IZHAKIELLA AUSTRALIENSIS SP. NOV. ISOLATED FROM AN AUSTRALIAN DESERT SOIL

Mukan Ji, Sihui Tang and Belinda C. Ferrari*

Abstract

A Gram-stain-negative, rod-shaped, non-motile bacterium, designated D4N98T, was isolated from a desert soil near Glendambo, Australia. The taxonomic position of strain D4N98T was investigated using a polyphasic approach. Strain D4N98T shared 97% 16S rRNA gene sequence similarity with the only reference strain of the genus Izhakiella (Izhakiella capsodis N6P06T), and less than 96% similarity with other species of genera in the family Enterobacteriaceae with validly published names. Phylogenetic analysis, based on 16S rRNA, rpoB, atpD, gyrB genes and a concatenated sequence comprising 37 single copy marker genes, as well as chemotaxonomic data (major polar lipids: phosphatidyl ethanolamine, phosphatidylglycerol, diphosphatidylglycerol, a neutral lipid, and a glycolipid. Major fatty acids (>10%): C16:0, C17:0 cyclo, summed feature 2 (C14:0 3-OH and/or iso-C15:0 11), summed feature 3 (C16:1ω7c and/or iso-C15:0 2ω6c), summed feature 8 (C18:1ω7c and/or C18:1ω6c) support the affiliation of this strain to the genus Izhakiella. The results of in silico DNA–DNA hybridisation plus physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain D4N98T from the other species of the genus Izhakiella with validly published names. Therefore, strain D4N98T represents a novel species, for which the name Izhakiella australiensis sp. nov. is proposed. The type strain is D4N98T (LMG 30066T=DSM 105030T).

The genus Izhakiella is a recently recognised genus of the family Enterobacteriaceae [1]. Currently, there is only one species, Izhakiella capsodis N6P06T, with a validly published name, which was isolated from the mirid bug Capsodes infuscatus [1]. Here, we describe a second species within the genus Izhakiella, isolated from an arid soil in Australia. Strain D4N98T was isolated from a desert soil collected near Glendambo, in the far north of South Australia (S31° 1’ 54”, E135° 48’ 24.48”). Approximately 1 g soil was suspended in 10 ml sterile 0.9% saline and vortexed vigorously to separate bacterial cells from detrital material. An aliquot (100 µl) of the suspension was then spread onto 0.05x RAVAN broth at the mid-exponential to end-exponential phase. Following sequencing, 1.235 Gb of DNA sequence was generated with an average coverage of approximately 110 fold, yielding 2 469 569 paired-end reads, with an average sequence length of 250 bp. The resulting sequence data was quality assessed, trimmed and assembled de novo, using SPAdes Genome Assembler v3.6.1 [4] with UNSW Sydney’s Katana supercomputing cluster. After assembly, only contigs longer than 1 kb were retained.

The assembled D4N98T draft genome contained 60 contigs with an N50 value of 200 759 bp. The D4N98T genome was at least 4 969 540 bp long with a G+C content of 54.03%, and one plasmid was present that was 45 894 bp in size. Open reading frames were predicted and annotated using the RAST and the JGI servers [5, 6] and RNAmmer 1.2 [7] was used to identify the rRNA genes. There were 4903 potential genes predicted, 4762 of which were protein coding. Based on JGI annotation, 141 RNA genes were identified, 16 of which were rRNA genes (eight 5S rRNA, three 16S rRNA, five 23rRNA and 66 tRNA genes).

To determine the phylogenetic position of D4N98T, the 16S rRNA, rpoB, gyrB and atpD genes were analysed. The sequences of these genes were extracted from the draft genome and aligned against sequences from closely related species within the family Enterobacteriaceae, based on the
results from a BLAST search [8], an EzTaxon server search [9] and a recent publication [1]. Multiple sequence alignments were performed using CLUSTAL_X [10]. Additionally, a more comprehensive phylogenetic analysis was performed based on a concatenated sequence comprising 37 highly conserved single copy essential genes identified using PhyloSift v1.0.1 [11]. The vast majority of these 37 marker genes are associated with the translation processes (ribosomal protein subunit genes, translation initial factor and rRNA synthesis related genes) and the rest are involved in protein
metabolism, haem biosynthesis and purine nucleotide synthesis [12]. This concatenated sequence was compared with genome data from related Enterobacteriaceae that were retrieved from the GenBank and JGI databases. Phylogenetic trees were reconstructed using an approximately-maximum-likelihood method in FastTree [13], with bootstrap values based on 1000 replications. Maximum parsimony phylogenetic trees were reconstructed using the dnapers program within the Phylip package [14], with 1000 bootstrap replications. Pair-wise sequence identity values between strain D4N98T and related taxa were computed using the dnadist program within the Phylip package [14].

**Fig. 2.** Approximate maximum-likelihood tree, based on a concatenated sequence comprising 37 conserved single copy essential genes [11] of the genus Izhakiella and phylogenetically related species. Genomes of Enterobacter aerogenes KCTC 2190, Enterobacter cloacae subsp. cloacae ATCC 13047T and Citrobacter freundii ATCC 8090T were included as outgroups. Bootstrap values (≥50%) resulting from 1000 replicates are indicated at branch nodes. (See Table S1 for genomes annotation project accession numbers.) Bar, 0.01 substitutions per nucleotide position.
The whole genome shotgun project of strain D4N98T has been deposited into the GenBank and JGI databases with project accession IDs of PRJNA356803 and Ga0101823, respectively.

A complete full length 16S rRNA gene sequence (1 547 bp) was retrieved from genome sequence data. We aligned this 16S rRNA gene sequence to the available Enterobacteriaceae taxa, of which only 1 347 bp were available in the databases. Strain D4N98T exhibited 97% sequence identity with I. capsodis N6PO6T and shared less than 96% identity with species from other genera within the family Enterobacteriaceae. Analysis of the phylogenetic tree based on 16S rRNA genes (Fig. 1) showed that strain D4N98T formed a cluster with I. capsodis N6PO6T, and consistent clustering patterns between strain D4N98T and I. capsodis N6PO6T were also observed in the phylogenetic trees inferred from partial atpD (623 bp), rpoB (637 bp) and gyrB (710 bp) genes (Figs S1, S2 and S3, available in the online Supplementary Material). In all cases, strain D4N98T clustered with I. capsodis N6PO6T, indicating that strain D4N98T does indeed belong to the genus Izhakiella, in the family Enterobacteriaceae. Furthermore, the phylogenetic tree reconstructed using concatenated sequences comprising 37 conserved single copy essential genes (Fig. 2) confirmed that D4N98T clustered with I. capsodis N6PO6T, but was distinct from the genera Pantoea, Erwinia and Tatumella.

To confirm that I. capsodis N6PO6T and D4N98T are different species, in silico genome-genome comparison hybridisation was calculated using the recommended settings of the Genome-to-Genome Distance Calculator (GGDC) web server version 2.0 [15]. This in silico DNA–DNA hybridisation test revealed highly dissimilar genomes with 26.6% (24.3–29.1%) similarity between strain D4N98T and I. capsodis N6PO6T, and only 20.6% (18.4–23.1%) between strain D4N98T and Pantoea calida PSNH2T.

Gram-staining was performed on cells grown for one day at 30 °C on LB agar, as described by Gerhardt et al. [16].

Table 1. Major fatty acids present in I. australiensis sp. nov. D4N98T and I. capsodis N6PO6T.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18:0</td>
<td>33%</td>
<td>37.5%</td>
</tr>
<tr>
<td>C17:0 cyclo</td>
<td>10.7%</td>
<td>14.9%</td>
</tr>
<tr>
<td>Summed features*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summed feature 2</td>
<td>12.6%</td>
<td>10.1%</td>
</tr>
<tr>
<td>Summed feature 3</td>
<td>10.2%</td>
<td>10.9%</td>
</tr>
<tr>
<td>Summed feature 8</td>
<td>13%</td>
<td>11.9%</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI System. Summed feature 2 contains C16:0 3-OH and/or iso-C15:0 1 I; summed feature 3 contains C18:1ω7c, iso-C15:0 2-OH and/or C16:1ω6c; summed feature 8 contains C18:1ω7c and/or C18:1ω6c.

Table 2. Biochemical comparison of the two type strains of species of the genus Izhakiella, I. australiensis sp. nov. D4N98T and I. capsodis N6PO6T.

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Arginine dihydrolase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from Inositol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Cell morphology was observed under a BX61 motorised microscope with a DP71 digital camera attachment (Olympus) (Fig. S4a). Transmission electron microscopy was used to investigate further the cell morphology and cell size. For this, strain D4N98T was cultured in LB medium without shaking for 48 h at 20 °C, centrifuged and suspended in 0.9% sterile saline. The sample was fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffered saline (1 ml), then placed onto a Cu-coated grid (Electron Microscope Unit, UNSW) and stained with 2% (v/v) uranyl acetate. Samples were imaged on a Tecnai G2 20 TEM (FEI) (Fig. S4b).

The results of the chemotaxonomic analysis are given in the species description. The following analytical procedures were carried out: fatty acid extraction and analysis were performed according to the recommendations of the commercial identification system MIDI (Microbial Identification System), and the whole fatty acid composition was determined using an Agilent 6890 N gas chromatograph; polar lipids were extracted from lyophilized biomass using the two-stage method and separated using two-dimensional silica gel TLC, as described by Tindall [17].

The major cellular fatty acids of the novel isolate are given in Table 1, all of the major fatty acids were also present in I. capsodis N6PO6T [1]. In addition, fatty acids C12:0, C19:0 cyclo, C14:0 and C12:0 2-OH were also identified, albeit at lower abundances (<10%; Table S2). The dominant polar lipids of strain D4N98T were phospholipids, which included phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol; a neutral lipid and a glycolipid were also identified (Fig. S5).

For phenotypic characterisation of strain D4N98T, LB agar was used as the growth medium. Growth at 4, 10, 20, 25, 30, 35, 37 and 41 °C was measured. Salt tolerance was determined at 30 °C using Biolog phenotype microarray plates (Biolog). The ability to grow on MacConkey agar...
(Oxoid) was also measured. Growth under anaerobic conditions was determined after incubation in anaerobic conditions generated using a GasPak EZ Gas Generating Container Systems (BD) at 30 °C. Biochemical and enzymatic tests were performed by using Biolog (Biolog), API ZYM, 50CH and 20E identification systems (BioMérieux), according to the manufacturer’s instructions, except that the incubation temperatures used were 30 °C for 3 days. Evidence for catalase activity was from bubble production in a 3% (v/v) hydrogen peroxide solution, while oxidase activity was determined using BactiDrop Oxidase ampoules (Thermo Fisher Scientific). The phenotypic traits of strain D4N98T are described in the species description and are compared with I. capsodis N6PO6T in Table 2.

Being Gram-stain-negative, facultatively anaerobic, chemoheterotrophic, oxidative-negative, catalase-positive, glucose-fermentative and rod-shaped, D4N98T shares many characteristics with the other member of the genus Izhakiella (Table 2). Strain D4N98T was distinguishable from I. capsodis N6PO6T as it is non-motile, unable to produce urease or ferment inositol, but was capable of producing β-galactosidase and arginine dehydroarase.

The results of this study support the recognition of a novel species within the genus Izhakiella, for which the name Izhakiella australiensis sp. nov. is proposed.

**DESCRIPTION OF IZHAKIELLA AUSTRALIENSIS SP. NOV.**

Izhakiella australiensis [aus.tr.a.li.en'sis N.L. fem. adj. australi-ens of or belonging to Australia, the country where the strain was isolated from].

A chemoheterotrophic facultatively anaerobic bacterium. After 24 h of incubation on LB agar at 30 °C, colonies are circular, smooth, transparent and colourless. Cells are rod-shaped (0.54–0.79 μm in width × 10–1.62 μm in length), Gram-stain-negative and non-motile. Growth occurs between 10 and 37 °C, but not at 40 °C, and the optimum temperature for growth is 25–30 °C. Growth is observed on MacConkey agar, but no colour change is observed. The major cellular fatty acids (>10%) are: C₁₆:₀, C₁₇:₀ cyco, summed feature 2 (C₁₄:₀ 3-OH and/or iso-C₁₆:₁ I), summed feature 3 (C₁₆:₁ ω7c, iso-C₁₅:₀ 2-OH, and/or C₁₆:₁ω6c), and summed feature 8 (C₁₈:₁ω7c and/or C₁₈:₁ω6c). The dominant polar lipids are phospholipids including phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, a neutral lipid and a glycolipid.

The following characteristics are observed after 48 h of incubation at 30 °C: Voges-Proskauer-positive; unable to use citrate as the sole carbon source; can assimilate D-glucose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, malic acid and L-rhamnose; can ferment glucose, D-mannitol and trehalose; cannot ferment lactose, sorbitol, sucrose and L-arabinose; positive for catalase, arginine dihydrolase, cystine arylamidase, acid phosphatase, naphthol phosphohydrolase, β-galactosidase and lysine decarboxylation; negative for decarboxylation of ornithine, tryptophan deaminase, gelatinase activities, cytochrome c oxidase and unable to produce urease or ferment inositol; does not reduce nitrate to nitrogen or produce H₂S. Acid production is positive for D-glucose, D-ribose, D-galactose, D-fructose, D-mannose, L-rhamnose, D-mannitol, N-acetyl-glucosamine, trehalose, D-arabitol and weakly positive for glycerol, erythritol, DL-arabinose and D-tagatose. Acid production does not occur for DL-xyllose, D-adonitol, methyl-β-D-xlypyranoside, L-sorbose, dulcitol, inositol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, amygdalin, D-arabinose, arbutin, aesculin ferric citrate, salicin, D-cellobiose, maltose, lactose, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xyitol, gentiobiose, turanose, D-lyxose, D-fucose, L-fucose, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate.

The type strain, D4N98T (=LMG 30066T=DSM 105030T), was isolated from an Australian desert soil.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**References**

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