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Original article

Diversity of rhizospheric and endophytic bacteria isolated from dried fruit of Ficus carica

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ABSTRACT

There is currently an increasing demand for the characterization of endophytic bacteria isolated from different parts of plants (rhizosphere, roots, fruit, leaf) in order to improve the organic agriculture practices. The current research was performed to identify both rhizospheric bacteria isolated from the rhizosphere of *Ficus carica* in three different sites in the north of Tunisia and endophytic bacteria isolated from dried figs. We then characterized them for a diversity of plant growth-promoting (PGP) activities. A collection of 120 isolates from rhizospheric soil and 9 isolates from dried figs was obtained and purified. 16SrDNA gene amplification of rhizospheric bacteria revealed significant diversity and allowed for the assigning of the isolates to 6 phyla: *Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Firmicutes, Actinobacteria,* and *Bacteroidetes*. Representative strains of the collection (90 strains) were tested for numerous PGP activities and resistance to abiotic stresses. The most common PGP trait for all bacteria from the three regions was siderophore production (62%), followed by cellulase (38%), then protease activity (37%), then by lipases activity (17%) and lastly by solubilization of phosphates (9%). Twenty - three strains that showed most PGP traits were selected, 8 strains presented 12 or more, and 15 strains displayed between 7 and 11 of 17 PGP activities. The majority of the isolates manifested a possible adaptation to abiotic stress and unfavorable environments. PCR-DGGE analysis of soil rhizosphere of the three sites allowed also for the acquisition of a Cluster analysis of rhizospheric bacterial communities. Our current study identified and characterized for the first time in *Tunisia* rhizospheric and endophytic bacteria from dried fruit of *Ficus carica.*

1. Introduction

More than 800 species of trees, epiphytes, and shrubs belong to the genus Ficus (Moraceae), which is one of the most numerous angiosperm genera on the planet (Singh et al., 2011; Badgujar et al., 2014). Among them, the fig tree (*Ficus carica*) is a common fruit species in Mediterranean countries, and its fruit, which possesses high nutritive and pharmaceutical values, can be eaten fresh or dried (Yang et al., 2009; Lazreg et al., 2011).

Mediterranean countries are among the world's largest producers of fresh figs (Sadder and Ateyyeh, 2006; Barolo et al., 2014). Fresh figs are available only in one season, so during the rest of the year the fruit is marketed for consumption after drying. The two most common types of dried figs are sun-dried figs and tray-dried figs (Crisosto et al., 2010). Dried figs are a good source of carbohydrates, sugars, proteins, vitamin A, vitamin C, potassium, calcium and iron; in addition, they are fat free, sodium free and cholesterol free (Vinson, 1999; Slatnar et al., 2011). They also contain high amounts of polyphenols and crude fiber (Mawa et al., 2013). Dried figs are used as a food supplement by diabetics and, because of the high amount of sugars they contain, it is also consumed as a sweet (Badgujar et al., 2014). In Tunisia dried figs

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(called chriha in the Tunisian dialect) are often used as a tonic (Le Floc'h, 1983; Leporatti and Ghedira, 2009). In biotechnology dried figs can be used as an alternative natural support for the immobilization of yeast cells and also as a biocatalyst in brewing (Bekatorou et al., 2002). During storage, dried figs form sugar efflorescence in which yeast frequently occurs; in fact, a new species of yeast (saccharomyces delphensis) was isolated from South African dried figs (Lansky and Paavilainen, 2010). These days dried figs are collected from shops in order to investigate the contamination of fungi (Saadullah and Abdullah, 2015).

Bacterial endophytes of dried fruit of *Ficus carica* are poorly documented and previous studies have focused only on root-associated endophytes with plant growth-promoting effects (Lai et al., 2006; Choubane et al., 2016). In Tunisia, people planted fig trees in ancient times and its distribution extends from the north to the south of the country; that is to say, it grows both in cold, humid regions and in hot, dry regions (Mars et al., 2009). In more recent times, with the aim to increase crop yield, people have excessively adopted the use of agrochemical products, such as herbicides, insecticides, fungicides, fertilizers, which is having a negative influence on the environment as well as on human health. Due to the problems arising from the use of agrochemicals products, scientists are exploring alternative biological methods. Among them, microorganisms known as "Plant Growth Promoting Rhizobacteria" (PGPR) are very promising, as they can be used as biofertilizer to ameliorate plant growth or improve crop production (Agbodjato et al., 2016). PGPR is able to facilitate the absorption and availability of nutrients via several mechanisms, such as phosphate solubilization, nitrogen fixation and phytohormones production (Arora et al., 2012). Several genera were described to be PGPR such as: *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus*, and *Serratia* (Prasad et al., 2019). In addition, biofertilization is considered as a green tool to increase harvest and improve the quality of figs, making it a good substitute to chemical fertilizer (Mishra et al., 2013). A study was conducted in Egypt to investigate the combined effects of organic manure and bio-*N*-fertilization on the growth and yield of fig trees. The results showed that poultry manure + *Azotobacter* and poultry manure + *Azospirillum* treatments resulted in the best growth, productivity and fruit quality (Osman and Abd El-Rhman, 2010).

The aims of the current research were to: (i) isolate and identify endophytic bacteria from dried fruit of *Ficus carica*, (ii) isolate and identify bacteria from the rhizosphere of *Ficus carica* in three different regions in the north of Tunisia, (iii) evaluate the bacterial community dynamics in the rhizosphere of three regions using PCR-DGGE analysis, (iv) identify plant growth-promoting bacteria (PGP) traits and abiotic stress resistance.

2. 2.Materials and methods

2.1. Soil sampling

Three farms in Tunisia were used to gather the samples; their geographic locations were along a gradient from 36° to 37° N latitude and from 9° to 11° E longitude. The rhizosphere soil of three fig trees of similar age was separately sampled at a depth of 30 cm. The soil surrounding the roots was collected after removing the roots. The sampling was conducted under sterile conditions. Then the samples were stored at −20 °C.

2.2. Collection of plant material

Thirty samples of dried figs were taken from two southern regions of Tunisia: Tataouine and Djerba. Fifteen figs from Djerba (tradionnally were sun -dried and soaked in olive oil) (Mars et al., 2008) and 15 figs from Tataouine (tradionnally were sun-dried) (Mars et al., 2008; Nikolidaki, et al., 2017). Then the samples were stored in Ziplock bags at room temperature (20 °C).

2.3. Bacteria isolation from Ficus carica rhizosphere

Bacteria isolation was carried out by the suspension of one gram of rhizosphere soil in 9 ml of sterile physiological solution, then the tubes for each sample were shacked for 24 h at 400 rpm. Then, suspensions were diluted with physiological water in tenfold series and plated on PCA (Plate Count Agar), TSA (Tryptic Soy Agar), and KB (King'B agar) media. After cultivating for 5 days at 30 °C, different colonies were picked and plated in triplicate (Ferjani et al., 2015).

2.4. Bacteria isolation from dried figs

The surface of the collected samples was cleaned with 2 percent sodium hypochlorite for 1 min before being washed with sterile distilled water. In the next step the samples were crushed with sterile equipment. The samples were placed in a sterile stomacher bag (10 g for one sample), 190 ml physiological saline peptone solution was added, then the samples were deposited in a stomacher (Ntuli et al., 2017). The suspensions were then tenfold diluted with physiological water, plated on TSA, and cultured at 30 °C for seven days (Alvarez-Pérez et al., 2012).

2.5. DNA extraction and PCR amplification of 16 SrRNA and identification of isolates

Using a boiling lysis method previously described by Ferjani et al. (2015) genomic DNA was recovered from bacterial isolates. The 16S rRNA genes were amplified using PCR with 1 U of DreamTaq Green DNA Polymerase (Fermentas), primers forward 8F (AGAGTTTGATCMTGGCTCAG) and reverse1507R (TACCTTGTTACGACTT). Federici et al. (2011) defined the cycle parameters. The PCR products were cleaned using the Sigma-Aldrich GenElute PCR Clean-Up kit before being transferred for sequencing. The BLAST tool (https://www.ncbi.nlm.nih.gov/BLAST/) was used to determine which isolates were present. The neighbor-joining phylogenetic tree was produced by MEGA v7.0 (Kumar et al., 2016) with bootstrap values based on 1000 replications. *Escherichia coli* was used as outgroup.

2.6. Soil DNA Extraction, PCR-DGGE analysis of bacterial communities.

The PowerSoil DNA Isolation kit was used to extract DNA from soil samples (MoBio Laboratories Inc). PCR amplification was performed as previously described by Federici et al. (2011) in a final volume of 50 µL using primers forward 341F (ATTACCGCGGCTGCTGG) and reverse 534R (ATTACCGCGGCTGCTGG). Electrophoresis in 2 percent (w/v) agarose gels colored with ethidium bromide was used to examine PCR products. Federici et al. (2011) previously reported on bacterial community profiling using DGGE analysis. On a 6 percent polyacrylamide-bis-acrylamide (37.5:1) gel with a 40–60 percent urea-formamide denaturing gradient, the amplification products were resolved. At 60 °C, the gel was run at 100 V for 16 h. Gels were stained with SBYR Gold (Invitrogen) for 30 min after electrophoresis and photographed under UV light.The GelJ v.2.0 program was used to analyze digitalized DGGE fingerprints (Heras et al., 2015). The dendrograms were generated using a UPGMA and Dice's coefficient of similarity (Federici et al., 2011). To analyze the bacterial diversity present in the rhizosphere of *Ficus carica* in three regions in northern Tunisia, the Shannon– Wiener (H), evenness (E), richness (S), and Simpson (D) indices were computed.

2.7. Plant growth promoting (PGP) traits and abiotic stress tolerance

Eighty-two identified bacterial strains were tested for several PGP activities and resistance to numerous abiotic stresses. Resistance to osmotic stress was tested by adjoining 30 % of polyethylene glycol (PEG 8000) into tryptic soy broth (TSB) medium. Salt resistance was detected by adding 10 %, 13 %, 16 %, 20 % of NaCl to the culture media and cultivated at 30 °C for five days.

In order to study the tolerance to pH, culture medium was adjusted either by a solution of NaOH (3 M) to reach the target val-

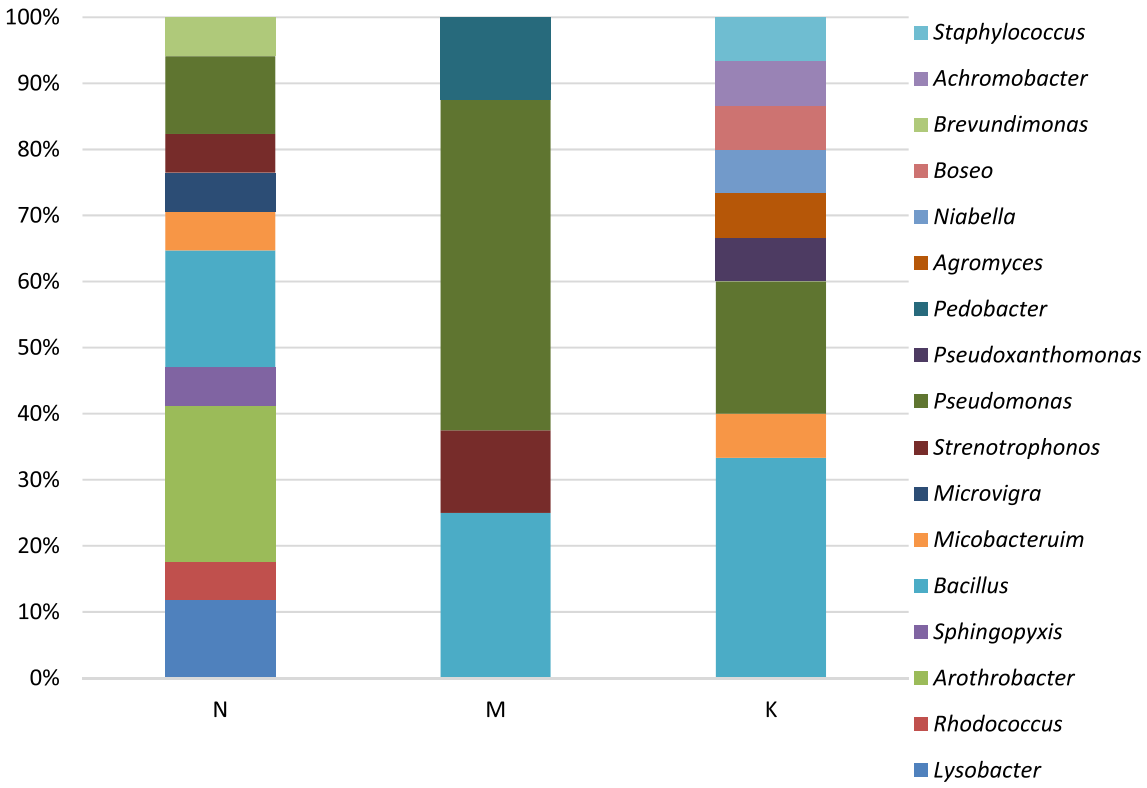

Fig. 1. Diversity of rhizospheric bacteria of *Ficus carica*. Phylogenetic association of the isolates from rhizosphere of *F. carica* at genus levels. N = Nabeul; M = Mateur; K = Kerkouane.

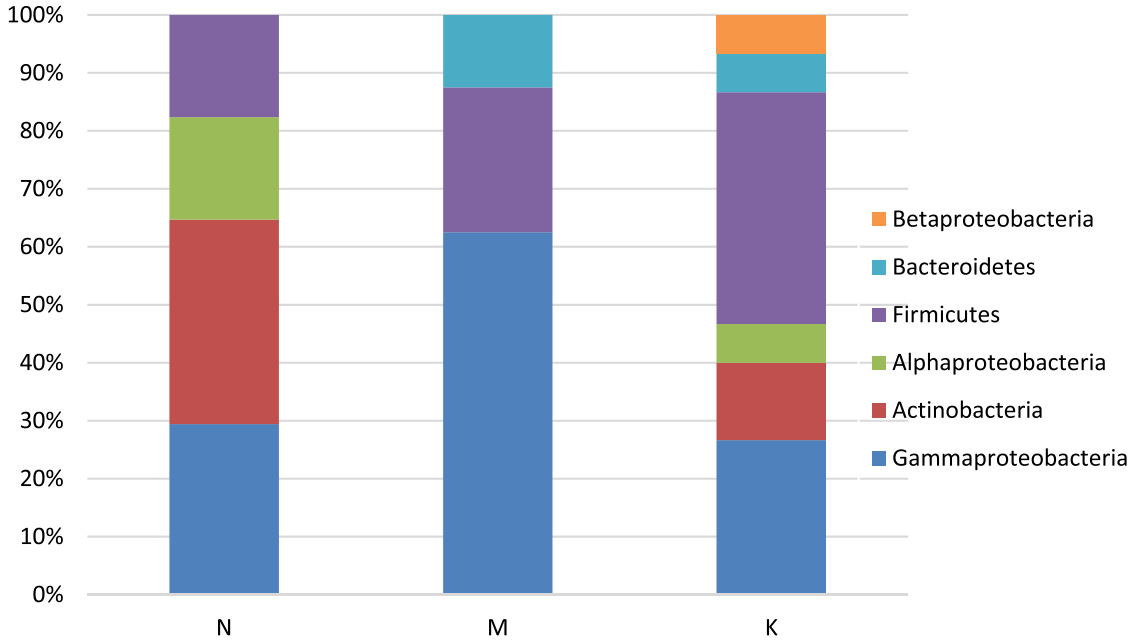

Fig. 2. Diversity of rhizospheric bacteria of Ficus carica. Phylogenetic association of the isolates from rhizosphere of F. carica at class levels. N = Nabeul; M = Mateur; K = Kerkouane.

ues of 10 and 12 or by a concentrated HCl solution (12 N) to attain the values of 3 and 4. The ability to grow at 45 °C, 50 °C and 55 °C was assessed in TSA by incubating at each temperature for five days (Ferjani et al., 2015). Mineral phosphate solubilization was checked by the appearance of a clear halo around the bacterial colonies grown on Pikovskaya medium (Nautiyal, 1999). The strains were spotted on CMC agar and skimmed milk media to evaluate Cellulase and protease activities separately, as described by Wang et al. (2015). Lipase activity was tested using the medium detailed by Omidvari (2008). The strains were deposited on this specific medium and incubated at 27 °C for 48 h. The observation of depositions around the bacterial colonies proved the presence of activity. The production of siderophore was identified on CAS (chrome azurols) agar medium as detailed by Wang et al. (2015). The color change around the colonies from blue into orange was noted as positive ones (Perez-Miranda et al., 2007).

3. Results

3.1. Cultivable bacteria isolated from Ficus carica rhizosphere

A total of 120 bacterial strains associated with *Ficus carica* rhizosphere were isolated from three sampling sites in different geographic locations in the north of Tunisia. Among them, 82 isolates were identified using partial 16S rRNA gene sequencing. A high

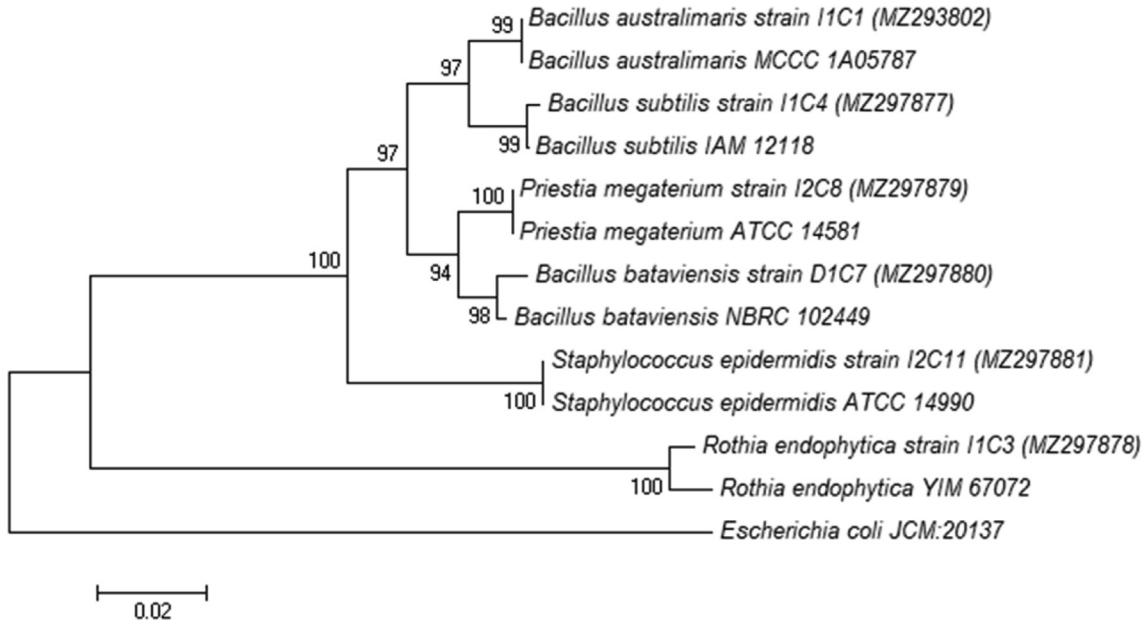

Fig. 3. The phylogenetic relationship between strains IC1, IC8, IC4, DC7, IC11, and IC3 using Neighbor-joining tree based on 16S rDNA sequences. At the nodes, bootstrap values greater than 70 percent (reported as percentages of 1000 replications) are provided.

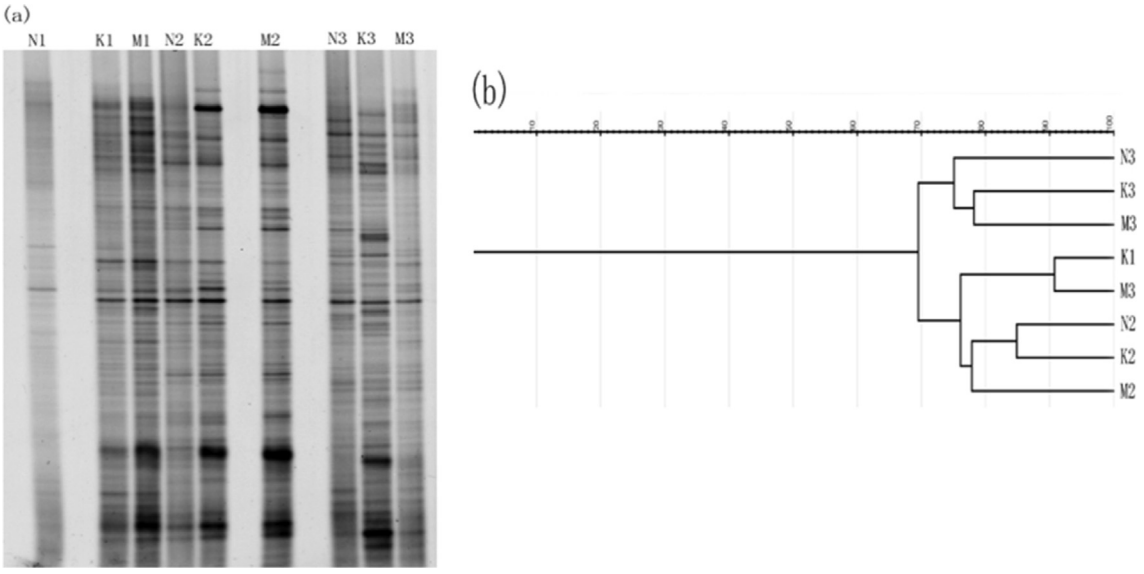

Fig. 4. (a) DGGE analysis of PCR products from DNA extracted from Ficus carica soil rhizosphere samples in three Tunisian regions. (b) Dendrogram illustrating the genetic similarity of soil rhizosphere after UPGMA cluster analysis with GelJ.

	Richness (S)	Shannon diversity index (H)	Simpson index of dominance (D)	Evenness (E)
N1	23			
N2	50	0.802625215	0.02898555	0.22110234
N3	53			

	Richness (S)	Shannon diversity index (H)	Simpson index of dominance (D)	Evenness (E)
K1	64			
K2	53	1.051298387	0.046619379	0.26121425
K3	52			

	Richness (S)	Shannon diversity index (H)	Simpson index of dominance (D)	Evenness (E)
M1	62			
M2	54	1.020260074	0.041767742	0.258078817
M3	43			

Fig. 5. Diversity indices between soil rhizosphere samples of Ficus carica in three regions in Tunisia. S: Richness, H: Shannon–Weaver index, D: Simpson index of dominance, E: evenness.

diversity of bacterial communities associated with *Ficus carica* rhizosphere soil was detected, especially in two regions of Beni Khiar and Kerkouane. Phylogenetic analysis of the isolates demonstrated that species were divided into six phyla, 17 different bacterial genera (Fig. 1), indicating a large genetic diversity in *Ficus carica* rhizosphere. *Bacillus* and *Pseudomonas* were the most frequently observed genus in the collection at 25 % and 22.5 % respectively. The phylogenetic result also showed a slight predominance of Gram-positive bacteria (52.5 %) belonging to *Firmicutes* (27.5 %), *Actinobacteria* (20 %) and *Bacteroidetes* (5 %). For Gram-negative bacteria (47.5 %), *Gammaproteobacteria* (35 %), *Alphaproteobacteria* (10 %) and *Betaproteobacteria* (2.5 %) were present (Fig. 2).

3.2. Cultivable bacteria isolated from dried fruit of Ficus carica

Nine bacterial strains were isolated from dried figs. A low diversity of bacterial communities associated with dried figs was observed, with *Bacillus* being the most frequent genus detected. BLAST analysis of the 16SrDNA sequences revealed that isolate IC1 has 100 % homology with *Bacillus australimaris* MCCC 1A05787(NR_148787.1), isolate IC8 with *Priestia megaterium* ATCC 14581(CP035094.1), IC11 with *Staphylococcus epidermidis* NBRC 100,911 (AP019721.1); 99 % homology of isolate IC4 with *Bacillus subtilis* IAM 12118(MK267098.1), isolate DC7 with *Bacillus bataviensis* NBRC 102,449 (NR_114093.1); and 98 % homology of isolate IC3 with *Rothia endophytica* DSM 26247(NR_109752.1). The 16S rDNA sequences were placed in the GenBank database, below the accession numbers MZ293802 for I1C1, MZ297877 for I1C4, MZ297879 for I2C8, MZ297880 for D1C7, MZ297881 for I2C11 and MZ297878 for I1C3 (Fig. 3).

3.3. Study of bacterial communities by DGGE

Denaturing gradient gel electrophoresis (DGGE) analysis was used to determine the bacterial diversity of soil rhizosphere samples, with each band on the gel representing a separate bacterial community (Muyzer et al., 1993). The DGGE profiles were reproducible between triplicate independent DNA extractions from the combined samples of the rhizosphere soil of *F. carica* from Nabeul (N), Kerkouane (K) and Mateur (M) (Fig. 4(a)). A Cluster analysis of bacterial populations obtained from DGGE profiles fixed on the median similarity matrix (UPGMA dendrogram, Fig. 4(b)) was also possible using PCR-DGGE analysis. For three regions, community diversity indexes were determinated: Nabeul (N), Kerkouane (K) and Mateur (M) (Fig. 5). The rhizosphere soil collected from the region of Kerkouane samples displayed the largest number of bands (richness score), the highest Shannon–Weaver index and also the highest Simpson index values. The value of evenness was quite constant and was defined by low E values (varying from 0.221 to 0.261).

3.4. PGP potential and abiotic stress of bacteria isolates

Eighty-two isolates were characterized for various PGP activities. Most isolates demonstrated several PGP traits, which can promote plant growth in different ways. The most common PGP trait for all bacteria from the three regions was siderophore production (62 %), followed by cellulase (38 %), then protease activity (37 %), then by lipases activity (17 %) and lastly by solubilization of phosphates (9 %). Besides, more studies were completed to assess the resistance of these strains to abiotic stresses (Fig. 6).

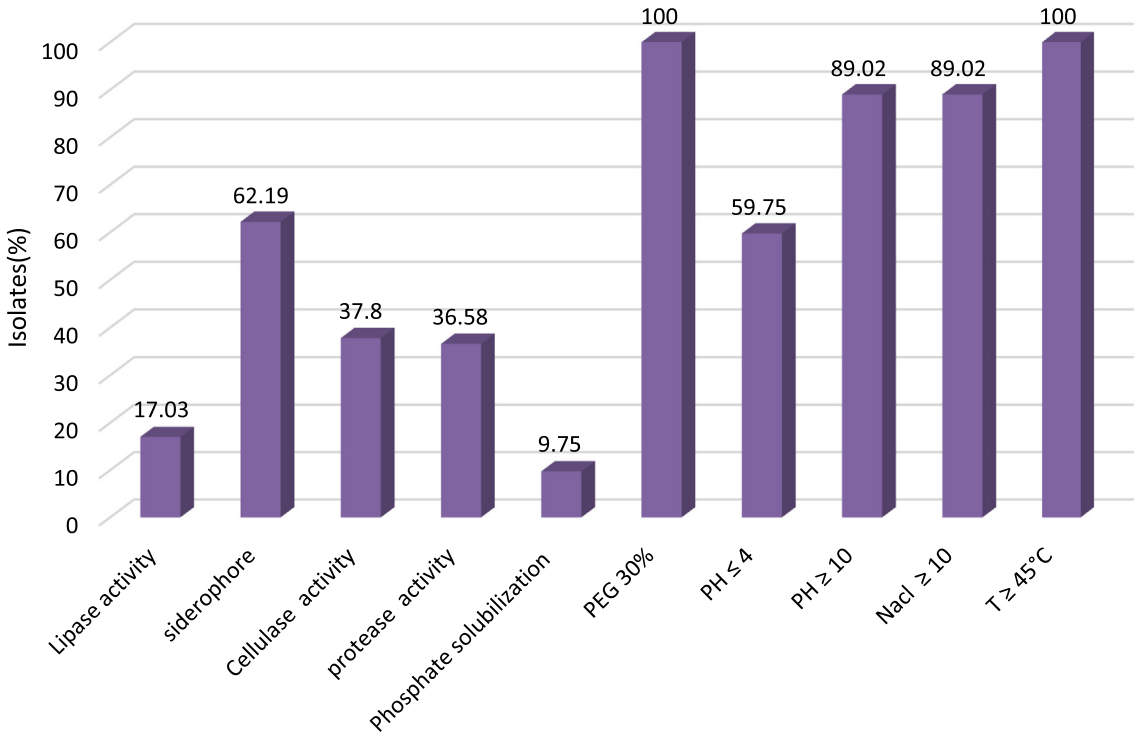

Fig. 6. PGP activity and abiotic stress tolerance of the isolates from rhizosphere of Ficus carica.

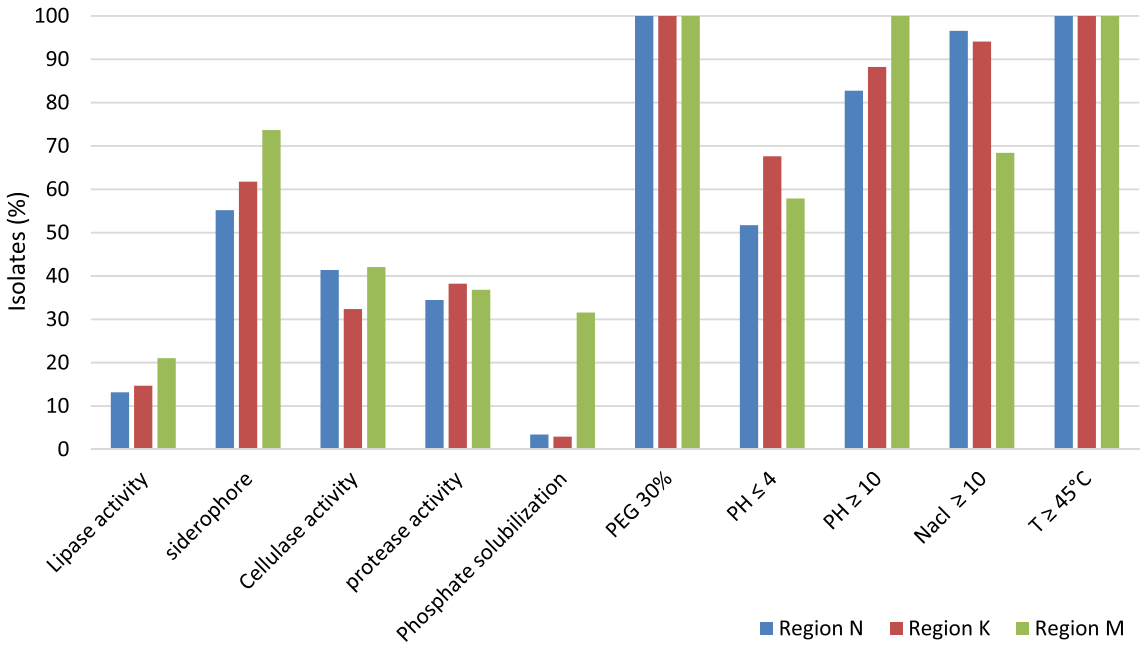

Fig. 7. Percentage of the strains associated with the rhizosphere of Ficus carica showing PGP traits and abiotic stress of isolated from three regions (Nabeul, Kerkouane and Mateur).

The results revealed that all strains showed stress resistance, and they all could grow in the presence of a significant concentration of PEG. All strains were capable to grow at 45 °C; among those, 27 % could also grow at 50 °C, and only 13 % could tolerate higher temperatures (55 °C). 89 % of isolates showed moderate tolerance to salt (10 % NaCl), 72 % could grow in 13 % NaCl, 52 % could exist in 16 % NaCl, and only 30 % could tolerate 20 % NaCl. The ability of the isolates to grow in a broad pH range was also tested. It was found that 85 % of the strains were facultative alkalophiles, which are capable to grow (until pH = 12), while 63 % of them could grow in acidic media (pH = 4). 29 % of the isolates were facultative acidophiles, which are capable to grow at pH = 3. The details of PGP

		PGP activities					Abiotic stress resistance											Isolates identification			
Origins	Strains	Prot.	Cell.	Sid.	Lip.	P sol.	PEG	T 45°	T 50°	T 55°	10% Nacl	13% Nacl	16% Nacl	20% Nacl	PH=3	PH=4	PH=10	PH=12	PGP score	ACC num	%	Closest described relative (BLAST)
R	N1	■		■			■	■			■	■	■	■		■	■	■	11	MZ820059	99%	*Lysobacter soli*
R	N2	■	■				■	■			■	■	■	■		■			9	MZ820060	99%	*Bacillus simplex*
R	N3	■			■		■	■	■	■	■	■	■	■	■		■	■	13	MZ820061	100%	*Rhodococcus wratislaviensis*
R	N6	■	■	■			■	■			■	■	■	■	■	■	■	■	13	MZ820062	99%	*Arthrobacter siccitolerans*
R	N10						■	■			■	■	■	■		■	■	■	9	MZ820075	99%	*Sphingopyxis panaciterrae*
R	N11						■	■			■	■	■			■	■	■	7	MZ820076	99%	*Arthrobacter pascens*
R	N12						■	■			■	■	■	■		■	■	■	8	MZ820077	99%	*Bacillus nealsonii*
R	N13	■	■	■		■	■	■			■	■	■	■	■	■	■	■	13	MZ820078	99%	*Bacillus simplex*
R	N14	■					■	■			■	■	■	■		■	■	■	9	MZ820079	99%	*Arthrobacter pascens*
R	N16		■	■			■	■			■	■	■	■	■	■	■	■	12	MZ820080	99%	*Arthrobacter siccitolerans*
R	N26			■			■	■			■	■				■	■	■	7	MZ820081	99%	*Lysobacter soli*
R	N30		■				■	■			■	■	■	■		■	■	■	9	MZ820082	98%	*Microvirga zambiensis*
R	45N						■	■			■	■	■	■		■	■	■	8	MZ823807	97%	*Bacillus nealsonii*
R	3K	■		■			■	■	■	■	■	■	■	■	■	■	■	■	14	MZ823808	99%	*Bacillus thuringiensis*
R	4K	■	■				■	■	■	■	■	■	■	■	■	■	■	■	12	MZ823810	98%	*Bacillus cereus*
R	28K		■				■	■			■	■	■	■		■	■	■	8	MZ824302	97%	*Pedobacter sp.*
R	44K						■	■			■	■	■	■		■	■	■	8	MZ824303	94%	*Bacillus pumilus*
R	55K						■	■			■	■	■	■		■	■	■	8	MZ824304	99%	*Bacillus thuringiensis*
R	12 M		■	■		■	■	■			■	■	■	■		■	■	■	11	MZ824410	98%	*Bacillus velezensis*
R	19M		■				■	■			■	■	■	■		■	■	■	8	MZ824411	97%	*Brevundimonas sp.*
F	DC7	■				■	■	■			■	■	■	■	■	■	■	■	12	MZ297880	99%	*Bacillus bataviensis*
F	IC4	■		■		■	■	■			■	■	■	■	■	■	■	■	12	MZ297877	99%	*Bacillus subtilis*
F	IC8		■				■	■			■	■	■	■		■	■	■	9	MZ297879	100%	*Priestia megaterium*

Fig. 8. PGP potential of the isolates is demonstrated as a PGP score, determinated by the sum of the number of PGP activity by each strain.R = rhizosphere;F = fig; Prot. = protease;activity;Cell. = cellulose activity; Sid. = siderophore production; Lip. = Lipase.

traits and abiotic stress of the isolated strains were presented in Fig. 7. The most potentially beneficial rhizobacteria were represented in Fig. 8, which show their biocontrol traits. 23 selected strains showed most PGP traits, 8 strains presented 12 or more, and 15 strains displayed between 7 and 11 of 17 PGP activities. None of the isolates possess all PGP traits. The majority of the isolates manifested a possible adaptation to abiotic stress and unfavorable environments. All strains exhibited resistance to low moisture, and all strains could grow at 45 °C. All strains could exist in media with 10 % NaCl added, 18 strains could tolerate 20 % NaCl added, and the majority of the strains could grow in basic media (pH = 10 and pH = 12). Seven strains manifested siderophore activity, 10 strains displayed a positive protease activity, 10 strains presented cellulase activity and 2 strains had lipase activity. Also, only 2 strains among these selected bacteria showed phosphate solubilization activity.

4. Discussion

To our knowledge the identification and the characterization of rhizospheric and endophytic bacteria from dried fruit of *Ficus carica* is studied for the first time in Tunisia. Rhizospheric bacteria species were isolated in non-specific media in order to obtain the greatest number of genera as possible. In this research, we characterized the biodiversity via PCR-16S rRNA analysis and then sequencing partial 16 S rRNA genes. We found that the cultivable bacterial community was principally constituted of *Pseudomonas, Bacillus, Arthrobacter, Lysobacter, Microbacterium* and *Stenotrophomonas*. Among them, the genera *Pseudomonas* and *Bacillus* were the most frequent endophytic bacteria for their PGP activities as described in the literature (Zhang et al., 2017; Chaudhary and Sharma, 2019). It is well-known that soil microbial communities are one of the most significant reservoirs of biological diversity in the world receiving no less than one quarter of the living organisms on earth (Decaëns et al., 2006; Tibbett et al., 2020). 17 different genera have been identified by this study, and the high genetic diversity is probably due to multitude elements (Macdonald and Singh, 2014; Nwachukwu and Babalola, 2021). PGPR are agriculture bioresources that enhance both plant growth and productivity, these Plant microbiomes have a direct impact on noxious microbes in the rhizosphere, and many beneficial microorganisms isolated from soil could boost the defensive capacity of the plant (Zamioudis and Pieterse, 2012; Babalola et al., 2021). The studies of bacteria strains naturally associated with dried fruit are limited (Nyanga et al., 2007; Ribeiro et al., 2014) and the fruit flora commonly includes yeasts and molds (Qadri et al., 2015). For dried figs, natural sun-drying had a lot of disadvantages as a result of the uncontrolled time and temperature, as well as the lack of meshes. All these factors can generate infection by fungi (Trucksess and Scott, 2008). *Aspergillus* spp., *Fusarium spp*., and *Penicillium* species are the largest fungal contaminants in dried figs (Turkoz Bakirci, 2020; Javanmard, 2010). The study of bacterial communities in dried figs has not been previously explored. In our study, 9 bacteria were isolated from dried figs, and *Bacillus* was the most frequent genus detected. In fact, in order to investigate the safety of dried fruits this genus was found in dried figs: *Bacillus Cereus (*Akbas and Ozdemir, 2008) and *Bacillus subtilis (*Hamanaka et al., 2002). The genus *Bacillus* is also very common in dried vegetables *(*Kudjawu et al., 2011). We identified *Rothia endophytica*, which has been isolated from healthy Maize roots (Elbahnasawy et al., 2021) and from wheat (Yousaf et al., 2017). We also found *Staphylococcus epidermidis*, which has been detected in the nectar of *Nicotiana glauca* (Fridman et al., 2012).

After its initial adoption in 1993, DGGE has been universally employed in recent years to investigate microbial communities (Muyzer et al., 1993; Ferreira et al., 2008). Genetic diversity was evaluated as bands on DGGE profile. To evaluate differences in the bacterial population in soils, the Shannon–Wiener index of diversity (H), richness (S), and evenness (E) were used.

Few studies have applied the index of diversity to compare between same-plant rhizosphere soils in different regions. To our

knowledge, rhizosphere soil of *ficus carica* has not been investigated by PCR-DGGE analysis to assess bacterial community dynamics. The DGGE profile of *ficus carica* rhizosphere from the region of Kerkouane was the most diverse followed by that of Mateur, with the least diverse being from Nabeul the values of richness (S) and Shannon–Wiener index (H) of each soil confirms the previous result. Some studies reported the study of rhizospheric bacteria using PCR-DGGE analysis in different plant bacteria in avocado (Yang et al., 2001), and peach palm (Almeida et al., 2005). The dendrogram representing the bacterial communities at different sampling dates were drawn (Fig. 5b) and N3, K3, M3 were grouped together with an average similarity of 75 %. K1, M3, N2, K2, M2 were in the same group with 76 % similarity. This result demonstrated that there is not a huge difference between the three regions concerning the bacterial community analysis.

The potential PGPR of rhizospheric and endophytic bacteria displayed numerous plant growth-promoting activities, such as siderophore production, phosphate solubilization, cellulase activity and protease activity (Kumar et al., 2014; Vinayarani and Prakash, 2018). In this research, we tested our isolates for various plant growth promoting properties, and we observed that *Bacillus* then *Pseudomonas* gave positive reactions for phosphate solubilization, production of siderophore, protease, cellulose and lipase. The role of siderophore is to chelate and to extract ferric iron from mineral or organic complexes present in the environment, and thus make it accessible to microorganisms and plants (Neilands, 1995; Ghosh et al., 2020). The production of siderophores is an ecological advantage in the rhizosphere and many rhizobacteria acquire this ability especially the following genera: *Pseudomonas, Bacillus, Rhodococcus,* and *Enterobacter* (Tian et al., 2009; Kour et al., 2019). Only few rhizobacteria displayed the ability to solubilize phosphate. Some studies reported that the activities of different soil enzymes were severely lowered in saline soils, which influenced the capacity of the soil (Batra and Manna, 1997, Dagar et al., 2004). *Bacillus* and *Pedobacter Pseudomonas* displayed the most efficiency in phosphate solubilization in our collection. *Azospirillum*, *Burkholderia, Pseudomonas*, *Rhizobium* and *Enterobacter* are reported in the literature as having highly efficient PSB (De Zutter et al., 2021). Phosphate solubilizing bacteria (PSB) are microorganisms that represent many functional characteristics associated to abilities of phosphate solubilization, they could show an encouraging substitute method to decrease the need of phosphate mineral fertilization to improve soil management and sustainable agriculture (Calvo et al., 2014; Alori et al., 2017; Amy et al., 2022). Other cell wall-degrading enzymes were also explored. We observed that 37 %, 36 %, and 17 % of the strains isolated from the rhizosphere have protease, cellulose and lipase activity, respectively. The rhizosphere bacterial species, such as *Pseudomonas*, *Bacillus*, were active against soil fungi by producing lytic enzymes, such as cellulase, protease, and lipase (Mabood et al., 2014; Chandran et al., 2021). In particular, *Pseudomonas* is known to produce lytic enzymes. These strains were active at suppressing fungal plant pathogens in tomatoes (Lugtenberg et al., 2001). Also, cell wall-degrading enzymes are produced by *Bacillus spp* such as proteases and chitinases. (El-Bendary et al., 2016; Schönbichler et al., 2020). The main factors limiting the biological activity of bacteria in the soil are: water deficit, extreme pH and temperatures, and nutrient deficiencies (Pandey et al., 2017). The frequent interactions between these different constraints influence the growth and survival capacity of microorganisms in arid soils (Lal et al., 2007). Our bacterial strains were tested for resistance to numerous abiotic stresses to assess their adaptability to all these naturals constraints. All the strains could grow at 45 °C, and even 13 % could tolerate a higher temperature of 55 °C; most *Pseudomonas* strains present this ability (Scheldeman et al., 2006). Also, it has been reported that *Bacillus thermoamylovorans* can survive at up to 60 °C (Choonut et al., 2020). All the strains isolated could grow on a dehydrated medium with a concentration of 30 % PEG 8000. It has been proven that inoculation with rhizobacteria allows an improvement in the water status of plants under limited irrigation conditions (Eid et al., 2021). The percentage decreases proportionally with the increase of the salt concentration; the tolerance to salinity is 89 %, 72 %, 52 % and 30 % for a concentration of NaCl of 10 %, 13 %, 16 % and 20 %, respectively. Salt stress tolerance is an attractive PGPR trait and is important for several soil types that suffer from salinity problems (Etesami and Maheshwari, 2018).

The study of abiotic stress tolerance for bacteria seems very interesting, for it can aid in the understanding of the adaptation mechanisms of microorganisms living in an environment and the selection of a large number of PGPR to improve the performance of plant tolerance under environmental stress conditions. Bacterial endophytes were known to be a source of new secondary metabolites for a potential therapeutic purpose (Tan and Zou, 2001; Shukla et al., 2014).

5. Conclusion

The results of this study might allow for the selection of several candidate strains that could be applied to promote plant growth when they are exposed to nutrient or environmental stress. Rhizospheric and endophytic bacteria from dried fruit of *Ficus carica* showed numerous PGP activities that potentially enhance plant growth. The selected strains will be further tested in future in vivo against phytopathogens. These plant growth-promoting bacteria may have a good impact in organic agriculture practices.

CRediT authorship contribution statement

Lamis Abid: Conceptualization, Methodology, Writing – original draft. **Marwa Smiri:** . **Ermanno Federici:** Conceptualization, Methodology, Resources. **Bart Lievens:** Methodology, Resources. **Mohamed Manai:** Supervision, Resources. **Yunjun Yan:** Resources. **Najla Sadfi-Zouaoui:** Supervision, Methodology, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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