environmental microbiology reports

Environmental Microbiology Reports (2015) **7**(4), 668–678 doi:10.1111/1758-2229.12304

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

Hanene Cherif,[1†] Ramona Marasco,[2†] Eleonora Rolli,[3†] Raoudha Ferjani,[1] Marco Fusi,[2] Asma Soussi,[2] Francesca Mapelli,[3] Ikram Blilou,[4] Sara Borin,[3] Abdellatif Boudabous,[1] Ameur Cherif,[5] Daniele Daffonchio[2,3*] and Hadda Ouzari[1]**

[1]*Laboratoire de Microbiologie et Biomolécules Actives, Département de Biologie, Faculté des Sciences de Tunis, Campus Universitaire, Tunis, 2092, Tunisia.*

[2]*Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology, Thuwal, 23955-6900, Kingdom of Saudi Arabia.*

[3]*Department of Food Environmental and Nutritional Sciences, Università degli Studi di Milano, Milano, 20133, Italy.*

[4]*Plant Developmental Biology, Wageningen UR, Wageningen, The Netherlands.*

[5]*Laboratory BVBGR, ISBST, University of Manouba, La Manouba, 2010, Tunisia.*

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

Received 6 October, 2014; accepted 27 May, 2015. For correspondence. *E-mail daniele.daffonchio@kaust.edu.sa; Tel. +966 (2) 8082884. **E-mail imene.ouzari@fst.rnu.tn; Tel. (+216)70860553; Fax (+216)70860553.
[†]Equal contribution.

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.

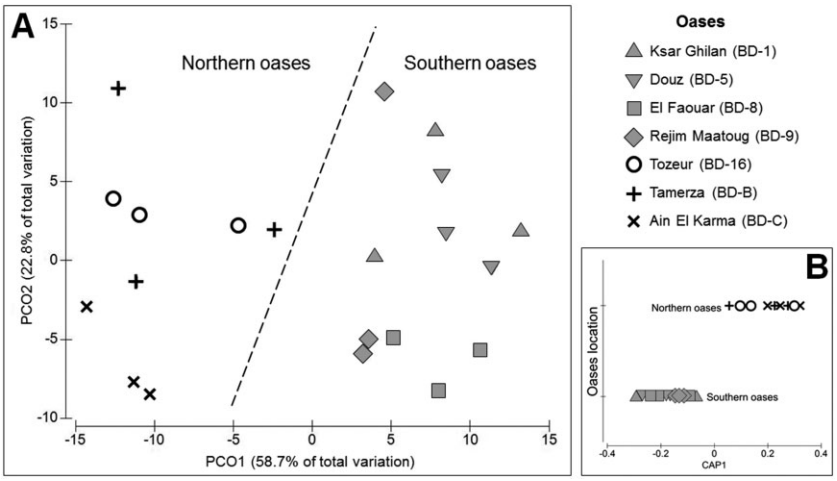


Fig. 1. Analysis of the bacterial community structure associated with date palm root tissues in different oases of Tunisia.
A. Principal coordinate analysis represents the structural diversity of endophytic bacterial communities associated with date palm root tissues. The endophytic communities of date palm roots from the northern (black symbols) and southern (grey symbols) oases compared with the Chott El Jerid saline lake exhibit two different groupings that explain 81.5% of the total variation.
B. The canonical analysis of principal coordinates confirms the separation between northern (black circles) and southern (grey circles) oases.

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

(A) Marginal test

Variables	*F*	*P*	Prop
Lat N	**16.256**	**0.0001**	**0.46**
Long E	**11.218**	**0.0001**	**0.37**
Alt (m)	**3.8354**	**0.0204**	**0.17**
T min (°C)	**8.0295**	**0.0008**	**0.30**
T max (°C)	1.9707	0.1239	0.09
Rainfall min (mm)	**7.9872**	**0.0007**	**0.30**
Rainfall max (mm)	**3.6725**	**0.0230**	**0.16**

(B) Sequential test

Variables	AIC	*F*	*P*	Prop	Cumul	Res df
+Lat N	**95.567**	**16.256**	**0.0001**	**0.46108**	**0.46108**	**19**
+Long E	95.781	1.5973	0.1725	0.04393	0.50501	18
+Alt (m)	97.382	0.32608	0.8771	0.00932	0.51432	17
+T min (°C)	**94.856**	**3.8485**	**0.0102**	**0.09417**	**0.60849**	**16**
+T max (°C)	**93.349**	**2.7265**	**0.0363**	**0.06022**	**0.66871**	**15**
+Rainfall min (mm)	92.706	1.8776	0.1068	0.03918	0.70789	14
+Rainfall max (mm)	92.706	0	1	0.00000	0.70789	14

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.

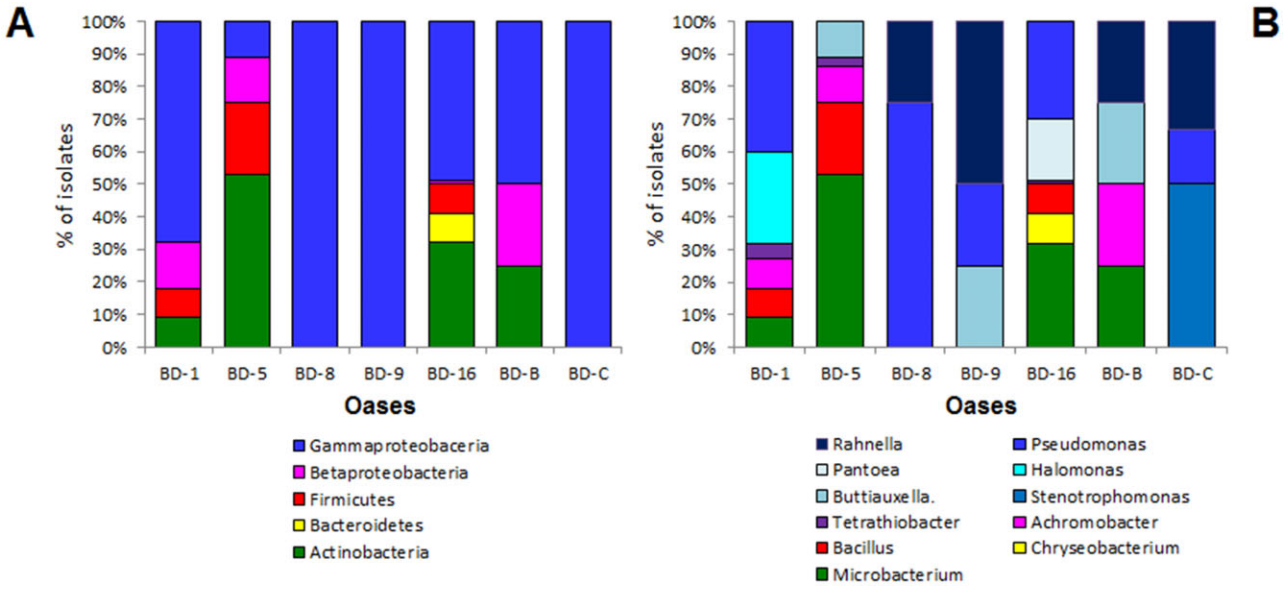
Isolates were assigned to five phyla, *Proteobacteria* (76%), consisting of *Betaproteobacteria* (6%) and *Gammaproteobacteria* (70%), *Actinobacteria* (17%), *Firmicutes* (6%) and *Bacteroidetes* (1%) (Fig. 2A), consistent with the cultivation-independent approach (Table S3). Phylogenetic analysis performed 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



C

Plant promoting and protecting potential of bacteria: ■ Yes □ No

Oases	Strains	PGP activities: P Sol.	Sid.	N fix.	EPS	IAA	Amm.	Prot.	ACCd	Abiotic stress resist.: 5% NaCl	8% NaCl	10% NaCl	42°C	4°C	50°C	10% PEG	20% PEG	Bioc.: *B. cinerea*	*A. niger*	PGP score	Isolate identification: Closest described relative (BLAST)	Acc. N°	%
BD-1	E102	■	□	■	□	■	□	□	■	□	□	□	□	■	□	■	■	□	■	8	*Pseudomonas frederiksbergensis*	(HQ824922)	99
BD-1	E103	□	□	■	□	■	□	□	■	■	□	□	□	■	□	■	■	■	■	9	*Microbacterium foliorum*	(JN99375)	99
BD-16	E633	■	□	■	□	■	□	□	□	■	□	□	□	■	□	■	■	■	■	9	*Microbacterium foliorum*	(JN99375)	100
BD-16	E634	□	■	□	□	■	■	■	□	□	□	□	□	■	□	■	■	■	■	9	*Chryseobacterium* sp.	(FJ65416)	97
BD-8	E813	■	□	■	□	■	□	■	■	■	□	□	■	■	□	■	■	□	□	10	*Buttiauxella* sp.	(HQ662633)	99
BD-8	E814	■	■	■	□	■	□	■	■	■	□	□	□	■	□	■	■	□	□	10	*Microbacterium foliorum*	(EU834263)	100
BD-5	E517	■	□	■	□	■	□	□	■	■	■	■	□	■	□	■	■	□	□	10	*Microbacterium xinjiangensis*	(DQ923131)	99
BD-5	E515	■	□	□	□	■	■	□	■	■	■	■	□	■	□	■	■	□	□	10	*Pantoea septica*	(HM854257)	99
BD-1	E123	□	□	■	□	■	■	□	□	■	■	■	■	■	□	■	■	□	□	10	*Halomonas* sp.	(FJ444981)	97
BD-16	E638	■	□	■	□	■	□	□	■	■	□	□	□	■	□	■	■	■	■	10	*Microbacterium foliorum*	(EU834263)	100
BD-1	E112	■	□	■	□	□	■	□	■	■	□	□	□	■	□	■	■	■	■	10	*Achromobacter piechaudii*	(EU239469)	99
BD-1	E115	■	□	■	□	■	■	□	□	■	■	■	□	□	□	■	■	■	■	11	*Halomonas nitroreducens*	(EF613114)	98
BD-1	E111	■	■	□	□	■	□	□	■	■	□	□	■	■	□	■	■	■	■	11	*Pseudomonas* sp.	(DQ77773)	99
BD-9	E922	□	■	■	□	■	□	□	■	■	□	□	□	■	□	■	■	■	■	11	*Rahnella* sp.	(JQ864392)	99
BD-16	E637	■	■	■	□	■	□	■	□	□	□	□	■	■	□	■	■	■	■	11	*Stenotrophomonas* sp.	(JN886733)	99
BD-5	E511	■	□	■	□	■	■	□	■	■	■	□	■	■	□	■	■	□	■	12	*Tetrathiobacter* sp.	(JN86352)	99
BD-5	E513	■	□	■	□	■	■	□	□	■	■	■	■	□	□	■	■	■	■	12	*Bacillus* sp.	(FJ6743)	99
BD-16	E636	■	■	■	□	■	□	■	■	■	■	■	□	■	□	■	■	■	■	12	*Stenotrophomonas* sp.	(JN886733)	99
BD-8	EB13	■	□	■	□	■	■	□	■	■	■	■	□	■	□	■	■	■	■	12	*Buttiauxella* sp.	(JX82298)	95
BD-16	E632	■	■	■	□	■	■	□	■	■	■	■	□	■	□	■	■	■	■	12	*Microbacterium* sp.	(JF7475)	99
BD-8	E822	■	□	■	■	■	■	□	■	■	■	■	□	■	□	■	■	■	■	12	*Rahnella* sp.	(JF262562)	99
BD-5	E516	■	□	■	□	■	■	■	□	■	■	■	■	□	□	■	■	■	■	13	*Bacillus aquimaris*	(JQ3919)	99
BD-16	E635	■	■	■	■	■	■	□	□	■	■	■	□	■	□	■	■	■	■	13	*Pantoea* sp.	(FJ426593)	99
BD-1	E142	■	■	■	□	■	■	□	■	■	■	■	□	■	□	■	■	■	■	13	*Pseudomonas* sp.	(JX47434)	99
BD-8	E821	□	■	■	■	■	■	■	■	■	■	□	■	■	□	■	■	■	■	13	*Pseudomonas brassicacearum*	(JN6283)	97
BD-8	E823	■	□	■	■	■	■	■	■	■	■	■	□	■	□	■	■	■	■	13	*Pseudomonas brassicacearum*	(JN65747)	99
BD-1	E141	■	□	■	■	■	■	■	■	■	■	■	□	■	□	■	■	■	■	13	*Pseudomonas brassicacearum*	(JN6283)	99
BD-8	E825	■	□	■	■	■	■	■	■	■	■	■	□	■	□	■	■	■	■	13	*Pseudomonas* sp.	(JN2157)	96

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.

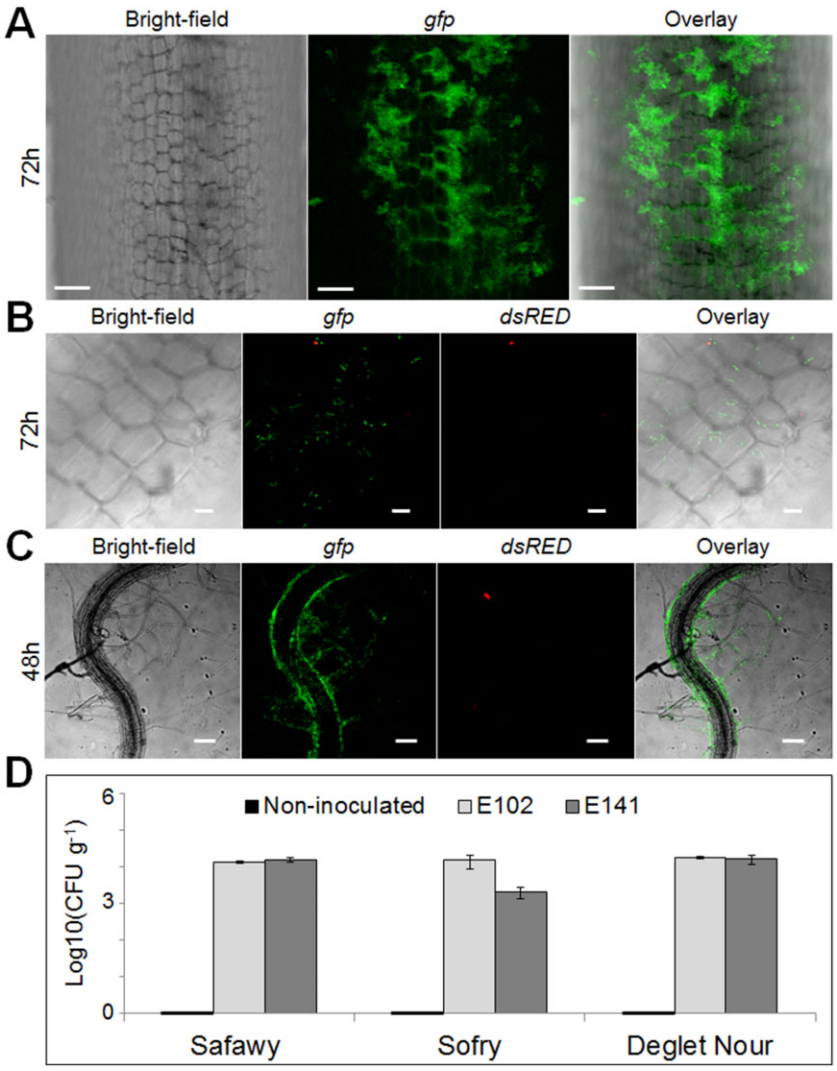


Fig. 3. Rhizocompetence and root recolonization ability of date palm root endophytic bacteria on different plant models. Colonization of date palm root after 72 h by a *gfp*-labelled E141 strain alone (A) or supplied together with a ds*red*-labelled *E. coli* (B). *Arabidospis thaliana* roots after 48 h exposure to a *gfp*-labelled E141 strain together with a ds*red*-labelled *E. coli* (C). The scale bars correspond to 100 μm in (A) and (C) and 10 μm in (C). The *gfp* (A–C) and ds*red* (B and C) fluorescences are visible in green and red respectively. Images were obtained with a Zeiss (A) or a Leica (B and C) confocal microscope (see *Experimental procedures*). (D) Re-isolation experiments showing the ability of both *P. Brassicacearum* E141 and *P. Frederiksbergensis* E102 to actively colonize root tissues of different date palm cultivars including the original Tunisian cultivar (Deglet Nour) from which the strains were isolated and two Saudi Arabian cultivars (Safawy and Sofry). CFU g^{-1} are expressed as mean ± SD.

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 10^4 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 10^3 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

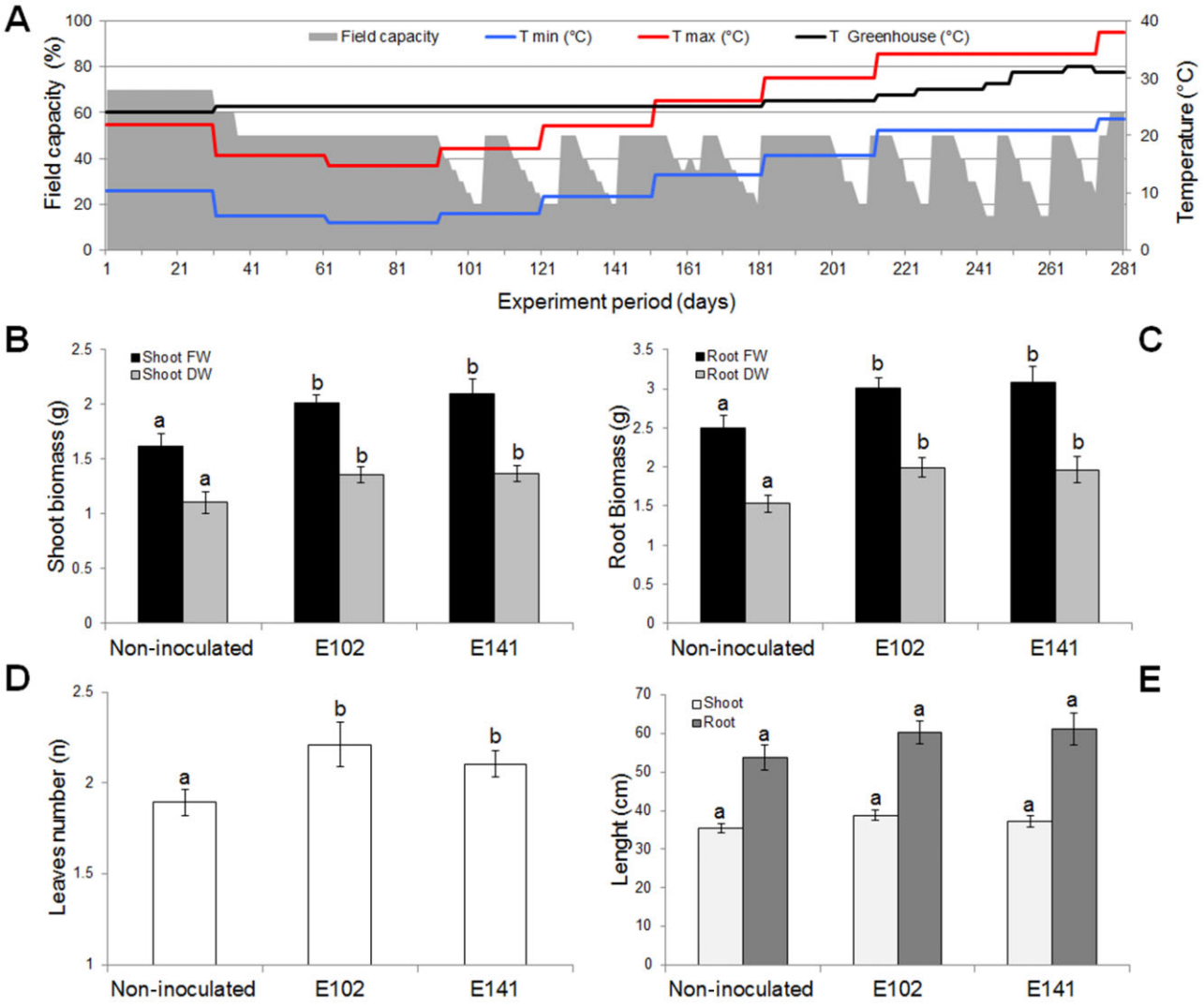


Fig. 4. Date palm root endophytic bacteria promote the growth of date palms exposed to drought.
A. Levels of controlled drought stress during a 9-month period of date palm growth. Controlled drought periods were induced by interrupting irrigation for periods of time ranging between 6 and 12 days. Field 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 (*P. Brassicacearum* E141 and *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.

50% while during the water stress periods, it was decreased down to 15–20% by interrupting irrigation (Fig. 4A). Bacteria-treated date palm plantlets had significantly more fresh aerial and root biomasses than non-inoculated plants (Fig. 4B and C; Tables S4 and S5). Both strains increased fresh biomass of date palm shoot and root between 25–30% and 20–23% respectively (Fig. 4B and C). Dry biomass (Fig. 4B and C) and number of leaves (Fig. 4D) confirmed that bacterial inoculation significantly increased plant growth (Table S4). No statistical differences were observed for root or shoot length (Fig. 4E; Tables S4 and S5).

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., 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 arboreal 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. (Argandonña *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 Merlino 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 $< 10^1$. Seeds were coated once with the bacterial suspension strains E102 and E141 at a concentration of 10^8 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 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'.

Acknowledgements

The authors declare an absence of competing interests. This research was supported by the EU project BIODESERT (European Community's Seventh Framework Programme CSA-SA REGPOT-2008-2 under grant agreement no. 245746), King Abdullah University of Science and Technology (baseline research funds to D.D.) and the BIOGESTECA project (no. 15083/RCC 'Fondo per la promozione di accordi istituzionali', Regione Lombardia, Italy). ER and FM acknowledge support by Università degli Studi di Milano, DeFENS, the European Social Fund (FSE) and Regione Lombardia (contract 'Dote Ricerca').

References

Ahmad, F., Ahmad, I., and Khan, M.S. (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol Res* **163:** 173–181.

Ali, S.Z., Sandhya, V., and Rao, L.V. (2013) Isolation and characterization of drought-tolerant ACC deaminase and exopolysaccharide-producing fluorescent *Pseudomonas* sp. *Ann Microbiol* **64:** 493–502.

Andrade, L.F., de Souza, G.L.O.D., Nietsche, S., Xavier, A.A., Costa, M.R., Cardoso, A.M.S., *et al*. (2014) Analysis of the abilities of endophytic bacteria associated with banana tree roots to promote plant growth. *J Microbiol* **52:** 27–34.

Angel, R., Soares, M.I., Ungar, E.D., and Gillor, O. (2010) Biogeography of soil archaea and bacteria along a steep precipitation gradient. *ISME J* **4:** 553–563.

Argandonña, M., Fernaàndez-Carazo, R., Llamas, I., Martínez-Checa, F., Caba, J.M., Quesada, E., and Moral, A. (2005) The moderately halophilic bacterium *Halomonas maura* is a free-living diazotroph. *FEMS Microbiol Lett* **244:** 69–74.

Bachar, A., Al-Ashhab, A., Soares, M.I.M., Sklarz, M.Y., Angel, R., Ungar, E.D., and Gillor, O. (2010) Soil microbial abundance and diversity along a low precipitation gradient. *Microb Ecol* **60:** 453–461.

Bakhtiar, Y., Yahya, S., Sumaryono, W., Sinaga, M.S., and Budi, S.W. (2013) Adaptation of oil palm seedlings inoculated with arbuscular mycorrhizal fungi and mycorrhizal endosymbiotic bacteria *Bacillus subtilis* B10 towards biotic stress of pathogen *Ganoderma boninense*. *Path Microbiol* **6:** 157–164.

Balloi, A., Rolli, E., Marasco, R., Mapelli, F., Tamagnini, I., Cappitelli, F., *et al*. (2010) The role of microorganisms in bioremediation and phytoremediation of polluted and stressed soils. *Agrochimica* **54:** 353–369.

Barreveld, W.H. (1993) *Date Palm Products. Agricultural Services Bulletin No. 101*. Rome, Italy: FAO.

Berard, A., Ben Sassi, M., Renault, P., and Gros, R. (2012) Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. *J Soils Sediments* **12:** 513–518.

Berg, G., and Smalla, K. (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol Ecol* **68:** 1–13.

Berg, G., Zachow, C., Müller, H., Philipps, J., and Tilcher, R. (2013) Next-generation bio-products sowing the seeds of success for sustainable agriculture. *Agronomy* **3:** 648–656.

Brader, G., Compant, S., Mitter, B., Trognitz, F., and Sessitsch, A. (2014) Metabolic potential of endophytic bacteria. *Curr Opin Biotechnol* **27:** 30–37.

Chaparro, J.M., Badri, D.V., Bakker, M.G., Sugiyama, A., Manter, D.K., and Vivanco, J.M. (2013) Root exudation of phytochemicals in *Arabidopsis* follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PLoS ONE* **8:** e55731.

Cleary, D.F.R., Smalla, K., Mendonca-Hagler, L.C.S., and Gomes, N.C.M. (2012) Assessment of variation in bacterial composition among microhabitats in a mangrove environment using DGGE fingerprints and barcoded pyrosequencing. *PlosONE* **7:** e29380.

Compant, S., Van Der Heijden, M.G.A., and Sessitsch, A. (2010) Climate change effects on beneficial plant–microorganism interactions. *FEMS Microbiol Ecol* **73:** 197–214.

Daffonchio, D., Borin, S., Frova, G., Manachini, P.L., and Sorlini, C. (1998) PCR fingerprinting of whole genomes: the spacers between the 16S and 23S rRNA genes and of intergenic tRNA gene regions reveal a different intraspecific genomic variability of *Bacillus cereus* and *Bacillus licheniformis*. *Int J Syst Bacteriol* **1:** 107–116.

Daffonchio, D., Hirt, H., and Berg, G. (2015) Plant–microbe interaction and water management in arid and saline soils. In *Principles of Plant–Microbe Interactions*. Lugtenberg, B. (ed.). Switzerland: Springer International Publishing, pp. 265–276.

De Grenade, R. (2013) Date palm as a keystone species in Baja California peninsula, Mexico oases. *J Arid Env* **94:** 59–69.

De Zelicourt, A., Al-Yousif, M., and Hirt, H. (2013) Rhizosphere microbes as essential partners for plant stress tolerance. *Mol Plant* **6:** 242–245.

Ding, G.C., Piceno, Y.M., Heuer, H., Weinert, N., Dohrmann, A.B., and Carrillo, A. (2013) Changes of soil bacterial diversity as a consequence of agricultural land use in a semi-arid ecosystem. *PLoS ONE* **8:** e59497.

Downer, J. (2004) Palm tree management. Part I: selecting the right palms. *Landscape Notes* **18:** 1–5.

El-Juhany, L.I. (2010) Degradation of date palm trees and date production in Arab countries: causes and potential rehabilitation. *Aust J Basic Appl Sci* **4:** 3998–4010.

Favia, G., Ricci, I., Damiani, C., Raddadi, N., Crotti, E., Marzorati, M., *et al*. (2007) Bacteria of the genus *Asaia* stably associate with *Anopheles stephensi*, an Asian malarial mosquito vector. *Proc Natl Acad Sci USA* **104:** 9047–9051.

Ferjani, R., Marasco, R., Rolli, E., Cherif, H., Cherif, A., Gtari, M., *et al*. (2014) The date palm tree rhizosphere is a niche for plant growth promoting bacteria in the oasis ecosystem. *BioMed Res Int* **2015:** 153851. http://dx.doi.org/10.1155/2015/153851. accepted for publication, in press.

García, A., Jimena, A., Martínez, M.M., and Gutiérrez, V. (2012) Effect of phosphate-solubilizing bacteria and compost on the nutritional characteristics of the oil palm crop (*Elaeis guineensis* Jacq.) in Casanare, Colombia. *Agronom Colomb* **30:** 274–281.

George, P., Gupta, A., Gopal, M., Thomas, L., and Thomas, G.V. (2013) Multifarious beneficial traits and plant growth promoting potential of *Serratia marcescens* KiSII and *Enterobacter* sp. RNF 267 isolated from the rhizosphere of coconut palms (*Cocos nucifera* L. *World J Microbiol Biotechnol* **29:** 109–117.

Gobet, A., Boetius, A., and Ramette, A. (2014) Ecological coherence of diversity patterns derived from classical fingerprinting and next generation sequencing techniques. *Environ Microbiol* **16:** 2672–2681.

Hardoim, P.R., van Overbeek, L.S., and Elsas, J.D.V. (2008) Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol* **16:** 463–471.

Jha, B., Gontia, I., and Hartmann, A. (2012) The roots of the halophyte *Salicornia brachiata* are a source of new halotolerant diazotrophic bacteria with plant growth-promoting potential. *Plant Soil* **356:** 265–277.

Lau, J.A., and Lennon, J.E. (2011) Evolutionary ecology of plant–microbe interactions: soil microbial structure alters selection on plant traits. *New Phytologist* **192:** 215–224.

Lodewyckx, C., Vangronsveld, J., Porteous, F., Moore, E.R., Taghavi, S., Mezgeay, M., and der Lelie, D.V. (2002) Endophytic bacteria and their potential applications. *Cr Rev Plant Sci* **21:** 583–606.

Mapelli, F., Marasco, R., Balloi, A., Rolli, E., Cappitelli, F., Daffonchio, D., and Borin, S. (2012) Mineral–microbe interactions: biotechnological potential of bioweathering. *J Biotechnol* **157:** 473–481.

Mapelli, F., Marasco, R., Rolli, E., Barbato, M., Cherif, H., Guesmi, A., *et al*. (2013) Potential for plant growth promotion of Rhizobacteria associated with *Salicornia* growing in Tunisian hypersaline soils. *BioMed Res Int* **2013:** 248078.

Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S., *et al*. (2012) A drought resistance-promoting microbiome is selected by root system under desert farming. *PLoS ONE* **7:** e48479.

Marasco, R., Rolli, E., Fusi, M., Cherif, A., Abou-Hadid, A., El-Bahairy, U., *et al*. (2013a) Plant growth promotion potential is equally represented in diverse grapevine root-associated bacterial communities from different biopedoclimatic environments. *BioMed Res Int* **2013:** 491091.

Marasco, R., Rolli, E., Vigani, G., Borin, S., Sorlini, C., Ouzari, H., *et al*. (2013b) Are drought-resistance promoting bacteria cross-compatible with different plant models? *Plant Signal Behav* **10:** e26741.

Marlet, S., Bouksila, F., and Bahri, A. (2009) Water and salt balance at irrigation scheme scale: a comprehensive approach for salinity assessment in a Saharan oasis. *Agric Water Manag* **96:** 1311–1322.

Marzorati, M., Borin, S., Brusetti, L., Daffonchio, D., Marsili, C., Carpani, G., and de Ferra, F. (2006) Response of 1,2-dichloroethane-adapted microbial communities to *ex-situ* biostimulation of polluted groundwater. *Biodegradation* **17:** 41–56.

Mekki, I., Jacob, F., Marlet, S., and Ghazouani, W. (2013) Management of groundwater resources in relation to oasis sustainability: the case of the Nefzawa region in Tunisia. *J Environ Manag* **121:** 142–151.

Merlino, G., Rizzi, A., Schievano, A., Tenca, A., Scaglia, B., Oberti, R., *et al*. (2013) Microbial community structure and dynamics in two-stage *single-stage* thermophilic anaerobic digestion of mixed swine slurry and market bio-waste. *Water Res* **47:** 1983–1995.

Mia, M.A., Shamsuddin, Z.H., Wahab, Z., and Marziah, M. (2010) Rhizobacteria as bioenhancer and biofertilizer for growth and yield of banana *Musa* spp. cv. 'Berangan'. *Sci Hortic* **126:** 80–87.

Muyzer, G., De Waal, E.C., and Uitterlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59:** 695–700.

Puente, M.E., Li, C.Y., and Bashan, Y. (2004) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. *Plant Biol* **6:** 643–650.

Qin, S., Miao, Q., Feng, W.-W., Wang, Y., Zhu, X., Xing, K., and Jiang, J.H. (2015) Biodiversity and plant growth promoting traits of culturable endophytic actinobacteria associated with *Jatropha curcas* L. growing in Panxi dry-hot valley soil. *Appl Soil Ecol* **93:** 47–55.

Roca, A., Pizarro-Tobías, P., Udaondo, Z., Fernández, M., Matilla, M.A., Molina-Henares, M.A., *et al*. (2014) Analysis of the plant growth-promoting properties encoded by the genome of the rhizobacterium *Pseudomonas putida* BIRD-1. *Environ Microbiol* **15:** 780–794.

Rodríguez-Navarro, D.N., Dardanelli, M.S., and Ruíz-Saínz, J.E. (2007) Attachment of bacteria to the roots of higher plants. *FEMS Microbiol Lett* **272:** 127–136.

Rolli, E., Marasco, M., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M.L., *et al*. (2015) Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environ Microbiol* **17:** 316–331. doi:10.1111/1462-2920.12439.

Schlaeppi, K., Dombrowski, N., Oter, R.G., van Themaat, E.V.L., and Schulze-Lefert, P. (2014) Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives. *Proc Natl Acad Sci USA* **111:** 585–592.

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012) Nih image to imageJ: 25 years of image analysis. *Nature Meth* **9:** 671–675.

Sessitsch, A., Reiter, B., Pfeifer, U., and Wilhelm, E. (2002) Cultivation-independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and Actinomycetes-specific PCR of 16S rRNA genes. *FEMS Microbiol Ecol* **39:** 23–32.

Souza, S.A., Xavier, A.A., Costa, M.R., Cardoso, A., Pereira, M.C., and Nietsche, S. (2013) Endophytic bacterial diversity in banana *Prata Anã* (Musa spp.) roots. *Genet Mol Biol* **36:** 252–264.

Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X., and Song, W. (2008) Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. *Microb Ecol* **55:** 415–424.

Taghavi, S., Garafola, C., Monchy, S., Newman, L., Hoffman, A., Weyens, N., *et al*. (2009) Genome survey and charac-

terization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. *Appl Environ Microbiol* **75:** 748–757.

Van Dorst, J., Bissett, A., Palmer, A.S., Brown, M., Snape, I., Stark, J.S., *et al*. (2014) Community fingerprinting in a sequencing world. *FEMS Microbiol Ecol* **89:** 316–330.

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; $P < 0.05$).