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Occurrence and Density of Alkalophilic Bacteria and Fungi in Saline Soils of Makkah District, Saudi Arabia

A.A. Khodair, A.S. Ramadani and A.M. Aggab

Biology Department, Faculty of Applied Sciences, Umm Al-Qura University, P.O. Box 3711, Makkah, Saudi Arabia

ABSTRACT. All soil samples were inhabited by alkalophilic, including alkalotolerant, bacteria in counts ranging from 3.7 to 37.2 $(x10^4)g^{-1}$ oven-dried soil. The density of alkalophilic fungi was significantly lower and their occurrence was restricted to only three sites out of four. The total alkalophilic bacteria of samples in vicinity of sea water were higher than in other inland samples. In presence of low organic matter, salinity appeared of more drastic effect on alkalophilic fungal population than bacteria. Increased content of soil organic matter appeared as a factor, beside others, which plays a probable role in minimizing the inhibitory effect of high salinity.

The genus *Bacillus* predominated in frequency, Gram negative rods were of moderate occurrence, while Gram positive micrococci and Gram positive asporogenous rods were of low frequency. Only 3.4 to 33.3% of *Bacillus* isolates together with all Gram positive asporogenous rods were obligate alkalophiles; the remaining isolates were either facultative or alkaline-tolerant.

Eight fungal genera could be identified as alkalophilic fungi. *Aspergillus* was the highest in occurrence but *Fusarium* was the highest in density at only one site.

Over recent decades, a very limited number of microorganisms growing at high pH values have appeared (Meek and Lipman 1922, Gibson 1934, Kushner and Lisson 1959). Recently, Horikoshi and Akiba (1982) and Krulwich and Guffanti (1983) described a new microbial world which includes aerobic and anaerobic spore-forming bacteria, actinomycetes, fungi and phages. Most of the work in this field was focused on the physiology, molecular biology, enzymology and industrial applications of such organisms.

Major studies on soil microflora in Saudi Arabia concentrated on thermophiles (Sabek and Abu-Zinada 1983, Abdulla and Gindy 1987), psychrophiles and mesophiles (Sejiny *et al.* 1980, Kashkari 1987), halophiles (Zaki *et al.* 1980) and on distribution and activity of microflora (Abu-Zinada *et al.* 1977).

The aim of the present investigation was to study the occurrence and density of alkalophilic microorganisms in Saudi Arabian saline soils of Makkah district.

Materials and Methods

Sites of study

Two saline localities were chosen: Red Sea shore at Sheaiba (70 km south to Makkah) and lowland at Al-Ka'keyah (outskirits of Makkah city). Soil samples were collected from 4 sites, two at each locality. At Sheaiba, the first site (I) represents fallow soil, while in the second site (II) the soil was covered with a halophytic vegetation dominated by *Zygophyllum album*. From each site, 2 composite soil samples were collected, one from spots in the vicinity of the sea (sample A) and the other from spots 100 m distant from the sea (sample B). At the Ka'keyah locality, one site (III) represents soil under reclamation (sample C) while the other (IV) represents soil harbouring a halophytic plant association (sample D) dominated with *Suaeda monoica*.

Soil sampling

Samples were collected according to the method described by Johnson *et al.* (1960). Soil samples were taken at random from each site, brought together into one composite sample which was mixed thoroughly and kept in polyethylene bags.

Soil analysis

Moisture content, total water soluble salts, organic matter and pH values were determined in replicate samples according to the techniques quoted by Jackson (1958).

Isolation methods

The dilution plate method (Johnson *et al.* 1960) was used for isolation and counting of alkalophilic bacteria. This method was not successful with fungi and therefore the Warcup technique (1950) was adopted.

Isolation media

For bacteria, the isolation medium was that recommended by Horikoshi and Akiba (1982). It was constituted of (g/l): glucose, 10; peptone, 5; yeast extract, 5; KH_2PO_4, 1; $MgSO_4$. $7H_2O$, 0.2; Na_2CO_3, 10 and agar, 15. Sodium carbonate solution was sterilized separately to give, after mixing with the remaining components, a final pH of 10.0-10.5.

For fungi, preliminary trials revealed that Sabouraud's agar was more suitable than Czapek-dox agar or the above-mentioned medium. It is composed of (g/l. w/v): glucose, 40; peptone, 10; agar, 15. Separately sterilized Na_2CO_3 was added to a final concentration of 1% together with chloroamphenicol (50 mg/l medium) to supress bacterial contamination. The final pH of this medium ranged from 10.0-10.5.

For bacteria, the inoculated plates were incubatd for 3 days at 30 ± 1°C after which a colony count was performed. Numbers of morphologically distinct bacterial colonies were also considered. After counting, all developed colonies were selected for identification and pure cultures were established on the same medium.

For fungi, incubation was at 25 ± 1°C, usually for 5 to 7 days, thereafter the developing colonies were counted per plate. The average count of either bacteria or fungi per gram of oven-dry soil in the original soil sample was calculated and recorded.

Characteristics of bacterial isolates

Preliminary identification of bacterial isolates was done according to the procedure previously described by Khodair *et al.* (1982) where Gram and spore stains were performed on 24 and 72 h cultures, respectively.

Amylolytic activities of isolates were also determined qualitatively using starch instead of glucose in the above mentioned medium, buffered at pH 9. The zone of hydrolysis was noted following addition of iodine solution after 48 h incubation at 30 ± 1°C.

The pH range of bacterial growth was determined using the following buffers: Na_2PO_4 - NaOH (pHs 7.0 & 8.2) and Na_2CO_3 - $NaHCO_3$ (pHs 9 & 10). The buffers were separately sterilized before addition, in suitable aliquots, to the previously mentioned agar medium devoid of Na_2CO_3. The carbonate buffer was sterilized by membrane filtration. The final buffer concentration did not exceed 0.2 M. The inoculated plates were incubated at 30 ± 1°C for 48 h after which the growth was visually detected.

Identification of fungal isolates

The developing fungal colonies were identified up to the genus level by microscopic examination and use of approved keys (Domsch *et al.* 1980, Domsch and Games 1972, Barnett and Hunter 1972, Booth 1971, Barron 1968, Raper and Fennel 1965, Raper and Thom 1949).

Results and Discussion

The characteristics of the collected soil samples are shown in Table 1. At the Sheaiba locality, a normal pattern of high salinity levels (9.57 & 10.06%) and low moisture content (3.95 & 3.83%) was observed in the inland soil samples (B1 & B2, 100 m offshore) as compared to those of sites neighbouring sea water (A1 & A2). The observed differences may be due to the effect of the wind factor which repeatedly transfers huge amounts of sea water to the nearest land thus raising its moisture content and washing out accumulated salts. On the contrary, the inland sites received droplets of sea water, the salts of which accumulated in the surface layer consequent to evaporation of the water. Fallow soil (site I) was found to contain organic matter (0.33 and 0.74%) lower than soil harbouring the *Zygophyllum album* association (1.17 and 1.48%).

Table 1. Soil properties of samples collected from different sites at Sheaiba (I & II) and Al-Ka'keyah (III & IV) localities

Site No.	Soil sample	Moisture content % (wt/wt)	Total soluble salts %	Organic matter (wt/wt)	pH value
I	A1	10.11	1.33	0.33	9.0
	B1	3.95	9.57	0.74	8.13
II	A2	5.01	4.10	1.17	8.57
	B2	3.83	10.06	1.48	8.10
III	C	3.17	2.27	1.16	8.75
IV	D	9.09	11.20	1.64	8.96

A: Sea vicinity.
B: 100m inland (*Zygophyllum album* association).
C: Land under reclamation.
D: *Suaeda monoica* association.

In soil samples collected from the Ka'keyah locality, the values of moisture content, total soluble salts and organic content were lower in soil under reclamation (site III) than in soil harbouring the *Suaeda monoica* association (site IV). The relatively higher level of soil organic matter recorded in the site covered with *Suaeda monoica* (site IV, sample D) compared to that in site III (sample C) is presumably due to decayed plant remains added naturally to soil. It has to be mentioned here that reclamation in site III (sample C) was in the form of primitive processes of cultivation usually resulting in few sparse plants. However, remarkable differences in organic matter content can be noticed in soil samples A2, B2, C & D (either reclaimed or inhabiting wild plants) if compared with samples free from any plant (A1 & B1).

Concerning soil reaction, the results obtained (Table 1) reveal that alkalinity is one of the distinctive features of soils under investigation. The pH values ranged between 8.1 and 9.0. This is in accordance with the findings of Daubenmire (1959) who stated that "in warm dry climates, soils are usually circumneutral to strongly basic because there is insufficient rainfall to leach away the bases as soon as they are released in weathering, and few acidic materials are produced there by natural process of decay".

The total alkalophilic bacterial and fungal counts, are included in Table 2. All soil samples analyzed were inhabited with alkalophilic bacteria in counts ranging from 3.7×10^4 up to 37.2×10^4 per g oven-dried soil. On the other hand, the density of alkalophilic fungi was significantly lower than bacteria and their occurrence was only restricted to sites I (sample A1), III and IV in count values ranging from 0.52×10^2 to 16.7×10^2 per g oven-dried soil.

The bacterial counts in samples from the Sheaiba locality (sites I & II) were appreciably higher than those of Al-Ka'keyah (sites III & IV). The data also revealed that the total alkalophilic bacterial counts of samples in the vicinity of sea water (A1 & A2) were remarkably higher than all other examined samples. On the other hand, the two compared sites at the Ka'keyah locality showed lowest counts of alkalophilic bacteria in soil under reclamation (3.7×10^4/g) than in soil covered with *Suaeda monoica* association (6.1×10^4/g). On the contrary, the alkalophilic fungal population was significantly higher in soil under reclamation (C) than in soil covered with *Suaeda monoica* association (D).

Although the aim of the present investigation was not to define the influence of independent environmental factors on the occurrence and density of alkalophilic bacteria and fungi, it is however of special importance to point out which of these factors may be influential when alkalophilic organisms have to be evaluated.

The data obtained showed that both pH and salinity level exert a noticeable effect on the density of alkalophilic soil bacteria and fungi (Tables 1 & 2). Thus at the Sheaiba locality, the relatively poor bacterial flora in inland spots (samples B1 & B2) as compared with their neighbouring spots (samples A1 & A2) can be attributed, in part, to their high salinity levels and lower pH values.

At the same locality (Sheaiba), salinity appeared of more drastic effect on alkalophilic fungal population which was missed completely on isolation plates from samples B1, A2 and B2 and was only recovered in very low density from soil sample A1 which contained the least concentration of total soluble salts (1.33%).

This explanation does not match with the relatively high counts of fungi in sample D at the Ka'keyah locality which revealed the highest salinity among all soil samples. Hence, other factor(s), such as the high soil moisture content prevailing at this site together with its high organic matter content, may play a role in minimizing the deleterious effect of salinity.

At the Ka'keyah locality, the relatively higher number of bacterial cells observed in site IV than in site III may indicate that the increased soil organic matter content in the former neutralizes the inhibitory effect of the highest level of salinity recorded there (11.2%). The suggested beneficial effect exerted by soil organic content can be also substantiated by comparing the density of bacteria in sites harbouring wild vegetation (II & IV) with that inhabiting fallow soils (I & II) in both localities. More evidence in that direction can be revealed by comparing fungal counts in site I (low organic matter and low salinity) with those in sites III

Table 2. Counts of alkalophilic microorganisms (per g oven-dried soil) inhabiting soil samples collected from different sites at Sheaiba (I & II) and Al-Ka'keyah (III & IV) localities

Site No.	Soil sample	Counts of	
		Bacteria ($10^4 g^{-1}$)	Fungi ($10^2 g^{-1}$)
I	A1	22.4	0.52
	B1	14.9	–
II	A2	37.2	–
	B2	15.4	–
III	C	3.7	16.67
IV	D	6.1	0.86

and IV (high organic matter and higher salinity). However, the competitive ability of fungi over bacteria in soil with low moisture content can not be ignored. This factor interprets the high fungal counts in site III together with the lowest bacterial count in the same site. In competition with bacteria, fungi are able to flourish under such conditions. This is in agreement with the findings of Campbell (1985) who stated that the critical factor for all heterotrophic bacteria and fungi is the quantity of organic matter, and to some extent its quality. Similarly, Salama *et al.* (1971) in their studies on the fungal flora of Egyptian soils found that the frequency of occurrence of fungal isolates was higher in soil samples collected from salt marshes than sand dunes in spite of the considerably higher percentage of total soluble salts in the former than the latter locality. They interpreted their results on the basis of the fact that soil samples representing salt marshes contained higher levels of organic matter and moisture content than those of sand dunes.

Increased numbers of alkalophilic bacteria recorded in samples A1 & A2 (Table 2) were coupled with a high frequency of star-shape colonies, amounting to 56.5% and 24.5% respectively. Chromogenic colonies were not recorded in site I but were weakly represented in other samples with percentages ranging between 1.8 and 7.1. Finally, in the present study, actinomycetes were not encountered on the isolation plates used.

The above findings indicate that the alkalophilic bacterial population investigated comprised several genera and/or species, and hence identification of the developed bacteria seems very important. Full identification of numerous isolates needs great effort. Therefore, preliminary identification of bacterial colonies appearing on counting plates was carried out according to the technique described by Khodair *et al.* (1982). In this method, Gram staining of 24 h subcultures from colonies appearing together with cell shape, size and endosporogenesis were investigated. Bearing in mind that the bacterial isolates were inhabiting virgin soil and that they were counted on semisynthetic medium under aerobic conditions, preliminary identification could be accomplished by exclusion of the unlikely parts of Bergey's manual (Buchanan and Gibbons 1974). The tentative identification method, mentioned above, has the advantage of simplicity as a mean of describing any physiologically distinct bacterial group such as alkalophiles.

The data included in Table 3 show that a total of 193 bacterial cultures were isolated, purified and tentatively identified. The genus *Bacillus* (Gram positive, sporeforming, aerobic and facultatively anaerobic rods) was predominant in all soil samples with frequency of 70.7 to 93.3%. Unidentified Gram-negative,

nonsporeformers, aerobic and facultatively anaerobic short rods (designated here as pseudomonads) were also isolated from all soil samples but with moderate frequency ranging from 6.6 to 26.8%. Gram-positive cocci, too small in size (designated here as *Micrococcus*) and Gram-positive asporogenous rods were of low frequency; the former disappeared from samples of Al-Ka'keyah and the latter was detected only in one soil sample (sample A2) at Sheaiba.

Regarding the effect of initial pH of the medium on growth of isolates (Table 3), the results indicate that the bacterial isolates tested can be grouped into two categories. Isolates of the first category showed better growth on both neutral and slightly alkaline media (pH 7.0 & 8.2), and most of them showed relatively poor growth at pH 9.0. No strains of this group could grow on highly alkaline medium (pH 10.0). This group can be designated as alkaline-tolerant strains (Horikoshi and Akiba 1982) or facultative alkalophilic strains (Guffanti *et al.* 1986). On the other hand, the second category includes organisms capable of growth only on highly alkaline media (pH 9.0 & 10.0), and thus considered as obligate alkalophiles (Table 3).

The data showed that facultative alkalophiles predominated in the isolates tested. The isolated *Micrococcus* strains and all pseudomonads were alkaline-tolerant. *Bacillus* isolates recovered from the Ka'keyah locality (sites III & IV) were facultative alkalophiles, while those isolated from the Sheaiba locality (sites I & II) comprised some obligate alkalophiles (3.4% to 33.3%). By contrast, all

Table 3. Percentages of alkalotolerant and/or alkalophilic bacteria appearing on count plates

Site No.	Soil sample	Bacillus %	Obligate alkalophilic isolates %	Pseudomonads %	Gram + ve asporogenous	Obligate alkalophilic isolates %	Micrococcus Gram + ve	Total No. of isolates
I	A1	70.7	3.4	26.8	0.0	0.0	2.4	41
	B1	75.0	8.3	18.7	0.0	0.0	6.2	32
II	A2	70.7	10.3	24.3	2.4	1.0	2.4	41
	B2	71.4	33.3	19.0	0.0	0.0	9.5	21
III	C	92.8	0.0	7.1	0.0	0.0	0.0	28
IV	D	93.3	0.0	6.6	0.0	0.0	0.0	30
								193

Gram-positive asporogenous rods isolated were obligate alkalophiles. In agreement with these results, previously described facultative and obligate alkalophiles include species and strains related to the genera, *Bacillus* (Boyer *et al.* 1973, Ohta *et al.* 1975, Guffanti *et al.* 1980, Ando *et al.* a,b, 1981, Koyama *et al.* 1983), *Micrococcus* (Akiba and Horikoshi 1976), *Pseudomonas* (Hale 1977), *Clostridium* (Souza *et al.* 1974), *Flavobacterium* (Souza *et al.* 1974), *Arthrobacter* (Shimao *et al.* 1989), and Corynoform bacterium (Souza and Deal 1977).

The amylolytic activity of the bacterial isolates (untabulated data) revealed that all members of the genus *Bacillus* together with the group of Gram-positive asporogenous rods produced amylase(s), a behaviour leading to the conclusion that alkalophilic bacteria not only represent a stable population in the analyzed soils but also share in the cycling of nutrients there.

Eight genera in addition to Mycelia Sterilia (hyaline & coloured) could be identified as alkalophilic fungi on isolation plates (Table 4). The number of genera varied with site variation, being clearly higher in site C (7 genera), followed by site D (2 genera), while site A1 was inhabited by only one genus.

The genus *Aspergillus* (moderate occurrence, recorded 2 times out of 3) constituted 31.52% and 80.0% of the total number of this genus isolates in site C and D respectively. This high density of aspergilli in site C was only surpassed by the genus *Fusarium* which accounted for 39.13% of the total genus count at that site. Since *Fusarium* isolates were recovered from only site C (one case of isolation) it was considered of low occurrence at the studied locality. *Curvularia* was third in order of density at site C. It comprised 9.78% of total genus count at that site irrespective of being also of low occurrence. Relative densities ranging from 5.43 to 6.52% were indexed by *Geotrichum, Cephalosporium* and sterile mycelium, all being of low frequency of occurrence. The genus *Rhizopus* was recovered on isolation plates from only site D (low occurrence) where it accounted for 20% of total alkalophilic fungi at that site.

Since the genus *Fusarium* constituted the highest percentage of total alkalophilic fungal count at site C, it may be expected that this genus has prominent alkalophilous tendencies. Johnson (1923) reported that *Penicillium variables, Fusarium bullatum* and *F. oxysporum* could grow at a pH value of 11 indicating its alkaline tolerance.

It is premature to state that the fungi isolated through this study are all alkalophilic. Whether such fungi are obligate, facultative alkalophiles and/or alkaline-tolerant species will be the objective of a next more critical investigation.

Table 4. Genus range, relative density (as % of total count) and frequency of occurrence of alkalophilic fungi in soil samples collected from different sites at Sheaiba (I & II) and Al-Ka'keyah (III & IV) localities

Genus	Site*						Frequency of occurrence
	I		III		IV		
	A1		C		D		
	Count / 3 plates	% of total count	Count / 3 plates	% of total count	Count / 3 plates	% of total count	
Aspergillus	–	–	29	31.52	4	80.0	M
Cephalosporium	–	–	6	6.52	–	–	L
Curvularia	–	–	9	9.78	–	–	L
Cylindrocarpon	–	–	1	1.09	–	–	L
Fusarium	–	–	36	39.13	–	–	L
Geotrichum	–	–	5	5.43	–	–	L
Humicola	3	100	–	–	–	–	L
Rhizopus	–	–	–	–	1	20.0	L
Sterile mycelium	–	–	6	6.52	–	–	L

*Fungal isolates could not be recovered on plates from soil samples B1 (site I), A2 & B2 (site II).
Occurrence remarks:

H = high (isolated from 3 sites)
M = moderate (isolated from 2 sites)
L = low (isolated from 1 site)

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مدى تواجد البكتريا والفطريات المحبة للقلوية وكثافتها في تربة ملحية بمنطقة مكة المكرمة ـ المملكة العربية السعودية

عبدالفتاح عبدالعزيز خضير و أحمد سراج رمضاني و عبدالعزيز مصطفى عقاب

قسم الأحياء ـ كلية العلوم التطبيقية ـ جامعة أم القرى
ص. ب: ٣٧١١ ـ مكة المكرمة ـ المملكة العربية السعودية

تم اختيار منطقتين تتصفان بتربتهما المالحة هما: ساحل البحر الأحمر عند الشعيبة (٧٠ كم جنوب مكة) والأرض المنخفضة عند الكعكية (ضواحي مدينة مكة). وجمعت عينات التربة من ٤ مواقع، موقعان في كل منطقة.

وأوضحت الدراسة أن كل عينات التربة التي فحصت تستوطنها بكتريا محبة للقلوية ومقاومة لها بأعداد تتراوح من ٣,٧ إلى ٣٧,٢ ($\times ١٠^{٤}$) لكل جرام من التربة الجافة، بينما كانت كثافة الفطريات المحبة للقلوية أقل من ذلك كثيراً، وتواجدت في ثلاثة مواقع فقط (١، ٢، ٣) من أربعة بتعداد يتراوح من ٠,٥٢ إلى ١٦,٧ ($\times ١٠^{٢}$) لكل جرام من التربة الجافة. وكان التعداد الكلي للبكتريا المحبة للقلوية في عينات التربة المجاورة لماء البحر أعلى من تلك الموجودة في عينات التربة البعيدة قليلاً عن ماء البحر (١٠٠ متر).

وتدل النتائج التي تم التحصل عليها من منطقة الشعيبة على أن ملوحة التربة لها تأثير مدمر على تعداد الفطريات المحبة للقلوية، حيث أمكن عزل هذه الفطريات بكثافة منخفضة جداً من عينة واحدة فقط اتصفت تربتها بإحتوائها على أقل قدر من الأملاح الذائبة (١,٣٣ ٪).

ولعل ما لوحظ في منطقة الكعكية من إزدياد التعداد البكتري في الموقع الرابع (ذو المحتوى الأعلى من المادة العضوية) عنه في الموقع الثالث (ذو المحتوى الأقل من المادة العضوية) على الرغم من المحتوى الملحي الأعلى (٢ , ١١ ٪) في الموقع السابق عن اللاحق، يدل على الدور الذي قد تلعبه المادة العضوية، بجانب عوامل أخرى مثل الرطوبة المرتفعة في التربة، في التخفيف من الأثر الضار للملوحة العالية.

ويدعم هذا الاقتراح ما لوحظ من إزدياد كثافة البكتريا في عينات التربة المغطاة بالكساء الخضري البري عنه في التربة غير المزروعة نتيجة لزيادة المحتوى العضوي في التربة التي تتلقى طبيعياً ـ أوراقاً متساقطة.

وأدت الدراسة إلى الحصول على ١٩٣ عزلة بكترية نقية، ساد فيها جنس الباسيلوس على غيره، بينما كانت العزلات التي أشير إليها كسيدومونات متوسطة التواجد، في حين أظهرت عزلات الميكروكوكساى، بالاضافة إلى العصويات الموجبة لصبغة جرام وغير المنتجة للجراثيم، تواجداً منخفضاً وكانت جميع العصويات الموجبة لصبغة جرام وغير المنتجة للجراثيم وكذلك ٤ , ٣ إلى ٣ , ٣٣ ٪ فقط من عزلات الباسيلوس محبة للقلوية اجباراً، بينما كانت بقية العزلات محبة للقلوية اختياراً أو تتحمل القلوية.

وقد أمكن التعرف على ثمانية أجناس فطرية محبة للقلوية، كان الاسبرجللس أكثرها تواجداً، بينما كان الفيوزاريوم أعلاها كثافة في موقع واحد فقط من المواقع الأربعة التي درست. وسيكون هدفاً ـ لدراسة لاحقة ـ التعرف على الأنواع الفطرية المحبة للقلوية اجباراً، أو اختياراً أو تلك المتحملة للملوحة.