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


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A bacterial strain, designated HOR6-4T, was isolated from the epidermal tissue of a root of a Phalaenopsis orchid. Strain HOR6-4T was characterized using a polyphasic approach. The cells were aerobic, Gram-stain-positive, non-motile coccoids. Phylogenetic analysis of its 16S rRNA gene sequence revealed a clear affiliation with the family Dermacoccaceae. Strain HOR6-4T showed relatively low 16S rRNA gene sequence similarity (below 95.9 %) to type strains of species with validly published names, showing the highest sequence similarities to the type strains of Flexivirga alba (95.8 %) and Yimella lutea (95.5 %). The DNA G+C content of strain HOR6-4T was 64.7 mol%. Strain HOR6-4T had anteiso-C17 : 0 (19.3 %), 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. Polar lipids were diphasphatidylglycerol, phosphatidylglycerol, three aminophospholipids, two phospholipids and an aminolipid. The peptidoglycan type was A4a, 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. Mycolic acids were not present. On the basis of comparative analysis of phenotypic and phylogenetic traits, strain HOR6-4T is considered to represent a novel species in a new genus in the family Dermacoccaceae, for which the name Rudaeicoccus suwonensis gen. nov., sp. nov. is proposed. The type strain of Rudaeicoccus suwonensis is HOR6-4T (=KACC 12637T = DSM 19560T).

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-C17:0 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 phosphatidylinositol (PI); phosphatidylinositol 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, HOR6-4T, 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/v) 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 fD1 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). Distances were calculated using distance options from Kimura’s two-parameter model (Kimura, 1983) and clustering was performed 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 model (Felsenstein, 1985) and maximum-parsimony (Fitch, 1971) methods. Bootstrap values were based on 1000 replicates (Felsenstein, 1985). 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 analysed 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 % Na₂SO₄, 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 coccoïds (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 freezedried 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 peptides 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-heptfluorobutyryl amino acid isobutyl esters (MacKenzie, 1987; Groth et al., 1996). Polar lipids were
**Table 1. Differential characteristics of strain HOR6-4^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</td>
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<td></td>
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<td></td>
<td></td>
<td>plate</td>
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<tr>
<td>Cell morphology</td>
<td>Coccoid</td>
<td>Coccoid</td>
<td>Short rod</td>
<td>Coccoid or short</td>
<td>Coccoid</td>
<td>Coccoid to</td>
<td>Coccoid to short</td>
<td>Coccoid</td>
<td>Coccoid</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rod</td>
<td></td>
<td>comma-shaped</td>
<td>rod</td>
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<td></td>
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<tr>
<td>Colony pigmentation</td>
<td>White</td>
<td>Pale yellow</td>
<td>Creamish white</td>
<td>White to pale</td>
<td>Orange, yellow</td>
<td>White</td>
<td>Yellow, white</td>
<td>Cream to bright</td>
<td>Orange</td>
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<td>Oxidase</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Temperature for growth (°C)</td>
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<tr>
<td>Major menaquinone(s)†</td>
<td>8(H4), 8(H4)</td>
<td>8(H4), 8(H4)</td>
<td>8(H4), 8(H4)</td>
<td>8(H4), 8(H4)</td>
<td>8(H4), 8(H4)</td>
<td>8(H4), 8(H4)</td>
<td>8(H4), 8(H4)</td>
<td>7, 8, 9, 10</td>
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</tr>
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<td>Polar lipid(s)†</td>
<td>DPG, PI, APL, PL,</td>
<td>PI, PG, DPG, PL,</td>
<td>PI, PG, DPG, PL,</td>
<td>PI, PG, DPG, PL,</td>
<td>PI, PG, DPG, PL,</td>
<td>DPG, PG, PI</td>
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<td></td>
<td>AL</td>
<td>PL</td>
<td>PL</td>
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<td>PL</td>
<td>PE, PL</td>
<td>PG</td>
<td>PE, PL</td>
<td>PL</td>
</tr>
<tr>
<td>Major fatty acid(s) (&gt;10 %)‡</td>
<td>i-C17:0, C17:1</td>
<td>i-C16:0, C17:0</td>
<td>i-C16:0, C17:0</td>
<td>i-C16:0, C17:0</td>
<td>i-C16:0, C17:0</td>
<td>C17:0</td>
<td>i-C15:0</td>
<td>C15:0</td>
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<tr>
<td></td>
<td>(10–Me)</td>
<td>(10–Me)</td>
<td>(10–Me)</td>
<td>(10–Me)</td>
<td>(10–Me)</td>
<td>(10–Me)</td>
<td>(10–Me)</td>
<td>(10–Me)</td>
<td>(10–Me)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>65</td>
<td>68</td>
<td>77</td>
<td>66</td>
<td>66</td>
<td>66</td>
<td>66–69</td>
<td>68.2</td>
<td>65.8</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, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PS, phosphatidylserine; 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.
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 peptide 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 (tubercolostearic
acid; 13.5 %), 10-methyl C17:0 (11.7 %), iso-C16:0 (8.9 %),
iso-C17:0:9c (8.9 %), iso-C17:1:6c (6.4 %), anteiso-C17:1:9c
(4.6 %), C18:1:9c (4.6 %), C17:1:8c (4.0 %), iso-C15:0
(3.0 %), C16:1 2-OH (3.0 %), summed feature 6
(C19:1ω11c and/or C19:1ω9c) (2.0 %), summed feature 3
(iso-C15:0 2-OH and/or C16:1:9c) (1.6 %), C17:0 (1.5 %),
C16:0 (1.3 %), C17:1:9c (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-OH,
isooC18:0, iso-C19:0, C15:1:9c, 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, interpep-
tide bridge and amino acid composition of the peptido-
glycan, 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.e.i.coc’cus. N.L. fem. n. Rudae a
arbitrary name, derived from the abbreviation RuDA
(Rural Development Administration); N.L. masc. n. coccus
(from Gr. n. kokkos a grain or berry) 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
menaquinins 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, order
Micrococcinae, suborder Micrococcales. 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-
rhamnose, sucrose, sodium acetate, L-alanine, L-serine, salicin,
melibiose, propionic acid, valeric acid, L-histidine, 3-
hydroxybutyric acid and 1-proline, but not L-arabinose,
D-mannitol, potassium glutonate, capric acid, adipic acid,
malic acid, trisodium citrate, phenylacetic acid, D-
rhamnose, inositol, itaconic acid, suberic acid, sodium mal-
onate, lactic acid, potassium 5-ketoglunonate, potassium 2-
ketoacetic acid, glycerol, 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-xylene, D-adonitol, methyl β-D-xylopyranoside, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, trehalose, inulin, melezitose, raffinose, starch, glycerogen, xylitol, 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, χ-galactosidase, β-galactosidase and z-glucosidase, but negative for activities of alkaline phosphatase, lipase (C14), trypsin, χ-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, χ-mannosidase and z-fucosidase (API ZYM). The G+C content of the DNA is about 65 mol%.

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

Acknowledgements

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References


