Phycicoccus soli sp. nov., isolated from soil

Hina Singh,†† KyungHwa Won,†† Hien T. T. Ngo,† Juan Du,† MooChang Kook‡ and Tae-Hoo Yi†

†Department of Oriental Medicine Biotechnology, College of Life Science, Kyung Hee University, 1 Seocheon, Kihung Yongin, Gyeonggi 446-701, Republic of Korea
‡Department of Marine Biotechnology, Anyang University, Incheon 417-833, Republic of Korea

The genus Phycicoccus was described by Lee (2006) with a single species, Phycicoccus jejuensis. Phylogenetically, the genus belongs to the family Intrasporangiaceae and order Micrococcales (Stackebrandt et al., 1997). The description of this genus Phycicoccus was emended by Zhang et al. (2011). At the time of writing, the genus Phycicoccus comprised eight species with effectively published names, P. jejuensis (Lee, 2006), P. aerophilus (Weon et al., 2008), P. bigeumensis (Dastager et al., 2008), P. dokdonensis (Yoon et al., 2008), P. ginsenosidimutans (Wang et al., 2011), P. cremeus (Zhang et al., 2011), 'P. ochangensis' (Kim et al., 2012a) and P. badiiscoriae (Lee, 2013); the name 'P. ochangensis' has not yet been validly published. Members of the genus Phycicoccus are characterized as Gram-positive, coccus-shaped bacteria with meso-diaminopimelic acid as the diamino acid of the peptidoglycan, MK-8(H₄) as the predominant isoprenoid quinone and C₁₀₀₈₀, iso-C₁₅₅₀, iso-C₁₅₇₀, C₁₅₀ and C₁₇₀ as the major fatty acids. This paper describes the characterization of a novel strain belonging to the genus Phycicoccus, THG-a₁₄ᵀ, by using a polyphasic approach. On the basis of the results obtained in this study, we propose that it should be placed in the genus Phycicoccus as the type strain of a novel species.

Strain THG-a₁₄ᵀ was isolated from a soil sample collected from Gyeeyang mountain (37° 33' N 126° 42' E) in Incheon, Republic of Korea. One gram of the soil sample was suspended in 10 ml sterile 0.85 % (w/v) NaCl (saline solution), vortexed and serially diluted, and 100 µl aliquots of the serial dilutions were spread on R2A agar (Difco) plates and incubated at 28°C for 1 week. Single colonies were purified by culturing them on R2A plates. One of the colonies purified was found to belong to the genus Phycicoccus and was preserved in R2A broth (Difco) supplemented with glycerol (25 %, w/v) at −80°C.

Genomic DNA was extracted and purified using a commercial genomic DNA extraction kit (Solgent). The 16S rRNA gene was amplified with the universal bacterial primers 27F (5'-TACCAGGGTATCTAATCC-3') and 1492R (5'-GGTTACCTTGGTACGACTT-3') (Weisburg et al., 1991) and the purified PCR products were sequenced by Solgent Co. Ltd (Daejeon, Korea). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database and EzTaxon e-server (http://eztaxon-e.ezbiocloud.net/).
The 16S rRNA gene sequence of strain THG-a14\textsuperscript{T} determined in this study was a continuous stretch of 1405 bp. According to the EzTaxon e-server, strain THG-a14\textsuperscript{T} exhibited high 16S rRNA gene sequence similarity to \textit{P. aerophilus} 5516T-20\textsuperscript{T} (97.7\%), \textit{P. ginsenosidimutans} BXN5-13\textsuperscript{T} (97.6\%), ‘\textit{P. ochangensis}’ L1b-b9 (97.4\%) and \textit{P. bigeumensis} MSL-03\textsuperscript{T} (97.2\%). Lower sequence similarity (<97\%) was found with all other recognized species of the family \textit{Intrasporangiaceae}. The phylogenetic tree showed that strain THG-a14\textsuperscript{T} clustered within the genus \textit{Phycicoccus} (Fig. 1 and Figs S1 and S2, available in the online Supplementary Material).

The morphological, physiological and biochemical characteristics of strain THG-a14\textsuperscript{T} were investigated after culturing on R2A agar at 28°C for 3 days. Gram-staining was determined using a Gram stain kit (bioMérieux) according to the manufacturer’s instructions. Cell morphology was observed at ×11000 magnification with a transmission electron microscope (model JEM1010; JEOL), using cells grown for 3 days at 28°C on R2A agar (Fig. S3). Growth was tested using several bacterial culture media including nutrient agar (NA; Difco), tryptone soya agar (TSA; Oxoid), R2A agar and MacConkey agar (Oxoid) at 28°C. Growth at 4, 10, 15, 18, 25, 28, 30, 35, 37 and 42°C was assessed after 4 days of incubation on R2A agar and growth at pH 4.0–10.0 (at intervals of 0.5 pH units) was determined in R2A broth after 4 days of incubation at 28°C. For the pH experiments, three different buffers were used (final concentration, 100 mM): acetic acid for pH 4.0–4.5, acetate buffer for pH 5.0–6.5 and phosphate buffer for pH 7.0–10.0. The pH of the R2A broth was confirmed after autoclaving. Salt tolerance was tested after 4 days of incubation at 28°C in R2A broth supplemented with 0–5\% (w/v) NaCl (at 0.5\% intervals). Growth was estimated by monitoring the OD\textsubscript{600}. Anaerobic growth was tested in serum bottles containing R2A broth supplemented with thioglycolate (0.1\%) in which the air was replaced by nitrogen gas. Motility was assayed on sulfide-indole-motility (SIM) medium (Difco). Catalase activity was determined from bubble production in 3\% (v/v) H\textsubscript{2}O\textsubscript{2} and oxidase activity was determined using 1\% (w/v) N,N,N’,N’-tetramethyl 1,4-phenylenediamine reagent. Hydrolysis of the following compounds was tested after 4 days of incubation at 28°C in R2A broth supplemented by nitrogen gas (0.1\%). For the pH experiments, three different buffers were used (final concentration, 100 mM): acetic acid for pH 4.0–4.5, acetate buffer for pH 5.0–6.5 and phosphate buffer for pH 7.0–10.0.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{phylogenetic_tree}
\caption{Neighbour-joining phylogenetic tree based on 16S RNA gene sequences showing the relationships of strain THG-a14\textsuperscript{T} with related members of the genus \textit{Phycicoccus}. Filled circles at nodes indicate generic branches that were also recovered by using the maximum-parsimony algorithm. Bootstrap values (expressed as percentages of 1000 replications) over 70\% are shown at branching points. \textit{Arsenicicoccus bolidensis} CCUG 47306\textsuperscript{T} was used as an outgroup. Bar, 0.005 substitutions per nucleotide position.}
\end{figure}

\begin{tabular}{|c|c|c|c|}
\hline
\textbf{MENTS} & \textbf{NAMES} & \textbf{ORIGIN} & \textbf{SCHC} \\
\hline
Terrabacter carboxydovorans & PY2 & FJ717334 & 86 \\
Terrabacter aerolatus & 5516T-36\textsuperscript{T} & EF212039 & 94 \\
Terrabacter ginsenosidimutans & Gsoil 3082\textsuperscript{T} & EU332827 & 96 \\
Terrabacter terrae & PPLB\textsuperscript{1} (AY944176) & 100 \\
Terrabacter tumescens & DSM 20308\textsuperscript{1} (X83812) & 77 \\
Terrabacter terrigena & ON10\textsuperscript{1} (FJ423552) & 86 \\
Humibacillus xanthopallidus & KV-663\textsuperscript{1} (AB282888) & 94 \\
\hline
janibacter limosus & DSM 11140\textsuperscript{1} (Y08539) & 100 \\
janibacter terrae & CS12\textsuperscript{1} (AF176948) & 77 \\
onrhithinibacter aureus & HB09001\textsuperscript{1} (FJ796074) & 77 \\
\textit{Phycicoccus jejuensis} & KSW2-15\textsuperscript{1} (DQ345443) & 71 \\
\textit{Tetrasphaera veronensis} & Ver1\textsuperscript{1} (Y14595) & 94 \\
\textit{Phycicoccus cremeus} & V2M29\textsuperscript{1} (FJ529696) & 83 \\
\textit{Phycicoccus ochangensis} & L1-b-b9 (GQ344405) & 94 \\
\textit{Phycicoccus ginsenosidimutans} & BXN5-13\textsuperscript{T} (EU332824) & 100 \\
\textit{Phycicoccus aerophilus} & 5516T-20\textsuperscript{T} (EF493847) & 96 \\
\textit{Phycicoccus soli} & THG-a14\textsuperscript{T} (KF999706) & 77 \\
\textit{Phycicoccus bigeumensis} & MSL03\textsuperscript{T} (EF466128) & 100 \\
\textit{Phycicoccus dokodensi} & DS-8\textsuperscript{1} (EF555583) & 96 \\
\textit{Phycicoccus badispori} & Sco-B23\textsuperscript{1} (FN386744) & 83 \\
\textit{Arsenicicoccus bolidensis} & CCUG 47306\textsuperscript{T} (AJ558133) & 96 \\
\hline
\end{tabular}
Cells of strain THG-a14T were Gram-stain-positive, aerobic, non-motile, coccus-shaped, oxidase- and catalase-positive. Colonies grown on R2A agar plates for 2 days were smooth, circular, white, convex and 0.5–1.0 mm in diameter. The isolate could hydrolyse urea, DNA, CM-cellulose, gelatin, ascusculin, casein, Tweens 20 and 80, starch and l-tyrosine, but could not hydrolyse chitin. Other physiological and biochemical characteristics of strain THG-a14T are summarized in the species description, and a comparison of selective characteristics of strain THG-a14T and related type strains is given in Table 1.

For determination of the DNA G+C content, genomic DNA of strain THG-a14T and its closest relative P. aerophilus KACC 20658T was extracted, purified by the method of Moore & Dowhan (1995) and degraded enzymically into nucleosides (nuclease P1 and alkaline phosphatase; Sigma). The nucleosides were analysed using a reversed-phase HPLC system (Alliance 2690 system; Waters) as described previously (Collins & Jones, 1981; Tamaoka et al., 1983; Hiraishi et al., 1996). Polar lipids of strain THG-a14T and the closest reference strain P. aerophilus KACC 20658T were extracted (Minnikin et al., 1977, 1984), examined by two-dimensional TLC using Kieselgel 60 TLC plates (10 × 10 cm; Merck) and identified as described previously (Collins & Jones, 1981). Chromatograms were developed in the first dimension with chloroform/methanol/water (65 : 25 : 4, by vol.) and in the second dimension with chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.) as solvent systems. Total lipids were detected by spraying the TLC plates with 5 % molybdatophosphoric acid followed by charring at 120 °C. Other lipids were detected by spraying with 0.2 % ninhydrin (aminolipids; Sigma), molybdenum blue reagent (phospholipid; Sigma) and 1-naphthol/sulfuric acid (glycolipids; Sigma) followed by charring at 120 °C for 5 min except for phospholipids, which were detected by charring at room temperature. Peptidoglycan and whole-cell-wall sugars of strain THG-a14T and P. aerophilus KACC 20658T were determined by using cellulose plates for TLC as described by Schleifer & Kandler (1972) and Staneck & Roberts (1974), respectively.

For cellular fatty acid analysis, strain THG-a14T and reference strains were grown on R2A agar plates for 3 days at 28 °C and biomass from the third quadrant for each strain was used for preparation. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). Fatty acids were analysed by gas chromatography (Hewlett Packard 6890) and using the Microbial Identification software package with the Sherlock system MIDI 6.1 and the Sherlock Aerobic Bacterial Database (TSBA 6.1) (Sasser, 1990).

The only respiratory quinone detected in strain THG-a14T was menaquinone MK-8(H4), in line with all other members tested on R2A agar supplemented as follows: casein (2 % skimmed milk; Oxoid), starch (1 % starch; Difco), aesculain [0.1 % aesculin and 0.02 % ferric citrate (Difco)], Tweens 20 and 80 [0.01 % CaCl2\, 2H2O and 1 % Tween 20 or Tween 80 (Sigma)], chitin (1 % chitin from crab shell; Sigma), l-tyrosine (0.5 % l-tyrosine; Sigma), CM-cellulose (0.1 % CM-cellulose; Sigma) and DNA (DNase agar; Scharlau) (DNase activity was revealed by flooding the plates with 1 M HCl). Tests were evaluated after 4 days of incubation at 28 °C. Carbon-source utilization, enzyme activities and other tests were conducted using API 20NE, ID 32 GN and API ZYM test kits according to the instructions of the manufacturer (bioMérieux). The API kits were incubated at 28 °C, and results were obtained after 24–38 h except for the API ZYM kit, results of which were recorded after 10 h.
of the family Intrasporangiaceae. Strain THG-a14\textsuperscript{T} contained meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. Whole-cell sugars found in strain THG-a14\textsuperscript{T} were glucose and ribose, and major fatty acids detected were iso-C\textsubscript{16:0} (17.2\%), iso-C\textsubscript{15:0} (16.6\%) and C\textsubscript{17:1}\textsubscript{\textit{\alpha}} (14.5\%). Results of carbon assimilation tests (API 20 NE and API 32 GN) and enzyme activity tests (API ZYM) are listed in Table 1. The fatty acid profiles of this strain and the type strains of related species of the genus Phycicoccus are shown in Table S1, and the polar lipid profiles of strain THG-a14\textsuperscript{T} and the closest reference strain \textit{P. aerophilus} KACC 20658\textsuperscript{T} are shown in Fig. S4.

In conclusion, the characteristics of strain THG-a14\textsuperscript{T} were consistent with the description of the genus Phycicoccus with regard to morphological, biochemical and chemotaxonomic properties. On the basis of the phylogenetic distance between strain THG-a14\textsuperscript{T} and recognized species of the genus \textit{Phycicoccus} indicated by 16S rRNA gene sequence comparisons, peptidoglycan type, quinone system, polar lipid profile and the combination of unique phenotypic characteristics, it is demonstrable that strain THG-a14\textsuperscript{T} should be assigned to the genus \textit{Phycicoccus} as a representative of a novel species, for which the name \textit{Phycicoccus soli} sp. nov. is proposed.

Table 1. Differential characteristics of strain THG-a14\textsuperscript{T} and related type strains

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>White</td>
<td>White</td>
<td>Yellow</td>
<td>White</td>
<td>Yellow</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NaCl range for growth (% w/v)</td>
<td>0–4.5</td>
<td>0–7.0</td>
<td>0–5.0</td>
<td>0.5–8.0</td>
<td>0–0.5</td>
</tr>
<tr>
<td>Indole reduction</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td>-</td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>-</td>
<td>W</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>+</td>
<td>w</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>w</td>
<td>w</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salcin</td>
<td>w</td>
<td>w</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Hydroxybutyrate</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Malate</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Itaconate</td>
<td>+</td>
<td>-</td>
<td>w</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>w</td>
<td>w</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>w</td>
<td>w</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-Serine</td>
<td>-</td>
<td>w</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>71.6</td>
<td>71.2</td>
<td>72.0\textsuperscript{a}</td>
<td>73.6\textsuperscript{b}</td>
<td>73.4\textsuperscript{c}</td>
</tr>
<tr>
<td>Cell-wall sugars\textsuperscript{f}</td>
<td>Glc, Rib</td>
<td>Glc, Rib</td>
<td>ND</td>
<td>Glc, Rib\textsuperscript{b}</td>
<td>ND</td>
</tr>
</tbody>
</table>

\*Data from: \textsuperscript{a} Wang \textit{et al.} (2011); \textsuperscript{b} Kim \textit{et al.} (2012a); \textsuperscript{c} Dastager \textit{et al.} (2008).

\textsuperscript{f}Glc, Glucose; Rib, ribose.
**Description of Phycicoccus soli sp. nov.**

*Phycicoccus soli* (so’li, L. gen. n. soli of soil, the source of the type strain).

Cells are Gram-stain-positive, catalase- and oxidase-positive, aerobic, non-motile cocci, 0.5–1.0 μm in diameter. Colonies on R2A agar plates after 3 days of incubation at 28°C are smooth, circular, white and convex. Growth occurs in 0–4.5% (w/v) NaCl at 10–35°C and at pH 5.5–8.5. Optimum growth occurs in 0–3% (w/v) NaCl, at 28°C and at pH 6.5–7.5. Growth occurs on R2A agar and TSA but not on MacConkey agar. Can also grow slowly on NA. Positive for indole production and nitrate reduction. Hydrolyses urea, DNA, casein, aesculin, CM-cellulose, starch, gelatin, L-tyrosine and TWEENS 20 and 80, but not chitin. Positive (in API 20 NE and API ID 32GN strips) for assimilation of D-glucose, maltose, malate, gluconate, melibiose, L-fucose, D-sorbitol, L-rhamnose, N-acetylglucosamine, sucrose, L-arabinose, L-histidine, 3-hydroxybutyrate, D-ribose, inositol, itaconate and glycerogen. Weakly positive results are obtained for D-mannose, salicin, sodium acetate and L-alanine and negative results are obtained for assimilation of D-mannitol, L-serine, L-proline, 4-hydroxybenzoate, propionate, trisodium citrate, caprate, valerate, 2-ketogluconate, 5-ketogluconate, suberate, sodium malonate, lactate, 3-hydroxybenzoate, adipate and phenylacetate, acidification of glucose and activity of arginine dihydrolase. According to API ZYM tests, for the following enzyme activities are positive: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, cystine arylamidase, valine arylamidase, z-mannosidase, z-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, z-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase and β-glucosidase. Negative for the following activities in API ZYM tests: z-fucosidase, trypsin and N-acetyl-β-glucosaminidase. The predominant quinone is menaquinone MK-8(H4), and iso-C16:0, iso-C15:0 and C17:0 3OH are the major cellular fatty acids. The polar lipids include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphoaminoethylglycocolipids, unidentified phospholipids and unidentified lipids. Among them, the major polar lipids are diphosphatidylglycerol, phosphatidylinositol and unidentified phospholipid PL1. The cell-wall peptidoglycan contains meso-diaminopimelic acid as the diamino acid. The whole-cell-wall sugars are glucose and ribose.

The type strain, THG-α14T (≡KACC 17892T=JCM 19837T), was isolated from soil from Gyeyang mountain, Incheon, Republic of Korea. The genomic DNA G+C content of the type strain is 71.6 mol%.

**Acknowledgements**

This work was funded by the Ministry of Trade, Industry & Energy (MOTIE, Korea) under the industrial infrastructure program for fundamental technologies (no. N0000888).

**References**


