Draft genome sequence of Promicromonospora panici sp. nov., a novel ionizing-radiation-resistant actinobacterium isolated from roots of the desert plant Panicum turgidum

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Corresponding Author	Family Name	**Normand**
	Particle	
	Given Name	**Philippe**
	Suffix	
	Division	
	Organization	Université de Lyon, Université Lyon 1
	Address	Lyon, France
	Division	
	Organization	CNRS, UMR 5557, Écologie Microbienne, UMR1418, INRA
	Address	69622, Villeurbanne Cedex, France
	Phone	
	Fax	
	Email	philippe.normand@univ-lyon1.fr
	URL	
	ORCID	
Author	Family Name	**Guesmi**
	Particle	
	Given Name	**Sihem**
	Suffix	
	Division	
	Organization	National Agronomy Institute of Tunisia
	Address	Avenue Charles Nicolle, 1082, Tunis, Mahrajène, Tunisia
	Division	Laboratory “Energy and Matter for Development of Nuclear Sciences” (LR16CNSTN02), National Center for Nuclear Sciences and Technology
	Organization	Sidi Thabet Technopark
	Address	2020, Sidi Thabet, Tunisia
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Nouioui**
	Particle	
	Given Name	**Imen**
	Suffix	

	Division	School of Natural and Environmental Sciences
	Organization	Newcastle University
	Address	Ridley Building 2, Newcastle upon Tyne, NE1 7RU, UK
	Division	
	Organization	Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures
	Address	Braunschweig, Germany
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Pujic**
	Particle	
	Given Name	**Petar**
	Suffix	
	Division	
	Organization	Université de Lyon, Université Lyon 1
	Address	Lyon, France
	Division	
	Organization	CNRS, UMR 5557, Écologie Microbienne, UMR1418, INRA
	Address	69622, Villeurbanne Cedex, France
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Dubost**
	Particle	
	Given Name	**Audrey**
	Suffix	
	Division	
	Organization	Université de Lyon, Université Lyon 1
	Address	Lyon, France
	Division	
	Organization	CNRS, UMR 5557, Écologie Microbienne, UMR1418, INRA
	Address	69622, Villeurbanne Cedex, France
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Najjari**
	Particle	
	Given Name	**Afef**

	Suffix	
	Division	
	Organization	Université de Tunis el Manar, Faculté des Sciences de Tunis, LR03ES03 Microorganismes et Biomolécules Actives
	Address	2092, Tunis, Tunisia
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Ghedira**
	Particle	
	Given Name	**Kais**
	Suffix	
	Division	Laboratory of Bioinformatics, Biomathematics and Biostatistics-LR16IPT09
	Organization	Institut Pasteur de Tunis, Université de Tunis El Manar
	Address	1002, Tunis, Tunisia
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Igual**
	Particle	
	Given Name	**José M.**
	Suffix	
	Division	
	Organization	Instituto de Recursos Naturales y Agrobiología de Salamanca, Consejo Superior de Investigaciones Científicas (IRNASA-CSIC)
	Address	c/Cordel de Merinas 40-52, 37008, Salamanca, Spain
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Cherif**
	Particle	
	Given Name	**Ameur**
	Suffix	
	Division	
	Organization	University Manouba, ISBST, BVBGR-LR11ES31,
	Address	Biotechpole Sidi Thabet, 2020, Ariana, Tunisia
	Phone	
	Fax	
	Email	
	URL	

	ORCID	
Author	Family Name	**Klenk**
	Particle	
	Given Name	**Hans-peter**
	Suffix	
	Division	School of Natural and Environmental Sciences
	Organization	Newcastle University
	Address	Ridley Building 2, Newcastle upon Tyne, NE1 7RU, UK
	Phone	
	Fax	
	Email	
	URL	
	ORCID	
Author	Family Name	**Sghaier**
	Particle	
	Given Name	**Haïtham**
	Suffix	
	Division	Laboratory "Energy and Matter for Development of Nuclear Sciences" (LR16CNSTN02), National Center for Nuclear Sciences and Technology
	Organization	Sidi Thabet Technopark
	Address	2020, Sidi Thabet, Tunisia
	Division	
	Organization	University Manouba, ISBST, BVBGR-LR11ES31,
	Address	Biotechpole Sidi Thabet, 2020, Ariana, Tunisia
	Phone	
	Fax	
	Email	
	URL	
	ORCID	

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Abstract

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Keywords (separated by '-')	Ionizing-radiation resistant - Genome sequence - Ksar Ghilane oasis - Polyphasic taxonomy - *Promicromonospora panici* - Oxidative stress
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Sihem Guesmi[1,2] · Imen Nouioui[3,4] · Petar Pujic[5,6] · Audrey Dubost[5,6] · Afef Najjari[7] · Kais Ghedira[8] · José M. Igual[9] · Ameur Cherif[10] · Hans-peter Klenk[3] · Haïtham Sghaier[2,10] · Philippe Normand[5,6]

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Keywords Ionizing-radiation resistant · Genome sequence · Ksar Ghilane oasis · Polyphasic taxonomy · *Promicromonospora panici* · Oxidative stress

Abbreviations

DSBs	Double-strand breaks
ROS	Reactive oxygen species
ANI	Average nucleotide identity
isDDH	In silico DNA-DNA hybridization
IR	Ionizing radiation
kGy	Kilogray
TSA	Tryptic soy agar
TSB	Tryptic soy broth

Introduction

The genus *Promicromonospora*, belonging to the family *Promicromonosporaceae*, was first proposed by Krasil'nikov et al. (1961). This genus is phylogenetically closely related to the genera *Cellulosimicrobium* and *Oerskovia* (Schumann et al. 2001; Busse et al. 2003). Members of the genus are aerobic and Gram-positive actinomycetes. Their major characteristics are a branched substrate mycelium that fragments into rod-shaped or coccoid elements, iso- and anteiso-branched as the predominant cellular fatty acids, MK-9(H_4) as the major menaquinone and a DNA G + C content of 70–75% (Schumann and Stackebrandt 2012).

Promicromonospora genus contains 18 species that have been isolated from various biotopes, such as air in the medieval Vienna (Busse et al. 2003), medicinal plant *Maytenus austroyunnanensis* in Yunnan Province (Qin et al. 2012), the surface-sterilized root of a pine tree in Australia (Kaewkla et al. 2017), soil in Mount Song (Zheng et al.

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✉ Philippe Normand
philippe.normand@univ-lyon1.fr

Extended author information available on the last page of the article

2017), etc. However, it is important to note that the number of validly named species is 14 as of August 08, 2020, available at https://lpsn.dsmz.de/genus/promicromonospora, including *Promicromonospora soli* (Zheng et al. 2017), *Promicromonospora kermanensis* (Mohammadipanah et al. 2017), *Promicromonospora callitridis* (Kaewkla and Franco 2017), *Promicromonospora alba* (Guo et al. 2016) and *Promicromonospora iranensis* (Mohammadipanah et al. 2014), among the most recently described species.

Promicromonospora strains have industrially and environmentally valuable applications owing to the production of various bioactive molecules (Gabani and Singh 2013; Thomas et al. 2016), such as antibiotics (Izumikawa et al. 2011), xylanase (Kumar et al. (2011), cellulase (Thomas et al. 2016), xylanilyticolides (Jin et al. 2018), etc. Furthermore, *Promicromonospora* genus showed interesting biological properties, such as antimicrobial activity against bacteria and yeast pathogens (Zothanpuia et al. 2018), bioremediation by the removal of cadmium from polluted waste (Hamedi et al. 2015), plant growth-promoting rhizobacterium through the production of gibberellins (Kang et al. 2012, 2014), etc.

Despite the potential applications of *Promicromonospora* species, there have been a limited number of reports that investigated genomic and phenotypic aspects such as multi-resistance to extreme conditions (radiation, desiccation, oxidative stress, etc.) of this genus members. The number of related genomes available at the National Center for Biotechnology Information (NCBI) and the Genomes OnLine Database is limited to 25 as of August 08, 2020.

In this study, we report the phenotypic, phylogenetic and genomic characterization of a novel actinomycete species, *Promicromonospora panici* sp. nov., with insights into its tolerance to ionizing radiation (IR), desiccation and oxidative stress.

Materials and methods

Strain isolation and cultivation

AQ6 PT9^T was isolated from irradiated roots of *P. turgidum*, which were collected in May 2015 from the oasis Ksar Ghilane (Tunisia, coordinates N 32°59.557′, E 9°36.941′, 221.9 m above sea level) at the gates of the Sahara Desert. One-gram root sample aliquots were exposed to levels of IR at a dose of 10 kilogray (kGy) with a mean rate dose of 25.6 kGy/h at room temperature using a cobalt-60 gamma irradiator at the National Center for Nuclear Sciences and Technology (CNSTN), Tunisia. After gamma irradiation, the 1 g root samples were mixed with 9 mL of 0.9% NaCl solution (w/v) and shaken on a rotary shaker at 250 rpm, 30 °C for 1 h (h). Then, 100 µL sample of the suspension was serially diluted (10^{-5}–10^{-9}) and spread onto tryptic soy agar (TSA) medium (contains 15 g/L of tryptone, 5 g/L of soy peptone, 5 g/L of sodium chloride and 15 g/L of bacteriological agar, at pH 7). After 7 days of aerobic incubation at 30 °C, colonies were transferred and purified on liquid medium using tryptic soy broth (TSB) and maintained as 20% (v/v) glycerol suspensions at − 20 and − 80 °C (Rainey et al. 2005).

For routine work, PT9^T was cultured aerobically on solid TSA medium and liquid TSB medium for 3 days at 30 °C. Isolate PT9^T was deposited in the BCCM collection (Belgian Coordinated Collections of Microorganisms) and the DSMZ collection (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) under the deposit numbers LMG 31,103 and DSM 108,613, respectively.

Morphological and phenotypic characteristics

Colony morphology of isolate PT9^T was observed by using cells grown on TSA at 30 °C for 2 days. Gram staining was assessed using the standard Gram stain method and spore motility was carried out by optical light and epifluorescence microscopy (Leica Microsystems, Germany) observation of cells suspended in phosphate buffer (at pH 7). For the observation of morphological characteristics, bacterial cells growing on TSA medium at 30 °C for 72 h, were harvested with 2% glutaraldehyde in saline solution (0.9% of NaCl) and then fixed cells were dried in a graded ethanol series (50, 70, 90 and 100% of ethanol) (Kaewkla and Franco 2019). Observations were done via a scanning electron microscope (JSM 5400.JEOL, Japan).

Growth of isolate was checked on Luria–Bertani (LB) (sodium chloride 10 g/L, tryptone 10 g/L, yeast extract 5 g/L), nutrient agar (meat extract 10 g/L, peptone 10 g/L, sodium chloride 5 g/L, agar 15 g/L at pH 7.3), R2YE medium (is a variation of R2 containing yeast extract) (sucrose 10.3 g/L, K2SO4 0.25 g/L, MgCl2·6H2O 10.12 g/L, glucose 10 g/L, casamino acid 0.1 g/L, yeast extract 5 g/L, agar 15 g/L), soya flour mannitol agar (soya flour 2 g/l, mannitol 20 g/L, agar 20 g/L), starch casein agar (starch 10 g/L, casein powder 10 g/L, agar 15 g/L at pH 7.2) and in TSA medium at 30 °C for 72 h. The optimal temperature for growth was tested by checking growth on TSA over a range of 10–60 °C with 5 °C intervals. The pH range for growth was examined over a range pH 4–10 with 1 pH unit intervals in TSB over 3 days of incubation at 30 °C. Tolerance of NaCl was evaluated in TSB supplemented with 0–15.0% (w/v) NaCl (at 2.0% intervals) after incubation at 30 °C for 72 h.

Catalase activity was determined by monitoring generation of bubbles upon the addition of a drop of 3% hydrogen peroxide (H_2O_2) solution (Smibert and Krieg 1994).

Oxidase activity was assessed by the oxidation of 0.2% (w/v) oxidase reactive by monitoring the colour change.

For chemotaxonomic characterization, cells of PT9^T were maintained on yeast extract—malt extract agar (International Streptomyces Project medium 2; ISP2) medium which had been shaken at 180 rpm and incubated for 7 days at 28 °C. After incubation, the biomass of isolate PT9^T was harvested from ISP2 broth cultures. Then, cells were washed three times with sterile distilled water and freeze-dried. Whole-cell sugars were extracted according to the procedures of Lechevalier and Lechevalier (1970) and identified using thin layer chromatography (TLC) as described by Staneck and Roberts (1974). Whole-organism diaminopimelic acids were identified using the protocols of Schleifer and Kandler (1972). Polar lipids pattern was determined following the modified protocol of Minnikin et al. (1984) by Kroppenstedt and Goodfellow (2006). Cellular fatty acids were extracted according to the protocol of Miller (1982) with minor modification from Kuykendall et al. (1988). Fatty acid patterns were determined from the biomass of isolate PT9^T using the Standard Microbial Identification (MIDI) system Version 4.5 and the ACTIN6 database (Sasser 1990).

Genome sequencing, molecular and phylogenetic analyses

Genomic DNA extraction

Genomic DNA extraction from isolate PT9^T was performed as described previously by Marmur (1961) with a phenol/chloroform extraction step (Li et al. 2007). Cells from 50 mL cultures grown on TSB were collected by centrifugation (Eppendorf France) and the pellets were solubilized in 8 mL of 10 mM Tris–HCl at pH 8, 10 mg/mL of lysozyme and 1 mM of ethylenediaminetetraacetic acid (EDTA). Cells lysis was done on the ice during one hour, then 8 mL of 2% w/vol sodium dodecyl sulfate (SDS) and 2.8 mg/mL of proteinase K were added to the mix and incubated at 55 °C for 5 h. Proteins and cell debris were removed by extraction with 16 mL of phenol, chloroform and isoamyl alcohol 25:24:1 (v/v/v) at pH 8.2. After centrifugation, the aqueous phase was transferred into a new tube and genomic DNA was precipitated by adding 1.5 mL of 3 M sodium acetate and two volume of absolute ethanol. The DNA pellet was washed in 70% ethanol, air-dried and solubilized in TE buffer (Tris–EDTA buffer from Invitrogen™, 10 mM Tris and 0.1 mM EDTA) at pH 8. The quality of extracted DNA was assessed by measuring the A_{260}/A_{280} ratio using a Nanodrop (Implen NP80, Thermo Fisher Scientific, USA) spectrophotometer.

Genome sequencing, assembly and annotation

The genome sequencing of PT9^T was performed on the Illumina MiSeq™ platform (Illumina, USA) by Biofidal society, Vaulx-en-Velin, France (https://www.biofidal-lab.com) using NextGen High Throughput Sequencing. Thus, a standard Illumina shotgun library "Nextera XT DNA Library Prep" was constructed and sequenced using the Illumina MiSeq technology by synthesis. A paired-end sequencing strategy was used with an average size of 2 × 300 bp in length generated and a total number of reads of 25,425,220 bp obtained. Quality control of raw data was assessed using the FastQC tool (https://www.bioinformatics.babraham.ac.uk/projects/fastqc). The Unicycler software was used for de novo assembly. Genome annotation was performed with the MicroScope platform version 3.10.0 using the integrated microbial genomes non-redundant database, KEGG and COG databases (Vallenet et al. 2017). Reads were processed and assembled as described by Castro-Wallace et al. (2017). The final draft assembly had an average depth coverage of 6.9 Mbp and 59 contigs. In addition, the percentage of G + C in the complete genome sequence was calculated.

Phylogenetic analysis and genomic comparisons

Analysis of the 16S rDNA gene used the complete sequence retrieved from the genome using MAGE platform (Vallenet et al. 2009). This gene was identical to the 16S rDNA gene sequence originally deposited in GenBank (MG712873) after the initial single colony isolation and amplification by polymerase chain reaction using the universal 27F and 1492R primers (Sun et al. 2010). The resulting 16S rDNA gene sequence was compared with those of all validly named species using the EzTaxon database EzBioCloud (https://www.ezbiocloud.net/identify/) (Yoon et al. 2017) and the web tool leBIBI(QBPP) available at https://umr5558-bibiserv.univ-lyon1.fr/lebibi/lebibi.cgi (Flandrois et al. 2015). The phylogenetic tree highlighting the position of PT9^T relative to the type strains of the other species within the genus *Promicromonospora* was constructed using W-IQ-TREE software accessed at https://iqtree.cibiv.univie.ac.at/, (Trifinopoulos et al. 2016) based on the Shimodaira–Hasegawa approximate likelihood ratio (SH-aLRT) (%) (Guindon et al. 2010) and the ultrafast bootstrap (UFboot) (%) (Minh et al. 2013).

These 16S rDNA sequence data have been submitted to the DDBJ/EMBL/GenBank databases through https://submit.ncbi.nlm.nih.gov/ under accession number MG712873. Genome based phylogenetic tree was constructed using a high throughput wed server, Type Strain Genome server (TYG) available in the Genome to Genome Distance Calculator webserver (Meier-Kolthoff and Göker 2019). AQ7

Average nucleotide identity (ANI) values between the genome of isolate PT9^T and those of the type strains of *Promicromonospora* species were calculated using best hits and reciprocal best hits between two genomic datasets as described by Yoon et al. (2017) available at (https://www.ezbiocloud.net/tools/ani). The top hits of the 16S rDNA-related strains of PT9^T were identified via the in silico calculation of digital DNA-DNA hybridization (dDDH) values between strains determination, using the Genome-to-Genome Distance Calculator (GGDC) web server (https://ggdc.dsmz.de/distcalc2.php) (Goris et al. 2007; Meier-Kolthoff et al. 2013).

Growth under extreme conditions

The resistance of isolate PT9^T to extreme conditions was assessed under IR, desiccation and hydrogen peroxide stress. The tolerance to gamma irradiation was assayed using cultures of PT9^T and *Escherichia coli* grown in TSB medium and LB agar, respectively at 30 °C for 3 days. Thus, *E. coli* DH5α was used as an IR-sensitive bacterium (Beblo-Vranesevic et al. 2018) for comparative analyses. Briefly, bacterial cells were collected, washed twice with a 0.9% NaCl solution, homogenized and subsequently resuspended in the same solution of NaCl. Survival rates after exposure to a range of gamma radiation doses (1–5 kGy) were determined using the 3-(4, 5-dimethyl imidazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Mosmann 1983). Bacterial cells were exposed to levels of gamma irradiation at a dose ranging from 1 to 5 kGy using a cobalt-60 gamma irradiator at the National Center for Nuclear Sciences and Technology (CNSTN), Tunisia. A volume of 10 µL of MTT solution (5 mg/mL) was added to 100 µL of irradiated cells and kept in the dark for 3 h at 37 °C. Results are represented with the corresponding controls not exposed to gamma irradiation, normalized to 100% (Morini et al. 2017). After incubation, the absorbance in each well was measured, including blanks, at 570 nm using a microtiter plate reader (Shimadzu, UV-1800). The value of D_{10} (kGy), which is the dose necessary for a 90% reduction in Colony Forming Units, was also calculated via RadioP1 (www.radiop.org.tn) (Benhamda et al. 2015) based on our experimental survival data.

The desiccation tolerance assay was performed in TSB medium according to Mattimore and Battista (1996). Briefly, 200 µL of cell suspensions were pipetted into sterile Petri dishes and dried 28 days in a desiccator at 30 °C. *E. coli* DH5α was used as a desiccation-sensitive bacterium (Beblo-Vranesevic et al. 2018) for comparative analyses. After 28 days, samples were revived by washing the cells off the plate with 200 µL of saline solution (0.9% NaCl) to rehydrate the cells. Cell viability was examined using MTT assay, then 100 µL of cell suspensions was transferred to a 96-well microplate and a volume of 10 µL of MTT solution (5 mg/mL) was added. The microplate was incubated at room temperature in the dark for 3 h at 37 °C. After incubation, the absorbance of each well, including blank (without cells) which was normalized to 100%, was measured at 570 nm using a microtiter plate reader (Shimadzu, UV-1800). The measured absorbance at OD 570 nm is proportional to the number of viable cells. The survival rate represents the absorbance at 570 nm after drying with a reference before drying. The package lethal (https://github.com/hofnerb/lethal) was used also to estimate the survival of PT9^T following exposure to desiccation.

Growth of isolate PT9^T with various concentrations of H_2O_2 (v/v) was also performed as described by Gholami et al. (2015). *E. coli* DH5α was used as a proteome-oxidation-sensitive bacterium (Krisko et al. 2010) for comparative analyses. Fifty µL of bacterial suspensions were added to 100 µL of TSB supplemented with 0–5% (v/v) of H_2O_2 using 96-well microplates. Plates were kept in the dark at 30 °C for 48 h with agitation at 120 rpm. The optical density was determined at 630 nm using a microplate reader TECAN (Infinite® 200 PRO, Germany).

Prediction of secondary metabolite clusters and genes related to oxidative stress response

Clusters for secondary metabolites of isolate PT9^T were predicted using the antiSMASH (https://antismash.secondarymetabolites.org/#!/start) version 4.0.2 software (Weber et al. 2015). Besides, in silico analyses of the genome PT9^T were done and genes implicated in scavenging of reactive oxygen species (ROS) were determined.

Nucleotide sequence accession number

The whole-genome shotgun sequence of isolate PT9^T was submitted to GenBank/ENA under BioProject accession number PRJEB29453 (ERP111755), BioSample number SAMEA5056529 and SRA accession number ERS2867704. Contig sequences of the de novo assembly were deposited at EMBL under the accession numbers UWYY01000000–UWYY01000056.

Results and discussion

Cultural and phenotypic characteristics

PT9^T was isolated from the irradiated roots of *Panicum turgidum*, which were collected from the oasis of Ksar Ghilane in the south of Tunisia. The isolate is an aerobic, Gram-positive, non-motile and non-spore-forming actinomycete. Colonies are white-pigmented on TSA medium (Fig. S1), like *P. xylanilytica*, which was isolated from the medicinal

plant *Maytenus austroyunnanensis* (Qin et al. 2012). Cells of PT9^T developed a branched substrate mycelium with a wrinkled and shining surface, the septate hyphae of which broke, at a later stage, into fragments of various sizes and rod-shaped elements (Fig. 1). This feature is shared by all members of the genus *Promicromonospora* (Krasil'nikov et al. 1961; Schumann and Stackebrandt 2012).

This actinobacterial isolate had an optimal growth on nutrient agar, R2YE (medium specific for *Streptomyces* genus), soya flour mannitol agar and TSA media after incubation at 30 °C for 72 h. It grew moderately well on LB agar and starch casein agar media. Growth of isolate PT9^T was observed at 28–37 °C (optimum at 30 °C) and pH 6.0–9.0, like *P. xylanilytica* YIM 61515 ^{T} (Qin et al. 2012). No growth was observed in the presence of 2% NaCl. Catalase and oxidase activities of PT9^T were positive, similar to *P. xylanilytica* YIM 61515 ^{T} (Qin et al. 2012). *Promicromonospora sukumoe* was shown to have a very high endo-cellulase activity (Ventorino et al. 2015).

Chemotaxonomic characteristics

Chemotaxonomic features of isolate PT9^T matched those described for members of the genus *Promicromonospora*. Whole-organism hydrolysates of isolate PT9^T contained DL-2,6-Diaminopimelic acid (DL-DAP) and galactose, glucose and ribose as whole cell sugars. These traits are the same as the close phylogenetic neighbor, *P. soli* CGMCC 4.7398^T (Zheng et al. 2017).

Polar lipid profiles of PT9^T consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylinositol (PI) and unidentified lipid (L), phospholipid (PL), phosphoglycolipid (PGL) and glycolipids (GL_{1-3}). These features are coherent with those *P. soli* CGMCC 4.7398^T (Zheng et al. 2017) (Fig. S2). The major fatty acids (> 25%) of PT9^T are *anteiso*-$C_{15:0}$ (56.4%) and *iso*-$C_{15:0}$ (26.9%) (Table S1), which are similar to those of *P. soli* CGMCC 4.7398^T (Zheng et al. 2017).

The chemotaxonomic features of isolate PT9^T are in line with the genus *Promicromonospora* but are not enough to distinguish isolate PT9^T from its close neighbor. For this reason, phylogenetic analyses based on genomic comparisons were necessary to determine the taxonomic affiliation of isolate PT9^T.

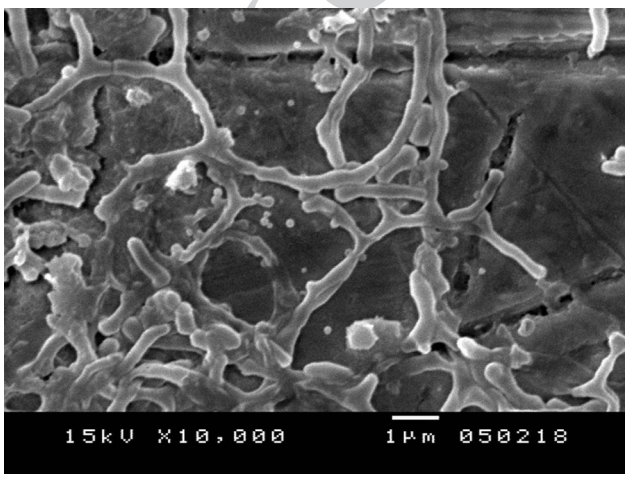

Fig. 1 XXX

Phylogeny and genomic comparisons

Blast of the complete 16S rDNA gene sequence (1508 bp) of isolate PT9^T showed similarity values of 99.3% and 99.0% with *P. xylanilytica* YIM 61515^T, *Promicromonospora aerolata* DSM 15943^T and *P. iranensis* DSM 45554^T, respectively. However, it decreased to 98.9%, 98.8%, and 98.7% with *Promicromonospora vindobonensis* DSM 24547^T, *Promicromonospora thailandica* DSM 26652^T and *P. alba* DSM 100490^T and *P. callitridis* DSM 103339^T (Table 1), respectively.

Comparison of the sequences of isolate PT9^T and type strains belonging to genus *Promicromonospora* revealed identity differences that are marginally within the cut-off value of > 1.3% (Stackebrandt and Ebers 2006).

The results of the 16S rDNA gene sequence similarity are not in line with the phylogenetic position of strain PT9^T in the 16S rDNA gene based tree (Fig. S3). Strain PT9^T occupied a poorly supported distinct branch loosely associated to a subclade housing several *Promicromonospora* spp. including the ones cited above and next to the type strain of *P. soli*. Thus, further information is required to determine the reliable phylogenetic position of isolate PT9^T.

In the genome based phylogenetic tree (Fig. 2), strain PT9^T was found to form a well-supported subclade closely related to the type strain of *P. soli* which was associated to the branch of *Promicromonospora umidemergens* DSM 22081^T.

16S rDNA gene sequence similarities between PT9^T and closely related type strains indicated the need to confirm the genomic distinctness of the type strain as representing a novel species by genomic relatedness indices. These analyses are necessary to further differentiate PT9^T from other species affiliated to the *Promicromonospora* genus.

Therefore, pairwise comparisons between isolate PT9^T and the available reference genomes were determined based on the proposed thresholds for recognition of genomic species, ANI and isDDH values. ANI values between *P. panici* PT9^T and closely related species ranged from 84.6 with *P. sukumoe* DSM 44121^T to 86.95% with *P. soli* CGMCC 4.7398^T, which were below the cut-off level (95–96%) for species delineation (Goris et al. 2007; Richter and Rossello-Mora 2009). Indeed, strain PT9^T

Table 1 Identities of the strain PT9^T 16S rDNA gene to those of *Promicromonospora* type strains using EZbiocloud

Rank	Name	Strain	Accession	16S rDNA pairwise similarity(%)
1	*Promicromonospora xylanilytica*	YIM 61515^T	FJ214352	99.3
2	*Promicromonospora aerolata*	DSM 15943^T	AJ487303	99.3
3	*Promicromonospora iranensis*	DSM 45554^T	JN038073	99.0
4	*Promicromonospora vindobonensis*	DSM 24547^T	AJ487302	98.9
5	*Promicromonospora thailandica*	DSM 26652^T	AB560974	98.8
6	*Promicromonospora alba*	DSM 100490^T	KP784765	98.7
7	*Promicromonospora callitridis*	DSM 103339^T	GU434237	98.7
8	*Promicromonospora kroppenstedtii*	DSM 19349^T	KI911710	98.7
9	*Promicromonospora endophytica*	JCM 19560^T	GU434253	98.7
10	*Promicromonospora soli*	CGMCC 4.7398^T	KY352348	98.6
11	*Promicromonospora sukumoe*	DSM 44121^T	AB023375	98.6
12	*Promicromonospora citrea*	DSM 43110^T	X83808	98.5
13	*Promicromonospora kermanensis*	DSM 45485^T	KJ780745	98.5
14	*Promicromonospora umidemergens*	DSM 22081^T	FN293378	98.3

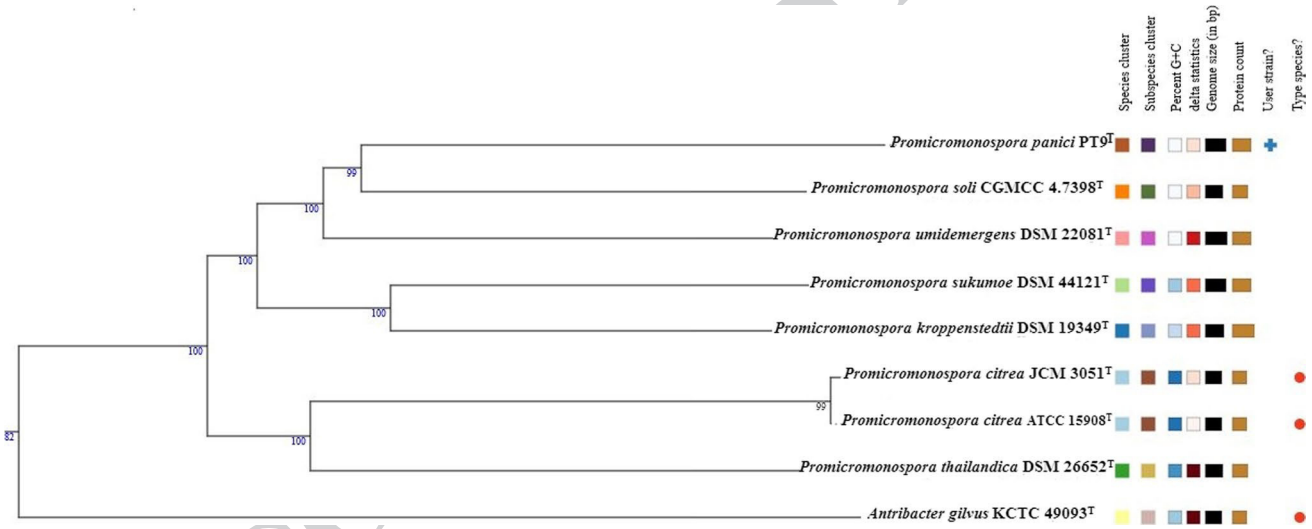

Fig. 2 XXX

displayed isDDH value of 32.8% with its closest neighbor *P. soli* CGMCC 4.7398^T and 31.3% with *Promicromonospora kroppenstedtii* DSM 19349^T. DNA-DNA relatedness of 28.8% for isolate PT9^T against *P. sukumoe* DSM 44121^T. These values are well below the 70% cut-off value recommended for the circumscription of bacterial genomic species (Wayne et al. 1987; Chun et al. 2018).Thus, isDDH and ANI values indicated that PT9^T represents a novel species within the genus *Promicromonospora*.

Based on the morphological, phenotypic and genetic characteristics, it is evident that isolate PT9^T should be assigned to a novel species in the genus *Promicromonospora*, for which the name *Promicromonospora panici* sp. nov. is proposed.

Growth under extreme conditions

The survival rate of PT9^T after exposure to IR was assessed. PT9^T showed a marked survival profile following treatment with increasing doses of gamma radiation (1−5 kGy), when compared to *E. coli* DH5α (Fig. 3).

As shown in Fig. 3, there is a significant difference between survival profiles of PT9^T and the IR-sensitive strain DH5α. Indeed, the D_{10} value of PT9^T was around 2.6 kGy

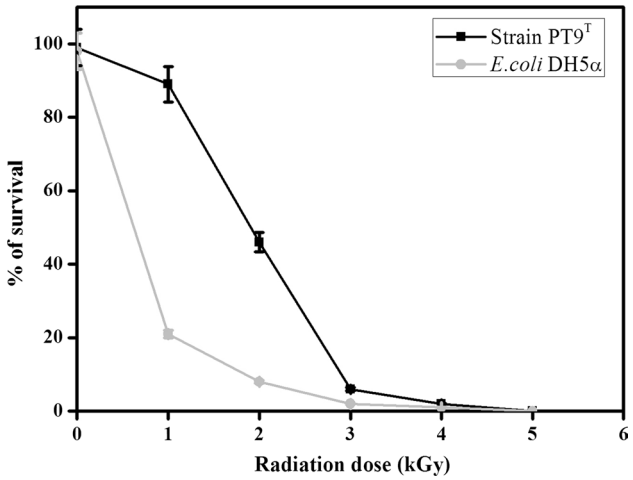

Fig. 3 XXX

(Fig. S4). To be considered an IR-resistant prokaryote, a bacterium should have a D_{10} value greater than 1 kGy (Sghaier et al. 2008). Accordingly, PT9^T (D_{10} = 2.6 kGy) was described as an IR-resistant actinobacterium. Furthermore, the desiccation of isolate PT9^T for 28 days resulted in an overall loss of culturable cells (CFU/mL) of approximately 32% (Fig. S5). Also, the survival rate for this Saharan actinobacterium after 28 days of desiccation was 68% (Fig. S6). Thus, it is legitimate to conclude that PT9^T is also tolerant to desiccation.

The evaluation of resistance to H_2O_2 of PT10 was also assessed at various concentrations from 1 to 5% (v/v) (Fig. 4).

As shown in Fig. 4, PT9^T is tolerant to H_2O_2 at concentrations ranging from 1 to 3% (v/v), when compared to *E. coli* DH5α. However, the growth of PT9^T was almost completely inhibited at a concentration of 5% (v/v) of H_2O_2.

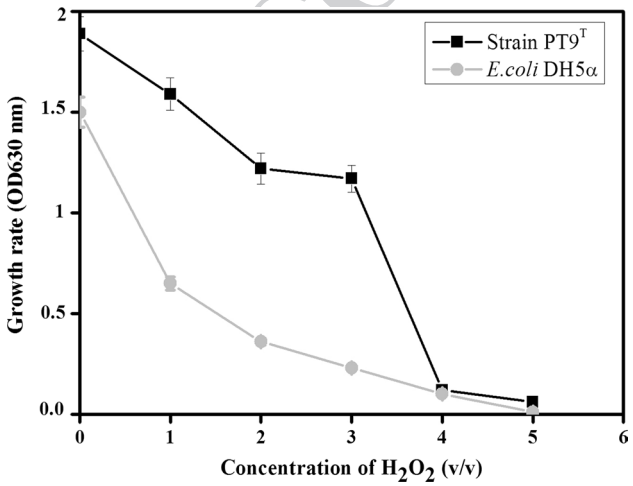

Fig. 4 XXX

Table 2 Genome features of *P. panici* PT9^T

Features	Chromosome
Genome size (bps)	6,582,650
Contig numbers	39
G+C content (%)	71.2
Number of Scaffolds	59
Number of Contigs	59
Average CDS length (bp)	984.18
Protein coding density (%)	91.9
Number of genomic objects	6368
Protein coding genes CDSs	6291
rRNA genes	3
tRNA genes	51

This is the first report that examined the growth under extreme conditions of a strain belonging to genus *Promicromonospora*. Isolate PT9^T is resistant to gamma radiation, desiccation and oxidative stress (H_2O_2). The convergence and the similarity among desiccation and gamma radiation stress response pathways in *Deinococcus radiodurans* were explained by the common production of ROS, which induces oxidative damage to cells (Slade et al. 2011; Lim et al. 2019). Thus, IR-resistant prokaryotes can also be desiccation tolerant (Slade et al. 2011). However, it is necessary to note 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 multiresistance of isolate PT9^T may be originating from its oxidative response mechanisms. Thus, the genome sequencing of PT9^T was performed to identify these mechanisms and the strategies used to thrive under extreme conditions, such as gamma radiation, desiccation, salt concentration, H_2O_2, etc.

Genome sequence analysis and oxidative stress response

Genomic features

The complete genome of *P. panici* PT9^T consists of 6,582,650 bps in size and has an average G+C content of 71.2%. The size of the PT9^T chromosome is not similar to this of the most closely related strain *P. soli* CGMCC 4.7398^T (5,378,526 bps). De novo assembly of the genome sequencing data of PT9^T resulted in 59 contigs and an average CDS length of 984.18 bp. The genome was predicted to contain 6368 protein-coding genes, with a 91.9% protein-coding density, 51 tRNA and 3 rRNA genes. General

features for the complete genome of PT9^T are shown in Table 2.

For 6291 protein-coding genes, 75.08% (4723 CDSs) are classified in at least one Clusters of Orthologous Genes (COG) group. The most abundant categories are general function prediction (910); carbohydrate transport and metabolism (808); transcription (744); amino acid transport and metabolism (610); and inorganic ion transport and metabolism (405). The identification also of several categories involved in the defense of microorganisms, which are cell wall/membrane/envelope biogenesis (270); replication, recombination and repair (200); secondary metabolites biosynthesis, transport and catabolism (175); chaperones (151); defense mechanisms (116); and more others (Supporting information presented in Table S2).

Prediction of genes related to oxidative stress response

The genome sequence of PT9^T contains several genes implicated in response to oxidative stress (Table 3).

Several proteins detected in isolate PT9^T were reported to have a role in IR and desiccation resistance-associated mechanisms such as GyrAB, NucS, PolA, RadA, RecA, RecD, RecO, SodA, SoxR, SSB and UvrD (Lim et al. 2019). PT9^T contains also various proteins implicated in defense mechanisms related to oxidative stress response, such as universal stress protein UspA, class III stress response-related ATPase ClpC, Fe-S center for redox-sensing, putative ATP-dependent helicase DinG, putative ABC transporter YclH, ferric enterobactin transport ATP-binding protein FepC, daunorubicin/doxorubicin resistance ATP-binding protein DrrA. The proteins observed in response to oxidative stress of *D. radiodurans* were DNA repair proteins (PolA, RadA, RecA, RecF, RecO), DNA protein binding (ArgF, DnaE, HisS, RecN), ribosomal proteins (RplB, Rpsl, RpsR) and oxidation–reduction process proteins (GltA, NuoC) (Slade et al. 2009; Ujaoney et al. 2017; Gao et al. 2020).

Isolate PT9^T seems to possess genes involved in the protection of its cytosolic proteins from oxidation (such as *mntA*, *mntB*, *sodA*) and the repair of DNA double-strand breaks (DSBs) after exposure to IR (*dinG*, *hup*, *recA*, *recQ*, *ssbA*) (Lim et al. 2019). Several DNA repair genes, which were reported to contribute to radiation and oxidative stress resistance in *D. radiodurans*, such as *gyrAB*, *ligD*, *ligA*, *recC* (Bellenberg et al. 2019; Lim et al. 2019), were also detected in the genome of isolate PT9^T. Some genes of *D. radiodurans*, such as *recF* and *recO*, are extremely sensitive to gamma rays, which are required for recombination repair by an incomplete genome reconstitution, a reduced level of DNA breakdown and an absence of DNA synthesis (Bentchikou et al. 2010). *RecF* and *RecO* genes play an essential role in DNA double-strand-break repair pathways through an extended synthesis-dependent strand annealing process (ESDSA) (Bentchikou et al. 2010), which might be involved in the repair of DSBs and the DNA synthesis step of ESDSA for isolate PT9^T.

Furthermore, the treatment of *D. radiodurans* R1 with IR (and also desiccation) induced the over-expression of several genes in responses to oxidative stress damages, such as *recA* and *uvrD*, which encode proteins that have been previously associated with DNA repair (Tanaka et al. 2004).

PT9^T contains also many DNA ligase paralogs (such as *ligA*, *ligC*, *ligD*) and putative DNA ligases. For instance, *ligD* gene is involved in the bacterial non-homologous DNA end joining (NHEJ) pathway according to the study of Ishino and Narumi (2015). *D. radiodurans* possesses efficient oxidative damage protection mechanisms, such as NHEJ and CNDEJ (condensed nucleoid-dependent end joining) repair pathways, involving radiation-inducible proteins (Ishino and Narumi 2015). These DNA repair proteins, such as ATP-dependent DNA ligase that was detected in the genome of PT9^T, play a principal role in the CNDEJ pathway, which represents a DSBs repair system specific to radioresistant bacteria with a high degree of genome condensation.

PT9^T contains also desiccation tolerance-associated proteins, such as ferritin BfrB, pleiotropic regulatory protein DegT, HTH-type transcriptional regulator RcdA, manganese-containing peroxidase XpdC (Mn_catalase) (Lim et al. 2019).

Various enzymes involved in oxidative stress responses, such as catalase, superoxide dismutase, NADH-dependent oxidases, manganese-containing antioxidants, which provide an important role to protect radioresistant microorganisms (Cárdenas et al. 2012; Bellenberg et al. 2019). Catalase KatA, superoxide dismutase [Mn] SodA and manganese MntA were detected in the genome of PT9^T.

Radioresistant microorganisms have various mechanisms of defense against ionizing radiation, desiccation, oxidative stress-generating agents and environmental stresses that can damage cell components, including the production of scavenging ROS, detoxification and repair systems (Lim et al. 2019). *Deinococcus radiodurans*, has developed efficient enzymatic and non-enzymatic antioxidant systems, involving different physiological determinants and well-regulated molecular mechanisms for tolerance to radiation, desiccation and oxidative stress (Timmins and Moe 2016; Bellenberg et al. 2019). *D. radiodurans* employs various strategies to prevent oxidative stress, including an efficient DNA repair system, ROS-scavenging enzymes (catalase and superoxide dismutase), non-enzymatic antioxidants and transcriptional regulators (OxyR) (Munteanu et al. 2015; Chen et al. 2019). The genome sequence of *D. radiodurans* revealed the presence of homologues of most well-known prokaryotic DNA repair proteins involved in base excision repair, nucleotide excision repair, mismatch repair and recombination repair.

Table 3 List of genes involved in response to oxidative stress

Label	Length (pb)	Gene	Functional
PROM_v1_370013	1239	*recF*	DNA replication and repair protein RecF
PROM_v1_20039	1449	*katA*	Scavenge (Catabolism) vegetative catalase 1
PROM_v1_40259	2232	*katG*	Catalase/hydroperoxidase HPI(I)
PROM_v1_70135	606	*recR*	RecA filament-DNA complex stabilisation factor
PROM_v1_470015	510	*dps*	DNA protection during starvation protein
PROM_v1_210103	1848	*recQ*	ATP-dependent DNA helicase RecQ
PROM_v1_10004	549	*ssbA*	Single-strand DNA-binding protein SSB
PROM_v1_10142	2628	*clpB*	Protein disaggregation chaperone
PROM_v1_50101	963	*drrA*	Daunorubicin/doxorubicin resistance ATP-binding protein DrrA
PROM_v1_330032	1779	–	UMUC domain protein DNA-repair protein
PROM_v1_270047	3621	*mfd*	Transcription-repair-coupling factor
PROM_v1_230011	1755	*recN*	DNA repair protein RecN
PROM_v1_20182	1224	*recA*	Multifunctional SOS repair factor
PROM_v1_40053	747	*recO*	DNA repair protein RecO
PROM_v1_40152	2496	*ligD*	Multifunctional non-homologous end joining DNA repair protein LigD
PROM_v1_240013	2412	*ligA*	DNA ligase
PROM_v1_10007	237	–	Ribosomal protein S18 RpsR
PROM_v1_170101	375	–	Ribosomal protein S12 (BS12) RpsL
PROM_v1_170093	837	–	Ribosomal protein L2 (BL2) RplB
PROM_v1_90068	1578	*lig*	Putative DNA ligase
PROM_v1_120124	1182	*ligC*	DNA ligase C
PROM_v1_40293	1518	*obgE*	ppGpp-binding GTPase involved in cell portioning; DNA repair and ribosome assembly
PROM_v1_90030	465	*atl1*	Alkylated DNA nucleotide flippase Atl1; Ada-like DNA-binding domain: nucleotide excision repair
PROM_v1_140011	1119	–	DNA alkylation repair protein
PROM_v1_190139	1443	*radA*	DNA repair protein
PROM_v1_20165	2106	*dinG*	putative ATP-dependent helicase DinG homolog
PROM_v1_220099	447	*rnhA*	DNA replication, recombination, and repair; Degradation of proteins, peptides, and glycopeptides
PROM_v1_10046	456	–	Nucleotide-binding universal stress protein: UspA family
PROM_v1_140049	510	–	Universal stress protein UspA
PROM_v1_190003	2562	*clpC mecB*	Class III stress response-related ATPase, AAA + superfamily
PROM_v1_200143	489	*soxR marC*	DNA-binding transcriptional dual regulator, Fe-S center for redox-sensing
PROM_v1_28004	837	–	Ser/Thr protein kinase RdoA involved in Cpx stress response
PROM_v1_350012	396	–	Universal stress protein MSMEG_4207
PROM_v1_380020	420	–	Stress response protein
PROM_v1_480007	660	–	Putative stress-induced transcription regulator
PROM_v1_20114	204	*cspD*	Cold-shock protein, molecular chaperone, RNA-helicase co-factor
PROM_v1_50089	2478	*gyrA*	DNA gyrase subunit A
PROM_v1_250073	2211	*gyrB*	DNA gyrase subunit B
PROM_v1_10073	282	*hup*	DNA-binding protein HB1
PROM_v1_120020	2637	*polA*	DNA polymerase I
PROM_v1_50212	3378	*recB*	RecBCD enzyme subunit RecB
PROM_v1_50213	3402	*recC*	RecBCD enzyme subunit RecC
PROM_v1_50211	1866	*recD*	RecBCD enzyme subunit RecD
PROM_v1_30191	2067	*uvrD*	ATP-dependent DNA helicase UvrD2
PROM_v1_20292	810	*feuV*	Iron(III)-siderophore transporter: ATP binding component
PROM_v1_50182	882	*xpdC*	Phage PBSX; manganese-containing peroxidase
PROM_v1_150073	873	*mntA*	Manganese transport system ATP-binding protein MntA
PROM_v1_150072	864	*mntB*	Manganese transport system ATP-binding protein MntB

Table 3 (continued)

Label	Length (pb)	Gene	Functional
PROM_v1_290076	894	*mntC*	Manganese transport system ATP-binding protein MntC
PROM_v1_20214	1230	–	Mn^{2+} and Fe^{2+} transporters of the NRAMP family
PROM_v1_230090	1308	*mntH*	Divalent metal cation transporter MntH
PROM_v1_250040	630	*sodA*	Superoxide dismutase [Mn]
PROM_v1_70011	753	–	Fe(3+)-transporting ATPase
PROM_v1_180005	1566	*fbpB*	Fe(3+)-transport system permease protein SfuB
PROM_v1_150099	789	*fepC*	Ferric enterobactin transport ATP-binding protein FepC
PROM_v1_80082	1320	–	Mn^{2+} and Fe^{2+} transporters of the NRAMP family
PROM_v1_20146	825	*fecE*	Iron-dicitrate transporter subunit
PROM_v1_20290	1020	*fecD*	Iron-dicitrate ABC transporter (permease)
PROM_v1_240047	1308	*gltA*	Glutamate synthase (large subunit, NADP-dependent)
PROM_v1_80125	762	*nuoC*	NADH-quinone oxidoreductase subunit C
PROM_v1_120112	1380	*hisS*	Histidine–tRNA ligase
PROM_v1_20071	3552	*dnaE*	DNA polymerase III subunit alpha
PROM_v1_20016	948	*argF argC*	Ornithine carbamoyltransferase
PROM_v1_400015	696	*nucS*	Endonuclease NucS

Thus, the DNA repair machinery of *D. radiodurans* seems similar to that of other bacteria, such as isolate $PT9^T$, with specificities contributing to radiation resistance and protection of proteins against oxidative damages (Makarova et al. 2001; Daly 2012; Lim et al. 2019).

To conclude, oxidative stress resistance of $PT9^T$ against damages caused by gamma radiation, desiccation and oxidative stress generated by H_2O_2 was explained by the presence of a combination of enzymatic anti-oxidants (ROS-scavenging enzymes, such as catalase, superoxide dismutase) and non-enzymatic (like carotenoids and manganese Mn^{2+}) defense mechanisms (Shashidhar et al. 2010; Slade and Radman 2011). These proteins are mainly associated with DNA repair and response to oxidative stress. Further functional analyses of these new oxidative stress-induced proteins will enhance our understanding of the regulatory mechanism underlying extreme resistance to oxidative stress in isolate $PT9^T$. However, sufficient proteome protection is crucial for survival after irradiation because protein activity is required for essential processes including transcription, translation and DNA repair (Bellenberg et al. 2019).

Secondary metabolites prediction

The genome analysis of isolate $PT9^T$ harbors multiple secondary metabolites related to defense mechanisms and strategies developed to survive under extreme conditions (Table 4).

As shown in Table 4, five biosynthesis gene clusters for secondary metabolites were identified in the genome of isolate $PT9^T$—oligosaccharides, t3pks, lassopeptides, siderophores and ectoine.

Various microbial metabolites, such as oligosaccharides, carotenoids and compatible solutes (ectoine) were demonstrated to protect cells against IR (Beblo-Vranesevic et al. 2017). Cupriabactin is a carboxylate-type siderophore extracted from *Cupriavidus necator* JMP134, which demonstrated important roles in iron scavenging, bacterial motility, biofilm formation and oxidative stress resistance. Physiological analyses revealed that this system induced an increase

Table 4 AntiSMASH secondary metabolites of *P. panici* $PT9^T$

Cluster	Cluster type	Most similar known cluster	Length (bp)
1	Oligosaccharide	Oligosaccharide	51,475
2	t3pks	Arylomycin biosynthesis Alkylresorcinol, Clarexpoxcin, Polyketide	41,116
3	Lassopeptide	SapB biosynthesis	22,426
4	Siderophore	Desferrioxamine B biosynthesis	12,643
5	Ectoine	Ectoine biosynthesis	8697

of the resistance of *C. necator* JMP134 to stress caused by aromatic compounds (Li et al. 2019). In general, oxidative stress responses of bacteria have been observed to be part of complex phenomena such as biofilm formation, antibiotic resistance and pathogenicity (Cárdenas et al. 2012). Also, a secondary defense barrier consists of the induced expression of specific ROS degrading enzymes and repair systems for damaged macromolecules (Cabiscol et al. 2000).

Altogether, our findings allow us to develop an integrated understanding of the molecular basis of recovery from desiccation and other protein and DNA-damaging agents in PT9^T and roots-associated bacteria, particularly in the extreme environment of the Tunisian desert.

Description of *Promicromonospora panici* sp. nov.

Promicromonospora panici [pa'ni.ci. N.L. gen. n. *panici* referring to the isolation source, the xerophytic plant *Panicum*].

Aerobic, Gram-staining positive, non-spore-forming and non-motile actinobacterium. Forms well-developed branching septate hyphae that breaks up into fragments of various sizes and rod-shaped elements on the media tested after incubation at 30 °C for 72 h. Colonies are white to yellowish-white with characteristic wrinkly surfaces. Cells grow well on soya flour mannitol, nutrient agar and tryptic soy agar media, but no growth was observed on LB agar medium. Optimal growth occurs at 28–37 °C (optimum at 30 °C) and pH 6.0–9.0. No growth in the presence of 2% NaCl. Positive for catalase and oxidase.

The major fatty acids are anteiso-C15:0 and iso-C15:0. The polar lipid pattern is diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphoglycolipid (PGL), unidentified glycolipids (GL_{1-3}), lipid (L) and phospholipid (PL).Whole-organism hydrolysates contain DL-2,6-diaminopimelic acid (DL-DAP), galactose, glucose and ribose as whole cell sugars.

The genome of the type strain is 6.5 Mb with a GC content of 71.2 mol%. The GenBank/ENA accession number for the draft genome sequence of isolate PT9^T is PRJEB29453.

The type strain PT9^T (LMG 31103^T = DSM 108613^T) was isolated from irradiated roots of *P. turgidum* collected from the Ksar Ghilane oasis, southern Tunisia.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Basu B, Apte SK (2012) Gamma radiation-induced proteome of *Deinococcus radiodurans* primarily targets DNA repair and oxidative stress alleviation. Mol Cell Proteom 11(M111):011734

Beblo-Vranesevic K, Galinski EA, Rachel R, Huber H, Rettberg P (2017) Influence of osmotic stress on desiccation and irradiation tolerance of (hyper)-thermophilic microorganisms. Arch Microbiol 199:17–28

Beblo-Vranesevic K, Bohmeier M, Perras AK et al (2018) Lack of correlation of desiccation and radiation tolerance in microorganisms from diverse extreme environments tested under anoxic conditions. FEMS Microbiol Lett 365(6):fny044

Bellenberg S, Huynh D, Poetsch A, Sand W, Vera M (2019) Proteomics reveal enhanced oxidative stress responses and metabolic adaptation in *Acidithiobacillus ferrooxidans* biofilm cells on pyrite. Front Microbiol 10:592

Benhamda C, Benkahla A, Ben Miled S, Ouled- Haddar H, del Carmen MC, Gtari M, Cherif A, Hofner B, Gjedira K, Sghaier H (2015) The RadioP1–an integrative web resource for radioresistant prokaryotes. Intech Open, London, pp 89–105

Bentchikou E, Servant P, Coste G, Sommer S (2010) A major role of the RecFOR pathway in DNA double-strand-break repair through ESDSA in *Deinococcus radiodurans*. PLoS Genet 6:e1000774

Blin K, Medema MH, Kazempour D, Fischbach MA, Breitling TRE, Weber T (2013) AntiSMASH 2.0-a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res 41:W204–W212

Busse HJ, Zlamala C, Buczolits S, Lubitz W, Kämpfer P, Takeuchi M (2003) *Promicromonospora vindobonensis* sp. nov. and *Promicromonospora aerolata* sp. nov., isolated from the air in the medieval 'Virgilkapelle' in Vienna. Int J Syst Evol Microbiol 53:1503–1507

Cabiscol E, Tamarit J, Ros J (2000) Oxidative stress in bacteria and protein damage by reactive oxygen species. Internal Microbiol 3:3–8

Cárdenas JP, Moya F, Covarrubias P, Shmaryahu A, Levicán G, Holmes DS et al (2012) Comparative genomics of the oxidative stress response in bioleaching microorganisms. Hydrometallurgy 12:162–167

Castro-Wallace SL, Chiu CY, John KK, Stahl SE, Rubins KH et al (2017) Nanopore DNA sequencing and genome assembly on the international space station. Sci Rep 7:18022

Chen Y, Xue D, Sun W, Han J, Li J, Gao R, Zhou Z, Zhang W, Chen M, Lin M et al (2019) SRNA OsiA stabilizes catalase mRNA during oxidative stress response of *Deincoccus radiodurans* R1. Microorganisms 7:e422

Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466

Daly MJ (2012) Death by protein damage in irradiated cells. DNA Repair (Amst) 11:12–21

Flandrois JP, Perriere G, Gouy M (2015) leBIBIQBPP: a set of databases and a web tool for automatic phylogenetic analysis of prokaryotic sequences. BMC Bioinform 16:251

Gabani P, Singh OV (2013) Radiation-resistant extremophiles and their potential in biotechnology and therapeutics. Appl Microbiol Biotechnol 97:993–1004

Gao L, Zhou Z, Chen X, Zhang W, Lin M, Chen M (2020) Comparative proteomics analysis reveals new features of the oxidative stress response in the polyextremophilic bacterium *Deinococcus radiodurans*. MDPI J Microorgan 8:451

Gholami M, Etemadifar Z, Bouzari M (2015) Isolation a new strain of *Kocuria rosea* capable of tolerating extreme conditions. J Environ Radioact 144:113–119

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57(1):81–91

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. System Biol 59(3):307–321

Guo L, Liu C, Zhao J, Li C, Guo S, Fan J, Li J, Wang X, Xiang W (2016) *Promicromonospora alba* sp. nov., an actinomycete isolated from the cuticle of *Camponotus japonicas* Mayr. Int J Syst Evol Microbiol 66:1340–1345

Hamedi J, Dehhaghi M, Mohammdipanah F (2015) Isolation of extremely heavy metal resistant strains of rare actinomycetes from high metal content soils in Iran. Int J Environ Res 9(2):475–480

Izumikawa M, Takagi M, Shin-Ya K (2011) Isolation of a novel macrocyclic dilactone-JBIR-101-from *Promicromonospora* sp. RL26. J Antibiot 64:689–691

Jin L, Zhao J, Jiang S, Zhao Y, Han X, Guo X, Wang X, Xiang W (2018) *Promicromonospora viridis* sp. nov., a novel actinomycete isolated from soil. Antonie van Leeuwenhoek.

Kaewkla O, Franco CMM (2017) *Promicromonospora callitridis* sp. nov., an endophytic actinobacterium isolated from the surface-sterilized root of an Australian native pine tree. Int J Syst Evol Microbiol 67:3559–3563

Kaewkla O, Franco CMM (2019) *Actinomycetospora callitridis* sp. nov., an endophytic actinobacterium isolated from the surface-sterilised root of an Australian native pine tree. Antonie Van Leeuwenhoek 112:331–337

Kang SM, Khan AL, Hamayun M, Hussain J, Joo GJ, You YH, Kim JG, Lee IJ (2012) Gibberellin-producing *Promicromonospora* sp. SE188 improves *Solanum lycopersicum* plant growth and influences endogenous plant hormones. J Microbiol 50:902–909

Kang SM, Khan AL, Waqas M, You YH, Kim JH, Kim JG, Hamayun M, Lee IJ (2014) Plant growth promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. J Plant Interact 9:673–682

Krasilnikov NA, Kalakoutskii LV, Kirillova NF (1961) A new genus of Actinomycetales, *Promicromonospora* gen. nov. vol 1. Bull Acad Sci USSR (Ser Biol).

Kroppenstedt RM, Goodfellow M (2006) The family Thermomonosporaceae: Actinocorallia, Actinomadura, Spirillispora and Thermomonospora. Archaea, Bacteria, Firmicutes, Actinomycetes. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes: a handbookon the biology of bacteria. Springer, New York, pp 682–724

Kumar M, Joshi A, Kashyap R, Khanna S (2011) Production of xylanase by *Promicromonospora* sp. MARS with rice straw under non sterile conditions. Process Biochem 46:1614–1618

Lechevalier MP, Lechevalier HA (1970) Chemical composition as a criterion in the classification of aerobic actinomycetes. Int J Syst Bacteriol 20:435–443

Li WJ, Xu P, Schumann P, Zhang YQ, Pukall R, Xu LH, Stackebrandt E, Jiang CL (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. Int J Syst Evol Microbiol 57:1424–1428

Li C, Zhu L, Pan D, Li S, Xiao H, Zhang Z, Shen X, Wang Y, Long M (2019) Siderophore mediated iron acquisition enhances resistance to oxidative and aromatic compound stress in *Cupriavidus necator* JMP134. Appl Environ Microbiol 85:e01938-e2018

Lim S, Jung JH, Blanchard L, de Groot A (2019) Conservation and diversity of radiation and oxidative stress resistance mechanisms in *Deinococcus* species. FEMS Microbiol Rev 43(1):19–52

Makarova KS, Aravind L, Wolf YI, Tatusov RL, Minton KW, Koonin EV, Daly MJ (2001) Genome of the extremely radiation-resistant bacterium *Deinococcus radiodurans* viewed from the perspective of comparative genomics. Microbiol Mol Biol R 65:44–79

Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. J Mol Biol 3:208

MattimoreBattista VJR (1996) Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J Bacteriol 178:633–637

Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R (2011) antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res 39:W339–W346

Meier-Kolthoff J, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182

Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60

Minh BQ, Nguyen AT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol 30(5):1188–1195

Minnikin D, O'Donnell A, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Mohammadipanah F, Montero-Calasanz MD, Schumann P, Sproer C, Rohde M, Klenk HP (2017) *Promicromonospora kermanensis* sp. nov., an actinobacterium isolated from soil. Int J Syst Evol Microbiol 67:262–267

Morini J, Babini G, Barbieri S, Baiocco G, Ottolenghi A (2017) The interplay between radioresistant Caco-2 cells and the immune system increases epithelial layer permeability and alters signalling protein spectrum. Front Immunol 8:233

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Munteanu A, Uivarosi V, Andries A (2015) Recent progress in understanding the molecular mechanisms of radioresistance in *Deinococcus* bacteria. Extremophiles 19:707–719

Qin S, Jiang JH, Klenk HP, Zhu WY, Zhao GZ, Zhao LX, Tang SK, Xu LH, Li WJ (2012) *Promicromonospora xylanilytica* sp. nov., an endophytic actinomycete isolated from surface-sterilized leaves of the medicinal plant *Maytenus austroyunnanensis*. Int J Syst Evol Microbiol 62:84–89

Richter M, Rossello-Mora R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA 106:19126–19131

Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol Rev 36:407–477

Schumann P, Stackebrandt E (2012) The family *Promicromonosporaceae*. Bergey's manual of systematic bacteriology. Springer, New York

Schumann P, Weiss N, Stackebrandt E (2001) Reclassification of *Cellulomonas cellulans* (Stackebrandt and Keddie 1986) as

Cellulosimicrobium cellulans gen. nov., comb. nov. Int J Syst Evol Microbiol 51:1007–1010
Sghaier H, Narumi I, Satoh K, Ohba H, Mitomo H (2007) Problems with the current deinococcal hypothesis: an alternative theory. Theory Biosciences 126:43–45
Sghaier H, Ghedira K, Benkahla A, Barkallah I (2008) Basal DNA repair machinery is subject to positive selection in ionizing-radiation-resistant bacteria. BMC Genom 9:297
Shashidhar R, Kumar SA, Misra HS, Bandekar JR (2010) Evaluation of the role of enzymatic and non-enzymatic antioxidant systems in the radiation resistance of *Deinococcus*. Can J Microbiol 56:195–201
Slade D, Radman M (2011) Oxidative stress resistance in *Deinococcus radiodurans*. Microbiol Mol Biol R 75:133–191
Slade D, Lindner AB, Paul G, Radman M (2009) Recombination and replication in DNA repair of heavily irradiated *Deinococcus radiodurans*. Cell 136:1044–1055
Smibert R, Krieg N (1994) Phenotypic characterization. In: Gerhardt P (ed) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, pp 607–654
Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards. Microbiol Today 33:152–155
Staneck JL, Roberts GD (1974) Simplified approach to identification of aerobic actinomycetes by thin layer chromatography. J Appl Microbiol 28:226–231
Sun LN, Zhang YF, He L, Chen ZJ, Wang QY, Qian M, Sheng XF (2010) Genetic diversity and characterization of heavy metal-resistant-endophytic bacteria from two copper-tolerant plant species on copper mine wasteland. Bioresour Technol 101:501–509
Tanaka M, Earl AM, Howell HA, Park MJ, Eisen JA, Peterson SN, Battista JR (2004) Analysis of *Deinococcus radiodurans*'s transcriptional response to ionizing radiation and desiccation reveals novel proteins that contribute to extreme radioresistance. Genet Soc Am 168:21–33
Thomas L, Ram H, Kumar A, Singh VP (2016) Production, optimization, and characterization of organic solvent tolerant cellulases from a lignocellulosic waste-degrading actinobacterium, *Promicromonospora* sp. VP111. Appl Biochem Biotechnol 179:863–879
Timmins J, Moe E (2016) A decade of biochemical and structural studies of the DNA repair machinery of *Deinococcus radiodurans*: major findings, functional and mechanistic insight and challenges. Comput Struct Biotechnol J 14:168–176
Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res 44:232–235
Ujaoney AK, Padwal MK, Basu B (2017) Proteome dynamics during post-desiccation recovery reveal convergence of desiccation and gamma radiation stress response pathways in *Deinococcus radiodurans*. Biochim Biophys Acta Proteins Proteom 1865:1215–1226
Vallenet D, Calteau A, Cruveiller S, Gachet M, Lajus A, Josso A, Mercier J, Renaux A, Rollin J, Rouy Z, Roche D, Scarpelli C, Médigue C (2017) MicroScope in 2017: an expanding and evolving integrated resource for community expertise of microbial genomes. Nucleic Acids Res 45:517–528
Ventorino V, Aliberti A, Faraco V, Robertiello A, Giacobbe S, Ercolini D, Amore A, Fagnano M, Pepe O (2015) Exploring the microbiota dynamics related to vegetable biomasses degradation and study of lignocellulose-degrading bacteria for industrial biotechnological application. Sci Rep 5:8161
Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Truper HG (1987) Report of the Ad Hoc Committee On Reconciliation Of Approaches To Bacterial Systematics. Int J Syst Evol Microbiol 37:463–464
Weber T, Blin K, Duddela S et al (2015) antiSMASH 3.0-a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 43:237–243
Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617
Zheng W, Li D, Zhao J, Liu C, Zhao Y, Xiang W, Wang X (2017) *Promicromonospora soli* sp. nov., a novel actinomycete isolated from soil. Int J Syst Evol Microbiol 67:3829–3833
Zothanpuia PAK, Leo VV, Chandra P, Kumar B, Nayak C, Hashem A, Abd-Allah EF, Alqarawi AA, Singh BP (2018) Bioprospection of actinobacteria derived from freshwater sediments for their potential to produce antimicrobial compounds. Microb Cell Fact 17:68

Affiliations

Sihem Guesmi[1,2] · Imen Nouioui[3,4] · Petar Pujic[5,6] · Audrey Dubost[5,6] · Afef Najjari[7] · Kais Ghedira[8] · José M. Igual[9] · Ameur Cherif[10] · Hans-peter Klenk[3] · Haïtham Sghaier[2,10] · Philippe Normand[5,6]

1 National Agronomy Institute of Tunisia, Avenue Charles Nicolle, 1082 Tunis, Mahrajène, Tunisia

2 Laboratory "Energy and Matter for Development of Nuclear Sciences" (LR16CNSTN02), National Center for Nuclear Sciences and Technology, Sidi Thabet Technopark, 2020 Sidi Thabet, Tunisia

3 School of Natural and Environmental Sciences, Newcastle University, Ridley Building 2, Newcastle upon Tyne NE1 7RU, UK

4 Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

5 Université de Lyon, Université Lyon 1, Lyon, France

6 CNRS, UMR 5557, Écologie Microbienne, UMR1418, INRA, 69622 Villeurbanne Cedex, France

7 Université de Tunis el Manar, Faculté des Sciences de Tunis, LR03ES03 Microorganismes et Biomolécules Actives, 2092 Tunis, Tunisia

8 Laboratory of Bioinformatics, Biomathematics and Biostatistics-LR16IPT09, Institut Pasteur de Tunis, Université de Tunis El Manar, 1002 Tunis, Tunisia

9 Instituto de Recursos Naturales y Agrobiología de Salamanca, Consejo Superior de Investigaciones Científicas (IRNASA-CSIC), c/Cordel de Merinas 40-52, 37008 Salamanca, Spain

[10] University Manouba, ISBST, BVBGR-LR11ES31,,
Biotechpole Sidi Thabet, 2020 Ariana, Tunisia