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Antibacterial Activity of *Lyngbya* and *Chroococcus* Species Isolated from Koya (Hizoop River)

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Abstract: In the study cyanobacterial strains were isolated from different sites of Hizoop rivers, Koya-Iraq and identified according to their morphological characters by using microscope, two genera which were in filamentous form identified as *Chroococcus* sp. and *Lyngbya* sp.. After identification of genera their optimum growth condition studied by using the effect of temperature and pH to their dry weight. In the result, the optimum temperature and pH for both filamentous cyanobacteria were 25 °C and pH 7.5. Both cyanobacterial strains were extracted with ethanol, methanol and diethyl ether at various concentrations (0.2 g/mL, 0.1 g/mL, 0.005 g/mL) which exhibited the antibacterial activities against *Staphylococcus aureus*, *E. coli* and *B. subtilus*. Inhibition activities of the two cyanobacterial extracts were more effective at high concentration against the tested pathogens at the low concentration, especially those of *Lyngbya* sp. The higher inhibition zone showed with extract by ethanol.

Key words: Cyanobacteria, pH, temperature, antibacterial activity.

1. Introduction

Cyanobacteria are the most prokaryotic algae and they are found in virtually every type of environment including terrestrial, fresh water, marine habitats. Since cyanobacteria are prokaryotes, they lack membrane bound organelles however the external structure can change from unicellular or colonial to branched or unbranched and filamentous [1]. Like rhodophytes, the cyanophytes possess no flagellated or ciliated cell at any stage of their life cycle. They are heavily pigmented with chlorophyll a, beta carotene, and several xanthophylls [2, 3].

The bacterial infections are still the major problem in the world today, because these bacteria which causes disease will eventually develop ways to resist the drugs as well. To help preventing and treating these illnesses, many researchers have studied the antimicrobial effects of various plants extracts, as well as antimicrobial activity of algae.

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In general, isolation of bioactive compounds from cyanobacteria is done to discover new compounds. A number of cyanobacteria produce toxins that may have potential pharmaceutical application [4]. The authors had found that various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial, antialgal, antifungal and antiviral activity [5-8]. As an efficient strategy of investigation, organic solvents have been used to extract the possible lipid soluble active principles from microalgae [8, 9]. The antimicrobial substances involved may target various kinds of microorganisms, prokaryotes as well as eukaryotes. The properties of secondary metabolites in nature are not completely understood [6, 10].

2. Materials and Methods

2.1 Isolation and Identification

Samples were collected from sites along the Hizoop rivers Fig. 1. Water samples diluted and plated onto plates of BG11 medium solidified with %1.5 agar-agar. According to Rippka and Castenholz et al.,

the cultures were incubated under continues light at pH 7.2 and 23 °C. Two weeks later, following the growth of colonies on the agar media, the colonies were removed with pasture micropipettes and were gently blown into liquid medium then incubated at 23 °C, at pH 7.2 [11, 12].

After 15 days single cells and filamentous removed with pasture micropipette and examine under light microscope and identified as described by [10, 13, 14].

2.2 Effect of Environmental Factors

Strains were cultivated at different temperature 20 °C, 23 °C, 25 °C & 30 °C and different pH 6, 6.5, 7, 7.5, 8 & 8.5 [15, 16].

2.3 Analytical Method

2.3.1 Cell Dry-Weight and Fresh-Weight Estimation

According to Oswald, samples grown at 100 mL BG11 medium After 2 weeks 10 mL of samples were taken and cells harvested after centrifugation for 10 min. To determine the cell dry weight, cells were harvested after centrifugation for 10 min, the collected sample were dried in oven at 80 °C and weighed quickly after drying [17].

2.3.2 Preparation of Cyanobacterial Extracts

Cyanobacterial cells were dried at 70 °C, and then the cells were grinded in sterile tubes. As described by Thummajitsakul, cells were mixed with ethanol, diethyl ether and methanol for 1 mL with shaking for 10 min and kept in room temperature for 10 h. After that, the solvent was removed by incubation at 60 °C and redissolved in water (ratio 0.2 g/mL, 0.1 g/mL and 0.05 g/mL) and kept at 4 °C until use for further assay [18].

2.3.3 Antibacterial Bioassay

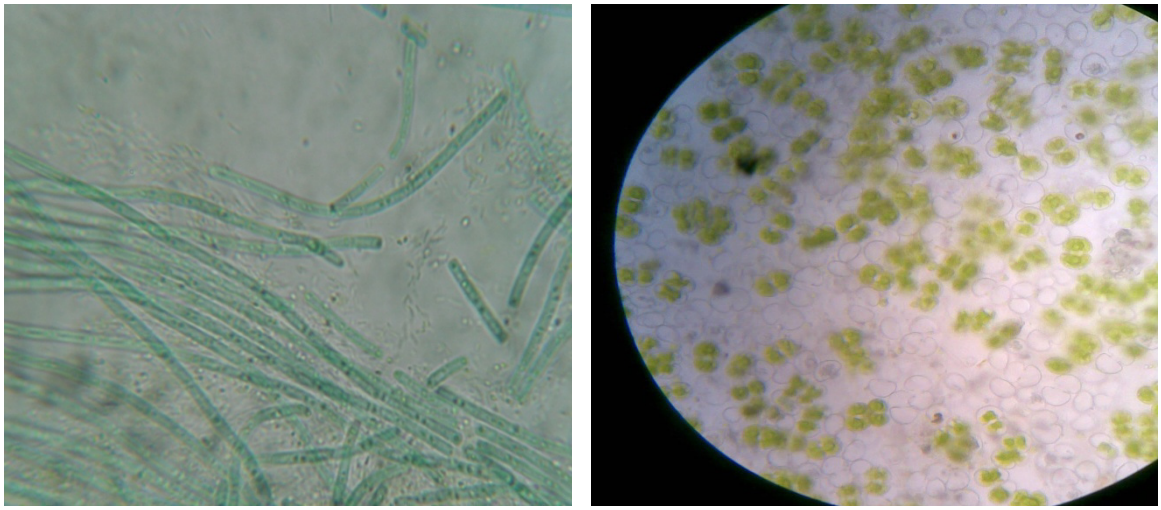
Staphylococcus aureas, *E. coli* and *Bacillus subtilus*, were used as test microorganisms. Antibacterial activity was determined by the disc method as described by ghasemi et al., Filter paper disc were saturated with 20 μL of test bacteria and dried under laminar air flow and placed on nutrient agar, the plates were incubated at 37 °C for 24-28 h. Ampicilin, gentamicin and amphoterin were used as positive control. The diameter of inhibition zones were determined and used as an indication of antibacterial activity [19].

3. Results

Growing cells were observed and photographed with light microscope, and the mode of division of strain examined in slide culture showed the following characters:

Filament erect or less curved, rarely solitary, ends not constricted and not attenuated, cross walls marked with one or two large granules on either side; and is identified as *Lyngbya* sp. Spherical or ovate colony of 2-4 spherical cells. Evenly arranged cell sheath usually is well defined with colorless lamellate *Chroococcus* sp. (Fig. 2).

Fig. 1 Hizoop River.



(a)—*Lyngbya* sp. (b)—*Chroococcus* sp.

Fig. 2 Isolated unicellular cyanobacteria.

3.1 Effect of pH to Growth Rate

The higher cell dry weight was at pH 7.5 for two cyanobacteria and the lowest growth rate shown at pH 8.5 for both cyanobacteria isolates (Fig. 3).

3.2 Effect of Temperature to Growth Rate

In Fig. 4, the temperature role in the growth of cyanobacterial strain, at 20 °C and 35 °C, the higher growth shown at 25 °C for both cyanobacterial strains, while in 20 °C, the cyanobacteria strains showed low growth.

3.3 Antibacterial Activity of Chroococcus sp. and Lyngbya sp.

The effect of ethanol, diethyl ether and methanol extractions of cyanobacteria *Lyngbya* sp. and *Chroococcus* sp. on the inhibition of tested pathogens was shown in Tables 1-3. The results showed that ethanol, diethy ether and methanol extracts of *Lyngbya* sp. and *Chroococcus* sp. at various concentrations (0.2 g/mL, 0.1 g/mL and 0.005 g/mL) exhibited the antibacterial activities against *Staphylococcus aureus*, *E. coli* and *B. subtilus*. Inhibition activities of the two cyanobacterial extracts were more effective at high concentration against the tested pathogens at the low concentration, especially those of *Lyngbya* sp. The higher inhibition zone showed with extract by Ethanol.

4. Discusion

Cyanobacteria grow well within the temperature range of 25-30 °C, these temperatures displays a short exponential phase, along linear phase and stationary phase from about 14 days on. In this study the optimum temperature for the growth of filamentous and unicellular cyanobacteria were around 20-35 °C, the highest growth found at 25 °C, and the lowest growth found at 35 °C which show denaturized of pigment after 10 days of cultivation. This result shows that increase of temperature from 20 °C to 30 °C caused increase of chlorophyll and cell dry weight but higher this value caused decreasing of chlorophyll-a and cell density with cell dry weight. This result is agreed with finding of Donmez et al. [21-23].

The effect of pH on growth rate and chlorophyll content was shown in Fig. Cyanobacteria grow at all pH values just the best growth determined at pH 7.5 for both cyanobacterial strain. This pH is near the pH of isolated place.

Several different organic solvents have been used to screen cyanobacteria for antibacterial activity. The species *Lyngbya* and *Chroococcus* demonstrated the effective extracts for antibacterial activity against

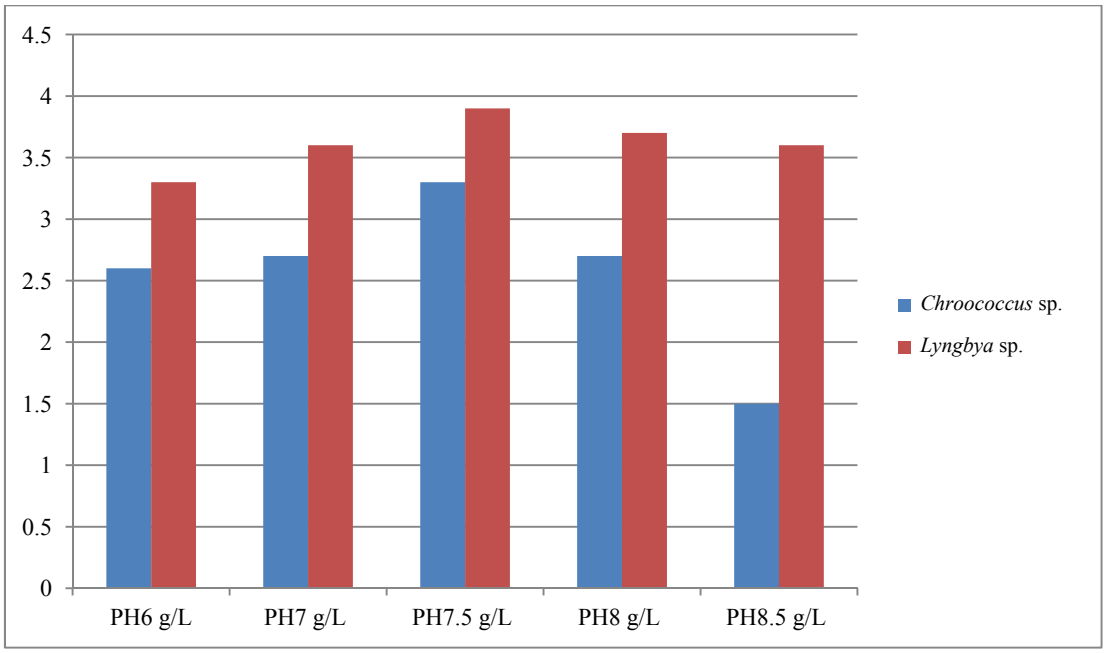


Fig. 3 Effect of pH to dry weight of cyanobacterial strains.

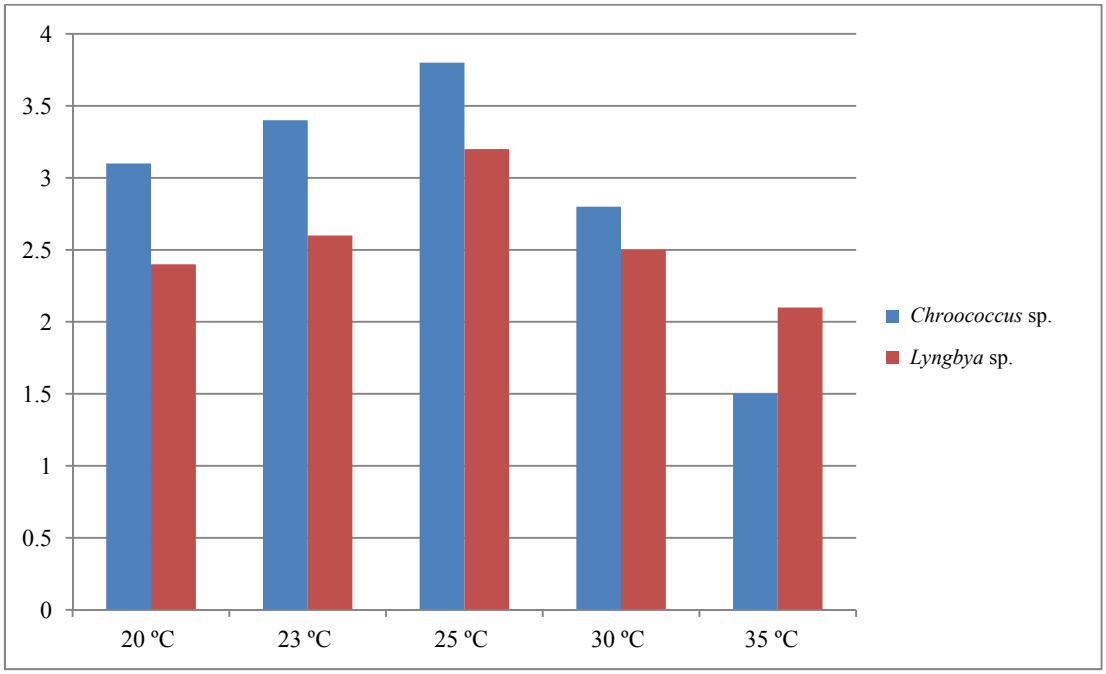


Fig. 4 Effect of temperature to dry weight of cyanobacterial strains.

Table 1 Antibacterial activity of *Lyngbya* sp. and *Chroococcus* sp. extracted with ethanol (diameter of inhibition zone in mm).

Bacterial species	*Lyngbya* sp. (g/mL)			*Chroococcus* sp. (g/mL)		
	0.05	0.1	0.2	0.05	0.1	0.2
Escherichia coli	4	7	10	5	7	9
Staphylococcus aureus,	4	9	11	6	9	9
B. subtilus	5	6	9	5	8	10

Table 2 Antibacterial activity of *Lyngbya* sp. and *Chroococcus* sp. extracted with diethyl ether (diameter of inhibition zone in mm).

Bacterial species	*Lyngbya* sp. (g/mL)			*Chroococcus* sp. (g/mL)		
	0.05	0.1	0.2	0.05	0.1	0.2
Escherichia coli	7	9	8	3	6	7
Staphylococcus aureus	9	9	9	5	6	7
B. subtilus	7	9	8	4	7	8

Table 3 Antibacterial activity of *Lyngbya* sp. and *Chroococcus* sp. extracted with methanol (diameter of inhibition zone in mm).

Bacterial species	*lyngbya* sp. (g/mL)			*Chroococcus* sp. (g/mL)		
	0.05	0.1	0.2	0.05	0.1	0.2
Escherichia coli	5	7	9	5	7	8
Staphylococcus aureus,	5	9	8	4	6	7
B. subtilus	6	9	8	6	7	8

Escherichia coli, Staphylococcus aureus and *Bacillus subtilus*. The extracts of *Lyngbya* sp. shows the highest inhibition zone to *Staphylococcus aureus* (11 mM) at 0.2 g/mL which showed more sensitivity to the cyanobacteria extracts than those of *Escherichia coli* and *Bacillus subtilus.* The cyanobacterial extracts showed more effective inhibition activities by increasing their concentration. It was reported by Crosby, that correlation between the extracts concentration and the inhibition zone sizes in the logarithm revealed the linear relationship [20].

5. Conclusions and Recommendation

After isolation of cyanobacteria from Hizoop rivers and determination of optimum pH and temperature. It was concluded that the extracts of *Lyngbya* and *Chroococcus* species indicated the potential of antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilus*. Therefore, the basic knowledge may be useful in various applications such as pharmaceutics and agricultures, and for further investigations.

The authors suggested that further work should be performed on the isolation and characterization of the active components responsible for the antibacterial activities need to be evaluated.

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