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Characterization and Antibacterial Effects of *Microcystis aeruginosa* Extracts

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MICROCYSTIS aeruginosa MDEG1 strain from El-Manzala Lake (Damietta, Egypt) was identified classically by its morphological characteristics. Its identification was confirmed using 16S rRNA gene sequencing. Different concentrations (1, 10, 25 and 50mg/ml) of petroleum ether and chloroform of *M. aeruginosa* MDEG1 extracts were examined for their antibacterial activity by well diffusion technique. Petroleum ether extract had a high activity against *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. Chloroform extract exhibited higher activity against *K. pneumoniae*. Gas chromatography, mass spectrophotometry (GC-MS) and H-proton nuclear magnetic resonance (^{1}H-NMR) analysis revealed the presence of Hexadecanoic acid (Palmitic acid) and its methyl ester derivative as major constituents of petroleum ether and chloroform algal extract, that might be involved in their antibacterial activity. The cytomorphology using scanning electron microscopy (SEM) for treated *K. pneumonia* (Nosocomial infection bacteria) with *M. aeruginosa* extract (Chloroform, 50mg/ml) showed appearance of giant cell formation, while transmission electron microscope (TEM) showed ruptured bacterial cell wall damage with extrusion of cytoplasmic content indicating its bactericidal effect.

Keywords: Cyanobacteria, *M. aeruginosa*, Antimicrobial activity, SEM, TEM.

Introduction

The cyanobacteria have been recognized to provide chemical and pharmacological novelty and diversity. They are a rich source of bio-fertilizers, food, feed, antimicrobial agents and they produce compounds that have potential biotechnological application (Muruga et al., 2014). They provide a safer and cost effective way of treating bacterial infections (Pradhan et al., 2014). Cyanobacteria are especially abundant in shallow, warm, nutrient rich or polluted water low in oxygen and can grow to form thick scums that could color the water, creating blooms (Stotts et al., 1993). *M. aeruginosa* is one of the most widespread and bloom-forming species in the fresh and brackish water (Rzymski et al., 2014). *Microcystis* (order Chrococcales and class Cyanophyceae) occurs in the form of characteristic colonies that can be classified as different morphological types (morphotypes), each of which is equivalent to a species (morphospecies) (Komárek & Anagnostidis, 1999). *Microcystis* colonies differ in shape and size, but also in the appearance of their mucilage (Via-Ordorika et al., 2004). Molecular identification of *Microcystis* had been established by using 16S rRNA gene sequencing to differentiate between its species (Neilan et al., 1997; Otsuka et al., 1998).

Although, antibiotics have been widely used in last decades, the development of microbial resistance to them is also increased (Mohamed et al., 2018a). In order to overcome this problem, the exploring of new antibacterial substances from bioactive algal compounds especially from cyanobacteria is as an alternative solution and an important necessity (Tajbakhsh et al., 2011). This is due to their wide biological activities, low cost and higher safety compared to synthetic

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drugs (Grabley & Thiericke, 1999). Fatty acids (FAs) are ubiquitous molecules typically bound to other compounds such as glycerol, sugars or phosphate head groups to form lipids. FAs can be released from lipids, typically by enzyme action, to become free fatty acids (FFAs) which have diverse and potent antibacterial activities (Desbois et al., 2009). The antibacterial activity of each FFA is influenced by its structure and shape. This, in turn, is a function of the carbon chain length and the presence, number, position and/or orientation of the double bonds (Zheng et al., 2005).

Usually, the antimicrobial compounds have a severe effect on the exposed microorganisms' cells. Generally, the morphological abnormalities of the bacterial cells were observed by scanning electron microscopy (Mohamed et al., 2018b). On the other hand, formation of extracellular capsular polysaccharides, changes in cell size, and cell division alterations were also determined by analysis of transmission electron microscopy (Alexander et al., 2006).

The aim of the current work is to determine the biological capacity of *Microcystis aeruginosa* isolate in relative to its antibacterial activity and its cytomorphological effect on *Klebsiella pneumoniae*.

Materials and Methods

Collection of the algal sample

Algal bloom of *Microcystis aeruginosa* (order Chrococcales and class Cyanophyceae) has highest cell density (99%) during late autumn months (November) as recorded at El-Manzala Lake (brackish water). The bloom sample was collected with plankton net of 10µm mesh size. The samples were then spread on string nets allowed to dry in air. Air- dried samples were weighed to evaluate the dry wt., grounded and stored in stoppered bottles in refrigerator.

Genomic DNA extraction

Chromosomal DNA was extracted by phenol/ chloroform technique according to procedure modified from (Ausubel et al*.,* 1996). 1.5ml of fresh cell suspension was centrifuged at 6.500rpm for 5min. The pellet was suspended in 1ml Sucrose-EDTA-Tris-HCL (SET buffer) (20% sucrose, 50mM Tris-HCl, PH 7.6, 50mM EDTA) for washing by centrifugation at 6.500rpm for another 5min. The pellet was re-suspended in 100µl SL buffer (SET buffer + Lysozyme), vortex and followed by the addition of 2µl RNase. After incubation at 37°C for 10min, 500µl TE buffer (10mM Tris HCl, pH 7.6, 0.2mM EDTA) and 50µl of 10% sodium dodecyl sulfate were added. DNA was extracted with 500µl of phenol/ chloroform isoamyl alcohol solution (25:24:1), centrifuged at 6.500rpm for 5min and precipitated with cold isopropanol. DNA was harvested by centrifugation at 6.500rpm for 10min, washed with 500µl of 75% cold ethanol and the DNA pellet was suspended in 1/10 TE buffer and stored at -20°C until used for PCR amplification.

Primers and PCR conditions

The 16S rRNA gene was amplified by using a pair of universal primers (Neilan et al. (1997), specific for prokaryotes (27 F: AGAGTTTGATCCTGGCTCAG and 1494 R: TACGGCTACCTTGTTACGAC). The PCR product was sequenced by an automated sequencer (Macrogen, South Korea) using the same previous primers.

Agarose gel electrophoresis

The detection of genomic DNA and size of PCR products were examined by agarose gel electrophoresis. 1% agarose was dissolved in TAE buffer pH8.0 (0.04M Tris-acetate, 0.001M EDTA). For DNA visualization, 2µl ethidium bromide (10mg/ml) were added and the gel was examined by UV transilluminator. Usually 2µl of loading buffer (0.25% Bromophenol blue, 70% Glycerol, 10mM Tris-HCl pH 7.0), 1Kb DNA Ladder (Fermentans) were used as molecular marker to indicate the size of the DNA fragments.

Alignment and phylogenetic analysis

BLAST (Altschul et al., 1997) was performed for the resulting 16S rDNA sequence to match the best similarities with other related sequences on database. The best DNA sequence similarities obtained from NCBI GenBank were aligned with our 16S rDNA sequence using CLUSTAL Omega. Unalignable regions were excluded manually and the sequences from the same species and unidentified organisms were discarded. MEGA version 4 was used for phylogenetic tree analysis and viewing. The neighbor-joining was performed using the maximum composite likelihood methods with confidence levels estimated by 1000 bootstrap replicates (Tamura et al., 2007).

Algal extraction

About 200g of air dried *M. aeruginosa* were soaked in methanol and left overnight at room temperature, then filtered off. The marc was washed several times with methanol then the filtrate was evaporated under reduced pressure to afford the crude extract, and then stored in dark containers at 4°C until usage (Perumal et al., 2012). Successive extraction to exhaustion was done with petroleum ether (60-80°C) followed by chloroform. The marc after each extraction was dried until freed from the solvent, before extraction with the next solvent. The obtained extracts were separately concentrated under reduced pressure, dried to constant weight in vacuum desiccators, weighed and reserved in the refrigerator until further investigation.

GC-MS analysis

The compounds identification was performed by an Agilent 6890 Gas Chromatograph (GC) equipped with an Agilent Mass Spectrometric (MS) detector, with capillary interface and fused silica capillary column PAS 5ms (30m × 0.32mm × 0.25mm film thickness) at Cairo Agricultural Research Center. 1ml of algal extract sample was injected under helium as a carrier gas at approximately 1ml/min, pulsed split less mode and solvent delay was 3min. The mass spectrophotometric detector was operated in electron impact ionization mode with energy of 70e.v. scanning from m/z 50 to 500. The instrument was manually tuned using perfluorotributy l amine (PFTBA).

^{1}H NMR spectra analysis

H-Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectra were obtained using 300MHz Bruker NMR instrument. Chemical shifts are given in δ (ppm) relative to TMS as internal standard material at Faculty of Science, Kafr El-Sheikh University.

Antibacterial assay

The total extracts of petroleum ether and chloroform were decolorized on activated charcoal and then filtered. Each filtrate was taken to dryness under reduced pressure at 40°C. Part of the obtained thick tarry residues was dissolved in dimethyl sulfoxide (DMSO) to make 1, 10, 25 and 50mg/ml concentrations, and then screened for the presence of antibacterial activities against bacteria (*Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosae, Klebsiella pneumoniae* and *Proteus marbilis*), which were kindly provided from the Laboratory of Bacteriology, Faculty of Science, Damietta University, Egypt.

Pore plate method was performed on medium contains 5g glucose, 5g peptone, 5g sodium chloride, 3g beef extract and 15g agar, dissolved in distilled water up to 1L (Hornsey & Hide, 1974). 0.3ml of algal extract was introduced into 10mm well diameter. For the control, 0.3ml of DMSO was used. Inhibition zones were read one day after incubation at 37°C. Each assay was repeated three times and the mean values were recorded.

Scanning electron microscopy

Agar cultures with bacterial colonies were first excised and trimmed to approximately 10mm x 10mm specimens as thin as possible (1-2mm), and fixed in a buffered (0.1M phosphate buffer, pH 6.5-7.0) fixative 2-3% glutaraldehyde for periods ranging from 5min to 24hr before they were further reduced into smaller (about 5mm x 5mm) specimens (Glauert, 1975; Erdos, 1986). Then, the specimens were post-fixed in 1% osmium tetroxide in the same buffer, dehydrated in a graded series of acetone. Critical-point dried specimens were coated with gold palladium in a polaron E500 sputter coater (Polaron Equipment Ltd., England) and viewed in Scanning Electron Microscope (JEOL JSM 35C) at the EM unit, Faculty of Science, Alexandria University.

Transmission electron microscopy

The tested bacteria were inoculated in nutrient broth media containing algal extract at final concentration (50mg/ml). Also, control untreated bacteria were included. After incubation time, the bacteria were centrifuged, and then fixed with formalin-glutaraldehyde fixative ($_{4}F_{1}G$) in 0.1M phosphate buffer pH 7.4. After rinsing in the buffer, samples were post-fixed in 2% osmium tetroxide for 2hr at 4°C in the same buffer. The cells were washed and dehydrated at 4°C through a graded series of ethanol. Cells were then treated with propylene oxide solution and embedded in a mixture of 1:1 of Epon-Araldite for 1hr. Polymerization was done in the oven at 65°C for 24hr. Ultrathin sections (50µm) were cut on ultratome (Model LKB), then mounted on copper grids, double stained with uranyl acetate and lead citrate and investigated on a JEOL 100CX TEM at the EM unit, Faculty of Science, Alexandria University.

Results

Morphological characteristics of Microcystis

The colony morphology, size and mucilage characteristics of the *Microcystis* isolate were performed. The colonies were more or less spherical, and the cells usually 4-6μm in diameter, sparsely to densely agglomerated and contained gas vesicles. The mucilage was colorless, structureless and did not form a very wide margin around the cells. According to Komárek & Anagnostidis (1999), this morphospecies can assign to *Microcystis aeruginosa* MDEG1 isolate.

Molecular identification of Microcystis

The 16S rRNA gene sequencing of the PCR product for the *Microcystis* isolate MDEG1 resulted 1428bp (Accession No. MG979399). Its DNA sequence alignment revealed high similarity with some different species of *Microcystis* including *M. aeruginosa*. The best identity reached 100% with *M. aeruginosa* (Accession No. D89031). The other species were closely clustered as one group with less identity with the studied *M. aeruginosa* isolate, while *M. elabens* (AB001724) and *M. holsatica* (Accession No. D89036) were clustered away in separate clade (Fig. 1).

Bioactivity of M. aeruginosa extract

The antibacterial activity of *M. aeruginosa* MDEG1 strain against *S. aureus*, *B. cereus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *P. marbilis* was evaluated. Generally, 50mg/ml algal extract concentration showed the highest effect against all bacterial species (Table 1). Petroleum ether extract of *M. aeruginosa* MDEG1 possessed a good activity against most of the Gram negative bacteria (*K. pneumoniae* and *P. aeruginosa*) and also Gram positive bacteria (*S. aureus)*. On the other hand, the chloroform extract exhibited higher activity against Gram negative bacteria *(K. pneumoniae)* and showed lower activity against the other tested bacteria. Furthermore, all concentrations of both extracts showed no activity against *Bacillus subtilis*.

Compounds identification

The petroleum ether extract of *Microcystis aeruginosa* contained 19 compounds. They comprised 18 aliphatic compounds (10 fatty acids, 4 alkanes, 2 monoterpenoids, 1 fatty alcohol and 1 hexadecanoic acid), and only one was aromatic. The major constituent with a peak area 48.43% and retention time (R_t) 21.71 was for hexadecanoic acid (palmitic acid). The compound 2, 2-dimethyl-7-methoxy-2, 3-dihydro-2H-benzo[b]thiene gave the peak area 0.13% and retention time (R_t) 15.41 (Table 2). The algal chloroform extract revealed 16 compounds mainly aliphatic fatty acids and fatty acid methyl ester, only three of them were heterocyclic compounds (β-pinene. loliolide, and 2-hydroxy-5,6-epoxy-β-ionone). The main constituent was palmitic acid, methyl ester with 78.98% peak area and R_t at 39.81.

Cytomorphological effect of M. aeruginosa extracts

The most sensitive strain (*K. pneumoniae*, Nosocomial infection bacteria) was chosen to study the effect of 50mg/ml *M. aeruginosa* chloroform extract on its cytomorphology and ultrastructure using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The SEM revealed that the exposure of *K. pneumoniae* cells to chloroform extract of *M. aeruginosa* resulted in giant cell formation and appearance of elongated filament like structure, compared with normal straight rod cells arranged singly or in pairs for the untreated bacteria (Fig. 2). The TEM micrographs of treated *K. pneumoniae* revealed successive bactericidal effects including electron dense granules and electron lucent vacuoles formation in the cytoplasm**,** weakening and damaging of the boundary cell wall**,** cell malformation, loose attachment of cell membrane, twisted cell were clearly visible and finally granular cell contents exudes from the damaged membrane. The untreated cells exhibited normal appearance with polysaccharide capsule of considerable thickness which was closely adhering to the cell wall (Fig. 3).

Discussion

Microcystis is a coccoid cell shape with tendency to form aggregates of colonies having gas vesicles and amorphous mucilage or a sheath (Komárek & Anagnostidis, 1999). Based on the previous criteria, ten species of *Microcystis* had been characterized in Europe including *M. aeruginosa* (Kützing) Kützing, *M. botrys* (Teiling), *M. firma* (Kützing) Schmidle, *M. flos-aquae* (Wittrock) Kirchner, *M. ichthyoblabe* (Kützing), *M. natans* (Lemmermann) ex Skuja, *M. novacekii* (Komárek) Compère, *M. smithii* (Kützing and Anagnostidis), *M. viridis* (A. Braun in Rabenhorst) Lemmermann and *M. wesenbergii*

(Komárek) Komárek in Kondratieva. Many other species had been also identified outside Europe. The morphospecies *M. aeruginosa* have been identified in brackish water blooming from El-Manzala Lake, Damietta, Egypt. Some species including *M. aeruginosa* are often found in North African freshwater habitats (Nasri et al., 2004) and drinking water sources (Chiu et al., 2017).

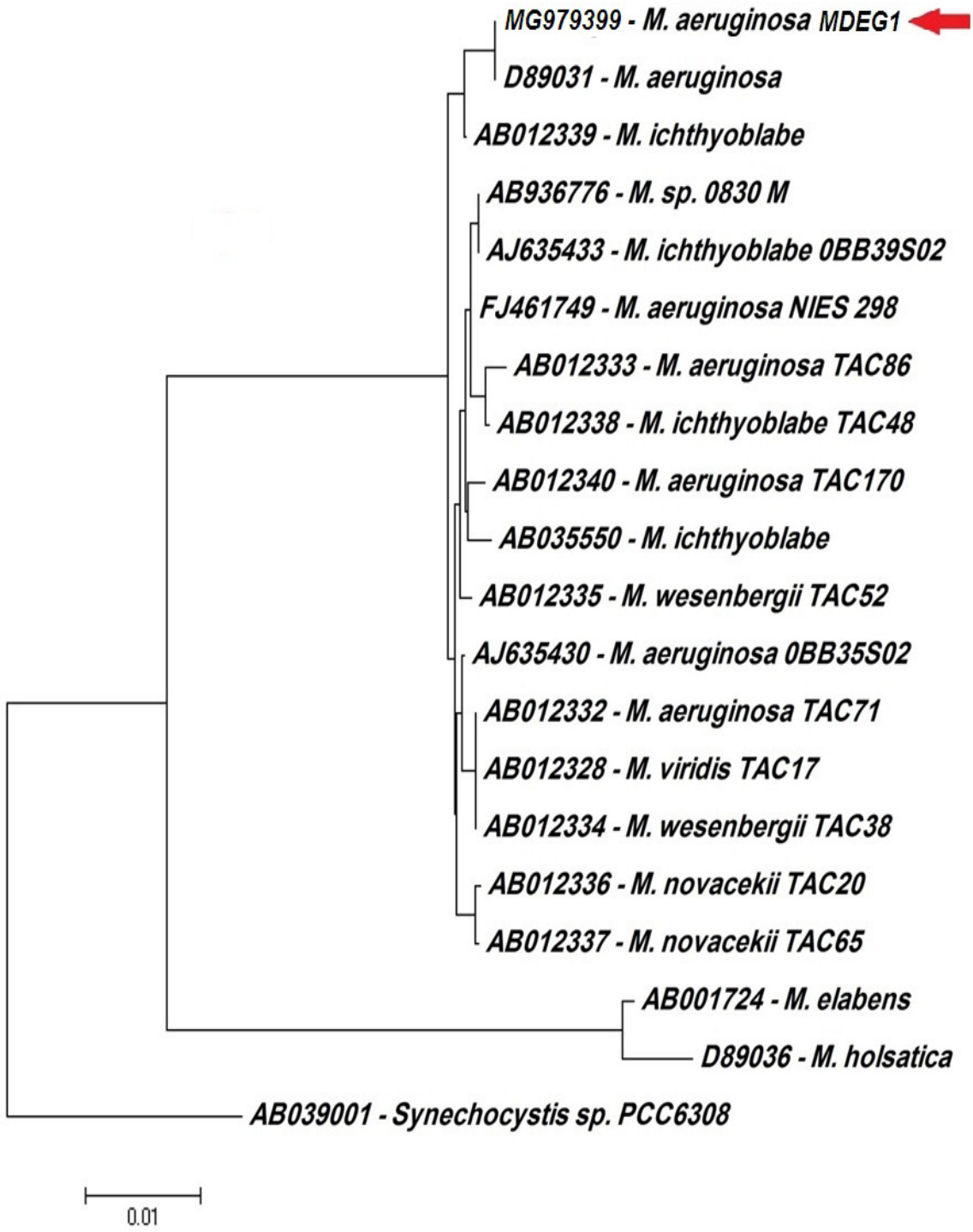

Fig. 1. Phylogenetic tree based on the 16S rDNA sequence for *M. aeruginosa* MDEG1 (AC: MG 979399) with other related members of *Microcystis* [The rooting of the tree was achieved by *Synechocystis* sp, PCC6308 (AC: AB0 39001)].

TABLE 1. Antibacterial activity (as inhibition zone diameter mm) of different extracts of *Microcystis aeruginosa* MDEG1.

Tested bacteria	Petroleum ether extract (mg/ml) in DMSO				Chloroform extract (mg/ml) in DMSO			
	1	10	25	50	1	10	25	50
S. aureus	3±0.6	7±.6	10±0.3	13±0.6	1±0.3	2±0.3	3±0.9	5±0.9
B. cereus	1±0.3	5±0.6	6±0.6	7±0.6	2±0.6	2±0.6	3±0.6	5±0.6
B. subtilis	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
E. coli	2±0.6	3±0.6	4±0.3	5±0.6	1±0.3	1±0.3	1±0.3	2±0.6
P. aeroginosa	6±0.3	7±0.6	9±0.6	12±0.3	1±0	2±0.6	2±0.6	2±0.6
K. pneumoniae	5±0.6	12±0.6	15±0.6	16±0.6	10±0.6	12±0.6	15±0.6	16±0.3
P. marbilis	Nil	1±0	3±0.6	5±0.6	1±0.3	2±0.6	2±0.6	2±0.6

TABLE 2. Identified compounds based on GC-MS and ^{1}H-NMR analysis for petroleum ether and chloroform extracts of *Microcystis aeruginosa* MDEG1.

Petroleum ether			Chloroform		
Compound name	**R_t**	**Area %**	**Compound name**	**R_t**	**Area %**
Nonanoic acid	10.46	0.08	β-Pinene	10.40	0.97
Hexanedioic acid mono methyl ester	11.22	0.04	Loliolide	34.06	0.74
Iso-octadecanoic acid	11.81	0.08	Tetradecanoic acid, methyl ester	34.81	0.35
Nonanoic acid, methyl ester	12.94	0.06	Tetradecanoic acid, 12-methyl-, methyl ester	36.40	0.54
Oleyl alcohol	13.55	0.14	2-hydroxy-5,6-epoxy-β-ionone	36.57	0.24
Dodecanoic acid	15.09	0.13	Octadecane	36.60	0.28
2,2-dimethyl-7-methoxy-2,3-dihydro-2H-benzo[b]thiene	15.41	0.13	2-pentadecanone, 6,10,14-trimethyl	37.80	5.08
3-oxo-ß-ionone	16.66	0.25	Heptadecanoic acid	39.09	0.24
n-heptadecane	16.94	3.25	Hexadecanoic acid (Palmitic acid)	39.80	5.54
Tetradecanoic acid, methyl ester	17.20	0.40	Palmitic acid, methyl ester	39.81	78.98
2-methyl-heptadecane	17.68	0.25	Palmitic acid, ethyl ester	41.48	0.71
Octadecane	18.25	2.82	Oleic acid	41.90	2.17
2-pentadecanone, 6,10,14-trimethyl	18.87	1.60	3-hydroxytetradecanoic acid methyl ester	44.07	4.85
Nonadecane	19.49	0.53	Octadecanoic acid, methyl ester	44.49	2.59
Hexadecanoic acid, methyl ester	20.08	13.9	Octadecanoic acid	45.97	0.31
Hexadecanoic acid (Palmitic acid)	21.71	48.43	9-octadecenamide	46.10	1.34
Heptadecanoic acid	22.11	1.04			
Iso-octadecanoic acid	22.82	1.02			
Octadecanoic acid	23.08	2.26			

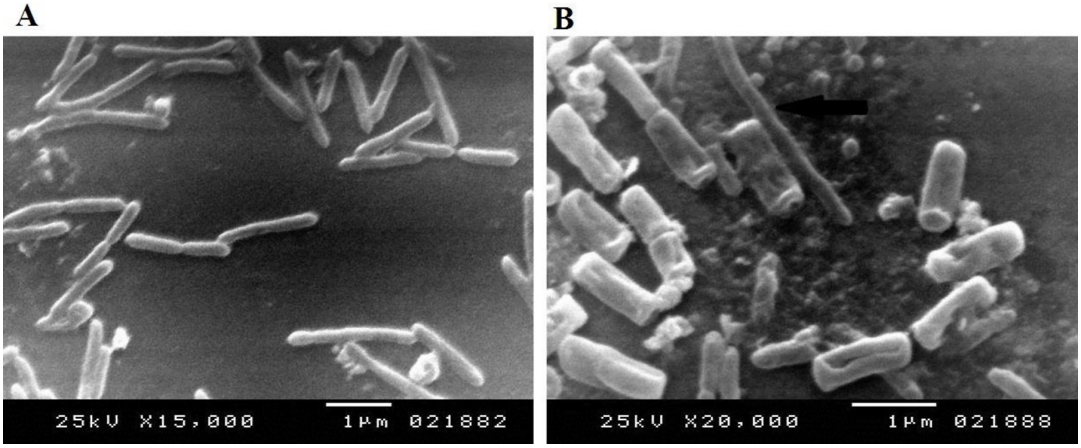

Fig. 2. SEM micrographs of *K. pneumoniae;* (A) Normal untreated cells with normal cytomorphology (rod cells arranged singly, in pairs or short chains), (B) Bacterial cell elongation with filament-like structure (giant cell) formation due to the effect of chloroform extract of *M. aeruginosa.* Scale bar = 1.0 µm.

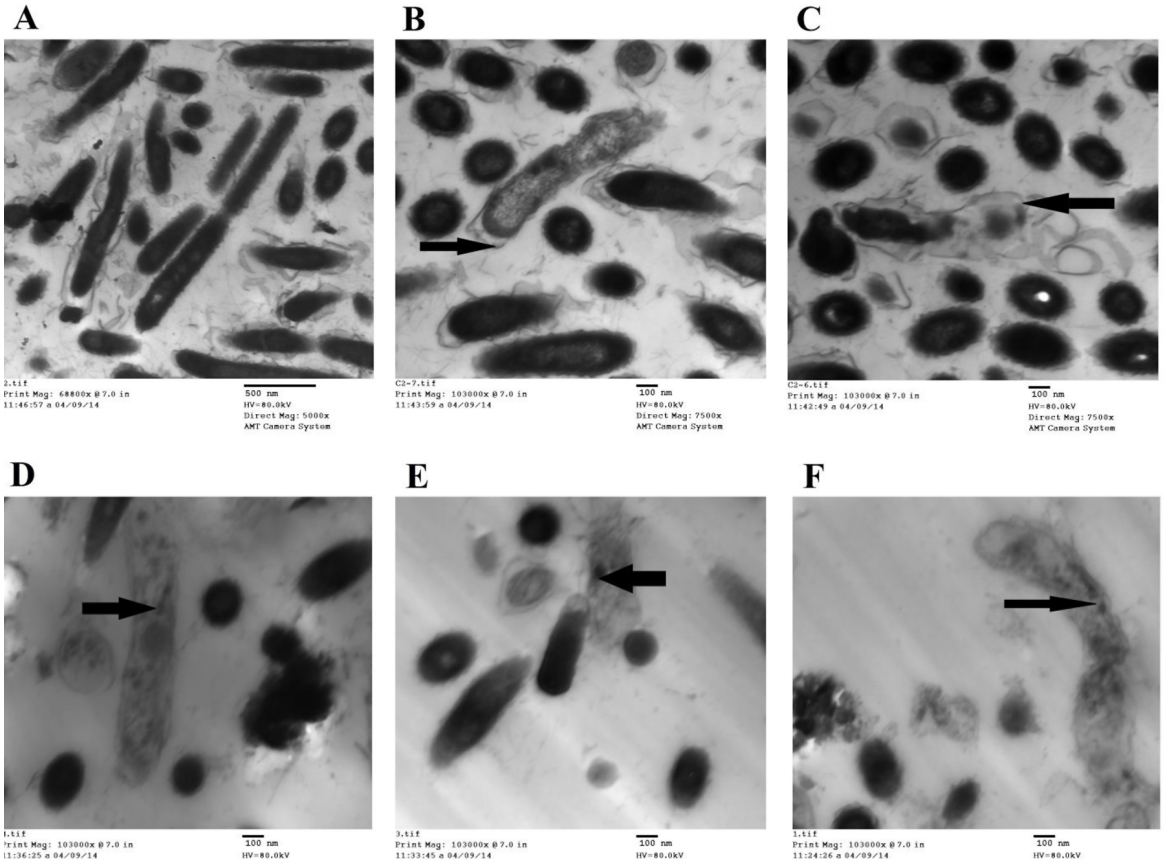

Fig. 3. TEM micrographs of *K. pneumoniae* showed normal cell ultrastructure for untreated bacteria; (A) The treated bacteria with 50mg/ml chloroform extract of *M. aeruginosa* revealed abnormalities including loose attachment of cell membrane and cell enlargement (arrow) in B & C; granular cell contents exudes from the damaged membrane (arrow) in D, E & F [Scale bar = 1.0µm].

The phylogenetic tree based on the 16S rDNA analysis confirmed the identification of the *M. aeruginosa* MDEG1 isolate as it exhibited high identity reached 99% -100% in one cluster with some *Microcystis* species including other *M. aeruginosa* strains. Several previous studies based on 16S rDNA sequence analysis had shown that different *Microcystis* species can be clustered

together (Neilan et al., 1997; Lyra et al., 2001; Rinta-Kanto et al., 2005). Furthermore, Otsuka et al. (1998) found that five *Microcystis* species: *M. aeruginosa*, *M. ichthyoblabe*, *M. wesenbergii*, *M. viridis* and *M. novacekii*, were so closely related in terms of 16S rDNA sequence that they can be grouped as a single species and concluded that the 16S rDNA sequence is insufficiently variable to be used for phylogenetic analysis of these organisms at species level. Moreover, Neilan et al. (1997) reported that minor and variable morphometric parameters might have led to the identification of *M. wesenbergi* and *M. viridis*, although it was difficult to justify their separation from *M. aeruginosa* on the basis of the results of 16S rRNA gene analysis. The difference in resolution from 16S rRNA in *Microcystis* matches the reported average sequence diversity of less than 1% in this gene (Otsuka et al., 1998; Boyer et al., 2001).

Abundant bioactive substances are produced by algae. These substances could have medicinal properties including antibacterial effect. In the present study, we attempted to evaluate the antibacterial activity of *M. aeruginosa* MDEG1 from El-Manzala Lake against *S. aureus*, *B. cereus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *P. marbilis*. The petroleum ether extract of *M. aeruginosa* MDEG1 revealed a considerable high activity against some of the studied Gram negative bacteria (*K. pneumoniae* and *P. aeruginosa*) and Gram positive bacteria (*S. aureus*). Meanwhile, chloroform extract exhibited higher activity against *K. pneumoniae* and showed less activity against the other species. Antibacterial substances possessed different solubility according to the solvent used for extraction. The resulting extracts effects may also have differed due to the efficiency of the extraction methods to recover the active metabolites (Tuney, 2006), susceptibility of strains, assay methods and variation in seasonal sampling (Sasidharan et al., 2009). For example, Madhumathi et al. (2011) indicated that ethanol, acetone, diethyl ether and methanol extracts from *M. aeruginosa* and other cyanobacteria possessed different antibacterial activity profiles towards some Gram positive and negative bacteria, and also different antifungal profile activity. The methanolic extract of *M. aeruginosa* showed significant anticyanobacterial activity against *Anabaena* and *Nostoc* strains and antialgal activity against a green alga *Bracteacoccus* with no antibacterial effect (Yadav et al., 2012). An antimicrobial activity was observed also in the hexanic extract of *M. aeruginosa* (RST 9501 strain) against *Mycobacterium tuberculosis* (Ramos et al., 2015). The highest antifungal activity against all the tested mycotoxigenic fungi was observed when diethyl ether extract of *M. aeruginosa* was applied (Marrez & Sultan, 2016). The microbiocidal effect of the *M. aeruginosa* MDEG1 extracts might be attributed to the presence of aliphatic fatty acids such as hexadecanoic acid methyl ester which is the major component in chloroform extract, and also hexadecanoic acid which is the major constituent in the petroleum ether extract. Choi & Jang (2014) stated that some fatty acids like hexanedioic acid have antibacterial activities. It was also reported that dodecanoic acid had bioactivity against Gram positive *S. aureus* and Gram negative *E. coli* bacteria (Kumar et al., 2011). Butylated hydroxytoluene and hexadecanoic acid were also detected in *M. aeruginosa* that might be involved in its antifungal activity (Marrez & Sultan, 2016).

The exposure of *K. pneumoniae* cells to 50mg/ml chloroform extract of *M. aeruginosa* resulted in giant cell formation and appearance of elongated filament like structure. The enlargement reduces the relative contact surface. Therefore, bigger cells can tolerate the stress conditions better than normal cells of the same species (Elliott & Greenwood, 1983). In addition, TEM of the treated *K. Pneumoniae* with the chloroform extract of *M. aeruginosa* MDEG1 revealed successive events of bactericidal effect. These events started with the appearance of loose attachment of bacterial capsules, presence of electron dense granules and electron lucent vacuoles formation in the cytoplasm**,** weakening and damaging of the boundary cell wall**,** cell malformation, loose attachment of cell membrane, twisted cell were clearly visible and finally ruptured bacterial cell wall damage with extrusion of cytoplasmic content**.** Similar successive events that lead to cell death were also observed by Alexander et al., (2006) as an effect of nisin against *Bacillus subtilis*. Furthermore, the *M. aeruginosa* MDEG1 chloroform extract possessed the same effect of amoxicillin/clavulanic acid (Augmentin 30µg) on *K. Pneumoniae* (Badawy, 2014).

Conclusion

The petroleum ether and chloroform extracts of the identified *Microcystis aeruginosa* MDEG1

strain exhibited highest antibacterial against *K. Pneumoniae* and some other Gram negative bacteria. The chloroform extract totally deformed the *K. Pneumoniae* cells. This might be attributed to the presence of antibacterial compounds such as hexadecanoic acid, hexadecanoic acid methyl ester and dodecanoic acid as revealed by the GC-MS analysis and ^{1}H NMR spectra. More study should be performed in order to purify and identify the exacted antibacterial agent.

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ميكروسيستس إيروجينوزا كمنتج لمضاد بكتيرى

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فى تلك الدراسة تم تعريف سلالة ميكروسيستس إيروجينوزا (*Microcystis aeruginosa* MDEG1) من بحيرة المنزلة (دمياط – جمهورية مصر العربية) وذلك بالطرق التقليدية المعتمدة على الصفات المظهرية، كما تم تأكيد التعريف بالطرق الحديثة المعتمدة على تحليل التتابع النيوكليوتيدى للحمض النووى الريبوزى لجين 16S rRNA، تم أيضا تقييم تلك السلالة لإمتلاكها نشاط ضد بكتيرى لأنواع مختلفة من البكتيريا الموجبة لصبغ جرام (*Staphylococcus aureus*، *Bacillus cereus*، *Bacillus subtilis*) و السالبة لصبغ جرام (*Escherichia coli*، *Pseudomonas aeruginosa*، *Klebsiella pneumonia*، *Proteus marbili*).

تم إختبار تركيزات مختلفة (1، 10، 25 و 50 مليجرام/مللي) من مستخلصات الإثير البترولى والكلوروفورم لسلالة MDEG1 *M. aeruginosa* على السلالات البكتيرية السبقة، وقد أظهرت النتائج أن مستخلص الإثير البترولى كان له نشاط جيد ضد السلالات البكتيرية *K. pneumonia* و *P. aeruginosa* و *S. aureus*، أما مستخلص الكلوروفورم فقد أظهر نشاط أعلى ضد *K. pneumonia*، كما تم التعرف على المركبات الموجودة في كلا المستخلصين بواسطة جهازى ال GC-MS و ^{1}H-NMR اللذين أظهرا وجود مركبات يمكن أن يكون لهم دور فى النشاط ضد البكتيرى، و كان أكثرهم تواجدا بشكل رئيسى هو حمض البالميتك و مشتقه إستر الميثيلى فى مستخلصى الإثير البترولى والكلوروفورم على التوالى.

أظهر الفحص المجهرى بالميكرسكوب الإلكترونى الماسح و القاطع (النافذ) لبكتيريا *K. pneumonia* المعالجة بمستخلص الكلوروفورم (50 مليجرام/مللي) أن الخلايا البكتيرية أصبحت أكبر في الحجم مع ظهور فجوات عديدة في السيتوبلازم البكتيري، بالإضافة إلى ظهور تمزق للحافظة وجدار الخلية وأخيرا قذف المحتويات الحشوية منها كدليل على التأثير المميت لهذا المستخلص.