

ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF CYANOBACTERIA.

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ABSTRACT

*The concept of biological control for health maintenance has received worldwide attention during the last few decades. Therefore, the main objective of this work was to look for active substances that could be used as antibacterial agents. To achieve this target different extracts (Methanol, Petroleum ether, Dichloromethan, Ethyl acetate) from *Anabaena oryzae*, were examined. The algal extracts were tested in vitro for its antibacterial effects against three Gram +ve and Gram -ve bacteria (*Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumonia*) using Agar well diffusion method and concentrations from 50 mg up to 150 mg were taken tested bacteria showed inhibition in growth by these extracts.*

*Conclusively, the results obtained in the present investigation clearly suggest that the production of pharmacologically products from *Aphanocapsa*, could represent a viable and environmentally friendly alternative to reduce the use of synthetic chemicals because of their unintended side effects for the control of pathogenic microorganisms.*

Key words: Antibacterial, cyanobacteria, methanol extract.

INTRODUCTION

Pharmaceutical drug discoveries, for most of the past 40 years, have depended heavily on the process of empirically screening of large number of pure compounds to provide new leads ,Cyanobacteria are a very old screening method of cyanobacteria for antibiotics, and other pharmacologically active compounds have recently received considerable

attention (Borowitzka, 1995). Algae are rapidly proving to be an extremely important source of biologically active secondary metabolites which could be used for the biological control of pathogens. Cyanobacteria (blue green algae) are one of the richest sources of biomedical relevant compounds with extensive therapeutic pharmaceutical applications (Tan, 2007; Gademann and Portmann, 2008; Martins *et al.*, 2008). Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial (Mundt *et al.*, 2003; Rao *et al.*, 2007; Kaushik and Chauhan, 2008), antifungal (MacMillan *et al.*, 2002), cytotoxic (Luesch *et al.*, 2000).

Most species of cyanobacteria are free-living, freshwater, marine or terrestrial which could be planktonic, or benthic and comprise major components of microbial mats. There are many reports on the inhibition of human pathogens by algal extracts. Research to identify antimicrobial compounds produced by microalgae against human pathogens as well as other pathogens, has recently received considerable attention as a new source of novel antimicrobial substances (Ladygina *et al.*, 2006). Certain systematic groups of microorganisms are characterized by specific composition of intracellular hydrocarbons; in particular, cyanobacteria are unique in their ability to produce 7 and 8-methyl heptadecanes (Ladygina *et al.*, 2006). Ethyl acetate extract of *Spirulenaplantensis* consisted of heptadecane and tetradecane which can inhibit some Gram +ve and Gram -ve bacteria and *Candida albicans* (Ozdemir *et al.*, 2004). El-Sheekh *et al.*, (2006) showed that phenolic compound from *Nostocmuscorum* exhibited antagonistic activity against Gram +ve and Gram -ve bacteria. Ghasemi *et al.*, (2004) isolated substances belonging to groups of peptides, polypeptides, amides and alkaloids from *Fischerellaambigua*. *Anabaena sp.* produce a number of bioactive compounds, mostly lipopeptidases that have antibiotic, antialgal, anticancer, anti-inflammatory, cytotoxic and enzyme-inhibiting effects (Burja *et al.*, 2001; Fujii *et al.*, 2002). *Oscillatoria sp.* can produce fatty acids, tetraamine, spermine and piperazine derivatives which show antimicrobial activity (Mundt *et al.*, 2003; Shanab, 2007). Thillairajasekar *et al.*, (2009) showed the presence of fatty acids from hexane and ethyl acetate extract of *Trichodesmium erythraeum* exhibited antimicrobial activity. These developments and the associated increase in bacterial infections intensified the search for new, safer, and more efficacious agents to combat serious bacterial infections.

Therefore, this study was designed to investigate the antibacterial activity of cyanobacterium species *Anabaena oryzae* and phytochemical analysis for its antibacterial active extract.

MATERIALS AND METHODS

Isolation and Purification of cyanobacteria:

Cyanobacterium, *Anabaena oryzae*, was isolated from soil. Isolation and purification of cyanobacteria was done according to the method described by Rippka, (1988). Cyanobacteria were isolated after repeated light migrations on solid BG11.

Cultivation of cyanobacteria:

Cyanobacteria were grown in Erlenmeyer flasks, (250 ml) on liquid BG11 medium in axenic conditions. The cultures were incubated at the room temperature ($25\pm 2^{\circ}\text{C}$) and a light intensity 2500 lux provided by cool, and white, fluorescent tubes under continuous illumination.

Harvesting the cyanobacteria :

Cyanobacterial cells were collected by centrifugation in the exponential growth phase (after 12 days), the pellets were washed with distilled water and dried in oven at 60°C .

Preparation of cyanobacterium extract:

0.1 gm of cyanobacterium was soaked in petroleum ether for 48h and filtrated. The residues were soaked again in petroleum ether for 48h and filtrated. The precipitate was soaked in Dichloromethane for 48h and repeated after 48h soaked and filtrate and then by methanol. The filtrate was taken and concentrated in vacuum until drying. The concentrated residue re-dissolved in 2 ml dimethylsulphoxide (DMF) to form different concentration (50,100,150 $\mu\text{g/ml}$). DMF is used as control.

Microbial indicators and growth conditions

Gram +ve and Gram -ve bacteria were used in this study. *Escherichia coli*, *klebsiella pneumonia* (Gram -ve), *Bacillus cereus* (Gram +ve). Bacterial strains were kindly provided from Microbiology Laboratory, Botany Department, Faculty of Science, Tanta University, Egypt. All bacterial strains were maintained on nutrient agar slants and incubated at 30°C . Bacterial biomass was prepared by inoculating 100 ml of nutrient broth medium. Bacterial cultures were shaken (250 rpm) at 30°C for 24 h. Different inoculates were used at a logarithmic phase of growth.

Testing antibacterial activity by the agar-well diffusion method

The antagonistic activity of the extract of cyanobacterial cells was determined using cut-diffusion technique in which cut (5 mm) was

punched upon the surface of agar plates previously inoculated with each of the above mentioned indicator strains. Each well bottom was sealed with two drops of sterile water agar. About 100 mg of algal extract were transferred into each well. Wells loaded with the extracting solvents were used as controls; plates were then incubated at 37°C for 24 h. After incubation, the diameters of the inhibition zones were measured with calipers and the results were recorded in mm (Attaie *et al.*, 1987). All tests were performed under sterile conditions in duplicate and repeated three times.

Electron microscopic examination:

In order to visualize the morphological changes in the bacterial cells after treatment with the methanol extract of *Anabaena oryzae*, scanning electron microscope studies were carried out. Bacterial cells were harvested, washed with phosphate buffer and fixed with 2% glutaraldehyde followed by 1% osmium tetroxide. After completion of fixation, samples were washed in buffer solution and the washed samples were dehydrated in ascending order of ethanol concentrations. The samples were dried completely and finally coated with gold in JEOL-JSM 5300, The Scanning Electron Microscope for samples, Using SEM Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K.V., magnification 14x up to 1000000 and resolution at the ministry of petroleum the Egyptian mineral resources authority central laboratories sector.

Statistical analysis:

Each single treatment was replicated three times and the mean value \pm standard error was considered.

RESULTS:

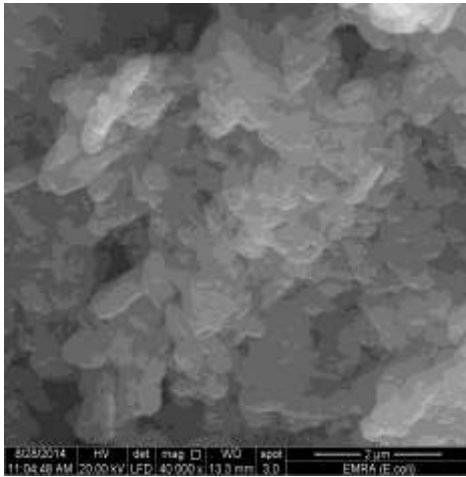
Antibacterial activities of cyanobacterial extract

The results showed that methanol extracts notably inhibited nearly the whole panel of the tested bacteria with the widest inhibition zone (17.3 mm) against *Escherichia coli*, and smaller inhibition zone (15.3 mm) against *Klebsiella pneumoniae*, and no inhibition zone against *Bacillus cereus* (Table 1).

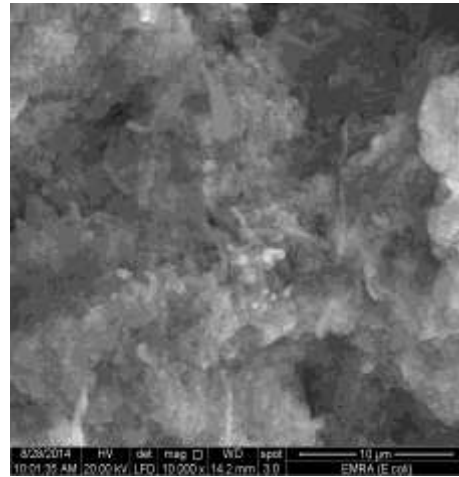
Electron microscopic examination:

Electron microscopic examination of *Escherichia coli*, *Bacillus cereus*, *klebsiella pneumoniae* before and after treatment with methanol extract of

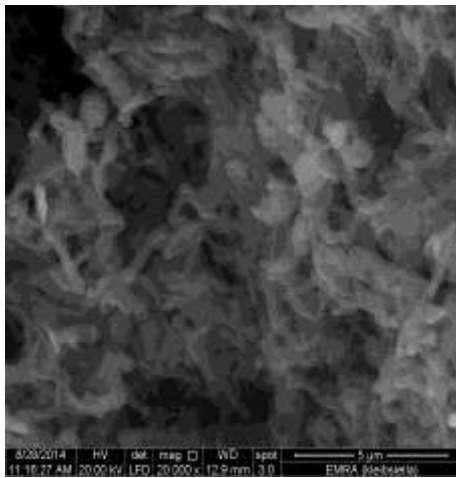
Anabaena oryzae, demonstrated morphological alteration and they were greatly transformed by the effect of algal extract as shown in Figure 1.



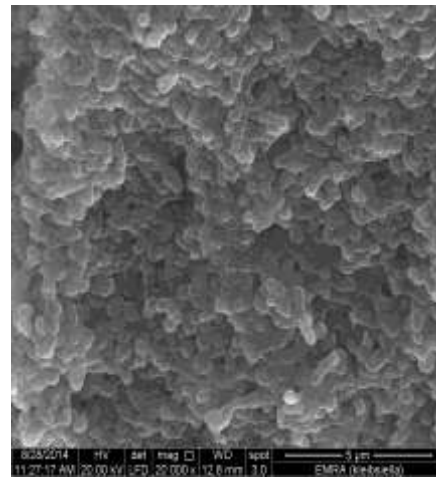
E. coli treated with algal extract



E. coli untreated with algal extract



K. pneumonia treated with algal extract



K. pneumonia untreated with algal extract

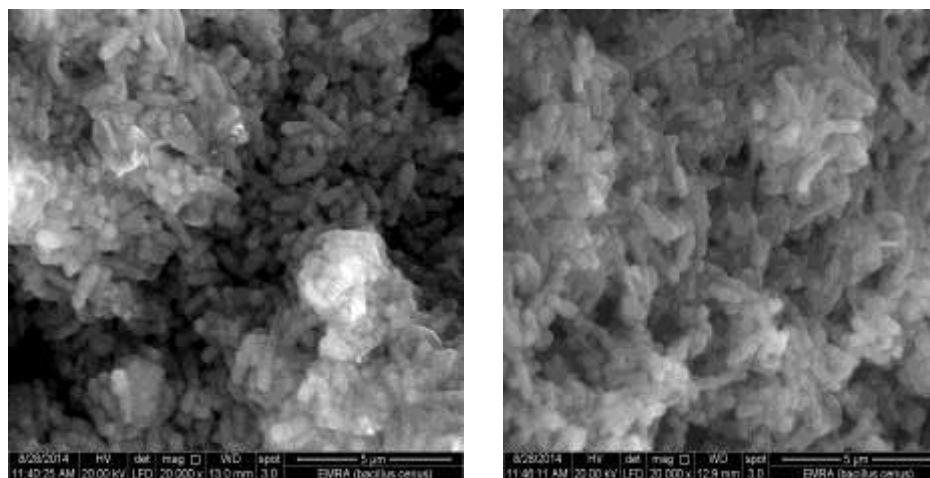
*B. cereus* treated with algal extract*B. cereus* untreated with algal extract

Figure (1): Electron microscopic examination of *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumonia* before and after treatment with methanol extract of *Anabaena oryzae*.

DISCUSSION:

The goal in this study is to reveal the biological production of bioactive compounds by some cyanobacterial species. This study confirms that the methanol extract of cyanobacterial species have high activities against the tested microorganisms. The cluster analysis showed that, not all the target strains tested were equally susceptible to antibacterial metabolites produced from cyanobacterial species. These differences could be attributed to the strain of bacteria used could affect the results significantly (Philip *et al.*, 2009). Our results are in agreement with Piccardi *et al.* (2000) who evaluated 50 *Nostoc* isolates from different habitats for their activity against two fungi (*Penicillium expansum* and *Rhizoctonia solani*), and three bacterial species (*Agrobacterium vitis*, *Escherichia coli* and *Staphylococcus epidermis* and *Artemiamauplii*). The screening has shown that 24 of the isolates showed activity against at least one of the target organisms. it can be concluded that algal activity against Gram -ve bacteria was common as well as against Gram +ve and the tested fungi. These results go in harmony with those obtained by Feiet *al.* (2002); Walters *et al.* (2003) and Ghazalaet *al.* (2004) in their studies against Gram +ve, Gram –ve bacteria and pathogenic fungi. The ability to

produceagents may be significant not only as a defensive mechanism for the algal strains but also as a good source of the new bioactive compounds from a pharmaceutical point of view.

Conclusively, the results obtained in the present investigation clearly suggest that the production of pharmacologically products from *Aphanocapsasp*, could represent a viable and environmentally friendly alternative to reduce the use of synthetic chemicals because of their unintended side effects for the control of pathogenic microorganisms.

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مواد مضاده للبكتريا من مستخلصات مختلفه من الطحالب الخضراء المزرقة

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السادات -مصر

أجريت هذه الدراسة لاختبار الواسمات الجزيئة الخاصة بجينات المقاومة لفيروس
اصفرار و تجعد الاوراق فى اربعة عشر صنف من الطماطم المحلية و الهجن و هى
Jubilee, Peto 86, Super Strain B, Advanteg , Edkawi , Super Bader,
Tempo(LA 2050), Azteca, G.5, G.S, Nada, 9062, 9065 and 9064.
تم زراعتها فى الحقل المفتوح و الصوبة وتم عمل فحص لجين TY2 المقاوم
للفيروس. جميع الاصناف تحت الدراسة اظهرت الحزمه ٨٠٠ قاعدة نيكلو تيديه و
التي تدل على ان هذه الاصناف حساسة للفيروس، ماعدا الصنف ٩٠٦٤ الذى اظهر
الحزمة ٨٠٠ قاعدة و ٩٠٠ قاعدة نيكلو تيديه و اللتان تدل على ان هذا الصنف مقاوم
للفيروس.

التوصية: أوصت هذه الدراسة ان:

* استخدام طرق الواسمات الجزيئية للتعرف على الأصناف المقاومة و الحساسة
للفيروسات.

* استخدام الطرق الجزيئية الحديثة للتعرف على الأصناف المحلية لدى مربى
النبات و ذلك قبل إدخالها فى برامج التربية لتوفير الوقت و الجهد و المال.