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Research Article

Ferro-chelatase enzyme activity of blue green algae from Yeşilırmak

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Abstract

Blue green algae are microscopic photosynthetic bacteria, naturally in ponds, rivers, lakes and streams. Tetra pyrroles can be classified based on the presence, position and substituents of a chelated metal in the pyrrole ring. Heme and chlorophyll, which are the most common tetra pyrolle in nature, are synthesized by blue green algae. Heme is an essential cofactor for virtually all forms of life and the last step of heme biosynthesis is catalyzed by ferrochelatase enzyme. In this study, blue green algae, photosynthetic bacteria, isolated from Yeşilırmak were used. Eight morphologically different isolates were obtained. The highest specific activity belongs to isolate 8 as 0.217 Umg^{-1}.

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Blue green algae,
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1. Introduction

Tetra pyrroles and their derivatives play an important role in all living organisms. They are involved in many metabolic processes such as energy transfer, catalysis and signal transduction.

Ferrochelatase (EC 4.99.1.1, protoHemeferrolyase) enzyme is an enzyme that shows a catalytic effect in the in vivo synthesis of the proto Heme (Heme) structure (Dailey, 1990). Heme, which is formed by the binding of the Fe^{2+} to protoporphyrin IX substrate under the enzyme catalysis of the ferrochetase enzyme, is a compound found in all organisms other than a few bacterial species and extremophile. Protoporphyrin IX is tetrapirol precursors for iron chelation in heme biosynthesis. Although it is involved in oxygen binding and transport, electron transport, anaemia is observed in synthesis disruptions or disorders and protoporphyrins accumulating in the body have a toxic effect It is found in mitochondrial

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membrane in mammalian and yeast cells, while in bacteria it is present in the cytoplasmic membrane (Al-Karadaghi et al., 1997; Hardison, 1999).

Blue green algae are versatile tetrapyrrole synthesizers that can produce end products (heme, chlorophyll, phycobilins and cirrhome), which represent all the main branches of the tetrapyrrole biosynthetic pathway. In addition, phylogenetic studies show that tetrapyrrole synthesis genes in plants originate from blue green algae (Suzuki et al., 2002). Although blue green algae are not characterized as much as in plants, recent studies of the biochemistry and molecular genetics of this pathway have begun to take advantage of the oxygenic prokaryotic organisms (Beale, 1994).

2. Materials and Methods

2.1. Isolation, Purification and Cultivation of Blue-Green Algae

Water samples were taken from Yeşilırmak in Amasya province in 2018. 100 mL of water sample was inoculated in 100 mL of 2X concentrate BG11 medium and incubated for 4-6 week at 28°C in orbital shaker. Subsequent cultures were incubated in the BG11 solid medium (contain 1 % agar). Purified cultures were examined under light microscope (Leica DM500) morphologically.

Purified isolates were incubated in 250 mL volume flasks for 1 month for large volume production, then the flask volume was increased to 1 lt and incubated for 2 months in fresh medium. During incubation, ventilation was provided from the vacuum arm of Nuche flasks with CO_2 and O_2 gases at certain intervals.

2.2. Protein Extraction

The density of the cells was adjusted to 0.5 absorbance at 750 nm. The cells were centrifuged at 10000 g for 10 minutes, the supernatant was removed and the pellet was washed with PBS buffer. After re-centrifugation, the pellet was resuspended in 200 µL PBS. Cells were frozen at -80°C and rapidly thawed at 37°C. Cell wall was broken with ultrasonication probe in ice. Tube walls were washed with 100 µL PBS buffer and collected

by centrifugation at 7000 g for 10 minutes (Ivleva and Golden, 2007). Then the supernatant was used as crude protein extract.

2.3. Determination of Protein Concentration

The protein contents of the extracts were determined according to the Bradford method using bovine serum albumin as standard (Bradford, 1976).

2.4. Determination of Ferro-Chelatase Activity

1 mL of protein extract was incubated at 37°C in a Thunberg tube in a 4.2 mL reaction volume containing 200 µmol porphyrin substrate, 400 µmol $FeSO_4$, 40 µmol GSH and 200 µmol phosphate buffer (pH 7.8). After incubation, the reaction was stopped by adding 1 mL of pyridine, 0.5 mL of 1 N NaOH and 1 mL of water. The reaction mixture was divided into two equal portions. In the first part, 2 mg of solid $Na_2S_20_4$ and in the second part, 0.05 mL of 3 mM $K_3Fe(CN)_6$ were added and analyzed in UV-VIS spectrophotometer (Thermo Genesis 10S) (Porra and Jones, 1963). One unit of ferrochelatase activity is the amount of enzyme that catalyzes the formation of 1 nmol metalloprotoporphyrin at 1 h at 37°C.

3. Results and Discussion

As a result of sampling from Yeşilırmak, blue green algae with 8 different morphologies were isolated. As a result of the light microscopy examination of the isolates; It has been determined that there are filamentous members with heterocyst fixating nitrogen (Figure 1).

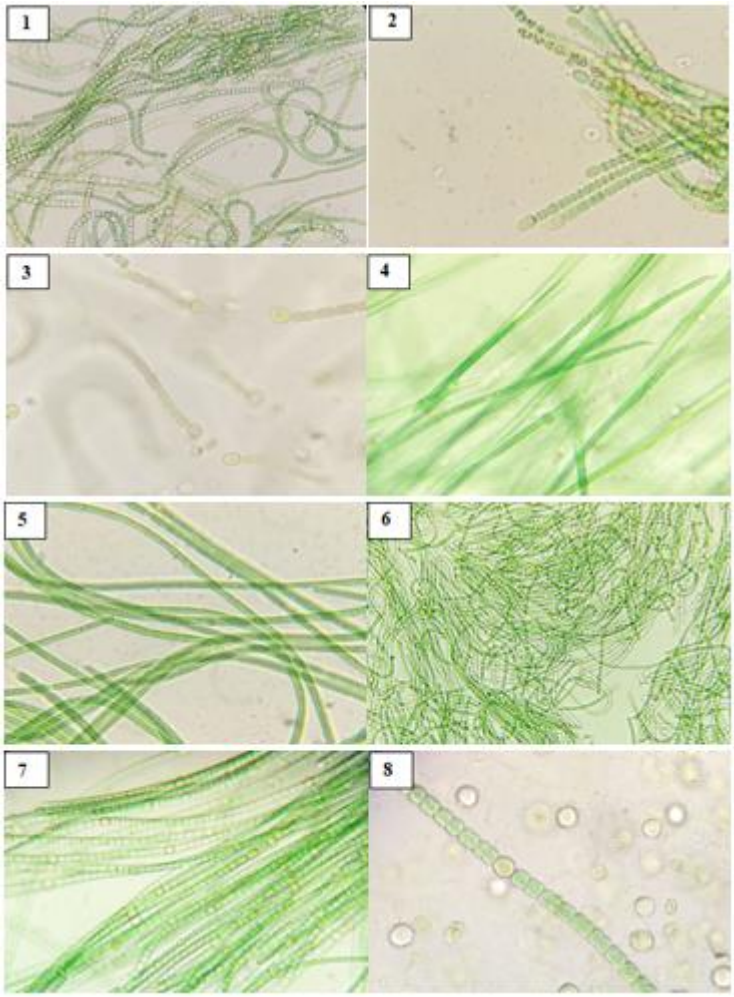

Figure 1. Light microscopy photographies of pure isolates (1-8)

As a result of protein extraction from 8 isolates protein content were found to be 26.1, 24.2, 26.2, 24.3, 23.3, 24.4, 28.1, 24.6 $mgmL^{-1}$, respectively. The highest protein content was found in isolate 7 (28.1 $mgmL^{-1}$) and the lowest protein content was found in isolate 5 (23.3 $mgmL^{-1}$). The ferrochelatase activity of the isolates is 5.12, 4.96, 4.44, 4.82, 4.54, 5.23, 3.91, 5.34 UmL^{-1}, and the specific ferroschetase activity is 0.196, 0.205, 0.169, 0.198, 0.195, 0.214, 0.139, 0.217, respectively (Table 1). It was calculated as Umg^{-1}. The highest specific activity belongs to the isolate 8 as 0.217 Umg^{-1}.

Table 1. Ferro-chelatase activity of isolates

Sample	Protein content ($mgmL^{-1}$)	Activity (UmL^{-1})	Specific activity (Umg^{-1})
Isolate 1	26.1	5.12	0.196
Isolate 2	24.2	4.96	0.205
Isolate 3	26.2	4.44	0.169
Isolate 4	24.3	4.82	0.198
Isolate 5	23.3	4.54	0.195
Isolate 6	24.4	5.23	0.214
Isolate 7	28.1	3.91	0.139
Isolate 8	24.6	5.34	0.217

The ferrochelatase activity of the wild and mutant strains of *Spirillum itersonii* on the crude membrane was found to be 44 Umg^{-1} in the presence of mesoporphyrin substrate (Dailey and Lascelles, 1974).

The specific activity of the ferrochelatase enzyme isolated from the *Saccharomyces cerevisiae* mitochondrial membrane was found to be 15 Umg^{-1} (Camadro and Labbe, 1988).

Ferrochelatase enzyme activity of pig liver tissue homogenate was found as 2.1 Umg^{-1}. As a result of the purification process, the specific activity was calculated as 977 Umg^{-1} (Cánepa and Llambías, 1988).

Myamato et al. (1994) expressed the enzyme ferrochelatase in *Escherichia coli*. The specific activity of the enzyme in the cell was found to be 56.6 Umg^{-1}.

Purification of *Rhodopseudomonas sphaeroides* membrane-bound ferrochelatase, the protein concentration in the crude membrane was found as 21 $mgmL^{-1}$, and the specific activity of the ferrochetase enzyme was found to be 0.98 Umg^{-1}. These results are similar to the protein and specific activity values of our isolates. The specific activity after purification was calculated as 1600 Umg^{-1} and it is thought that we can achieve similar results by applying similar purification steps (Dailey, 1982).

In this study, the ferrochelatase activity of 8 different blue green algae isolates was examined and similar activity results were obtained. The specific enzyme activity in homogenate is low due to its high protein content. Therefore, the enzyme must be purified for

high specific activity. However, there are limited data on the purification of the ferrochelatase enzyme. This is due to the enzyme not being stabilized outside the cell. Although the ferrochelatase enzyme has been studied in other organisms, bacteria, yeast, mammalian cells and plants (Jones, 1968; Goldin and Little, 1969; Dailey, 1982; Martinge et al., 1994; Franco et al., 1995), there is no comprehensive study with blue-green algae. Consequently, further studies are needed to elucidate the ferrochelatase enzyme activities of blue green algae.

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