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Age-based study of the root-associated microbiota of two Algerian date palm cultivars

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Investigating Saharan soil microbiomes via microbial community inventorying is crucial to our general understanding of the phylogenetic diversity of root-associated microbial communities and assemblages. This paper investigates the qualitative distribution of root-associated microorganisms of two date palm cultivars, one of which is resistant (Takerbucht) and the other susceptible (Aghamu) to *Fusarium oxysporum* f. sp. *albedinis* (F.o.a.) infection. Species richness comprised 314 bacterial strains belonging to four distinct taxonomical groups (*Bacillus*, Burkholderiales, *Pseudomonas*, and Coryneforms). Overall, 55, 141 and 118 isolates affiliated to 10, 12 and 13 different species were identified in control, young and old date palm rhizospheres, respectively. To better visualise the patterns of microbiome association, we combined a principal component method with a hierarchical-partitional clustering analysis, which allowed and facilitated interpretation of root assemblages by clustering comparable items together. *In vitro* antifungal activity showed that the isolates that had a strong antagonistic effect were the ones that predominated in the endorhizosphere of the Takerbucht cultivar. These bacteria were almost exclusively members of the genus *Bacillus*, especially *B. subtilis*, exhibiting notable antifungal activity against *Fusarium oxysporum* f. sp. *albedinis* in arid environments.

Keywords: Saharan oasis, date-palm, root-associated microbial communities, Correspondence Analysis (CA), Hierarchical Cluster Analysis (HCA)

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INTRODUCTION

The Algerian Sahara Desert is known for being a very dry and hostile environment. Climatic changes due to altered precipitation patterns and temperature fluctuations result in extended drought periods and severe dry periods that hinder crop production, slow the dynamics of nutrient buildup and lead to a loss of biodiversity over time. Moreover, soil degradation and limited vegetation cover lead to high rates of soil erosion and low nutrient content that threaten the livelihoods of people living in these areas and force many to migrate (Bashan et al., 2012; Ahkami et al., 2017; Ye et al., 2019). Nonetheless, the Algerian Sahara Desert is endowed with extraordinary biological resources, which are characterised by an outstanding capacity to adapt to drought and salinity (co-evolved traits of a ternary structure composed of plants, soil microbiota, and their immediate environment) (Naylor & Coleman-Derr, 2018).

The date palm, *Phoenix dactylifera* L., is one of these extraordinary biological resources, and constitutes the core of any oasis system framework. The date palm enables the creation of a favourable environment for people (source of food, currency, windbreaks, construction materials and artisanal handcrafts) and livestock. Through the cover provided by its canopy, the latter attenuates the adverse effects of dry air and hot winds, increases hygrometry and reduces evaporation, consequently promoting the

development of underlying crops (fruit trees, vegetables, forage, etc.) (Djerbi, 1994).

Unfortunately, date palms face a significant threat caused by pathogenic *Fusarium oxysporum* f. sp. *albedinis*, which has devastated millions of trees. This soil-borne micro-fungus is classified on the EPPO 'A2' list of quarantine organisms and on the 'A' list of pathogens against which monitoring and control are mandatory in Algeria (Decree, no. 95–387, 1995; OEPP/EPP, 1982). Therefore, the characterisation of the date palm soil microbiome through comprehensive microbial community inventorying is crucial for gaining a better understanding of soil microbial taxonomic and functional abundances, as well as of the phylogenetic diversity of root-associated microbial communities (Gans et al., 2005; Tringe et al., 2005).

Soil ecosystems are home to some of the most complex and diverse microbial communities on Earth, which generate large-scale data that are challenging to visualise and analyse. For this purpose, we have combined a principal component method with a hierarchical-partitional clustering analysis. In this context, correspondence analysis is used in a preprocessing step for denoising data (dimensionality reduction, balancing the influence of groups of observations). Then, the classification method (hierarchical clustering analysis) is applied to the first dimensions (largest proportion of the total inertia) of

the clustering analysis by taking a group of objects as input and searching for delineating characteristics and features that are common and discriminative among the group's objects. Both methodologies allow for better data visualisation and facilitate interpretation of association patterns by clustering comparable items together at close χ^2 distances (Husson et al., 2010).

This study aims to identify the microbial communities that live on the rhizosphere of fusariosis-resistant and fusariosis-sensitive date palm cultivars, and to determine the association patterns that are present in both young and old roots to construct a comprehensive inventory of the endemic phylogenetic resources that are unique to this ecosystem. Moreover, our findings may lead to the identification of rhizocompetent bacteria and novel physiologically active compounds for the management of fusariosis (Bayoud) disease.

MATERIALS AND METHODS

Study site and climate

The classification of the site's climate followed the Köppen climate classification, as outlined by Kottek et al. (2006). However, the study site itself is a representative Saharan oasis situated in southwestern Algeria at coordinates 28° 00' 54" N, 0° 27' 05" W, within the administrative division of Bouda-Adrar (Figure 1A). The oasis features well-maintained date palm groves, and an annual soil amendment practice takes place in October. This amendment involves the application of N-P-K fertilisers in the form of ammonium nitrate and natural manure. Importantly, the distribution of these fertilisers across the oasis exhibits heterogeneity.

Physico-chemical characteristics of soil samples

The samples were collected from the surface soil (top 30 cm) of the palm groves. Five sampling areas were delineated, approximately 30 metres apart. Among these, a mixture comprising three samples of soil was analysed, and the respective physico-chemical characteristics were assessed (pH, total calcium carbonate, organic matter, cation exchange capacity, electrical conductivity, soluble salt rate, as well as the sand, loam and clay content); soil moisture at sampling sites did not exceed 10%.

Date palm rhizosphere sampling

Date palm trees from the cultivars Takerbucht and Aghamu, which are resistant and sensitive, respectively, to date palm fusariosis (caused by

Fusarium oxysporum f. sp. *albedinis*; Bayoud) were sampled. Several trees in this date palm plantation displayed fusariosis symptoms and were severely affected (Figure 1B). The trials were carried out in a 145 × 45-metre date palm grove containing more than 250 trees. Five sampling areas consisting of plots of three date palm trees arranged in a triangle were defined. In each, a resistant Takerbucht date palm tree, in addition to healthy (asymptomatic) and diseased Aghamu date palm trees, was selected for rhizospheric sampling. Diseased Aghamu had symptoms of fusarium wilt that were subsequently confirmed in the laboratory (identification of *F. oxysporum* f. sp. *albedinis* from rachis) (Leslie & Summerell, 2008).

Isolation and enumeration of bacterial rhizospheric microflora

From each date palm tree specimen, rhizosphere soil and the less tightly adherent root surrounding soil were collected at a depth of 25–40 cm using sterile equipment. For the control samples (bulk soil), these were taken near the roots of the date palms, and the free soil (bare soil, absence of vegetation) that was not near the roots was mixed with them (Ferjani et al., 2015). Two categories of root age (young or old) were considered; the distinction was made based on observable characteristics such as colour, texture, size, branching patterns and proximity to the base. Young roots, being lighter in colour and having a smoother texture, were identified closer to the base, while older roots, with darker hues and a more fibrous texture, extended further into the soil. A dilution plating technique was used for the isolation of bacteria. One gram of each sample was washed and disinfected in 10 ml (quantity sufficient to prepare) of sterile distilled water supplemented with 3.5% aqueous calcium hypochlorite solution. Each sample was separately ground, vortexed and filtered, and the resulting suspension underwent serial decimal dilutions (10^{-1} to 10^{-5}) (Rapilly, 1968). For the enumeration of bacteria, 0.2 ml aliquots of each dilution were spread on the surface of nutrient agar media supplemented with 50 mg/l of cycloheximide (Hayakawa and Nonomura, 1987).

Microbiome identification

Taxonomic determination of isolates was conducted after 14 days of incubation at 30°C on the basis of (i) macro and micro-morphological observations under optical microscopy at 100× and 400× magnification (Parte et al., 2012), (ii) cultural characteristics and (iii)

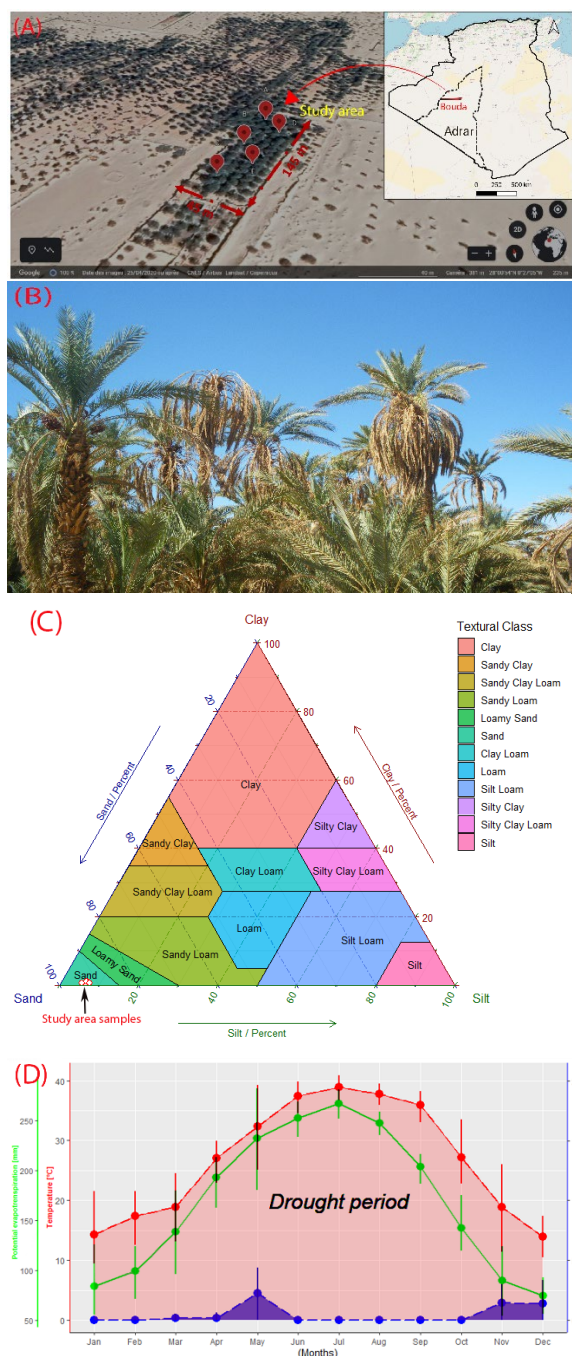


Figure 1. (A) Geographic map indicating the sampling site's location (A-E, red pin icons). (B) Bayoud's disease on date palm trees. (C) USDA textural classification chart of five sandy habitat sampling areas. (D) Ombrothermic diagrams of Bagnouls and Gausson applied to meteorological data originating from Bouda-Adrar, located in the Algerian Sahara Desert.

chemical component characteristics (pigment production, flagellum staining, oxidase (Bactident oxidase Merck 1.13300), accumulation of polyhydroxybutyrate (PHB), PHB particle staining, PHB hydrolysis, carbon source utilisation, ortho or

meta protocatechuate cleavage, arginine dihydrolase (ADH) and production of levane) (Fisher & Conn, 1942; Ottow & Zolg, 1974; Chen et al., 1982; Han & Clarke, 1990; Merrick et al., 1999; Ahmad et al., 2012).

Antagonistic activity against *F. oxysporum* f. sp. *albedinis*

Most representative bacteria were tested against *F. oxysporum* f. sp. *albedinis*. The experiments were carried out by streaking a straight line of each bacterial inoculum across the surface of the culture medium (Czapek Dox Agar). After incubation for 7 days at 30°C, the bioassay organisms were streaked at right angles to the bacterial isolates (straight line). The plates were incubated at 30°C and observed for antibiosis over 1 week. The extent of growth inhibition of the bioassay organisms was estimated by measuring the length of the inhibition range extending away from the tested isolates.

Statistical analysis

All graphics and data were processed in R for Windows v4.1.0 (Charif & Lobry, 2007) using the R Studio GUI v1.4.1717. Multivariate data analysis was conducted using correspondence analysis, followed by hierarchical clustering analysis (Euclidean distance associated with Ward's agglomerative method) in order to distinguish homogeneous groups using abstract and product terms of the FactoMineR, Factoextra, Corrplot and Ggplot2 packages (Lê et al., 2008; Wickham, 2016; Kassambara & Mundt, 2017; Wei & Simko, 2021).

RESULTS AND DISCUSSION

Climatic characteristics

According to the Köppen climate classification system, the study area falls within an arid (dry) zone characterised by a hot (h), desertic (W) and arid (B) climate, denoted as 'BWh' (Kottek et al., 2006). This climate type is marked by substantial inter- and intra-annual variability in precipitation, which occurs solely in the form of rainfall. The region experiences wide temperature ranges, significant evapotranspiration, severe and prolonged droughts, and occasional extreme weather events such as heat waves and violent wind episodes, as noted by Le Houérou (1990) and Ozenda (2004). The ombrothermic diagram developed by Bagnouls and Gausson illustrates that the period of drought and intense evapotranspiration (ETP) significantly surpasses the amount of rainfall throughout the entire year (Figure 1D). In 2021, the total rainfall did not exceed 20 mm, and there were

fewer than 20 effective rainy days. The hottest month in this arid climate is July, characterised by an average maximum temperature of 47°C. Conversely, the coldest month is January, with an average high temperature of 14.6°C and an average low temperature of 2.4°C. This climate pattern highlights the challenging environmental conditions in the region, emphasising the aridity and temperature extremes characteristic of the study area.

Soil physico-chemical characteristics

Granulometric analysis of soil particles showed that the studied date palm grove soil is, according to the USDA textural soil classification diagram, a sandy soil (Figure 1C). In such loosely aggregated coarse soil, the rate of soil degradation speeds up and induces a reduction in organic carbon, acceleration of soil erosion, reduction in soil fertility and loss of biodiversity (Li et al., 2018). In this nutrient-restricted environment (<5% organic matter), microbial development and activities are hampered, affecting the biogeochemical cycling of nutrients (Bastida et al., 2015). The analysed samples were homogenous and non-calcareous, with an alkaline pH ranging between 8.2 and 8.7 and an electrical conductivity of less than 2 dS/m. In the case of cultivated soil, salinity, which is a crucial microbiome controlling factor, did not inhibit microbial development since it did not exceed 4 mS/cm for mycelial bacteria and 8 mS/cm for bacteria. Indeed, according to Mokrane et al. (2013), these values are threshold points at which bacteria start to be severely impacted, whether they are in the rhizosphere. Detailed physico-chemical characteristics of analyzed samples are in Table 1.

Diversity and qualitative distribution of microbial communities associated with date palm rhizosphere

Soil amendment with ammonitrates and other organic fertilisers has induced permanent alterations in the microbial equilibrium of oasis soils, favouring bacterial flora at the expense of other microbial groups such as micro-fungi, particularly the saprophytic *Fusarium*, known for its high antagonistic power, and actinomycetes. This shift has contributed to a consistent increase in disease occurrence (Belarbi, 1980; Amir & Amir, 1989). Paradoxically, the reasonably well-maintained grove, incorporating practices like ploughing, irrigation and organic amendment, has adversely impacted date palm cultivation. Despite being conducive to date palm growth, these conditions also facilitate the spread of Bayoud (Amir & Amir, 1989). In this context, the

structure of bacterial communities in the rhizospheres of control, young and old date palms reveal quantitative variability between cultivars, irrespective of their health status. (Figure 2A and B).

Depending on the specific root exudate profile, organic acids and sugars positively influence microbial richness in the rhizosphere. High levels of species diversity and richness contribute to enhanced functional redundancy within the soil microbial community (Vurukonda et al., 2016). Morphological and chemical analysis of cellular components allowed us to isolate, purify and identify a total of 314 bacterial strains belonging to four distinct taxonomic groups (*Bacillus*, Burkholderiales, *Pseudomonas* and Coryneforms). Overall, 55, 141 and 118 isolates belonging to 10, 12 and 13 different species were respectively identified in the control, young and old date palm rhizospheres (Table 2); rare species that occurred less than 5 times in the 5 studied lots were discarded from subsequent statistical analyses to improve the robustness of the results (*B. coagulans*, *B. polymyxa*, *B. licheniformis*, *P. stutzeri*, *P. palleronii*, *P. pickettii* and *Pimelobacter* sp.). Nonetheless, owing to their relative rarity, the sampling effort and methodology, identification technique, spatio-temporal variability and microbial turnover, one should not disregard the possible presence of undetected taxa (Fages & Mulard, 1988). The comparison of the distribution of different bacterial taxa regarding palm age indicated that their occurrence was higher in young date palms than in older or control samples. Burkholderiales and *Bacillus* constituted many isolated species, with 55.09% and 31.52% of total strains, respectively, followed by coryneform bacteria (8.9%) and *Pseudomonas* (4.45%), which were represented by a unique species, namely, *P. pseudoalcaligenes*.

The *Bacillus*, Coryneforms and *Pseudomonas* chiefly consist of plant growth-promoting rhizobacteria (PGPR) that promote plant growth and development (Kloepper & Beauchamp, 1992). These beneficial microorganisms, crucial for plant health, are recruited from the soil microbiome, contributing to host-specific communities with reduced diversity. Drought has a direct impact on the initial inoculum of the soil microbiome by favouring the selection of drought-tolerant taxa. Additionally, drought indirectly influences the soil microbiome through alterations in soil chemistry, diffusion rates, root exudate profile and morphology. These microorganisms have been proposed to use different mechanisms of alleviating

Table 1. Physico-chemical characteristics of the analyzed soil samples.

Physico-chemical character	Samples					Mean value	Standard deviation
	(A)	(B)	(C)	(D)	(E)		
Humidity (%)	2.60	2.60	7.80	3.40	9.60	5.20	3.27
pH* Water (1/2.5)	8.40	8.70	8.60	8.40	8.20	8.46	0.19
Organic matter (%)	3.17	6.28	4.22	1.05	2.11	3.37	2.01
CaCO ₃ * Total (%)	6.60	6.78	5.68	6.15	5.72	6.19	0.5
Electrical conductivity (30% [w/v]) dS/m	0.21	0.13	0.11	0.14	0.20	0.16	0.04
Soluble salts rate	0.07	0.04	0.04	0.05	0.06	0.05	0.01
CEC* (meq/100g)	24.95	24.95	24.80	24.80	24.95	24.89	0.08
Granulometry (%)							
Coarse sand	15.09	15.70	11.67	16.87	16.78	15.22	2.12
Fine sand	78.89	77.43	80.79	75.78	75.37	77.65	2.24
Coarse loam	2.80	3.09	5.42	5.75	5.19	4.45	1.39
Fine loam	2.69	3.78	1.06	0.53	2.13	2.04	1.29
Clays	0.53	0.00	1.06	1.07	0.53	0.64	0.44
Texture (USDA)	Sandy	Sandy	Sandy	Sandy	Sandy	Sandy	

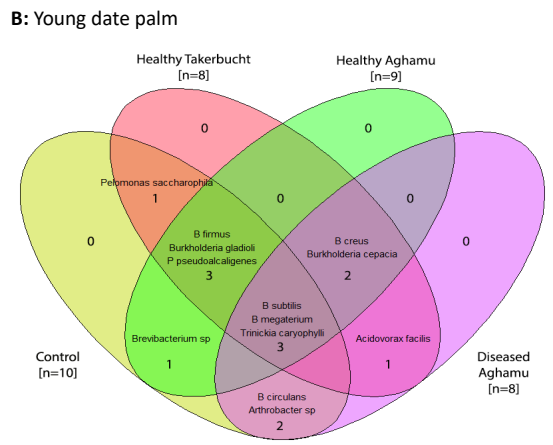
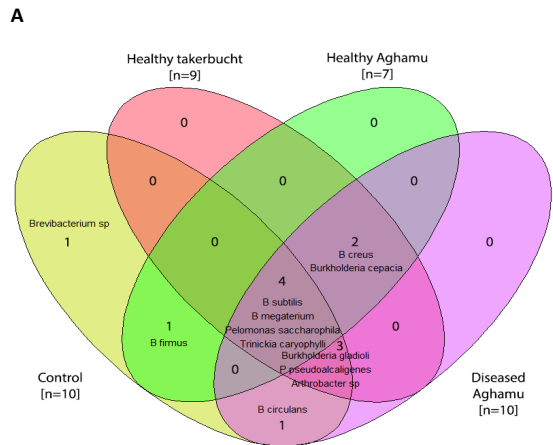
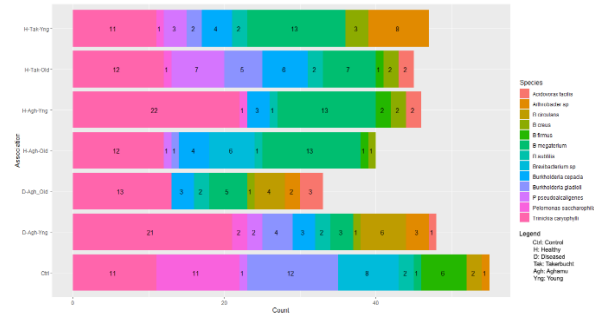
*CEC: Cation Exchange Capacity; pH: potential of hydrogen; CaCO₃: Calcium carbonate.

Table 2. Microbial distribution by genera (A) and date palm host (B) on control soil and rhizosphere.

(A) Microbial species	Samples			
	Control	Young	Old	Total (n,%)
<i>Bacillus</i>				
<i>B. cereus</i>	0	6	4	(10, 03.18%)
<i>B. firmus</i>	6	2	2	(10, 03.18%)
<i>B. subtilis</i>	2	5	5	(12, 03.82%)
<i>B. circulans</i>	2	6	4	(12, 03.82%)
<i>B. megaterium</i>	1	29	25	(55, 17.51%)
	11	48	40	(99, 31.52%)
<i>Burkholderiales</i>				
<i>Burkholderia gladioli</i>	12	6	6	(24, 07.64%)
<i>B. cepacian</i>	0	10	13	(23, 07.32%)
<i>Pelomonas saccharophila</i>	11	4	1	(16, 05.09%)
<i>Trinickia caryophylli</i>	11	54	37	(102, 32.48%)
<i>Acidovorax facilis</i>	0	3	5	(08, 02.54%)
	34	77	62	(173, 55.09%)
<i>Pseudomonas</i>				
<i>P. pseudoalcaligenes</i>	1	5	8	(14, 4.45%)
	1	5	8	(14, 4.45%)
Coryneforms				
<i>Arthrobacter</i> sp.	1	11	2	(14, 4.45%)
<i>Brevibacterium</i> sp.	8	0	6	(14, 4.45%)
	9	11	8	(28, 8.90%)
(B) Count species based on cultivars and health status				
Healthy Takerbucht	28	47	45	120
Healthy Aghamu	21	46	40	107
Diseased Aghamu	6	48	33	87
Total isolates	55	141	118	314

stress in plants. They promote the development of the root, thereby improving the absorption of water, as well as the production of plant hormones such as indole acetic acid, cytokinins, gibberellins and 1-aminocyclopropane-1-carboxylate deaminase, which confer tolerance to drought by reducing ethylene levels in plants (Gagné-Bourque et al., 2016). Other known mechanisms of action of PGPR organisms are carbon sequestration, nitrogen fixation, solubilisation of nutrients such as phosphate and potassium, iron

sequestration by bacterial siderophores and remediation of polluted soil (Glick, 2012; Olanrewaju et al., 2017; Niu et al., 2018). For a complete description of root-associated bacterial communities, refer to Table 2. The set of seven stacked histograms in Figure 2A provides a detailed representation of species distribution frequencies based on age, health status, date palm cultivar and sampling location (rhizosphere or control soil).



B: Old date palm

Figure 2. (A) Stacked histograms depicting abundances of isolated species on control, young and old Aghamu and Takerbucht's date palm rhizosphere. (B) Venn diagram with four sets; values of "n" reported between square brackets represent the total number of species in each date palm category, whereas numbers within the diagram are species shared exclusively between the corresponding groups.

The results indicated a notable increase in Burkholderiales and *Bacillus* abundance in the rhizosphere compared to the control group, suggesting their prevalence in this specific microbial community. Furthermore, it was observed that *Bacillus* and coryneform densities decreased from resistant to susceptible cultivars.

Conversely, *Pseudomonas* thrived within young susceptible cultivars regardless of their health status, indicating a specific association with susceptibility.

Regarding the relative abundance of isolated species, *Trinickia caryophylli* (onion rot pathogen) and *B. megaterium*, belonging to Burkholderiales and *Bacillus*, respectively, exhibited significantly higher relative abundances (4 and 5 times higher, respectively) than those of other related species within the same taxonomic groups. Furthermore, a marked reduction in community richness was observed in *Pseudomonas* and coryneform communities within the root rhizosphere (Figure 2A and Table 2). This decline in *Pseudomonas* and Coryneforms was primarily associated with the heightened dominance of Burkholderiales and *Bacillus*, as depicted in Figure 4B. The shift in population structure leading to this reduction was likely influenced by factors such as environmental changes, resource competition or the emergence of specific microbial species that affected the surrounding soil and the host plant (Naylor & Coleman-Derr, 2018). Notably, Burkholderiales and *Bacillus* emerged as the predominant taxa in the studied environment.

Venn species-specific associations

Specificity is often employed in the context of symbiotic relationships in microbiome research, where it defines the ability of a given species to associate with a small consortia of host species (Combes, 2001; Poulin et al., 2011; Shefferson et al., 2019). The four-set Venn diagram shown in Figure 2B represents the species distribution in the young and old date palm rhizospheres. This diagram was used to assess microbiome species-specific and cultivar-specific populations. The diagram shows that there were four (*B. megaterium*, *B. subtilis*, *T. caryophylli* and *P. saccharophila*) and three (*B. megaterium*, *B. subtilis* and *T. caryophylli*) common and omnipresent species, respectively, among the control soil and all other date palm modalities. No exclusive species were recorded for all date palm categories (i.e. all species were also recorded in other sites) except for control soil sampled from the young rhizosphere, which was characterised by the presence of *Brevibacterium* sp., which is considered to be a promising new biocontrol agent (Ahmed et al., 2015; Mohamad et al., 2018; Chopra et al., 2020); the count and remaining shared species are represented in Figure 2B.

Correspondence analysis results

Multivariate non-parametric correspondence analysis is a method that enables assessment and interpretation of multiple associations among qualitative or (categorised) quantitative variables using a χ^2 metric (Benzécri, 1973; Greenacre, 2017). In the present study, correspondence analysis was performed to visualise association patterns (Figure 3). Specifically, correspondence analysis was applied to count species data where each strain (active variable) was represented by two scores, for dimensions 1 and 2 (Dim1 and Dim2), respectively. When the scores of Dim1 and Dim 2 are projected onto the plane, each active variable is displayed as a point on the map (biplot), and its quality of projection on the plane is given by the Cos^2 coefficient, which ranges from 0 to 1, with 1 representing a perfect representation on the map (Figure 3). Two simple correspondence analyses were performed on the entire dataset, one with and one without control data. For both, strong and significant Pearson's chi-squared values were found in the associated contingency tables (with control: $\chi^2=218.88$, $df=72$, $p\text{-value}=2.2e^{-16}$; without control: $\chi^2=135.4$, $df=60$, $p\text{-value}=9.43e^{-08}$). In these analyses, the inertia of the first three dimensions expressed 81.5% and 80.72% of the total inertia for the with- and without-control data, respectively, meaning that the planes explained more than 80% of the total cloud variability of rows (or columns). Therefore, the combinations of the first three factorial axes provided an acceptable two-dimensional order that captured most of the multidimensional information (main association patterns). The graphical summaries of these associations are shown in Figure 3A, B and C.

Overall, the two dimensions distinguish healthy from diseased date palm trees and the control from the other groups. Indeed, the biplot in Figure 3A first shows the presence of two separate clusters on opposite sides of the second dimension (Dim 2). The eccentric control group (left-hand side) is associated with *P. saccharophila*, *B. gladioli* (pathogen, rot agent with three pathovars causing gladiolus, onion and rapid soft rot of cultivated mushrooms) (Hildebrand et al., 1973; Young et al., 1978; Lincoln et al., 1991), *B. firmus* and *Brevibacterium* sp. The two bacterial species depicted in red indicate a good quality of projection on the plane ($\text{cos}^2 > 0.7$; total species contributions $> 75\%$). The remaining health- and age-based date palm association groups (right-hand side) are far away from the control group and lie close together in Figure 3A, suggesting that they may share similar isolate groups (misleading representation). To

address this issue, a biplot based on a subset consisting solely of these overlapping groups (excluding the control group) was used to further investigate specific differences or eventual association patterns (Figure 3B). The six health- and age-based date palm association groups fell into three clusters depending on their health status: the healthy young and old Takerbucht, the healthy young and old Aghamu, and the diseased young and old Aghamu, were all closely related (Figure 3D, correlation matrix with clusters).

Brevibacterium sp. and *B. megaterium*, as well as *Arthrobacter* sp., were associated with healthy old Aghamu and young Takerbucht date palm trees, respectively (i.e. the 1st and 3rd dimensions of the biplot). Additionally, it is worth noting that Coryneforms were the primary contributors to their respective dimensions (*Brevibacterium* sp.: 47.38%; *Arthrobacter* sp.: 56.79%). *B. circulans* and *T. caryophylli* were better projected into the second dimension. However, *B. circulans* was exclusively associated with diseased old Aghamu, while *T. caryophylli* was more closely related to healthy young Aghamu than to diseased old Aghamu. Finally, *P. pseudoalcaligenes* and *B. gladioli* lay in between the second and third dimensions and were both closely related to healthy old Takerbucht (Figure 3). *B. cereus*, *B. firmus*, *B. cepacia*, *A. facilis*, *P. saccharophila* and *B. subtilis* were all poorly projected on the biplot since they contributed less than 5% to the construction of the three first dimensions. Nonetheless, their ordination on the different maps suggests that they were respectively associated with healthy young Takerbucht, healthy old Aghamu, healthy old Takerbucht, diseased old Aghamu and, for the remaining two species, with the diseased young Aghamu.

Hierarchical clustering analysis results

The pattern displayed in Figures 3B and 3C was summarised in a factorial map and a dendrogram (Figure 4A and B), in which six separate clusters were formed from the thirteen isolates. The first and largest cluster contained six isolates, including *P. saccharophila*, *B. subtilis*, *B. cepacia*, *T. caryophylli*, *B. megaterium* and *B. cereus*; the latter was divided into three main subgroups. The species pair *A. facilis* and *B. circulans*, along with *P. pseudoalcaligenes* and *B. gladioli*, constituted separate groups that clustered together. Finally, *B. firmus*, *Arthrobacter* sp. and *Brevibacterium* sp. were unique species that each formed unique clusters.

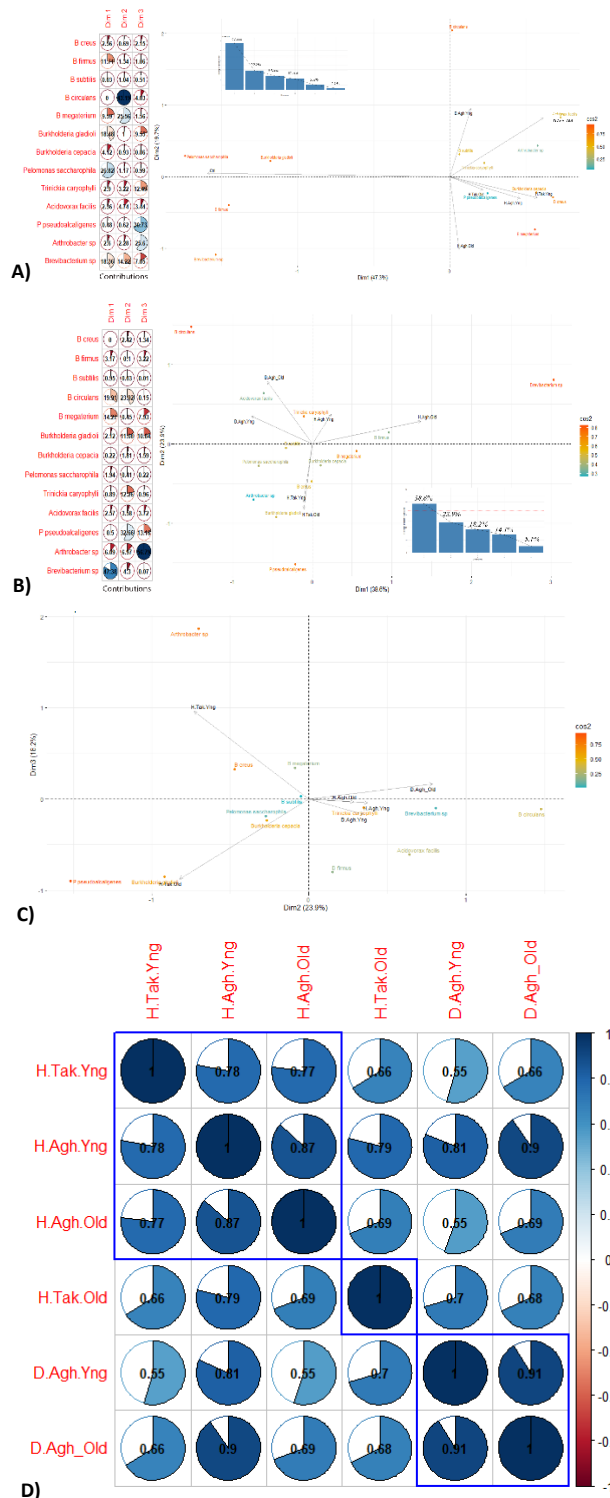


Figure 3. Correspondence analysis biplot of species score and Cultivar's health state/age. Cultivar groups: 1 [healthy Takerbuch], 2 [healthy Aghamu], 3 [diseased Aghamu] and age groups: 1 [young], 2 [old]. (A) Control group included. (B) Control group not included Dim 1 and 2. (C) Control group not included Dim 2 and 3. (D) Group correlations.

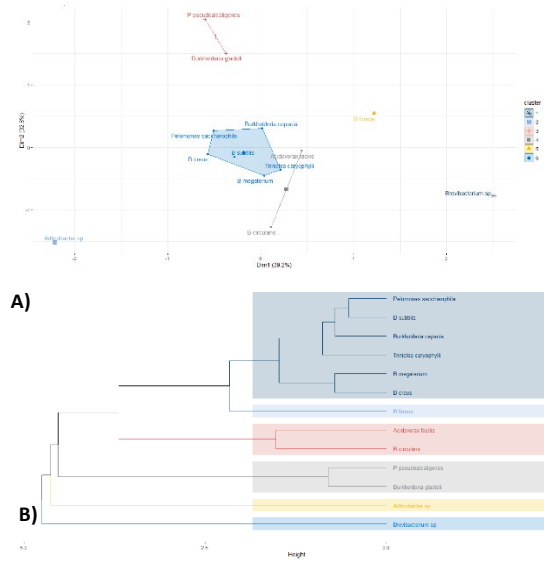


Figure 4. (A) Hierarchical cluster analysis factorial map. (B) HCA Dendrogram.

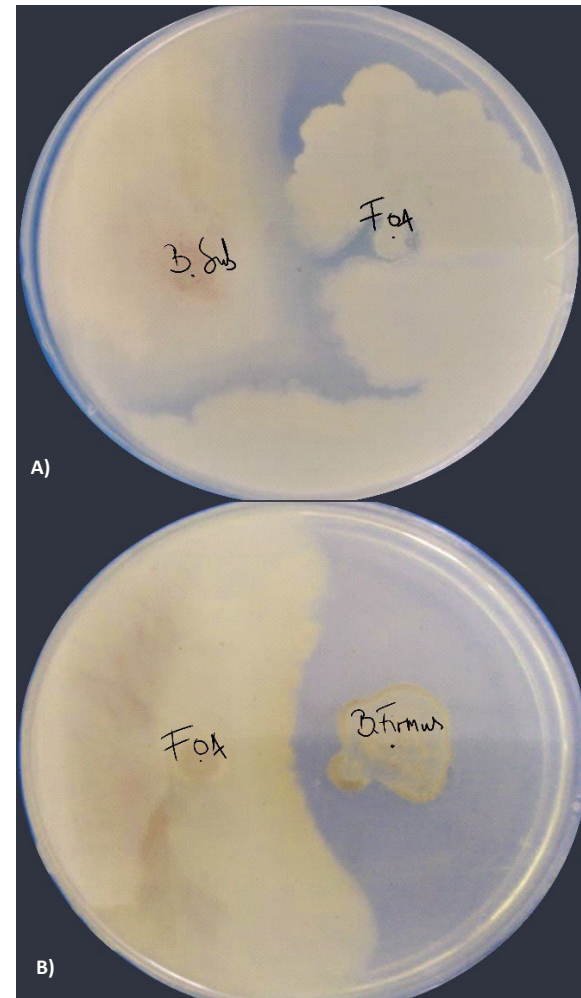


Figure 5. Emergence of antagonistic interaction between *B. subtilis* (A) and *B. firmus* (B) and *F.o.a* After 7 days of culture.

Antifungal activity against *Fusarium oxysporum* f. sp. *albedinis*

In our study, the isolates that showed a strong antagonistic effect were those that predominated in the endorhizosphere of the cultivar Takerbucht. These microorganisms were almost all members of the species *B. subtilis*, which has been identified by many authors to be the main species in the rhizosphere with antifungal activity against *F. oxysporum* f. sp. *albedinis* in arid regions (Figure 5) (Köberl et al., 2011). Indeed, Takerbucht's resistance to pathogenic fungi has been shown to be proportional to the amount and nature of the cultivar's root exudates (organic acid, phenolic compounds) that inhibit spore germination. Thus, Takerbucht might promote the development of fewer pathogenic microorganisms (e.g. *F. oxysporum* f. sp. *albedinis*, *B. gladioli* and *T. caryophylli*) than sensitive cultivars (Aghamu) that support the proliferation of pathogenic organisms through the formation of readily assimilable compounds (glucides, amino acids, proteins, lipids and various minerals). Furthermore, this situation is accompanied *in situ* by an increase in disease severity caused by *B. circulans* and *T. caryophylli*, which stimulate pathogenic micro-fungi by producing vitamins (thiamine, biotin) that enhance their virulence. Regrettably, the cultivar Takerbucht is only of interest in the southern Sahara, since it does not mature in septentrional regions (i.e. M'zab) (Bennaceur, 1981; Schroth & Becker, 1990). Conclusively, as per Moustiri (1992), the efficacy of bacterial isolates hinges on their ability to colonise roots rather than relying solely on direct antibiosis or effective *in vitro* suppression of pathogens found in limited quantities within roots. Consequently, while bacterial genera like *Bacillus*, *Pseudomonas*, *Streptomyces* and *Burkholderia* are acknowledged as biocontrol organisms, it is crucial to recognise that beneficial plant-microbe interactions are specific; that is, the reported broad-spectrum antagonists remain limited in number (Buée et al., 2009; Köberl et al., 2013).

CONCLUSION

The results of this study constitute an inventory providing information on the composition and association patterns of different microbial communities that grow in young and old date palm rhizospheres in Algerian sandy-soil oases. Species richness in these habitats comprised 314 bacterial strains belonging to four distinct taxonomical groups (*Bacillus*, Burkholderiales, *Pseudomonas* and Coryneforms). Overall, 55, 141 and 118 isolates belonging to 10, 12 and 13 different species were

respectively identified in control, young and old date palm rhizospheres. This microbial diversity is appreciable when considered in the context of a xeric environment. The application of correspondence analysis in conjunction with hierarchical clustering analysis allowed us to highlight the presence of strong and significant associations between young and old root microbiota of all date palm modalities, as well as enabling the capture of most of the multidimensional information (>80%), with direct visualisation of hidden structures within the data (main association patterns). Continuing from the present work, the future project aims to delve into intricate interactions within root-associated microbiomes and assess entire communities in the Algerian date palm rhizosphere. This exploration will involve microbial community profiling, metagenomic analysis and experiments designed to elucidate ecological dynamics.

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