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MICROBIAL DIVERSITY OF EL-KHADRA LAKE

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ABSTRACT

The microbial diversity of El-Khadra lake at Wadi-El-Natroun was studied, The lake water, shore and bottom soil and rhizosphere of adjacent plants were tested for total count of bacteria, proteolytic, lipolytic, cellulytic bacteria and azotobacter. The isolated bacteria from the lake were identified and it was almost as bacilli and to some extent cocci. The lake also has green cakes floating on the surface, and bacteria producing protease, lipase but less of cellulase and N-Fixing Azotobacter.

The identification of the free microflora in water, showed the dominance of the genus *Bacillus* over other aquatic microflora. The result showed that *Pseudmonas* and "xanthomonas" produce gelly yellow as a growth promotor.

INTRODUCTION

Aquatic bacteria are classified according to their salt optima for growth as non-halophilic, slightly, moderately or extremely halophilic and halo tolerant according to El-Sheikh (1998). Although NaCl is the major soluble salt which determines changes in salt osmolarity in the natural environment. It has been shown that other less penetrating solutes like potassium chloride, ammonium and potassium sulphate, potassium and sodium phosphate induce the mechanisms of osmosis in organisms including *Azotobacter chroococcum* and *Klebsiella pneumonia* (Madkour et al., 1990, Miller and Wood ,1996 and Malin et al., 1996), *Azospirillum brasilense* (Shahaby, 1997) cyanobacteria (Apte and Thomas, 1997). In conventional media *Escherichia coli* and *Salmonella typhyimurium* as well as *Rhizobium* have intracytoplasmatic solute concentrations of about 3.0-3.5 bar, mainly by accumulation of potassium and metabolically produce anions (Botsford and Lewis, 1990; Shahaby, 1996). Osmotic pressure in fresh water testing media are around O.1 bar (Imhoff, 1986).

Many species of bacteria respond and adapt hyperosmotic conditions in environment by the intracellular accumulation of low molecular weight organic solutes called osmolytes (Csonka and Hanson, 1991). The accumulates of osmolytes is thought to counteract the dehydrating effect of low water activity in the media, but not interfere with macromolecular structure or function. These solutes contribute to the positive turgor pressure which is required for cell expansion and growth(Reed et al., 1989).

This work aims to study of the presence of microorganisms adjacent to the lake, and the rhizosphere of weeds around as well as in the lake water.

MATERIALS AND METHODS

Sampling:

i) water samples were collected from under the surface of beach-lake water at 30 cm depth in sterilized plastic bottles of 1 litre
ii) mud samples were collected from the bottom of beach lake of 100 cm depth in sterilized plastic containers of 1 litre
iii) rhizophere of plants *(Zygophyllum dunosum)* around the lake were collected by digging around the plant deeply 60 cm and collected in plastic containers
iv) goat and camel faces samples were collected from around the lake when the goats were ranching by using sterilized plastic bags.
v) wet stone were collected from the top soil around the lake in the places which found the wet holes full with Algae mass by using sterilized plastic bags.
vi) algae cakes were collected from the floating mass productions of algae in mid lake using sterilized plastic bags

All samples were transported to N.R.C. in ice tanks within 3 hours.

Bacterial counts:-

The Serial dilutions plate count method according to Allen (1953) was used for counting :-

Total Colony counts:- using nutrient agar medium according to Topping (1938).

Casein soil extract medium for proteolytic bacteria counts:- (casein 30.0g, agar 20.0 g, soil extract 1 litre, pH 6.8-7.2. Casein was dissolved in 15 ml 0.1 N NaOH before incorporation into medium ingredient).

Lypolytic bacterial counts:- using nutrient agar amendment with 1% oil and incubated for 7 days at 30°C. Positive colonies were characterized by blue-green color after treating with 10ppm $CuSO_4$ solution.

The serial dilution MPN technique according to Allen (1953) was used for counting :

Asymbiotic nitrogen fixing bacteria (Azotobacter), using Ashby's medium (Allen, 1953)

Cellulytic bacteria counts, on Dobos's medium.(Allen,53)

Isolation and Identification

Identification of Aquatic bacteria according to Bergey's Manual (1984) was done on 5 day cultures, grown on nutrient slants agar.

Microbiological Tests

The following tests were done on 5 days cultures grown on either slants or nutrient broth:

- Motility
- Morphology and gram stain
- Fermentation of sugars(sucrose, maltrose, ..)

- The presence, shape and position of spores
- Catalase production
- V.P. test
- Methyl red reaction
- Indole production

Starch hydrolysis

Plates of starch agar were streaked by the organisms and incubated at 30°C for 5 days. Starch hydrolysing bacteria were recognized by the appearance of clear zones around their growth when the plates were floated by iodine solution.

Gelatin liquifaction

Nutrient gelatin tubes were inoculated and incubated at 30°C for 7 days. After chilling the liquid tubes indicated the ability of the microorganisms to liquified gelatin.

RESULTS AND DISCUSSION

Table (1) shiows the high occurrence of bacteria in the lake water and rhizosphere of *Zygophyllum Jumosun* around the lake, which contains total aerobic bacterial count of 285 x 10^6 CFU/L, The grasses grazed by goats supplied the animals faces with proteolytic bacteria in MPN of about 109 x 10^6 and 4 x 10^6 of lypolytic and 0.17 x 10^4 of cellulytic bacteria and negligible amount of azotobacter.

Table (1):Presence of microbial groups in the ecosystem of El-Khadra lake

Locations	Total count CFU	Proteolytic bacteria MPN	Lipolytic bacteria MPN	Cellulytic bacteria MPN	Azobacter CFU	PH
Water	285 × 10^6	301× 10^3	0.23× 10^3	0.01× 10^3	0.8 10^3	8.2
Goat (faces)	5 x 10^7	109 x 10^5	4x 10^6	0.17 x 10^4	0.2 x 10^4	9.05
Camel (faces)	7.6 x 10^8	91 x 10^8	9.1x 10^7	0.32 x 10^5	0.8x 10^4	8.7
Mud	393 x 10^7	180 x 10^7	33 x 10^6	0.02 x 10^4	0.02 x 10^4	8.70
Wet stone	26.3 x 10^7	5.2 x 10^6	13 x 10^2	0.02 x 10^4	0	9.95
Rhizosphere	285 x 10^7	31.6 x 10^6	12 x 10^6	16x 10^5	4.5 x 10^4	9.55
Algae cakes	310 x 10^7	180 x 10^7	25 x 10^6	1.1 x 10^4	0.02 x 10^4	9.15

The faces of camels showed more bacterial counts than faces of goat, proteolytic and cellulytic bacteria groups as the pH was less alkaline than goat faces but there was similarity in the counts with the algae cake which can be eaten by grazing animals since those get nutrition from the lake cakes They are very healthy and have massive bodies and brown color which may be due to the â-carotene, found in cakes and the high protein ratio, according to Aly, (2000). The osmotolerancy of the present organisms

allowed there passage through the digestive tract of the animal till they become in their faces which was showing alkali reaction of 8.7 , 9.05 in camels and goats respectively.

The floating mass contains high proliferating bacteria groups which produce protease, lipase and cellulases which may be helpful for these animals in the digestive tract and can be taken in the future production of Spirulina as a source of these enzyme production.

The identification of isolated bacteria of the El-Khadra Lake is given in table (2). It is clear that bacteria belonging to genus *Bacillus* were about 10 isolates/ 35 total isolates from the lake. Eleven isolates were belonging to cocci genera, which represents 31.8 % of total isolates.

Table (2): G⁻ rods oxidase negative Bacteria in water samples

Tested Genus	Methyl red	V.P.	Citrate	Indole production	Gelantin Liquefac-tion	44°C
Escherichia coli	+	-	-	+	-	-
Citrobacter	+/-	-	+	-	-	-
Klebsella	-	+	+	-	-	-
Enterobacter	-	+	-	-	-	-

Table (3) G⁻ rods oxidase positive Bacteria in water samples

Test Genus	Anaerobic decarboboxy	Agrinine dihydrolase	H_2S	Indol	xylose	dextrose	Urea	Citrate
Achrombacter	-/-	-	-	-	-	-	+	+
Pseudomonas	-/-	+	-	-	-	+	-	+
Aeromonas	+/+	+	-	+	-	+	-	+
Xanthomonas	-/-	-	-	-	-	-	+	-

Table (4): Differential characteristics key of Bacillus group Bergeys (1994)

Rod shaped young cultures	+
Motile	+
Endospores produced	+
Gram stain	+
Strict anaerobes	-
Sulphate activity reduced to sulphide	-
Catalase	+
Marked acidity from glycose	+
Nitrate reduction to nitrite	-

The detailed analysis of these genera is given in table (5) were 4 isolates belong to *Streptococcus*, 4 to *Micrococcus*, and 3 to *Staphylococcus*. However, fourteen isolates belong to gram (-) rods, which represents 40 % of total isolates.These groups (table 2) were classified as Pseudomonas (8.6%), Aeromones (5.7%), Achromobacter (2.9%), Enterobacter (8.6%), Citrobacter (5.7%) and Klebsiella (8.6%).

It is clear from the tabulated characteristics of the identified isolates of bacteria from El-Khadra lake that *Bacillus* were dominant in the water of this

lake, as it represented about 28.6 % of the total isolates followed by *Micrococcus sp.* and *Streptococcus* sp. represents 11.4 % for each one, *Aeromonas sp., Citrobacter* sp., 5.7% for each.Then the lowest percentage was 2.9% represented by *Achromobacter sp.*

Table (5): Total number of bacteria isolated from water of El-Khadra Lake

Genus	Total number	% of the total
Streptococcus	4	11.4
Micrococcus	4	11.4
Staphylococcus	3	8.6
Pseudomonas(table 3)	3	8.6
Aeromonas (table 3)	2	5.7
Achrombacter (table 3)	1	2.9
Bacillus (table 3)	10	28.6
Enterobacter (table 2)	3	8.6
Citrobacter (table 2)	2	5.7
Klebsella (table 2)	3	8.6
Total isolates	35	100

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REFERENCES

Aly , M.S. (2000) Eclolgical studies of phytoplanktons in closed lakes at Wadi – El- Natroun , Egypt . phycol. Vol ., 1.2000.

Allen , O.N. (1953) . Experiments in Soil Bacterilogy, 1 st . Ed. Burgess pub 1., USA

Bergey's Manual of systematic Bacteriology 1984, Williams & Wilkins, Baltimore, USA.

- Vol. 1 . Krieg, N.R. (ed.) . ordinary gram negative bacteria.
- Vol. 2 . Sneath, P.H.A. (ed.) . ordinary gram positive bacteria.

Apte, S.K. and Thomas, J. (1997). Possible amoleriation of coatal soil salinity using

halotolerant nitrogen-fixing cyanobacteria. *Plant soil.* 189(2): 205-211.

Botsford, J.L. and Lewis, T.A. (1990). Osmoregulation in *Rhizobium meliloti* production of glutamiatic acid in response to osmatic stress. *Applied Environmental Microbiology.* 56(2): 488-494.

Csonka, L.N. and Hanson, A.D. (1991). Procaryotic osmoregulation: Genetics and physiology. *Am. Rev. Microbiology.* 45: 569-606.

El-Sheikh, E.A.E. (1998) Effects of salt on rhizobia and bradyrhizobia: A review. *Annl.Applied Biology.* 132(3): 507-524.

Imhoff, J.F. (1986) Osmoregulation and compatible solutes in bacteria. *FEMS. Microbiol. Rev.* 39 : 57-66.

Madkour, M.A., Smith, L.T. and Smith G.M. (1990) Preferential osmolyte accumulation: A mechanism of osmotic stress adaptio in diazotrophic bacteria. *Applied Environmental Microbiology*.56: 2876-2881.

Malin, G. and Lapidot, A. (1996) Induction of synthesis of tetrahydropyramidine derivates in *streptomyces* strains and their effect on *Escherichia coli* in response to osmotic and heat stress. *J. bacterial.* 178(2): 258-395.

Miller, K.J. and Wood, J.M. (1996) Osmoadaptation by rhizosphere bacteria. *Ann. Rev. Microbial.* 50: 101-136.

Reed, R.H.; Borowitzka, L.J.; Mackay, M.A.; Chudek, J.A.; Foster, R.; War, S.R.C.;

Moore, D.J. and Stewart, W.D.P. (1989) Organic solutes accumulation in osmotically stressed cyanobacteria. *Microbial. Rev.* 39: 51-56

Shahaby, A.F. (1996) Osmoadaptation of growth and nitrogen fixation in *Rhizobium meliloti* in response to osmotic shocks. *J.Agric. Science.* Mansoura University. 21(12): 4359-4371

Shahaby, A.F. (1997) Osmoregulation *Azospirillum brasilense*: Production of glutamic acid and prolin response to stress. *J.Agric. Science.* Mansoura University, 22(12): 4817-4833

Topping, L.B. (1938) The predominant microorganisms in soils II: The relative abundance of different types of organism obtaines by plating and the relation of the plate to total counts. Zbi.Bakt. Abt.II, 98;93

التنوع الميكروبى فى بحيرة الخضرا

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تم دراسة تنوع الميكروبات فى بحيرة الخضرا فى وادى النطرون و ذلك فى شـــاطىء البحيرة و تربة القاع و تربة المحيط الجذرى للنباتات المجاورة و درس فى ذلـــك العـــدد الكلـــى للبكتريا و عدد محللات البروتين و الدهون و السيليولوز و بكتريا الأزوتوباكتر و تم ايضا تعريف و تمييز أنواع البكيريا و وجد أن معظمها عصويات و إلى حد ما كرويات. و قد وجد فى البحيرة أقراص خضراء عائمة على سطح الماء حول الشواطىء و وجد فيها بكتريا منتجة للبروتيـــاز و الليبز و قليلا من السيليلوز و الأزوتوباكتر و أظهر تميز البكتريا أن معظمها من جنس باســـيلس مع وجود ميكروفلورا مائية.

و أظهرت النتائج أن ميكروبات بسيدومونس وزانسوموناس التى تنتج صبغات صفـــراء غروية تمثل منشطات للنمو.