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

Amira L. Hanna, Hanan H. Youssef, Wafaa M. Amer, Mohammed Monib, Mohammed Fayeza, Nabil A. Hegazi

Department of Microbiology, Faculty of Agriculture, Cairo University, Giza, Egypt
Department of Botany, Faculty of Sciences, Cairo University, Giza, Egypt

Received 3 July 2011; revised 3 November 2011; accepted 23 November 2011
Available online 10 January 2012

KEYWORDS
North Sinai;
Desert ecosystems;
Xerophytes;
Culturable bacteria;
Rhizospheric microorganisms (RMOs);
Diazotrophs;
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 sakazakii, Enterobacter cloacae, Serratia adorifera, Serratia liquefaciens and Klebsiella oxytoca. The non-Enterobacteriaceae population was rich in Pantoeae spp., Agrobacterium radiobacter, Pseudomonas vesicularis, Pseudomonas putida, Stenotrophomonas maltophilia, Ochrobactrum anthropi, Sphingomonas paucimobilis and Chryseomonas luteola. Gluconacetobacter diazotrophicus were reported inside root and shoot tissues of a number of tested plants. The dense bacterial populations...
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 N₂-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 openplain 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.
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 Waring 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 subsamples 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 forceps (naked root/rhizoplane) and (d) surface-sterilized roots (endorhizo- sphere) 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), shaken (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 pour plate method [14]. Diazotrophs were cultured using the surface-inoculated plates and the N-deficient combined carbon sources medium (CCM)
For each suitable using the most probable number (MPN) and the semi-solid [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 C2H4 culture−1 h−1 were considered positive, streaked on CCM agar plates and incubated for 48–72 h. at 30 °C. For further isolation of all selected isolates, single colony isolation was performed on agar plates of CCM. Pure

### 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 |
|-----|------------|--------|-------------|--------|
| 1   | Cyperus laevigatus L b | Cyperaceae | I Site 2 | 2005 |
| 2   | Pancratium maritimum L | Amaryllidaceae | II Site 3 | 2005 |
| 3   | Thymelaea hirsuta (L.) Endl | Thymelaceae | II Site 1 | 2005 |
| 4   | Astragalus kaliricus DC | Fabaceae | III Site 5 | 2004 |
| 5   | Corinula caica monantha Delile | Chenopodiaceae | III Site 3 | 2004 |
| 6   | Fagonia arabica L | Zygophyllaceae | III Site 1 | 2004 |
| 7   | Fagonia mollis (Labiill.) H.L. Wendl | Zygophyllaceae | III Site 1 | 2004 |
| 8   | Haloxylon salicornicum (Moq.) Bunge ex Boiss | Chenopodiaceae | III Site 1 | 2004 |
| 9   | Heliotropium digynum (Forssk.) Chir | Boraginaceae | III Site 3 | 2004 |
| 10  | Panicum turgidum Forsk | Poaceae | III Site 4 | 2004 |
| 11  | Stigmasteris scoparia (Trin. & Rupr.) de Winter b | Poaceae | III Site 2 | 2004 |
| 12  | Zilla spinosa (L.) Prantil | Brassicaceae | III Site 8 | 2004 |
| 13  | Zygophyllum alhum L. var. amblyocarpum (Baker) Hadidi | Zygophyllaceae | III Site 3 | 2004 |

**Annual**

| No. | Host plant | Family | Area-site a | Season |
|-----|------------|--------|-------------|--------|
| 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  | Polycarpa repens (Forssk.) Asch. & Schwein | Caryophyllaceae | I Site 3 | 2005 |
| 18  | Silene scolcaentula Forsk | Caryophyllaceae | I Site 3 | 2005 |
| 19  | Trachynia distachya (L.) Link = Brachypodium distachyum (L.) P. Beauv b | Poaceae | I Site 1 | 2005 |
| 20  | Anclusa humilis (Desf.) I.M. Johnst | Poaceae | II Site 4 | 2005 |
| 21  | Bromus madritensis L b | Poaceae | II Site 2 | 2004 |
| 22  | Bromus scopy anus 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 Rett | Poaceae | II Site 4 | 2005 |
| 27  | Polycarp succulentum (Delile) J. Gay | Poaceae | II Site 4 | 2005 |
| 28  | Pseudoloura pumila (L.) Grande | Caryophyllaceae | II Site 1 | 2005 |
| 29  | Senecio glaucus (L.) Var. coronifolius (Maire) C. Alexander | Apiaceae | 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  | Cusandra nemaphatica (Spreng.) K. Rich b | Poaceae | III Site 2 | 2004 |
| 34  | Cyperus capitatus Vand b | Poaceae | III Site 2 | 2004 |
| 35  | Eremobium aegyptiacum (Spreng.) Asch. & Schwient. var. aegyptiacum | Brassicaceae | III Site 11 | 2005 |
| 36  | Erodium oxyrhynchum M. Bieb | Geraniaceae | III Site 4 | 2004 |
| 37  | Euphorbia retusa Forsk | 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 | Poaceae | III Site 3 | 2004 |
| 41  | Oligomeris linifolia (Hornem.) J.F. Macbr | Resthaceae | III Site 10 | 2005 |
| 42  | Striga parviflora (Delile.) Webb | Brassicaceae | III Site 4 | 2004 |
| 43  | Trigonella stellata Forsk | Leguminosae | III Site 9 | 2005 |

---

**Notes:**

- **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.
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–CaCO3 (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; K₂HPO₄, 0.4; KH₂PO₄, 0.6; MgSO₄, 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; CuSO₄, 0.08 mg; ZnSO₄, 0.25 mg; MnSO₄, 0.01; yeast extract, 0.2; fermentol (a local product of corn-steep liquor), 0.2; KOH, 1.5; CaCl₂, 0.02; FeCl₃, 0.015; Na₂ MoO₄, 0.002. Sodium lactate was included as 0.6 ml (50% v/v).

LGI medium [15]: It contains (g l⁻¹): K₂HPO₄, 0.2; KH₂PO₄, 0.6; MgSO₄·7H₂O, 0.2; CaCl₂·2H₂O, 0.02; Na₂MoO₄·H₂O, 0.002; FeCl₃·H₂O, 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 Na₂MoO₄·H₂O, 0.1 mg of biotin, 0.2 mg of pyridoxal HCI 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 CaCO₃, GYC [21,15]: It contains (g l⁻¹): glucose, 100; yeast extract, 10; CaCO₃, 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 culturable 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 meteorological 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 10⁵ to 10⁹ cfu g⁻¹ dwt of endorhizosphere and phyllosphere. The plant species Eremobium aegypticum, 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 culturable 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 >10⁶–10⁸ cfu g⁻¹ dwt. For the endorhizosphere, E. aegypticum 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 (>10⁶ cfu g⁻¹) were E. aegypticum, C. murale and N. procumbens. Four plants supported populations less than 10⁵ 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 Cyperus 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 kaoeoides, 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. 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 was 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) and rhizoplane the highest. Of interest is 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 (>10⁶ 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 10⁵ to 10⁹ 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 diazotrophs. 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 >10⁵ to 10⁶ cfu g⁻¹ dwt; representing 50–85% of the microbial population Fig. 5 B. Compared to the free sand (10⁵–10⁶ cfu g⁻¹ dwt), the sand sheath and the root surfaces (rhizoplane) harbored higher populations (10⁶–10⁷ 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 >10⁶ to 10⁹ cfu g⁻¹ dwt, representing 50–97% of total endophytic bacterial community.
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),...
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. agglomerans, E. sakazakii), Serratia spp. (S. adorifera, S. liquefaciens), Agrobacterium spp. (A. radiobacter), Klebsiella spp. (K. oxytoca), Pseudomonas spp./Brevundimonas spp. (P. vesicularis, P. putida), Chryseomonas spp. (C. luteola), Stenotrophomonas spp. (S. maltophilia), Ochrobactrum spp. (O. anthrophi) 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

| Host plant | Area | Isolate code | Sphere | N₂-ase activity (nmol C₂H₄ 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 | Good |
| T. distachya | I | B 18/B/48 | Sand sheath | 12.26 | B. polymyxa | Good |
| T. distachya | I | B 17/B/48 | Root (endorhizosphere) | >41.88 | B. megaterium | Excellent |
| I. spicata | II | B 45/B/48 | Root | 17.95 | B. megaterium | 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 | II | B 142/B/48 | Root (endorhizosphere) | 22.44 | B. megaterium | V. good |
| T. koeleriodes | 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. tenaxfolius | III | B 60/B/48 | Root | 26.33 | B. pumilus | V. good |
| C. cineria | III | B 87/B/48 | Root | 28.42 | B. megaterium | Good |
| C. cineria | 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. marinum | III | B 129/B/48 | Root (endorhizosphere) | 14.96 | B. megaterium | V. good |
| C. capitanus | III | B 165/B/48 | Root (endorhizosphere) | >41.88 | B. megaterium | V. good |
| A. tenaxfolius | III | B 61/B/48 | Shoot | 6.58 | B. pumilus | V. good |
| E. retusa | III | B 65/B/48 | Shoot | 19.44 | B. megaterium | Good |
| 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 |

- Rhizo-sheathed plants.

| Host plant | Area | Isolate code | Sphere | N₂-ase activity (nmol C₂H₄ 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 agglomerans | Excellent |
| P. pumila | II | B 50/NE/24 | Root | 13.46 | Brevundimonas (Pseudomonas) vesicularis | Good |
| P. marinum | II | O 94/NE/24 | Root not determined | Ochrobactrum anthrophi | V. good |
| P. marinum | II | E 91/E/48 | Root | 27.52 | Enterobacter cloacae | Good |
| P. marinum | II | E 92/E/48 | Root | 22.44 | Enterobacter sakazaki | V. good |
| M. parviflora | C | 115/NE/24 | Shoot | 29.92 | Chryseomonas liquefaciens | 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. tenaxfolius | III | B 58/NE/24 | Root | 26.92 | Brevundimonas (Pseudomonas) vesicularis | Good |
| H. marinum | III | E 123/E/24 | Root | 14.96 | Enterobacter agglomerans | Good |
| H. marinum | III | P 131/NE/48 | Root | 35.9 | Pseudomonas putida | V. good |
| Z. album | II | S 147/NE/24 | Root | >41.88 | Stenotrophomonas maltophilia (Xantho. maltophilia) | Excellent |
| Z. album | II | 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 |
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

![Diagram showing 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 | P. agglomerans$^b$ | P. ananas$^b$ | P. terrea$^b$ | P. punctata$^a$ | P. citrea$^a$ | P96 | P92 | P89 | P88 | P65 |
|--------------------------|----|--------------------|---------------|---------------|----------------|---------------|-----|-----|-----|-----|-----|
| Indole production        | –  | V                  | +             | –             | –              | –             | +   | +   | +   | –   | –   |
| Citrate utilization      | +  | +                  | +             | –             | +              | +             | +   | +   | +   | –   | –   |
| Acid production in sorbitol | + | +                  | +             | –             | –              | –             | +   | +   | +   | –   | –   |
| Acid production in sucrose | + | +                  | +             | +             | –              | +             | +   | +   | +   | –   | –   |
| Acid production in inositol | – | –                  | +             | V             | V              | –             | +   | +   | +   | +   | +   |
| 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]).

---

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.
The richest plants, both in roots and shoots (>10^7), were *Heliotropium dignum*, *Malva parviflora*, *Svignya parviflora*, *N. procumbens* and *S. succulenta* while the poorest (≤10^5) were *E. oxyrychnum*, *F. mollis*, *Thymelaea hirsuta*, *C. monacantha*, *Astragalus 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 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-

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

| Plant            | Area | Isolate code | Sphere tested | N2-ase activity (nmol C2H4 h^-1 5 ml culture^-1^) | Proposed position | Identification |
|------------------|------|--------------|---------------|-------------------------------------------------|-------------------|---------------|
| *L. perenne*     | III  | S 14/E/24    | Root          | 2.69                                            | *Serratia puthymica* | 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 agglomeranence* | Good         |
| *S. succulenta* | I    | E 43/E/24    | Shoot         | 10.17                                           | *Enterobacter agglomeranence* | Good         |
| *P. punila*     | II   | A 49/E/24    | Root          | 39.04                                           | *Aeromonas sobria* | Excellent     |
| *A. tenifolius* | III  | E 52/E/24    | Shoot         | 41.88                                           | *Enterobacter agglomeranence* | 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 agglomeranence* | Good         |
| *P. maritimun*  | 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     |
| *L. capitata*   | 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                                           | *Chrysomonas luteola* | Good         |
| *L. capitata*   | I    | A 25/NE/24   | Shoot         | 29.92                                           | *Agrobacterium radiobacter* | Good         |
| *C. marule*     | 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                                           | *Chrysomonas luteola* | Good         |
| *P. minor*      | II   | C 97/NE/24   | Root          | 20.94                                           | *Chrysomonas luteola* | V. good      |

ND, not detected.
tions of ca. $10^6$ 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 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 ($10^7$–$10^9$ 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. polymyxa_ , _B. macerans_ , _B. circulans_ and _Bacillus licheniformis_. The family Enterobacteriaceae was represented by _Enterobacter agglomerans_ , _Enterobacter sakazakii_ , _Enterobacter cloacae_ , _Serratia_ _adgorfera_ , _Serratia liquefaciens_ and _Klebsiella oxytoca_. Among non-Enterobacteriaceae were _Pantoaea_ spp. _Agrobacterium radiobacter_ , _Pseudomonas vesicularis_ , _Pseudomonas putida_ , _Stenotrophomonas maltophilia_ , _Ochrobactrum anthropi_ , _Sphingomonas paucimobilis_ and _Chrysemonas luteola_. The taxonomic profile of _Pantoaea_ 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 endoppyte originally isolated from sugarcane roots and stems [15]. It has been estimated that _G. diazotrophus_ 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 phyllo-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. \( \geq 10^{6} \text{ cfu. g}^{-1} \text{ dwt} \)) while Bromus scoparius (ca. \( \leq 10^{5} \text{ cfu. g}^{-1} \text{ dwt} \)) 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 [34] 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).

References

[1] Othman AA, Shawky ME, Amer MW, Fayezy M, Monib M, Hegazi NA. Biodiversity of microorganisms in semi-arid soils of North Sinai deserts. Arch Agron Soil Sci 2003;49:241–60.

[2] Othman AA, Amer MW, Fayezy M, Monib M, Hegazi NA. Biodiversity of diazotrophs associated to the plant cover of North Sinai deserts. Arch Agron Soil Sci 2003;49:683–705.

[3] Othman AA, Amer MW, Fayezy M, Hegazi NA. Rhizosheath of Sinai desert plants is a potential repository for associative diazotrophs. Microbiol Res 2004;159:285–93.

[4] Ali MS, Hamza AM, Amin G, Fayezy M, El-Tahan M, Monib M, et al. Production of biofertilizers using baker’s yeast effluent and their application to wheat and barley grown in north Sinai deserts. Arch Agron Soil Sci 2005;51(6):589–604.

[5] Ali MS, Amin G, Fayezy M, El-Tahan M, Monib M, Hegazi NA. Production of rhizobia biofertilizers using baker’s yeast effluent and their application to Leucaena leucocephala. Arch Agron Soil Sci 2005;51(6):605–17.

[6] Volkens G. Die Flora der aegyptisch arabischen Wueste auf der Grundlage anatomisch-physiologischer Forschungen. Berlin: Gebrueder Borntraeger; 1887, p. 156.

[7] Martin JK. Factors influencing the loss of organic carbon from wheat roots. Soil Biol Biochem 1977:9:1–7.

[8] El-Haddi MN. Observations on the flora of the Sinai mountain region. Bull Soc Geogr Egypt 1969;40:123–55.

[9] Hoffmann V. Studies of Flora of Egypt. Cairo University: Beirut Publishing; 1974, p. 888.

[10] Gibbali MA. Studies on the Flora of Northern Sinai. M.Sc. Thesis, Fac. Agric. Cairo Univ; 1988, p. 393.

[11] Boullos L. Flora of Egypt, monocotyledons (Alismataceae – Orchidaceae), vol. 4. Cairo (Egypt): Al Hadara Publishing; 2005, p. 617.

[12] Youssef HH, Fayezy M, Monib M, Hegazi NA. Gluconacetobacter diazotrophicus: a natural endophytic diazotroph of Nile Delta sugar cane capable of establishing an endophytic association with wheat. Soil Fert Soils 2004;6:391–7.

[13] Hegazi NA, Hamza AM, Osman A, Ali S, Sedik MZ, Fayezy M. Modified combined carbon N-deficient medium for isolation, enumeration and biomass production of diazotrophs. In: Kauser Malik A, Sujjad Mirza M, editors. Nitrogen fixation with non-legumes. Kluwer Academic Publishers; 1998, p. 247–53.

[14] Parkinson D, Gary TRG, Williams ST. Methods for study the ecology of soil micro-organisms, vol. 19. IBP Handbook; 1971, p. 679.

[15] Cavalcante VA, Dobereiner J. A new acid-tolerant nitrogen-fixing bacterium associated with sugar cane. Plant Soil 1988;108:23–31.

[16] Meynell GC, Meynell EW. Theory and practice in experimental bacteriology. London: Cambridge University Press; 1965, p. 287.

[17] Hegazi NA, El-Mallawani AA, Monib M, Azospirilla and other symbiotic nitrogen fixing bacteria in rhizosphere of some plants prevailing in Egyptian desert. In: Proceedings of the IV conference on microbiology, vol. 1. Cairo. Soil, Food and Industrial Microbiol Egypt. Society for Applied Microbiology; 1980, p. 119–24.

[18] Krieg NR, Holt JG. Bergy’s manual of systematic bacteriology. 1st ed. Baltimore: Williams and Wilkins; 1984, p. 548.

[19] Logan NA, Berkeley RCW. Identification of Bacillus strains using the API system. J Can Microbiol 1984;130:1871–82.

[20] Pan B, Kevin JV. Response of the endophytic diazotroph Gluconacetobacter diazotrophicus on solid media to changes in atmospheric partial O₂ pressure. Appl Environ Microbiol 2001;67(10):4694–700.

[21] Micales BK, Johnson JL, Claus GW. Deoxyribonucleic acid homologues among organisms in the genus Gluconobacter. Int J Syst Bacteriol 1985;35:79–85.

[22] Kule SP, Raghu K. Relationship between microbial numbers and other microbial indices in soil. Bull Environ Cont Tox 1989;43:941–5.
