Chryseomicrobium palamuruense sp. nov., a haloalkalitolerant bacterium isolated from a sediment sample

Pavan Kumar Pindi,1 Kodaparthi Ashwitha1,2 and A. Swaroopa Rani2

1Department of Microbiology, Palamuru University, Mahabubnagar-509 001, T.S, India
2Centre for Biotechnology, IST, JNTUH, Kukatpally, Hyderabad-500 085, T.S, India

A novel Gram-stain-positive, rod shaped, motile bacterium, designated strain PU1T, was isolated from a sediment sample collected from a drainage near hostel of Palamuru University, Mahabubnagar district, T.S, India (16° 43' 23"N 77° 58' 49"E). Cells of strain PU1T are positive for catalase, oxidase, phosphatase, lipase and urease, and negative for gelatinase, amylase, protease, cellulase, lysine decarboxylase and ornithine decarboxylase. The fatty acids were dominated by saturated fatty acids (82.7 %), with a high abundance of iso-C15:0 (48.8 %), anteiso-C15:0 (7.3 %), iso-C16:0 (11.9 %), C16:1ω7c alcohol (11.8 %) and iso-C17:0 (5.3 %). Strain PU1T contained MK-8 as the major respiratory quinone and diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine make up the phospholipid composition. The cell-wall peptidoglycan contains meso-diaminopimelic acid as the diamino acid and cell-wall sugars are d-glucose and d-galactose. 16S rRNA gene sequence analysis indicated Chryseomicrobium imtechense and Chryseomicrobium amylolyticum, members of family Planococcaceae within the phylum Firmicutes, are the closest related species with 16S rRNA gene sequence similarities of 99 %. Other members of the family Planococcaceae had sequence similarities of 99 %, and DNA–DNA relatedness values between strain PU1T and Chryseomicrobium imtechense MW 10T, Chryseomicrobium amylolyticum JC16T were 38 and 32 % respectively. The G+C content of DNA of strain PU1T is 48.5 mol%. Based on the above-mentioned phenotypic and phylogenetic characteristics, strain PU1T represents a novel species of the genus Chryseomicrobium for which the name Chryseomicrobium palamuruense sp. nov. is proposed. The type strain is PU1T (=CCUG 59101T=JCM 16712T=KCTC 13722T=NBRC106750T).

Chryseomicrobium aureum (Arora et al., 2011; Raj et al., 2013; Deng et al., 2014). In this paper, we describe a Gram-stain-positive, rod-shaped, motile, non-endospore-forming bacterium (strain PU1T) that shows 99 % 16S rRNA gene sequence similarity with the type strains of other species of the genus Chryseomicrobium.

Strain PU1T was isolated from a sediment sample collected from drainage near hostel of Palamuru University, Mahabubnagar district, T.S, India (16° 43’ N 77° 05’ E) on 17 August 2009. The sediment sample that yielded strain PU1T had a pH of 8. For isolation of bacteria, 100 µl sediment sample was plated on nutrient agar medium adjusted to pH 6–8 with salt percent ranging from 7–9, and incubated at room temperature for 15 days. Based on the colony morphology, a creamish colony was selected and characterized in the present study. Purification of the isolate was achieved by streaking on tryptone soy agar (TSA) plates repeatedly.

The family Planococcaceae comprises 14 genera at the time of writing: Planococcus, Lysinibacillus, Caryophanon, Solibacillus, Ureibacillus, Psychrobacillus, Kurthia, Viridibacillus, Rummelibacillus, Sporosarcina, Filibacter, Paenisporosarcina, Planomicrobium and Bhargavaea. Phylogenetic examinations strongly suggest that the recently described genus Chryseomicrobium (Arora et al., 2011) comprises the fifteenth genus of this family. Members of the genus Chryseomicrobium are aerobic, Gram-positive, non-endospore-forming rods and, at the time of writing, are represented by only three species with validly published names, Chryseomicrobium imtechense, Chryseomicrobium amylolyticum, and Chryseomicrobium aureum. The GenBank/NCBI accession number for the 16S rRNA gene sequence of strain PU1T is FN555708.

Four supplementary figures and a supplementary table are available with the online Supplementary Material.
Cell morphology and motility were studied using a light microscope. Motility was assessed on TSA medium containing (l-1): pancreatic digest of casein (17 g), papaic digest of soyabean meal (3 g), sodium chloride (5 g), dipotassium hydrogen phosphate (2.5 g), dextrose (2.5 g) and agar (0.4 g). Growth on TSA medium at different temperatures, salt tolerance, biochemical characteristics, carbon assimilation, H2S production and the sensitivity of the culture to different antibiotics were determined by previously described methods (Lanyi, 1987; Smibert & Krieg, 1994). Biochemical characteristics were also double-checked with Hi25 Enterobacteriaceae identification kit (cat. #KB003; HiMedia) and HiCarbohydrate kit parts A, B and C (cat. #KB009; HiMedia) according to the manufacturer’s protocol. Growth of strain PU1T at different pH was checked on NA medium buffered either with citric acid-NaOH (for pH 5 and 6), phosphate (for pH 7 and 8), glycine-NaOH (for pH 9 and 10) or Tris buffer (for pH 11 and 12).

Fatty acid methyl esters were prepared and analysed by using the Sherlock Microbial Identification System (MIDI) according to the protocol described by Agilent Technologies. For this purpose, all strains were grown on TSA medium at 30°C for 2 days. Peptidoglycan was prepared according to the method described by Rosenthal & Dzierski (1994). The peptidoglycan obtained was then hydrolysed with 2 M HCl at 60 min. The hydrolysate was extracted into acetate buffer and subjected to amino acid analysis on an automated amino acid analyser. The composition of the peptidoglycan was determined according to the method of Schleifer & Kandler (1972). Polar lipids were analysed by lyophilizing cell pellets free of medium by the method of Minnikin et al. (1975) and identified by TLC. Cell-wall components were extracted and analysed according to the method described by Komagata & Suzuki (1987). Menaquinones and polar lipids were determined in freeze-dried cells. Menaquinones were extracted as described by Collins et al. (1977) and were analysed by HPLC (Groth et al., 1997). DNA was isolated according to the procedure of Marmur (1961) and the G+C content was determined from melting point (Tm) curves (Sly et al., 1986) obtained by using a Lambda2 UV-Vis spectrophotometer (Perkin Elmer) equipped with the Templab 2.0 software package (Perkin Elmer). Escherichia coli K-12 strain was used as a standard in determining the mol% G+C content of the DNA. For 16S rRNA gene sequencing, DNA was prepared using the Mo Bio microbial DNA isolation kit (Mo Bio Laboratories) and sequenced as described previously (Lane, 1991). Taq polymerase supplied by Merck was used for PCR, which was started with the primers 16S F 5'-GTTTGATCCTTGTCAG-3' and and 16S R 5'-AAGGAGGTGATCAGCCGCA-3'. The resultant almost-complete sequence of the 16S rRNA gene of strain PU1T contained 1502 nucleotides. This sequence was subjected to BLAST sequence similarity search (Altschul et al., 1990) and analysis using the EzTaxon server (Kim et al., 2012) to identify the nearest taxa. All the 16S rRNA gene sequences belonging to the family Planococcaceae were downloaded from the GenBank database (http://www.ncbi.nlm.nih.gov), aligned using the CLUSTAL X program (Thompson et al., 1997) and the alignment corrected manually. Phylogenetic trees were reconstructed using two tree-making algorithms, maximum-likelihood (ML) using the PhyML program (Guindon & Gascuel, 2003) and neighbour-joining (NJ) (Saitou & Nei, 1987) using the PHYLIP package, version 3.5 (Felsenstein, 1993), and the resultant tree topologies were evaluated by bootstrap analysis based on 1000 resamplings using the SEQBOOT and CONSENSE programs in the PHYLIP package. Pair-wise evolutionary distances were computed using the DNADIST program with the Kimura 2-parameter model as developed by Kimura (1980).

The genomic relationship between strain PU-1T, Chryseobacterium amylolyticum JC16T, Chryseobacterium aureum BUT-2T and Chryseobacterium intenches MW 10T was examined by DNA–DNA hybridization using a membrane-filter technique (Tourova & Antonov, 1988). Hybridization was performed with three replicates for each sample. A nick translation kit was used for labelling the probe with a-P32 dCTP. The DNA immobilized on the blots (nylon membranes) was probed with labelled DNA and then exposed to a phosphor imaging screen (Amersham Biosciences). A TYPHOON (3480) variable mode imager was used to scan and quantify the phosphor-imaging screen.

Cells of strain PU1T are rods of 0.6–0.7 µm in width and 1.6–2 µm in length, and multiply by binary fission. Cells are Gram-positive (Fig. 1). Colonies are circular, 1–2 mm in diameter, smooth, creamish in colour, opaque, crateriform and entire on nutrient agar. The strain is aerobic and grows chemoheterotrophically. Growth is observed from 18 to

![Fig. 1. Electron micrograph of negatively stained cells of strain PU1T, grown on nutrient agar medium at pH-8 and temperature of 37°C for 30 h of incubation period. Bar, 0.5 µm.](image-url)
40 °C with optimum growth at 37 °C. Growth is observed at salinities from 0 to 9 % (NaCl, w/v), with an optimum between 8–9 % (NaCl, w/v). Growth occurs from pH 7 to pH 10, with optimum growth at pH 8. The cellular fatty acid composition of strain PU1\textsuperscript{T} showed a spectrum of 22 fatty acids with a pronounced dominance of saturated fatty acids (82.7 %), including a high abundance of iso-C\textsubscript{15:0}, anteiso-C\textsubscript{15:0}, iso-C\textsubscript{16:0}, C\textsubscript{16:1}ω7c alcohol and iso-C\textsubscript{17:0} (Table 1). When compared with \textit{Chryseomicrobium imtechense} MW 10\textsuperscript{T} and \textit{Chryseomicrobium amylolyticum} JC16\textsuperscript{T}, the number of fatty acids detected in strain PU1\textsuperscript{T} was lower but the composition differed considerably (Table 1). The menaquinone present in strain PU1\textsuperscript{T} is MK-8 (Fig. S1, available in the online Supplementary Material) (100 %) and diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine (Fig. S2) make up the phospholipid composition. The cell-wall peptidoglycan contains meso-diaminopimelic acid as the diamin acid and cell-wall sugars are D-glucose and D-galactose. Other characteristics are listed in the species description. The DNA G+C content of strain PU1\textsuperscript{T} is 48.5 mol%.

The phylogenetic relationship of strain PU1\textsuperscript{T} was ascertained based on the 16S rRNA gene sequence similarity of PU1\textsuperscript{T} with other reported species using BLAST sequence similarity search (NCBI-BLAST/EzTaxon). The results indicated that at the 16S rRNA gene sequence level, strain PU1\textsuperscript{T} was close to the phylogenetic neighbours, \textit{Chryseomicrobium imtechense} MW 10\textsuperscript{T} and \textit{Chryseomicrobium amylolyticum} JC16\textsuperscript{T} with pairwise sequence similarity of 99 % each. Phylogenetic analyses based on ML (Fig. 2), and NJ (Fig. S3) trees further indicated that strain PU1\textsuperscript{T} clustered with \textit{Chryseomicrobium imtechense} MW 10\textsuperscript{T} and \textit{Chryseomicrobium amylolyticum} JC16\textsuperscript{T} with a phylogenetic distance of 99 % each and distinct from the other species of the genus \textit{Chryseomicrobium}. Despite the high 16S rRNA gene sequence similarity, it is observed that, at the whole genome level, when strain PU1\textsuperscript{T} was radioactively labelled, the DNA–DNA relatedness between strain PU1\textsuperscript{T} and \textit{Chryseomicrobium imtechense} MW 10\textsuperscript{T} and \textit{Chryseomicrobium amylolyticum} JC16\textsuperscript{T} is only 38 and 32 % (reciprocal reaction %) indicating that strain PU1\textsuperscript{T} could be assigned to a novel species.

In addition, strain PU1\textsuperscript{T} could be phenotypically differentiated from the phylogenetically closely related species of \textit{Chryseomicrobium imtechense} and \textit{Chryseomicrobium amylolyticum} (Tables 2 and S1). For instance strain PU1\textsuperscript{T} differs from both with respect to cell size, salt tolerance, growth temperature range, hydrolysis of starch and urea, citrate utilization, major fatty acid composition, cell wall and DNA G+C content (Table 2). It is interesting to see that the cell wall (peptidoglycan type) in two of the reference strains examined in the present study contained L-Orn–D-Glu which

### Table 1. Fatty acid composition of strain PU1\textsuperscript{T} and closely related species of the genus \textit{Chryseomicrobium}

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{14:0}</td>
<td>0.4</td>
<td>2.8</td>
<td>4.0</td>
<td>1.7</td>
</tr>
<tr>
<td>iso-C\textsubscript{15:0}</td>
<td>48.7</td>
<td>68.57</td>
<td>57.7</td>
<td>60.5</td>
</tr>
<tr>
<td>anteiso-C\textsubscript{15:0}</td>
<td>7.3</td>
<td>2.52</td>
<td>4.2</td>
<td>6.9</td>
</tr>
<tr>
<td>C\textsubscript{16:0}</td>
<td>1.1</td>
<td>1.51</td>
<td>9.8</td>
<td>1.3</td>
</tr>
<tr>
<td>iso-C\textsubscript{16:0}</td>
<td>11.9</td>
<td>10.4</td>
<td>11.3</td>
<td>11.8</td>
</tr>
<tr>
<td>C\textsubscript{16:1}ω7c alcohol</td>
<td>11.8</td>
<td>10.88</td>
<td>0.9</td>
<td>5.9</td>
</tr>
<tr>
<td>C\textsubscript{16:1}ω9c</td>
<td>ND</td>
<td>ND</td>
<td>0.2</td>
<td>ND</td>
</tr>
<tr>
<td>C\textsubscript{16:1}ω11c</td>
<td>2.2</td>
<td>2.0</td>
<td>2.5</td>
<td>2.2</td>
</tr>
<tr>
<td>iso-C\textsubscript{17:0}</td>
<td>5.3</td>
<td>3.00</td>
<td>6.8</td>
<td>5.6</td>
</tr>
<tr>
<td>anteiso-C\textsubscript{17:0}</td>
<td>2.4</td>
<td>ND</td>
<td>2.8</td>
<td>4.7</td>
</tr>
<tr>
<td>iso-C\textsubscript{17:1}ω10c</td>
<td>2.4</td>
<td>2.43</td>
<td>5.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Summed feature(s)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>11.0</td>
<td>9.6</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>ND</td>
<td>ND</td>
<td>1.6</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>0.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Summed features represent groups of two or more fatty acids that could not be separated using the MIDI system. Summed feature 3 contained C\textsubscript{16:1}ω7c/C\textsubscript{16:1}ω6c; summed feature 4 contained iso-C\textsubscript{17:1}ω7c/aniso-C\textsubscript{17:1}ω7c; summed feature 5 contained anteiso-C\textsubscript{18:1}ω7c/C\textsubscript{18:1}ω6c; summed feature 7 contained C\textsubscript{19:1}ω7c/C\textsubscript{19:1}ω6c; summed feature 8 contained C\textsubscript{18:1}ω7c; summed feature 9 contained C\textsubscript{16:1}ω10-methyl.
Description of Chryseomicrobium palamuruense sp. nov.

Chryseomicrobium palamuruense (pa.la.mu.ru.en’se. N.L. neut. adj. palamuruense belonging to Palamuru).

Cells are Gram-stain-positive, motile, rod-shaped (0.6–0.7 µm in width and 1.6–2 µm in length), occur singly and multiply by binary fission. Colonies on nutrient agar are circular, 1–2 mm in diameter, smooth, cream, opaque, crateriform and entire. Cells grow from 18 to 40 °C with an optimum temperature of 37 °C and tolerate up to 9.0% NaCl (w/v). Growth occurs in a pH range of 7 to 10. Catalase, oxidase, phosphatase, lipase and urease are positive, but gelatinase, amylase, protease, cellulase, β-galactosidase, lysozyme decarboxylase and ornithine decarboxylase are negative. Phenylalanine deamination, nitrate reduction, H2S production, Voges-Proskauer reaction, methyl red reaction and tryptophan deamination are negative. Aesculin and Tween 80 are not hydrolysed. Cells produce acid from L-tryptophan, maltose, cellobiose, D-glucose, methyl α-D-mannoside, melibiose, creatin, starch, sorbose, citric acid, sodium gluconate, phenylalanine, rhamnose, erythritol, inositol, D-mannitol and D-raffinose after one week of incubation at optimum temperature and pH. Assimilates xylose, glucose, rhamnose, glucosamine, mellibiose, cellobiose, D-arabinose, citrate, glycerol, malonate and ribose, but not galactose, inulin, sodium gluconate, salicin, dulcitol, fructose, inositol, L-arabinose, lactose, maltose, mannose, sorbitol, sucrose, mannitol, adonitol, methyl α-D-glucoside, melezitose, methyl α-D-mannoside, raffinose, rhamnose, saccharose, trehalose, xylitol, ONPG, aesculin and sorbose. Susceptible to (µg per disc) co-trimoxazole (25), lincomycin (2), ampicillin (10), amikacin (30), cephotaxime (30), clarithromycin (2), aztreonam (10), amoxicillin (10), ampicillin (10), and resistant to (µg per disc) kanamycin (30), tetracycline (30), and nalidixic acid (30). DNA G+C content also differentiates the three reference strains with 52 to 57.4 mol% from the novel strain with 48.5 mol%.

Thus the cumulative differences that strain PU1T exhibits with the above three closely related species unambiguously supports the creation of a novel species for which the name Chryseomicrobium palamuruense sp. nov. is proposed.
The strain strain PU1<sup>T</sup> (=CCUG 59101<sup>T</sup>=JCM 16712<sup>T</sup>=KCTC 13722<sup>T</sup>=NRBC106750<sup>T</sup>) was isolated from a sediment sample collected from drainage near hostel of Palamuru, Mahabubnagar district, T.S., India. The genomic DNA G+C content of the type strain is 48.5 mol%, and the cellular fatty acid composition is given in Table 1.

Acknowledgements

We thank Dr S. Shivaji, Centre for Cellular and Molecular Biology (CCMB, HYD) and Prof. G. Bagyanarayana, Vice-Chancellor and Prof. I. Pandu Ranga Reddy, Registrar, Palamuru University, Mahabubnagar, T.S., India for their encouragement and support.

References


