

Examination the growth of blue green alga, *Chroococcus turigidus* in different traditional media formulations

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ABSTRACT

Chroococcus turigidus. One-celled, or an association of 2-32 spherical, hemispherical or ovate individuals, either free floating, adhering to submerged substrates, or forming expansions in moist aerial habitats; cell contents homogeneous or granular, not vacuolated, light to bright blue-green, olive-green, or yellowish.

Chroococcus one of the members of blue green algae is the richest source of chlorophyll which is widely used as a health food and feed supplement, as well as in the pharmaceutical and cosmetics industry. *Chroococcus* has been produced commercially in several countries for its use as food and medicinal purpose due to its valuable contents particularly pigments and proteins. The aim of this work is to evaluate the effect of three different traditional inorganic medium such as Chu-10 medium, BG-11 medium, and BG-11 with minerals medium, on the growth of *Chroococcus turigidus*. The best growth was obtained in BG-11 with minerals medium as compared to other medium.

KEYWORDS: blue green alga ,*Chroococcus*, and different media.

INTRODUCTION

The prevalence of diseases such as cancer, human immunodeficiency virus (HIV) – acquired immune deficiency syndrome, hematological and autoimmune disorders is increasing rapidly. In recent years, the pharmaceutical industry focusing on the discovery of new bioactive compounds as antiviral agents and the main focus in recent decades for pharmaceutical discovery from natural products has been on microbial sources (bacterial and fungal), dating back to the discovery of penicillin from the mould fungus *Penicillium notatum* in the first half of the twentieth century (1). The first investigation of the antibiotic activity of algae was carried out by (2).

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Evidence of phycochemical and pharmacological studies on algae is available in the literature with special references to terpenoids and steroids (3 and 4). Several screening studies have been carried out over the past years with the aim to discover new antibiotic or cytotoxic metabolites of microalgae especially green algae and cyanobacteria (5 and 6) as well as to discover cyanobacteria which were toxic to other cyanobacteria or green algae (7) and as enzyme inhibitions (8). Cyanobacteria have unique food storage compounds, myxophycean starch and cyanophycin. Besides the immune effect, blue-green algae improves metabolism, where blue green algae have cholesterol - lowering effect in animals and humans.

There have been extensive studies on the growth of algae in culture media with different concentration of different carbon sources (9 and 10). Variation in the elemental composition of algae under different conditions and different stages of growth has been reported(11 and 12). The objective of the present study is to evaluate the influence of different traditional media on the growth *Chroococcus turigidus* of to find out best defined inorganic media for cultivation of *Chroococcus turigidus*.

MATERIALS AND METHODS

During October 2015 two isolates of *Chroococcus turigidus* were isolated from Al-Wathba drinking water treatment station located in the center of Baghdad. This station located on longitude 20°44'45.58"E and latitude 20°33'33.55"N. cultured on Ch-10 medium and maintained on the same medium by regular subculturing in every two weeks (13) and identified by(29) . Water temperature was measured immediately in the field by placing a precise clean mercury thermometer (range 10 to 60 C°) graduated up to 0.1C° ,while the pH were measured by using pH meter (HANNA Instruments).Samples for nutrient analysis (NO₂, NO₃ and PO₄)were collected from 10-20 cm depth of water(14). Experiments to evaluate the effect of different traditional media on two isolates of *Chroococcus turigidus* were carried out in the departmental laboratory.

In order to find out the best culture medium, cultures were subjected to three different traditional media provided from (HIMEDIA) Trade mark of different chemical compositions and pH, as mentioned below:-

- 1- BG11 Broth w/Minerals (15 and 16).
- 2- BG11 Broth (15 and 16).
- 3- Chu's Medium No. 10 (17 and 18).

Three test tube sets of each medium containing 10 ml of medium and 2 ml of freshly growing culturesof each isolates were subjected to different medium and there growth were followed through optical density (OD). All

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medium in the flask and test tubes were sterilized in autoclave at 121°C for 20 min. before inoculation. The cultures were incubated under controlled conditions for algal growth (200) $\mu\text{E}/\text{m}^2/\text{s}$ and $26 \pm 2 \text{ C}^\circ$. Observations were carried out over a period of five weeks after initial readings. Growth was followed through optical density, Optical density was recorded by using Spectrophotometer at 540 nm (19). Cultures were shaken gently thrice a day to avoid clumping and accelerate the growth process. Experiment for each medium was carried out in triplicates.

RESULTS AND DISCUSSION

Growing cells were observed and photographed with light microscope, and the mode of division of strain examined in slide culture showed the following characters: Spherical or ovate colony of 2-4 spherical cells. Evenly arranged cell sheath usually is well defined with colorless lamellate *Chroococcus turigidus* figure. 1

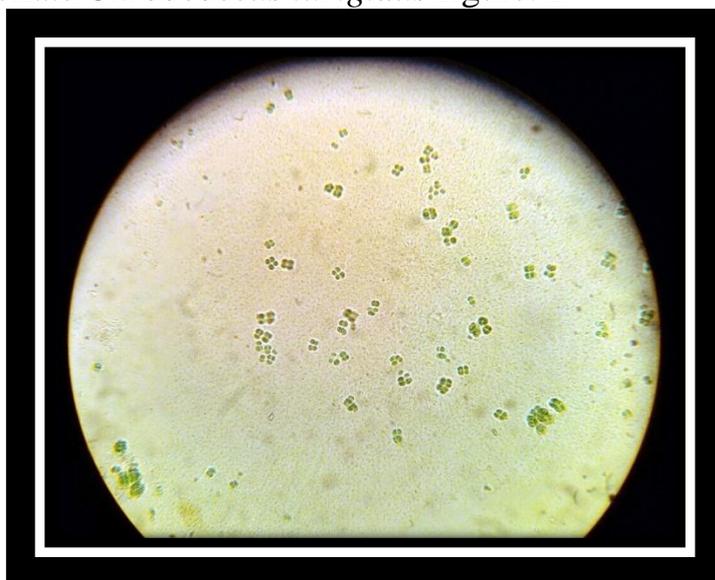


Figure (1): Photomicrographs of *Chroococcus turigidus* isolated from drinking water treatment stations in Baghdad which grown on ch-10 broth (40X) .

All the values of water properties were recorded in the collection's period of water showed in the table. 1

Table (1):some water properties were measured in drinking water treatment stations in Baghdad. Each value is Mean \pm SD (n=3),

Factors	Water temp.	pH	NO ₃ - $\mu\text{g}/\text{l}$	NO ₂ - $\mu\text{g}/\text{l}$	PO ₄ - $\mu\text{g}/\text{l}$
Values	(30 \pm 2)	(8.7 \pm 0.8)	(8.4 \pm 1.8)	(0.32 \pm 0.1)	(2.4 \pm 0.57)

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Estimation of growth of each isolates from *Chroococcus turigidus* in different media shows different growth pattern, among the all three media, BG11 Broth w/Minerals shows maximum growth followed by BG11 Broth, and minimum growth was observed in Chu's Medium No. 10.

Optical density clearly indicated that the best growth of *Chroococcus turigidus* was obtained in BG11 Broth w/Minerals as compared to that in other media. Optical density had increased by 6.1 times and 6 times of the initial record respectively, after a period of five weeks (Table -2 and Table -3). BG11 Broth was next in promoting the growth of the two isolates *Chroococcus turigidus*. Growth was increased about 6 times in terms of isolate 1 and 6 times in terms of isolate 2, after a period of five weeks (Table -2 and Table -3). Chu -10 Medium OD was increased 5.1 and 5 times, respectively, of the initial record, after a period of five weeks (Table -2 and Table -3).

Table(2): growth Optical density of *Chroococcus turigidus* (1) at different media each value is Mean±SD (n=3).

<i>Chroococcus turigidus</i> (1)			
(540) nm			
	BG-11 Minerals	BG-11	Ch-10
Week	AB	AB	AB
Initial	0.05±0.01	0.05±0.01	0.05±0.01
1st	0.06±0.02	0.09±0.03	0.09±0.03
2nd	0.11±0.03	0.043±0.02	0.025±0.03
3rd	0.25±0.03	0.073±0.03	0.074±0.03
4th	0.49±0.02	0.34±0.02	0.17±0.01
5th	0.391±0.02	0.355±0.04	0.292±0.03

Table(3): growth Optical density of *Chroococcus turigidus* (2) at different media each value is Mean±SD (n=3).

<i>Chroococcus turigidus</i> (2)			
(540) nm			
	BG-11 Minerals	BG-11	Ch-10
Week	AB	AB	AB
Initial	0.05±0.01	0.05±0.01	0.05±0.01
1st	0.208±0.03	0.05±0.01	0.02±0.01
2nd	0.218±0.01	0.031±0.03	0.116±0.03
3rd	0.227±0.02	0.121±0.03	0.244±0.04
4th	0.38±0.03	0.17±0.01	0.17±0.03
5th	0.382±0.02	0.379±0.01	0.250±0.01

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The growth rate of the algae seemed to be directly associated with morphological configurations. The normal morphological configuration was observed in BG11 Broth w/Minerals. Most of the cells were healthy, bright green and having intact chloroplast up to five weeks. Five week onwards certain cells were noted to be unhealthy for the two isolates of *Chroococcus turigidus* figure -2. BG11 Broth was second best medium for promoting the growth of *Chroococcus turigidus*. In this culture, healthy appearance of the cells was maintained up to four weeks with the exception of the few cells. Subsequent observations revealed unhealthy for the two isolates of *Chroococcus turigidus* figure -2. Chu -10 Medium was found third best medium in terms of growth. In this medium cells were having green, intact chloroplasts, but some unhealthy cells were also seen in the third week figure -2.

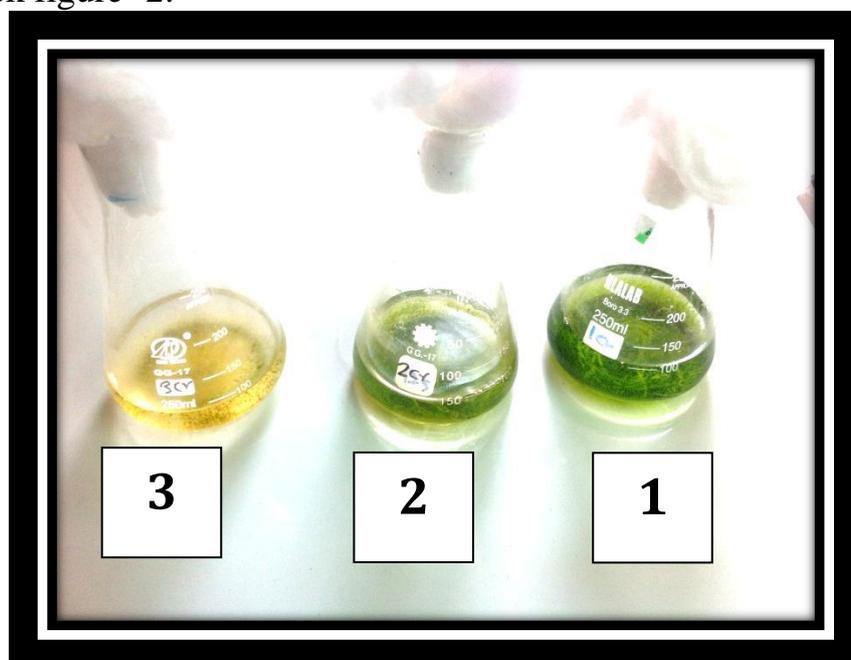


Fig1: Growth of *Chroococcus turigidus* (1) in different media after five weeks

- 1- BG11 Broth w/Minerals.
- 2- BG11 Broth.
- 3- Chu -10 Medium.

All the values of water properties were recorded in the collection's period of water agreed with (20) who study this station at same period.

Three different traditional inorganic medium varying in their chemical composition and pH, in which, BG11 Broth w/Minerals proposed best for growth (OD), pigment complex and morphology. Similar observations were also reported by many scientists i.e. nutritional studies

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with variations in the amounts of essential elements in the solution may show that BG11 Broth w/Minerals will result in a faster rate and greater amount of growth of many of the algae (15 and 16).

BG11 Broth w/Minerals had K_2HPO_4 was the source of phosphate, it may be responsible for the rapid growth of the alga under experiment, as has earlier been reported in *Selenastrum* and this phenomenon assigned to the enhanced dark reaction (21), while other nutrients were limiting. It is often assumed that the limiting nutrients are nitrogen and phosphorus (elements that comprise higher percentages in the cellular composition).

Nitrogen being important constituent of the cell protein was needed for algal growth, either in combined or in molecular form. In BG11 Broth w/Minerals $Ca(NO_3)_2 \cdot 4H_2O$ at higher pH led to precipitate formation in the medium but lower pH of the medium prevent the precipitation. The gradual rise in pH in cultures using nitrate only, though troublesome because of reduction in the solubility of phosphate salts at the higher pH (22), $Ca(NO_3)_2 \cdot 4H_2O$ also found in Chu -10 Medium and BG11 Broth but less (concentration) than which found in BG11 Broth w/Minerals. As nitrogen deficiency develops the amount of chlorophyll in the cells decreases faster than the nitrogen content in algae cultures (23).

BG11 Broth w/Minerals had $MgSO_4$ was the source of magnesium, it is permitted the maximum growth of the present alga and magnesium deficiency interrupted cell division which results in abnormally large cell formation (24). Increase in magnesium alone in the medium resulted in higher cell number, although increase in nitrogen alone did not make much difference that means cells need magnesium to synthesize chlorophyll (23). The process of multiplication requires a larger concentration of magnesium in the medium than does the production of cell material²⁵. $MgSO_4$ also found in Chu -10 Medium and BG11 Broth but less concentration than which found in BG11 Broth w/Minerals.

Iron uptake is strictly required for phytoplankton development, because in the absence of iron, retardation of growth, reduction of photosynthetic activity and chlorophyll content is observed(26). Ferric citrate and citric acid combination was the source of iron in BG11 Broth w/Minerals by substituting an organic source of iron, ferric citrate. This improvement is due to increased iron availability (27) and an equal amount of citric acid with the ferric citrate stabilized the concentration of reactive iron in the nutrient solution.

Trace metal mixture and Na_2EDTA were included in the composition of BG11 Broth w/Minerals. The research establishing requirements of

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various species of algae for manganese, zinc, calcium, boron, and possibly copper but trace metals can act as a nutrient but at an elevated concentration they can interact with proteins and can change the structure and enzymatic activities within the cell of an aquatic organism and can display its toxic effects at the whole organism level (28).

EDTA increases the density to which a population can be carried and that without it some factor, presumably availability of microelements, soon becomes severely limiting. Consideration of the function of a chelating agent shows that it will be a very great aid in mass culture work. It is a serious problem to provide adequate amounts of the various microelements, and to maintain their availability, without exceeding the limits of toxicity. The easily reversible chelate complex provides a buffer system that will maintain ionic concentrations at desirable levels throughout the life of a culture.

It has mentioned above that the water body has pH 7.3 from where the algae have been isolated. The pH of modified BG11 Broth w/Minerals was maintained between 7.1 to 7.2, thus this medium favours the growth of algae.

The performance of different species in different media may also be conditioned by the previous history of the cells(22). However, in our experiments, the BG11 Broth w/Minerals supports the growth of(*Chroococcus turigidus*) blue green alga.

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الخلاصة

يكون طحلب *Chroococcus turigidus* احادي الخلية او على شكل تجمع من 2-32 من الافراد الكروية او المخروطية ، والتي اما ان تكون طافية في المياه او ملتصقة بمواد طافية او ممكن تواجدها في البيئات الرطبة .المحتوى الخلوي متجانس او حبيبي وليس على شكل فقاعات، لونها يكون اخضر مزرق ناصع او اخضر زيتوني او مصفر .

يعد طحلب *Chroococcus* واحد من المصادر الغنية بالكلورفيل العائدة الطحالب الخضر المزرق ذات الاستعمال الواسع الغذاء الصحي ومكملات الغذاء وايضا تستعمل في الصناعات الدوائية ومواد التجميل . يتم انتاج طحلب *Chroococcus* في عدد من الدول صناعيا لاجل استعماله كغذاء و استعمالات طبية نظرا لمحتوياتها الثمينة خصوصا الصبغات والبروتينات . هدف هذه الدراسة لتقييم تاثير ثلاث من الاوساط الزرعية العضوية التجارية وهي Chu-10 medium و BG-11 medium و BG-11 with minerals medium في نمو طحلب *Chroococcus turigidus*. افضل نمو تم ملاحظته مع وسط BG-11 with minerals medium مقارنة مع الوسطين الاخرين.