THESIS ABSTRACT

Non Chlorophyll Accessory Pigments in Cyanobacteria: Simultaneous Extraction, Seperation and Maximization

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Aim of the thesis

The impartial objective of this study is to characterize, using the same criteria mentioned above, the cyanobacteria species located in areas around Ankara hot-springs. Then, morphology studies have been applied to identify cyanobacteria strains. After the specific identification, other measurements took place to specifically characterize these species according to some recent studies. After this characterization, we identified which ones were recorded in gene bank and which ones were not. The non-recorded species were further investigated and recorded to the gene bank as they are considered novel.

Hypothesis of the thesis

Cyanobacteria get energy through prokaryotes that receive sunlight as a source of energy using chlorophyll and various dyes. The presence of essential elements such as oxygen, nitrogen and carbon play a crucial role in aquatic environments. Until today, the picture remains unclear about the emergence of microbes like cyanobacteria

on Earth, and many researchers verified their current diversity more than two billion years ago. Cyanobacteria are the new revolution in modern science and caught the attention of a lot of scientists in the fields such as biotechnology, pharmacology, food industry. This attention was due to the unusual and specific conditions of living that these species were shown. Also, most of them are not yet reported in the literature. In this study, the idea is to collect some of these algae species from the some hot-spring areas in Ankara province/Turkey and characterize them specifically using methods obtained from other studies. This characterization will give us information about specific conditions. This study will also contribute development to the using of cyanobacteria in the areas mentioned above.

Materials and methods

Cyanobacteria are considered as a sustainable feedstock for the production of biochemically active compounds such as phycobiliproteins (PBPs). In this study, 12 different cyanobacteria isolated from hot-spring water resources in Ankara province were evaluated for their PBP production efficiency. Morphological analysis and Sanger sequencing revealed that 5 of the strains belong to *Nostoc* genus, 3 of them belong to *Anabaena*, and others belong to genus *Trichormus, Nodularia, Chlorogloeopsis, and Nodosilinea.* Isolated strains have been maintained in nitrogen lacking BG-11 growth medium during the thesis study. Total PBP content of *Trichormus sp.* IMU26 and *Anabaena variabilis IMU8* were found as 23.2% and 17.3% while it was lower than 15% in all other strains studied.

Kits Used in the Experiment
Tools and Devices
The Sampling Resources
Types of Media
DNA Experiment
DNA Extraction
Gel Electrophoresis
Polymerase Chain Reaction (PCR)
Sequence Information of Cyanobacteria Species
Extraction and Estimation of Phycobiliproteins
Extraction of Carotenoids and Chlorophyll a
Harvesting Biomass

Extraction of Carotenoids and Chlorophyll a
Measurement of Carotenoids and Chlorophyll a
Fourier Transform Infrared Spectroscopy (FT-IR) Analysis
Growth Measurements Content
Harvesting Culturing Biomass
Measurement of Total Protein Content
Extraction of Total Protein
Protein Determination
Measurement of Total Saccharide
Harvesting Biomass.
Extracting of Saccharide from the Samples
Measurement of Total Saccharide
Glucose Calibration
Analysis in High-Performance Liquid Chromatography (HPLC)
Carotenoid Analysis by HPLC
Vitamin Analysis B2 by HPLC
Fluorescence Imaging of Cyanobacterial lipids (Nile Red)

Results

The following studies were carried out to see the impact of nitrogen and phosphorus availability on PBP production on diazotrophic cyanobacteria isolated from hot-spring water resources. For this aim, the strains *Trichormus sp. IMU26* and *Anabaena variabilis IMU8* were incubated in N-replete, N-lacking, and N-P-lacking BG-11 growth medium. Results showed that nitrogen supply resulted in higher soluble protein and saccharide content but a lower growth rate and PBP production in *Trichormus sp. IMU26* and *Anabaena variabilis IMU8*. Short term (6 days) N-P-deprivation induced PBP production with no clear change in growth while growth and PBP content decreased in the longer incubation period (12 days). Fourier transforms infrared spectroscopy results refer that membrane-bound oligosaccharides may have regulatory roles for PBP production in *A.variabilis IMU8* during long term diazotrophic cultivation. Moreover, rapid induction of zeaxanthin and β-carotene production and a slight reduction of echinenone and canthaxanthin levels might be associated with increased PBP levels in short term N-P-deprivation of *Trichormus sp. IMU26.*

Conclusion

The thermal diazotrophic cyanobacteria *Trichormus sp. IMU26* and *Anabaena variabilis IMU8* were introduced to the literature as potential candidates for pilot scale PBP production. Isolated from hot-spring water resources, which differ from sea and spring waters with their unique mineralization and temperature levels, these two cyanobacteria grow well in N-lacking growth medium under room temperature and the contamination risk is fairly low.