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# Detection of Iron Chelates from Cyanobacteria

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**Abstract:** Four axenic cultures of cyanobacteria, *Synechococcus mundulus, Oscillatoria lemnitica and Arthrospira platensis, Nostoc carneum* were investigated for the production of siderophores. These cyanobacterial isolates were grown in low iron concentration for inducing siderophore production into the nutrient medium using the Chrome Azoral Sulphonate(CAS) agar universal test plates. The maximum siderophores production was recorded for *Synechococcus nidulans* (73.8 %).

**keywords**: *Synechococcus mundulus*, Iron, Siderophores, Chrome Azoral S

## 1.Introduction

Some microorganisms and higher plants are able to secrete siderophores, which are low-molecular-weight chelating compounds that promote iron uptake.

Iron is a necessary nutrient element that is difficult to get due to its limited solubility in physiological conditions. In addition to being the most important transition metal ion for almost living systems [9]. The chelator compound that excreted by aerobic and facultative anaerobic microorganisms living in toxic aquatic conditions is known as siderophore (from the Greek term "iron carrier"). It has the ability to build extremely stable iron complexes.

These siderophore molecules characterized by their great affinity for iron, forming highly stable compounds with iron in the trivalent state having good solubility in water providing iron in more available form for microorganisms. Siderophores have been recommended as one of the virulence factors of microbial pathogens owing to their ability to attack transferrin which is the iron-protein of the host. Whereas siderophores have the potentiality to eliminate iron in one step [11] or within proteolytic action [4,26].

Characteristics of siderophore are summarized as follows: (1) It has a low molecular weight (500-1000 Da); (2) it is a ferric-specific ligand; (3) iron levels in the medium influence its manufacture; and (4) high formation constant [17].

Cyanobacteria among all microorganisms inhabiting the aquatic ecosystems introduce the greatest-characterized examples for iron uptake. It has been documented that iron uptake system of some cyanobacteria includes the extracellular secretion of siderophores which bind and transport iron to the iron-depleted cells [10, 23,25]

Iron is a vital nutrient element involved in many significant cellular metabolic operations including DNA synthesis, cellular respiration in addition to antioxidant potentiality. Although its presence extensively on Earth, iron relatively difficult to be transported to microbial cells according to its ferric state ($Fe^3$) that is mostly insoluble in water. So that, most microorganisms have developed many mechanisms to assimilate iron from environment [12].

Iron chelators including different functional groups recognizing hydroxamate- and catecholate-type siderophores that have been found in marine bacterial strains as well as cyanobacteria [4,13, 25].

Bacteria and fungi that live in low iron environments producing siderophores, which are ferric ion specific chelating agents with a relatively low molecular weight. These molecules scavenge iron from the habitat

converting it available to the microorganism [18]. Low-iron environments is one processes that forming the physiologic scenario of adaptation for microorganisms inhabiting aquatic ecosystems as cyanobacteria that are among the aquatic microbes providing the ideal models for iron uptake excreted externally as documented by [2,7,16] who found extracellular siderophores secretion that combined and transport iron to the iron-stressed cells.

It was reported that certain siderophore (schizokinen) belonging to dihydroxamate-type have been secreted by *Anabaena* sp. PCC 6411 was secreted by Bacillis megaterium [6,22] as well as natural cyanobacterial populations [1].

This study aimed to evaluate the growth of four cyanobacteria in absence of iron and their production of siderophores.

## 2. Materials and methods

### 2.1- Cyanobacteria isolates and growth conditions.

Axenic cultures of *Synechococcus mundulus, Oscillatoria lemnitica* and *Arthrospira platensis, Nostoc carneum* were provided from the microalgal collection of Botany Department of Mansoura University. Cyanobacterial isolates were grown on BG-11 [20] at 25°C under continuous illumination with intensity of 100 ~mol. $m^{-2}$. $s^{-1}$ for 18 days. Growth was estimated as dry biomass for two days intervals. Cyanobacterial biomass was collected by centrifugation at 3500 rpm for 4 min. The obtained pellets were dried at 65 °C for constant weight.

For experimental work, BG-11 medium was modified by substituting ferric ammonium citrate component by $FeC1_3.6H_2O$, in addition to exclude citric acid which may be function as siderophore in some microorganisms [8]. Tris-HC1 was added to maintain pH at 7.4. De-ionized water was used in preparation to ensure absence of any residual metals. Iron as ferric chloride was added to a final concentration of 5.1 x $10^{-9}$ M to ensure that cells are iron restricted. The iron-sufficient control is $4.2 \times 10^{-5}$ M $FeCl_3$ .

### 2.2- Modified Chrome Azoral Sulphonate(CAS) agar universal test plates

Chrome Azoral Sulphonate (CAS) assay was modified in order to investigate the potentiality of different cyanobacteria to release siderophore in the solid nutrient medium.

### 2.3- Preparation of CAS-blue agar

CAS-blue agar was prepared according to the protocol of [21]**.** An aliquot of CAS (60.5 mg) was liquefied in (50 ml) de-ionized $H_2O$, then 10 ml iron (III) solution (1m M $FeCl_3.6H_2O$, 10 m M HCl) was mixed. The previously prepared solution was added slowly under stirring to 72.9 mg of hexadecyltrimethyl ammonium bromide (HDTMA) dissolved in 40 ml de-ionized water, whereas a dark blue coloration was attained.

For solidifying CAS reagent appropriate of 3.1g agar and 2g D-glucose dissolved in 100 ml de-ionized water was added, then pH was adjusted at 6.8 using tris HCl, and autoclaved at 121°C for 15 min. Siderophores was measured by the following formula

Siderophore Units = (Ar-As)/Ar*100

**Where**: **Ar** = 630 nm reference absorbance (CAS reagent).**As** = Absorbance of sample at 630 nm.

**Table (1):** Growth of Cyanobacteria expressed as dry biomass (mg/L) after 18 days of incubation

| Days of incubation | Dry Biomass (mg/L) | | | |
|---|---|---|---|---|
| | ***S.mundulus*** | ***A.platensis*** | ***O.lemnitica*** | ***N.carneum*** |
| **2** | 44.8±5.1 | 81.4±7.2 | 78.8±8.7 | 86.3±9.6 |
| **4** | 53.8±8.4 | 129.3±12.9 | 126.6±14.4 | 138.4±13.4 |
| **6** | 67.3±13.1 | 258.6±16.3 | 251.5±17.6 | 269.4±19.3 |
| **8** | 107.0±15.9 | 392.5±19.7 | 321.2±21.6 | 381.3±20.6 |
| **10** | 210.5±17.6 | 489.4±19.3 | 4616.4±22.1 | 525.6±22.6 |
| **12** | 381.6±22.3 | 511.6±23.3 | 535.4±24.4 | 623.4±28.1 |
| **14** | 459.6±26.7 | 609.4±26.1 | 642.8±29.8 | 743.5±26.2 |
| **16** | 615.2±27.1 | 681.4±28.6 | 726.2±31.1 | 848.3±24.3 |
| **18** | 612.7±27.7 | 732.6±28.9 | 7803±30.8 | 869.8±22.8 |

## 3. Results

The four cyanobacterial cultures were let to grow for 18 days and growth was estimated as dry biomass each two days intervals (Table.1). In general, the maximum growth was reached after 16 days incubation period.

The color changes from blue to purple, orange, magenta, or yellow as observed in (Fig.1) whereas siderophore units (SU) were calculated. *S. mundulus* recorded the maximum SU reaching 73.8%, followed by 31.76% for *O. lemnitica*, 29.1% for *A. platensis and* finally 16.45 % for *N. carneum* as illustrated in Fig.1.

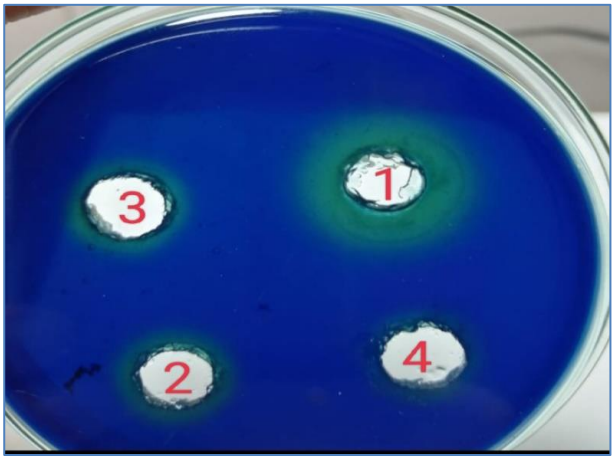


**Fig. 1:** The chrome azoral sulfonate (CAS) assay Siderophore production by cyanobactreria

(1) S. mundulus ( 2) O. lemnitica

( 3) A. platensis (4) N. carneum

Algal biomass increases progressively during the incubation period reaching maximum level at the range of $16^{th}$ – $18^{th}$ day of incubation for all the cultured cyanobacteria ( *Synechococcus mundulus* , *Arthrospira platensis*, *Oscillatoria lemnitica* and *Nostoc carneum*).

All cultured cyanobacteria exhibited the maximum biomass at the $18^{th}$ day of incubation except for *S. mundulus* exhibited the maximum biomass at $16^{th}$ day of incubation.

The chrome azoral sulfonate (CAS) assay was applied to investigate the presence of siderophore on agar surface. This method is depend upon change in color that involves the transfer of the ferric iron with its intense blue complex to siderophore. The isolates showed a definite zone on the CAS medium indicating the assembly of the siderophore.

## 4. Discussion

It was reported that most procedures for determining siderophores are carried out on the culture filtrate. Siderophores assaying protocols may be either unspecific, supported compounds or biological properties of the siderophores [10].

The most applied protocol is the chrome azurol S (CAS) adopted by [21]. This assay is often applied in both liquid and solid media [14] since it applied for quantifying approach. Then, phosphates and proteins interfere with the CAS assay giving inaccurate results [19].

Various previous studies documented the extracellular production of siderophores by cyanobacteria [3], Oscillatoria, [5], Arthrospira [15] and Nostoc **[**24**].**

This study indicated the ability of these isolates to produce siderophores with varying potentiality recording the maximum production for *Synechococcus mundulus* .This results pave the way for characterization of siderophore(s) produced by this strain and their application.

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