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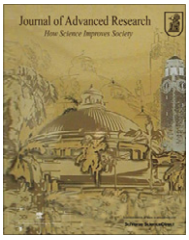

ORIGINAL ARTICLE

Diversity of bacteria nesting the plant cover of north Sinai deserts, Egypt

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Rhizosheath

Abstract North Sinai deserts were surveyed for the predominant plant cover and for the culturable bacteria nesting their roots and shoots. Among 43 plant species reported, 13 are perennial (e.g. *Fagonia* spp., *Pancratium* spp.) and 30 annuals (e.g. *Bromus* spp., *Erodium* spp.). Eleven species possessed rhizo-sheath, e.g. *Cyperus capitatus*, *Panicum turgidum* and *Trisetaria koelerioides*. Microbiological analyses demonstrated: the great diversity and richness of associated culturable bacteria, in particular nitrogen-fixing bacteria (diazotrophs); the majority of bacterial residents were of true and/or putative diazotrophic nature; the bacterial populations followed an increasing density gradient towards the root surfaces; sizeable populations were able to reside inside the root (endorhizosphere) and shoot (endophyllosphere) tissues. Three hundred bacterial isolates were secured from studied spheres. The majority of nitrogen-fixing bacilli isolates belonged to *Bacillus megaterium, Bacillus pumilus*, *Bacillus polymexa, Bacillus macerans, Bacillus circulans* and *Bacillus licheniformis*. The family Enterobacteriaceae represented by *Enterobacter agglomerans, Enterobacter sackazakii*, *Enterobacter cloacae*, *Serratia adorifera, Serratia liquefaciens* and *Klebsiella oxytoca*. The non-Enterobacteriaceae population was rich in *Pantoae* spp., *Agrobacterium rdiobacter*, *Pseudomonas vesicularis*, *Pseudomonas putida*, *Stenotrophomonas maltophilia*, *Ochrobactrum anthropi*, *Sphingomonas paucimobilis* and *Chrysemonas luteola*. *Gluconacetobacter diazotrophicus* were reported inside root and shoot tissues of a number of tested plants. The dense bacterial populations

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reported speak well to the very possible significant role played by the endophytic bacterial populations in the survival, in respect of nutrition and health, of existing plants. Such groups of diazotrophs are good candidates, as bio-preparates, to support the growth of future field crops grown in deserts of north Sinai and irrigated by the water of El-Salam canal.

Introduction

The semi-arid deserts of north Sinai represent a very important agricultural extension to the Nile Valley. Governmental plans are underway to develop agriculture productivity, especially through the mega project of El-Salam (Peace) canal. The canal brings Nile water, mixed with the Delta drainage water (1:1, v/v), to reclaim 150,000 ha. This long-term planning project is confronted with a number of ecological concerns, in respect of upsetting the long-established biodiversity of flora and microflora, and possible erosion and salination of soils. Therefore, and since 1995, the microbe–plant–soil systems of north Sinai are under investigations through a number of successive research projects. As a result, the existing microflora–flora interactions were documented in a number of publications [1–3]. Special attention was given to prevailing N_2-fixers (diazotrophs) and future manipulation of their representatives as biofertilizers [4,5]. In addition, efforts were devoted to specific plant–microbe models of ecological importance, e.g. fixing sand dunes and inhabiting salt-affected areas. In this respect, Othman et al. [3] demonstrated the richness of the plant–soil system with various groups of rhizospheric microorganisms (RMOs). They also drew the attention towards a potential group of plants possessing sand sheath encasing roots of plants, a phenomenon that was actually reported years ago [6]. It appeared that the rhizosheath in itself acts as additional compartments under the effect of plant roots, being chemically and physically enriched and subsequently nourishing functional populations of microorganisms [1]. In particular, it is reported to be a potential repository for the nitrogen fixing bacteria [7]. Aware of the ecological and economical importance of associated microflora, it was of rather interest to further explore the flora of north Sinai for rhizospheric microorganisms (RMOs), nesting the interior of roots (endorhizosphere) and shoots (endophyllosphere), as well as the unique root adjacent compartment known as rhizosheath. Special efforts are given to the prevailing groups of nitrogen-fixing (diazotrophs) community prevailing under the extremely harsh and variable environmental semi-arid conditions of north Sinai deserts.

Material and methods

Experimental sites

The studied region extends 160-km eastwards of the Suez Canal into north Sinai, from Rummanah (30°58′35.94″N-32°45′35.94″E) to Wadi El-Arish (30°43′49.80″N-34°25′10.68″E). Based on the records of the regional meteorological station of El-Arish, the climatic data of the studied areas is outlined in Table 1. The summer months (July and August) are the hottest, and the mean temperature was highest in August (32.9 °C) and lowest in January (8.0 °C). Very narrow variation in relative humidity is reported throughout the whole year, ranged from 70% in April to 76.0% in August. The total mean of annual rainfall was 157.11 mm during the period 1995 to 2005. The wind velocity reached its mean maximum (10.0 knot) in January and minimum (4.0 knot) in May till October.

The study covers three potential areas Fig. 1. The first area is ''Rummanah-Bir El Abd'' characterized by an open plain of gravely desert having scanty quantities of rainfall with very few inland salines. Seven plant samples were collected from three sites. The second area is ''Rafah-El Arish'' coastal area with scattered semi-stable dunes and coastal salines to the north. A number of 13 plant samples were obtained representing four sites. ''Wadi (Valley) El-Arish'' is representing the third area with 23 plant samples. It covers a virtual triangular with sides of ca. 29 km, 39 km and base of 40 km, and respective apices at Bir Lahfan, Abu Ujaylah and Gebel (heights) Libni. The area contains stable and semi-stable sandy fields, supported with relatively higher amounts of rainfall (ca. 100 mm/year) and low soil salinity that permits agricultural activities. The environmental conditions prevailing in the studied areas are presented in Table 1.

Sampling of flora

Sinai lies in the semi-arid regions of the world. Its natural flora is mainly xerophytes and dominated by Mediterranean elements; in addition to Saharo-Arabian and Irano-Turanian elements in the second position. Plants were sampled during their optimum growth in the rainy seasons (October–May) of 2004 and 2005, and identified at Cairo University Herbarium (CAI) based on the authentic herbarium specimens and available literature [8–11]. Each plant sample is a composite of at least three plants exists in the sampling site. The identified specimens were deposited as herbarium specimens in the ''Research Center for Agro-biotechnologies, Faculty of Agriculture, Cairo University'', Rafah, north Sinai.

Sampling of plant–soil systems

Bacteria closely associated to the surface layers of root tissues (named as rhizoplane or tentatively endorhizosphere) and shoots (endophyllosphere) of various plant–soil systems were examined for total culturable populations of bacteria and associated nitrogen-fixing bacteria (diazotrophs). Phyllosphere samples were obtained by first insertion and separation of the vegetation part of plant into plastic bags. Then, the root system (intact roots with closely-adhering soil) was removed and transferred to plastic bags. All samples were kept in a cold bow and brought within 24 h to the laboratory. Samples were kept in the refrigerator until analyses within 72 h of sampling.

Table 1 Metrological data of north Sinai based on recordings of El-Arish regional station 2003–2005.[a]

Item	January	February	March	April	May	June	July	August	September	October.	November	December	Mean
Mean air temp (°C)	13.9	14.5	20.1	18.5	21.5	23.9	26.0	26.5	25.2	23.3	19.9	15.8	20.7
Mean RH%	70.0	69.0	67.0	67.0	68.0	72.0	74.0	75.0	71.0	73.0	71.0	66.0	70.3
Mean wind speed (m/sec)	4.7	5.5	5.7	4.8	4.6	4.5	4.3	4.0	4.1	3.5	3.9	4.6	4.5
Sun shine duration (h)	6.2	6.0	7.1	7.9	9.8	11.9	11.4	10.5	8.8	7.7	6.9	6.0	8.4
Net solar radiation (Mj/m^2/day)	11.2	13.1	17.2	20.4	24.5	27.9	26.9	24.5	20.1	15.9	12.4	10.7	18.7
Rain (mm/month)	20.3	17.1	12.0	6.1	3.2	0.0	0.0	0.2	0.6	6.0	16.2	22.2	8.7
ETO (mm/day)	1.9	2.4	3.2	3.8	4.7	5.5	5.5	5.2	4.4	3.2	2.5	2.2	3.7

[a] Central Laboratory for Agricultural Climate (CLAC 2006). Annual Climatic Book. Pp. 21. Ministry of agriculture, Dokki, Giza, Egypt.

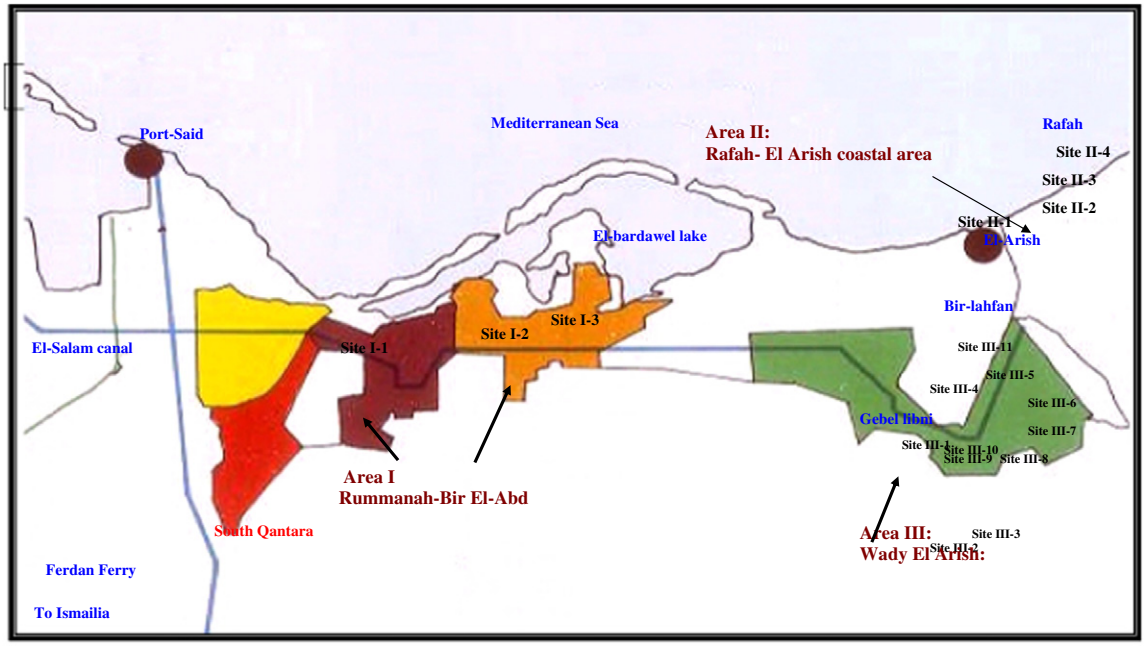


Fig. 1 Map illustrating areas and sites sampled in north Sinai based on GPS data obtained. Sites I-1 through 3, Rummanah-Bir El Abd area I: Bir al Rummanah 30°58′35.94″N-32°45′35.94″E; Bir al Abd 31° 1′35.94″N-33° 4′35.95″E; Bir al Abd 31° 2′35.94″N-33° 7′35.94″E; **sites II-1 through 4, Rafah-El Arish coastal area II:** Al Arish 31° 8′24.00″N-33°52′43.20″E; Rafah 31°17′6.00″N-34°13′12.00″E; Rafah 31°17′41.94″ N-34°12′3.00″E; Rafah 31°18′6.00″N-34°12′54.00″E. **sites III-1 through 11, Wady El Arish area III**: Wadi al Arish 30°41′3.84″ N-33°47′59.40″E; Wadi al Arish30°41′51.96″N-33°49′58.80″E; Wadi al Arish 30°47′35.76″N-33°58′7.80″E; Bir lahfan 30°54′17.28″ N-33°50′43.20″E; Wadi al Amr 30°59′21.60″N-34°14′56.94″E; Ayn al Qusaymah 30°43′49.80″N-34°25′10.68″E; Ayn l Qusaymah 30°40′49.80″N-34°21′10.68″E; Wadi al Arish 30°29′43.32″N-34° 7′50.40″E; Wadi al Arish 30°30′48.00″N-34°10′36.00″E; Wadi al Arish 30°55′35.94″N-34° 1′35.94″E; Wadi al Arish 30°57′40.20″N-33°58′35.98″E.

Preparation of samples for microbial analyses

Surface sterilization for either roots or shoots was carried [12], the intact shoot or root was carefully washed with tap water, treated with 95% ethanol for 30 s followed by 3% sodium hypochlorite for 30 min, then thoroughly washed five times with sterile distilled water. Sterility check was carried out by placing segments of sterilized plant materials on the surface of prepared nutrient agar plates. Finally, the plant materials were triturated for 5 min in Warring blender using sufficient amount of half strength basal salts of the N-deficient combined carbon sources medium (CCM) liquid medium [13] as a diluent. Further serial dilutions were prepared, using the same diluent, for enumerating bacterial groups in the roots and shoots.

Roots with encasing sand sheath were divided into sub-samples prepared for: (a) the loose free sand; (b) the encasing compact sand of the rhizosheath (sand sheath); (c) roots carefully deprived of their sand load by sterile forcipes (naked root/rhizoplane) and (d) surface-sterilized roots (endorhizosphere) using ethanol and sodium hypochlorite [12]. For each sub-sample, enough soil and/or plant material were used to prepare the first dilution in 100 ml glass bottles containing 45 ml diluent (the basal salt of CCM medium), shaked (150 rpm) for 60 min, then further serial dilutions were prepared for culturing representative groups of bacteria.

Bacteriological determinations

Suitable dilutions of prepared samples, three replicates for each plant sphere, were analyzed for total culturable bacteria using the nutrient agar and the pour plate method [14]. Diazotrophs were cultured using the surface-inoculated plates and the N-deficient combined carbon sources medium (CCM)

Table 2 Perennial and annual plants reported and sampled in the studied areas of north Sinai during the seasons 2004 and 2005.

No.	Host plant	Family	Area-site [a]	Season
	Perennial			
1	*Cyperus laevigatus* L[b]	Cyperaceae	I Site 2	2005
2	*Pancratium maritimum* L	Amaryllidaceae	II Site 3	2005
3	*Thymelaea hirsuta* (L.) Endl	Thymeliaceae	II Site 1	2005
4	*Astragalaus kahiricus* DC	Fabaceae	III Site 5	2004
5	*Cornulaca monacantha* Delile	Chenopodiaceae	III Site 3	2004
6	*Fagonia arabica* L	Zygophyllaceae	III Site 1	2004
7	*Fagonia mollis* (Labill.) H.L. Wendl	Zygophyllaceae	III Site 1	2004
8	*Haloxylon salicornicum* (Moq.) Bunge ex Boiss	Chenopodiaceae	III Site 1	2004
9	*Heliotropium dignum* (Forssk.) C. Chr	Boraginaceae	III Site 3	2004
10	*Panicum turgidum* Forssk[b]	Poaceae	III Site 4	2004
11	*Stipagrostis scoparia* (Trin. & Rupr.) de Winter [b]	Poaceae	III Site 2	2004
12	*Zilla spinosa* (L.) Prantl	Brassicaceae	III Site 8	2004
13	*Zygophyllum album* L. var. amblyocarpum (Baker.) Hadidi	Zygophyllaceae	III Site 3	2004
	Annual			
14	*Centaurea pallescens* Delile	Asteraceae	I Site 1	2005
15	*Chenopodium murale* L	Chenopodiaceae	I Site 1	2005
16	*Launaea capitata* (Spreng.) Dandy	Asteraceae	I Site 1	2005
17	*Polycarpaea repens* (Forssk.) Asch. & Schweinf	Caryophyllaceae	I Site 3	2005
18	*Silene succulenta* Forssk	Caryophyllaceae	I Site 3	2005
19	*Trachynia distachya* (L.) Link = *Brachypodium distachyum* (L.) P. Beauv [b]	Poaceae	I Site 1	2005
20	*Anchusa humilis* (Desf.) I.M. Johnst	Boraginaceae	II Site 4	2005
21	*Bromus madritensis* L[b]	Poaceae	II Site 2	2004
22	*Bromus scoparius* L[b]	Poaceae	II Site 2	2004
23	*Erodium crassifolium* L' Hér	Geraniaceae	II Site 4	2005
24	*Iflago spicata* (Forssk.) Sch. Bip	Asteraceae	II Site 1	2005
25	*Malva parviflora* L	Malvaceae	II Site 4	2005
26	*Phalaris minor* Retz	Poaceae	II Site 4	2005
27	*Polycarpon succulentum* (Delile) J. Gay	Caryophyllaceae	II Site 4	2005
28	*Pseudorlaya pumila* (L.) Grande	Apiaceae	II Site 1	2005
29	*Senecio glaucus* L. subsp. *coronopifolius* (Maire) C. Alexander	Asteraceae	II Site 4	2005
30	*Trisetaria koelerioides* (Bornem and Hackel) Meldris[b]	Poaceae	II Site 4	2005
31	*Asphodelus tenuifolius* Cav	Liliaceae	III Site 9	2005
32	*Cotula cinerea* Delile	Asteraceae	III Site 10	2005
33	*Cutandia memphatica* (Spreng.) K. Richt[b]	Poaceae	III Site 2	2004
34	*Cyperus capitatus* Vand[b]	Cyperaceae	III Site 2	2004
35	*Eremobium aegyptiacum* (Spreng.) Asch. & Schwienf. var. aegyptiacum	Brassicaceae	III Site 11	2005
36	*Erodium oxyrhynchum* M. Bieb	Geraniaceae	III Site 4	2004
37	*Euphorbia retusa* Forssk	Euphorbiaceae	III Site 9	2005
38	*Hordeum murinum* L [b]	Poaceae	III Site 6	2004
39	*Lolium perenne* L.[b]	Poaceae	III Site 7	2004
40	*Neurada procumbens* L	Neuradaceae	III Site 3	2004
41	*Oligomeris linifolia* (Hornem.) J.F. Macbr	Resedaceae	III Site 10	2005
42	*Svignya parviflora (Delile.) Webb*	Brassicaceae	III Site 4	2004
43	*Trigonella stellata* Forssk	Leguminosae	III Site 9	2005

[a] For detailed information on sites, please refer to the detailed map (Fig. 1); I, II and III are the major three studied areas; 1, 2–11 are the number of sites within each area.

[b] Plants possessed sand sheath and subjected to further microbial analyses.

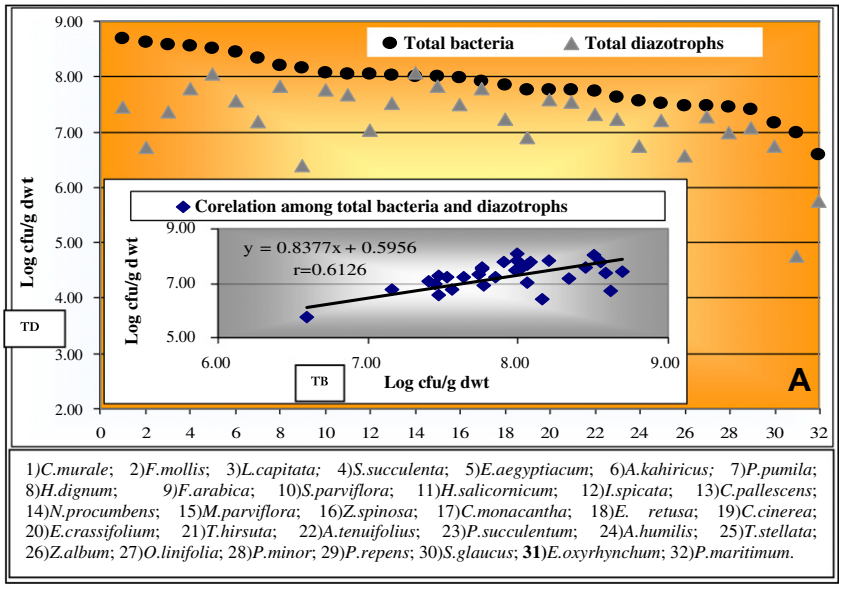
[13]. Incubation took place at 30 °C, and the developed c.f.u were counted during 2–7 days of incubation [1,2].

The *Gluconacetobacter*-like populations were enumerated using the most probable number (MPN) and the semi-solid N-deficient LGI culture medium [12,15]. For each suitable dilution, 1 ml aliquots were transferred to five tubes containing 5 ml of semi-solid LGI medium, incubated at 30 °C for 7 days. MPN estimates were derived using tables of Meynell and Meynell [16].

For the culturable spore-forming populations, just prior to plating, suitable dilutions were pasteurized at 80 °C for 15 min. In general, bacterial populations were calculated on dry matter (105 °C for soils and 75 °C for plant materials) basis.

Isolation, purification and identification of representative isolates of diazotrophs

Representative colonies developed on CCM agar plates were selected for single colony isolation. In addition, sets of semi-solid CCM medium inoculated with 0.5 ml aliquots of suitable dilutions were also prepared, incubated for 48–72 h. at 30 °C. Acetylene reducing activity [17] was measured for tubes exhibiting good growth, and cultures produced more than 5 nmoles C_2H_4 culture^{-1} h^{-1} were considered positive, streaked on CCM agar plates and incubated for 48–72 h. at 30 °C. For further purification of all selected isolates, single colony isolation was performed on agar plates of CCM. Pure



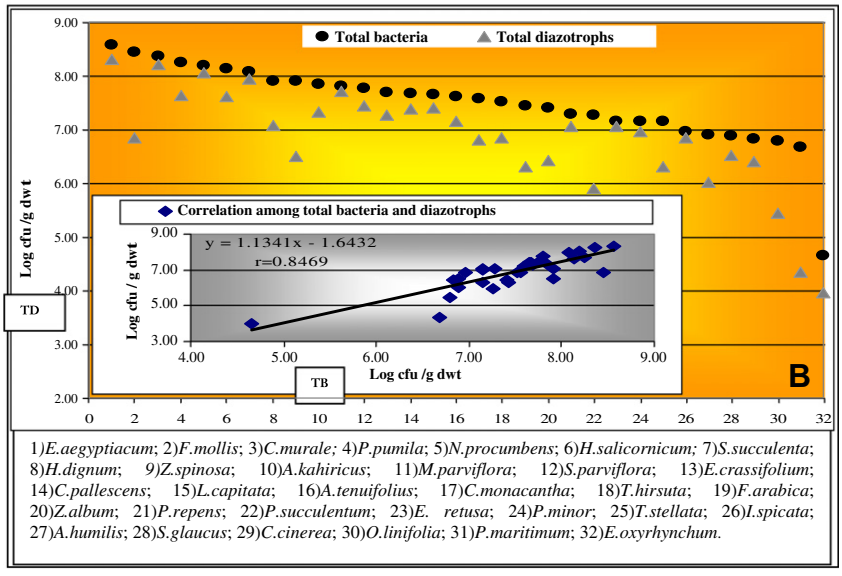


Fig. 2 Ranking of total culturable endophytic total bacteria (TB) and total diazotrophs (TD)in roots (endorhizosphere, A) and shoots (endophyllosphere, B) of sampled plants during the seasons 2004/2005. Inserted are the calculated correlation coefficients and linear regression among either populations.

isolates were re-examined for acetylene-reducing activity, colony morphology and cell characteristics according to Bergey's Manual of Systematic Bacteriology [18]. Representative isolates were also examined for growth and cultural characteristics based on API microtube systems gallery [19]; API 20E for Enterobacteriaceae; API 20 NE for non-Enterobacteriaceae and API 50CHB for bacilli.

For *Gluconacetobacter*-like diazotrophs, the MPN tubes of LGI medium showing typical dark-orange surface pellicle and clear colorless medium below were considered positive. Representative isolates were obtained by single-colony isolation on agar plates of the same medium. After 7–10 days, pure orange colonies were transferred into LGIP medium. For more purification, isolates were streaked on potato agar [15], modified LGIP medium [20] and glucose–yeast–$CaCO_3$ (GYC) [21,15] agar plates. Pure isolates were re-examined for acetylene reducing activity, colony morphology and cell characteristics and identified according to Bergey's Manual of Systematic Bacteriology [18]. The API microtube systems 20E and 20NE were further used as a standardized micro-method [19]. The *Gluconacetobacter diazotrophicus* type culture (ATCC 49037) was used as a reference strain.

Culture media

Nutrient agar [14]: It contains ($g\,l^{-1}$): beef extract, 3.0; peptone, 5.0; glucose, 1.0; yeast extract, 0.5; agar, 15; pH, 7.2.

N-deficient combined carbon sources medium, CCM [13]: It comprises of ($g\,l^{-1}$): glucose, 2.0; malic acid, 2.0; mannitol, 2.0; sucrose, 1.0; K_2HPO_4, 0.4; KH_2PO_4, 0.6; $MgSO_4$, 0.2; NaCl,

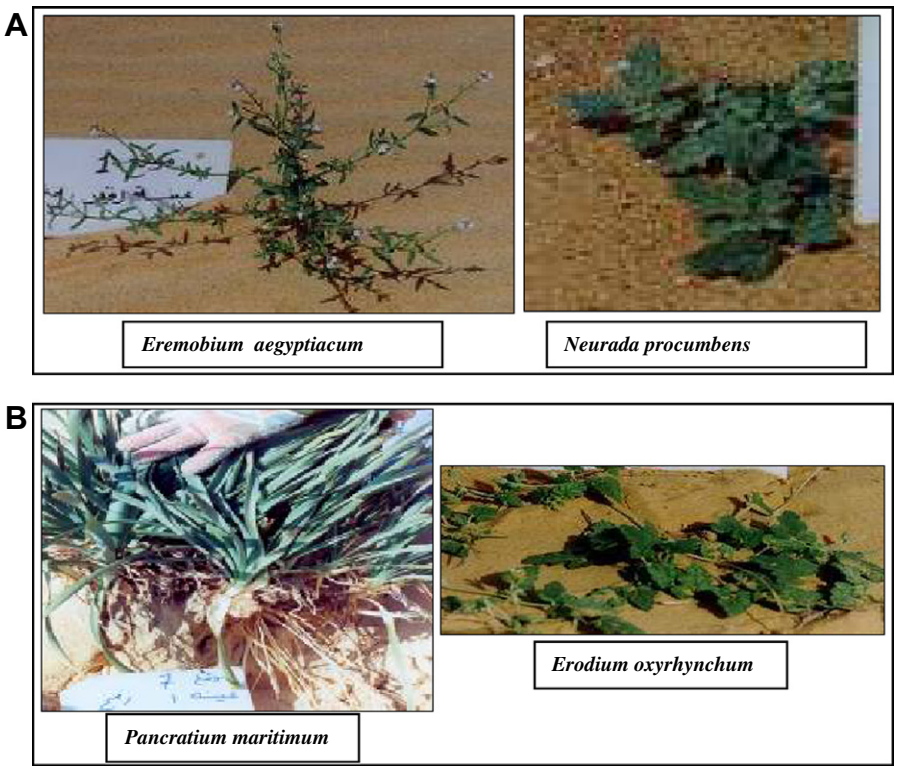


Fig. 3 Representatives of the richest (A) and the poorest (B) north Sinai plant cover in respect of endophytic culturable populations.

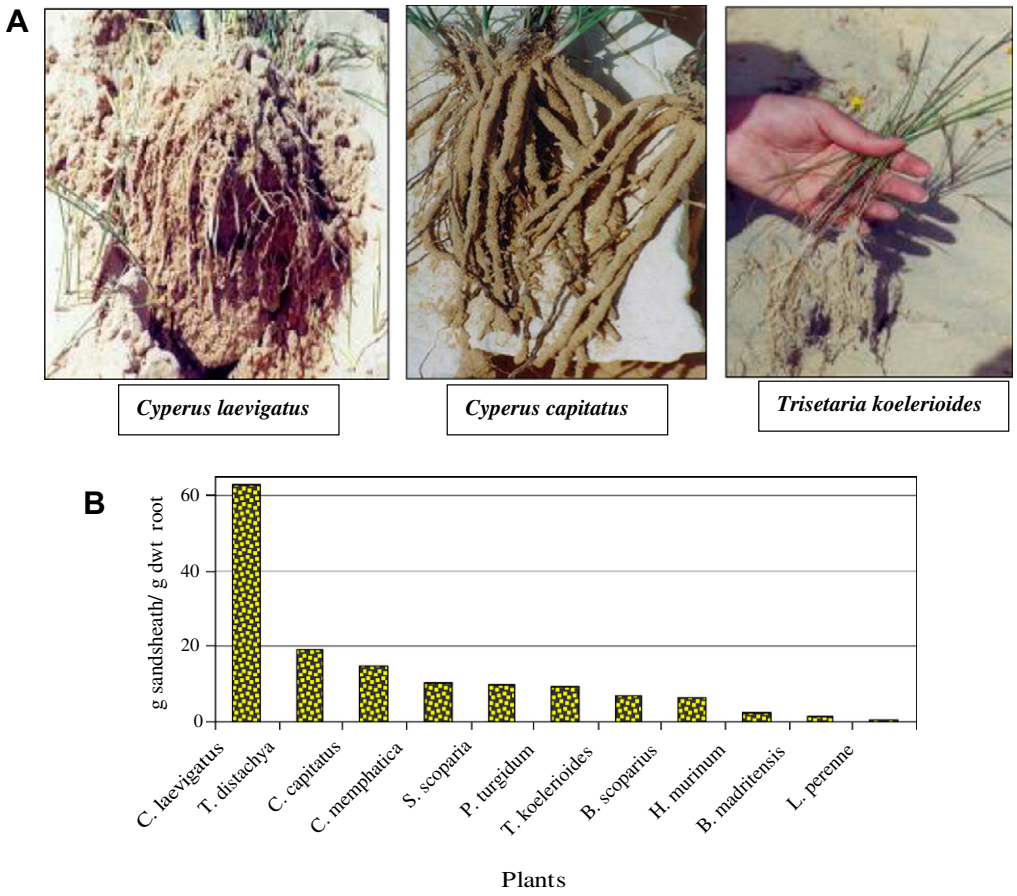


Fig. 4 Representatives of sand-sheathed plants (A) and the specific sand load (g sand g^{-1} root) on their roots (B).

0.1; $CuSO_4$, 0.08 mg; $ZnSO_4$, 0.25 mg; $MnSO_4$, 0.01; yeast extract, 0.2; fermentol (a local product of corn-steep liquor), 0.2; KOH, 1.5; $CaCl_2$, 0.02; $FeCl_3$, 0.015; Na_2 MoO_4, 0.002. Sodium lactate was included as 0.6 ml (50% v/v).

LGI medium [15]: It contains (g l^{-1}): K_2HPO_4, 0.2; KH_2PO_4, 0.6; $MgSO_4{\cdot}7H_2O$, 0.2; $CaCl_2{\cdot}2H_2O$, 0.02; $Na_2MoO_4{\cdot}H_2O$, 0.002; $FeCl_3{\cdot}H_2O$, 0.01; bromothymol blue 0.5% solution in 0.2 N KOH, 5 ml; agar, 1.8; crystallized cane sugar, 100; PH, 6.0.

Modified LGIP medium [20]: It contained per liter: 0.02 g of $Na_2MoO_4{\cdot}2H_2O$, 0.1 mg of biotin, 0.2 mg of pyridoxal HCl l and 5 ml of sugarcane juice (pressed from fresh sugarcane stem). The final pH was adjusted to 5.5 using 1% acetic acid. For single colony isolation, diluted cells were spread on solid LGIP agar medium (15 g of agar per liter plus 50 mg of yeast extract per liter).

Potato agar [15]: It comprises of (l^{-1}): potato extract 200 ml; sucrose 100 g, agar 15 g. Glucose yeast extract $CaCO_3$, GYC [21,15]: It contains (g l^{-1}): glucose, 100; yeast extract, 10; $CaCO_3$, 20; agar, 15; distilled water, 1000; pH 6.8.

Statistical analysis

Data obtained were statistically analyzed using STATISTICA 6.0 (StatSoft, Inc., Tulsa, USA). Analysis of variance (ANOVA) was used to examine the independent effects as well as possible interactions. Correlation coefficient and linear regression were also computed.

Results

Diversity of total cultlurable bacteria and diazotrophs in the endorhizosphere and endophyllosphere of tested plants

The studied region is extending eastward from Rummanah-Bir El Abd to Wadi (Valley) El-Arish Fig. 1. Sampling was carried out during the rainy seasons of 2004 and 2005. Forty-three species, 30 annuals and 13 perennials, were collected and showed the highest dominance and frequency as well as adaptation to north Sinai environment. Based on the data collected at El-Arish metrological station during the period 2003/2007 Table 1, it is documented that the environmental conditions are extremely harsh and variable, being reflected on the vegetation and associated microflora. Under such environment, it was of rather interest to report on the diversity of culturable bacteria nesting the naked surfaces and their lining tissues of plant roots and shoots, tentatively referred to in this study as endorhizosphere and endophyllosphere respectively.

Table 2 summarizes the botanical status of plants sampled throughout the study.

The endorhizospheric and phyllospheric populations of total culturable bacteria and diazotrophs are reported and ranked in Fig. 2. Majority of plant roots and shoots (96%) were nested with populations ranged from 10^6 to 10^8 cfu g $^{-1}$ dwt of endorhizosphere and phyllosphere. The plant species *Eremobium aegyptiacum, Neurada procumbens, Fagonia mollis*, *Chenopodium murale*, *Pseudorlaya pumila*, *Haloxylon salicornicum* and *Silene succulenta* were particularly the richest in associated endophytic microflora compared to *Erodium oxyrhynchum* and *Panicum maritimum* Fig. 3.

Total cullturable diazotrophs, nitrogen-fixing bacteria, did positively correlate with the total bacterial populations Fig. 2. Their populations in roots and shoots of majority of plants were in the range of $>10^6$–10^8 cfu g^{-1} dwt. For the endorhizosphere, *E. aegyptiacum* and *N. procumbens* were top ranked Fig. 3a compared to *P. maritimum* and *E. oxyrhynchum* the very poorest Fig. 3b. The wealthiest plants in endophyllosphere ($>10^8$ cfu g^{-1}) were *E. aegyptiacum*, *C. murale* and *N. procumbens*. Four plants supported populations less than 10^6 cfu g^{-1} dwt, with *E. oxyrhynchum* being the poorest.

The study areas were inhabited with 11 plants characterized by having a sand sheath closely adhering to the plant root Table 2. The specific sand load (g sand/g dwt root) did vary among plants, being extremely thick (62 g) for *Cypreus laevigatus*, because of its intensive root biomass and network, and very thin (0.7 g) for *Lolium perenne* Fig. 4. Besides the free sand, the successive root spheres of sand sheath, rhizoplane and endorhizosphere were analyzed for their microbial load of total culturable bacteria, diazotrophs, total sporeformers and spore-forming diazotrophs. ANOVA analysis indicated the significant independent effects of plant type, sphere and microbial groups tested Fig. 5. Among plants, the poorest in total culturable microbial communities were *Trisetaria koelerides, Stipagrostis scoparia and C. laevigatus*, being statistically inferior to the remaining eight plants among which differences were not significant except for *B. madritensis*, the richest of all Fig. 5. As to spheres, the free sand was statistically the poorest and rhizoplane the highest. Of interest is that the microbial load differences among sand sheath and rhizoplane of all tested plants were insignificant. It appears that the microbial communities in the root spheres were active and mobile in order to migrate and/or invade the root interiors (endorhizosphere) with substantial populations ($\geqslant 10^5$ cfu g^{-1} dwt). Differences among culturable bacterial groups were significant, following the descending order of total bacteria, total diazotrophs, total spore-forming bacteria and spore-forming diazotrophs.

The various combinations of 2-way interactions are illustrated in Fig. 5B. The total culturable bacteria ranged from 10^5 to 10^9 cfu g^{-1} dwt, significantly enriched in the root region, being highest on the rhizoplane followed by sand sheath, being lowest in the free sand Fig. 5B3. The total culturable diazotrophs followed a similar trend, and were found abundant in the root spheres, representing more than 70% of the total population. The interaction between plants and bacterial groups Fig. 5B1, again indicated the statistical inferiority of *S. scoparia*, *C. laevigatus* and *T. koelerides*, together with the descending order of total bacteria, total diazotrophs, total spore formers, and spore-forming diazotrops. Irrespective of bacterial groups Fig. 5B2, the tested microbial communities were highest in the rhizoplane and sand sheath, with insignificant differences among them, compared to the free sand. The above conclusions were further confirmed by 3-way interaction.

The spore-forming bacteria, either diazotrophic or not, did occupy a significant niche, with populations ranged from $>10^3$ to 10^6 cfu g^{-1} dwt; representing 50–85% of the microbial population Fig. 5 B. Compared to the free sand (10^3–10^5 cfu g $^{-1}$ dwt), the sand sheath and the root surfaces (rhizoplane) harbored higher populations (10^6 to 10^7 cfu g^{-1} dwt) reported for 8 out of 11 tested rhizosheathed plants. The spore-forming bacteria were able to taxi and nest the interiors of plant roots (endorhizosphere) with substantial populations of $>10^3$ to 10^5 cfu g^{-1} dwt, representing 50–97% of total endophytic bacterial community.

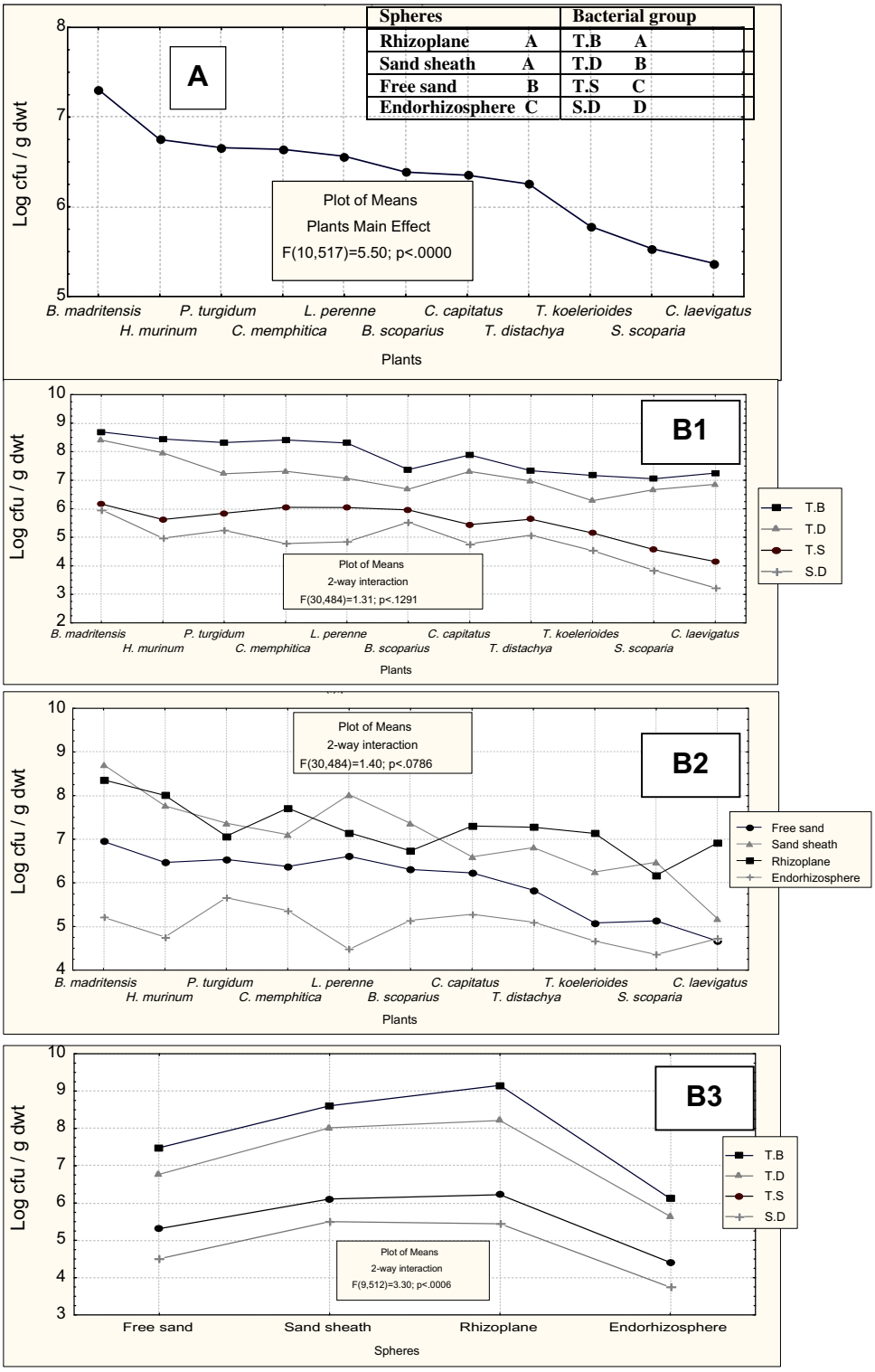


Fig. 5 Total culturable bacteria and diazotrophs reported for rhizo-sheathed plants. (A) The independent effect of plants; the inserted table demonstrates the effect of both spheres and culturable bacterial groups reported by ANOVA analyses. (B) The Two-way interactions computed during ANOVA analysis: B1, Plants and bacterial groups; B2, Plants and root spheres; B3, Spheres and bacterial groups. (T.B., Total Bacteria; T.D., Total Diazotrophs; T.S., Total Spore-formers; S.D., Spore-forming Diazotrophs).

Endophytic nitrogen-fixing isolates reported

Special attention was given to the nitrogen-fixing pure isolates nested the roots and shoots of xerophytic plants. Forty-one pure isolates were secured and subjected to taxonomic analyses. The spore-forming diazotrophs were predominant and well represented by the genus *Bacillus* (23 isolates), particularly the species *Bacillus megaterium* (14), *Bacillus pumilus* (4),

Table 3 Taxonomic position of endophytic spore-forming isolates of diazotrophs obtained from roots and shoots of tested xerophytes (based on API 50CHB).

Host plant	Area	Isolate code	Sphere	N_2-ase activity (nmoles C_2H_4 h^{-1} 5 ml $culture^{-1}$)	Proposed position	Identification
C. pallescens	I	B 1/B/48	Root	>41.88	*B. megaterium*	Excellent
L. capitata	I	B 15/B/48	Root	31.41	*B. macerans*	Good
S. succulenta	I	B 36/B/48	Root	>41.88	*B. pumilus*	V. good
T. distachya[a]	I	B 18/B/48	Sand sheath	12.26	*B. polymyxa*	V. good
T. distachya[a]	I	B 17/B/48	Root (endorhizosphere)	>41.88	*B. megaterium*	Excellent
I. spicata	II	B 45/B/48	Root	17.95	*B. megaterium*	V. good
I. spicata	II	B 46/B/48	Root	23.03	*B. megaterium*	Excellent
M. parviflora	II	B 116/B/48	Root	14.96	*B. megaterium*	Excellent
B. scoparius[a]	II	B 142/B/48	Root (endorhizosphere)	22.44	*B. megaterium*	V. good
T. koelerioide[a]s	II	B 5/B/48	Sand sheath	6.58	*B. circulans*	Excellent
M. parviflora	II	B 117/B/48	Shoot	17.95	*B. polymyxa*	Good
A. tenuifolius	III	B 60/B/48	Root	26.33	*B. pumilus*	V. good
C. cinerea	III	B 87/B/48	Root	28.42	*B. megaterium*	Good
C. cinerea	III	B 89/B/48	Root	41.29	*B. megaterium*	Good
Z. spinosa	III	B 145/B/48	Root	>41.88	*B. licheniformis*	V. good
H. salicornicum	III	B 168/B/48	Root	25.13	*B. megaterium*	Excellent
H. murinum[a]	III	B 129/B/48	Root (endorhizosphere)	14.96	*B. megaterium*	V. good
C. capitatus[a]	III	B 165/B/48	Root (endorhizosphere)	>41.88	*B. megaterium*	V. good
A. tenuifolius	III	B 61/B/48	Shoot	6.58	*B. pumilus*	V. good
E. retusa	III	B 65/B/48	Shoot	19.44	*B. megaterium*	Excellent
E. aegyptiacum	III	B 71/B/48	Shoot	>41.88	*B. megaterium*	Excellent
O. linifolia	III	B 79/B/48	Shoot	19.44	*B. megaterium*	V. good
Z. spinosa	III	B 144/B/48	Shoot	>41.88	*B. pumilus*	Good

[a] Rhizo-sheathed plants.

Table 4 Taxonomic position of endophytic non-spore-forming isolates of diazotrophs obtained from roots and shoots of tested xerophytes (based on API 20E and 20NE).

Host plant	Area	Isolate code	Sphere	N_2-ase activity (nmoles C_2H_4 h^{-1} 5 ml $culture^{-1}$)	Proposed position	Identification
S. succulenta	I	S 39/NE/24	Root	31.14	*Sphingomonas paucimobilis*	V. good
P. pumila	II	E 53/E/48	Root	>41.88	*Enterobacter agglomerance*	Excellent
P. pumila	II	B 50/NE/24	Root	13.46	*Brevundimonas* (*Pseudomonas*) *vesicularis*	Good
P. maritimum	II	O 94/NE/24	Root	not determined	*Ochrobactrum anthropi*	V. good
P. maritimum	II	E 91/E/24	Root	27.52	*Enterobacter cloacae*	Good
P. maritimum	II	E 92/E/48	Root	22.44	*Enterobacter sakazaki*	V. good
M. parviflora	II	C 115/NE/24	Shoot	29.92	*Chrysemonas luteola*	Good
S. glaucus	II	A 28/NE/24	Shoot	>41.88	*Agrobacterium radiobacter*	Excellent
E. aegyptiacum	III	K 78/E/48	Root	29.92	*Klebsiella oxytoca*	Good
F. Arabica	III	S 155/E/24	Root	28.42	*Serratia adorifera*	Good
F. Arabica	III	S 156/E/24	Root	>41.88	*Serratia adorifera*	Good
A. tenuifolius	III	B 58/NE/24	Root	26.92	*Brevundimonas* (*Pseudomonas*) *vesicularis*	Good
H. murinum	III	E 123/E/24	Root	14.96	*Enterobacter agglomerance*	Good
H. murinum	III	P 131/NE/48	Root	35.9	*Pseudomonas putida*	V. good
Z. album	III	S 147/NE/24	Root	>41.88	*Stenotrophomonas maltophilia* (Xantho. maltophilia)	Excellent
Z. album	III	S 148/E/24	Root	17.95	*Serratia liquefaciens*	V. good
E. oxyrhynchum	III	A 138/NE/24	Shoot	29.32	*Agrobacterium radiobacter*	Excellent
H. salicornicum	III	A 170/NE/48	Shoot	>41.88	*Agrobacterium radiobacter*	Excellent

Bacillus polymyxa (2), *Bacillus macerans* (1), *Bacillus licheniformis* (1) and *Bacillus circulans* (1) Table 3.

The non-sporing population was represented by 18 isolates. They belonged to the genera *Enterobacter* spp. (*E. cloacae, E. agglomerance, E. sakazaki*), *Serratia* spp. (*S. adorifera, S. liquefaciens*), *Agrobacterium* spp. (*A. radiobacter*), *Klebsiella* spp. (*K. oxytoca*), *Pseudomona spp./Brevundimonas* spp. (*P. vesicularis, P. putida*), *Chrysemonas* spp. (*C. luteola*), *Stenotrophomonas* spp. (*S. maltophilia*), *Ochrobactrum* spp. (*O. anthropi*) and *Sphingomonas* spp. (*S. paucimobilis*) Table 4.

Both spore- and non-spore forming diazotrophs were present endophytically in roots or in the shoots of plants, but one *B. circulans* and one *B. polymyxa* were found in sand sheath layers Table 3. In general, the specific load of spore-forming community in the sand sheath differed among tested plants. Five plants, belonged to Gramineae (Poaceae), harbored in

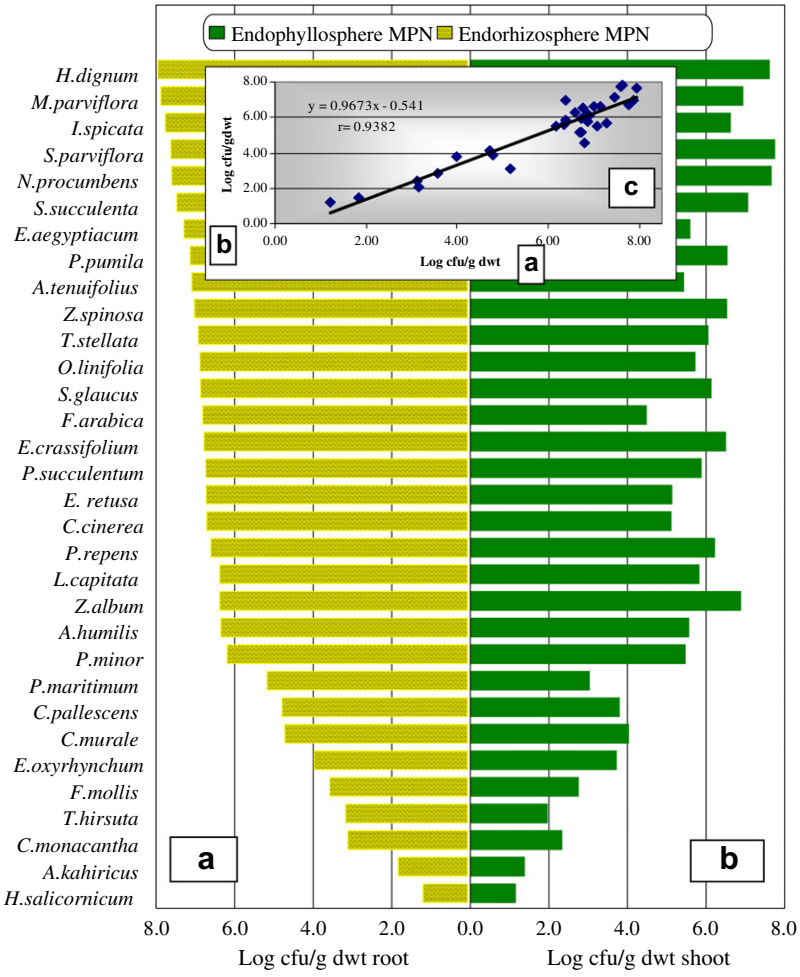


Fig. 6 MPN of culturable endophytic *Gluconacetobacter diazotrophicus*-like populations reported in shoots (a) and roots (b) of tested xerophytic plants, and computed correlation coefficients and regression lines (c) in between.

Table 5 Taxonomic position of *Pantoae* spp. isolates obtained during the present study in relation to representatives of those reported in literature.

Characteristics	9C[a]	*P. agglomerans*[b]	*P. ananas*[b]	*P. terrea*[b]	*P. punctata*[c]	*P. citrea*[c]	*P*96	*P*92	*P*89	*P*88	*P*65
Indole production	–	V	+	–	–	–	+	+	+	–	–
Citrate utilization	+	+	+	+	+	–	+	+	+	–	–
Acid production in sorbitol	+	–	+	–	–	–	+	+	+	–	–
Acid production in sucrose	+	+	+	+	+	–	+	+	+	–	–
Acid production in inositol	–	–	+	–	–	–	+	+	+	+	–
Nitrate reduction	–	+	V	V	+	+	–	–	–	–	–
Gelatine liquefaction	–	+	+	–	–	–	–	+	+	–	+
Motility	+	+	+	+	–	–	+	+	+	–	+

[a] *Pantoae* isolates (Ref. [56]).
[b] *P. agglomerans* and *P. ananas* (Ref. [30]); V, variable reaction.
[c] *P. terrea*, *P. punctata* and *P. citrea* (Ref. [31]).

their sand sheath populations exceeded 10^6 cfu g^{-1} dwt. They followed the descending order *B. madrietensis, L. perenne, B. scoparius, P. turgidum* and *H. murinum*. The load of *C. laevigatus*, of the family Cyperaceae, was particularly the lowest ($< 10^6$ cfu g^{-1} dwt) Fig. 5A. A trend that is very much comparable to the spore-forming community nesting the intact root surfaces (rhizoplane).

Gluconacetobacter diazotrophicus

The endophytic *Gluconacetobacter diazotrophicus*, present inside roots or shoots, were abundant in the selective LGI semi-solid culture medium. For the majority of plants (75–80%), their culturable populations in shoot and root tissues ranged from 10^4 to 10^7 cfu g^{-1} Fig. 6. Among the

Table 6 Taxonomic position, based on API 20 E and 20 NE, of endophytic isolates of diazotrophs other than *Gluconacetobacter* spp. developed in LGI semi-solid medium.

Plant	Area	Isolate code	Sphere tested	N_2-ase activity (nmoles C_2H_4 h^{-1} 5 ml $culture^{-1}$)	Proposed position	Identification
L. perenne	III	S 14/E/24	Root	2.69	*Serratia plymuthica*	Good
L. perenne	III	E 15/E/24	Root	8.68	*Enterobacter sakazaki*	Good
E. aegyptiacum	III	E 16/E/24	Root	3.29	*Enterobacter sakazaki*	Good
C. pallescens	I	E 21/E/24	Shoot	3.74	*Enterobacter agglomerance*	Good
S. succulenta	I	E 43/E/24	Shoot	10.17	*Enterobacter agglomerance*	Good
P. pumila	II	A 49/E/24	Root	39.04	*Aeromonas sobria*	Excellent
A. tenuifolius	III	E 52/E/24	Shoot	41.88	*Enterobacter agglomerance*	Good
E. aegyptiacum	III	E 61/E/24	Root	2.99	*Enterobacter sakazaki*	Good
O. linifolia	III	E 62/E/24	Root	5.68	*Enterobacter agglomerance*	Good
P. maritimum	II	E 76/E/24	Root	ND	*Erwinia* spp.	V. good
C. capitatus	III	E 87/E/24	Root	ND	*Enterobacter sakazaki*	Good
C. cinerea	III	P 65/E/24	Shoot	3.74	*Pantoae* spp.	Good
C. capitatus	III	P 88/E/24	Shoot	2.99	*Pantoae* spp.	Good
C. capitatus	III	P 89/E/24	Shoot	10.50	*Pantoae* spp.	Good
C. capitatus	III	P 92/E/24	Root	18.7	*Pantoae* spp.	Excellent
P. minor	II	P 96/E/24	Shoot	2.24	*Pantoae* spp.	Excellent
S. parviflora	III	B 2/NE/24	Shoot	1.5	*Bukholderia* (*Pseudomonas*) *cepacia*	Excellent
L. capitata	I	B 4/NE/24	Root	2.24	*Bukholderia* (*Pseudomonas*) *cepacia*	Excellent
L. capitata	I	B 6/NE/24	Root	20.94	*Bukholderia* (*Pseudomonas*) *cepacia*	Good
P. turgidum	III	B 19/NE/24	Sand sheath	10.62	*Bukholderia* (*Pseudomonas*) *cepacia*	Good
C. pallescens	I	A 22/NE/24	Shoot	20.94	*Chrysemonas luteola*	Good
L. capitata	I	A 25/NE/24	Shoot	29.92	*Agrobacterium radiobacter*	Good
C. murale	I	X 57/NE/24	Root	6.73	*Stenotrophomonas* (*Xanthomonas*) *maltophilia*	Excellent
E. aegyptiacum	III	B 59/NE/24	Shoot	15.71	*Bukholderia* (*Pseudomonas*) *cepacia*	Good
E. aegyptiacum	III	C 60/NE/24	Shoot	15.71	*Chrysemonas luteola*	Good
P. minor	II	C 97/NE/24	Root	20.94	*Chrysemonas luteola*	V. good

ND, not detected.

richest plants, both in roots and shoots ($>10^7$), were *Heliotropium dignum, Malva parviflora*, *Svignya parviflora*, *N. procumbens* and *S. succulenta* while the poorest ($\leqslant 10^4$) were *E. oxyrhynchum*, *F. mollis*, *Thymelaea hirsute, Cornulaca monacantha, Astragalaus kahiricus* and *H. salicornicum*. Highly significant correlation coefficient ($r = 0.9382$) was reported between populations harbored the shoots and roots of plants.

The taxonomic profile using API 20E and API 20NE (data not shown) of 10 pure isolates was comparable to the reference type culture strain (ATCC 40379). Of interest is that the selective LGI culture medium did also support the growth of another group of isolates that did not match with the taxonomic profile of *Gluconacetobacter diazotrophicus* but *Pantoea* spp. Tables 5 and 6 and other species of diazotrophs, namely *Enterobacter agglomerance*, *Enterobacter sakazaki*, *Serratia plymuthica*, *Aeromonas sobria*, *Erwinia* spp., *Bukholderia* (*Pseudomonas*) *cepacia*, *Chrysemonas luteola*, *Agrobacterium radiobacter* and *Stenotrophomonas* (*Xanthomonas*) *maltophilia* Tables 6. All diazotrophic isolates were present endophyticaly in roots or in the shoots of plants, but one *Bukholderia* (*Pseudomonas*) *cepacia* was found in the sand sheath layers Table 6.

Discussion

The major goal of this study was to document the diversity of bacteria associated to the plant cover of north Sinai deserts. This necessitated surveying the predominant plant species and assaying the culturable bacteria associated to the plant canopy and root systems. Since microflora might be used as bioindicators of plant–soil health, suitability and perturbation; the size, composition and nature of microbial populations are used as indicators of biological status of soil/plant health and nutrition. However, a lot of problems are encountered with culturable population of bacteria, either total or specific groups [22]. Although Frankenberger and Dick [23] concluded that plate count technique is not reliable measure of microbial growth and activity in plant–soil system, there is evidence that this technique is useful in comparative ecological studies of specific microbial population [24].

Within the studied areas, 30 annual and 13 perennial plants were encountered and selected for microbiological analyses. This number is rather limited compared to those recorded earlier in north Sinai. Gibbali [10] in his extensive survey reported more than 300 species. It is expected that the number of existing plant species are declining along the years because of low rainfall as well as the on-going human interaction through rural and agricultural developments and activities.

As to the xerophyte–microbe–environment panorama; several factors are expected to support the microbial establishment and growth in this particular environment, e.g., beneficial root exudates, shedding of plant parts to improve soil fertility, presence of shade to reduce the direct sun-rays, favorable pH, low soil salinity, plant stability among soil layers, limited fluctuations in rainfall and temperature, absence of allelopathic and/or bacteriostatic plant compounds and wide root/shoot ratio [2].

Both endorhizosphere and endophyllosphere of xerophytes tested accommodated high total culturable bacterial popula-

tions of ca. 10^8 cfu g^{-1} dwt, which proves many more bacterial infections of inner plant tissues. Similarly, associative diazotrophs were extraordinary reported in both plant niches. Due to definition of James et al. [25], endophytes are heterotrophic microorganisms that are able to invade and penetrate plant organs encompassing roots, stems and leaves. The studies of Reis et al. [26] have shed the light upon the invasion process and indicated that the endophyte first colonizes the root surfaces and then infects the roots via lateral root junctions and/or root tips. The endophyte, thereafter, enters the root vascular system from whence it translocates to the lower stem in the xylem. In addition to the possibility of infection at lateral root junctions, James et al. [27] suggested that there are at least two other potential sites of infection; wounds and stomata. In either location, the bacteria elicited a localized host defense response in the form of a polymeric matrix material that surrounded them. The invasion process appears not always to be detrimental to plant nutrition and health but may even be confer some growth benefits [26]. In accordance, Chanway [28] reported that some endophytic bacteria are thought to produce compounds that render plant tissues less attractive to herbivores, while other strains may increase host plant drought resistance.

Endophytic bacteria comprise only part of the non-pathogenic microflora exist naturally inside plant tissues. Work with plant species of agricultural and horticultural importance indicates that some endophytic bacterial strains stimulate host plant growth by acting as biocontrol agents, either through direct antagonism of microbial pathogens or by inducing systemic resistance to disease-causing organisms. Other endophytic bacterial strains may protect crops from parasitic nematodes and insects. In Brazil, the N_2-fixing endophytes of sugarcane, *Acetobacter diazotrophicus*, (now *Gluconacetobacter diazotrophicus*), and *Herbaspirillum* spp. colonize internal root, stem and leaf tissues, and are thought to provide up to 80% of the host plant's nitrogen needs [28]. Other endophytic bacteria stimulate plant growth via mechanisms yet to be elucidated.

As reported by Olivares and James [29], at early stage of the plant–microbe interaction, the numbers of endophytes inside plant tissues appear to be quite high (10^7–10^8 cells g^{-1} fresh weight), although it should be noted that such numbers certainly include many surface-dwelling bacteria that have survived via tight adherence to plant surfaces within mucus and/or a preference for colonizing cracks and crevices. This applies very well to the present results of dense endophytic populations reported for the tested xerophytic plants of north Sinai.

Three hundred bacterial isolates were secured from endorhizosphere and endophyllosphere of tested plants. Among those, 41 isolates were further purified and identified based on colony and cell morphology as well as API (20E, 20NE and 50CHB) profiles. Of the forty one identified strains, 23 were BNF *Bacillus* spp. The majority of bacilli strains were *B. megaterium* followed by *B. pumilus*,*B. polymexa, B. macerans, B. circulans* and *Bacillus licheniformis*. The family Enterobacteriaceae was represented by *Enterobacter agglomerans*, *Enterobacter sackazakii*, *Enterobacter cloacae*, *Serratia adorifera, Serratia liquefaciens* and *Klebsiella oxytoca*. Among non-Enterobacteriaceae were *Pantoae* spp. *Agrobacterium rdiobacter*, *Pseudomonas vesicularis*, *Pseudomonas putida*, *Stenotrophomonas maltophilia*, *Ochrobactrum anthropi*, *Sphingomonas paucimobilis* and *Chrysemonas luteola*. The taxonomic profile of *Pantoae* spp. isolates is most likely matches with the reported *P. anans* (2 isolates) and *P. citrea* (two isolates) [30,31]. Similarly, other workers have reported isolation of indigenous endophytic bacteria from yellow dent type corn [32], sweet corn [33] and alfalfa [34].

The present study presents original data on the indigenous bacterial endophytes isolated from the natural plant cover of deserts, in particular north Sinai. The endophytic microorganisms were recovered based on the method of surface sterilization with ethanol and sodium hypochlorite followed by triturating of plant organs. Other methods such as Scholander pressure bomb was proposed [35] for releasing endophytes. They mentioned that crushing method mainly recovers the endophytes that residing the root cortex particularly Gram positive species as *Bacillus* spp. while the pressure bomb procedure detects vascular colonists such as *Agrobacterium radiobacter* and less common species. Genera like *Pseudomonas* and *Phyllobacterium* were recovered with equal frequencies using both techniques.

In fact, bacilli, particularly N_2-fixing species, have already been found in association with grass roots. Among those, *Bacillus polymyxa* is well documented colonizer of wheat rhizosphere [28], while *Bacillus circulans* was identified in maize rhizosphere by [36]. The present study, as well as of Othman et al [2], are among the original reports on these species as endophytic diazotrophs to xerophytic plants.

Gluconacetobacter diazotrophicus, previously known as *Acetobacter diazotrophicus* [37], is a strict aerobic N_2-fixing endopyte originally isolated from sugarcane roots and stems [15]. It has been estimated that *G. diazotrophous* can fix up to 150 kg N ha^{-1} $year^{-1}$ in sugarcane [38]. Such high levels of N_2-fixation have not been reported in any other system outside legume-*Rhizobium* symbiosis. The bacterium has subsequently been isolated from sweet potato [39], sorghum [40], coffee [41], some tropical grasses [42], finger millet [43] and pineapple [44]. The bacterium was also able to establish an endophytic association with wheat [12].

This bacterium is of special interest because, besides fixing atmospheric dinitrogen in the presence of KNO_3 and at low pH values < 3.0, it can secrete up to 50% of the fixed N_2 in a form potentially available to plants [45]. Such an endophytic diazotroph was also isolated from sugarcane in Mexico, Cuba, Australia and Egypt [46,47,12]. The occurrence of the microorganism was reported in roots, tubers and stems of sweet potato which confirm the endophytic nature of this particular diazotroph [48].

So far, no information is available in literature on the natural endophytic occurrence of this particular diazotroph in xerophytic plants. Therefore, this work presents original data on the endophytic existence of *Gluconacetobacter diazotrophicus* in the rhizo- and plyllo-spheres of a number of desert plants in Sinai environment. But in several instances, difficulties in finding this bacterium may be related to methods used for surface sterilization and isolation. Here, Youssef et al. [12] reported that surface sterilization of plant organs by combined treatment with ethanol alcohol and sodium hypochlorite was very successful and efficient for the elimination of contaminants and hence facilitated the isolation of the diazotroph.

The root system of plant is as complicated as the shoot in its diversity, in its reactions with the matrix of substances and with the myriad organisms that surround it [49]. This complexity was illustrated in the much studied corn root system, covering the changes along the framework roots: the surface tissues and their interactions with the soil, the water conducting xylem, whose gradual elaboration dictates the water status

of the root. A conspicuous manifestation of the changes is the rhizosheath, whose microflora differs from those on the bare zones. The multitude of fine roots is the most active part of the system in acquiring water and nutrients, with its own multitude of root tips, sites of intense chemical activity, that strongly modify the soil they contact, mobilize reluctant ions, immobilize toxic ions, coat the soil particles with mucilage and select the microflora. Therefore, it was of rather interest to study in this work the phenomenon of rhizosheath formation during the ecological study on the plant community of Sinai deserts. Microbiological analysis indicated, generally, the richness of sand grain sheath compared to the surrounding free sand soil. In addition, microbial populations in rhizosheaths were comparable to those reported on the intact roots of tested plants. This indicates that the rhizosheath environment extends the root continuum that favors microbial activity and consequently magnifies plant–microbe interactions [3].

As to xerophytes examined, the rhizoplane of *Bromus madritensis* accommodated extraordinary bacterial loads of both total bacteria and diazotrophs (ca. $\geqslant 10^9$ cfu. g^{-1} dwt) while *Bromus scoparius* (ca. $\leqslant 10^7$ cfu. g^{-1} dwt) was the poorest. This emphasizes that plant effect is among the major biotic criteria that governs the plant–microbe interaction in desert environments. In addition, the richness of sand sheath in microbial populations of total bacteria and diazotrophs, with bacterial load corresponding to ca. 93% of the root surfaces, is a distinguished phenomenon of ecological importance in the studied desert environment of north Sinai.

Indeed, the cylindrical sand grain sheaths encasing the root of grasses were first reported for the Egyptian desert flora [6]. Such sand grains are thought to be cemented by various bonding agents including secretions of mucilage and root cap tissues [50]. Microbial exudates might also be involved [51]. Exopolysaccharides either capsular or hydrosoluble, are produced by a group of microorganisms in the root zone of different xerophytes encompassing *Leuconostoc mesenteroides*, *Rhonella aquatilis* and *Enterobacter amnigenus* [52] and *Agrobacterium* sp. [53]. An explanation for the high microbial load in sheath zones of xerophytes tested in the present study is the possible richness in organic products [7], greater moisture content [54] and possible reduction of oxygen concentration [55] throughout the rhizosheath. This would create an environment which favors the activity of associative diazotrophs [51], and significantly high linear acetylene reducing activity was recorded in either intact-soil-plant cores or disintegrated rhizosheath.

In conclusion, the present study demonstrated the great diversity of culturable endophytic bacteria, particularly diazotrophs, in the plant–soil systems of north Sinai deserts. Their prevalence with dense populations suggests their very possible contribution to the survival of such xerophytic plants under the stress conditions of north Sinai deserts. The successful colonization of such bacterial endophytes to other plant species strongly suggests their possible future application as bio-preparates for plant nutrition (biofertilizers) and health (bio-pesticides).

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