CYANOBACTERIA AS BIOPRODUCERS OF ECONOMIC COMPOUNDS

By

BASSANT ESSAM AHMED SAYED
B.Sc. Agric. Sci. (Biochemistry/Microbiology), Fac. Agric., Cairo Univ., 2012

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences
(Agricultural Microbiology)

Department of Agricultural Microbiology
Faculty of Agriculture
Cairo University
EGYPT

2018

APPROVAL SHEET

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Name of Candidate: Bassant Essam Ahmed **Degree:** M.Sc.
Title of Thesis: Cyanobacteria as Bioproducers of Economic Compounds

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ABSTRACT

Cyanobacteria are known as a potential source of several metabolic compounds like phytohormons, phenols, amino acids, fatty acids, alkans as well as pharmaceuticals. These compounds are reported to be used in agriculture, biology and medicine. This study was performed to isolate *Anabeana*, *Nostoc* and *Fischerella* sp, from river Nile, Egypt. Also, their potential to produce some bioactive compounds were evaluated.

Results indicated that *Fischerella* BS1-EG isolated during this study as well as other Anabeana and Nostoc isolates have a considerable antifungal activity against *Aspergillus, Fusarium* and *Penicillum* sp. In general, the determined fungal activities by means of inhibition zone ranged from 6.8-16 mm.

Regarding the cell cytotoxicity of *Anabaena*, *Nostoc* and *Fischerella* BS1-EG cultures on liver cancer (HepG-2), lung cancer (A549), colon cancer (HCT-116), breast cancer (MCF-7), data indicated that all cyanobacterial crude extracts exhibited a variable influence on all tested cell lines. GC-MS analysis showed that 31 different compounds were detected and identified as fatty acids, alkaloids, phenols, amino acids. The most important 14 compounds were identified as anticancer, antimicrobial, antiinflammatory agents. Also 26 different compounds and the most important 15 compounds from *Anabeana* Qr and 22 compounds from *Nostoc* Sh1 and the most important 9 compounds having different biological activites.

On the other hand, results revealed that all tested cyanobacterial cultures proved to have anti hyper glycemia activity through inhibition of α-glucosidase activity. These results may indicate that, for the first time, *Fischerella* BS1-EG. is recorded to have different avenues as human anticancer and antidiabetic.

Keywords: Cyanobacteria, *Fischerella* , GC-MS, antidiabetic, anticancer.

DEDICATION

Special deep thanks and appreciation are given to my ***Lovely Family … Father " ESSAM AHMED", Mother " SAFAA EL REFEAA"*** *and* ***Sister " SAHAR ESSAM"*** *for all the love and endless support they offered in whole life during my study.*

ACKNOWLEDGEMENT

Foremost, I wish to express my sincere thanks, deepest gratitude and appreciation to ***Dr. Aziz Mohamed Aziz Higazy*** *Professor of Microbiology, Faculty of Agriculture, Cairo University, for his continuous support of my graduate studies, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. Without his supervision, guidance and persistent help this thesis would not have been possible.*

My sincere thanks are also due to ***Dr. Mona Hussein Badawi*** *Assistant Professor of Microbiology, Faculty of Agriculture, Cairo University for her supervision, continued assistance, and guidance through the course of my study and for her revision of the manuscript of this thesis. She was always willing to help and give her best suggestions.*

Great appreciation is also extended to ***Dr. Soha Sayed Mostafa****, Head Researcher of Microbiology, Soil, Water and Environment Research Inst., ARC, Giza for sharing in supervision, Kind help and continued suggestions during some of my experimental studies.*

I am so much grateful to ***Dr. Tarek Sayed Ragab****, Lecturer of Microbiology, Fac. Agric., Cairo University for his kind help and assistance in PCR experiments*

Words of appreciation must go to ***Dr. Diaa Attia Gab-Allah Marrez****, Researcher of Microbiology, NRC, Dokki for help and support during some practical trials.*

I would like to thank ***Dr. Mamoun S.M. Abd El-Kareem****, GC-MS Lab, Nuclear Research Center, Egyptian Atomic Energy Authority, Cairo, Egypt, for GC-MS Analyses.*

Great appreciation is also extended to all staff members and colleagues of the Microbiology Department, Faculty of Agriculture, Cairo University for their kind help and facilities they provided. Besides, I am grateful to my lab mates, ***Mr. Bahei Essam, Ms. Amal Ahmed, Ms. Eman Selim, Ms. Marwa Esmail, Mr.Amr Mostafa, Ms. Safinaz Magdi, Ms. Sherene Farouk*** *for all the help and great time we have had in the last three years.*

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INTRODUCTION

Despite significant progress in the fields of cancer diagnosis and chemotherapy, cancer remains one of the greatest causes of death worldwide. Novel approaches to cancer management often fail due to frequent genetic alterations and mutations in cancer genomes. Because of the high frequency of side effects caused by chemotherapy, metastatic cancers still need new and more effective chemotherapeutics. There is emerging interest in developing drugs to tackle these problems by using natural compounds, which may affect multiple targets with lower side effects and greater efficiency (Aung *et al.*, 2017).

In this respect, natural compounds from various sources including plants, animals and microorganisms were reported to offer a great opportunity for discovery of novel therapeutic candidates for the treatment of cancer (Newman *et al.*, 2003).

There are many therapies for cancer treatment, which may consist of a single or combination of classical treatments such as surgery, chemotherapy, radiotherapy, immunotherapy and monoclonal antibody therapy. Chemotherapy is a treatment that uses anticancer drugs to damage DNA in unhealthy and rapidly dividing cancer cells. Also, chemotherapy with a defined dosage is usually used to trigger cancer cell cytotoxicity at desirable apoptotic rates. The effectiveness of chemotherapeutic agents depends on their type, dosage and any adverse reactions in patients. There are several anticancer drugs used alone or in combination with other agents to kill cancerous cells.

Chemotherapeutic drugs that include synthetic, semi-synthetic and naturally occurring compounds are cytotoxic and can destroy both cancerous cells and rapidly dividing normal cells (Aung *et al*., 2017). On the other hand, treatment of cancer by natural compounds and their semi-synthetic analogues both *in vitro* and *in vivo* shows promising results against different malignancies (Manson, 2003; Prakash *et al*., 2013). Natural compounds such as sesquiterpenes, flavonoids, alkaloids, diterpenoids, saponins and polyphenolic compounds can be substituted for, or applied in combination with, existing drugs (Chai *et al*., 2010 and Millimouno *et al*., 2014).

Cyanobacteria, promising photoautotrophic prokaryotes, found in various freshwater and marine environments, are now well recognized as biosources for several pharmaceutical compounds. These compounds are necessary for treatment of different human diseases and disorders. In this respect, many species of cyanobacteria are known to have important role in treatment of various human diseases *e.g.* antibacterial (Burja *et al*., 2001), anti-HIV (Rajeev and Xu, 2004), antifungal (Burja *et al*., 2001), antiviral (Ramaknshnan, 2013), antiinflammatory (Shizuma, 2003), antioxidant and coenzyme (Plavisc *et al*., 2004), antidiabetic (Priatni *et al*., 2016) and anticancer (Sutharsana *et al*., 2016). It is reported that, in general, cyanobacteria are still unexplored as natural source offering a large amount of chemicals for original compounds discovery and new drugs (Singh *et al*., 2005). Traditional antibacterial and anticancer drugs producers like actinomycetes and hyphomycetes have been in the focus of pharmaceutical research for decades. Since the discovery rate of interesting compounds in these classical source organisms is decreasing, it is time to turn to cyanobacteria and exploit their potential. In this regard, cyanobacteria are well known to produce antitumor, anticancer, antiviral and antifungal compounds. Many of the pharmaceutically important compounds in cyanobacteria are peptides, including cyanobacterial toxins and important agents for anti-cancer drugs (Singh *et al*., 2017). Recently, several authors reported that *Fischerella* sp. (Devi and Mehta, 2016), *Anabeana* sp. (Hamouda *et al*., 2017), *Nostoc* sp. (Nowruzi *et al*., 2018) and *Oscillatoria* sp. (Wijesekara and Manage, 2017), produced several compounds such as fischerindole L, fischerellin A, ambiguine isonitriles A-F, ambigol A and B, tjipanazole D, cyanovirin-N, calothrixin A, lyngbyatoxin A and cryptophycin.

This study was designed to isolate and identify the dominant species of cyanobacteria from River Nile at Giza, Red Sea, Mediterranean Sea and Lake Quaron at Fayoum. All the selected cultures were characterized and evaluated for their potential capacity to produce secondary metabolites that may have antifungal, anticancer and antidiabetic activities.

REVIEW OF LITERATURE

1. Cyanobacteria

Cyanobacteria are morphologically diverse group of Gram-negative photosynthetic prokaryote. They are unique in their cosmopolitan distribution found in almost every conceivable habitat on earth (Nubel *et al*., 2000). They are able to survive in extreme environments such as rocky shores, hot springs as well as under desiccation, osmotic, salinity and UV stresses, photooxidation, heat and cold shock, anaerobiosis, nitrogen starvation *etc*. (Zehr *et al*., 2000). Cyanobacteria occupy a central position in global nutrient cycling especially due to their inherent capacity to fix atmospheric CO_2 and N_2 through Rubisco and nitrogenase enzymes respectively (Sinha *et al*., 1995). Some cyanobacteria were reported to fix atmospheric nitrogen in both fresh water and marine environments (Paerl, 2017). Until past few decades of research, cyanobacteria were of academic interests only and were mostly ignored as nuisance but, now they are proved as potential candidates for much biotechnological utilization (Singh *et al*., 2017). Cyanobacteria are ecologically and economically important microorganisms. From ecology point of view, they contribute significantly to the primary production of various ecosystems, especially freshwater and marine ecosystems, and play significant role in carbon, oxygen and nitrogen cycling (Waterbury *et al*., 1979; Tomitani *et al*., 2006).

Applications of cyanobacteria in biotechnology are reported in diverse areas, such as agriculture, aquaculture, pollution control *i.e.*

bioremediation, bioenergy and biofuels, and nutraceuticals have been well-documented (Patterson, 1996; Abed *et al*., 2009 and Pandey, 2010). Moreover, they are known to produce a wide range of pharmacologically important bioactive compounds *e.g.* antibacterial, antifungal, antiviral, anticancer, muscle relaxants) and commercially important high-value products, such as polyunsaturated fatty acids (PUFA) and phycobiliproteins (Skulberg, 2000; Pandey *et al*., 2007 and Tan, 2007; Barrios-Llerena *et al*., 2007; Sekar and Chandramohan, 2008 and Eriksen, 2008).

2. Ecology and taxonomy of cyanobacteria

The global importance of cyanobacteria is well known from their worldwide distribution and abundance as well as their contribution to atmospheric oxygen. In spite of a long history of cyanobacterial research in microbiology and botany, only a small portion of their high diversity has been recovered and only recently been addressed by molecular and phylogenetic methods (Rott *et al*., 2018).

Field-based biodiversity studies are still required due to the fact that the current knowledge on cyanobacteria taxa richness worldwide remains relatively restricted because 50 % of species are left to be described according to recent estimates (Nabout *et al*., 2013). However, major progress can only be reached by integration of field observations that report ecotypes adapted to a specific habitat with analysis of derived laboratory strains that allow evaluation of phenotype plasticity during the life cycle and at varied cultivation conditions while linking it to a stable genotype.

Morphological and ultra-structural studies are strongly suggested as a useful complement to laboratory investigations based on strains *e.g.* biochemical analyses, large-scale DNA sequencing (Willmotte *et al.*, 2017). In ecological studies, single cell- or colony-based approaches are applicable to a variety of cyanobacteria that resist cultivation. These techniques are useful for molecular identification and barcoding of special morphotypes that are manifested exclusively under natural conditions, plus their phenotype plasticity assessment and ecological niche estimation.

Cyanobacterial populations occupying specific niches in the environment and forming the key components of distinct microbial communities *e.g.* macroscopic biocalcified mats, epilithic growth in streams require development of new specific molecular markers with high resolution to enable understanding of their genetic identity and the identification of niche-specific ecotypes (ecomorphs). Elucidation of functional genes connected with species-specific traits and tracking of their expression is necessary to make progress in understanding the ecological roles of cyanobacterial components of microbial communities and consortia (Rott *et al.*, 2018).

Cyanobacteria are major contributors to global biogeochemical cycles. The genetic diversity among cyanobacteria enables them to thrive across many habitats. (Walter *et al.,* 2017) and therefore form a challenging group for the microbiologists. Their traditional taxonomy