Distribution and Relative Dominance of Species of the Genus *Bacillus* in Two Regions of El-Jamahiriya

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ABSTRACT

Fifty isolates of the genus *Bacillus* were obtained from soil samples from two different locations in El-Jamahiriya: Tripoli and Wadi El-Shatti representing northern and southern regions, respectively. All isolates were purified and characterized to the species level. Eight different species were designated as: *B. subtilis, B. cereus, B. megaterium, B. coagulans, B. licheniformis, B. circulans, B. lentus,* and *B. firmus.* Among the species isolated from the northern region, *B. cereus* appeared to dominate the bacillus component in this soil. On the other hand both *B. subtilis* and *B. circulans* shared the dominance in the soil of the southern region.

INTRODUCTION

The aerobic, spore-forming bacilli constitute an important segment of the soil heterotrophic population. Being capable of endospore formation with the resultant tolerance of adverse soil conditions, the genus *Bacillus* makes up between 7 and 67% of the total soil bacteria (1).

In their active growth, several species participate in an immense number of activities in the soil. *Bacillus megaterium* was reported to be active in the mineralization of organic phosphorous in the soil (5). *Bacillus megaterium* and *B. circulans* participate in the reduction of ferric and manganese oxides in soils and hence increase the availability of these two elements (3). *Bacillus sphaericus,* was found to be highly active in cometabolizing certain pesticides (Chlorobenzoate), and being able to decompose several phenylurea herbicides (12, 13).

Seed, as well as root bacterization with *B. subtilis* had been successfully used in controlling seedling diseases in certain plants (4). Production of growth promoting substances by *B. megaterium* and other species of the bacilli, were found to allow more germination, elongation of stems, enlargement of leaves and earlier flowering and fruiting in certain crops (5). Synthesis of extracellular polysaccharides by *B. subtilis, B. megaterium,* and *B. polymyxa* were found to interact with soil clays and contribute to the stabilization of soil aggregates (7, 8).

The intensity and dominance of certain species of the genus *Bacillus* were reported

to increase from northern to southern regions as a result of high thermal conditions prevailing in the latter region (9). Out of the sixteen soil species listed in Bergey's Manual, seven were found to grow optimally at 37C, nine grow well at 30C, and only five were able to grow at both temperatures (2).

The objectives of this work were to study the distribution and relative dominance of species of the soil bacilli in samples from the northern and southern regions of El-Jamahiriya, and to establish stock cultures of isolates for further research, particularly in the sense of inducing soil aggregates to reduce wind erosion.

MATERIALS AND METHODS

Soil samples from Tripoli (northern region) and Wadi El-Shatti (southern region) were collected, air-dried and seived to pass a 2mm screen. Original samples were stored in covered plastic containers in the laboratory, and subsamples were taken as needed. Selective isolation of aerobic, spore-forming bacteria (genus: *Bacillus*) was carried out by pasteurization of soil suspensions at 80 to 85 C for 10 minutes (11). Subsequent platings were made on three different media, nutrient-agar (NA), dextrose-agar (DA), and dextrose-nitrate agar (DNA). The growth on the first two media was used to characterize and differentiate species (2). On the other hand, the dextrose-nitrate agar medium was selectively included since it allows sporulation of certain species more easily, and could be used as a secondary criterion for species differentiation.

A set of morphological as well as physiological tests and measurements were carried out and the results obtained were used to key down the various isolates to the species level (6).

RESULTS AND DISCUSSION

Fifty isolates were collected and purified through successive plate transfers on one of the three media that showed originally the best growth. Both Gram's and spore-stains were made on the initially purified cultures to acertain their belonging to the genus *Bacillus*. Based on the morphological and physiological studies, the results of which are indicated in Tables 1 and 2, the fifty isolates were grouped in eight different species designated as: *B. subtilis, B. cereus, B. circulans, B. megaterium, B. lentus, B. licheniformis, B. firmus,* and *B. coagulans.*

The distribution and relative dominance of these species in the two regions are summarized in Table 3. It is evident from the results obtained that both *B. subtilis* and *B. circulans* dominate the soil of Wadi El-Shatti (southern region). At elevated temperatures, characteristic of this area where reocrds in excess of 40C, for most of the year, are not uncommon it would be expected so. Both species are known to grow optimally at a temperature range between 30 and 40C, with a maximum heat tolerance of 55C. This is in agreement with the findings reported from another zone of the southern region, namely El-Kufra (10). The soil sample from Tripoli (northern region) was dominated by a single species designated as *B. cereus.* This is expected since this bacillus grows optimally at 30C, with a maximum temperature tolerated by most strains between 37 and 45C.

Table 1.a — Cultural and physiological characteristics of Wadi El-Shatti isolates.

Culture No	Growth on DA as compared to NA	Action on glucose Acid/Gas		Acetoin Production	MR Test	Indole Test	Growth on 7% NaCl	Starch Hydrolysis	Casein Hydrolysis
1	good	+	−	+	+	−	+	+	+
2	»	+	−	+	+	−	+	+	+
3	»	+	−	+	+	−	+	+	VW
4	»	+	−	+	+	−	+	+	VW
5	»	+	−	+	+	−	+	+	−
6	»	+	−	+	+	−	+	+	+
7	»	+	−	+	+	−	+	+	+
8	»	+	−	+	+	−	+	+	+
9	»	+	−	+	+	−	+	+	−
10	not good	+	−	+	+	−	+	+	VW
11	good	+	−	+	+	−	+	+	+
12	»	+	−	+	+	−	+	+	+
13	»	+	−	+	+	−	−	+	+
14	»	+	−	+	+	−	+	+	+
15	»	+	−	+	−	−	+	+	+
16	»	+	−	+	+	−	+	+	+
17	»	+	−	+	+	−	+	+	+
18	»	+	−	+	+	−	−	+	−
19	»	+	−	+	+	−	−	+	−
20	»	+	−	−	+	−	+	VW	VW
21	»	+	−	+	+	−	+	+	+
22	»	+	−	+	+	−	+	+	+
23	not good	VW	−	−	−	−	−	+	+
24	good	+	−	+	−	−	−	+	+
25	»	+	−	+	+	−	+	+	+
26	»	+	−	+	+	−	+	+	−
27	»	+	−	+	+	−	−	+	+
28	»	+	−	+	+	−	+	+	+
29	»	+	−	+	+	−	+	+	−

W, weak; VW, very weak.

Table 1.b — Cultural and physiological characteristics of Tripoli isolates.

Culture No	Growth on DA as compared to NA	Action on glucose Acid/Gas		Acetoin Production	MR Test	Indole Test	Growth on 7% NaCl	Starch Hydrolysis	Case in Hydrolysis
30	good	+	−	+	+	−	+	+	−
31	»	+	−	+	+	−	+	+	+
32	»	VW	−	−	−	−	−	−	−
33	»	VW	−	−	−	−	−	−	−
34	»	+	−	+	+	−	+	+	−
35	»	+	−	−	+	−	+	VW	VW
36	»	+	−	+	+	−	−	+	VW
37	»	+	−	+	+	−	−	+	+
38	»	VW	−	−	−	−	−	−	−
39	»	VW	−	−	−	−	−	−	−
40	»	+	−	−	+	−	−	VW	−
41	»	VW	−	−	−	−	−	−	−
42	»	VW	−	−	−	−	−	−	−
43	»	+	−	−	+	−	+	VW	VW
44	»	VW	−	−	−	−	−	−	−
45	»	+	−	−	+	−	+	−	VW
46	»	+	−	+	+	−	+	+	+
47	»	+	−	+	+	−	+	+	−
48	»	VW	−	−	−	−	+	−	−
49	»	+	−	+	+	−	+	+	−
50	»	−	−	+	+	−	−	+	−

Table 2.a — Protoplasm appearance, and some morphological characteristics of vegetative cells, and spores of Wadi El-Shatti isolates.

Culture NO	Protoplasm	Vegetative cell		Spore			Sporangium
		width (Mm)	length	location	stain	shape	
1	NV	<0.9	1.5-2.5	CT	ne	E	NS
2	NV	<0.9	2.0-3.0	C	ne	E	NS
3	NV	<0.9	1.5-2.5	C	ne	E	NS
4	NV	<0.9	1.5-2.5	CT	ne	E	NS
5	NV	<0.9	2.0-3.5	CT	ne	E	NS
6	NV	<0.9	2.0-3.5	CT	ne	E	NS
7	V	<0.9	1.5-2.5	C	e	E	S
8	V	<0.9	1.5-2.5	C	e	E	S
9	NV	<0.9	2.0-3.5	CT	ne	E	NS
10	NV	<0.9	1.5-2.5	C	e	E	S
11	NV	>0.9	2.0-5.0	T	ne	E	NS
12	NV	<0.9	1.5-2.5	T	e	E	S
13	NV	<0.9	2.5-3.5	CT	ne	E	S
14	NV	<0.9	2.0-4.0	C	e	E	NS
15	NV	<0.9	1.5-2.5	C	e	E	S
16	NV	<0.9	1.5-3.0	T	e	E	NS
17	NV	<0.9	1.5-2.5	C	e	E	NS
18	V	>0.9	2.5-6.0	CT	e	E	S
19	V	>0.9	2.5-5.0	T	ne	E	NS
20	V	>0.9	3.5-7.0	T	ne	E	NS
21	NV	<0.9	1.5-3.0	C	ne	E	S
22	NV	<0.9	1.5-3.5	C	ne	E	NS
23	V	>0.9	2.5-5.0	T	ne	E	NS
24	NV	<0.9	1.5-2.5	CT	ne	E	NS
25	NV	<0.9	2.0-3.0	CT	ne	E	NS
26	NV	<0.9	2.0-3.0	CT	ne	E	NS
27	NV	<0.9	1.5-3.5	CT	ne	E	S
28	NV	<0.9	2.5-5.0	CT	ne	E	S
29	NV	<0.9	2.5-3.5	CT	e	E	S

V, vacuolated; NV, not vacuolated; C, central; CT, central to terminal; T, terminal; ne, not easily stained; e, easily stained; E, elliptical; NS, not swollen; S, swollen.

Table 2.b — Protoplasm appearance, and some morphological characteristics of vegetative cells, and spores of Tripoli isolates.

Culture NO	Protoplasm	Vegetative cell		Spore			Sporangium
		width (Mm)	length	location	stain	shape	
30	NV	<0.9	1.0-2.5	T	ne	E	S
31	NV	<0.9	1.0-2.5	CT	e	E	NS
32	V	>0.9	1.0-2.5	T	ne	E	NS
33	V	>0.9	1.0-4.5	T	ne	E	NS
34	NV	<0.9	1.5-3.5	T	ne	E	S
35	V	>0.9	2.5-3.5	CT	ne	E	NS
36	NV	<0.9	1.0-3.0	CT	ne	E	NS
37	NV	<0.9	1.0-2.5	C	ne	E	S
38	V	>0.9	3.0-7.0	C	ne	E	NS
39	V	>0.9	3.0-7.0	CT	ne	E	NS
40	V	>0.9	2.5-3.5	CT	ne	E	NS
41	V	>0.9	1.5-3.5	CT	ne	E	NS
42	V	>0.9	2.0-4.0	CT	ne	E	NS
43	V	>0.9	2.5-3.5	CT	ne	E	NS
44	V	>0.9	1.0-5.0	CT	ne	E	NS
45	V	>0.9	2.5-3.5	CT	ne	E	NS
46	NV	<0.9	1.5-5.0	T	e	E	NS
47	NV	<0.9	1.5-2.5	CT	ne	E	S
48	V	>0.9	1.5-5.0	CT	ne	E	NS
49	NV	<0.9	1.5-3.0	C	e	E	S
50	V	>0.9	1.5-7.0	T	e	E	NS

Table 3 - Species designation and their relative dominance.

	Isolate number	Species designation	Relative dominance (%) of the total isolates
Wadi El-Shatti	1,2,5,6,9,11, 14,16,22,25 and 26	*Bacillus subtilis**	38
	7,8,10,12,13, 15,17,21,27, 28 and 29	*B. circulans**	38
	18,19, and 23	*B. lentus*	10
	3 and 4	*B. firmus*	7
	20	*B. meqaterium*	3.5
	24	*B. coagulans*	3.5

	32,33,38,39, 41,42,44,48, and 50	*B. cereus**	43
	35,40,43, and 45	*B. megaterium*	19
Tripoli			
	30,37,47, and 49	*B. circulans*	19
		B. licheniformis	14
	31,34, and 46	*B. coagulans*	5
	36		

* Dominant species.

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التوزيع والسيادة النسبية لأنواع جنس *Bacillus* في منطقتين من الجماهيرية

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المستخلص

تم الحصول على خمسين عزلة لجنس *Bacillus* في التربة من منطقتين في الجماهيرية هما طرابلس ووادي الشاطىء يمثلان المنطقة الشمالية والمنطقة الجنوبية على التوالي. من التجارب المعملية التي أجريت على العزلات للتعرف والتصنيف تم حصر المجموعة المعزولة في ثمانية أنواع، هي:

B. subtilis, B. cereus, B. megaterium, B. coagulans, B. licheniformis, B. circulans, B. lentus, B. firmus

لقد وجد أن السيادة بين الأنواع المصنفة في تربة المنطقة الشمالية تقتصر على نوع واحد هو *B. cereus*، بينما كانت السيادة في تربة المنطقة الجنوبية مشتركة بين نوعين هما *B. circulans, B. subtilis*.