Aliicoccus persicus gen. nov., sp. nov., a halophilic member of the Firmicutes isolated from a hypersaline lake

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A novel Gram-staining-positive, moderately halophilic bacterium, designated strain A76T, was isolated from a brine sample of the hypersaline lake Aran-Bidgol in Iran. Cells were strictly aerobic, coccus-shaped, non-motile, non-sporulating, and catalase- and oxidase-positive. Strain A76T grew between pH 7.0 and 10.0 (optimal growth at pH 8.0), between 20 and 45 ºC (optimal growth at 35 ºC) and at salinities of 0.5 to 12.5 % (w/v) NaCl (optimal growth at 7.5 %, w/v, NaCl). On the basis of 16S rRNA gene sequence analysis, strain A76T was shown to belong to the phylum Firmicutes with sequence similarities of 94.1, 93.1 and 91.1 %, to the type species of the genera Jeotgalicoccus, Salinicoccus and Nosocomiicoccus, respectively. The DNA G+C content of this new isolate was 38.8 mol%. The major cellular fatty acids of strain A76T were anteiso-C15 : 0 and iso-C15 : 0, and its polar lipid pattern consisted of diphosphatidylglycerol, phosphatidylglycerol, a glycolipid, an unknown lipid and two unknown phospholipids. The isoprenoid quinones were MK-6 (94 %), MK-5 (3 %) and MK-7 (3 %). The amino acid constituents of the cell wall were Lys, Asp, Gly, Glu and Ala. The physiological, biochemical and phylogenetic differences between strain A76T and type strains of taxa with validly published names suggest that this strain represents a novel species in a novel genus within the family Staphylococcaceae, for which the name Aliicoccus persicus gen. nov., sp. nov. is proposed. The type strain of Aliicoccus persicus is strain A76T (= CECT 8508T = DSM 28306T = IBRC-M 10081T).

Moderately halophilic bacteria are characterized by their inability to grow in the absence of salt and they grow optimally in media containing 0.5–2.5 M salt. Moderate halophiles are known from eight different phyla, with most species belonging to the phyla Proteobacteria or Firmicutes (de la Haba et al., 2011). The coccus-shaped moderately halophilic bacteria have been classified in the following genera: Halobacillus (Spring et al., 1996), Jeotgalicoccus (Yoon et al., 2003), Nesterenkonia (Stackebrandt et al., 1995), Salinicoccus (Ventosa et al., 1990) and Tetragenococcus (Collins et al., 1990). The aim of the present study was to determine the taxonomic position, using a polyphasic approach, of a moderately halophilic coccus-shaped bacterium, strain A76T, isolated from a hypersaline lake in Iran. The results indicated that this isolate represents a novel moderately halophilic species in a novel genus of the phylum Firmicutes. Jeotgalicoccus coquinae CCM 7682T and Jeotgalicoccus aerolatus CCM 76791T, obtained from the Czech Collection of Microorganisms and Salinicoccus

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain A76T is HQ433462.

Three supplementary figures are available with the online version of this paper.
Strain A76\textsuperscript{T} was isolated from a brine sample of the hypersaline lake Aran-Bidgol, which is located near Kashan city in the centre of Iran. The pH of the lake is neutral (about pH 7.0–7.5) and its salinity reaches saturation. The lake, with a surface area of about 650 km\textsuperscript{2} in dry seasons, can be considered as halotolerant based on the salt composition (Makhdoumi-Kakhki et al., 2012). At the time of sampling, the temperature of the water was 32 °C and the pH 7.0.

The strain was isolated by diluting the brine sample in sterile 10 % (w/v) salt solution, plating on 7.5 % HM medium and incubating at 35 °C aerobically. The 7.5 % HM medium contained (g 1\textsuperscript{–1}): NaCl, 60.75; MgCl\textsubscript{2}.6H\textsubscript{2}O, 5.25; MgSO\textsubscript{4}.7H\textsubscript{2}O, 7.2; CaCl\textsubscript{2}.2H\textsubscript{2}O, 0.27; KCl, 1.5; NaHCO\textsubscript{3}, 0.045; NaBr, 0.0195; proteose-peptone no. 3, 5; yeast extract, 10; and glucose, 1 (Ventosa et al., 1982). The pH of this medium was adjusted to pH 7.5. The strain was subsequently purified three times by plating on the same medium and maintained on the same 7.5 % HM medium, and at −80 °C in this medium without agar but supplemented with 30 % (v/v) glycerol.

The genomic DNA of the isolate was extracted with a DNA extraction kit (High Pure PCR Template Preparation kit; Roche) according to the manufacturer’s protocol and the 16S rRNA gene was amplified using the bacterial universal primers 27F and 1492R (Lane et al., 1985). Direct sequence determination of the PCR-amplified DNA was conducted on an ABI 3730XL DNA sequencer at Macrogen (Seoul, South Korea).

The 16S rRNA gene sequence analysis was performed with the ARB software package (Ludwig et al., 2004). The 16S rRNA gene sequence was aligned with the published sequences of closely related bacteria and the alignment was confirmed and checked against both primary and secondary structures of the 16S rRNA molecule using the alignment tool of the ARB software package. For phylogenetic inference, phylogenetic trees were reconstructed using three different methods: maximum-parsimony (Fitch, 1971), neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms integrated in the ARB software. The 16S rRNA gene sequences used for phylogenetic comparisons were obtained from the GenBank database and their strain designations and accession numbers are shown in Fig. 1.

An almost complete 16S rRNA gene sequence of strain A76\textsuperscript{T} (1435 nt) was obtained. The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e tool (Kim et al., 2012).

The 16S rRNA gene sequence analysis revealed that strain A76\textsuperscript{T} is a member of the family Staphylococcaceae (Schleifer & Bell, 2009); however, its similarity was as low as 94.1, 93.1 and 91.1 % to representatives of the most closely related type species Jeotgalicoccus halotolerans YKJ-101\textsuperscript{T}, Salinicoccus roesei DSM 5351\textsuperscript{T} and Nosocomiicoccus ampullae TRF-1\textsuperscript{T}, respectively. Phylogenetic analysis using the maximum-parsimony algorithm revealed that the novel strain clustered with the halophilic members of this family but represented a separate lineage (Fig. 1). The phylogenetic position was also confirmed in trees generated using the neighbour-joining and maximum-likelihood algorithms (Figs S1 and S2, available in the online Supplementary Material).

Cell morphology was examined using an Olympus BX41 microscope equipped with phase-contrast optics. Gram staining was performed by the Burke method (Murray et al., 1994) and the result was confirmed by the KOH test (Baron & Finegold, 1990). Motility was analysed by the wet-mountain method (Murray et al., 1994). The presence of endospores was investigated by using the Schaeffer–Fulton staining method (Murray et al., 1994). Catalase, oxidase and urease activities, nitrate reduction, hydrolysis of aesculin, production of indole, methyl red and Voges–Proskauer tests were done as recommended by Smibert & Krieg (1994). Hydrolysis of Tween 80 was examined as described by Harrigan & McCance (1976). Determination of acid production from carbohydrates, as well as utilization of carbon and nitrogen sources, was performed as recommended by Ventosa et al. (1982). Antibiotic susceptibility tests were performed on Mueller–Hinton agar plus 7.5 % (w/v) sea salts (Ventosa et al., 1982) seeded with a bacterial suspension containing 1.5 × 10\textsuperscript{8} c.f.u. ml\textsuperscript{−1} using discs (HiMedia) impregnated with various antimicrobial compounds. The plates were incubated at 35 °C for 48 h and the inhibition zone was interpreted according to the manufacturer’s manual. To determine the optimal temperature and pH for growth of the strain, broth cultures were incubated at temperatures of 15–50 °C at intervals of 5 °C and at pH 6–11 at intervals of 0.5 pH units. pH 6–9 and pH values above 9 were obtained using Tris/HCl and glycine/sodium hydroxide buffers, respectively. Growth at different NaCl concentrations (0.5, 2.5, 5, 7.5, 10, 12.5, 15 and 20 %, w/v) was tested on HM medium at pH 8.0. Growth was monitored by turbidity at OD\textsubscript{600} using a spectrophotometric method (model UV-160 A; Shimadzu). Other physiological and biochemical tests were performed as described previously (Mata et al., 2002; Quesada et al., 1984; Ventosa et al., 1982).

Strain A76\textsuperscript{T} was a Gram-staining-positive, non-motile, non-sporulating and strictly aerobic coccus. Colonies were pale yellow, circular and 1.6–1.8 mm in diameter after incubation at 35 °C for 48 h on 7.5 % HM. This new isolate was moderately halophilic, showing optimal growth at 7.5 % (w/v) NaCl and being able to grow in media with 0.5 to 12.5 % (w/v) NaCl. It was not able to grow in media without NaCl. Strain A76\textsuperscript{T} was sensitive to amikacin (30 μg), ampicillin (30 μg), amoxicillin (30 μg), bacitracin (10 μU), carbenicillin (100 μg), cefalotin (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), nitrofurantoin (300 μg),
penicillin G (10 U), polymyxin B (100 U), rifampicin (5 μg), streptomycin (10 μg), tetracycline (30 μg) and tobramycin (10 μg) but resistant to kanamycin (30 μg), erythromycin (5 μg) and nalidixic acid (30 μg). Other phenotypic features are listed in Table 1 as well as in the genus and species descriptions.

For determination of DNA base composition, DNA was isolated, purified by hydrofluoric acid treatment and the values described for type species of the genera Salinicoccus (Ventosa et al., 1993), while strain A76T differs from the type species of the genus Jeotgalicoccus by the presence of a glycolipid (Yoon et al., 2003).

The major isoprenoid quinone of strain A76T was MK-6 (94 %), while MK-5 (3 %) and MK-7 (3 %) were also present. This chemotaxonomic property is similar to those of the species belonging to the genus Salinicoccus (Ventosa et al., 1990) and essentially differs from the members of the genus Jeotgalicoccus, which have a menaquinone with several isoprene units as the major lipoquinone (Yoon et al., 2003).

The whole-cell fatty acid composition of strain A76T was determined according to the standard protocol of the Microbial Identification System (Sherlock version 6.1; MIDI). Extracts were analysed using a Hewlett Packard model HP6890A gas chromatograph equipped with a flame-ionization detector as described by Kämpfer & Kroppenstedt (1996). Fatty acid peaks were identified using the TSBA40 database. The fatty acid profile of strain A76T was characterized by the fatty acids anteiso-C\textsubscript{15:0}
Table 1. Differential characteristics between strain A76<sup>T</sup> and the type species of phylogenetically related genera within the family Staphylococcaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Colony pigmentation</td>
<td>Hypersaline lake</td>
<td>Fermented seafood</td>
<td>Solar saltern</td>
<td>Surface of used saline bottle</td>
</tr>
<tr>
<td>Growth temperature (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>20–45</td>
<td>4–42</td>
<td>15–40</td>
<td>30–45</td>
</tr>
<tr>
<td>Optimum</td>
<td>35</td>
<td>30–35</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Growth at absence of NaCl</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Growth with 20% NaCl</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>W</td>
</tr>
<tr>
<td>Mannitol</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Casein</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Presence of glycolipid</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Predominant menaquinone(s)</td>
<td>MK-6</td>
<td>MK-7</td>
<td>MK-6</td>
<td>MK-7 (49.5%), MK-8 (44.5%)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>38.8</td>
<td>42</td>
<td>51</td>
<td>33.5</td>
</tr>
<tr>
<td>16S rRNA gene similarity to strain A76&lt;sup&gt;T&lt;/sup&gt; (%)</td>
<td>100</td>
<td>94.1</td>
<td>93.1</td>
<td>91.1</td>
</tr>
</tbody>
</table>

(43.8 %), iso-C<sub>15</sub>:0 (25.3 %), anteiso-C<sub>17</sub>:0 (9.1 %), iso-C<sub>16</sub>:0 (6.1 %), iso-C<sub>17</sub>:0 (5.6 %), anteiso-C<sub>16</sub>:0 (2.7 %), iso-C<sub>14</sub>:0 (1.7 %), iso-C<sub>17</sub>:0<sup>10</sup> (1.2 %) and anteiso-C<sub>17</sub>:0 (1.0 %). The major fatty acids pattern of the new taxon is similar to those of the members of the genera Salinicoccus (Ventosa et al., 1990) and Jeotgalicoccus (Yoon et al., 2003), while it differs from those of members of the genus Nosocomiicoccus, which have C<sub>14</sub>:0 and C<sub>16</sub>:0 as the major fatty acids (Alves et al., 2008).

In conclusion, the 16S rRNA gene sequence analysis revealed that strain A76<sup>T</sup> belonged to the family Staphylococcaceae, constituting a separated branch with respect to the other closely related genera. Strain A76<sup>T</sup> was distinguished by some chemotaxonomic characteristics, such as fatty acids, polar lipid and menaquinone composition, from the related genera Jeotgalicoccus and Salinicoccus. Also, several phenotypic features such as NaCl concentration range and temperature range for growth, and acid production from carbohydrates (Table 1), as well as DNA base composition clearly support that strain A76<sup>T</sup> represents a genus different from other related genera within the family Staphylococcaceae. On the basis of these data, we propose to place strain A76<sup>T</sup> in a novel species in a new genus with the designation *Alicoccus persicus* gen. nov., sp. nov.

Description of *Alicoccus persicus* sp. nov.

*Alicoccus persicus* (per’si.cus. L. masc. adj. persicus of Persia).

Description is as for the genus with the following additional characteristics. Cells are coccus-shaped, 0.7–0.8 μm in diameter. Colonies are circular, entire, smooth and pale yellow, 1.6–1.8 mm in diameter on 7.5 % HM agar medium after 48 h of incubation at 35 °C. Moderately halophilic, growing with 0.5–12.5 % (w/v) NaCl, with optimal growth at 5–7.5 % (w/v) NaCl. No growth occurs in the absence of NaCl. Grows at 20–45 °C (optimally at 35 °C) and pH 7.0–10.0 (optimally at pH 8.0). Nitrate is not reduced to nitrite. Negative for decomposition of aesculin, casein, DNA, gelatin, starch and Tweens 20, 40, 60 and 80. Acid is not produced from arabinose, D-fructose, D-glucose, galactose,}

**Description of *Alicoccus persicus* gen. nov.**

*Alicoccus* [A.li.i.coc’cus. L. n. alius other, another; N.L. masc. n. coccus a coccus (from Gr. n. kokkos a grain, berry); N.L. masc. n. *Alicoccus* the other coccus].

Cells are Gram-staining-positive, strictly aerobic, non-motile, non-sporeulating and coccus-shaped. Catalase- and oxidase-positive. Moderately halophilic. The polar lipid pattern consists of diphosphatidylglycerol, phosphatidylglycerol, one unknown glycolipid, two unknown phospholipids and one unknown lipid. The predominant menaquinone is MK-6. Major fatty acids are anteiso-C<sub>15</sub>:0 and iso-C<sub>15</sub>:0. The amino acids of the peptidoglycan are Lys, Asp, Gly, Glu and Ala. DNA G+C content of the type strain of the type species, *Alicoccus persicus*, is 38.8 mol% (HPLC method). Phylogenetically related to the genera *Jeotgalicoccus*, *Salinicoccus* and *Nosocomiicoccus* within the family *Staphylococcaceae*. The type species is *Alicoccus persicus*. 

[Alicicoccus persicus gen. nov., sp. nov.](http://ijs.sgmjournals.org)
lactose, mannitol, D-mannose, maltose, melibiose, ribose, sucrose, trehalose or D-xylene. Citrate utilization is positive. Indole and H$_2$S are not produced. Methyl red, Voges-Proskauer, urease, β-galactosidase, ornithine decarboxylase and phenylalanine deaminase tests are negative. The following compounds are utilized as sole sources of carbon and energy: arabinose, cellobiose, D-fructose, galactose, maltose, mannitol and sucrose. The following compounds are not utilized as sole source of carbon and energy: D-glucose, mannose, melibiose, ribose, raffinose, starch, trehalose, glycogen, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, glycerol, L-histidine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-tyrosine and L-valine. The predominant isoprenoid quinone is MK-6, with MK-5 and MK-7 as minor quinones. Cellular fatty acids are anteiso-C$_{15:0}$, iso-C$_{15:0}$, anteiso-C$_{17:0}$, iso-C$_{16:0}$, iso-C$_{17:0}$, anteiso-C$_{16:0}$, iso-C$_{14:0}$ and iso-C$_{17:0}$ 10c and anteiso-C$_{14:0}$.

The type strain is A76$^T$ (=CECT 8508$^T$=DSM 28306$^T$=IBRC-M 10081$^T$), isolated from Aran-Bidgol hypersaline lake in Iran. The DNA G+C content of the type strain is 38.8 mol%.

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