CYANOBACTERIAL METABOLITES IN RELATION TO SOME PATHOGENS

By

RASHA KAMAL OSMAN HAMAD
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2003

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

2016

APPROVAL SHEET

CYANOBACTERIAL METABOLITES IN RELATION TO SOME PATHOGENS

M.Sc. Thesis
In
Agric. Sci. (Agricultural Microbiology)

By

RASHA KAMAL OSMAN HAMAD
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2003

APPROVAL COMMITTEE

Dr. KHALED ABDEL FATAH EL-DOUGDOUG ………………...
Professor of Microbiology, Fac. Agric., Ain Shams University

Dr. SAMIR ABDEL WAHED EL-GIZAWY ……………………....…
Professor of Microbiology, Fac. Agric., Cairo University

Dr. AZIZ MOHAMED AZIZ HIGAZY…………………………………..
Professor of Microbiology, Fac. Agric., Cairo University

Date: 22 / 2 /2016

SUPERVISION SHEET

CYANOBACTERIAL METABOLITES IN RELATION TO SOME PATHOGENS

M.Sc. Thesis
In
Agricultural Sci. (Agricultural Microbiology)

By

RASHA KAMAL OSMAN HAMAD
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2003

SUPERVISION COMMITTEE

Dr. AZIZ MOHAMED AZIZ HIGAZY
Professor of Microbiology, Fac. Agric., Cairo University

Dr. HANAN MOHAMED ABDEL- LATIEF
Lecturer of Microbiology, Fac. Agric., Cairo University

ACKNOWLEDGEMENT

I wish to express my sincere thanks, deepest gratitude and appreciation to Prof. ***Dr. Aziz Mohamed Aziz Higazy*** *Professor of Microbiology, Faculty of Agriculture, Cairo University, for suggesting the problems, supervision, continued assistance, and guidance through the course of my study and for his revision of the manuscript of this thesis.*

Sincere thanks are also due to Dr. ***Hanan Mohamed Abdel – Latief*** *lecturer of Faculty of Agriculture, Cairo University for sharing in supervision. Kind help and useful suggestions during my work. Experience that she does not skimp on me amultitude of thanks and appreciation for her.*

Great appreciation is also extended to all staff members of the Microbiology Department, Faculty of Agriculture, Cairo University. Special deep appreciation is given to my father and mother.

CONTENTS

CONTENTS (continued)

Name of Candidate: Rasha kamal Osman Hamad **Degree:** M.Sc.
Title of Thesis: Cyanobacterial Metabolites in Relation to Some Pathogens
Supervisors: Dr. Aziz Mohamed Aziz Higazy
Dr. Hanan Mohamed Abdel-Latief
Department: Microbiology
Approval: 22 / 2 / 2016

ABSTRACT

Increasing the microbial resistance to the current drugs is the one of the major problems nowadays. Therefore, there have been tremendous efforts towards finding new metabolites for the development of new antimicrobial drugs. The aim of this study was evaluating the antimicrobial activity of different cyanobacterial genera isolated from the River Nile. Also, food biopreservation via cyanobacterial extract was assessed in milk as a food model. Many species of cyanobacteria were detected in the River Nile. Among them, three representative genera were isolated and identified as *Fischerella, Oscillatoria* and *Anabaena*. Cyanobacterial filtrates and organic extracts from each filtrate and mass were examined for the inhibitory activity against seven tested microorganisms; *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 33018, *Escherichia coli* ATCC 8739, *Pseudomonas aeroginosa* ATCC 9027, *Salmonella typhimurium* ATCC 14028, *Aspergillus niger* NRRl 326 and *Candida albicans* ATCC 1023.The methanol extract showed more potent activity than hexane extract. The methanol extract from mass of *Oscillatoria* scored a high inhibitory effect against *E. coli*, *Ps. aeruginosa*, *B. cereus* and *S. aureus*. The broth microdilution assay gave minimum inhibitory concentrations (MIC) values ranging from 0.016 to 0.5 $\mu l\ \mu l^{-1}$. The antibacterial activity of methanol extract from both cyanobacterial filtrate and mass was decreased or completely lost after boiling for 10, 20, 30 min. On the other hand, the activity was stable or enhanced after pasteurization at 80°C/15 min. The methanol extract of *Oscillatoria* mass was evaluated for its applicability as biopreservative in milk. The milk was pasteurized, treated with 25 % (v/v) of the extract and stored at 4±1°C/20 days. After twenty days of storage, the decreasing percentage reached 96.3 % of *E. coli*, 97.8 % of *Ps. aeroginosa*, 99.6 % of *B. cereus* and 99.9 % of *S. aureus*. On the other hand, after four days of storage, the total viable count increased by 81.82% and 30.30 % in control and treated samples, respectively. That means the increasing rate of total viable count in the presence of *Oscillatoria* extract was lower than that in the control samples. After eight days, the initial population of spore-forming bacteria in treated sample decreased to 9.4×10^2 cfu/ml and reached 8.4×10^2 cfu/ml after twenty days (reduction percentage of 30.0%).

Key words: Cyanobacteria, Antimicrobial activity, Successive extraction, Thermal stability, *Oscillatoria*, biopreservation, Milk

INTRODUCTION

Increasing the microbial resistance to the obtainable drugs and spreading the multi-drug resistant microorganisms as methicillin resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumonia* (PRSP), vancomycin resistant *Enterococcus* (VRE), multi-drug-resistant *Mycobacterium tuberculosis* (MDR-TB) and *Pseudomonas aeroginosa* indicate the loss of conventional antibiotics efficiency. These factors lead to incessant search for discovery of new antimicrobial agents from new sources specially there are a large number of unexploited microbes, which produce potent bioactive compounds. In this respect, the natural products from cyanobacteria are most likely offering newer source of antibiotics (Kumar, 2014).

Cyanobacteria (blue-green algae) are a group of extraordinary diverse Gram-negative prokaryotes that originated 3.5 billion years ago (Kaushik *et al*., 2009). They are characterized by their capacity to perform biological nitrogen fixation and oxygenic photosynthesis. Cyanobacteria represent an exceptionally diverse but highly specialized group of microorganisms adapted to various ecological habitats. They can be found in terrestrial, glaciers, marine, brackish and fresh water environments and they are very resistant to extreme environmental conditions. For example, they were reported to tolerate the high temperature up to 50º C (Malathi *et al*., 2014). They are considered as a main component of phytoplankton in many freshwater and marine ecosystems (Dixit and Suseela, 2013).

Cyanobacteria produce one or a range of bioactive compounds, which are potentially rich source of a vast array of products with applications in feed, food, nutritional, cosmetic and pharmaceutical applications (Tan, 2007). Due to their high chemical stability and water solubility, these compounds have important implications. In this respect, Gerwick *et al*. (2008) found that secondary metabolites extracted from cyanobacteria were from the members of oscillatoriales (49%), followed by nostocales (26%), chroococcales (16%), pleurocapsales (6 %) and stigonematales (4%). Cyanobacteria such as *Anabaena*, *Nostoc*, *Microcystis*, *Lyngbya*, *Oscillatoria*, *Phormidium* and *Spirulina* produce variety of high value compounds such as carotenoids, fatty acids, lipopeptides, polysaccharides and other bioactive compounds. Also, Ghasemi *et al*. (2003) found that *Fischerella* species had ability to produce broad-spectrum of antimicrobial substances.

Most species of cyanobacteria are known to produce bioactive compounds with diverse biological activities in two ways either within the cell biomass *i.e.* intracellular metabolites or towards the environments *i.e.* extracellular metabolites. Generally, these activities may include cytotoxic, anticancer, antimalarial, anticardiotoxic, immunosuppressive, hypocholesterolemic, antihepatoxic, antialgal, antibacterial, antifungal and antiviral activities (Noaman *et al*., 2004, Dahms *et al*., 2006, El-Sheekh *et al*., 2008, Kaushik *et al*., 2009, Malathi *et al*., 2014 and Mandal and Rath, 2015).

The biomass of cyanobacteria shows bioactivity when extracted with organic solvents. Methanol extracts from *Tychonema bourrellyi, Aphanizomenon flos-aquae* and *Cylindrospermopsis raciborskii* were considered as antibacterial agents (Østensvik *et al*., 1998). The methanol extract of *Chroococcus dispersus* has both antifungal and antituberculosis activities (Ghasemi *et al*., 2007). Bioassays of methanol extracts from the genera of *Anabaena* and *Nostoc* were found to be antifungal and antibacterial (Drobac-Åik *et al*., 2007). Methanol extracts of *Oscillatoria* sp. exhibited inhibitory effect against fungal pathogens, followed by extracts in n-propanol, petroleum ether and water (Pawar and Puranik, 2008). Extractions of bioactive compounds from *Phormidium* sp. in different solvents (hexane, ethanol and water) were found to have antifungal and antibacterial activities (Meizoso *et al*., 2008). Methanolic extract of *Fischerella* sp. showed antibacterial activity (Asthana *et al*., 2006).

One of the most significant applications of natural antimicrobial agents is food biopreservation, as the microbial food contamination is the major food safety concern. In addition, in the recent years, the consumer demand towards high quality and safe food is growing. The antimicrobial agents have been successfully applied as food additives designed to reduce or eliminate pathogens or spoilage organisms in food increasing the food shelf life (Crozier-Dodson *et al*., 2004 and Balasubramanian *et al*., 2009).

Accordingly, the main objective of the present study was evaluation of the antimicrobial activities of different cyanobacterial genera isolated from the River Nile and drinking water treatment plant in Giza Governorate. Also, food biopreservation via cyanobacterial extract was assessed in milk as a food model.

REVIEW OF LITERATURE

1. Cyanobacteria

Cyanobacteria are a simple, but primitive and diverse group of microorganisms, with characteristics in common to both bacteria and algae. Their success as a group in a wide range of habitats has been attributed to their unique physiological characters and high adaptive ability under a wide range of environmental conditions (Kumar, 2014). Their species display a remarkable diversity in cell morphology or form. The unicellular cyanobacteria have spherical, ovoid or cylindrical cells that can occur single-celled or may aggregate into irregular colonies morphology. A slimy matrix secreted during the growth of the colony holds it together. Some cyanobacteria aggregate into regular colonies, or filaments. Filamentous morphology is the result of repeated cell divisions occurring in a single plane at right angles to the main axis of the filament or divide in more than one plane. The multicellular structure consisting of a chain of cells is called a trichome. Cell size and shape show great variability among the filamentous cyanobacteria (Rippka *et al*., 1979; Mur *et al*., 1999 and Newcombe, 2009).

Photosynthesis capture sunlight for energy using chlorophyll a and various accessory pigments. In addition to their remarkably long persistence as free-living organisms, cyanobacteria also form symbiotic associations with more complex biota (Vincent, 2009). Cyanobacteria are an extremely diverse group with different sets of traits, and will respond to different aspects of climate change *e.g*. increased stratification, altered nutrient availability (Carey *et al*., 2012). Many species are motile through gliding movement, which allow them to position themselves under optimal conditions (Whitton and Potts, 2002).

They lack nuclei and other organelles and they have a peptidoglycan cell wall that is typical of gram-negative Eubacteria. They also possess several features that set them apart from other bacteria, especially their photosynthetic apparatus and oxygen production. All cyanobacteria contain chlorophyll a and most contain the blue phycobiliproteins phycocyanin and allophycocyanin, giving the cells their characteristic blue-green color. Many taxa also contain the phycobiliprotein phycoerythrin, making the cells red, or sometimes black. Many filamentous cyanobacteria produce different cell types that play specific physiological, reproductive, or ecological roles. The most well known of these is the heterocyte (often called a heterocyst, although it is not a cyst) and is the location of the enzyme nitrogenase for nitrogen fixation, another specialized cell type is the akinete, a structurally reorganized cell that is formed under unfavorable conditions (Vincent, 2009).

Many cyanobacterial genera exhibit optimal growth rates and bloom potentials at relatively high water temperatures; hence global warming plays a key role in their expansion and persistence (Paerl and Otten, 2013). From ancient time cyanobacteria were used as food and fodder. However, it is notorious due to their toxin production and fouling of ponds as it form water blooms. They produce various metabolites that are antibacterial, antifungal, antimalarial, anticancerous, antitumor, antialgal, antiviral, UV protectants, inhibitors of enzymes, hepatotoxins and neurotoxins (Kumar, 2014). Cyanobacteria are also used in aquaculture, wastewater treatment, fertilizers, production of secondary metabolites including exopolysaccharides, vitamins, enzymes (Abed *et al*., 2009) and several phytohormones like indole-3-acetic acid and cytokinins (Schmülling, 2004; Ahmed *et al*., 2010 and Babu *et al*., 2013).

Intracellular and extracellular compounds from various cultured and field cyanobacteria, with diverse biological activities and a wide range of chemical classes, have considerable potential for development of pharmaceuticals and other biomedical applications (Tuyet, 2010).

2. River Nile ecology and pollution

River Nile is the longest river in the world, flowing south to north 6,850 kilometres, over 35 degrees of latitude (Karyabwite, 2000). It running from the heart of Africa to the Mediterranean and crossing the borders of ten countries comprising more than 300 million people (Tvedt, 2008). River Nile forms the main water resource of Egypt. The actual release from the High Aswan Dam shows very little yearly variation. Annual variation of the release of water from the High Aswan Dam depends mainly on irrigation needs. The release from the high Aswan Dam ranges from approximately 800 m^3/s during the winter closure period to approximately 2760 m^3/s during the summer months. In the Nile Valley and Nile Delta, groundwater resource use account for approximately 4.4 billion m^3/yr., mainly being recharged from the Nile and from seepage from irrigated agriculture.

River Nile supplies 65 % of the industrial water needs and receives more than 57 % of its effluents (Wahaab and Badawy, 2004). Millions upon millions look to the river for their drinking water, for the irrigation of crops, as a source of power, and as an object of cultural veneration and pride (Tvedt, 2008).

Fig. 1. Map of River Nile (Elgohary, 2012)