Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 3 (8): 539-551, 2008
ISSN 1816-4935

Effect of Biotic and Abiotic Factors on Pathogenic Gram-Negative Bacteria in Lake Qarun, Egypt

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Abstract: In addition to El-Bats and El-Wadi drains, the main sources of drainage water, six stations were selected and distributed all over Lake Qarun. Total and the most common pathogenic Gram-negative bacteria; *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella choleraesuis*, *Proteus vulgaris* and *Serratia liquefaciens* as well as actinomycetes were enumerated and identified. Twenty-one actinomycetes were isolated and screened for their antibacterial activities. All actinomycetes isolated were identified as *Streptomyces* and eleven of them showed antibacterial activities against the pathogenic Gram-negative bacteria isolated from the same tested water sample. The most antibacterial active isolate was subjected to morphological, physiological and biochemical studies and identified as *Streptomyces calvus*. The antimicrobial activity of the identified *Streptomyces calvus* against some indicator organisms was also performed. In addition, the effects of some abiotic factors on the tested bacteria were discussed.

Key words: Lake Qarun, abiotic variables, Gram-negative pathogens, actinomycetes antibacterial activity

INTRODUCTION

Lake Qarun is of great importance as a protected area in addition to its touristic and fisheries importance. Although fishing in Lake Qarun contributes only 1% to inland fisheries of Egypt, it is the most important source of fish (65%) in Fayoum Governorate. However, Lake Qarun has been subjected to pollution by agricultural drainage water and raw domestic sewage. Physical and chemical variables of Lake Qarun water have been studied by Sabae and Ali (2004).

Lake Qarun is considered as a highly eutrophic body of waters (Abdel-Malek and Ishak, 1980). The biodiversity and phytoplankton communities were previously studied by Fathi and Flower (2005), while abundance and diversity of zooplankton in Lake Qarun has been investigated by Kalifa and El-Shabrawy (2007). Seasonal and long-term changes of macrobenthos has been studied by El-Shabrawy and Kalifa (2007). On the other hand, bacterial indicators of sewage pollution (Sabae, 1993; Sabae and Rabeh, 2000), bacteria involved in biogeochemical cycles (Rabeh, 2001) and bacterial biomass in Lake Qarun (Rabeh, 2003) has been studied. However, Gram-negative pathogens and actinomycetes in the Lake under investigation have not ever been examined.

Thus, the present study represents the first monitoring of Gram-negative pathogens and aquatic actinomycetes in Lake Qarun. The antibacterial activity of actinomycetes isolated from both Drain's and Lake's water against six Gram-negative bacterial pathogens isolated from the same tested water was also examined.

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MATERIALS AND METHODS

Study Area

Lake Qarun

Lake Qarun is the only enclosed saline lake among the inland lakes of Egypt. It occupies an area of about 228 km^{-2}, extending about 40 km from east to west, with mean breadth about 5.7 km from the north to south. The lake receives agricultural drainage water and sewage through two main drains, El-Batts and El-Wadi in addition to 11 small drains.

El-Batts and El-Wadi Drains

El-Batts Drain, extending about 50.9 km, receives agricultural drainage water as well as crude sewage from the eastern and north eastern part of El-Fayoum depression. The annual average of drainage water 207.6 millions m^3 which discharged into the eastern part of the lake. On the other hand, El-Wadi Drain (48.5 km long), receives drainage water from the middle region of El-Fayoum depression, which is discharged into the middle part of the lake. The annual average of its drainage water is 103.03 million m^3. As a result of the rising of water level in Lake Qarun, El-Wadi Drain partially delivers its water to Wadi El-Raiyan depression since 1973, forming Wadi El-Raiyan Lakes (Rabeh, 1999).

Sampling Stations

Subsurface water samples were collected seasonally (from winter to autumn 2006) from six Lake' stations in addition to El-Batts and El-Wadi Drains (one station each) (Fig. 1). Lake' stations were distributed at different locations, namely, Eastern part (30.3 to 34.27% salinity) of the Lake (represented by stations, 1 and 2), Middle part (30.42 to 34.51% salinity) of the Lake (represented by stations, 3 and 4) and Western region (31.42 to 35.08% salinity) of the lake (represented by stations 5 and 6).

Physical and Chemical Variables

Temperatures of subsurface water were measured using a digital thermometer, while pH was recorded using a pH meter. Water transparency was measured by black/white Sechi disc. On the other hand, water salinity was recalculated after Abd Ellah' study (personal communication).

Microbiological Analysis

Total Gram-Negative Bacteria

The total counts of Gram-negative bacteria in the studied pond system during different seasons were determined, using MacConkey agar supplemented with 0.001 g L^{-1} crystal violet (Rabeh and Azab, 2006).

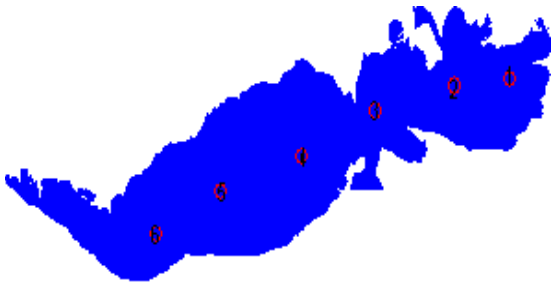

Fig. 1: Map showing the selected sampling stations

Enumeration and Isolation of Gram-Negative Pathogens

Morphologically similar isolates of the Gram-negative bacteria were grouped and counted. The Gram-negative pathogens were streaked on their corresponding specific media and then morphological and biochemical characterizations were carried out and finally the identification was confirmed by API 20E (Biomerieux). *E. coli* was subcultured on EMB medium (APHA, 1998), *Klebsiella* on BIND (Brilliant green- inositol- nitrate-deoxycholate) agar medium (Ohtomo and Saito 2003), *Pseudomonas* on King's B medium (King *et al*., 1954), *Salmonella* on SS medium (Oxoid Manual, 1981), *Proteus* on urea agar base medium (Oxoid Manual, 1981) and *Serratia* on *Serratia* differentiation (SD) medium (Gibson and Friedman, 1978).

Api 20 E Biochemical Characterization

Commercial kits (API 20 E) were used for confirming the identification of the isolates by referring to the API catalogue. The isolated Gram negative bacteria were compared with those described by Bergy *et al*. (1989).

Enumeration and Isolation of Actinomycetes

The tested water samples were concentrated separately in a water bath at 40°C and actinomycetes isolation and enumeration were carried out according to the method described by Hsu and Lockwood (1975).

Antimicrobial Activity of the Isolated Actinomycetes

The antibacterial activity of the actinomycetes isolated from the examined water samples against *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella choleraesuis*, *Proteus vulgaris* and *Serratia liquefaciens* isolated from the same examined water samples was performed using the well method as described by Holmalahti *et al*. (1994). Antimicrobial activity of the most active isolate against some indicators was also carried out. *Proteus vulgaris* 1753, *Serratia marscens* 921/79LR were kindly provided by Professor Martin, H.H, Institute of Microbiology, TH Darmstadt, Germany. *E. coli* 1357, *Staphylococcus aureus*, *Sarcina lutea* and *Candida albicans*, were obtained from the culture collection of the Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain-Shams University and Cairo, Egypt. *Penicillium italicum* was identified by Azab *et al*. (2006). *Bipolaris sorokiniana* (Saccxardo) was kindly provided by Associate Professor Gouda, M. I., Plant Pathology Research Institute ARCV, Giza, Egypt.

Characterization of the Most Active Actinomycete

The most active actinomycete was characterized by the methods developed by collaborators of International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966).

Cultural and Morphological Characteristics

The growth and colony character (color of aerial and substrate mycelium and soluble pigments) of the selected strain was observed as described by Williams *et al*. (1989), Thom and Raper (1945), Coppola and Giannattasio (1965), Pridham and Lyons (1961), Kuster (1959), Shirling and Gottlieb (1966) and Pridham *et al*. (1957). The cultured plates were incubated for 7, 14 and 21 days at 30°C. The color of colony and substrate mycelia was determined according to Tresner and Backus (1963) and Shirling and Gottlieb (1966), respectively.

The morphology of spore chain was examined by light microscope by inoculating the selected organism on glycerol-asparagine agar, inorganic salt starch agar, oatmeal agar and yeast extract-malt extract agar media (Shirling and Gottlieb, 1966). A thin layer of each used media was covered sterile microscope slide placed in a sterile Petri dish. Each slide was examined after the 7, 14 and 21 days of

incubation at 28°C (Pridham *et al*., 1958). The spore surface of the selected organism was examined by transmission electron microscopy (JEOL 1010), using 10 days culture in starch nitrate medium.

Physiological and Biochemical Characterization

The procedure of Becker *et al*. (1964) was followed for the hydrolysis of whole cells proceeding DAP analysis.

The temperature range and pH for growth was determined on Bennett's agar with glucose replaced by glycerol. The growth was determined by measuring the diameter of growing colonies (Williams *et al*., 1983a).

The ability of the tested organism to produce melanin pigment and other soluble colors other than melanoid was carried out by the method described by Shirling and Gottlieb (1966).

The selected organism was cultured onto starch nitrate broth supplemented with 0.01% tryptophan. The cultured was extracted after 7 days incubation at 30°C with ethyl acetate and evaporated under vacuum at 40°C (Wing *et al*., 1982). The indole acetic acid residue was detected by the method described by Langenbeck-Schwich and Grambow(1984). $FeCl_3$ (0.05% in sulphuric acid) used as a color reagent (Van Overbeck *et al*., 1957). The appearance of pink color indicated positive result.

The degradation of allantoin was examined by the method of Gordon (1968). The degradation of cellulose and carboxymethyl cellulose was determined using the medium described by Pridham and Gottlieb (1948) by the method. The degradation of tyrosine (0.5% w/v), xanthine (0.4% w/v), casine (1% w/v) by the method described by Jones (1949). Gelatin (0.4%) and starch (1.0%) degradation was detected according to Frazier(1926) and Cowan (1974) and scoring zone of clearing as positive.

The catalase production was determined by the method by Williams *et al*. (1983a), lecithinase activity was determined by the method of Nitsch and Kutzner (1969), lipase activity was detected as described by Sierra (1957) and urease activity was determined using the method of Gordon (1966).

Hydrogen sulfide formation was determined by the method described by Williams *et al*. (1983a).

Resistance to chemical inhibitors was tested using Bennett's agar plates with glycerol instead of glucose containing the inhibitors, sodium chloride (7% w/v), sodium azide (0.1% w/v), phenol (0.1% w/v) and potassium cyanide (0.02% w/v) (Williams *et al*., 1983a).

The selected actinomycetes was tested for its ability to growth in the presence of the antibiotic rifampicin (50 μg mL^{-1}), using freeze-dried filter paper disc method of Goodfellow and Orchard (1974). Filter-sterilized arabinose, glucose, glycerol, inositol, lactose, mannitol, mannose, ribose and sorbitol were added separately to a final concentration 1% to the basal medium. Changes in the basal medium color from purple to yellow-brown indicated acid production (Gordon, 1968).

Different carbon sources (L- arabinose, cellulose, D-fructose, D-galactose, D-glucose, glycerol, m-inositol, D-lactose, mannitol, D-mannose, D-raffinose, L-rhamnose, sucrose and D-xylose were used at concentration of 1% (w/v)) utilization was determined by the method described by Williams *et al*. (1983a).

Different nitrogen sources (ammonium chloride, ammonium phosphate, ammonium sulfate, L-asparagine, L-cystine, L-histidine, L-phenyl alanine, potassium nitrate and sodium nitrate) were added (0.1% w/v) separately to the basal medium of. Growth was scored after 15 day by comparing test plates with both negative and positive controls (Williams *et al*., 1983b).

Statistical Analysis

Cluster analysis for the classification of samples based on log Bray Curtis similarity index and the correlation coefficient (r) between the bacteria and the environmental factors using the computer programme Ms Microsoft Excel Ver., 2003 were carried out.

RESULTS AND DISCUSSION

Physical and Chemical Variables

In this study, the water temperature of Lake Qarun, total and pathogenic Gram-negative bacteria and seasonal changes in pH and salinity of Lake Qarun water were studied. Water temperature of Lake Qarun ranged between 13.5°C during winter and 30°C during summer. On the other hand, water temperature of El-Batts Drain ranged from 18.5 to 30.6°C, while they ranged from 18.5 to 30.5°C in El-Wadi Drain during winter and spring, respectively (Fig. 2a). Total and pathogenic Gram-negative bacteria were low during winter, high during spring, reaching maximal values during summer and decreased again during autumn. Statistically, total and pathogenic Gram-negative bacteria were positively correlated with water temperatures (r values ranged from 0.4 to 0.52).

Low water transparency was recorded in El-Batts (8-20 cm) and El-Wadi (10-25 cm) drains. Maximal bacterial counts were recorded in both El-Batts and El-Wadi Drains where low transparency (high suspended matter) was recorded. As regards Lake Qarun, the Sechi disc readings ranged from 50 cm at station 1 during spring and summer to 70 cm at station 6 during autumn (Fig. 2b). Accordingly, the minimal bacterial counts were recorded at station 6 in which lower suspended matter were recorded (r-values ranged from -0.36 to -0.57). The optimum pH for most micro-organisms varies between 6.5 and 8.5 which correspond to the pH range of most of the larger bodies of water (OöBagde and Varma, 1991). A similar situation was recorded in Lake Qarun where the pH ranged from 8.1 at station 3 during summer to 8.73 at station 3 during winter (Fig. 3a). The water salinity of Lake Qarun

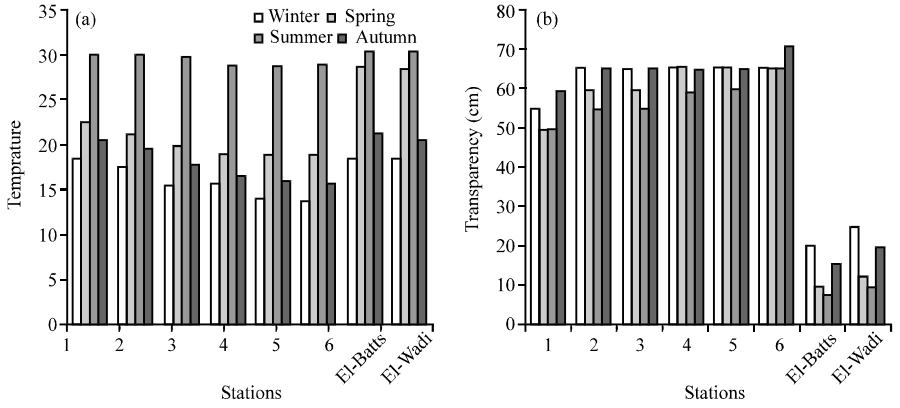

Fig. 2: Seasonal changes in (a) temperature and (b) transparency of lake Qarun water

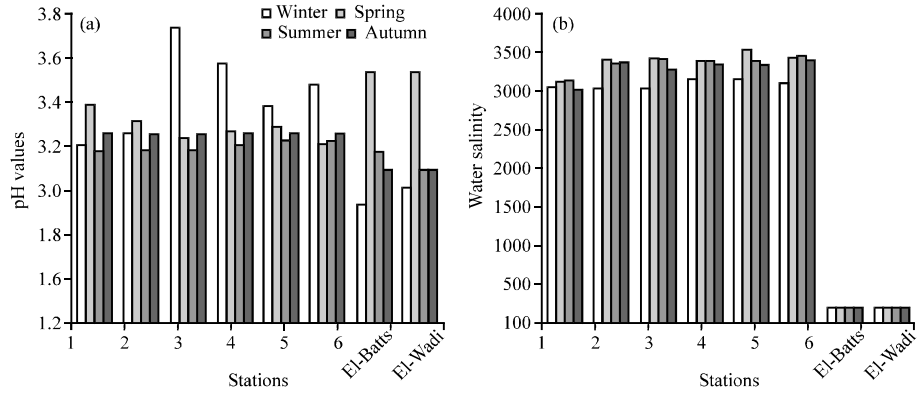

Fig. 3: Seasonal changes in (a) pH and (b) salinity of lake Qarun water

water ranged from 30.3-35.08‰ with gradual increase from Eastern to Western part direction (Fig. 3b). On the other hand, The tested bacteria decreased in the same direction (r = -0.51, -0.80, -0.79, -0.79, -0.82, -0.82 and -0.75, for total Gram-negative bacteria; *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella choleraesuis*, *Proteus vulgaris* and *Serratia liquefaciens*, respectively).

Several species of Gram-negative bacteria present in municipal wastewater are pathogenic. This pathogenisty is usually associated with certain components of the cell walls, in particular the lipopolysaccharide, also known as LPS or endotoxin, layer (Baron, 1996). Thus, identification of these pathogenic agents in water resources is beneficial for controlling and prevention planning of the infectious diseases.

During the present study, total counts of Gram-negative bacteria (GNB) ranged in Lake Qarun from 0.2×10^3 cfu/100 mL during winter to 6×10^3 cfu/100 mL during summer. As regards site-wise variations, station 1(Eastern Part) maintained the highest counts, while the lowest ones were at station 6(Western Part). This might be due to the high salinity in the Western part (r = -0.51). On the other hand, counts of Gram-negative bacteria in El-Batts and El-Wadi Drains ranged from 14×10^4 and 5×10^4 cfu/100 mL to 55×10^4 and 50×10^4 cfu/100 mL during winter and summer, respectively (Table 1). The highest counts of Gram-negative bacteria in both Drains may be due to: (1) the high amount of suspended matter which was reflected by the low values of Sechi disc (8-20 cm, 10-25 cm in El-Batts and El-Wadi Drains, respectively) (2) the lower salinity and the high concentration of nutrients, which are required for bacterial growth (Rabeh 2003; Sabae and Ali, 2004) and (3) fecal pollution caused by man and domestic animals (Sabae and Rabeh, 2000).

The most common Gram-negative bacteria isolated were identified to the species level and confirmed by using API 20E to be *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Salmonella choleraesuis*, *Proteus vulgaris*, *Serratia liquefaciens*.

E. coli is the main indicator of recent sewage pollution. The counts of this organism in the Lake in question and both Drains were presented in Table 2. Along the studied stations of Lake Qarun, the density of *E. coli* varied between 1×10^2 cfu/100 mL during winter to 2×10^3 cfu/100 mL during summer. Compared to Lake' stations the highest counts were recorded for El-Batts and El-Wadi Drains. The pathogenic bacterium, *Pseudomonas aeruginosa* fluctuated; either it was completely absent from stations 5 and 6 along the period of study excepting spring or reaching 2×10^3 cfu/100 mL at

Table 1: Total counts of Gram-negative bacteria (cfu/100 mL) in Lake Qarun

Seasons	Winter	Spring	Summer	Autumn
1	0.9×10^3	1.8×10^3	6×10^3	1.1×10^3
2	0.6×10^3	1.2×10^3	4.2×10^3	0.8×10^3
3	0.4×10^3	1×10^3	2.3×10^3	0.6×10^3
4	0.3×10^3	0.8×10^3	1.9×10^3	0.5×10^3
5	0.2×10^3	0.4×10^3	0.6×10^3	0.3×10^3
6	0.2×10^3	0.3×10^3	0.4×10^3	0.2×10^3
El-Batts Drain	14×10^4	50×10^5	55×10^5	18×10^4
El-Wadi Drain	5×10^4	44×10^4	50×10^4	8×10^4

Table 2: Counts of *E. coli* (cfu/100 mL) in Lake Qarun

Seasons	Winter	Spring	Summer	Autumn
1	0.2×10^3	0.5×10^3	2×10^3	0.3×10^3
2	0.2×10^3	0.3×10^3	1.2×10^3	0.3×10^3
3	0.1×10^3	0.3×10^3	0.6×10^3	0.2×10^3
4	0.1×10^3	0.3×10^3	0.5×10^3	0.2×10^3
5	0.1×10^3	0.1×10^3	0.3×10^3	0.1×10^3
6	0.1×10^3	0.1×10^3	0.2×10^3	0.1×10^3
El-Batts Drain	4.0×10^4	13×10^4	14×10^4	4.5×10^4
El-Wadi Drain	1.5×10^4	12×10^4	12×10^4	2×10^4

Table 3: Counts of *Pseudomonas aeruginosa* (cfu/100 mL) in Lake Qarun

Seasons	Winter	Spring	Summer	Autumn
1	0.2×10^3	0.4×10^3	1.2×10^3	0.2×10^3
2	0.1×10^3	0.3×10^3	0.8×10^3	0.2×10^3
3	0.1×10^3	0.2×10^3	0.4×10^3	0.1×10^3
4	0.1×10^3	0.2×10^3	0.3×10^3	0.1×10^3
5	0.0	0.1×10^3	0.0	0.0
6	0.0	0.1×10^3	0.0	0.0
El-Batts Drain	3×10^4	11×10^4	12×10^4	3.5×10^4
El-Wadi Drain	1×10^4	10×10^4	11×10^4	1.5×10^4

Table 4: Counts of *Klebsiella pneumoniae* (cfu/100 mL) in Lake Qarun

Seasons	Winter	Spring	Summer	Autumn
1	0.1×10^3	0.2×10^3	0.6×10^3	0.1×10^3
2	0.1×10^3	0.1×10^3	0.5×10^3	0.1×10^3
3	0.1×10^3	0.1×10^3	0.3×10^3	0.1×10^3
4	0.0	0.1×10^3	0.4×10^3	0.1×10^3
5	0.0	0.1×10^3	0.1×10^3	0.0
6	0.0	0.0	0.1×10^3	0.0
El-Batts Drain	1.8×10^4	7×10^4	7×10^4	2.5×10^4
El-Wadi Drain	0.7×10^4	6×10^4	8×10^4	1.25×10^4

Table 5: Counts of *Salmonella choleraesuis* (cfu/100 mL) in Lake Qarun

Seasons	Winter	Spring	Summer	Autumn
1	0.1×10^3	0.2×10^3	0.5×10^3	0.1×10^3
2	0.1×10^3	0.1×10^3	0.5×10^3	0.1×10^3
3	0.0	0.1×10^3	0.3×10^3	0.1×10^3
4	0.0	0.1×10^3	0.2×10^3	0.0
5	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
El-Batts Drain	1.4×10^4	5×10^4	5×10^4	2×10^4
El-Wadi Drain	0.5×10^4	4×10^4	4×10^4	1×10^4

Table 6: Counts of *Proteus vulgaris* (cfu/100 mL) in Lake Qarun

Seasons	Winter	Spring	Summer	Autumn
1	0.1×10^3	0.1×10^3	0.5×10^3	0.1
2	0.1×10^3	0.1×10^3	0.4×10^3	0.0
3	0.0	0.1×10^3	0.2×10^3	0.0
4	0.0	0.0	0.1×10^3	0.0
5	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
El-Batts Drain	1.0×10^4	3×10^4	4×10^4	1.75×10^4
El-Wadi Drain	0.3×10^4	3×10^4	3×10^4	0.75×10^4

station 1 during summer (Table 3). The absence of this pathogenic organism from the last stations (Western part) might be attributed to high salinity (34.88 and 35.08‰) (Abd Ellah's study, personal communication) (r = -0.79). Mostafa *et al*. (2001) revealed that *Pseudomonas aeruginosa* was not detected in Rashid estuary especially near the sea which might suggest a greater sensitivity of these bacteria to high salt concentrations. The counts of *Klebsiella pneumoniae* in Lake and both Drains under investigation were presented in Table 4. Gradual decrease in the numbers of this organism from the Drain to the Lake up to station 6 was recorded, where it was completely absent from station 6 during winter, spring and autumn. *Salmonella choleraesuis* was completely absent from station 3 during winter, station 4 during winter and autumn and from stations 5 and 6 during the whole period of the present study. Again here, both Drains maintained the highest counts of this pathogenic organisms compared with those recorded in Lake' stations (Table 5). *Proteus vulgaris* and *Serratia liquefaciens* in both Drains and Qarun Lake followed the same pattern like the above mentioned bacteria. Their highest counts (5×10^2 and 4×10^2 cfu/100 mL, respectively) at station 1 during summer (Tables 6, 7).

Table 7: Counts of *Serratia liquefaciens* (cfu/100 mL) in Lake Qarun

Seasons	Winter	Spring	Summer	Autumn
1	0.1×10^3	0.1×10^3	0.4×10^3	0.1
2	0.0	0.1×10^3	0.2×10^3	0.0
3	0.0	0.1×10^3	0.1×10^3	0.0
4	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
El-Batts Drain	0.8×10^4	2×10^4	3×10^4	0.75×10^4
El-Wadi Drain	0.1×10^4	1×10^4	2×10^4	0.25×10^4

Table 8: Counts of actinomycetes (cfu/ mL) in Lake Qarun

Seasons	Winter	Spring	Summer	Autumn
1	1.6×10^3	1.4×10^3	1.3×10^3	1.5×10^3
2	1.7×10^3	1.5×10^3	1.4×10^3	1.6×10^3
3	1.9×10^4	1.7×10^4	1.6×10^4	1.8×10^4
4	2.0×10^4	1.8×10^4	1.7×10^4	1.9×10^4
5	2.2×10^4	2.0×10^4	1.8×10^4	2.1×10^4
6	2.3×10^4	2.1×10^4	1.9×10^4	2.2×10^4
El-Batts Drain	1.4×10^3	1.2×10^3	1.1×10^3	1.3×10^3
El-Wadi Drain	1.5×10^3	1.3×10^3	1.2×10^3	1.4×10^3

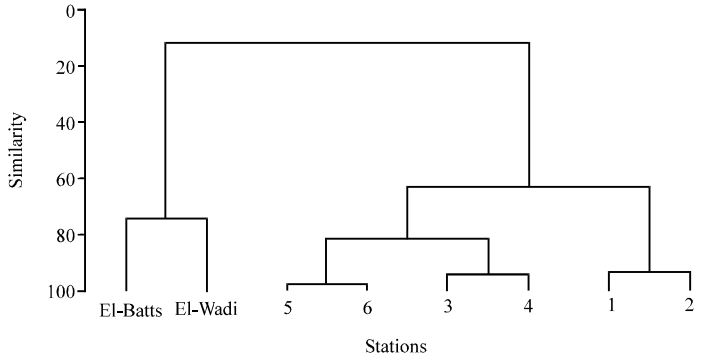

Fig. 4: Cluster analysis of some abiotic and biotic variables in Qarun Lake

Counts of Actinomycetes in the Tested Water

Counts of actinomycetes varied between 1.3 $\times10^3$ cfu mL^{-1} at station 1(the Eastern Part) during summer and 2.3 $\times10^4$ cfu mL^{-1} at station 6(the Western Part) during winter. Compared to Lake' stations, both El-Batts and El-wadi Drains maintained the lowest numbers of actinomycetes (Table 8).

The results of the classification of samples based on log Bray Curtis similarity index are shown in (Fig. 4). The classification showed two distinct groups A and B , group B is subsequently divided into smaller 3 clusters were observed. The cluster analysis separated two stations El-Wadi and El-Batts drains in one cluster. the other stations are grouped owing to its characteristics into three subgroups having similarity index about 98% then these subgroups merged in one group.

Antibacterial Activity of the Isolated Actinomycetes

Actinomycetes are a group of gram-positive microorganisms some of which can produce unique bioactive compounds. These micro-organisms are found in a wide range of aquatic and terrestrial environments and when, faced with harsh conditions have the ability to activate the metabolic pathways that ensure their survival. The unique bioactive compounds which may be produced through this process may have commercial applications. In the present investigation, twenty one isolates of

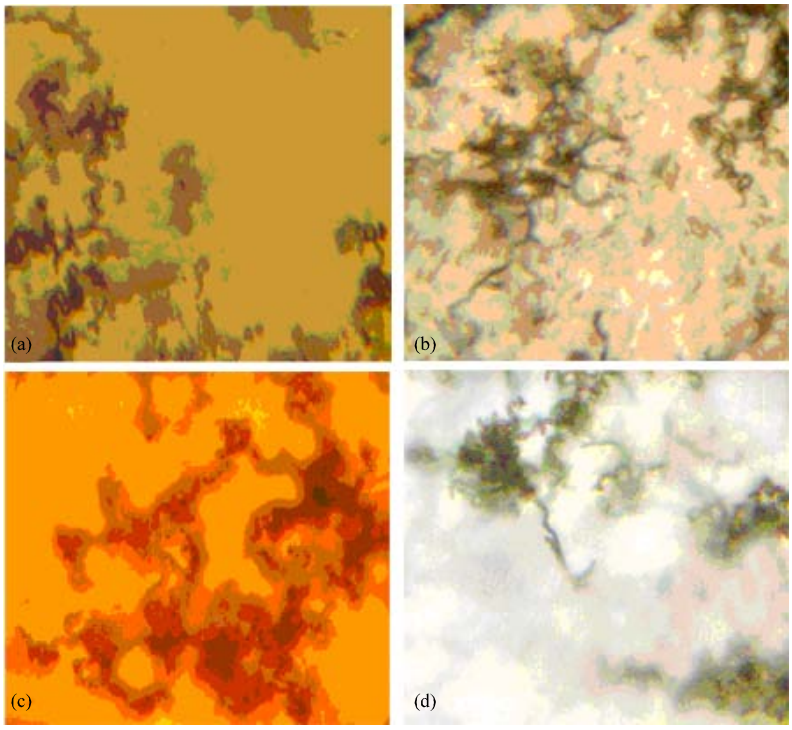

Fig. 5: The morphology of spores of the selected streptomyces No.8 examined by light microscope on (a) yeast extract-malt extract agar (b) oatmeal agar (c) glycerol-asparagine agar and (d) inorganic salts starch agar

actinomycetes were isolated and screened for their antagonistic activities against the pathogenic Gram-negative bacteria isolated from the same tested water sample. Out of these actinomycetes, isolates No. 1, 2, 3, 4, 5, 7, 8, 9, 10, 13 and 20 exhibited antagonistic activity against these organisms. Isolate No. 8 was the most active *Streptomyces* in suppression their growth, where the inhibition zones ranged from 1.5 to 2.5 cm.

Identification of the Most Antibacterial Active Actinomycete No. 8

The organism showed a highest antagonistic activity (No. 8) was aerobic, spore-forming with aerial mycelia containing L-form diamionopimelic acid (DAP) and glycine, Gram positive organism .The growth were good on inorganic salt-starch agar and starch agar but poor on Czapex's agar, glycerol asparagines agar, yeast extract-malt extract agar and moderate on the other tested media .The spore chains are short and poorly developed on yeast-malt agar, oatmeal agar and glycerol-asparagine agar. The aerial mycelium was poorly developed on most media with best sporulation and spiral developed on inorganic salt-starch media (Fig. 5). The color of aerial mycelium was in the gray color series. The electron microscopy of the organism grown in starch nitrate medium showed that the spores have spiny surface (Fig. 6). Melanin pigments are not found in peptone yeast-iron agar, tyrosine agar or tryptone-yeast broth, no distinctive pigment was found in medium in yeast-malt agar, oatmeal agar, salts-starch agar or glycerol asparagines agar.

As regarding the physiological and biochemical characteristics, the selected actinomycete grew well at 30°C and pH 7.0 but did not grow at 45°C. The results showed that whole cell hydrolysate contains LL-diaminopimelic acid. The organism did not produced melanin pigment and had the ability

Fig. 6: Spore view of the selected streptomyces, No. 8 on starch nitrate medium examined electron microscope

to decomposed allantoin, casine, tyrosine and xanthine. Also it had the ability to degrade urea, tween 80, gelatin and starch. It was not produce H_2S and not reduced nitrate. Decarboxylation of amino acids was recorded. It was not produce indole compounds. It was not tolerant to the toxic compound tested except sodium potassium cyanide. The organism had antagonistic activity against *Aspergillus niger*, *B. subtilis* and *P. solanacearum* 1274 and it was resistant to rifampicin.

Acid was formed from lactose, ribose, tyrosine, arabinose, mannose, glucose but not from sorbitol, glycerol and mannitol. The strain fairly to fully utilize the most used carbon sources. All of tested nitrogen sources were utilized but poor utilization of L-histidine, cystine and ammonium phosphate was recorded.

For the all cultural, physiological and biochemical results of the isolate No. 8, it was suggested to be belonging to the genus *Streptomyces* and it resembles *Streptomyces calvus*.

Antimicrobial Activity of Streptomyces Calvus

Streptomyces calvus isolated from the examined water samples exhibited antimicrobial activities against all indicator organisms used (inhibition zones ranged from 1.1 to 4.94 cm). In this connection, Saadoun *et al*. (1999) recorded the inhibitory effect of some aquatic actinomycetes especially *Streptomyces violaceusniger* against *E. coli, Aspergillus niger* and *Candida albicans* and no inhibitory effect against other gram-negative bacteria tested, filamentous fungi and yeast. Similarly, Rabeh *et al*. (2007) revealed that *Streptomyces viridiviolaceus* isolated from Lake Bardawil exhibited antibacterial activities against *Corynebacterium michiganese* B-33, *E. coli*, *Edwardsiella tarda*, *Pseudomonas solanacearum* B-3212 and *Staphylococcus*. No antimicrobial activities were recorded against the other tested bacteria (*Mycobaterium*, *B. cereus* and *Micrococcus*) and fungi (*Candida albicans*, *C. tropicalis*, *Aspergillus niger* and *Rhizopus nigricans*) under the experimental conditions used.

During the present investigation the reduction in numbers of total and pathogenic Gram-negative bacteria might be attributed to abiotic factors (pH, temperature, transparency, salinity and solar radiation) and biotic factors (zooplankton, bacteriophages and actinomycetes).

Statistical analysis showed a negative correlation between the counts of actinomycetes on one side and the counts of total gram-negative bacteria (-0.21), *E. coli* ((-0.33), *Pseudomonas aeruginosa*

(-0.32), *Klebsiella pneumoniae* (-0.33), *Salmonella choleraesuis* (-0.34), *Proteus vulgaris* (-0.34) and *Serratia liquefaciens* ((-0.31) on the other side. Such inverse relation between actinomycetes and all pathogenic Gram-negative bacteria isolated from the same examined water might be due to the antibacterial activities of actinomycetes against these bacteria.

Actually, the antagonistic activity of some actinomycetes isolated from Lake Qarun and both drains against all gram-negative pathogens isolated from the same water might explain the role of such actinomycetes in the self-purification in the Lake and both Drains under investigation. In this connection the antagonistic activity of aquatic actinomycetes against different bacteria was reported by Terkina *et al*. (2006) in Lake Baikal (Russia) and by Rabeh *et al*. (2007) in Lake Bardawil (Egypt). On the other hand, the antagonistic activity of some sewage actinomycetes against enteric bacteria was recorded in gravel bed hydroponic (GBH) system (Sahar, 1994) and in oxidation pond system (Rabeh *et al*., 2006).

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