Oasis desert farming selects environment-specific date palm root endophytic communities and cultivable bacteria that promote resistance to drought

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Summary

Oases are desert-farming agro-ecosystems, where date palm (Phoenix dactylifera L.) plays a keystone role in offsetting the effects of drought and maintaining a suitable microclimate for agriculture. At present, abundance, diversity and plant growth promotion (PGP) of date palm root-associated bacteria remain unknown. Considering the environmental pressure determined by the water scarcity in the desert environments, we hypothesized that bacteria associated with date palm roots improve plant resistance to drought. Here, the ecology of date palm root endophytes from oases in the Tunisian Sahara was studied with emphasis on their capacity to promote growth under drought. Endophytic communities segregated along a north–south gradient in correlation with geo-climatic parameters. Screening of 120 endophytes indicated that date palm roots select for bacteria with multiple PGP traits. Bacteria rapidly cross-colonized the root tissues of different species of plants, including the original Tunisian date palm cultivar, Saudi Arabian cultivars and Arabidopsis. Selected endophytes significantly increased the biomass of date palms exposed to repeated drought stress periods during a 9-month greenhouse experiment. Overall, results indicate that date palm roots shape endophytic communities that are capable to promote plant growth under drought conditions, thereby contributing an essential ecological service to the entire oasis ecosystem.

Introduction

Desert oases are fragile ecosystems, where environmental conditions are milder than those in the surrounding desert ensuring fertility and allowing desert farming (Mekki et al., 2013). Date palm (Phoenix dactylifera L.) is a keystone species in the oasis ecosystem because it conditions the oasis microclimate by controlling air temperature, humidity and soil–water dynamics, making it suitable for agriculture (de Grenade, 2013). However, date palm cultivation is threatened by low rainfall, high temperatures, water resources often high in salt content and high incidence of pests (Downer, 2004; El-Juhany, 2010; Barreveld, 1993).

Plant-associated microbes (rhizobacteria and endophytes) benefit the host by positively affecting paedogenesis and nutrient availability, stimulating growth, suppressing diseases, inducing abiotic stress tolerance and influencing crop yield and quality (Puente et al., 2004; Mapelli et al., 2012; Berg et al., 2013). Changes in the structure of plant-associated bacterial communities towards the selection of assemblages that are metabolically and physiologically adapted to abiotic stress, improve the resistance to stressors such as drought by providing different services to plants: (i) production of exopolysaccharides that protect roots from mechanical stress determined by dry soil compactness, (ii) promotion of osmolyte accumulation that contribute to reduce cell dehydration, (iii) enzymatic and non-enzymatic alleviation of oxidative stress and (iv) synthesis of hormone-like substances that modulate root development and hormone...
homeostasis (Ahmad et al., 2008; Balloi et al., 2010; Berard et al., 2012; Lau and Lennon, 2011; de Zelicourt et al., 2013).

Despite evidence that endophytic bacteria are capable of supporting plant growth under stress (Rolli et al., 2015), no data exist on the date palm endophytic bacteria and their role in the protection against drought conditions.

Here, we aim to assess the ecology, rhizocompetence and capability of date palm endophytic bacteria to support plant resistance to drought. We will address the following questions: (i) are geo-climatic factors involved in shaping the endophytic community structure in date palm root?, (ii) do endophytes on date palm roots have plant growth promoting (PGP) functional traits linked to drought resistance?, (iii) are cultivable endophytes rhizocompetent and do they present cross-colonization capacities in different plants? and (iv) do date palm root endophytes favour plant tolerance to drought stress?

Results

Inter-oases beta-diversity of the endophytic bacterial community in the root of date palm

The oases selected for this study (Fig. S1A) were private agriculture farms, characterized by a traditional management: old date palm tree plantations, organic fertilization and irrigation by submersion using deep aquifer fossil water. The oases located along north–south aridity transect were characterized by poorly fertile soils, with loess soils in the northern oases (BD-16, BD-B and BD-C) and sandy-silt soil in the southern ones (BD-1, BD-5, BD-8 and BD-9).

Similar numbers of 16S rRNA gene copies of endophytic bacteria were measured by quantitative real-time polymerase chain reaction (qRT-PCR) in the root tissues of date palms from seven Tunisian desert oases [permutational multivariate analysis of variance (PERMANOVA); df = 6, 20; F = 1.1; P = 0.4; Fig. S1B].

Multiple-band PCR-Denaturing Gradient Gel Electrophoresis (DGGE) profiles of the 16S rRNA gene were observed in the root tissues, demonstrating discrete endophytic bacterial communities inhabiting the date palm root tissues along the examined north–south aridity transect (Fig. S2). Principal coordinate analysis of PCR-DGGE gel-band profiles explained 81.5% of the total variability and indicated a north–south segregation pattern of bacterial communities relative to the latitudinal position of the oases (Table S1) with respect to the Chott El Jerid saline system (Fig. 1A); these results were confirmed by canonical analysis of principal coordinates, where a clear separation between northern–southern oases is evident (Fig. 1B). Endophytic bacterial communities segregated between oases located to the north (BD-16, BD-B and BD-C) and south (BD-1, BD-5, BD-8 and BD-9) of the Chott El Jerid as supported by PERMANOVA pair-wise test (PERMANOVA, df = 1, 20; F = 20.19; P < 0.001; Table S2). Among the environmental factors examined (Table S1), a marginal test revealed that latitude, longitude, altitude, temperature and rainfall were statistically significant driving forces determining the structure of root-associated bacterial communities (Table 1A). However, latitude and temperature proved to be the dominant geo-climatic variables shaping the structure of endophytic bacterial communities (Table 1B). We assessed the linkage of the environmental factors through multiple correspondence that showed they were related each other with the only exception for the minimum rainfall (Fig. S3).

PGP functionality of cultivable endophytic bacteria associated with date palm roots

A total of 120 bacterial strains were isolated from the root tissues of date palms cultivated in the oases sampled.
Isolates were assigned to five phyla, **Proteobacteria** (76%), consisting of **Betaproteobacteria** (6%) and **Gammaproteobacteria** (70%), **Actinobacteria** (17%), **Firmicutes** (6%) and **Bacteroidetes** (1%) (Fig. 2A), consistent with the cultivation-independent approach (Table S3). Phylogenetic analysis performed on the isolates showed that species belonged to 11 bacterial genera (Fig. 2B), where **Pseudomonas** were the most common, representing 27% of the collection (35% in the southern oases and 16% in the northern oases).

The 28 different genotypes identified following de-replication of the collection by internal transcribed spacer-PCR (ITS-PCR) fingerprinting were characterized for PGP activities in vitro (Fig. 2C). Potentially beneficial strains, according to their PGP scores indicating multiple PGP and biocontrol traits and resistances to different abiotic stresses, were recovered from all the date palm roots samples. While none of the isolates displayed all the assayed PGP traits, 13 strains presented 12 or more and 15 strains ranged between 8 and 11 PGP traits. All isolates showed potential adaptation to unfavourable environmental conditions typical of arid soils through halotolerance (26 strains grew in media with 5% NaCl added) and resistance to low-water availability (28 strains) and variable temperature ranges (10 strains). None of the strains grew at 50°C and only six strains tolerated 10% NaCl in the growth medium. Plant growth promotion potential properties, such as those related to nutrient provision (phosphate solubilization and siderophore release) and auxin synthesis were equally distributed among isolates. Exopolymers (EPS) production was primarily presented by **Pseudomonas** strains. Seventy five per cent of the strains were active against both **Botrytis cinerea** and **Aspergillus niger**.

Two strains with contrasting PGP scores (Fig. 2C), **Pseudomonas frederickbergensis** E102 and **Pseudomonas brassicacearum** E141 strains, were selected for further studies on the rhizocompetence and the in vivo PGP potential on date palm.

In vitro root adherence and co-colonization assay on date palm and Arabidopsis thaliana

Date palm root colonization by strain E141 was studied using a gfp-labelled mutant. Colonization was observed to persist after 72 h (Fig. 3A). Co-colonization competition assay with an exogenous bacterial competitor demonstrated a selective colonization capacity of strain E141. After 72 h exposure to dsRED-labelled **Escherichia coli** and gfp-labelled E141, only cells of the latter were detected on date palm root rhizoplane (Fig. 3B). Root colonization by the gfp-labelled E141 strain was also observed on **Arabidopsis** root. Along time course experiments, microscopic observations of **Arabidopsis** roots exposed for 1 h to the gfp-labelled **P. brassicacearum** strain E141 revealed green fluorescent cells along the

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**Table 1.** Environmental factors shaping the structure of date palm root endophytic bacterial communities from oases in southern Tunisia.

<table>
<thead>
<tr>
<th>(A) Marginal test</th>
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DistLM analysis was performed on individuate geo-climate variables influencing the structure of the bacterial community associated with date palm root tissues. Geo-climatic variables tested included latitude (Lat N), longitude (Long E), altitude (Alt); minimum temperature (T min), maximum temperature (T max), minimum rainfall (Rainfall min) and maximum rainfall (Rainfall max). (A) The marginal test considers each geo-climatic variable and its contribution to explaining the total variability. (B) Sequential test to explain the total variation from variables. F, statistic F; P, probability (statistically significant variables are shown in bold, P < 0.05); Prop, proportion of total variation explained; AIC, Akaike information criterion; Cumul, cumulative variation explained by the listed variables; Res df, residual degrees of freedom.
Fig. 2. Diversity and PGP potential of date palm root-associated cultivable endophytic bacteria. Phylogenetic allocation of the isolates from date palm root tissues at class (A) and genus (B) levels. (C) PGP potential of the isolates is indicated as a PGP score, determined by the sum of the number of PGP potential abilities exhibited by each strain. Abiotic stress resists. = abiotic stress resistance; Bioc. = biocontrol potential; P Sol. = inorganic phosphate solubilization; Sid. = siderophore production; N fix. = nitrogen fixation; EPS = exopolymers release; IAA = auxin production; Amm. = ammonia production; Prot. = protease activity; accd = ACC deaminase activity; PEG = polyethylene glycol. The strains marked in grey are those used for the in vivo date palm experiment.
primary root (Fig. S4A). After 3 h of exposure, the organization of bacterial cell microcolonies was observed both on primary roots and root hairs (Fig. S4B). Five hours after exposure to the strain, the expected arrangement of gfp-labelled cell clusters continued to adhere to the rhizoplane (Fig. S4C). The gfp-labelled E141 strain had considerably colonized the primary root after 24 (Fig. S4D) and 48 h, while no cells of the dsRED-labelled E. coli were observed on the root after 48 h from the initial bacterization (Fig. 3C). The experiment confirmed the ability of strain E141 to establish on the root system of a plant species other than its original host. Fluorescent gfp-labelled E141 cells were detected in abundance on the rhizoplane of both the studied plant root systems, and were also visible in longitudinal sections of the roots, suggesting its ability to penetrate root tissues (Fig. 3B and C).

In addition, closer quantitative evaluation showed that both Pseudomonas spp. strains E102 and E141 efficiently colonized the roots of all three date palm cultivars (Deglet Nour, Safawy and Sofry) at high density (Fig. 3D). The strain E102 maintained a constant population density at about 10⁴ CFU g⁻¹ of tissue on all three cultivars. Similar values were observed with E141-treated roots, with the exception of the Sofry cultivar that had a density of 10³ CFU g⁻¹ on root tissue. No kanamycin-resistant isolates other than the strains E102 and E141 were obtained from inoculated plants or non-inoculated control plants, indicating that no contamination by spontaneous kanamycin-resistant bacteria occurred.

**Date palm growth promotion under controlled drought stress**

Strains E102 and E141 were tested for their in vivo PGP potential during a 9-month-long growth experiment that included 10 successive periods of induced water stress. During the experiment, field capacity was maintained at
50% while during the water stress periods, it was decreased down to 15–20% by interrupting irrigation for periods of time ranging between 6 and 12 days. Field capacity is indicated in grey; actual temperature in the greenhouse is shown by a black line; annual min (blue line) and max (red line) temperatures in the Ksar Ghilane oasis at the same time as the greenhouse experiment. Date palm growth promotion after a 9-month period of fluctuating water stress following the initial inoculation with two strains (\textit{P. Brasicacearum} E141 and \textit{P. Frederiksbergensis} E102) isolated from date palm roots sampled in the Ksar Ghilane oasis. Plant biomass after 9-month growth, expressed as mean of shoot (B) and root (C) fresh and dry weights ± standard error; number of leaves (D) and root and shoot length (E) ± standard errors. The data (averages, SDs and number of plants are reported in Table S4) were statistically analysed through a PERMANOVA (Table S5); different letters represent statistically significant differences ($P < 0.05$) for each parameter.

**Discussion**

The environmental conditions of the oases were found to influence the diversity of the endophytic bacterial communities in the date palm roots, but not the cell abundance, differently from what was observed in the rhizosphere (Ferjani et al., 2014). The selection of endophytic bacteria by root tissues is a complex process controlled by several factors including, the plant physiological features, the soil geochemical characteristics, the agriculture management practices and the geo-climatic conditions (Hardoim et al., 2008; Berg and Smalla, 2009; Angel et al., 2010; Bachar et al., 2011; Siemons et al., 2013).
et al., 2010; Chaparro et al., 2013; Ding et al., 2013; Schlaeppi et al., 2014). We found that latitude and temperature influenced beta diversity of root-associated endophytic bacteria, such that they could clearly be separated into two groups, those South and those North of the Chott El Jerid saline lake in southern Tunisia. Results support the conjecture that beta diversity of date palm endophytic communities varies along a north–south environmental gradient and indicate that PCR-DGGE of 16S rRNA gene was able to detect such variations in beta diversity. Even though PCR-DGGE is not anymore adequate for assessing alpha diversity in comparison with next-generation sequencing (NGS), several recent studies have shown that community fingerprinting techniques, including PCR-DGGE, are still useful for analysing beta diversity, where they support the same conclusions as NGS techniques (Cleary et al., 2012; Gobet et al., 2014; Van Dorst et al., 2014).

To improve the effectiveness of isolation of root endophytes from the inner part of date palm root, we have initially tested a root-peeling approach (Sessitsch et al., 2002). However, since we were not able to obtain intact root cores, we have adopted a surface sterilization procedures with bleach and ethanol treatments (Sun et al., 2008) that did not affect the root structure. Cultivable date palm endophytic bacteria were primarily from the class Gammaproteobacteria. Gammaproteobacteria-rich root tissues have been documented in other arbo real plants growing in arid regions such as grapevines (Marasco et al., 2013a), banana trees (Souza et al., 2013) as well as trees grown in conventional environments (Lodewyckx et al., 2002; Taghavi et al., 2009). Gammaproteobacteria of the genus Halomonas were observed only in Ksar Ghilane, the southernmost oasis examined in this study. The majority of soil in arid and semiarid areas is salt affected, particularly in oases, where salt accumulation is caused by a combination of factors including poor quality of irrigated water, poor or no drainage, shallow saline water tables and salinization of soil and groundwater (Marlet et al., 2009). Halomonas strains with PGP properties have previously been observed in association with halophytes such as Salicornia spp. (Argandonha et al., 2005; Jha et al., 2012) including those in the same area of the oases studied here (Mapelli et al., 2013).

The most frequent endophytic bacteria among the isolates belonged to the genus Pseudomonas well known for its PGP properties (Ali et al., 2013; Roca et al., 2014). Pseudomonads from the date palm root endosphere were capable to enhance phosphate solubilization, release siderophores, fix nitrogen, produce phytohormones and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, produce EPS and grow at low water activities in presence of 20% polyethylene glycol (PEG). These features may support, directly or indirectly, plant growth under drought favouring water uptake (Compant et al., 2010; Daffonchio et al., 2015).

The above-mentioned isolates represent bacterial species capable of forming colonies after 48 h incubation on agar plates. However, these fast-growing bacteria represent a fraction of the root system microbiome (Hardoim et al., 2008). It should be considered that several slow-growing bacteria such as many Actinobacteria are also an important component of the root system microbiome, and they would deserve further studies in the date palm (Sessitsch et al., 2002; Qin et al., 2015).

We have demonstrated that date palm roots provide a suitable microenvironment for bacteria with multiple PGP traits. Fully developed metabolic interactions require physical contacts between plants and their endophytes (Brader et al., 2014). Before bacteria can express any PGP traits, endophytes must adhere to the rhizoplane, colonize the root system and finally spread throughout the plant’s tissues (Hardoim et al., 2008; Marasco et al., 2013b; Rodríguez-Navarro et al., 2007). The Pseudomonas strains E102 and E141 efficiently cross-colonized different plant species and date palm cultivars, confirming their endophytic lifestyle.

Under drought conditions, Deglet Nour date palms treated with Pseudomonas spp. E102 or E141 appeared healthier, with increased fresh and dry biomass of the roots and the whole plant compared with non-inoculated plants. Because of the increase in plant dry weight rather than in water content, bacteria can be accredited for a capacity of stimulating growth and increasing biomass. Plants with more root biomass can efficiently extract residual soil water during drought-stress periods, favouring overall plant growth as observed in shoot weights. Therefore, in addition to the nutritional and protective effects that have been shown for oil, banana and coconut palms (Andrade et al., 2014; Bakhtiar et al., 2013; García et al., 2012; George et al., 2013; Mia et al., 2010), endophytic bacteria also help palms cope with water stress and drought, two essential obstacles to desert farming. The versatility of these strains in root colonization, general PGP activities and specific promotion of resistance to drought suggests that such drought-protective activity likely takes place naturally in oasis ecosystems.

**Experimental procedures**

**Study sites and preparation of date palm root tissues**

During March 2010, root samples of date palm (Phoenix dactylifera L. cv. Deglet Nour) were collected from seven different oases located along an aridity-latitude gradient in Tunisia (Fig. S1A) characterized by different geo-climatic traits (Table S1). In each sampling station, the roots of three healthy Deglet Nour date palms of similar age were collected.
at 40–60 cm depth, where the root system is most dense and active. All root samples were collected using sterile tools. In the laboratory, the root tissues were immediately surface sterilized as described by Sun and colleagues (2008), and the efficacy of the sterilization method was verified by plating the water from the last washing step and by plating pieces of the sterilized root on Trypic Soy Agar (TSA). No colonies were obtained from all the control plates after 10 days incubation at 30°C. Recovered samples were stored at −20°C for molecular analysis and at 4°C for isolation.

**DNA extraction, RT-PCR and PCR-DGGE analyses**

Three grams of surface-sterilized roots were ground in liquid nitrogen using a sterile mortar and pestle to obtain a homogenous root tissue powder. Total DNA was extracted from 1 g of powdered root tissue using the DNeasy Plant kit (Qiagen), according to the manufacturer’s protocol. The DNA was quantified and stored at −20°C until use.

Quantitative RT-PCR was performed on a Chromo4 real-time detector (Bio-Rad) to measure the presence and concentration of bacterial populations in root tissues following the method described in Marfino and colleagues (2013).

Primers 907R and 357F with a GC-clamp were used in this study for the amplification of bacterial 16S rRNA genes (Muyzer et al., 1993). Denaturing Gradient Gel Electrophoresis (DGGE) fingerprint analysis was performed as described in Marasco and colleagues (2012). The DGGE bands were excised from the gels using a sterile cutter extracted from the gel plug and amplified as reported in Marzorati and colleagues (2006). Amplification products were sequenced by Macrogen (South Korea), and sequences were deposited in the GenBank database under accession numbers KM355719 to KM355744. The DGGE band patterns were converted into line plots using IMAGE J software to generate a matrix of x/y values (Schneider et al., 2012). Statistical analyses of the data are reported in the Supporting Material under the heading ‘Statistical Analysis’.

**Isolation, identification and in vitro PGP activities of cultivable endophytes**

One gram of sterilized root from each sampling station was ground in phosphate-buffer saline (10 mM \(K_2HPO_4\), 10 mM \(KH_2PO_4\), 0.14 M NaCl) using a sterile mortar and pestle. The suspension was diluted in 10-fold series and plated onto TSA medium. After incubation at 30°C for 48 h, fast-growing bacterial colonies representing different morphotypes were selected and spread three times on the original medium. A total of 120 purified isolates were frozen in 25% glycerol at −80°C until use.

Extracted was DNA from isolates by boiling lysis as described by Marasco and colleagues (2012). Isolates were de-replicated using the ITS-PCR fingerprinting protocol (Daffonchio et al., 1998). Representative isolates from each genotype were identified by partial sequencing of the 16S rRNA gene at Macrogen (South Korea) after PCR amplification according to Marasco and colleagues (2012). Sequences were deposited in the GenBank database under accession numbers KM355745 to KM355772.

The 28 isolates obtained after de-replication were screened for production of IAA, siderophores, mineral phosphate solubilization, nitrogen-fixation activity, EPS and ammonia production, protease activity and tolerance to drought, salt and temperature variation as described in the Method S1.

In vivo recolonization experiment of date palm and Arabidopsis root system

The isolates in the collection were transformed with the pHM2-gfp plasmid (Favia et al., 2007). Stable transformants were obtained with *P. frederikbergensis* E102 and *P. brassicacearum* E141 strains as described in the Method S2.

The gfp-labelled strain E141 was selected for colonization experiments on date palm and *Arabidopsis* following the protocol described in the Method S3. A co-colonization competition assay was performed using gfp-labelled E141 and dsRED-labelled *E. coli*, as described in the Method S4. At time intervals, bacterized roots of date palm and *Arabidopsis* seedlings were analysed by confocal laser-scanning microscopes (Leica TCSNT) with Leica Confocal Software, using BP530/30 GFP (excitation at 488 nm) and LP590 TRITC filters (excitation length at 568 nm). For some experiments (as explained in the figure legends) a Zeiss LSM 710 Upright Confocal Microscope has been used with GFP and Propidium Iodide (PI) lasers and Bright Field light (BF) and the software ZEN2009.

Quantification of the colonization of date palm roots by strains E102 and E141 were performed by selective plating of the strains transformed with plasmid pHM2-gfp as described in Method S5.

**The ability of Pseudomonas sp. to promote date palm growth in vivo under drought stress**

Date palm seeds (*Phoenix dactylifera* cv. Deglet Nour) were soaked in water for 48 h to soften the mesocarp, sterilized with 90% ethanol for 2 min and then 1% sodium hypochlorite for 15 min followed by three rinses in sterile distilled water. Surface-sterilized seeds were sown in a pot containing 3 Kg of sterilized soil mixture (3:1 soil to sand). The soil mixture was sterilized by the tyndallization method: three cycles of sterilization at 100°C for 60 min were alternated with overnight incubation at 30°C. After tyndallization, 1 g of the soil mixture was serially diluted and spread on a TSA Petri dish to evaluate the CFU. After 48 h at 30°C, the CFU g−1 of mixed soil had bacterial counts < 10⁷. Seeds were coated once with the bacterial suspension strains E102 and E141 at a concentration of 10⁶ cells g−1 of soil; non-inoculated seeds were watered with sterile tap water. Seeds were maintained at 30°C for 1 week and after germination, the pots were transferred to a growth chamber with ~100 µmol photons m−2 s−1 of light for 12 h during the day; the average temperature is reported in Fig. 4A. The in vivo experiment continued for approximately 9 months (281 days). Date palm plantlets experienced a variable degree of controlled water stress reported as a percentage of field capacity (Fig. 4A). After 281 days shoot and root fresh weight, dry weight and length and number of leaves were measured on each of the 19 replicate
plants per treatment. Statistical analyses of the data are reported in the Supporting Material under the heading ‘Statistical Analysis’.

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References


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**Supporting information**

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

- **Method S1**
- **Method S2**
- **Method S3**
- **Method S4**
- **Method S5**

**Statistical analysis**

**Fig. S1.** Quantification of endophytic bacterial communities associated with date palm roots in different oases of Tunisia. A. Date palm root tissue sample stations. The greyscale on the map indicates areas with different mean altitudes (m, a.s.l.). Filled symbols indicate the oases in the Tunisian Grand Erg Oriental in the Sahara, south of the Chott El Jerid saline lake; empty symbols indicate the oases north of Chott El Jerid.

B. A box plot quantifying 16S rRNA genes by RT-PCR. The number of copies is expressed as Log$_{10}$ for gram of root tissues.

**Fig. S2.** Bacterial community diversity associated to date palm roots. DGGE patterns of 16S rRNA gene fragments amplified from the root tissues DNA. Each sampling station (from BD-1 to BD-C) is represented by three replicates. Triangles indicate DGGE bands successfully sequenced (reported in Table S3).

**Fig. S3.** Multiple correspondence analyses (MCA) of oases geo-climatic factors.

**Fig. S4.** Time course colonization experiment of *Arabidopsis* roots using *gfp*-labelled *P. brassicacearum* E141. (A), (B), (C) and (D) respectively show root images after 1 h, 3 h, 5 h and 24 h after inoculation with strain E141. The scale bars correspond to 100 μm.

**Table S1.** Geo-climate features of the seven sampled oases.

**Table S2.** Post-hoc statistical analysis (PERMANOVA) of date palm root endophytic bacterial diversity (measured as 16S rRNA gene PCR-DGGE patterns) across locations. $t = $ post hoc pairwise test; $P =$ probability (in bold the variables statistically significant; $P < 0.05$).

**Table S3.** Identification of bands cut from the DGGE gels. We sequenced 27 DGGE bands (Fig. S2) and affiliated them with five different phyla, predominantly *Proteobacteria* and *Actinobacteria*, with 14 and 9 bands respectively. Two bands belonged to the phylum *Firmicutes* and one belonged to each of *Bacteroidetes* and *Fusobacteria*. No specific distribution pattern in relation to the location of the oases location was observed using DGGE band taxonomy.

**Table S4.** Plant growth promotion activity mediated by E102 and E141. The data are reported as average and standard deviation of 19 replicates. For each growth parameter analysed, the letters represent the significant difference between the treatments due to PERMANOVA results (Table S5).

**Table S5.** Post-hoc statistical analysis (PERMANOVA) of date palm growth promotion. $t = $ post hoc pairwise test; $P =$ probability (in bold the variables statistically significant; $P < 0.05$).