Streptomyces radiopugnans sp. nov., a radiation-resistant actinomycete isolated from radiation-polluted soil in China

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The taxonomic position of an actinomycete isolated from radiation-polluted soil collected in Xinjiang Province, north-west China, was determined by using a polyphasic approach. The isolate, designated strain R97T, had chemical and morphological properties characteristic of streptomycetes. An almost-complete 16S rRNA gene sequence of the isolate was generated and compared with corresponding sequences of representative streptomycetes. The 16S rRNA data not only supported the classification of the strain in the genus Streptomyces but also showed that it represented a distinct phyletic line that was most closely, albeit loosely, associated with three other thermotolerant organisms, namely Streptomyces macrosporus NBRC 14748T, Streptomyces megasporus NBRC 14749T and Streptomyces thermolineatus NBRC 14750T. Strain R97T could be distinguished from these organisms based on a range of phenotypic properties. It is proposed that R97T (CGMCC 4.3519T = DSM 41901T) be classified as the type strain of a novel species in the genus Streptomyces, Streptomyces radiopugnans sp. nov. The organism was shown to be resistant to 60Co gamma radiation at a dose of 15 kGy.

The first radiation-resistant micro-organism to be described, designated ‘Micrococcus radiodurans’, was isolated from irradiated meat (Anderson et al., 1956) and was subsequently reclassified as Deinococcus radiodurans (Brooks & Murray, 1981). Other notable radiation-resistant bacteria include Bacillus nealsoni (Venkateswaran et al., 2003), Hymenobacter actinosclerus (Collins et al., 2000), Trueperia radiovictrix (Albuquerque et al., 2005) and the actinobacteria Kineococcus radiotolerans (Phillips et al., 2002) and Rubrobacter taiwanensis (Chen et al., 2004). During a study on the bioremediation of radiation-contaminated soils in Xinjiang Province, China, a radiation-resistant streptomycete-like strain, designated R97T, was isolated. The present study was designed to determine the taxonomic status of this organism by using genotypic and phenotypic procedures. The resultant data show that the strain should be classified as representing a novel species of the genus Streptomyces.
DDBJ/EMBL/GenBank databases. The program MEGA version 3.1 (Kumar et al., 2004) was used for both multiple alignment and phylogenetic analyses. Phylogenetic trees were generated via the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) tree-making algorithms; evolutionary distances for the neighbour-joining algorithm were calculated with the Kimura two-parameter model (Kimura, 1980). The topologies of the resultant trees were evaluated in a bootstrap analysis (Felsenstein, 1985) based on 1000 replicates. It was apparent from the initial neighbour-joining analysis, including more than 500 related sequences of Streptomyces type strains, that isolate R97T represented a bona fide member of the genus Streptomyces (data not shown).

The new isolate was examined for a range of chemotaxonomic and morphological properties known to be characteristic of members of the genus Streptomyces (Williams et al., 1989). To this end, hyphal and spore chain arrangement were observed on modified Bennett’s and inorganic salts-starch agar plates after incubation at 28 °C for 14 days, by using the coverslip technique of Kawato & Shinobu (1959). Spore chain morphology and spore surface ornamentation were observed by examining gold-coated dehydrated specimens under an FEI QUANTA scanning electron microscope. The isomers of diaminopimelic acid and whole-organism sugars were analysed by using the procedures developed by Hasegawa et al. (1983) and Lechevalier & Lechevalier (1980). Polar lipids were examined and identified according to the method of Minnikin et al. (1984), and fatty acids were extracted, methylated and analysed via GC by using the standard Sherlock MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Menaquiones were extracted and purified following the method of Collins (1985) and then analysed by HPLC. The DNA G+C content of the strain was determined by using the thermal denaturation method of Marmur & Doty (1962) with Escherichia coli K12 as a control.

Morphological and chemical characteristics of isolate R97T were in line with its assignment to the genus Streptomyces (Williams et al., 1989; Manfio et al., 1995). The organism formed an extensively branched substrate mycelium, with aerial hyphae that differentiated into rough to warty, oval-shaped spores (0.70–1.0 × 0.92–1.4 μm) in spiral spore chains (Fig. 1). It contained major amounts of LL-diaminopimelic acid in whole-organism hydrolysates, hexahydrogenated and octahydrogenated menaquinones with nine isoprene units [MK-9(H6, H8)] as predominant isoprenologues and diposphatidylglycerol and phosphatidylethanolamine as major polar lipids (phospholipid type II sensu Lechevalier et al., 1977), but lacked mycolic acids and characteristic sugars. The fatty acid profile was rich in saturated straight-chain and iso- and anteiso-branched components (fatty acid type 2c sensu Kroppenstedt, 1985). The DNA G+C content was 72.7 mol%.

Strain R97T was compared with its closest phylogenetic neighbours by using the procedures described above. It was evident from the phylogenetic tree thus constructed (Fig. 2) that the organism formed a distinct phylectic line together with the type strains of Streptomyces macrosporus and Streptomyces megasporus, an association that was supported by both of the tree-making algorithms employed and by a 99 % bootstrap value in the neighbour-joining analysis. Strain R97T shared highest 16S rRNA gene sequence similarity with S. macrosporus NBRC 14748T (97.5 %), which corresponds to 35 nt differences at 1444 locations with gaps, and lower values with S. megasporus NBRC 14749T (96.7 %) and Streptomyces thermolineatus NBRC 14750T (97.1 %). DNA–DNA relatedness studies were not

**Fig. 1.** Scanning electron micrographs of strain R97T showing spiral chains of rough to warty spores after growth on inorganic salts-starch agar for 14 days at 28 °C. Bars, 5 μm.
carried out between strain R97<sup>T</sup> and these organisms as representatives of several *Streptomyces* species with much higher 16S rRNA gene sequence similarities have DNA–DNA relatedness values well below the 70% cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987), as exemplified in a study on neutrotolerant acidophilic streptomycetes (Xu *et al.*, 2006).

The organism was also examined for a range of phenotypic properties. Colonial and pigmentation features were observed on inorganic salts-starch agar (ISP medium 4), and yeast extract-malt extract agar (ISP medium 2; Difco), on modified Bennett’s agar, Gauze’s synthetic medium no. 1 agar (DSMZ medium no. 1048) and yeast-starch agar (DSMZ medium no. 1027) after incubation for 14 days at 28 °C. Similarly, peptone-yeast extract-iron and tyrosine

![Fig. 2. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain R97<sup>T</sup> and the type strains of phylogenetically close *Streptomyces* species. Asterisks indicate branches of the tree that were also recovered with the maximum-parsimony tree-making algorithm. Numbers at nodes are percentage bootstrap values based on 1000 resampled data sets; only values above 50% are given. Bar, 0.005 substitutions per nucleotide position.](image)

**Table 1.** Phenotypic properties that differentiate strain R97<sup>T</sup> from representatives of phylogenetically close *Streptomyces* species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain R97&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>S. macrosporus</em> NBRC 14748&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>S. megalosporus</em> NBRC 14749&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>S. thermolineatus</em> NBRC 14750&lt;sup&gt;T&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore chain morphology</td>
<td>Spiral</td>
<td>Spiral</td>
<td>Spiral</td>
<td>Rectiflexibles</td>
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<tr>
<td>Spore surface ornamentation</td>
<td>Rough to warty</td>
<td>Warty</td>
<td>Warty</td>
<td>Smooth</td>
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<td>Degradation of:</td>
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<tr>
<td>Elastin</td>
<td>–</td>
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<td>Xanthine</td>
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<td>Growth on sole carbon sources</td>
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<tr>
<td>Inositol</td>
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<td>+</td>
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<td>–</td>
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<tr>
<td>Inulin</td>
<td>+</td>
<td>+&lt;sup&gt;W&lt;/sup&gt;</td>
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<tr>
<td>Mannitol</td>
<td>+</td>
<td>–</td>
<td>+&lt;sup&gt;W&lt;/sup&gt;</td>
<td>–</td>
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<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>+&lt;sup&gt;W&lt;/sup&gt;</td>
<td>–</td>
<td>+&lt;sup&gt;W&lt;/sup&gt;</td>
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<tr>
<td>Sucrose</td>
<td>+</td>
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<td>Trehalose</td>
<td>–</td>
<td>+</td>
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<td>Xylitol</td>
<td>+</td>
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<tr>
<td>D-Xylose</td>
<td>+</td>
<td>+</td>
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<td>Sodium citrate</td>
<td>+</td>
<td>–</td>
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<td>Growth at:</td>
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<td>pH 5</td>
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<td>pH 11</td>
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<td>Growth on sole nitrogen sources</td>
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<tr>
<td>KNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>–</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>L-Cysteine</td>
<td>+</td>
<td>–</td>
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<td>–</td>
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<td>L-Phenylalanine</td>
<td>–</td>
<td>+</td>
<td>–</td>
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agars (Shirling & Gottlieb, 1966) were examined for the production of melanin pigments. Additional phenotypic properties were determined by using established media and methods (Williams et al., 1983; Kämper et al., 1991). The various media supported the growth of a white to grey aerial spore mass and a range of substrate mycelial pigments. Melanin pigments were not formed on either peptone-yeast extract-iron or tyrosine agars. It is evident from Table 1 that strain R97T can be distinguished from its closest phylogenetic neighbours based on a combination of phenotypic properties, although like them it is thermotolerant.

The survival rate of strain R97T in response to gamma radiation was compared with those of Deinococcus radiodurans DSM 20539T (radiation-resistant organism) and Escherichia coli K12 (radiation-sensitive organism) via an established procedure (Ferreira et al., 1997; Chen et al., 2004). The strains were grown in modified Bennett’s broth (containing glass beads to prevent mycelium formation) to exponential growth phase, at which point biomass was washed with sodium chloride (0.85%, w/v), centrifuged at 4 °C and resuspended in saline (0.85%, w/v) to give a concentration of 1 × 10⁶–1 × 10⁷ c.f.u. ml⁻¹. Each suspension was divided into 2-ml aliquots and exposed to a ⁶⁰Co source at a dose rate of 0.167 kGy min⁻¹ at room temperature (1 kGy = 10⁶ rads); the gamma radiation doses were from zero to 20.0 kGy in steps of 2.5 kGy. Treated samples were diluted and plated in triplicate onto modified Bennett’s agar plates and then incubated at 28 °C for 15 days, at which point the colony-forming units were counted. Viability was assessed by using non-irradiated suspensions of each strain under the same conditions as the controls.

Strain R97T was resistant to gamma radiation with a shoulder dose (the dose required before the number of colony-forming units began to decline) of 5 kGy, a result comparable with the shoulder dose for D. radiodurans DSM 20539T. Exposure of the two radiation-resistant strains to 15 kGy resulted in survival rates for strain R97T and D. radiodurans DSM 20539T of 1 and 2.6%, respectively (see Supplementary Fig. S1 in IJSEM Online). Consequently, strain R97T can be added to the taxonomically diverse group of thermotolerant/thermophilic bacteria that are able to resist gamma radiation (Mattiore & Battista, 1996; Ferreira et al., 1997, 1999). It is not clear how such organisms have acquired their ability to resist radiation damage, although there is evidence that it may be due to evolutionary processes resulting from environmental stress, particularly that caused by drought and heat (Mattiore & Battista, 1996).

The genotypic and phenotypic data presented clearly demonstrate that strain R97T represents a novel species of the genus Streptomyces, for which the name Streptomyces radiopugnans sp. nov. is proposed.

**Description of Streptomyces radiopugnans sp. nov.**

*Streptomyces radiopugnans* (ra.di.o.pug’nans. L. n. radius a beam or ray; N.L. pref. radio- pertaining to radiation; L. part. adj. pugnans fighting or resisting; N.L. part. adj. radiopugnans radiation-resisting).

Aerobic, Gram-positive, radiation-resistant actinomycete that forms an extensively branched substrate mycelium which carries aerial hyphae that differentiate into spiral chains of spores with rough to warty surfaces. Moderate to abundant, white to pale-grey aerial spore mass is formed on modified Bennett’s, Gauze’s synthetic medium no. 1, inorganic salts-starch, yeast extract-malt extract and yeast-starch agars. Substrate mycelium is yellowish brown on modified Bennett’s, Gauze’s no. 1 and yeast-starch agars and light pinkish yellow on inorganic salts-starch and yeast extract-malt extract agars. Diffusible pigments are not formed on any of the media, and melanin pigments are not formed on peptone-yeast extract-iron or tyrosine agars. Growth occurs between 20 and 50 °C, but not at 55 °C. Growth also occurs in the presence of 0.1% phenol, but not in the presence of 7% NaCl. Tween 60 is degraded, but Tweens 20 and 80 are not. L-Arabinose, L-melezitose and L-ribose are used as sole carbon sources for energy and growth, but not L-cellobiose, L-lactose, L-lactulose, L-melibiose, D-raffinose or trehalose (all at 1%, w/v). Similarly, L-cysteine, L-glycine, D-glutamate, sodium azelate, sodium isobutyrate and sodium malonate are used as sole carbon sources for energy and growth, but not D-glucosid acid, hydroxy-L-proline, DL-isoleucine, L-leucine, methyl D-glucopyranoside, methyl D-mannopyranoside, L-phenylalanine, sodium propionate, sodium pyruvate, sodium suberate or spermidine (all at 0.1%, w/v). Additional phenotypic properties are given in Table 1. The fatty acid profile consists of iso-C₁₆:₀ (34.5%), anteiso-C₁₅:₀ (15.4%), iso-H-C₁₆:₁ (14.2%), anteiso-C₁₇:₀ (9.1%), iso-C₁₄:₀ (5.6%) and anteiso-C₁₇:₀(9c) (5.2%). The DNA G+C content is 72.7 mol%.

The type strain, R97T (=CGMCC 4.3519ᵀ = DSM 41901ᵀ), was isolated from a radiation-contaminated soil sample collected from Xinjiang Province, north-west China. The species description is based on a single strain and hence serves as a description of the type strain.

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**References**


