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Studies on the Microbial Population of a Libyan Desert Soil

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ABSTRACT

The bacterial flora of the virgin soils of Kufra consists of a large percentage of actinomycetes, e.g. *Streptomyces*, and spore-forming bacteria present as spores. When this desert soil was intensively farmed, the bacterial population increased tremendously. The increase in bacterial spores paralleled that of the total bacterial count, but the relative abundance of actinomycetes dropped significantly, although the absolute numbers increased with cultivation.

Bacillus species changed both qualitatively and quantitatively as a result of cultivation and cropping. In the virgin soil, *B. subtilis* was the most abundant species constituting about 40% of the total isolated bacilli. Other predominant species in the virgin soil were *B. megaterium*, *B. circulans*, *B. cereus* and *B. sphericus*. After repeated cropping of the desert soil *B. subtilis*, *B. cereus* and *B. megaterium* showed a reasonable increase in numbers.

INTRODUCTION

The genus *Bacillus* has been isolated frequently from different soils, but studies on the ecology of spore-forming bacteria have been mostly concerned with occurrence and distribution (4, 5, 6, 7, 15, 16). Most studies were concerned with undisturbed forest soils and grassland in many parts of the world. Studies on their presence in desert soils have been relatively few (3, 8, 9, 14).

It has always been assumed that *Bacillus* spp. are more tolerant of unfavourable environmental conditions because of their endospore formation, and so it was thought worthwhile to study their occurrence in the harsh environment of hot desert soils. The study was carried out on soils from the Kufra oasis, located in the Sahara desert in the southeastern part of Libya at about 24°E longitude. The area is currently under intensive agricultural development due to the discovery of an abundant supply of excellent quality ground water. A comparative study between the bacterial populations of the virgin desert soil and that of the cultivated fields in the same area was also carried out to throw some light on the effect of cultivation.

Soil in the Kufra oasis is characterized by a very high summer temperature (44–47°C), and extremely low moisture content (<0.1%). The annual rainfall ranges between 1.3 and 2.9 mm. The soil exhibits no profile development and is devoid of

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organic matter (<0.05%). A complete description of the chemical and mineralogical properties of this soil was published by Abdelgawad, Page and Lund (1).

MATERIALS AND METHODS

The soils selected were obtained from three different areas in the Kufra oasis. One area of virgin soil was selected at a distance from the cultivated and irrigated fields, and the other two areas were located in currently cultivated fields. These fields were designated as B_3W and B_7W by those working on the Kufra Agricultural Project (Fig. 1). The fields have been cultivated for several years, and carried crops of barley (*Hordeum vulgare*). The pH of the virgin soil was 7.5–8.2, while that of the fields was 6.4–7.0.

Four soil samples were taken from each area, kept in an ice box and flown to the laboratory. Dilutions and plating were performed immediately on arrival to minimize the effect of storage on the microbial population. The time from collection of samples to preparation of dilutions and plating was about 24 hours. Ten-fold serial dilutions were prepared in quarter strength Ringer's solution. The pipetting procedure for the estimation of the total heterotrophic bacteria described by Goodfellow *et al.* (5) was used. Dilutions were plated by transferring 1.0 ml of each dilution to each of 8 petri dishes and the isolation medium was then poured into the plates and mixed with the inoculum. Plating of spore-forming bacteria was carried out by pipetting 10 ml of the 10^{-2} soil suspension into a sterile boiling tube. The suspension was pasteurized by heating in a water bath at 80°C for 10 minutes. Further dilutions were prepared and plated in the same manner as for the non-pasteurized soil suspensions. The plating medium used for total counts and spore counts was peptone yeast extract actidione agar (5).

For the estimation of actinomycetes, starch casein agar (11) was used. All plates were incubated at 25°C for 14 days.

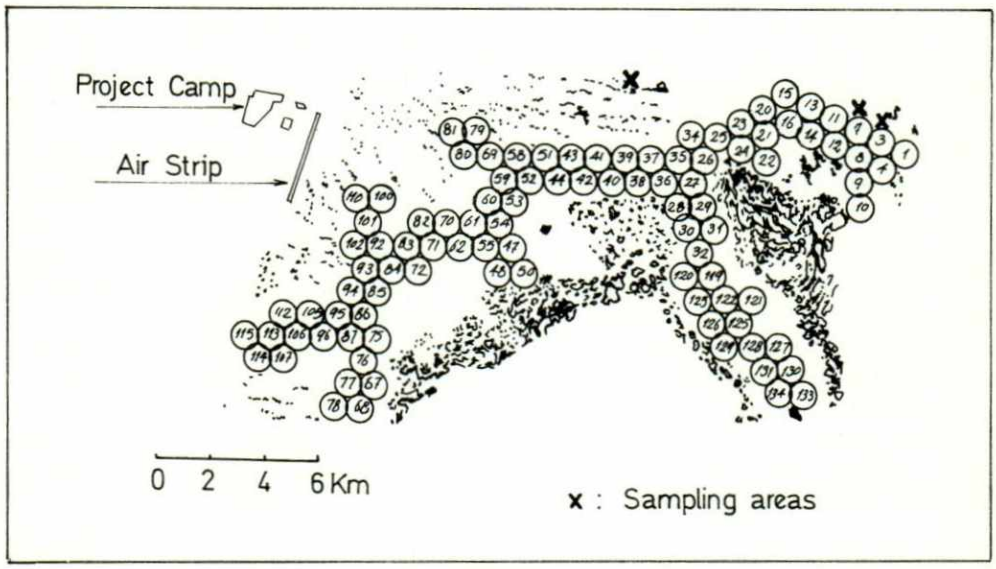

Fig. 1. Distribution and numbers of fields in the Kufra Agricultural Project.

To determine the distribution of *Bacillus* species in the three areas under study, isolations were made from the plates inoculated with the heated soil suspensions. Fifty isolates were taken from each area and subcultured into tubes of peptone yeast extract broth. The isolates were purified by streaking several times and maintained on peptone yeast extract agar at 5°C under oil. The isolated *Bacillus* species were identified according to Bergey's Manual (2).

RESULTS AND DISCUSSION

The virgin soil under study is characterized by a very low moisture content, it is also very poor in organic material. Estimation of the total bacterial population on peptone yeast extract agar gave a count of 3.1×10^5 cells per gram of oven-dried soil. When the same soil sample was plated on starch casein agar a count of 2.2×10^5 actinomycete colonies per gram of oven-dried soil was obtained (Table 1). This indicates that the ratio of actinomycetes to the total bacterial flora in the virgin soil was 1:1.4. The very low moisture content of the soil as well as the slight alkalinity (7.5–8.2) apparently favour the actinomycetes growth. It was previously reported that spores of actinomycetes seem to resist desiccation. Their relative abundance increased from 11 to 98% of the microflora after drought conditions in a Kenyan soil (13). In a dry alkaline soil (pH 9.0) Johnstone (10) also reported that 95% of the microflora were actinomycetes. In the cultivated fields, on the other hand, the total bacterial population showed significant increase (8.4×10^6 and 7.6×10^6 cells/gram oven-dried soil). The actinomycete count was about double that of the virgin soil. The increase in the bacterial population was probably a direct result of the improvement in the moisture status of the soil, as well as the incorporation of plant residues in the soil. The ploughing in of plant remains, and the presence of both dead and living plant roots in the soil caused an increase in utilizable organic matter. However, the increase in the number of actinomycetes did not parallel that of the total bacteria. This is due to the inability of actinomycetes to compete successfully with other bacteria when fresh organic material is added to the soil. This is evident from the sharp drop in the ratio of actinomycetes to the total bacterial flora in the two cultivated fields. This ratio was 1:15.8 and 1:11.3 in the B_3W and B_7W fields respectively.

Isolation of actinomycetes made from starch casein agar plates showed that all isolates were strains of *Streptomyces*. In a similar study on Tunisian desert soil, Johnson (8) reported that about 80% of his isolates were species of *Streptomyces*. Other

Table 1 The total bacterial count and the actinomycete count in the Kufra desert soil.

Soil	No. of bacteria/g oven-dried soil	No. of actinomycetes/g oven-dried soil	Ratio of actinomycetes to total bacteria
Virgin soil	3.1×10^5 (SD 1.0×10^5)[1]	2.2×10^5 (SD 0.6×10^5)	1:1.4
B_3W[2]	8.4×10^6 (SD 1.4×10^6)	5.3×10^5 (SD 1.6×10^5)	1:15.8
B_7W	7.6×10^6 (SD 1.2×10^6)	6.7×10^5 (SD 1.8×10^5)	1:11.3

[1] SD = Standard deviation.
[2] Kufra agricultural project designation.

Table 2 The number of bacteria in pasteurized and non-pasteurized soil suspensions of the Kufra desert soil.

Soil	No. of bacteria/g oven-dried soil		Presumed % of bacteria
	Non-pasteurized	Pasteurized	presented as spores
Virgin soil	3.1×10^5 (SD 1.0×10^5)[1]	7.4×10^4 (SD 1.8×10^4)	24.0
B_3W[2]	8.4×10^6 (SD 1.4×10^6)	2.6×10^6 (SD 0.6×10^6)	31.0
B_7W	7.6×10^6 (SD 1.2×10^6)	3.1×10^6 (SD 1.0×10^6)	33.0

[1] SD = Standard deviation.

[2] Kufra agricultural project designation.

workers (Lechevalier and Lechevalier, 12) also found that 95% of the actinomycetes isolated from 16 different soils were species of *Streptomyces*.

The population of spore-forming bacteria, present as spores, was estimated by plating soil suspensions after being pasteurized. The results are given in Table 2. The number of bacterial spores in the virgin soil, although much less than their numbers in the cultivated fields, made up about 24% of the total bacterial count. This result is very close to the results of Johnson and Cameron (9) who found species of *Bacillus* to account for about 25% of the isolates from hot desert soils collected from Argentina, Australia and Occupied Palestine. Cultivation of the desert soil caused an increase in both total bacterial and bacterial spore counts. The percentage of bacterial spores in the cultivated fields showed only a slight increase from that of the virgin soil. The marked increase in the spore-forming population after cropping suggests that these organisms take an active part in certain stages of the mineralization of organic residues in the soil. From the results reported here it is quite evident that the bacterial flora in the Kufra desert soil is made up mostly of strains of *Streptomyces* and various species of spore-forming bacteria.

In the present investigation, fifty spore-forming isolates were taken from each soil sampling area and identified. The results are given in Table 3. *Bacillus subtilis* was the

Table 3 Distribution of *Bacillus* species in the virgin and cultivated soils.

Bacillus species	Number of isolates			% of isolates		
	Virgin soil	B_3W[1]	B_7W	Virgin soil	B_3W	B_7W
B. subtilis	20	24	23	40	48	46
B. megaterium	8	11	10	16	22	20
B. cereus	6	8	9	12	16	18
B. circulans	7	4	5	14	8	10
B. sphericus	5	1	2	10	2	4
B. licheniformis	3	2	1	6	4	2
B. coagulans	1	—	—	2	0	0

[1] Kufra agricultural project designation.

most abundant species constituting from 40 to 48% of the isolated bacilli in the three soil areas. Other predominant species in the virgin soil were *B. megaterium*, *B. circulans*, *B. cereus* and *B. sphericus*. In their study on hot desert soils from different parts of the world, Johnson and Cameron (9) failed to isolate *B. licheniformis*. This species made up to 6% of the spore-forming isolates from the virgin soil in the present investigation. However, *B. licheniformis* is very similar to *B. subtilis*.

After cultivation all species present in the virgin soil were isolated with the exception of *B. coagulans*. However, the species which showed an increase after repeated cropping were *B. subtilis*, *B. megaterium* and *B. cereus*. The percentage of *B. circulans* and *B. sphericus* dropped slightly.

From these observations, the effect of cultivation of desert soils on the total spore-forming population and its influence on the species composition of *Bacillus* were clearly demonstrated.

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« دراسة على الأحياء الدقيقة بتربة صحراوية من ليبيا »

« عبد الرؤوف حموده سيالة »

« المستخلص »

لقد أجريت هذه الدراسة على التربة الصحراوية الموجودة بمنطقة الكفرة لمعرفة أعداد وأنواع الأحياء الدقيقة الموجودة بها ، والتغير الذى قد ينتج على هذه الأحياء الدقيقة بعد استصلاح وزراعة هذه التربة .

وجد أن التربة الصحراوية الغير مستصلحة تحتوى على عدد قليل من البكتريا وقد لوحظ أن نوع الاكتينومايسيت يمثل جزءا كبيرا من هذه الأحياء حيث كانت نسبة هذا النوع إلى مجموع البكتريا بالتربة الغير مزروعة ١ : ١,٤ . أما البكتريا المتجرثمة فكونت حوالى ٢٤٪ من المجموع الكلى .

بعد استصلاح التربة وزراعتها لسنوات متعددة وجد أن الأعداد الكلية للبكتريا قد زادت زيادة كبيرة ، أما بالنسبة لمجموعة الاكتينومايسيت فقد زادت زيادة بسيطة في التربة المزروعة ، بيد أن نسبتها للمجموع الكلى للبكتريا قد انخفضت انخفاضا كبيرا . كانت زيادة أعداد البكتريا المتجرثمة موازية للزيادة الكلية في أعداد البكتريا مما جعل نسبتها تزيد زيادة بسيطة فقط عن نسبتها في التربة الغير مزروعة .

وبدراسة أنواع البكتريا المتجرثمة في التربة وجد أن الأنواع السائدة في التربة الصحراوية الغير مستصلحة هى : B. subtilis, B. megaterium, B. cereus, B. circulans, B. sphericus أما بعد الاستصلاح والزراعة فالأنواع الثلاثة الأولى زادت نسبتها في التربة زيادة ملحوظة .