ANTIMICROBIAL ACTIVITY OF MICROORGANISMS PRODUCED PIGMENT ISOLATED FROM SOIL CULTIVATED BY MEDICINAL PLANTS

By

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B.Sc. Agric. Sci.,(Biotechnology), Fac. Agric., Cairo Univ., 2002

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APPROVAL SHEET

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DEDICATION

In the Name of Allah, the most gracious, the Most Merciful all praise is to Allah and prayers and peace. is upon Prophet Mohammed, His servant and messenger.

I dedicate this work to my dear and beloved thanks; to my parents, my husband Yaser, my brothers and my sister Hanaa, for their endless love, support and encouragement and to my son Ammar and my daughters Noran and Jana.

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ABSTRACT

The microbial communities of soil are among the most complex, diverse and important assemblages of organisms in the biosphere. They are an important source for the novel antimicrobial agents such as microbial pigments. Bacterial strains producing pigments like *Bacillus* sp., *Pseudomonas aeuroginosa, Pseudomonas fluorescence etc*., are prevalent in the soil environment, especially in root tubers and medicine plants.

The present study deals with the isolation of pigment-producing bacteria from soils cultivated with medicine plants in Giza Governorate. Also, extracted pigment was tested as an antimicrobial agent against fourteen pathogenic bacteria and fungi. The isolates were confirmed by the morphological and biochemical characterizations. The pure strain was grown in King meduim broth for green pigment and after the green pigment extracted with acetone was tested against *Bacillus cereus*, *Staphylococcus aureus*, *Salmomella Typhi* and *E. coli*. Results showed the high effect of green pigment was recorded with *E. coli* and *Salmonella Typhi* followed *Bacillus cereus* at 300 g/ml.

Keywords: Soil samples, green pigment, pigment production, antibacterial activities, antifungal activities.

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INTRODUCTION

The soil microbial communities are among the most complex, diverse and important assemblages of organisms in the biosphere. They are an important source for the search of novel antimicrobial agents and molecules with biotechnological importance such as microbial pigments that can be used as natural colorants as well as antimicrobial agents in place of antibiotic (Hackl *et al.*, 2004). The demand for new antibiotics continues to grow due to the rapid emergence of antibiotic-resistant pathogens causing life-threatening infections in spite of considerable progress in the fields of chemical synthesis and engineered biosynthesis of antimicrobial compounds (Carbonell *et al*., 2000). This changing pattern of diseases and the emergence of resistant bacterial strains to currently used antibiotics continuously put a demand on the drug discovery scientists to search for novel antibiotics such as bacterial pigments (Selvameenal *et al*., 2009).

Pigments of various colors are synthesized to protect the cells of micro-organisms from injurious effect of light rays of visible and near ultraviolet range (Mekhael and Yousif, 2009). These pigments are synthesized by various types of microorganisms as secondary metabolites and not often found in all types of organisms (Yokoyama and Miki, 1955). An important group of organic constituents of bacterial protoplasm is that of pigments. Some of these, like prodigiosin, pyocyanin, violacein, phenazine, pulcherrimin, iodinin, indigoidine and melanin are metabolic by-products formed under special circumstances (Giri *et al*., 2004). Microorganisms produce various pigments like carotenoids, melanin, flavones, quinines,

prodigiosins and more specifically monascins, violacein or indigo (Dufosse, 2006).

There are a few research papers concerned with isolated numbers of *Pseudomonas* that produce green diffusible pigment. The early reports (Hackl *et al.*, 2004) were concerned on isolated two strain of fluorescent *Pseudomonas* from water and soil environment as well as classified them as *Pseudomonas fluorescence* and *Pseudomonas putida*. These bacteria and many other strains of the fluorescent group of *Bacillus* were reclassified and renamed as fluorescent Pseudomonads. They suggested that *Pseudomonas* may excrete water-soluble fluorescent or green pigment into the culture media.

There is growing interest in microbial pigments due to their natural character and safety to use, medicinal properties, nutrients like vitamins, production being independent of season and geographical conditions, as well as controllable and predictable yield (Nakashima *et al.*, 2005). More over microbial pigments can be produced from waste material reducing water and environmental pollution (Joshi *et al.*, 2003).

This property is sometimes used as a taxonomic classification of different *Pseudomonas* (Carbonell *et al*., 2000). The biological activity of cultures of *Pseudomonas aeruginosa* and its inhibitory substances as well as the characterization of a metabolite responsible for antibacterial activity were also studied (Mekhael and Yousif, 2009 and Selvameenal *et al*., 2009).

Inspired by these facts, the aim of this research was to determine which bacteria producing pigment and evaluate the antimicrobial

activity of that pigments isolated from *Pseudomonas* against human pathogenic bacteria, fungi and to study antioxidant, cytotoxicity effect on human cell and assessment of purified pigment.

REVIEW OF LITERATURE

The increasing numbers in bacterial resistance to a number of antimicrobial agents are becoming a major health problem worldwide. Besides, the increasing use and misuse, of existing antibiotics in human, veterinary medicine and in agriculture have further aggravated the problem (Todar, 2004). Common species among them are methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant *Enterococcus* and *Mycobacterium tuberculosis* (Barrett and scott , 2003). It is further stated that, about 70% of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatment (Todar, 2004). Thus, there is an urgent need for the discovery of new classes of antimicrobial compounds to overcome existing resistance mechanisms and to effectively combat these human pathogens that can cause life-threatening infections. The importance of terrestrial microbes including bacteria as sources of valuable secondary bioactive metabolites is very well established and over 120 of the most important antibiotics in use today are obtained from terrestrial microorganisms (Alanis, 2005).

The new found awareness in human safety and environmental conservation has kindled fresh enthusiasm for natural sources of colors. Natural colorants or dyes derived from flora and fauna are believed to be safe because of non-toxic, non-carcinogenic and biodegradable in nature (Cristea and Vilarem, 2006). Traditional sources of colorants include natural products such as flavonoids and anthraquinones produced by plants and