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Saudi Journal of Biological Sciences 29 (2022) 1260–1268

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Roots of the xerophyte *Panicum turgidum* host a cohort of ionizing-radiation-resistant biotechnologically-valuable bacteria

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ARTICLE INFO

Article history:
Received 8 October 2020
Revised 6 September 2021
Accepted 9 September 2021
Available online 16 September 2021

Keywords:
Culture approach
Desiccation
Metabolic profiles
Metataxonomic
Panicum turgidum
Radioresistant

ABSTRACT

Bacterial communities associated with roots of *Panicum turgidum*, exposed to arid conditions, were investigated with a combination of cultural and metataxonomic approaches. Traditional culture-based techniques were used and 32 isolates from the irradiated roots were identified as belonging to Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria phyla. Four actinobacterial strains were shown to be ionizing-radiation (IR)-resistant: *Microbacterium* sp. PT8 (4.8 kGy (kGy)), *Micrococcus* sp. PT11 (4.4 kGy), *Kocuria rhizophila* PT10 (2.9 kGy) and *Promicromonospora panici* PT9^T (2.6 kGy), based on the D_{10} dose necessary for a 90% reduction in colony forming units (CFU). Concerning the investigation of microbial communities *in situ*, metataxonomic analyses of the diversity of IR-resistant microorganisms associated with irradiated roots revealed a marked dominance of Actinobacteria (46.6%) and Proteobacteria (31.5%) compared to Bacteroidetes (4.6%) and Firmicutes (3.2%). Gamma irradiation not only changed the structure of bacterial communities, but also affected their functional properties. Comparative analyses of metabolic profiles indicated the induction of several pathways related to adaptation to oxidative stress in irradiated roots, such as DNA repair, secondary metabolites synthesis, reactive oxygen species (ROS)-mitigating enzymes, *etc. P. turgidum* is emblematic of desert-adapted plants. Until now, there is no other work that has focused on the microbial profile of irradiated roots of this xerophyte.

1. Introduction

Resistance to IR has been correlated with tolerance to desiccation for more than four decades (Maxcy and Rowley, 1978; Rainey et al., 2005; Fredrickson et al., 2008; Arocha-Garza et al., 2017), the latter being a characteristic typically found in deserts organisms (Dion and Nautiyal, 2008). Maxcy and Rowley (1978) were the first to study radioresistant strains in natural microbiota using desiccation tolerance selection, without exposure to radiation sources. They suggested that there was a strong correlation between a prokaryote's capacity to survive regular episodes of dehydration and IR resistance (Maxcy and Rowley, 1978). Numerous previous studies demonstrated that prokaryotic strains resis-

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.09.020
1319-562X/

tant to IR are also tolerant to desiccation, such as *Methylobacterium* strains (Billi et al., 2000; Sghaier et al., 2005; Musilova et al., 2015). It was also shown that some desiccation-sensitive prokaryotes can possess resistance to IR (Beblo-Vranesevic et al., 2018). In this context, the 'radiation adaptation hypothesis' stipulates that desiccation tolerance could be a consequence to IR as gene products contributing to IR may have been recruited in some microbes to serve an additional function that is desiccation tolerance (Sghaier et al., 2007). The correspondence between the increase in the desiccation tolerance (days) and the rise in the (room temperature) irradiation survival (kGy) is 5 days and 1 kGy, respectively ((Musilova et al., 2015) and references therein). In this context, it is important to note that both IR and desiccation cause similar patterns of DNA damages (double-strand breaks (DSBs)) by accumulation of free radicals and ROS (Dose et al., 1992; Billi and Potts, 2002; Musilova et al., 2015). In addition, free-radical-mediated protein damages occur under both IR and desiccation exposure.

Dry biotopes offer promising environments to hunt for new IR-resistant prokaryotes (IRRP, IRRB and IR-resistant Archaea (IRRA)). Indeed, the tolerance to desiccation was utilized in the selection of new IR-resistant strains from non-radioactive sources (Rainey et al., 2005; Musilova et al., 2015). For instance, various phylogenetic groups of IRRP affiliated to Actinobacteria, Bacteroidetes, *Deinococcus-Thermus*, Firmicutes and Proteobacteria groups have been isolated from a wide range of arid biotopes, including the Sonoran desert (Rainey et al., 2005), the Sahara Desert in Morocco and Tunisia (de Groot et al., 2005), the Tataouine desert (Chanal et al., 2006), the Atacama desert (Azua-Bustos et al., 2012), the Taklamakan desert in Xinjiang (Yu et al., 2010; Liu et al., 2017), and the Lut desert of Iran (Mohseni et al., 2014; Shirsalimian et al., 2018). Conversely, radioactive environments have been mined for desiccation-tolerant prokaryotes (Fredrickson et al., 2004). Plant-associated IRRP nevertheless constitute a poorly studied group (Singh and Dubey, 2018). For instance, IRRP associated with *P. turgidum* — a desert perennial salt excluder bunchgrass of arid regions belonging to the *Poaceae* family and distributed in salt-affected areas and deserts with a height that may attain about 1 m in 25–30 days in summer (Khan et al., 2009) — are still unknown. Recently, the development of genomic tools, such as Next Generation Sequencing (NGS), has permitted analyses of the microbial diversity in extreme habitats and has been yielding potentially useful data for various applications. Thus, it is interesting to explore the diversity of microbial xerophytic species from arid zones.

In the present study, based on 16S rDNA marker gene sequencing, culture-based technique and metataxonomic analyses were used to study the diversity of IRRB associated with the irradiated roots of a xerophyte, *P. turgidum*.

2. Material and methods

2.1. Site description and sampling of P. Turgidum

Roots of *P. turgidum* were collected in May 2015 from an oasis at the gates of the Sahara Desert, Ksar Ghilane, Tunisia (GPS coordinates N32°59.557′, E9°36.941′, 221.9 m above sea level). The map showing the sampling location was generated using the software ArcGIS (https://www.arcgis.com/home/index.html) (Fig. 1). Samples were then either irradiated (R) or not (C), and both were stored at 4 °C until use. Roots of *P. turgidum* were homogenized and prepared for irradiation without drying and washing of soil particles. They were exposed immediately to gamma rays after collection without any treatment.

Fig. 1. Map showing the sampling location at Ksar Ghilane (Tunisia, GPS coordinates N32°59.557′, E9°36.941′, 221.9 m altitude).

2.2. Cultivable bacteria associated with irradiated roots

2.2.1. Recoverable CFU/g after exposure to different doses of gamma irradiation

The procedure of Rainey et al. (2005) was used with minor modifications. Briefly, 1 g aliquots of *P. turgidum* roots were exposed to levels of IR ranging from 0 to 15 kGy with a mean dose rate of 25.6 kGy/h at room temperature (25 °C) using a cobalt-60 gamma irradiator at the National Center for Nuclear Sciences and Technology (CNSTN), Tunisia. After exposure to IR, non-irradiated (C) and irradiated (R) samples were crushed in a sterile mortar with 9 mL of sterile distilled water and then transferred into 15 mL tubes with glass beads. The mixture was shaken for 1 h at room temperature (25 °C) using an orbital shaker at 150 rpm ; 1 mL of the resulting slurry was serially diluted (10^{-5}–10^{-9}) in a 0.9% NaCl solution. Then, 100 µL aliquots of each dilution were plated on tryptic soy agar (TSA) (Sigma). Growth of microorganisms and their appearance were recorded after 7 days of incubation at 30 °C, after which the cellular percentage of survival for each dose was determined. All manipulations were done in triplicates (3).

2.2.2. Isolation of bacteria and electron microscopy

For the isolation of IRRB, the dose of 10 kGy was chosen and 1 g of irradiated roots of *P. turgidum* was crushed as indicated in the previous step. After shaking in an orbital shaker, at 150 rpm for 30 min at room temperature, each mixture was serially diluted (10^{-1}–10^{-5}). A volume of 100 µL of each dilution was plated on TSA. After 7 days of incubation at 30 °C, different morphotypes were selected, purified, maintained in tryptic soy broth (TSB) and stored in 25% glycerol (vol/vol) at − 80 °C (Guesmi et al., 2019).

Morphological features of most IRRB were examined using a Scanning Electron Microscope (JSM 5400, JEOL, Japan) as described by Kaewkla and Franco (2019). After growth on TSA plates at 30 °C for 48 h, bacterial cells were fixed with 2% w/vol glutaraldehyde in 0.9% NaCl solution and then fixed cells were dried with graded (50, 70, 90 and 100%) ethanol solutions. All manipulations were done in triplicates (3).

2.2.3. Genomic DNA extraction

Genomic DNA extraction from 32 isolated strains was performed using a modified method from Marmur (1961). Briefly, the pellet of bacterial cells was re-suspended in 10 mL of lysis buffer containing 50 mM Tris/HCl, 10 mM ethylene diamine tetra acetic acid (EDTA), 100 µg/mL RNase A and 20 mg/mL lysozyme at pH 8. Then, 10 mL of 2% sodium dodecyl sulfate (SDS) wt/vol and 10 mg of proteinase K were added to the mixture and incubated for 4 to 6 h at 55 °C. Proteins and cell debris were removed

by extraction with 20 mL of phenol, chloroform and isoamyl alcohol (25:24:1 v/v/v) at pH 8.2 at room temperature. The mixture was sedimented by centrifugation at 5000 × g during 10 min (Avanti® J-E) and the upper aqueous phase containing DNA was carefully transferred into a new tube. An equal amount of isopropanol was added to precipitate DNA. The precipitate was sedimented by centrifugation at 5000 × g for 20 min and the supernatant was removed. Then, 2 mL of 75% ethanol vol/vol were added and the tubes were centrifuged again for 10 min. After drying, the DNA pellet was re-suspended in 200 µL of 10 mM Tris-HCl at pH 8. Total DNA was subjected to quality control by electrophoresis on 1% agarose gel and quantified by a NanoDrop spectrophotometer (Implen NP80, Thermo Fisher Scientific, USA) and Qubit assay (Quant-iT™ PicoGreen® dsDNA, Invitrogen).

2.2.4. Identification by 16S rDNA gene sequencing of pre-selected isolates and phylogenetic analysis

Bacterial cultures (isolates), which had survived the gamma irradiation preselected level of 10 kGy, were subjected to phylogenetic analysis by sequencing the 16S rDNA. The primers used were 27F (5′-AGAGTTTGATCMTGGCTCAG) as forward primer and 1492R (5′- TACGGYTACCTTGTTACGACTT) as reverse primer (Lane et al., 1985). Amplification was carried out in 50 µL reaction volumes — 5 µL of 27F (10 mM), 5 µL of 1429R (10 mM), 10 µL of 5X Taq buffer, 1 µL of deoxynucleoside triphosphates (dNTP, 10 mM), 2.5 µL $MgCl_2$ (25 mM), 0.25 µL of GoTaq DNA polymerase (2 U/µL) (Kit GoTaq® DNA Polymerase, Promega) and 5 µL of template DNA. The total volume was adjusted to 50 µL with distilled water (Fredrickson et al., 2004). Reactions were subjected to the following temperature cycling profile: 94 °C at 5 min as initial DNA denaturation step, followed by 30 cycles consisting of denaturation at 94 °C for 45 s (s), annealing at 55 °C for 45 s and extension at 72 °C for 90 s. A final extension step was performed after the amplification for 5 min at 72 °C. Following the manufacturer's instructions, the products were purified with a QIAQuick PCR purification Kit (Qiagen, Valencia, CA) then sequenced with the above-mentioned primers.

The 16S rDNA gene sequences, of 32 bacteria isolated in this study, were edited using BioEdit (Hall, 1999) and deposited in GenBank (https://submit.ncbi.nlm.nih.gov/) (Benson et al., 2017). Taxonomical identification was based on 16S rDNA relationships *via* EzBioCloud available at https://www.ezbiocloud.net/ (Yoon et al., 2017) and leBIBI^{QBPP} (Flandrois et al., 2015) available at https://umr5558-bibiserv.univ-lyon1.fr/lebibi/lebibi.cgi/. Phylogenetic analysis was performed using the default parameters of W-IQ-TREE (Trifinopoulos et al., 2016) available at http://iqtree.cibiv.univie.ac.at/ and displayed using the Interactive Tree of Life (iTOL; http://itol.embl.de) (Letunic and Bork, 2016).

2.2.5. Determination of the radiation survival response using the MTT assay

The survival of pre-selected strains, after exposure to IR, was determined using the 3-(4, 5-dimethyl imidazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay described by Mosmann (1983). Isolates were grown in TSB medium to the exponential phase. Cells were recovered by centrifugation, washed twice with 0.9% NaCl solution at pH 7 and re-suspended in the same buffer. Then, 1 mL of cell suspension (with density of 0.6) was transferred into sterile 1.5 mL Eppendorf tubes and subsequently exposed to 1–5 kGy of gamma irradiation at a dose rate of 25.6 kGy/h. Afterwards, 100 µL of cell suspensions were placed in 24-well plates and 10 µL of MTT solution at a concentration of 5 mg/mL were added and kept in the dark for 3 to 24 h at 37 °C, then formazan crystals were dissolved using dimethyl sulfoxide (Sigma).

Results are always shown with the corresponding controls unexposed to IR, which were normalized to 100% (Morini et al., 2017). The absorbance in each well was measured, including blanks, at 570 nm using a microtiter plate reader (Shimadzu, UV-1800). Numerical values of D_{10} (kGy) were determined using the RadioP1 database available at www.radiop.org.tn (Benhamda et al., 2015). The classification of an isolate as belonging to IRRB was done based on a previously proposed criterion (Sghaier et al., 2008) — D_{10} greater than 1 kGy. All manipulations were done in triplicates (3).

2.3. Metataxonomic approach

2.3.1. DNA extraction from roots of P. Turgidum

Non-irradiated and irradiated — 10 kGy — roots of *P. turgidum* were ground to a fine powder with a porcelain mortar and pestle. Extraction of genomic DNA was then carried out using Qiagen DNeasy Kit (Qiagen, Valencia, CA), quantified and checked for purity at a ratio 260/280 nm using a Nanodrop spectrophotometer (Implen NP80, Thermo Fisher Scientific, USA) and stored at − 20 °C.

2.3.2. NGS via an Illumina MiSeq platform

Genomic DNA was sent to Inqaba Biotechnical Industries, a commercial NGS service provider, for sequencing. Briefly, genomic DNA samples were PCR amplified using a universal primer pair (341F and 785R targeting V3 and V4 of the 16S rDNA gene). The resulting amplicons were gel purified, end-repaired and Illumina specific adapter sequences were ligated to extremities. Following quantification, samples were individually indexed and a bead based purification step (AmpureXP) was performed. Amplicons were then sequenced on an Illumina™ MiSeq platform (San Diego, Ca) using a MiSeq v3 (600 cycle) kit. Fifty Mb of data (2 × 300 bp long paired end reads) were produced for each sample.

2.3.3. Metataxonomic post-NGS in silico analyses

Reads were merged using the software FLASH (Magoč and Salzberg, 2011). Microbial diversity associated with non-irradiated and irradiated roots of *P. turgidum*, was quantified with One Codex (https://www.onecodex.com/). Taxonomic Units (OTUs) were determined at different levels; and taxa with relative abundance higher than 1% were retained for statistical and comparative analyses. The SILVAngs pipeline (SILVAngs 1.4, https://ngs.arb-silva.de/silvangs/) was used to create rarefaction curves based on rDNA amplicon reads from high-throughput sequencing (Wang et al., 2007; Quast et al., 2013). Functional inferences and comparisons, based on 16S rDNA datasets, were generated using the multi-modular web-platform iVikodak *via* global and local mapper modules (https://web.rniapps.net/iVikodak/local.php) (Nagpal et al., 2019).

2.4. Statistics and data illustration

Results were expressed statistically using standard deviation (SD). *P*-values less than 0.01 were considered as significant. Graphics illustrating these analyses were generated using Origin Lab (https://www.originlab.com/).

3. Results

3.1. Culture-based approach

3.1.1. Recoverable CFU/g following exposure to different doses of gamma irradiation

Initially, the survival of bacteria associated with roots of *P. turgidum* after exposure to IR at a dose range of 0–15 kGy was assessed (Fig. 2).

As shown in Fig. 2, there was a decrease in the percentage of microbial survival recovered from the roots of *P. turgidum* with the increase of IR dose. Approximately 99% of the culturable bacterial population was recovered after exposure of the sample to 1 kGy. Microbial survival decreased to 49% at a dose of 6 kGy. After exposure to 15 kGy, less than 1% of the original culturable population of bacteria could be recovered by dilution plating. The diversity — colony morphology, *etc.* — of recovered isolates decreased at higher irradiation doses. The majority of colonies on plates containing irradiated samples were pigmented.

3.1.2. Diversity and phylogenetic identification of IRRB based on 16S rDNA gene sequences

The diversity of the collection of 32 isolates, recovered from roots of *P. turgidum* after exposure to IR at a dose of 10 kGy, was studied. Sequences of 16S rDNA were used to assign each isolate to a taxonomic group (Table S1) and position them phylogenetically Fig. 3.

As shown in Fig. 3, obtained sequences were affiliated to four bacterial phyla — Firmicutes (56.25%), Actinobacteria (34.38%), Proteobacteria (6.25%) and Bacteroidetes (3.12%). Isolated microorganisms were assigned to nine different genera that have previously been shown to contain IRRB, namely *Bacillus* (13 isolates), *Microbacterium* (six isolates), *Staphylococcus* (five isolates), *Kocuria* (two isolates) and *Micrococcus* (two isolates). Four other strains, related to the genera *Acinetobacter*, *Olivibacter*, *Pantoea* and *Promicromonospora* were also detected.

3.1.3. Post-irradiation viability of representative isolates

Ten isolates representing each morphotype (*Bacillus mojavensis* PT1, *Bacillus subtilis* PT13, *Staphylococcus pasteuri* PT2, *Kocuria rosea* PT5, *Kocuria rhizophila* PT10, *Microbacterium* sp. PT8, *Promicromonospora panici* PT9^T, *Micrococcus* sp. PT11, *Pantoea eucrina* PT16 and *Olivibacter soli* PT19) were selected to evaluate their survival following irradiation at dose levels from 1 to 5 kGy (Fig. 4).

As shown in Fig. 4, survival rates following gamma irradiation decreased for all isolates at higher IR doses, when compared to non-irradiated controls. For instance, PT8 and PT11 exhibited 29.89 and 38.93% survival at a dose level of 3 kGy, respectively.

D_{10} values were calculated for the most IR-resistant strains (*Micrococcus* sp. PT11, *Microbacterium* sp. PT8, *Kocuria rhizophila* PT10 and *Promicromonospora panici* PT9^T) (Table S2).

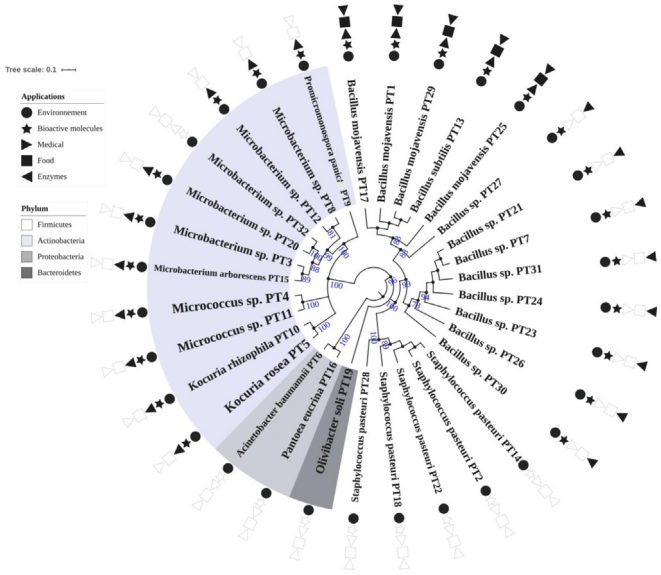

Fig. 3. Phylogenetic tree based on 16S rDNA gene sequences of 32 isolates from irradiated roots of *P. turgidum*. Support values are calculated from 1000 bootstrap replicates and only values superior to 70% are indicated.

3.2. Metataxonomic approach

3.2.1. NGS data, diversity indices, coverage and rarefaction curves

In this study, a total of 81,534 (74.89%) and 139,697 (54.50%) 16S rDNA high-quality reads with an average read length of 2 × 300 bps were generated from non-irradiated (C) and irradiated (R) root samples of *P. turgidum*, respectively. Merged reads were assigned to 1,014 and 799 OTUs to species level, and to 830 and 666 OTUs to genus level for irradiated and non-irradiated samples, respectively. Community diversity estimators of Shannon and Simpson and community richness estimators of Chao and Ace for irradiated (R) and non-irradiated (C) samples are summarized in Table S3. The sequence coverage was evaluated by rarefaction analysis at the 93% cut-off for the comparison of species richness (Fig. S1). None of the curves approached a plateau, suggesting that new bacterial taxa continued to emerge even after 81,534 and 139,697 reads in the un-irradiated and irradiated samples, respectively.

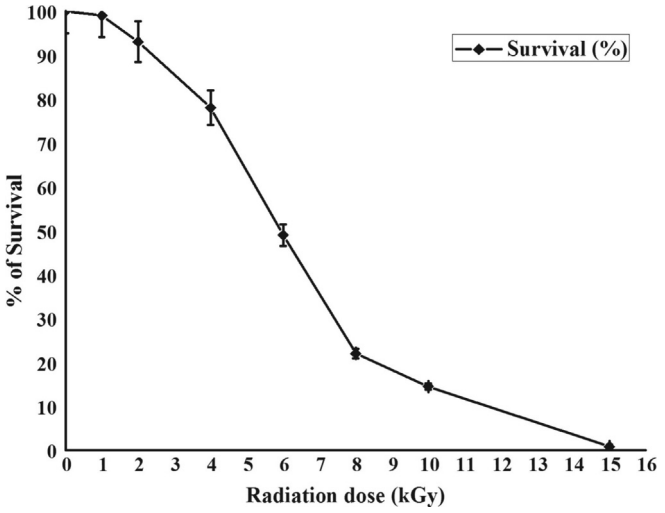

Fig. 2. Effect of gamma irradiation dose on the survival of culturable bacteria associated with roots of *P. turgidum*.

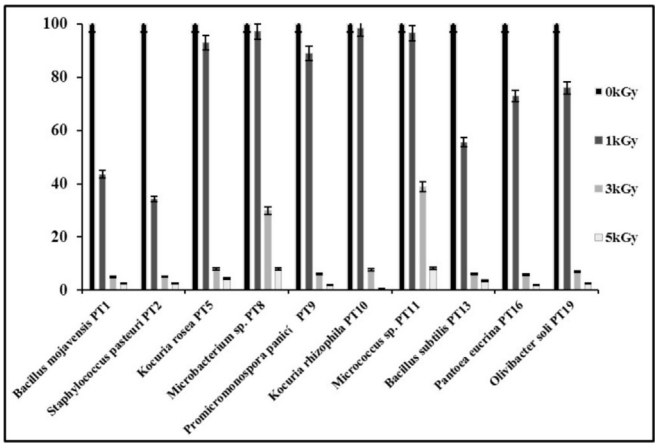

Fig. 4. Survival of representative pre-selected isolates, from irradiated roots of *P. turgidum*, exposed to 1, 3 and 5 kGy.

3.2.2. Taxonomic composition and distribution

The relative abundance of taxa associated with roots of *P. turgidum* varied between un-irradiated and irradiated samples. Bacteroidetes was dominant in non-irradiated samples. The phylum Actinobacteria was dominant in irradiated samples (Fig. 5 and Fig. S2). However, comparative analyses indicated the presence of four dominant phyla — Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria — associated with roots of *P. turgidum* in both non-irradiated and irradiated conditions (Fig. 5).

A significant increase in the relative abundance of Actinobacteria and Proteobacteria was observed following exposure to IR at a dose of 10 kGy. However, the relative abundance of both phyla Firmicutes and Bacteroidetes was significantly reduced after the treatment with IR (Fig. 5).

Indeed, IR affected the relative abundance of different families (Table 1) and genera (Fig. 6, Fig. S3) when compared to the control sample.

For instance, at the genus level, *Chitinophaga* and *Niastella*, belonging to Bacteroidetes, showed a decrease in their relative abundances after exposure to IR of *P. turgidum* roots (Supporting Information Table S4). Also, for example, relative abundances of major detected genera affiliated to Actinobacteria from non-irradiated and irradiated roots are shown in Fig. 6. Actinobacterial genera *Agromyces*, *Frankia*, *Kribella*, *Nocardioides*, *Pseudonocardia* and *Streptomyces* were more abundant following exposure to IR of roots of *P. turgidum*. Other genera, such as *Saccharothrix*, were significantly more abundant in the control than in the irradiated sample.

3.2.3. Strains abundance following gamma irradiation

As shown in Fig. 7, the abundance of some species affiliated to Actinobacteria increased following exposure to IR. These include *Geodermatophilus* sp., *Micrococcus luteus*, *Nocardioides alkalitolerans* and *Promicromonospora sukumoe*. Furthermore, two strains belonging to Bacteroidetes (*Hydrotalea flava* and *Ohtaekwangia koreensis*) and one strain belonging to Proteobacteria (*Steroidobacter denitrificans*) were more abundant in irradiated roots of *P. turgidum* than in the control.

Table 1
Percentage of increase and decrease of family abundance of irradiated roots of *P. turgidum*.

Family Up (%)		Family Down (%)	
Nocardioidaceae	12.27	*Pseudonocardiaceae*	9.30
Rhizobiaceae	3.69	*Bacillaceae*	8.79
Oxalobacteraceae	3.45	*Chitinophagaceae*	8.00
Microbacteriaceae	2.00	*Burkholderiaceae*	2.04
Sphingomonadaceae	1.95	*Oxalobacteraceae*	1.77
Streptomycetaceae	1.94	*Cytophagaceae*	1.76
Caulobacteraceae	1.39	*Comamonadaceae*	1.73

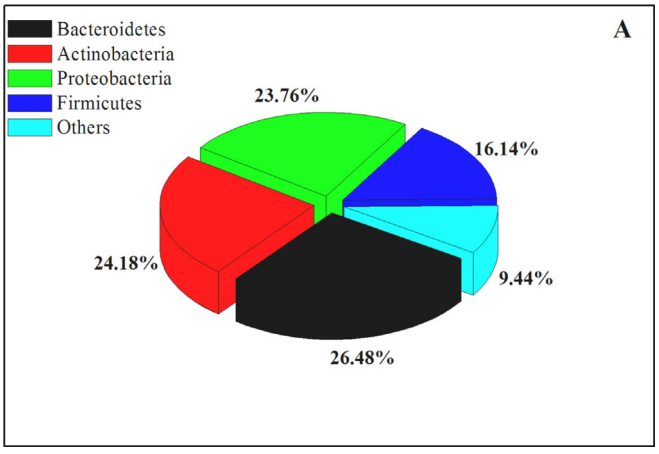

Fig. 5. Abundant bacterial phyla associated with roots of *P. turgidum*. A) Un-irradiated sample (0 kGy). B) Irradiated sample (10 kGy).

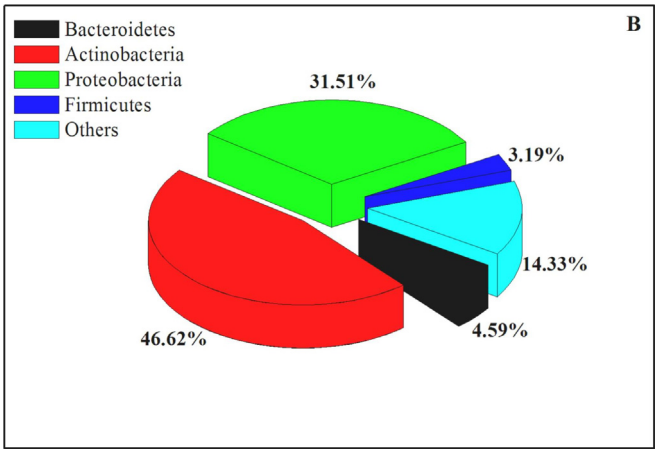

Fig. 5 (*continued*)

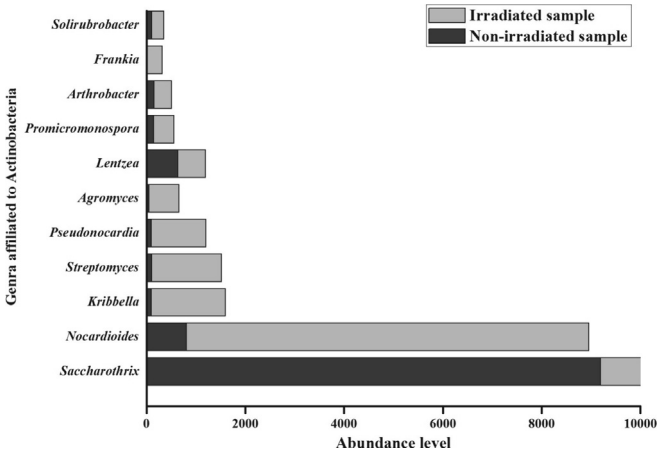

Fig. 6. Relative abundances of major genera affiliated to Actinobacteria associated with roots of *P. turgidum* in un-irradiated and irradiated samples.

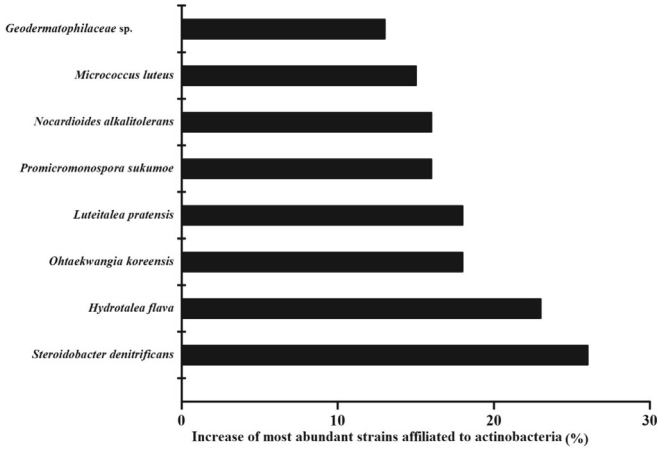

Fig. 7. Increase of abundance of major bacterial species associated with roots of *P. turgidum* after gamma irradiation.

3.2.4. Functional potential of microbial communities based on 16S rDNA gene sequencing datasets

The detailed indication of bacterial pathways present in studied (non-irradiated and irradiated) samples are shown in Fig. S5 using the "Pathway Abundance Profiles" functions in the iVikodak Global mapper (Nagpal et al., 2019). Some bacterial pathways associated with roots of *P. turgidum* were more abundant in the irradiated sample than in the control, such as carbohydrate metabolism (Fig. 8) and carbon fixation pathways (Fig. S6). However, IR also induced a decrease of the relative abundance of enzymes related to the capacity of the degradation of xenobiotic under oxidative stress conditions (Fig. 8).

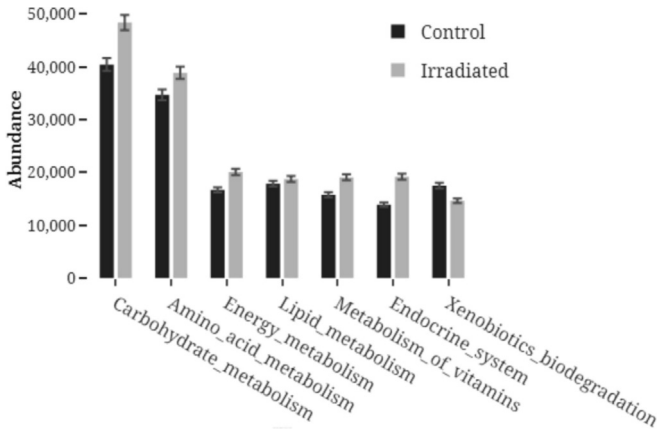

Fig. 8. Top bacterial pathways associated with un-irradiated and irradiated roots of *P. turgidum*.

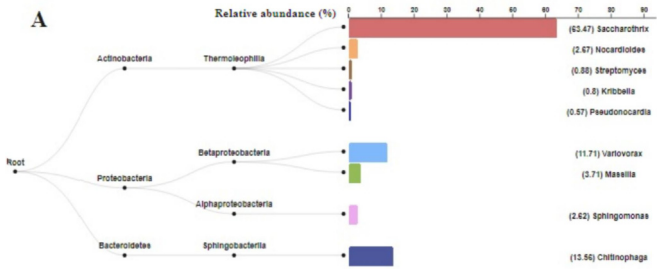

Fig. 9. Contributors to lipopolysaccharide biosynthesis. A) Un-irradiated sample. B) Irradiated sample.

Fig. S7 and Fig. S8 summarize changes in bacterial pathways due to IR treatment. Bacterial communities associated with irradiated roots were characterized by a higher abundance of pathways involved in DNA repair and secondary metabolic pathways. IR induced an increase of the relative abundance of the enzymes related to carbon fixation, nitrogen metabolism and biosynthesis of lipopolysaccharides. For instance, contributors to lipopolysaccharide biosynthesis are different in the control and irradiated samples (Fig. 9).

4. Discussion

This study explored the diversity of IRRB recovered from roots of *P. turgidum* following exposure to IR based on culture-dependent and metataxonomic approaches. Our results indicated that roots of *P. turgidum* harbour a variety of IRRB (Fig. 2). A total of 32 strains, affiliated to four phyla — Firmicutes (17 isolates), Actinobacteria (11 isolates), Proteobacteria (two isolates) and Bacteroidetes (one isolate) — were identified (Fig. 3, Fig. 4, Table S1, Table S2) with different morphotypes.

Our findings are concordant with previous works (Rainey et al., 2005; Chanal et al., 2006) that showed that many taxa of the bacterial community associated with arid soils are resistant to levels of IR that exceed the usual background levels in deserts and arid environments.

The diversity of IRRB associated with roots of the studied xerophyte is consistent with previous studies of desert soils in India (Sivakala et al., 2018), Namibia (Le et al., 2016), China (Yu et al., 2015) and Tunisia (Chanal et al., 2006). In particular, the abundance of Actinobacteria, Bacteroidetes and Proteobacteria groups has been widely reported, with the proportion varying with climates and niches(Le et al., 2016; Rao et al., 2016; Vikram et al., 2016). A real-time quantitative PCR-based approach developed by Rao et al. (2016), revealed the abundance of Actinobacteria and Proteobacteria in semi-arid soils collected from Thar Desert (India). Similarly, metagenomic analyses of the Namib Desert showed also the abundance of both Actinobacteria and Proteobacteria phyla (Le et al., 2016).

Our culture-dependent results are significant in at least two major respects. First, our data raise again the possibility that IRRB isolated from roots of xerophytes may be valorised in various biotechnological applications. Indeed, members of the Actinobacteria phylum were reported as producers of bioactive molecules, and also were proposed for multiple medical and environmental applications (Shivlata and Satyanarayana, 2015). Firmicutes isolates are producers of enzymes (protease, lipase, *etc.*) and sec-

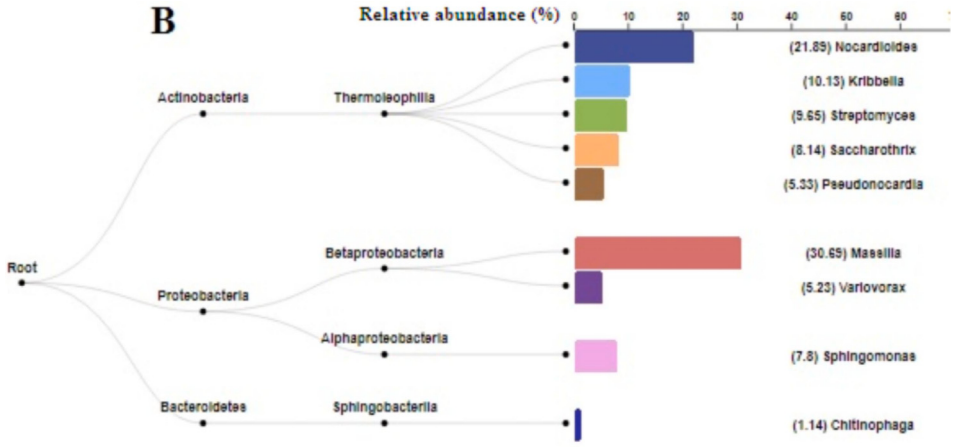

Fig. 9 (*continued*)

ondary metabolites (Kumar et al., 2013; Jasim et al., 2016). For example, *Bacillus* strains are implicated in food production as probiotic agents (Schultz et al., 2017). Proteobacteria and Bacteroidetes groups are used for environmental applications (Moghadam et al., 2016; Hong et al., 2018). Secondly, to the best of our knowledge, this is the initial report of the IR resistance of some taxa, like *Promicromonospora*, which we recently described in details (Guesmi et al., 2021). Similarly, the polyphasic characterization and the proteogenomic arsenal of IR-resistant *Kocuria rhizophila* PT10 were investigated (Guesmi et al., 2021). It can therefore be assumed that desert habitats represent suitable ecosystems, which could be used for bioprospecting extremophilic bacterial communities, like Actinobacteria, to query for novel metabolic capabilities and biotechnological potentials (Arocha-Garza et al., 2017; Singh and Dubey, 2018).

Bacterial community profiling of extreme and stressful environments is crucial for interpreting broader ecological patterns. In particular, hot desert environments are characterized by high spatial heterogeneity (Pajares et al., 2016) and possess endemic microbial communities (Ronca et al., 2015; Wei et al., 2016; Arocha-Garza et al., 2017), that play key roles in ecosystem processes (Le et al., 2016). Desert environments represent excellent model systems to explore microbial adaptation and response to various environmental stressors and to investigate the bacterial diversity (Arocha-Garza et al., 2017). In this context, culture-dependent methods remain insufficient to explore the total diversity of extremophilic microorganisms that colonize arid zones (Singh and Dubey, 2018). In order to enhance the repertoire of IRRB from roots of xerophytes, a culture-independent approach was also used in this investigation.

Our metataxonomic analyses showed four major phyla — Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria — with different distributions between non-irradiated and irradiated samples (Fig. 5, Table S4, Table S5). Exposure of roots of *P. turgidum* to IR induced an increase, of approximately 50%, in the abundance of Actinobacteria members. The Actinobacteria phylum was shown to comprise several species affiliated to well-known ecologically or biotechnologically important genera, such as *Frankia*, *Geodermatophilus*, *Micrococcus*, *Streptomyces*, *etc.* Arid and desert environments were reported to host an abundance of diversified xerotolerant microorganisms belonging to the phylum Actinobacteria (Yandigeri et al., 2012; Yu et al., 2015; Mohammadipanah and Wink, 2016). These strains demonstrated interesting capacities to survive under high temperatures, large radiation doses, elevated salt concentrations and drought conditions (Kurapova et al., 2012; Shivlata and Satyanarayana, 2015).

Furthermore, the functional potentials of microbial communities associated with roots of *P. turgidum* were predicted based on 16S rDNA gene sequencing datasets. The functional profiles were characterized by the presence of several pathways associated with environmental adaptations, such as polysaccharides and carotenoids biosynthesis, carbon fixation and xenobiotic-biodegradation pathways (Fig. 8). The bacterial communities associated with irradiated roots (50% of Actinobacteria) were characterized by a higher abundance of pathways involved in DNA replication and repair, such as mismatch and recombination repair, carbon fixation or nitrogen metabolism, pigment production and secondary metabolism biosynthesis (alkaloids, lipopolysaccharides and terpenes) (Fig. 9).

Several reports have shown that actinobacterial strains represent prolific sources of bioactive compounds, biomolecules and enzymes, with pharmaceutical, agricultural and biotechnological potentials (Ding et al., 2013; Shivlata and Satyanarayana, 2015; Singh and Dubey, 2018). The mechanisms of DNA repair (mismatch and recombination repair) and pigment production were mentioned as protective strategies against harmful rays in bacteria (Williams et al., 2007; Zhang et al., 2010). The antioxidant defences and repair systems contribute to the protection of proteins from oxidative damages and permit survival of radioresistant prokaryotes under extreme conditions and their function with great efficiency during recovery (Matallana-Surget et al., 2009; Asgarani et al., 2012).

The abundance of DNA replication and repair pathway, as well as the presence of signalling molecules, the abundance of secondary metabolites and the interaction pathways in the bacterial communities could be related to the suppression of ROS and the activation of cellular signalling pathways (Prabhakaran et al., 2016).

5. Conclusions

Our study reported the diversity of *P. turgidum*-associated IRRB, exposed to arid conditions, using a culture-based method and a metataxonomic approach. Thirty-two strains were isolated from irradiated roots of *P. turgidum* and were affiliated to Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. Metataxonomic analyses of the microbial community present in the irradiated roots of *P. turgidum* showed a marked dominance of Actinobacteria (46.62%) and Proteobacteria (31.51%) compared to Bacteroidetes (4.59%) and Firmicutes (3.19%). These findings have significant implications for the understanding of how plant roots exposed to IR will have taxonomically distinct bulk soil-associated, rhizospheric and endospheric microbiomes compared to un-irradiated samples. Moreover, considering that many of the microorganisms deciphered in this investigation are known to produce profitable primary and secondary metabolic products (*i.e.*, extremolytes and extremozymes), one of the more significant findings to emerge from this study is that xerophytes are pertinent targets to explore for new IR-resistant microorganisms with potential biotechnological and radioprotection applications.

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.

Acknowledgements

This work was supported by grants from the Tunisian Ministry of Higher Education and Scientific Research; without this generous and strong assistance, this study could not have been undertaken. It was performed under the auspices of the Tunisian National Center for Nuclear Sciences and Technologies (CNSTN) and Institut Pasteur (Tunisia) in collaboration with the Université de Lyon (France). We thank Betty Bigai, Corinne Sannaire and platform Genomique Environnementale PGE, UMR CNRS 5557 for technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.09.020.

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