latitude (N)	Longitude (E)
1	AL- khalidea	21 12.9994	40 27.2467
2	AL- wesam	21 13.7510	40 27.1506
3	AL sail	21 19.2795	40 25.8339
4	AL sail	21 19.3667	40 25.8060
5	Moawya Dam (Ekrema)	21 17.5914	40 29.6455
6	Wadi wigg	21 19.6727	40 28.4370
7	Wadi wigg	21 19.6066	40 28.4710
8	Lyah	21 22.3283	40 29.7546
9	Lyah	21 21.7423	40 30.3915
10	AL - qummria	21 22.4191	40 26.3863

Culturing

Fresh water samples were collected using a Van dorn bottle lowered 0.5 m below the water surface. 100 mL of unfiltered sample water was collected and preserved with 10 mL of 1% Lugol's solution in 120 mL glass jars (Smith, 1950). Cyanobacteria samples were inverted several times and 3 ml of subsample were settled overnight in sedimentation chambers. Ten random fields of view were counted with a Nikon microscope (Nikon, Melville, NY) at 200x ensuring that at least 300 organisms were counted per sample, and cyanobacteria were identified to the lowest practicable taxon based on preliminary examination of live material samples were diluted or concentrated accordingly to attain approximately 300 organisms for every ten random fields of view (Michael et al., 1991).

The sample was shaken to suspend sediment, and then triplicate 10- μ d aliquots were removed and diluted into 100 ml of sterile distilled water which was vacuum filtered through a sterile 47-mm-diameter polycarbonate membrane filter (0.4- μ m pore diamet Nuclepore) (Bourrelly, 1981). The filters were aseptically transferred, inoculum side up, onto plates of BG-11 medium (Rippka and Herdman 1993). The plates were incubated for 14 days, after which the cyanobacterial colonies growing on the surface of the membrane filters were counted with the aid of a dissecting microscope (x10 to x50 magnification) (Michael et al., 1991). Morphological identification was done using a microscopic method (Castenholz et al., 1989).

Physical and chemical characteristics

A pH Pen from Jenco Electronics (U.S.A.) was used to measure the pH. A conductometer (YSI Model 35 Yellow Springs, OH, U.S.A.) was used to test the electrical conductivity in water suspension; the conductivity readings were recorded in μ mhos. cm^{-1} (Merkle and Dunkle, 1972). It was Schwarzenbeck and Bederman who determined the elements magnesium and calcium (1948). The method used to determine orthophosphate was (Dewis and Freitas, 1970). As one of the growth markers, the optical density of the cyanobacteria suspension was measured at 680 nm, following the methodology of Lefort-Tran et al. (1988). One of the growth markers as mentioned by Lefort-Tran et al. (1988) allowed for the determination of dry matter (Whatman et al., 1975). The determination of dry matter was achieved by Whatman (1975).

3. Results and discussion

Distribution of cyanobacteria strains

One hundred and thirty-five samples of cyanobacteria were collected from ten locations. Of these, 46 were recorded as solitary colonies, 69 as filamentous without heterocysts and 20 as filamentous with heterocysts (Table 2).

(Table 2). Distribution of the cyanobacterial strains at the selected aera.

Sites	Species			Total number of species
	Solitary and colonial	Filamentous without heterocyst's	Filamentous with heterocyst's	
1	6	3	2	11
2	7	11	5	23
3	3	7	0	10
4	4	5	1	10
5	2	8	0	10

Sites	Species			Total number of species
	Solitary and colonial	Filamentous without heterocyst's	Filamentous with heterocyst's	
6	1	0	1	2
7	4	6	1	11
8	5	9	4	18
9	6	8	4	18
10	8	12	2	22

Identification of Cyanobacteria strains

After 135 samples were morphologically identified under a microscope, three families were found. The first family was identified as solitary colonists (Fig. 1). *Aphanocapsa* (Sandmann and Malkin, 1983), *Chroococcus* (Gama et al., 2019), *Merismopedia* (PRajaniemi-Wacklin, et al., 2006), *Microcystis* (Otsuka et al., 2006), *Arthrospira* (Otsuka, et al., 2006), and *Spirulina* (Chen and Chang, 2011) are among the families that are studied.

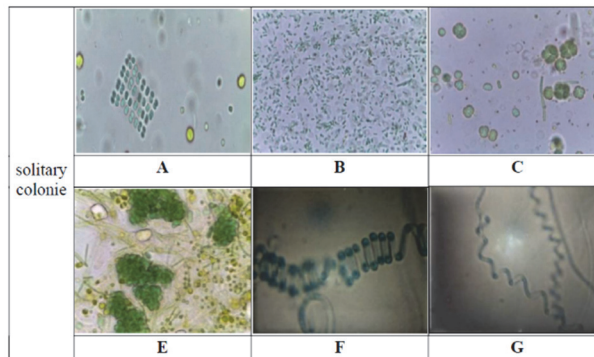


Fig.1. Solitary colonies include A (*Aphanocapsa* SP), B (*Chroococcus* SP), C (*Merismopedia*) D (*Microcystis*), E (*Arthrospira*), F (*Spirulina*). A microscope was used to identify the morphology. (Biology department, Taif university).

According to Figure 2, the second family was found to be filamentous without heterocyst. *Lyngbya* (Liu et al., 1998), *Oscillatoria* (Casamatta et al., 2005), *Anabaena* (Prasanna et al., 2006), and *Calothrix* (Berrendero et al., 2008) are some of the families that fall within this category (Fig. 2).

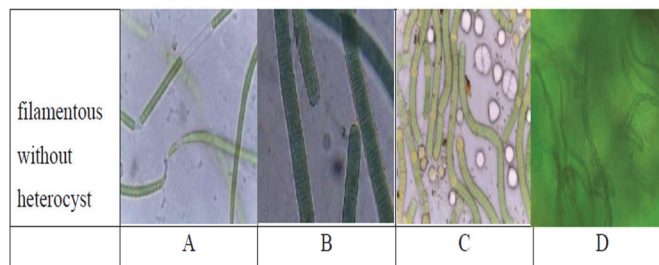


Fig. 2. Filamentous without heterocyst families include A (*Lyngbya*), B (*Oscillatoria*), C (*Anabaena*), D (*Calothrix*). A microscope was used to identify the morphology. (Biology department, Taif university).

A filamentous with heterocyst was found to be the third family (Fig. 3). *Tolypothrix* (Berrendero et al., 2008), *Scytonema* (Rodarte et al., 2014) and *Schizothrix* (Komarek et al., 2006) are some of these families.

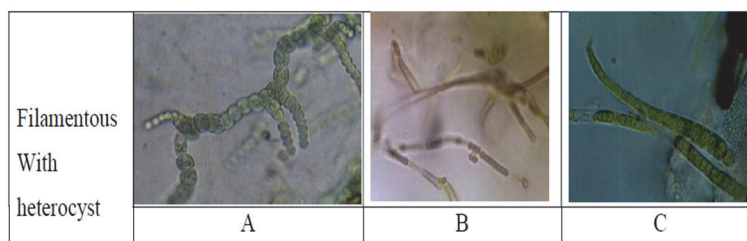


Fig. 3. Filamentous with heterocyst families include A (*Tolypothrix*), B (*Scytonema*) (B Rodarte, et al 2014), C (*Schizothrix*). A microscope was used to identify the morphology. (Biology department, Taif university).

Physical and chemical characteristics

Different locations have an average temperature of 29 Celsius. It was discovered that the pH values (8.46 and 9.5) were excessively alkaline (Harada et al., 1996). The calcium content was determined to be (11.77 ± 4.31) at position 1. The discovery of the Solitary and Colonial family at position number one is in line with the findings of (Rejmánková et al., 2005). Because of the low electrical conductivity of 225, the fifth location recorded Filamentous with heterocysts (Ormerod, 1992). At position number 10, the chloride content was measured to be (28.98 ± 0.80) . The discovery of the Filamentous family lacking heterocysts at site 10 is consistent with the findings of Jeffries et al. (1978). Nitrate concentrations are higher than those of other elements are because most cyanobacteria strains are capable of fixing nitrogen, which is consistent with (Howarth et al., 1988). At position number 10, the concentration of magnesium was recorded as 53.01 ± 2.50 (Table 3).

Conclusions

A total of 135 samples of Cyanobacteria were gathered from ten distinct locations across the western area of the Kingdom of Saudi Arabia. Three families were identified from all of the samples and Characteristics of the chemical and physical aspects were described.

(Table 3). Physical and chemical characterization of the tenth studies area.

Temp (°C)	pH	EC	P	NO3	Cl	Mg	Ca	
29.3	8.46±0.10	4450.33	28.45±0.11	37.18±23.44	2.53±0.89	3.98±2.14	11.77±4.31	1
29.4	8.80±0.12	3730.67	27.54±0.03	31.84±2.60	1.86±0.91	4.98±0.10	6.41±3.01	2
29.3	9.39±0.10	694.33	27.60±0.15	116.82±13.31	4.19±0.65	9.26±3.32	9.62±3.03	3
29.5	9.57±0.06	520	26.98±0.25	69.82±3.32	4.11±0.50	9.03±2.60	10.96±0.35	4
29.2	9.50±0.11	225	27.02±0.12	35.66±25.42	1.14±0.45	3.98±2.11	7.37±3.33	5
29.4	9.55 ±0.09	700	26.85±0.46	106.84±8.72	7.01±3.36	11.33±2.60	10.79±1.79	6
29.5	9.65±0.07	690	26.54±0.12	4.58±0.98	3.70±0.34	10.13±0.72	11.11±0.57	7
29.8	9.33±0.08	2660	26.28±0.10	42.80±0.48	14.99±1.19	22.98±5.39	23.67±9.35	8
29.7	9.36±0.10	2100	26.49±0.08	254.73±5.33	25.71±1.83	44.17±0.17	65.95±5.51	9
30	9.54±0.09	3400	26.61±0.10	289.04±19.73	28.98±0.80	53.01±2.50	77.80±1.41	10

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دراسات على سلالات البكتيريا الزرقاء في المياه العذبة من منظور بيئي في المنطقة الغربية للمملكة العربية السعودية

حسن ابراهيم المصعبي^١، وحاتم معتوق الياسي^١، وزكريا محمد^٢، وعلي حسن ابو عامر^٢

^١ قسم الأحياء، كلية العلوم، جامعة الطائف، المملكة العربية السعودية

^٢ قسم النبات والأحياء الدقيقة، كلية العلوم، جامعة سوهاج، مصر

المستخلص. يمكن العثور على نوع من البكتيريا الضوئية المؤكسدة المعروفة باسم البكتيريا الزرقاء، أو الطحالب الخضراء المزرقية، من القطب إلى القطب في البيئات المائية والهوائية والبرية. إنها بعض الأنواع الأكثر شيوعًا على وجه الأرض وتتنمي إلى واحدة من أقدم سلالات بدائيات النواة. تم إجراء قدر قليل فقط من الأبحاث حول سلالات البكتيريا الزرقاء في المناطق الوسطى والجنوبية. ويعتقد أن هذه الدراسة البيئية للبكتيريا الزرقاء هي الأولى من نوعها في المنطقة الغربية للمملكة العربية السعودية. ومن هذا المنطلق، فإن تحديد سلالات البكتيريا الزرقاء، وخصائصها الفيزيائية والكيميائية، في هذه المنطقة سيكون له الأولوية. هناك عنصران أساسيان للبحث الحالي. تم تعريف وعزل السلالات المعزولة في القسم الأول. يتم فحص الخصائص الفيزيائية والكيميائية في القسم الثاني.

الكلمات المفتاحية: البكتيريا الزرقاء، المياه العذبة، عزل السلالات، الخصائص الكيميائية والفيزيائية.

