

Research

Desert aridity recruits xerophyte microbiota of diverse structure and function

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Abstract

Drastic consequences of climate change necessitate exploring plant-microbiota of arid desert ecosystems for potential candidates recommended for future microbiota –mediate strategies of stressed agriculture. The prevalent xerophytes (Amaranthaceae, Compositae, Cyperaceae, Gramineae, Plantaginaceae, Scrophulariaceae, and Zygophyllaceae) were tested for culturable associated microbiota. Representative isolates were subjected to PGP functions, API-profiling and 16S rRNA genes sequencing. Xerophytes hosted dense populations of culturable microbiota, ecto-/endo-rhizosphere ($> 10^3$ – 10^7 CFUs g⁻¹ root). API microtube analysis confirmed the predominance of representative genera of Gram-positive/negative bacteria. Isolates expressed multi-functional PGP traits, e.g. nitrogenase activity, indole acetic acid production, P/K- solubilization, ammonia production, salt tolerance, and effective/wide enzymatic activities. Based on 16S rRNA gene sequencing, the dominant phyla were Firmicutes, Gammaproteobacteria and Alphaproteobacteria. Firmicutes represented in ecto-rhizosphere (*Bacillus rugosus*, *B. cereus*, and *B. altitudinis*) and endo- rhizosphere (*B. halotolerans*, *B. safensis* subsp. *safensis*, *B. siamensis*, *B. licheniformis* and *Brevibacillus parabrevis*). Gammaproteobacteria presented in ecto-rhizosphere as *Pseudomonas aeruginosa*, *Ps. hibiscicola*, *Ps. monteilii*, *Kosakonia cowanii* and *Serratia liquefaciens* and in endo-rhizosphere as *Pantoea vagans*, *P. conspicua* and *Stenotrophomonas maltophilia*. Alphaproteobacteria, *Azospirillum zeae*, *Azospirillum oryzae* and *Agrobacterium salinitolerans*, were present in ecto-rhizosphere. Conformity among API profiling and 16S rRNA gene sequencing on the genus level ($> 58\%$) indicated the reliability of API profiling as pre-identification scheme. It is concluded that the present study provides additional information regarding microbiota associated with xerophytes. Beside their taxonomic diversity, they are of potential/ functional impact that recommend their use as candidates of synthetic communities (SynCom) for the modification of economic crops introduced to arid deserts.

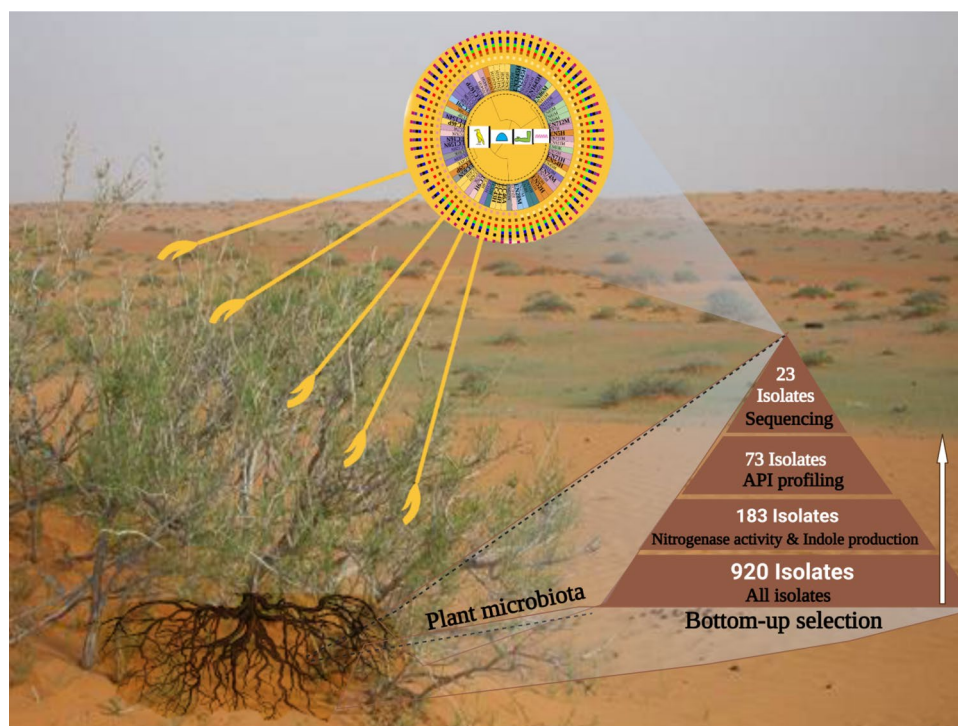
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Graphical Abstract



Keywords Semi/arid deserts · Xerophytes · PGPR/plant microbiota · Climate change/stress agriculture · Ecto-/endo-rhizosphere · API profiling/16S rRNA gene sequencing

1 Introduction

Arid and semi-arid regions occupy a significant domain of the planet and are expected to proportionally increase due to the current distressing scenarios in climate changes. Corollary, future agriculture is under tremendous abiotic stresses hindering further development and progress [1]. It is well established that drought is among the major abiotic factors that restrict plant development and crop production, and directly influence the activity of soil microbiota via its impact on growing plants and bio-mineralization. The altered root theater of microbial community structure aids to alleviate drought effect on plant through a number of mechanisms, *e.g.* production of exo-polysaccharides and 1-aminocyclopropane-1-carboxylate (ACC) deaminase [2], and initiation of protective rhizosheaths on roots [3, 4]. Salinity, as well, is one of the most significant abiotic stresses that limiting plant development and crop productivity in arid and semi-arid regions. It is coming more and more prominent as it currently represents almost 8.7% of the earth's surface area (<https://news.un.org/en/story/2021/12/1107172>), and expected to increase by 10% per year [5]. It appeared that the excessive salts in soil solution disrupt plant growth, and reduce *ca.* 70% of yields of a great number of cereal crops [6] and ultimately posing a serious threat to global food security [7].

Fortunately, a rate of drought- and salinity-tolerant strategies has been adopted, based on some biochemical and physiological changes. Recently, great attention is focused on the role played by plant growth-promoting rhizobacteria (PGPR) for supporting development and metabolic pathways of plants in drought- and salt- stressed environments due to their intimate association with plant roots. PGPR enhance plant establishment in stressed environments through various mechanisms, *e.g.* supplementary production of phytohormones that accelerate seed germination and seedling development, and of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme that causes the degradation of precursor of ethylene aiding to adaptation under rigorous environmental conditions. This is beside fixing atmospheric dinitrogen,

solubilizing phosphorus and modifying soil structure and fertility [8]. As well, they support plant adaptation to water-deficit conditions by the accumulation of osmolytes and production of extracellular polysaccharides (EPS), antioxidant and volatile compounds [9]. In this context, numerous genera of PGPR have been isolated and identified from different sources, e.g. *Bacillus* and *Paenibacillus* from finger millet, maize and sunflower; *Bacillus*, *Lysinibacillus*, *Paenibacillus* and *Planococcus* from wheat; *Pseudomonas* from sorghum [8, 10, 11]. In fact, growing in extreme pressures, plants select their root-associated microbial assemblages with adequate abilities to stimulate plant growth. And, the enormous microbial diversity in the root area plays a key function in the establishment of vegetation cover and conspicuously contributes in both soil and plant improvement.

These prokaryote-root interactions are considered pivotal for halophyte and xerophyte plant establishment, survival and production in rigorous environments under the ongoing scenarios of climate change and water scarcity [12–14].

Our previous publications extensively explored the diversity of culturable populations of microbiota associated to the drought-/salt-stressed plant cover of semi-arid deserts of north Sinai Peninsula, Egypt [3, 4, 15, 16]. The drought/heat stress in such arid environment was expressed in the predominance of spore-forming bacteria that did occupy a significant niche on root surfaces (ecto-rhizosphere) and even got access to the innermost roots (endo-rhizosphere). Their community was represented by quite diverse species of *Bacillus*. Non-spore forming members belonging to the genera *Sphingomonas*, *Brevundimonas*, *Ochrobactrum*, *Enterobacter*, *Chryseomonas*, *Agrobacterium*, *Klebsiella* and *Serratia* were also found. As well, the halophytes of the salt-affected sand dunes of western north coast of Egypt were examined for their associated microbiota [17]. And, the introduction of the plant-based-sea water culture medium enabled in situ recovery and in vitro cultivation of the plant associated halotolerant microbiota belonged to the genera *Bacillus*, *Halomonas* and *Kocuria*, the majority of which expressed multi-PGP functions, e.g. N_2 -fixation, IAA production and phosphate-solubilizing abilities.

Common to natural flora of semi-/arid- environment, the plant cover of arid deserts of Arabian Gulf, namely in Saudi Arabia deserts, is characterized by unique tolerance and resistance to developing drought and salinity [18]. And, it is expected, but not yet explored, that their indigenous associated microbiota is having specific adaptation mechanisms against the prevailing stresses and act in turn for the benefit of their host plants. Here, the need arises to explore such microbiota communities in this particular plant-soil environment to be further exploited for several purposes, particularly greening the deserts and crop improvement programs.

As the microbiota associated with xerophytes is still a black box and its study could help in future microbiota-mediated strategies [19], the present work dealt with wild plants naturally grown in Qassim deserts of Saudi Arabia. This is principally to explore the diversity and richness of their culturable microbiota associated to both ecto- and endo- rhizosphere (representing the inner tissues of surface-sterilized plant organs). API profiles and 16S rRNA gene sequencing were used for the identification and phylogenetic characterization of the representative isolates. For the possible future application of these bacteria as synthetic community (SynCom) for in situ modification, they were evaluated in relation to their potential PGP-functions to stimulate plant development and yield, e.g. N_2 -fixation, IAA production, P/K solubilisation and salt tolerance. In addition, the functional enzymatic diversity of the tested microbiota was explored using API-ZYM assay for more characterization of their metabolic activity. With the hope that the obtained results can help with answering the compelling question of: Are these micro-residents of natural xerophytes able to restore vegetation and degraded soil fertility in the arid stressed-region for future support of agricultural output, food security and ecosystem productivity/sustainability?

2 Materials and methods

2.1 Sampling sites and plant sampling

Samples of eleven wild xerophytic plant species were randomly collected from three sites of Qassim arid deserts to the east of Buraydah City, kingdom of Saudi Arabia, including site 1 located at N26.386.623 & E44.142.097, site 2 at N26.356.755 & E44.059.300 and site 3 at N26.192.695 & E44.296.101. The collected plant species were authenticated and identified at Cairo University Herbarium (CAI). The ID for the voucher specimens deposited in Cairo university herbarium (CAI) were outlined in Table 1. The taxonomic ranking, species distribution in Saudia Arabi and the ecological features were according to Migahid [20], Boulos [21], Ghazanfar [22] as outlined in Table 1.

Table 1 Taxonomic profile of collected plants of semi-arid desert environment of Qassim, Saudi Arabia: description, distribution and ecology

No	Scientific name/family	Species description	World Distribution	Distribution in Saudi Arabia	Ecological habitat	Voucher plant specimens ID
1	<i>Haloxylon salicornicum</i> (Moq.) Bunge ex Boiss* Family: Amaranthaceae	Perennial Shrub	Afghanistan, Algeria, Egypt, Gulf States, India, Iran, Iraq, Jordan, Kuwait, Lebanon, Syria, Libya, Oman, Pakistan & Palestine [20, 21]	NH, SH, NJe, N, S, NF & R [20]	The species grow in both temperate and tropical regions; it is tolerant to abiotic stresses such as drought and high temperature. Wide spread in saline-affected soil	CAL-911
2	<i>Artemisia monosperma</i> Delile Family: Compositae	Perennial low shrub	Libya, Egypt, Palestine, Lebanon, Syria, Iraq & India [21]	N, NJ, NF, E & R [21]	Widespread in desert sand dunes, and wadis with deep drifted sand	CAL-/1218
3	<i>Curatodia dichotoma</i> (Forssk.) Trab Family: Gramineae	Annual grass	Arabia, Egypt, Libya, Morocco, Palestine, Turkey, Algeria, Syria, Cyprus, Iraq & Iran [21]	Great sand dunes in Nafud sand area NJe [20]	Costal sand dune belt in Afro-Asian Mediterranean and Red Sea coasts	CAL-1042
4	<i>Hordeum marinum</i> Huds. Subsp. <i>gussonianum</i> (Parl.) Thell Family: Gramineae	Annual grass	Native to E. Central Europe to Mediterranean region, South west & Central Asia to W. Himalaya [21]	Great sand dunes in Nafud sand area NJe [20]	Fertile alluvial soil, road sides and edge of cultivations	CAL-/2026
5	<i>Scrophularia syriaca</i> Benth Family: Scrophulariaceae	Perennial shrub	Its native range is N Africa to W. & S Iran and Arabian [21]	NF [20]	Sandy banks of the desert wadis generally forming monospecific community	CAL-/1841
6	<i>Anthemis melampodina</i> Delile Family: Compositae	Annual herb	Its native range is E. Mediterranean. From Egypt to Iraq, Kuwait, Palestine & Arabian Peninsula [23]	N [25]	Sandy soil and alluvial desert plain	CAL-1882
7	<i>Gnaphalium luteoalbum</i> L Family: Compositae	Annual herb	Cosmopolitan [21]	NH, SH, S, and NJ [20]	Sandy fields, dune-slacks and waste ground, moist, disturbed areas along roads and streams	CAL-2349
8	<i>Schismus barbatus</i> (L.) Thell Family: Gramineae	Annual grass	Arabia, Algeria, Afghanistan, Egypt, France, Morocco, Namibia, Libya, Pakistan & South Africa, Tunisia [21]	N, NJ, NF https://powo.science.kew.org	Found mostly in disturbed areas, dry sandy and gravel deserts	CAL-599
9	<i>Cyperus conglomeratus</i> Rottb Family: Cyperaceae	Perennial sedge	It is indigenous to India, but is now found in Afghanistan, Algeria, Chad, Egypt, Eritrea, Ethiopia, Iraq, Mali, Mauritania, Senegal, Sudan, Palestine, Pakistan & South Africa [21]	N, NJ, NF & E [20]	Maritime sand and inland desert sandy plains [26]	CAL-2059
10	<i>Plantago psammophila</i> Agnew & Chal.-Kabi Family: Plantaginaceae	Annual herb	Its native range is Iraq to Arabian Peninsula [20]	NH, N, NJ & E [20]	Maritime sand and inland desert sandy plains	CAL-/1888
11	<i>Tetraena coccinea</i> (L.) Beier & Thulin Family: Zygophyllaceae	Perennial low shrub	Egypt, Jordan, Tunisia, Palestine, Somalia, South Africa and Greece & North Sudan [24]	Along the Red Sea coast NH, SH, N, S, NJw & E [20]	Found in the salt marshy habitats, coastal and inland saline sandy soils, dunes and sheets, and in saline depressions	CAL-938

N Northern region including Tabouk, Al-Jawf and Sakaka areas, NF Nefud region including the great northern Nefud area, Dahna and Al-Qasim area, NH Northern Hijaz, representing the western part of Saudi Arabia extends along Red Sea north of Jeddah, SH South Hijaz, representing the southern part of the western region extending south Jeddah to Yemen boundaries, S Southern region lying to the east of south Hijaz, includes Abha, Bisha and Najran regions, NJw Western Najd, NJe Eastern Najd, E Eastern region between Dahna and the Arabian Gulf, R: Al-Rub' Al Khali, including the southern and south-eastern part of Saudi Arabia

*All the tested plants were collected from Qassim area in February, 2022 in flowering stage

2.2 Quantification and isolation of bacteria

Samples of xerophytes were uprooted and loosely-adhered soil was removed by gentle shaking. The tightly-adhering soil to the root, regarded as rhizosphere soil, was collected and placed in a sterilized plastic bag for physico-chemical analyses (Table S1) according to the procedures described of Richard [27] and Jackson [28]. A composite sample of the two biological replicates obtained for each tested plant was prepared for further bacteriological analysis.

2.2.1 Quantification and isolation of culturable mesophilic/thermophilic bacteria in tested plant compartments

Samples of tested root compartments were prepared for microbiological analyses according to the methods described by Youssef et al. [29]. Initial dilutions of ecto-rhizosphere samples, that include both rhizosphere and rhizoplane bacteria, were prepared by transferring 5 g of plant roots with closely adhering soil into bottles containing 45 ml of half strength basal salts of CCM medium [30] minus carbon sources. Bottles were kept under gentle shaking for 60 min., and further serial decimal dilutions were prepared from the resulting suspension. The endo-rhizosphere samples were prepared from the inner tissues of surface-sterilized roots. Where 5 g of roots were initially washed with tap water to remove adhered soil particles, then roots were surface sterilized, with 95% ethanol for 5–10 s followed by 3% (w/v) sodium hypochlorite for 30 min, and then carefully washed with sterilized distilled water before crushing in Waring blender with adequate volume of basal salts of CCM medium according to Youssef et al. [31].

Aliquots of 200 µl of suitable dilutions prepared for both root compartments were used to surface-inoculate agar plates, three replicates for each dilution, of CCM culture medium. Incubation took place at 30 °C (for mesophiles) and 45 °C (for thermophiles) for 4–7 days and developed colony forming units (CFUs) were examined and counted. Suspended materials of roots were dried at 70 °C and weighed for calculations on dry basis of plant materials.

For counting spore-forming bacteria, the suitable dilutions prepared for both plant spheres were pasteurized/heated in a water bath at 80 °C for 15 min. Then, aliquots of 200 µl of suitable dilutions were surface inoculated on CCM agar plates. Four replicates were prepared from each suitable dilution, and incubated at 30, 45 and 55 °C. After 4–7 days, developed CFUs were examined and counted.

Representative plates were randomly selected for single colony isolation of predominant isolates. A total of 274 and 215 isolates was secured representing the mesophilic and thermophilic bacterial population, respectively (Table S2).

2.2.2 Quantification and isolation of culturable salt tolerant bacteria in tested plant compartments

For salt-tolerance population, aliquots of 500 µl of suitable dilutions, prepared from both plant compartments, were inoculated into MPN tubes containing 5 ml of semi-solid (1.8 g agar L⁻¹) CCM culture medium amended with 3 and 10% NaCl (w/v). Five tubes were prepared for each dilution, and incubated at 30 °C for 2–7 days. Positive tubes were characterized by growth in the form of turbidity, pellicle formation and confirmed by microscopy; and MPN estimates were derived using Cochran's Table-5 tubes [32]. Acetylene-reducing activities of positive culture tubes were measured according to the previously reported procedure of Hegazi et al. [33]. Culture tubes produced > 5 nmoles C₂H₄ culture⁻¹ h⁻¹ were considered true N₂-fixers, then streaked on CCM agar plates amended with 3 or 10% NaCl (w/v) and incubated for 2–7 days at 30°C. With single colony isolation, a number of 431 representative isolates was secured (Table S2).

2.3 Selection of functional bacterial isolates representing root compartments and various growth conditions

Representative agar plates and/or MPN tubes were selected for single colony isolation and purification of bacterial isolates developed under various growth conditions. More than 900 isolates were secured representing meso- and thermo-philic as well as salt-tolerant microbiota of both root compartments endo- and ecto-rhizosphere (Table S2).

2.3.1 Screening isolates by acetylene reducing activity

Initial pre-selection of isolates was based on positive acetylene reducing activity (> 5 nmoles C₂H₄ culture⁻¹ h⁻¹). The selected isolates (183 isolates) were maintained on agar slants and stored at + 4 °C for additional tests. Further bottom-up

selection resulted in a total of 73 isolates of multiple functions (acetylene-reducing activity, indole acetic acid production, salt tolerance, solubilization of P and K as well as ammonia production; Fig. 1) that were then subjected to API profiling and PGP traits (Table S3).

2.3.2 Indole-acetic acid (IAA) production

The test was carried out according to the procedures of Glickmann and Dessaux [34]. Erlenmeyer flasks containing 25 ml standard nutrient broth medium supplemented with L-tryptophan (100 mg l^{-1}) were inoculated with the tested isolates and incubated for 24–48 h at 30 and 45 °C for meso- and thermo-philic isolates respectively. The resulting liquid cultures were centrifuged at 6000 rpm for 10 min., and 8 ml of Salkovsky's reagent was added to 4 ml of the supernatant and incubated for 30 min to develop pink colour. Positive results were indicated by the change in colour from pink to deep purple and measured calorimetrically at 530 nm. IAA concentration was estimated against the prepared IAA standard curve.

2.3.3 Phosphate solubilizing activity

Tested isolates were inoculated into standard nutrient broth for 24 h., and incubated at 30 °C or 45 °C for mesophiles and thermophiles respectively. The bacterial cells were collected by centrifugation at 4000 rpm, washed twice with sterilized solution of sodium chloride (0.05% NaCl, w/v), and re-suspended to obtain a suspension equal to 0.2 of optical density (OD) at 600 nm. The prepared bacterial suspensions were used for determination of phosphate and potassium solubilization, as well as tolerance to salinity.

Phosphate dissolving ability was evaluated by the modified Pikovskaya's agar plates [35]. Aliquots of 4 μl of each bacterial suspension were inoculated in the center of agar plates of modified PVK agar medium containing $\text{Ca}_3(\text{PO}_4)_2$ as insoluble phosphate source; in three replicates. The plates were incubated at 30 °C for 7 days and formation of clearance zone is considered a positive result. Phosphate solubilization index (PSI) was calculated as follows: $\text{PSI} = (D + d)/d$; where d and D are the diameters of the colonies and halo zones around the colonies, respectively.

2.3.4 Potassium solubilizing activity

Potassium solubilization of the tested isolates was measured according to Priyanka and Sindhu [36] and Hu et al. [37] by using modified Aleksandrov medium supplemented with mica powder (2 g l^{-1}). Four μL of each bacterial suspension was spotted in the center of the prepared agar plates; in three replicates. The plates were incubated at 30 ± 2 °C for 3–7 days. The formation of clearance zone is considered a positive result. Potassium Solubilization Index (KSI) was calculated as follows: $\text{KSI} = (D + d)/d$; where the diameter of the colony (d) and halo zone (D) around the colony were measured.

2.3.5 Salt tolerance

Tolerance of bacterial isolates to salinity was evaluated by observing the bacterial growth on standard nutrient agar medium amended with various concentrations of NaCl (2, 4, 6, 8 and 10%, w/v). The previously adjusted bacterial suspensions were streaked on agar plates in 5 streaking lines, and incubated at 30 °C, with three replicates for each bacterial isolate. The bacterial growth was examined after 48–72 h., and compared with controls without salt addition [38]. The visual growth index recorded as follows: 1 = scant (discontinuous bacterial lawn, with scattered colonies); 2–3 = good (continuous bacterial lawn); and 4–5 = very good (continuous and denser bacterial lawn).

2.3.6 Ammonification

Production of ammonia was determined in peptone water [39]. Freshly grown cultures were inoculated into 10 ml peptone water then incubated at 30 °C for 48 h. After incubation, 0.5 ml of the Nessler reagent was added to the bacterial culture. Color changing from yellow to dark brown indicated ammonia production. Un-inoculated sterile medium was used as control.

2.4 API morpho- physiological identification of the selected isolates

According to Youssef et al. [29], biochemical test kits (bioMérieux API) were used for bacterial identification [40], including API 20E for Enterobacteriaceae, API 20NE for non-Enterobacteriaceae and API 50CHB for bacilli. Test results and constructed numerical profiles were submitted to the online database (<https://www.biomerieux-diagnostics.com/>) to identify the bacteria.

In addition, the API ZYM system was applied to detect of the enzyme activities, which has been used for characterization of a variety of bacteria [41]. The tested isolates were grown on nutrient agar plates, and a cell suspension was made in saline solution to obtain *ca.* 0.2 of optical density (OD) at 600 nm. Then, 65 µl of each adjusted bacterial suspension was placed in each cupule. After incubation at 37 °C for 4 h, 0.05 ml of each of API ZYM A and API ZYM B reagents (bioMérieux) was added to each cupule, and the gallery was kept in the dark for 5 min and then exposed for 30 s under a 750-W lamp to prevent non-specific yellowing of the colour reagent. Color reactions were estimated and scored from 0 to 5 according to the manufacturer's instructions. Values 1 to 5 were positive reactions with increasing strength depending on intensity, while 0 meant no reaction [41, 42].

2.5 Identification of selected isolates by 16S rRNA gene phylogeny

Genomic DNA was extracted from bacterial cells according to the cetyl trimethyl ammonium bromide (CTAB) protocol [43, 44]. DNA quality was checked using the Bio Drop µLITE spectrophotometer (Biochrom, Holliston, MA, USA). The 16S rRNA gene was amplified from genomic DNA extracted from selected isolates using the F-27 [5'-AGA GTT TGA TC (AC) TGG CTC AG-3'] and R1494-1514 [5'-CTA CGG (T/C) TAC CTT GTT ACG AC-3'] and R1494-1514 [5'-CTA CGG(T/C) TAC CTT GTT ACG AC-3'] primers.

The PCR reaction was carried out in a total volume of 25 µl contained 2 µl of each forward and reverse primers, 12.5 µl of PCR master mix (Fermentas, Thermo Scientific, MA, USA), 6.5 µl nuclease- free water and 2 µl DNA template in addition. Thermal cycling conditions were 5 min initial denaturing at 94 °C, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, extension at 72 °C for 2 min, and a final extension period of 10 min at 72 °C [45]. The PCR products were tested on 1.5% agarose gel to ensure single products of the expected size. QIAquick PCR Purification kit (Qiagen, Hilden, Germany) was used to purify the PCR products according to the instructions. Sequencing for purified PCR products was performed according to Sanger enzymatic sequencing (Eurofins MWG Operon, Ebersberg, Germany).

The acquired sequences were analyzed using the basic local alignment search tool (BLAST) (<http://www.ncbi.nlm.nih.gov/BLAST/>). A phylogenetic tree was constructed via a neighbour-joining method, comprising the sequences of the 22 bacterial isolates and 51 closest hits from the NCBI GenBank database, using MEGA v5.2 program. The 16S rRNA gene sequences were deposited at the Gen Bank under the accession numbers indicated in Tables (2).

2.6 Data analyses

MSTAT program [46] and STATISTICA (Statsoft, Inc. Tulsa, USA, Version 10.0 <http://www.statsoft.com>) were used for the analysis of variance (ANOVA) to examine the significant effects at the level of $p < 0.05$.

The multiple functions (indole acetic acid production, phosphate, and potassium solubilization, salt tolerance, and ammonium production) of the tested isolates were visualized in a dendrogram, using PAST 4.03 software (<https://past.en.lo4d.com/download>), and annotated using the online tool Interactive Tree of Life (iTOL) (<http://itol.embl.de>). R version 4.0.2 (<https://www.r-project.org/>), R-studio (<https://www.rstudio.com/>), R-project packages (cran.r-project.org), "ggbiplot" and "heatmap.2" were used for constructing PCA and heat maps.

3 Results

3.1 Floristic composition of the study area

The studied Qassim desert lies in the arid zone of Middle East and North Africa (MENA) region, and its natural flora is mainly of xerophytic nature. The area is flat plain and subjected to erosion and low rainfall. During the field trip of the present study, eleven plant species were collected, those showed the highest dominance and frequency as well as utmost

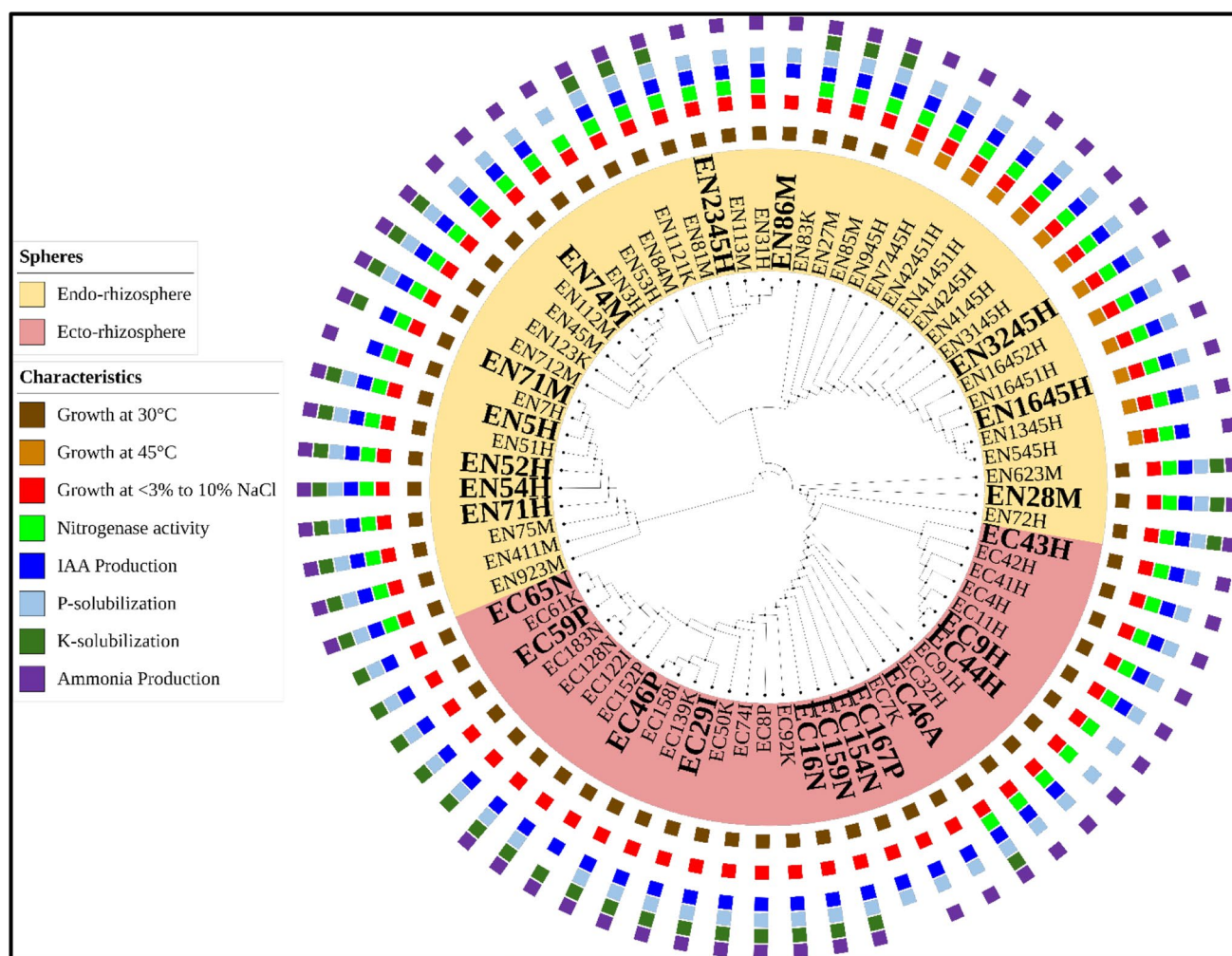


Fig. 1 UPGMA cluster analysis of tested bacterial isolates based on their PGP potential. Each square represents a positive result of the tested traits: nitrogen fixation measured as acetylene reduction, phosphate solubilization, indole acetic acid production, salt tolerance; in addition to the plant sphere of origin (ecto/endo-rhizosphere). Isolates in bold are those selected for further 16S rRNA gene sequencing

adaptation to the prevailing harsh environment. These secured xerophytic species are mainly Sahara-Arabian elements (Table 1). Representative soil samples in close contact to plant roots were taken as well, and mechano- and physico-chemical properties of soils are presented in Table (S1). The majority are of sandy loam textures besides those of sandy and loamy sand. Soils are very poor in organic matter (0.04–0.56%), varied in calcium carbonate (3.06–28.50%), non- to moderately saline (0.20–6.83 dSm⁻¹) with pH in the relatively alkaline side (7.90–8.86). Macro-nutrients are falling in the ranges 22.4–42.0 ppm for nitrogen, 1.5–19.0 ppm phosphorus and 89.7–371.0 ppm for potassium. Micro-nutrients are scantily present.

The tested xerophytes (Table 1) represent the following families: Compositae (*Artemisia monosperma* Del., *Anthemis melampodina* Del. and *Gnaphalium luteoalbum* L.), Gramineae (*Cutandia dichotoma*, Forssk. Trabut, *Hordeum marinum* Huds. and *Schismus barbatus* (L.) Thell.), Amaranthaceae (*Haloxylon salicornicum*, Moq. Bunge exBoiss.), Scrophulariaceae (*Scrophularia syriaca* Benth.), Cyperaceae (*Cyperus conglomeratus* Rottb.), Plantaginaceae (*Plantago psammophila* Agnew & Chal-Kabi), and Zygophyllaceae (*Tetraena coccinea* (L.) Beier & Thulin.).

3.2 Culturable population densities in root compartments of tested xerophytes as affected by varying growth conditions

All of the examined plants did accommodate dense populations of total culturable rhizobacteria. Significant differences were attributed to a number of single effects (Table S4). For host plants, and irrespective of root compartments and incubation conditions, the highest CFUs counts (> 5.0 log CFUs g⁻¹ root) were reported for *Artemisia monosperma*,

Gnaphalium luteoalbum and *Plantago psammophila*, followed by $> 4.5\text{--}4.9 \log \text{CFUs g}^{-1}$ root for *Haloxylon salicornicum*, *Hordeum marinum*, *Cyperus conglomerates* and *Tetraena coccinea*, and $> 4.0\text{--}4.6 \log \text{CFUs g}^{-1}$ root for *Scrophularia syriaca*, *Anthemis melampodina*, *Schismus barbatus* and *Cutandia dichotoma* (Fig. S 1). As to plant compartments, higher densities were significantly reported for the ecto-rhizosphere (ca. $5.0 \log \text{CFUs g}^{-1}$ root) compared to endo-rhizosphere (ca. $4.4 \log \text{CFUs g}^{-1}$ root) for almost all the tested plants (Fig. S 2). Populations of mesophiles (ca. $5.5 \log \text{CFUs g}^{-1}$ root), grown at incubation temperature of 30°C , were higher than those of thermophiles (ca. $3.8 \log \text{CFUs g}^{-1}$ root), developed at 45°C (Fig. S 3). Elimination of non-spore-forming bacteria by heat treatment/pasteurization resulted in the development of spore-forming rhizobacteria (ca. $3.9 \log \text{CFUs g}^{-1}$ root) (Fig. S4) representing a good percentage (ca. $> 70.0\%$) of the total rhizospheric bacterial community.

Four-way ANOVA analysis indicated significant differences among the interaction of parameters tested, i.e. plant species and various growth conditions (Table S 5). Of interest was the predominance of endophytic populations of both thermophiles and spore-forming communities in the roots of a group of tested xerophytes in this particular environment, namely *Haloxylon salicornicum*, *Artemisia monosperma*, *Hordeum marinum*, *Gnaphalium luteoalbum* and *Plantago psammophila*. Where a population of $> 3.8\text{--}4.77 \log \text{CFUs g}^{-1}$ root reported for thermophiles and $> 4.0\text{--}4.5 \log \text{CFUs g}^{-1}$ root for spore-formers. The thermophilic communities represented $> 60.0\text{--}95.0\%$, that about corresponding with the percentages of spore-formers of $65.0\text{--}90.0\%$. A similar trend was reported for the above-mentioned xerophytes regarding the populations of rhizobacteria in the ecto-rhizosphere; being in the range of $3.12\text{--}5.93 \log \text{CFUs g}^{-1}$ root for thermophiles and $3.3\text{--}5.07 \log \text{CFUs g}^{-1}$ root for spore-formers. Corresponding percentages were in the ranges of $40.0\text{--}95.0$ and $70.0\text{--}90.0\%$.

3.3 Culturable population densities in root compartments of tested xerophytes affected by salinity stress

Soil salinity is among the environmental stresses that affect the plant–microbe relationship that ultimately reflects on plant nutrition and health. ANOVA analysis (Table S6) revealed statistically significant differences attributed to the single effect of plant species, plant compartment and salt concentration. As to plant effect, *Artemisia monosperma* accommodated extraordinary population of total salt-tolerant rhizobacteria in its root compartments (average of $5.40 \log \text{MPN estimates g}^{-1}$), followed by *Haloxylon salicornicum* ($4.3 \log \text{MPN estimates g}^{-1}$). The majority of other plant species harboured rhizobacteria with lower densities ($2.7\text{--}4.4 \log \text{MPN estimates g}^{-1}$); as low as $2.7 \log \text{MPN estimates g}^{-1}$ were recorded for *Tetraena coccinea*. With respect to plant compartments, the ecto-rhizosphere was populated by higher salt-tolerant population (ca. $4.7 \log \text{MPN estimates g}^{-1}$) compared to the endo-rhizosphere ($3.6 \log \text{MPN estimates g}^{-1}$). In general, higher concentration of NaCl (10%) resulted in significant decreases in populations of salt-tolerant rhizobacteria ($3.7 \log \text{MPN estimates g}^{-1}$), approximated a one log decreases in numbers. Significant differences were attributed to interactions of plant compartments and salt concentration. In general, populations decreased by the increase of salt concentration and approximated 16% and 21% in the endo-rhizosphere and ecto-rhizosphere, respectively (Table S6). This applies to the majority of tested plants, in particular *Haloxylon salicornicum*, *Artemisia monosperma* and *Gnaphalium luteoalbum*.

3.4 Representative isolates of rhizobacteria: in vitro PGP functional diversity and taxonomic profiling (the graphical abstract)

3.4.1 Single colony isolation of representative isolates dominated in ecto-and endo-rhizosphere

A total of 920 isolates was single-colony obtained from CFUs developed on CCM culture medium, represented both plant compartments, ecto-rhizosphere (512) and endo-rhizosphere (408) of tested plants. Such isolates were developed under mesophilic (705) and thermophilic (215) growth conditions. In response to salt tolerance, 431 were particularly developed in the presence of $3\text{--}10\%$ NaCl in the culture media (Table S2).

Initial pre-selection of isolates was based on positive acetylene reducing activity ($> 5 \text{ nmoles C}_2\text{H}_4 \text{ culture}^{-1} \text{ h}^{-1}$). The selected isolates (183 isolates) were maintained on agar slants and stored at $+4^\circ\text{C}$ for additional tests. Further bottom-up selection resulted in a total of 73 isolates of multiple functions (acetylene reduction, IAA production, P-solubilization, K-solubilization, ammonia production and salt tolerance) (Fig. 1 and Table S3). Those isolates represented communities of ecto-rhizospheres (30 isolates) and endo-rhizospheres (43); and their taxonomic position was initially identified using API20E, API20NE and API50CH profiles (Tables S 7 and S 8). Irrespective of host plants and plant compartments, 48 isolates represented Gram positive spore-forming bacteria: *Bacillus circulans* (1), *Bacillus coagulans* (1), *Bacillus licheniformis* (21), *Bacillus megaterium* (5), *Bacillus pumilus* (4), *Bacillus subtilis* (3) *Bacillus cereus* (6), *Paenibacillus polymyxa* (5) and *Brevibacillus brevis* (2). It appeared that *Bacillus licheniformis* was dominant by 43.8% followed by *Bacillus cereus* (12.5%).

Among the 25 non-spore formers, the following species were reported: *Azospirillum* spp. (1), *Pseudomonas fluorescens* (2), *Stenotrophomonas maltophilia* (3), *Pseudomonas putida* (1), *Pseudomonas luteola* (1), *Sphingomonas paucimobilis* (1), *Klebsiella oxytoca* (4), *Klebsiella pneumonia* (2), *Klebsiella aerogenes* (1), *Pantoea* spp. (2), *Burkholderia cepacia* (4), *Serratia marcescens* (1), *Brevundimonas vesicularis* (1) and *Pseudomonas aeruginosa* (1).

3.4.2 Functional activities/diversity of tested isolates

The representative and designated isolates of either plant compartments were tested for a number of functions that would contribute to plant nutrition as plant growth promoters (PGBR) (Tables S 9 and S 10). Among the 30 isolates of ecto-rhizosphere, 10 isolates exhibited acetylene reducing activities of > 20 nmoles C_2H_4 culture⁻¹ h⁻¹, representing 33.3%. Among the most active isolates were *B. licheniformis*, *Ps. luteola* and *Azospirillum* spp. The acetylene-reducing activity was more common among isolates of endo-rhizosphere as the majority (93.0%) showed positive activities, and 28 isolates, representing 71.0%, gave activities > 50 nmoles C_2H_4 culture⁻¹ h⁻¹. The highest activities (> 100 nmoles C_2H_4 culture⁻¹ h⁻¹) were reported for the spore-forming *B. licheniformis*, *B. megaterium*, *Paenibacillus polymyxa* and the non-spore forming *Ps. fluorescens*, *Stenotrophomonas maltophilia* and *K. oxytoca*.

As to the production of IAA, the majority of isolates of ecto-rhizosphere (70.0%) produced the auxin within the range of > 2.0 – 9.9 mg L⁻¹. The highest production was reported for the spore-forming *B. licheniformis*, *B. cereus*, *Brevibacillus brevis* and the non-spore forming *K. pneumoniae*, *Burkholderia cepacia* and *Azospirillum* spp. Compared to ecto-rhizosphere, almost all isolates of endo-rhizosphere were more active in the production of IAA. The highest activities of > 10 mg L⁻¹ were particularly common among the non-spore forming species of *Pantoea* spp., *K. oxytoca*, *Ps. fluorescens*, followed by *Stenotrophomonas maltophilia*. Relatively lower activities (> 1.0 – 8.0 mg L⁻¹) were common among the spore-forming isolates, e.g. *Bacillus coagulans*, *B. licheniformis*, *B. circulans*, *B. subtilis*, *B. megaterium* and *B. pumilus*.

P and K solubilization was reported for the majority of tested isolates representing ecto- and endo-rhizosphere as the solubilization index (PSI) ranged from 1.0 to 5.5 for both P and K. Of interest is that $> 50\%$ of the isolates joined both activities. Among the most active ecto-rhizosphere isolates were *Klebsiella pneumonia*, *Burkholderia cepacia*, *Brevibacillus brevis*, *Azospirillum* spp., *Ps. fluorescens*, *Serratia marcescens* and *B. cereus*. For endo-rhizosphere, the highest values reported for the species *K. oxytoca*, *K. aerogenes*, *Stenotrophomonas maltophilia*, *Pantoea* spp., *Burkholderia cepacia*, *B. coagulans* and *B. licheniformis*.

The majority (83%) of isolates of ecto-rhizosphere tolerated the presence of 4% NaCl. The percentages of isolates tolerated elevated salt concentrations decreased by the increase of salt concentration. With the highest salt concentration of 10% NaCl, 7 isolates (23.3%) exhibited very good growth, namely *B. licheniformis*, *B. pumilus*, *Paenibacillus polymyxa*, *Brevundimonas vesicularis*, *Sphingomonas paucimobilis* and *Ps. luteola*. Compared to ecto-rhizosphere, the isolates of endophytes showed higher tolerance for increasing concentrations of salt, with percentages of 69.0, 54.8, and 35.7% at salt concentrations of 6, 8 and 10% NaCl, respectively. The very good growth at the highest salt concentration was common among isolates of *Bacillus licheniformis* in particular followed by *Paenibacillus polymyxa*, *B. pumilus*, *B. megaterium* and *B. subtilis*.

In general, the majority of isolates possessed the ability of mineralization of nitrogen in terms of ammonia production.

3.4.3 Identification of 16S rRNA sequenced multi-functional isolates

A group of the multi-function isolates, representing both plant compartments, were 16S rRNA sequenced (Fig. 1). The 16S rRNA gene sequences were applied to the NCBI GenBank database for identification and deposition of corresponding accession numbers (Tables 2 and S 11). The related phylogenetic tree was constructed (Fig. 2). It appeared that the most dominant phyla were Firmicutes followed by Gammaproteobacteria and Alphaproteobacteria. Representative genera of phyla Firmicutes were differently represented in the plant compartments, ecto- and endo-rhizosphere.

3.4.4 Enzymatic activity (API-ZYM) of multi-functions and 16S rRNA gene sequenced isolates

The enzymatic activities of 16S rRNA gene sequenced isolates were further tested using API-ZYM assay. Results indicated that each substrate of the test was degraded at least by one of the isolates tested. As to Gram positive bacilli (Fig. 3), the highest activities were reported for six enzymes: alkaline phosphatase, esterase lipase, luciferase, arylamidase, trypsin, acid phosphatase and naphthol-as-bi-phosphohydrolase. The lowest activities were reported for 7 enzymes: cystine arylamidase, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase,

Table 2 Matching of the taxonomic position of isolates obtained by both API 20E and 50 CH profiles and 16S rRNA gene sequencing

Isolates code	API 20E profile	% of similarity	Close match (BLAST)	% of identity	Accession number
Close matching at species level (3 out of 23 isolates)					
EC159N	<i>Serratia liquefaciens</i>	99.5	<i>Serratia liquefaciens</i>	98.94	OQ568848
EN86M	<i>Stenotrophomonas maltophilia</i>	97.4	<i>Stenotrophomonas maltophilia</i>	99.73	OQ568852
EN2345H	<i>Bacillus licheniformis</i>	99.9	<i>Bacillus licheniformis</i>	99.87	OQ568847
Close matching at genera level (13/23 isolates)					
EC154N	<i>Pseudomonas putida</i>	99.7	<i>Pseudomonas monteilii</i>	99.47	OQ568845
EC59P	<i>Pseudomonas fluorescens</i>	98.9	<i>Pseudomonas aeruginosa</i>	99.2	OQ568849
EC46A	<i>Azospirillum</i> spp.	ND ^a	<i>Azospirillum zeae</i>	99.44	OQ568850
EN54H	<i>Pantoea</i> spp.	99.6	<i>Pantoea vagans</i>	99.37	OQ568853
EC167P	<i>Bacillus licheniformis</i>	99.9	<i>Bacillus altitudinis</i>	99.19	OQ568857
EN71M	<i>Bacillus licheniformis</i>	99.7	<i>Bacillus halotolerans</i>	99.39	OQ568860
EN28M	<i>Bacillus circulans</i>	99.8	<i>Bacillus siamensis</i>	99.91	OQ568861
EN5H	<i>Bacillus pumilus</i>	99.9	<i>Bacillus safensis</i>	99.82	OQ568862
EN52H	<i>Bacillus coagulans</i>	99.9	<i>Bacillus safensis</i>	99.38	OQ568863
EC9H	<i>Bacillus megaterium</i>	99.8	<i>Bacillus rugosus</i> (<i>Bacillus halotolerans</i>)	99.91 99.27	OQ568864
EN1645H	<i>Bacillus licheniformis</i>	99.9	<i>Bacillus halotolerans</i>	99.82	OQ568865
EN3245H	<i>Bacillus megaterium</i>	99.2	<i>Bacillus licheniformis</i>	99.37	OQ568846
EC46P	<i>Bacillus pumilus</i>	99.9	<i>Bacillus rugosus</i>	99.13	OQ568866
None of genera/species matching (7/23 isolates)					
EC16N	<i>Klebsiella pneumoniae</i>	98.4	<i>Agrobacterium salinitolerans</i>	100	ND ^b
EC65N	<i>Serratia marcescens</i>	95.1	[<i>Pseudomonas</i>] <i>hibiscicola</i>	99.91	OQ568851
EN71H	<i>Burkholderia cepacia</i>	99.9	<i>Pantoea conspicua</i>	99.72	OQ568854
EC43H	<i>Brevundimonas vesicularis</i>	92.7	<i>Kosakonia cowanii</i>	99.71	OQ568855
EC44H	<i>Pseudomonas luteola</i>	99.9	<i>Bacillus rugosus</i>	99.8	OQ568856
EC29I	<i>Brevibacillus brevis</i>	99.9	<i>Bacillus cereus</i>	99.82	OQ568858
EN74M	<i>Bacillus megaterium</i>	98.9	<i>Brevibacillus parabrevis</i>	99.91	OQ568859

ND^a, not identified by API microtubes

ND^b, not determined as the sequence of this isolate was too short for data base submission and getting accession number; accordingly, it is not included in the phylogenetic tree (Fig. 2)

and α -fucosidase. The broadest activities were reported for the species of *B. rugosus*, *B. altitudinis*, *B. licheniformis* and *B. halotolerans*. For Gram negative bacteria (Fig. 4), the highest activities reported for five enzymes; alkaline phosphatase, leucine arylamidase, acid phosphatase, naphthol-as-bi- phosphohydrolase and β - glucosidase. The lowest activities were reported for the four enzymes: lipase, α - chymotrypsin, β - glucuronidase and N-acetyl- β -glucosaminidase. In particular, *Kosakonia cowanii* were clustered independently because of their strong activities for the particular enzymes of alkaline phosphatase, leucine arylamidase, acid phosphatase, naphthol-as-bi- phosphohydrolase, and β -galactosidase. In common, four enzymes were actively produced by representative species of both Gram positive and Gram negative bacteria, i.e. alkaline phosphatase, lucine arylamidase, acid phosphatase and naphthol-as-bi-phosphohydrolase. Compared to Gram positive species, the Gram negative ones showed broader enzyme activities, expressing variable activities with > 9–18 enzymes.

4 Discussion

In arid environments, drought is among the major abiotic stresses for agriculture production and development. It is emphasized that drought is negatively affecting future global agriculture yield and expected to extend in numerous areas across the globe, induced by dramatic climate changes [47]. In this respect, plants are placed into five ecological groups according to their drought or waterlogging tolerance as: hydrophytes, hygrophites, mesophytes, xerophytes,

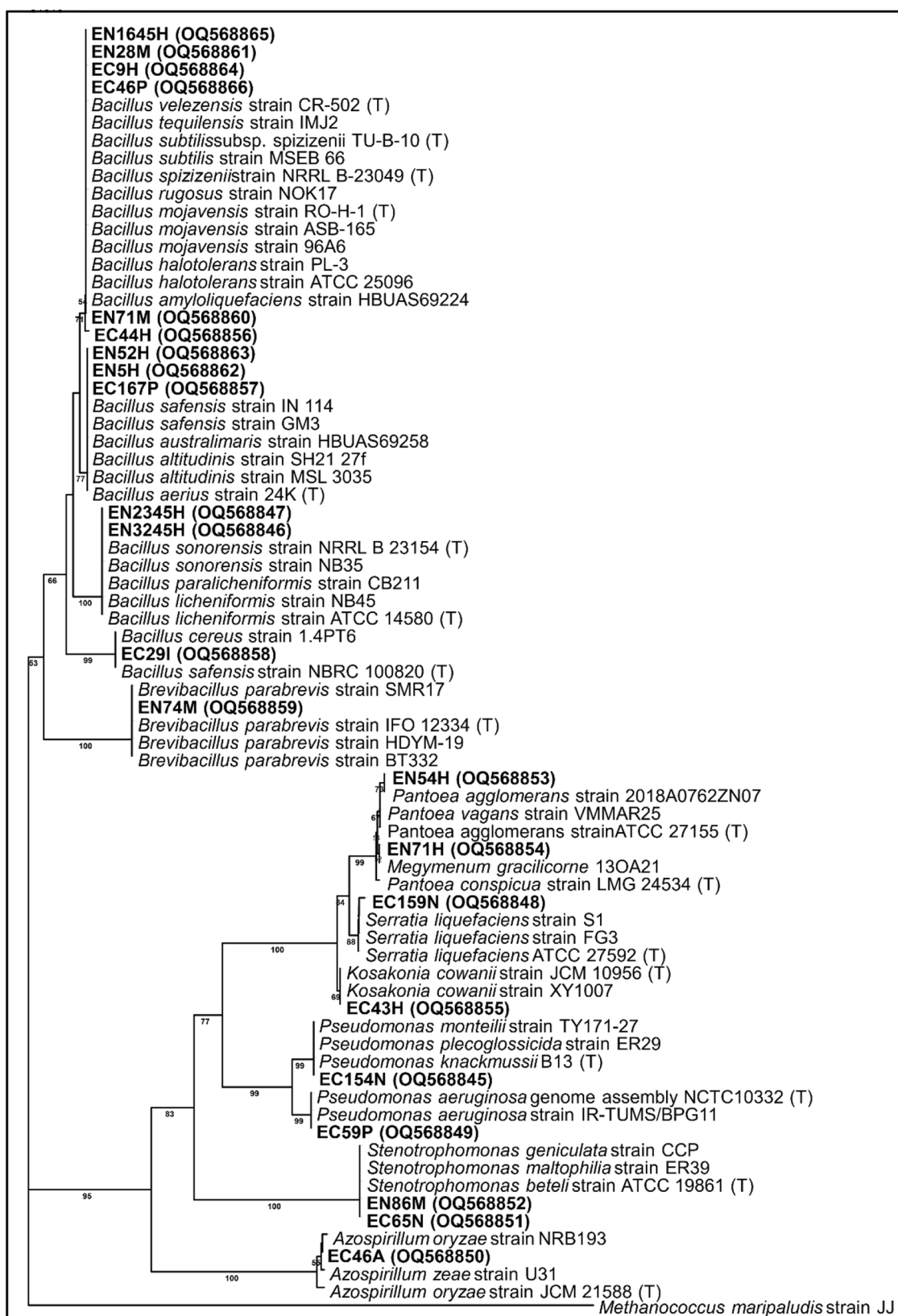


Fig. 2 Phylogenetic tree of the tested rhizobacteria isolates, constructed based on 16S rRNA gene sequences using Neighbour-Joining method with calculated Jukes-Cantor distances. The isolates from this study are indicated in bold followed by their accession numbers provided in brackets. Closest matches from the GenBank database are included, with type strains denoted by a capital "T" in brackets (T). *Methanococcus maripaludis* strain_JJ was included as an out-group strain to root the tree. Bootstrap values greater than 50% after 1000 replicates are shown at branch node

and halophytes [48]. Xerophytes are the plants that thrive in environments with low soil moisture availability, flourish in deserts and have unique adaptive features to stand desiccation such as reduced transpiration, high root osmotic pressure, deep roots, short vegetative period, low vegetation and intracellular CO₂ cycle [49]. Drought resistance of such xerophytes comprises of mainly four mechanisms: drought avoidance (DA), drought tolerance (DT), drought escape (DE) and drought recovery (DR) [50–52]. Specific traits are used to unravel prevalent plant drought resistance. They are mainly related to DA: *e.g.* moisture retention ability and water uptake and use efficiency, and to DT: mainly physiological parameters associated with osmotic adjustment, *e.g.*, osmotic potential, proline and soluble sugar content, abscisic acid concentration, and the alleviation of damage caused by drought, *e.g.* oxidative stress management through enzyme activity and chlorophyll content [52].

Arabian deserts as apart from the largest hot desert comprises one-third of the global desert area (9.4 million km²), just behind the cold deserts of Arctic and Antarctica [53]. The studied Qassim desert area lies in the arid zone of MENA region, and its natural flora is mainly of xerophytic nature [20, 54]. It is located at the center of the Arabian Peninsula known for its arid land that receives < 100 mm rain/year. As scarce information is available on the native desert plants and their associated microbiota, the present study primarily aims at filling such gap. Regarding the floristic composition, the present study showed that eleven plant species are of highest dominance and frequency as well as of utmost adaptation to the prevailing harsh environments. These xerophytic species are mainly Sahara-Arabian elements well adapted to their sand/loam/ sandy-loam soils, that are found to be very poor in organic matter (0.04–0.56%), with varied calcium carbonate contents (3.06–28.50%), non-to moderate salinity (0.20–6.83 dSm⁻¹), relatively alkaline pH (7.90–8.86); with low contents of macro-nutrients (22.4–42.0 ppm nitrogen, 1.5–19.0 ppm phosphorus and 89.7–371.0 ppm potassium) and micro-nutrients. Among the prevailing seven families, species of Compositae and Gramineae were more abundant followed by representatives of Amaranthaceae, Cyperaceae, Plantaginaceae, Scrophulariaceae and Zygophyllaceae. Such plant species are reported among the common species that grow in sand-dunes of the Afro-Asian arid deserts [18–21, 23]. They exhibited distinguished tolerance to abiotic stresses, including drought, water stress and high temperature.

In this respect, diversity of flora and associated microflora was extensively surveyed in the neighbouring MENA plant-soil ecosystems of north Sinai [4, 15, 16]. There, it is reported that communities of natural flora are found to be dominated by 31 perennial and 11 annual plant species. Perennials were represented by 17 families, among which the most abundant was reported for Amaranthaceae (20%) followed by Zygophyllaceae, Leguminosae, Compositae, Cruciferae, Boraginaceae and Gramineae (> 6–13%). Individual cases were reported for members of the families Polygonaceae, Rutaceae, Solanaceae, Geraniaceae, Nitrariaceae, Amaryllidaceae, Caryophyllaceae, Tamaricaceae and Thymelaeaceae. Among the 11 families reported for annuals, species of Compositae and Cruciferae were more abundant (20–40%) related to those of Amaranthaceae, Boraginaceae, Cleomaceae, Euphorbiaceae and Resedaceae. It is alarming that the number of reported species is limited compared to the total number of species recorded earlier, as it approached 350 species [55]. This is mainly due to climate changes in terms of the decreasing rainfall rate during the last decades, which is considered as one of the major abiotic limiting factors. Common with the xerophytic flora of the studied Qassim deserts and those reported for north Sinai were the species of *Haloxylon salicornicum* (Family Amaranthaceae) and *Artemisia monosperma* (Family Compositae). Members of the families Gramineae, Compositae and Zygophyllaceae, were common in both areas, but with different present species. Individual species of the families Scrophulariaceae, Cyperaceae and Plantaginaceae were only observed in the studied Qassim arid deserts.

Xerophytes don't stand alone in their harsh environment. Among their major partners are the microbiota, prokaryotic and eukaryotic, that are well-adapted to their host unique environments. They constitute one of the plant's inherent defense methods against both abiotic and biotic stresses [56–60]. Therefore, exploring the taxonomical/functional diversity and related mechanisms of associated microbiota under such stress conditions are among the potential gateways to improve agriculture productivity in dry environments [19, 57–61], among which is the Qassim desert area under investigation and that witness extensive projects of agricultural development. It is reported that multiple taxa of such endophytic microbiota are active in exercising multiple functions that improve a variety of parameters of plant growth and development, by direct and indirect mechanisms [61]. Further, augmentation through introduction/ inoculation practices with such bacteria supported plant growth via: (a) increasing the proline percentage in C4 plants [62], and phytohormones (as abscisic acid, auxin, ethylene, and gibberellin); (b) triggering the plant tolerance to drought stress by regulation of its morphological and physiological activities including biochemical and molecular processes; (c) further induce drought-stress responsible genes which play a crucial role to overcome the drought stress [63]. Specific examples of such introductions are reported and well-illustrated where: (a) *Azospirillum brasilense* increased the tolerance of *Arabidopsis* by increasing the abscisic acid (ABA) production [64]; (b) *Rhizophagus irregularis* efficiently colonized the root of lettuce and tomatoes and reduced the drought stress [65]; (c), the endophytic actinobacteria of *Streptomyces*

olivaceus strain DE10 colonized wheat and produced auxins to enhance its growth and tolerance [66]. As well, endophytic microbiota improved the drought tolerance by alteration of hormone profile in cucumber [67], *Lavandula dentata* [68], soybean [69], sugar cane [70] and wheat [71]. Certain endophytes enhanced the antioxidant-defense enzymes to increase the tolerance of host plants and enabling them to tolerate and survive the drought-induced oxidative stress [61, 72]. In addition, several thermo-tolerant endophytic bacteria act to alleviate the heat stress in several plants, as in soybean by *Bacillus cereus* strain SA1 [57], and in rice by *Bacillus amyloliquefaciens* [73]. Similar responses were reported for wheat and sorghum by strains of *Pseudomonas putida* [74, 75]. In addition, the literature is full of examples of consortia of mechanisms undertaken by endophytes to enrich/facilitate availability and uptake of nutrients (N, P, K, etc.) for stressed plants through transformation of major and minor elements to soluble forms [61, 76, 77]; and nitrogen fixation [15, 78]. Production of indole acetic acid (IAA) is among the mechanisms of endophytic bacteria that enabled their host plants to improve tolerance in semi-arid habitats, e.g. *Lysinibacillus* spp. and *Bacillus* spp. associated with *Prosopis strombulifera* [79] and *Gluconacetobacter diazotrophicus* together with *Saccharum officinarum* [70] and wheat [31].

In the present study, the culturable communities of microbiota, in terms of CFUs counts, nested the tested xerophytes with dense populations ($> 10^5$ CFUs g⁻¹ root). Statistically significant differences were attributed to plant species as well as compartment. Distinguished environment selection was expressed in the predominance of thermophiles and spore-formers, that are compatibly occupied > 40 – 90% of the culturable communities. Such environmental imprint that is coping with the prevailing harsh conditions was reported in the plant-soil environment of semi-arid deserts of north Sinai, Egypt [3, 4, 15, 16]. The spore-forming bacteria with their capabilities of withstanding the elevating high temperature and sun exposure did occupy a significant niche, having dense populations ($> 10^3$ to 10^6 CFUs g⁻¹ dwt) that represent > 50 – 85% of the cultural bacterial population, both on the root surfaces (rhizoplane) and sand sheath, if present. More adaptability was further expressed as the spore-forming bacteria were able to access the interiors of plant roots (endorhizosphere) with substantial populations ($> 10^3$ to 10^5 CFUs g⁻¹ dwt), representing 50 – 97% of total endophytic bacterial community [3].

Along the study, hundreds of isolates were obtained and documented the wide diversity of microbiota associated to the tested xerophytes of Qassim deserts. Identification of such isolates was compared based on both 16S rRNA gene sequencing and API profiles (Table 2). Of interest is that conformity among both techniques approached > 58 – 73% on the genus level; an indication on the reliability of using API profiling as pre-identification scheme for isolates of plant microbiota, at least on the genus level. Irrespective of plant compartments, tested xerophytes differed in hosting the detected genera of plant microbiota, where *Haloxylon salicornicum* followed by *Hordeum marinum*, *Gnaphalium luteoalbum* and *Scrophularia syriaca* were among the richest host plants (Fig. 5). Representatives of spore-formers, including *Bacillus licheniformis*, *Bacillus cereus*, *Paenibacillus polymyxa*, *Bacillus megaterium*, were the most common (65%), among which *B. licheniformis* was the most dominant (44%). As well, Gram-negative isolates (*Klebsiella pneumoniae*, *K. oxytoca*, *Pseudomonas putida*, *Ps. fluorescens*, *Ps. aeruginosa*, *Burkholderia cepacia*) were particularly enriched in the compartments of *Haloxylon salicornicum*. In this respect, reports for other species of *Haloxylon* (*H. ammodendron*) in Iran, documented that the culturable community in the plant rhizosphere was rich in representatives of genera belonging to Firmicutes and Proteobacteria, in particular *Bacillus* and *Pseudomonas* [14]. The secured isolates have different levels of tolerance to heat, salt, and drought stresses, and showed different capabilities of auxin production and phosphorous solubilization. Such PGP functions, experimentally and effectively enhanced drought tolerance in *Arabidopsis* as a model plant.

As to plant compartments, there is an indication on the predominance of Firmicutes that were differently represented in plant compartments. While *Bacillus rugosus*, *Bacillus cereus*, and *Bacillus altitudinis* reported for the ecto-rhizosphere, *Brevibacillus parabrevis*, *Bacillus halotolerans*, *Bacillus safensis* subsp. *safensis*, *Bacillus siamensis* and *Bacillus licheniformis* found in the endo-rhizosphere. Followed are members of Gammaproteobacteria which were variably represented in the ecto-rhizosphere by *Pseudomonas aeruginosa*, *Pseudomonas hibiscicola*, *Pseudomonas monteilii*, *Kosakonia cowanii* and *Serratia liquefaciens*; compared to the endo-rhizosphere being represented by *Pantoea vagans*, *Pantoea conspicua* and *Stenotrophomonas maltophilia*. The identified species of Alphaproteobacteria, *Azospirillum zeae*, *Azospirillum oryzae* and *Agrobacterium salinitolerans*, were present in the ecto-rhizosphere compartment. As well, the richness of such culturable taxa was reported in the rhizosphere of xerophytes of other arid deserts, e.g. north Sinai. Taking into consideration of the pooled data of all tested areas, cluster analysis of all xerophytes (65 xerophytes) against their associated bacterial genera (18 genera) (Fig. S5) indicated that: (a) the most predominant genera were *Bacillus* spp. followed by *Enterobacter* spp., (b) xerophytes of the families Amaranthaceae, Compositae and Gramineae hosted the highest populations and bacterial genera, particularly in the endo-rhizosphere and endo-phyllosphere. In other semi-arid deserts, e.g. Cholistan desert of Pakistan, existing xerophytes (e.g. *Haloxylon salicornicum*, *Panicum antidotale* and *Lasiurus scindicus*) were much enriched with *Bacillus subtilis*, *Bacillus licheniformis* and *Brevibacillus borstelensis* [80]. This is in addition to a low frequency

of some other bacterial species like *Paenibacillus dendritiformis*, *Pseudomonas fluorescence*, *Pseudomonas marginalis* and *Stenotrophomonas rhizophila*.

In north Africa deserts, microbiota associated to argan (*Argania spinosa*) was investigated based on both the cultivable fraction (based on the 16S rRNA gene sequences of the isolated strains.) and on the entire bacterial microbiota (based on the Illumina 16S rRNA gene dataset). In contrast to what is generally reported for conventional agricultural systems, the lower richness of the bacterial community inhabiting the bulk soil was reported in comparison to those colonizing the rhizosphere, and which is common in desertic areas [19]. In accordance with previous high-throughput taxonomic characterization of the endophytic bacterial communities, the microbiota of argan root system, was dominated by Proteobacteria, Bacteroidetes, and Actinobacteria. In conformity with our results, the cultivable endophytes of *A. spinosa* were rich in the genus *Pseudomonas* making the Class Gammaproteobacteria among the most abundant cultivable endophytes of *A. spinosa*, in accordance with the taxonomic classification of endophytes selected by other plants, i.e., date palms of desert-farming agro-ecosystems [19].

Functionally, the tested representative isolates exhibited multiple plant growth promoting functions that: (a) strongly indicate their potential contribution to xerophytes nutrition, health and survival under extreme arid conditions and (b) recommend their future manipulation to support growth and productivity of related plant genotypes introduced in desert farming. Nitrogen fixation, in terms of acetylene reducing activities ($> 20\text{--}70$ nmoles C_2H_4 culture $^{-1}$ h $^{-1}$) was commonly reported for isolates representing the endo-rhizosphere (93%) compared to the ecto-rhizosphere (33%). The highest activities (> 100 nmoles C_2H_4 culture $^{-1}$ h $^{-1}$) were reported for the spore-forming *B. licheniformis*, *B. megaterium*, *Paenibacillus polymyxa* and the non-spore forming *Ps. fluorescence*, *Stenotrophomonas maltophilia* and *K. oxytoca*. The majority of isolates actively produced IAA (> 10 mg L $^{-1}$), being rather common (> 70 and 100%) among isolates represented the ecto-rhizosphere and endo-rhizosphere respectively. The highest activities were particularly common among the non-spore forming species of *K. oxytoca*, *Stenotrophomonas maltophilia*, *Ps. fluorescence* and *Pantoea* spp. In addition, P and K solubilization (PSI ranged from 1.0 to 5.5) was common and co-shared among the majority ($> 50\%$) of isolates represented the ecto-rhizosphere and the endo-rhizosphere. Among the most active ecto-rhizosphere isolates were *K. pneumoniae*, *Burkholderia cepacia*, *Brevibacillus brevis*, *Azospirillum* spp., *Ps. fluorescence*, *Serratia marcescens*, and *B. cereus*. For endo-rhizosphere, the highest values reported for the species *K. oxytoca*, *K. aerogenes*, *Stenotrophomonas maltophilia*, *Pantoea* spp., *Burkholderia cepacia*, *B. coagulans* and *B. licheniformis*.

Of interest is the adaptive mechanisms that allow such functional community of tested microbiota to exhibit salt tolerance up to 10% NaCl, in particular those present in the endo-rhizosphere. It appeared that the very good growth at the highest salt concentration (10% NaCl) was common among isolates of *B. licheniformis*, *Paenibacillus polymyxa*, *B. pumilus*, *B. megaterium* and *B. subtilis*. Further, the majority of tested isolates possessed the ability of mineralization of nitrogen in terms of ammonia production. Progressively, the isolates expressed versatile metabolic activity that would support their growth and survival under the existing stressed environment. The applied API-ZYM assay is originally used and designed for fingerprinting of bacteria e.g. [81] and lately adapted to fungi [35]. It is considered a simple, rapid, and semi quantitative test of a variety of constitutively expressed carbohydrate, protein and lipid-degrading enzymes. The tested Gram-positive and Gram-negative isolates differed in their enzymatic spectra, as the Gram negative isolates showed broader enzyme activities, expressing variable activities with $> 9\text{--}18$ enzymes. Relating enzyme activities of isolates to their host plants, it appeared that the highest activities were reported for those isolates obtained exceptionally from the host plant of *Haloxylon salicornicum* followed by *Scrophularia syriaca*, *Cyperus conglomerates*, *Schismus barbatus*, *Cutandia dichotoma*, *Artemisia monosperma*, and *Anthemis melampodina* (Fig. 6). In spite of distinguished differences in metabolic capabilities among the bacterial species, all of the substrates tested were utilized by one or more of the tested isolates. Hence, it is proved that beside the taxonomic diversity of xerophyte-colonizing microbiota, functional diversity of their communities prevails. The broad spectra of enzyme production by the tested isolates of associated microbiota illustrate that they had different but complementary enzymatic processes. This is echoed in playing a significant role in nutrient mobilization and uptake in such arid soils that significantly contribute to the xerophytes growth/performance in order to survive and cope with existential threat of aridity. In fact, such taxonomical and functional diversity together with reported complementary enzyme spectra of root microbiota community of tested xerophytes speaks well to their integral membership among the 'plant holobiont', the plant per se and its microbiota [82, 83]. Now, it is widely recognized the major contribution of microbiota associated with specific plants living in the most diverse biomes, from

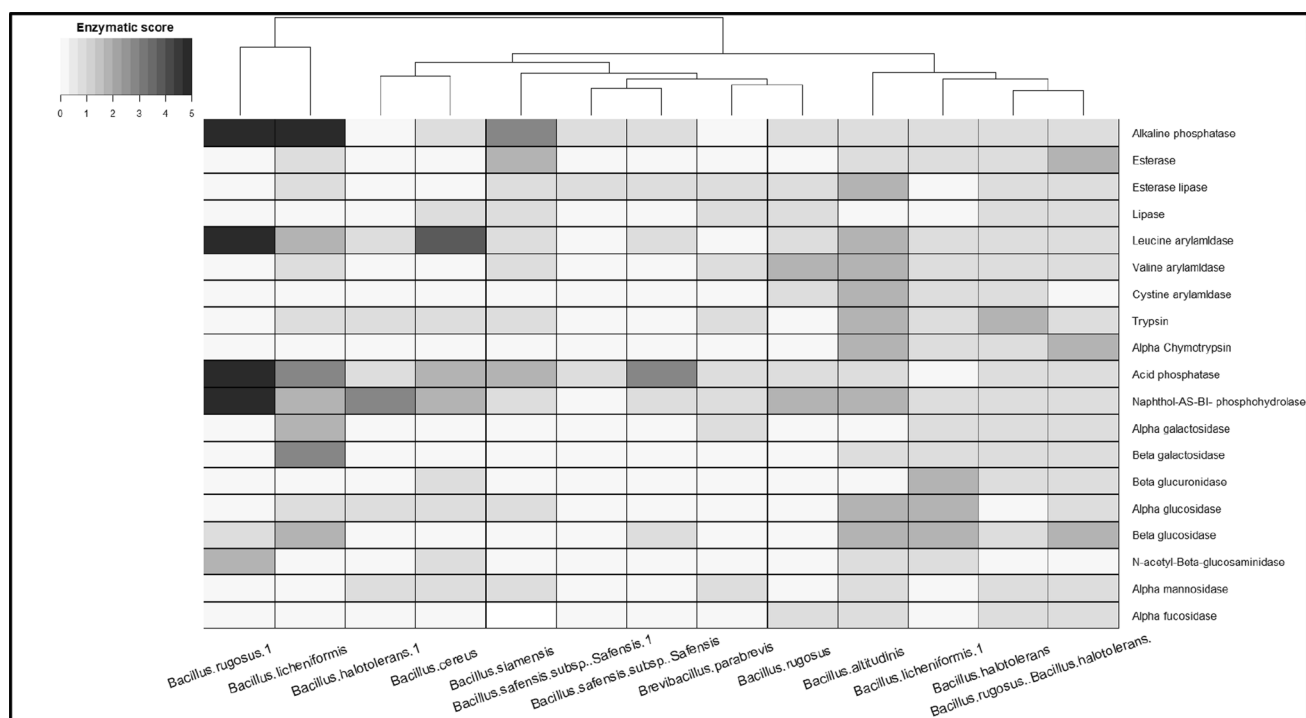


Fig. 3 Constitutive enzyme production of Gram-positive isolates of ecto/endo- rhizosphere of tested plants determined with the API-ZYM tests. Cells are shaded according to the intensity of enzymatic hydrolysis of the 19 substrates of the test from zero (null reaction) to five (strongest reaction). The maximum likelihood tree was constructed by using gplots package and R v.4.0.2 (<https://www.r-project.org/>) and R-studio (<https://www.rstudio.com/>)

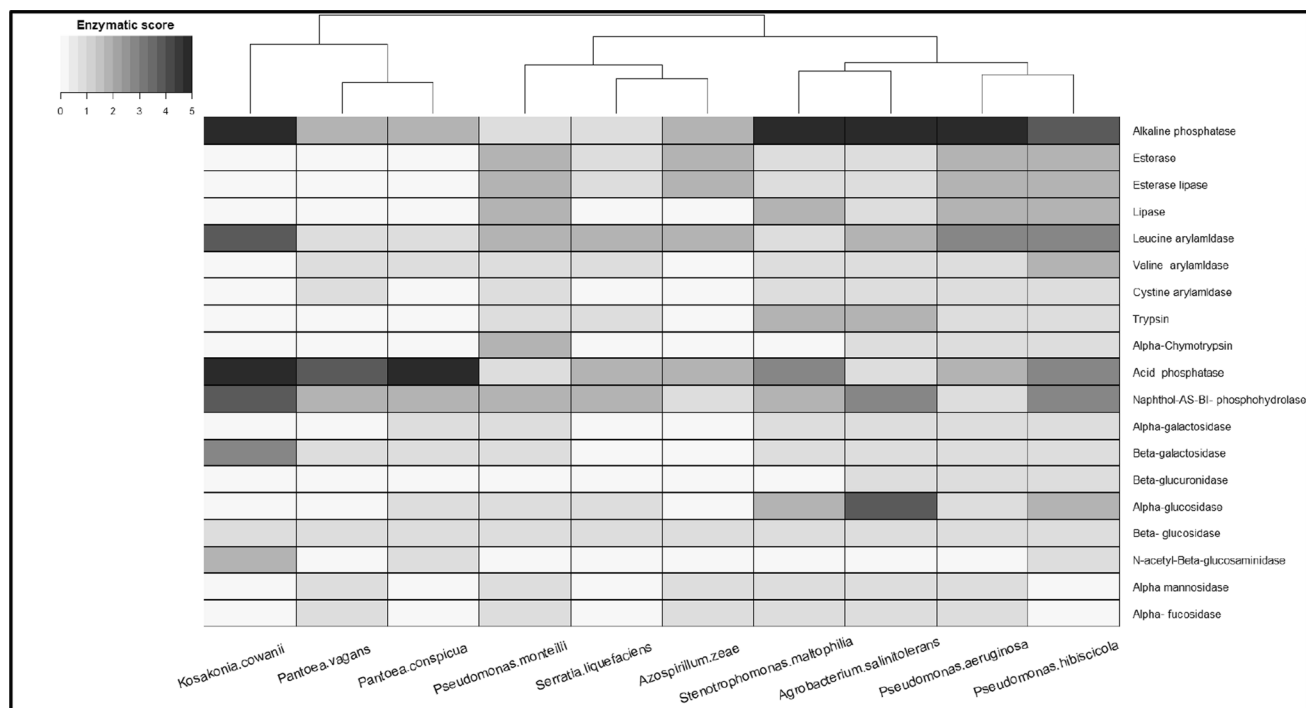


Fig. 4 Constitutive enzyme production of Gram-negative isolates of ecto/endo- rhizosphere of tested plants determined with the API-ZYM tests. Cells are shaded according to the intensity of enzymatic hydrolysis of the 19 substrates of the test from zero (null reaction) to five (strongest reaction). The maximum likelihood tree was constructed by using gplots package and R v.4.0.2 (<https://www.r-project.org/>) and R-studio (<https://www.rstudio.com/>)

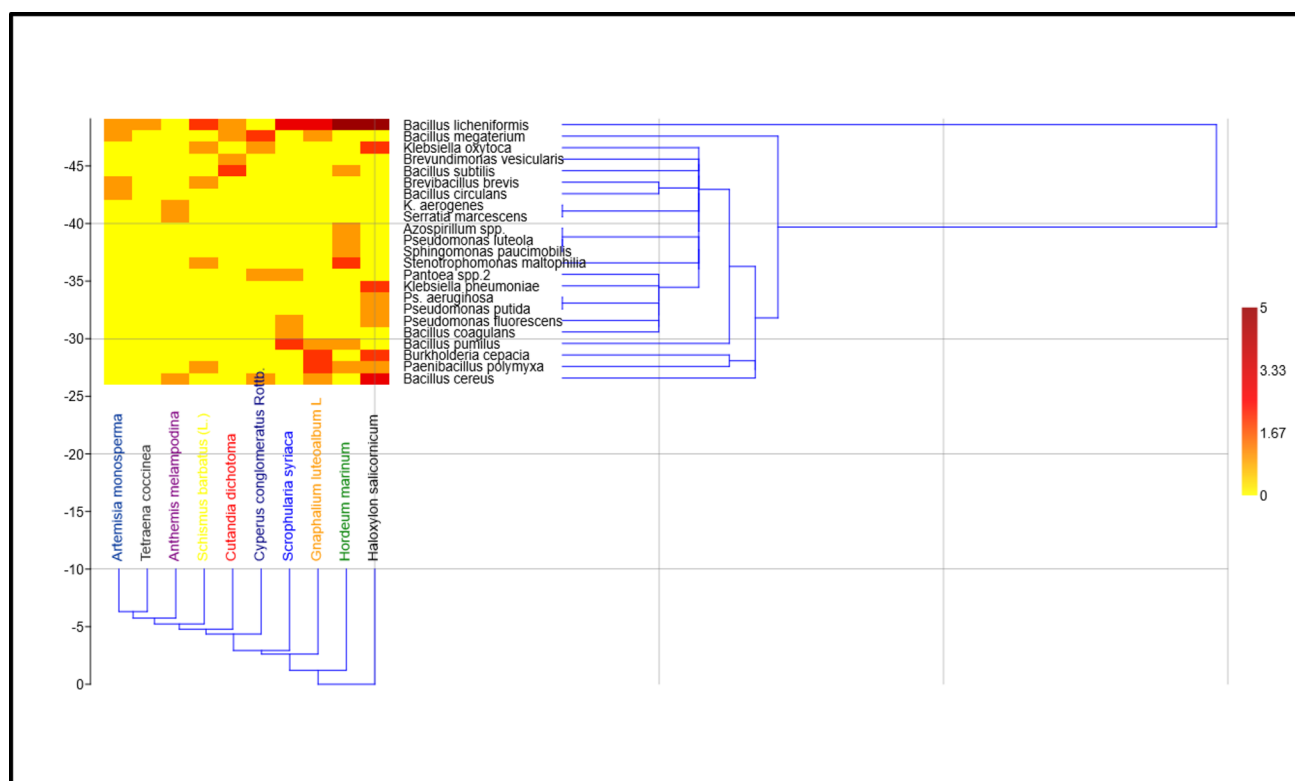


Fig. 5 Heatmap clustering representing the richness of tested xerophytes in the obtained species of culturable bacteria, irrespective of plant compartments (endo-/ecto-rhizosphere)

natural environments to intensively manage ecosystems. Further, recent studies raised fundamental questions about the regulation of plant–microbe interactions in the context of plant immune system, plant nutrition and development, and the factors that shape microbiota diversity [84]. This directed research towards the potential improvement of plant growth/productivity through application of microbiota-mediated strategies that depends on the introduction of diverse/functional synthetic communities (SynCom) of compatible microbiota to related plant genotypes, especially under stress conditions similar to those prevailing in arid deserts.

5 Conclusions

The obtained results confirm previous reports on xerophytes in arid deserts of the world, being not biotically- disconnected in their environment rather nested by rich, diverse and functional taxa of microbiota. Here, we document that the arid desert of Qassim represents a distinguished floristic biodiversity where representatives of more than 7 families of xerophytes flourish and prevail. They act as a unique platform of interacting xerophytes communities to associate with diverse and dense population of culturable microbiota. The prevailing xerophytes housed multiple genera of bacteria representing the dominant phyla of Firmicutes, Gammaproteobacteria and Alphaproteobacteria. In both ecto-/endo-rhizosphere of tested xerophytes, they are represented by composite species of *Bacillus* sp., *Brevibacillus* sp., *Pseudomonas* sp., *Serratia* sp., *Kosakonia* sp., *Pantoea* sp., and *Azospirillum* sp. Besides such awesome taxonomic diversity, they are equipped with array of PGP functions nominating them as potential microbial resources and future candidates of synthetic communities (SynCom) for microbiota-mediated strategies under drought stress. Such strategies are among the promising interventions that should be taken into consideration to support the on-going active agricultural development projects in arid deserts.

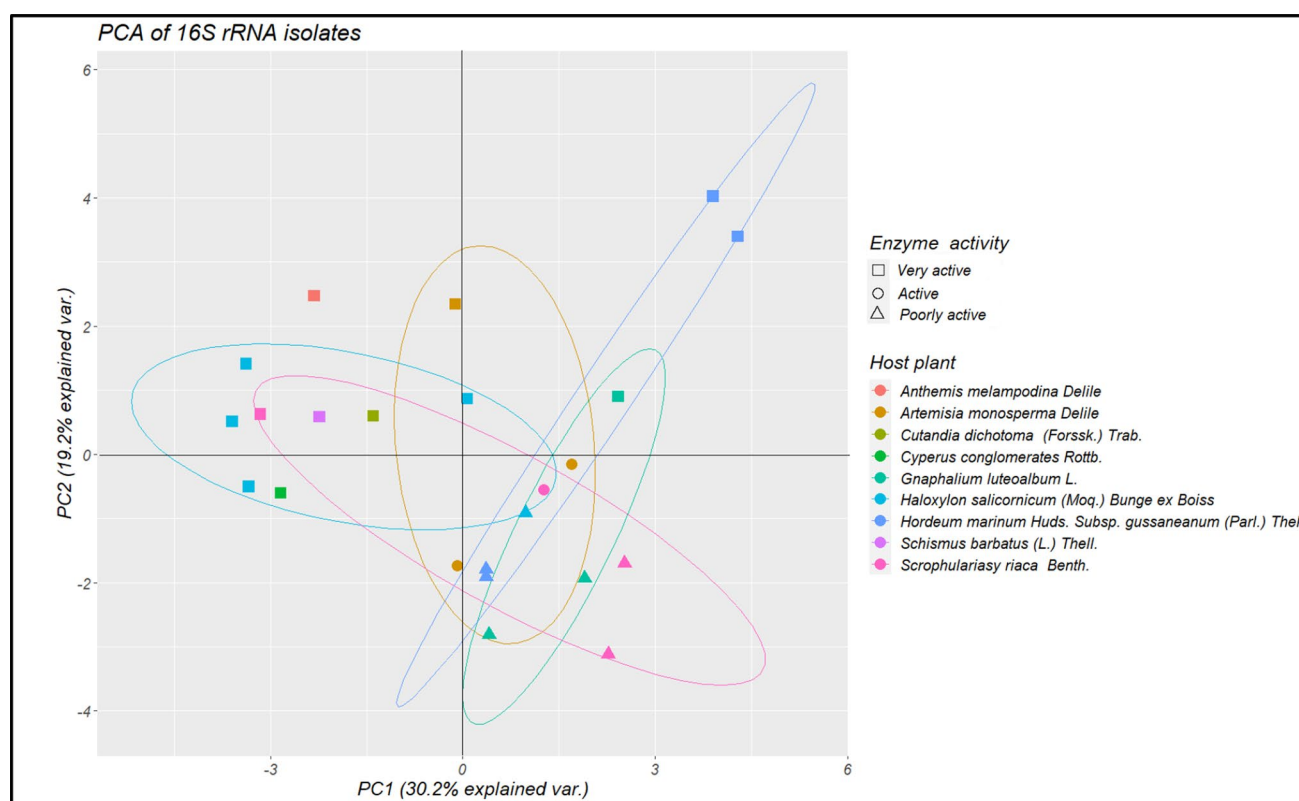


Fig. 6 Principal component analysis of the entire 16s rRNA gene sequencing isolates showing the relatedness of their enzymatic activity compared to host plants

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Plant guidelines The collection of plant material, complied with relevant institutional, national, and international guidelines and legislation.

Author contributions Mervat Hamza: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing—review & editing. Mohamed Abbas: Investigation, Validation, Writing—reviewing & editing. Hanan Youssef: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization. Eman Nour: Data curation, Formal analysis, Software. Mahmoud Abdelwahab: Data curation, Formal analysis, Software, Visualization, Roles/writing—original draft. Gylaine Tchakounté: Formal analysis, Methodology. Amal Othman: Methodology, Formal analysis, Roles/writing—original draft. Gehan Youssef: Methodology. Nada Moner: Methodology, Visualization. Randa Abdel-Fatah: Methodology, Visualization. Wafaa Amer: Conceptualization, Resources, Roles/writing – original draft, Writing—review & editing. Mohamed Fayed: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Roles/writing—original draft, Writing—review & editing. Ahmed I. Al-Turk: Conceptualization, Resources, Roles/writing—original draft, Writing—review & editing. Silke Ruppel: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Roles/writing—original draft, Writing—review & editing. Nabil Hegazi: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Roles/writing—original draft, Writing—review & editing. All authors have read and agreed to this version of the manuscript.

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Data availability All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate The plant specimens were collected from Qassim arid deserts, kingdom of Saudi Arabia, with permission of the Agricultural Directorate of Qassim province and under supervision of College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia c/o Prof. Dr. Ahmed I. Al-Turk, the Vice Dean. The voucher specimens identified by Prof. Wafaa M. Amer (Botany Department, Faculty of Science, Cairo University, Egypt) and deposited in Cairo University Herbarium (CAI). It was established from 1927, specimens ID are outlined in Table (1).

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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