**Rudaeicoccus suwonensis** gen. nov., sp. nov., an actinobacterium isolated from the epidermal tissue of a root of a *Phalaenopsis* orchid


1Korean Agricultural Culture Collection (KACC), National Academy of Agricultural Science, Rural Development Administration, Suwon, Republic of Korea
2DSMZ – German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, D-38124 Braunschweig, Germany
3Department of Molecular Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea

A bacterial strain, designated HOR6-4^T, was isolated from the epidermal tissue of a root of a *Phalaenopsis* orchid. The root was surface-sterilized with 6% sodium hypochloride, washed with sterile water and then homogenized with a homogenizer. The DNA G+C content of strain HOR6-4^T was 64.7 mol%. Strain HOR6-4^T had anteiso-C17:0 (19.3%) and 10-methyl C18:0 (tuberculostearic acid) (13.5%) and 10-methyl C17:0 (11.7%) as the major fatty acids and contained MK-8(H4) and MK-8(H6) as the predominant quinones. The peptidoglycan type was A4α, with an L-Lys–L-Thr–D-Glu interpeptide bridge with a glycine residue bound to the alpha-carboxyl group of D-Glu in position 2 of the peptide subunit.

The family *Dermacoccaceae* was first proposed by Stackebrandt & Schumann (2000), and its description was subsequently emended by Zhi et al. (2009) and Ruckmani et al. (2011). The family includes the genera *Branchiibius* (Sugimoto et al., 2011), *Calidifontibacter* (Ruckmani et al., 2011), *Demetria* (Groth et al., 1997), *Dermacoccus* (Stackebrandt et al., 1995), *Flexivirga* (Anzai et al., 2011), *Kytococcus* (Stackebrandt et al., 1995), *Luteipulveratus* (Ara et al., 2010) and *Yimella* (Tang et al., 2010). The members of the family *Dermacoccaceae* are characterized as Gram-positive-staining short rods or cocci that do not form endospores. Diverse menaquinones are present, such as MK-7(H2), MK-8(H2), MK-8(H4), MK-8(H6), MK-9(H2), MK-7, MK-8, MK-9 and MK-10. The major fatty acids include iso-C16:0, iso-C16:0 H and anteiso-C17:0. The peptidoglycan structure is of the A4z type with lysine as the diagnostic diamino acid. The common polar lipids are diphasphatidylglycerol (DPG), phosphatidylglycerol and phosphatidylglycerol (PG); phosphatidylglycerol mannoside, phosphatidylserine, a glucosamine-containing phospholipid and other unknown polar lipids are also present. Members of the family have been isolated from branchia of Japanese codling, fresh water, hot springs, sea water, deep-sea sediment, human blood, indoor air, soil and agar plates in the laboratory.

A bacterial strain, HOR6-4^T, was isolated from epidermal tissue of a root of a *Phalaenopsis* orchid. The root was surface-sterilized with 6% sodium hypochloride, washed with sterile water and then homogenized with a homogenizer AM-7 (Nihonseiki Kashima Ltd). The homogenized...
tissue was serially diluted with 0.85 % NaCl (w/w) and spread on R2A medium (Difco). Strain HOR6-4T was obtained after incubation of 10 days at 28 °C and is described in this study as a novel member of the family Dermacoccaceae.

Genomic DNA was isolated by the method of Ausubel et al. (1987), except that the lysates were extracted twice with chloroform to remove residual phenol. The 16S rRNA gene was amplified by using universal primers F1D and rP2 (Weisburg et al., 1991) and sequenced as described by Weon et al. (2005). Sequence alignment and analysis of the data were performed using the ARB software package (Ludwig et al., 2004) and the corresponding SILVA SSURef 100 database (release April 2011; Pruesse et al., 2007). Aligned nucleotide positions without filtering were used for tree reconstruction in MEGA version 4.0 (Tamura et al., 2007). Dendrograms were calculated using the neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Bootstrap values were based on 1000 replicates (Felsenstein, 1985). Maximum-likelihood analysis was performed with RAxML 7.0.4 using the GTR + Γ model (Stamatakis et al., 2005), and support for the maximum-likelihood tree was obtained by bootstrapping (1000 replicates) using CONSENSE in PHYLIP. To determine the closest phylogenetic neighbours of strain HOR6-4T, a continuous stretch (1463 bp) of its 16S rRNA gene sequence was obtained using the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). Strain HOR6-4T showed relatively low 16S rRNA gene sequence similarity (below 95.9 %) to type strains of species with validly published names. It showed the highest sequence similarities to the type strains of Flexivirga alba (95.8 %) and Yimella lutea (95.5 %). The neighbour-joining and maximum-parsimony trees clearly indicated that strain HOR6-4T was a member of the family Dermacoccaceae, and formed a cluster with Flexivirga alba (Fig. 1). The maximum-likelihood tree also showed strain HOR6-4T as a member of the family Dermacoccaceae, forming an independent clade (Fig. S1, available in IJSEM Online).

Cell morphology and motility were examined by means of light microscopy and transmission electron microscopy (LEO model 912AB) after 2 days of incubation at 28 °C on R2A. Growth was determined in R2A broth containing 0, 1, 3, 5, 7 and 10 % (w/v) NaCl. The pH range (pH 3.0–10.0 at intervals of 1.0 pH units) for growth was determined in R2A broth that was buffered with citrate/phosphate or Tris/HCl buffer (Breznak & Costilow, 1994). The temperature range for growth was checked at 4, 10, 15, 20, 25, 28, 30, 37 and 40 °C on R2A agar medium. Catalase activity was examined by bubble production in 3 % (v/v) hydrogen peroxide solution and oxidase activity was tested with 1 % (w/v) tetramethyl-p-phenylenediamine (bioMérieux). Casein, starch and tyrosine degradation was examined on R2A plates containing milk powder (5 %, w/v), starch (1 %, w/v) or tyrosine (0.1 %, w/v), respectively. CM-cellulose and Tween 80 hydrolysis was examined using R2A supplemented with 1 % (w/v) substrate. DNase activity was determined with DNase test agar (Difco). Anaerobic growth was investigated using incubation in the BBL GasPak anaerobic system (Difco) for 14 days at 28 °C on R2A agar containing 0.5 % NaSO₄, 0.5 % NaNO₃, 0.5 % NaHCO₃ or 0.02 % FeCl₃. Enzyme activities and other physiological and biochemical properties were determined by using API ZYM, API 20NE, API ID 32GN and API 50 CH test strips (bioMérieux) at 28 °C according to the manufacturer’s instructions.

Cells of strain HOR6-4T were aerobic, Gram-stain-positive cocci (Fig. S2), 0.6–0.8 μm in diameter. They did not form endospores. Strain HOR6-4T grew on R2A, Luria–Bertani (LB; Difco) agar, nutrient agar (NA; Difco) and trypticase soy agar (TSA; Difco), but not on MacConkey agar (Difco). Other physiological properties are given in the genus and species descriptions and in Table 1.

Chemotaxonomic studies were conducted with freeze-dried cells after cultivation in R2A medium for 2 days at 28 °C. Isoprenoid quinones were analysed by HPLC as described by Groth et al. (1996). Peptidoglycan preparations purified according to the method of Schleifer (1985) were obtained after disruption of cells by shaking with glass beads and subsequent trypsin digestion. The amino acids and peptidoglycans in cell-wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates by using previously described solvent systems (Schleifer, 1985). The molar ratio of amino acids was determined by GC and GC/MS of N-heptafluoroxyuranyl acid amino isobutyyl esters (MacKenzie, 1987; Groth et al., 1996). Polar lipids were
Table 1. Differential characteristics of strain HOR6-4\textsuperscript{T} and closely related genera of the family Dermacoccaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source(s)</td>
<td>Orchid root</td>
<td>Fish branchia</td>
<td>Warm spring</td>
<td>Compost soil</td>
<td>Deep-sea sediment</td>
<td>Soil</td>
<td>Air, human blood</td>
<td>Soil</td>
<td>Contaminated agar plate</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>Coccoid</td>
<td>Coccoid</td>
<td>Short rod</td>
<td>Coccoid or short rod</td>
<td>Coccoid</td>
<td>Coccoid to comma-shaped</td>
<td>Coccoid</td>
<td>Coccoid</td>
<td></td>
</tr>
<tr>
<td>Colony pigmentation</td>
<td>White</td>
<td>Pale yellow</td>
<td>Creamish white to pale yellow</td>
<td>Orange, yellow</td>
<td>White</td>
<td>Yellow, white</td>
<td>Orange</td>
<td>Yellow, white</td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>V</td>
<td>V</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cell-wall sugar(s)†</td>
<td>Glc, Rib</td>
<td>Ara, Gal, GlcN, Man, Rha</td>
<td>ND</td>
<td>Gal</td>
<td>Gal</td>
<td>Gal, Rib, GlcN</td>
<td>ND</td>
<td>GlcN, Man, Rha, Rib</td>
<td>Gal, Fuc</td>
</tr>
<tr>
<td>Major menaquinone(s)</td>
<td>8(H\textsubscript{4}), 8(H\textsubscript{4})</td>
<td>8(H\textsubscript{4}), 8(H\textsubscript{4})</td>
<td>8(H\textsubscript{4})</td>
<td>8(H\textsubscript{4})</td>
<td>7(H\textsubscript{2}), 8(H\textsubscript{2}), 9(H\textsubscript{2})</td>
<td>8(H\textsubscript{4}), 8(H\textsubscript{4})</td>
<td>7, 8, 9, 10</td>
<td>8(H\textsubscript{4}), 8(H\textsubscript{4})</td>
<td>8(H\textsubscript{4})</td>
</tr>
<tr>
<td>Polar lipid(s)‡</td>
<td>DPG, PI, APL, PL, AL</td>
<td>PI, PG, DPG, PL</td>
<td>PI, PG, DPG, PI, PL</td>
<td>PI, PG, DPG, PI, PL</td>
<td>DPG, PI, PS, PIM, PL</td>
<td>DPG, PI, PS, PIM, PL</td>
<td>DPG, PI</td>
<td>DPG, PI, AL</td>
<td>DPG, PI, AL</td>
</tr>
<tr>
<td>Major fatty acid(s) (&gt;10 %)§</td>
<td>ai-C\textsubscript{17}:0, C\textsubscript{18}:0</td>
<td>ai-C\textsubscript{17}:0, C\textsubscript{18}:0</td>
<td>i-C\textsubscript{16}:0, i-C\textsubscript{16}:1, H\textsuperscript{a}</td>
<td>C\textsubscript{18}:0, C\textsubscript{18}:0</td>
<td>i-C\textsubscript{16}:0, i-C\textsubscript{16}:1, H\textsuperscript{a}</td>
<td>i-C\textsubscript{16}:0, i-C\textsubscript{16}:1, H\textsuperscript{a}</td>
<td>i-C\textsubscript{16}:0, ai-C\textsubscript{17}:0, ai-C\textsubscript{17}:0</td>
<td>i-C\textsubscript{16}:0, ai-C\textsubscript{17}:0</td>
<td>i-C\textsubscript{16}:0, ai-C\textsubscript{17}:0</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>65</td>
<td>68</td>
<td>77</td>
<td>66</td>
<td>67.4</td>
<td>66–69</td>
<td>68.2</td>
<td>65.8</td>
<td></td>
</tr>
</tbody>
</table>

*Data from: a, Pathom-aree et al. (2006); b, Kämpfer et al. (2009); c, Ruckmani et al. (2011).
†Ara, Arabinose; Fuc, fucose; Gal, galactose; Glc, glucose; GlcN, glucosamine; Man, mannose; Rha, rhamnose; Rib, ribose.
‡DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidyglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PS, phosphatidylerine; AL, unknown aminolipid; APL, unknown aminophospholipid; GL, unknown glycolipid; GlcN-PL, glucosamine-containing phospholipid; PL, unknown phospholipid; L, unknown lipid.
§ai, Anteiso-branched; i, iso-branched; Me, methyl-branched.

| Genera: | 1, Rudaeicoccus gen. nov. (strain HOR6-4\textsuperscript{T}); 2, Branchiibius (unless indicated otherwise, data from Sugimoto et al., 2011); 3, Calidifontibacter (Ruckmani et al., 2011); 4, Demetria (Groth et al., 1997); 5, Dermacoccus (Stackebrandt et al., 1995; Pathom-aree et al., 2006); 6, Flexivirga (Anzai et al., 2011); 7, Kytooccus (Stackebrandt et al., 1995; Becker et al., 2002; Kämpfer et al., 2009); 8, Lateipulveratus (Ara et al., 2010); 9, Yimella (Tang et al., 2010); +, Positive; V, variable; −, negative; ND, no data available.
extracted and then examined by two-dimensional TLC (Minnikin et al., 1984). Sugar analysis of whole cells was carried out as described by Staneck & Roberts (1974). Mycolic acids were extracted and analysed as described by Minnikin et al. (1980). Cellular fatty acid methyl esters were prepared from cells grown in R2A medium for 2 days at 28 °C and were analysed by GC according to the instructions of the Microbial Identification System (MIDI). Fatty acid methyl esters were identified and quantified by using the TSBA 6 database (version 6.10) of the Sherlock Microbial Identification System (MIDI). The G+C content of the DNA was determined as described by Mesbah et al. (1989) by using a reversed-phase column (Supelcosil LC-18S; Supelco). Strain HOR6-4T contained MK-8(H4) (66 %) and MK-8(H6) (31 %) as the predominant quinones. The polar lipids were DPG, PI, three unknown aminophospholipids, two unknown phospholipids and an unknown aminolipid (Fig. S3). The peptidoglycan of strain HOR6-4T contained alanine, glycine, threonine, glutamic acid and lysine in the molar ratio of 1.7:1.1:0.9:2.2:1.0. The partial hydrolysate contained the following additional peptides: l-Ala–d-Glu, Gly–Glu and d-Ala–l-Lys–Thr. From these data, strain HOR6-4T was concluded to have the peptidoglycan type A4z, with an l-Lys–l-Thr–d-Glu interpeptide bridge with a glycine residue bound to the alpha-carboxyl group of d-Glu in position 2 of the peptidoglycan subunit. Whole-cell sugars were glucose and ribose. Mycolic acids were not present. The fatty acids of strain HOR6-4T were very complex, including anteiso-C17:0 (19.3 %), 10-methyl C18:0 (tuberculostearic acid; 13.5 %), 10-methyl C17:0 (11.7 %), iso-C16:0 (8.9 %), iso-C17:0 9c (8.9 %), iso-C17:0 7c (6.4 %), anteiso-C17:0 9c (4.6 %), C18:1 9c (4.6 %), C17:1 8c (4.0 %), iso-C15:0 (3.0 %), C16:1 2-0H (3.0 %), summed feature 6 (C19:1 11c and/or C19:1 9c) (2.0 %), summed feature 3 (iso-C15:0 2-0H and/or C16:0 7c) (1.6 %), C17:0 (1.5 %), C16:0 (1.3 %), C17:0 9c (1.1 %) and 10-methyl C19:0 (1.0 %) and other fatty acids in trace amounts (<1.0 %) such as 10-methyl iso-C16:1 H, anteiso-C15:0, C17:0 2-0H, iso-C18:0, iso-C19:0, C15:1 0c6c, anteiso-C16:0 and C19:0. The DNA G+C content was 65 mol%.

Phylogenetically, strain HOR6-4T was the member of the family Dermacoccaceae, forming a cluster with Flexivirga alba (Fig. 1). Strain HOR6-4T had lysine as the diagnostic diamino acid in the peptidoglycan structure, which also differentiates members of the family Dermacoccaceae from members of the closely related families Dermabacteraceae and Dermatophilaceae. In comparison with other genera of the family Dermacoccaceae, strain HOR6-4T can be differentiated from Flexivirga alba on the basis of catalase, nitrate reduction, the amino acid composition of the peptidoglycan and fatty acid composition. Strain HOR6-4T differs from members of the type genus of the family Dermacoccaceae, Dermacoccus, in colony colour, interpeptide bridge and amino acid composition of the peptidoglycan, menaquinone content, polar lipid pattern and fatty acid composition. Strain HOR6-4T can be differentiated from other members of the family Dermacoccaceae, particularly on the basis of chemotaxonomic properties (Table 1). Therefore, on the basis of chemotaxonomic and phylogenetic differentiation of the isolate from its closest neighbours in the family Dermacoccaceae, we propose that strain HOR6-4T represents a novel species in a new genus, Rudaeicoccus suwonensis gen. nov., sp. nov.

Description of Rudaeicoccus gen. nov.

Rudaeicoccus [Ru.da.ei.co.c’cus. N.L. fem. n. Rudaea arbitrary name, derived from the abbreviation RuDA (Rural Development Administration); N.L. masc. n. coccs (from Gr. n. kokkos a grain or berry) a coccus; N.L. masc. n. Rudaeicoccus a coccus named in honour of the Rural Development Administration].

Cells are aerobic, Gram-stain-positive coccoids. They do not form endospores. The peptidoglycan is of the type A4z, with an l-Lys–l-Thr–d-Glu interpeptide bridge with a glycine residue bound to the alpha-carboxyl group of d-Glu in position 2 of the peptide subunit. The predominant menaquinones are MK-8(H4) and MK-8(H6). The polar lipids are DPG, PI, three unknown aminophospholipids, two unknown phospholipids and an unknown aminolipid. Whole-cell sugars are glucose and ribose. Mycolic acids are not present. The major fatty acids are anteiso-C17:0, 10-methyl C18:0 and 10-methyl C17:0. Phylogenetically, the genus belongs to the family Dermacoccaceae, suborder Micrococcineae, order Actinomycetales. The type species is Rudaeicoccus suwonensis.

Description of Rudaeicoccus suwonensis sp. nov.

Rudaeicoccus suwonensis (su.wo.nen’sis. N.L. masc. adj. suwonensis referring to the Suwon region, Republic of Korea, where the type strain was found).

The following properties are observed in addition to those given in the genus description. Cells are 0.6–0.8 μm in diameter. Grows on R2A, LB agar, NA and TSA, but not on MacConkey agar. Colonies are white, regular, convex and round after 3 days on R2A. Growth is observed at 10–37 °C (optimum 28 °C) and at pH 4–9 (optimum pH 6–7). Does not require NaCl for growth and can tolerate up to 5 % NaCl (optimum 0–1 % NaCl). Hydrolyses casein, tyrosine and Tween 80, but not CM-cellulose, DNA or starch. Does not reduce nitrate to nitrite. Positive for urease, aesculin hydrolysis, gelatin hydrolysis and β-galactosidase (PNG), but negative for indole production, glucose fermentation and arginine dihydrolase (API 20NE test strip). Assimilates D-glucose, D-mannose, N-acetylglucosamine, maltose, D-ribose, sucrose, sodium acetate, l-alanine, l-serine, salicin, melibiose, propionic acid, valeric acid, L-histidine, 3-hydroxybutyric acid and L-proline, but not L-arabinose, 3-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-fucose,
D-sorbitol or 4-hydroxybenzoic acid (API 20NE and API 32GN test strips). Produces acids from D-glucose, D-mannose and aesculin ferric citrate and produces acids weakly from L-arabinose, D-ribose, D-galactose, D-fructose, L-sorbose, sucrose and turanose, but not from glycerol, erythritol, D-arabinose, D- or L-xyllose, D-adenitol, methyl β-D-xlylopyranoside, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglicosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, trehalose, inulin, melezitose, raffinose, starch, glycerogen, xyitol, gentiobiose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate (API 50CH). Positive for activities of esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase and β-glucosidase, but negative for activities of alkaline phosphatase, lipase (C14), trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-gluconidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM). The G+C content of the DNA is about 65 mol%.

The strain type is HOR6-4T (=KACC 12637T =DSM 19560T), isolated from epidermal tissue of a root of a Phalaenopsis orchid.

Acknowledgements

This study was carried out with the support of the National Academy of Agricultural Science, Rural Development Administration, Republic of Korea (project no. PJ008666). The authors thank Dr J. P. Ezébyé of the École Nationale Vétérinaire in Toulouse for advice concerning the naming of the novel taxon.

References


