Frondihabitans peucedani sp. nov., an actinobacterium isolated from rhizosphere soil, and emended description of the genus *Frondihabitans* Greene *et al.* 2009

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A novel actinobacterium, designated RS-15^T, was isolated from rhizosphere soil of a plant (*Peucedanum japonicum* Thunb.) on Mara Island, Jeju, Republic of Korea. Cells were strictly aerobic, Gram-stain-positive, oxidase-negative, catalase-positive, non-motile, irregular, short rods. The peptidoglycan in the cell wall was of the B2 β type with ornithine as the diagnostic diamino acid. The major menaquinone was MK-8; small amounts of MK-9 and MK-7 were also present. The polar lipids contained phosphatidylglycerol, five unknown glycolipids, three unknown phospholipids and two unknown lipids. Mycolic acids were not present. The predominant fatty acids were summed feature 7 (one or more of C_{18:1} ω 9c, C_{18:1} ω 12t and C_{18:1} ω 7c), anteiso-C_{15:0} and C_{14:0} 2-OH. The DNA G+C content was 68.3 mol%. 16S rRNA gene sequence analysis showed that strain RS-15^T belonged to the family *Microbacteriaceae* and formed a tight phylogenetic cluster with *Frondihabitans australicus* E1HC-02^T (98.5 % sequence similarity). The DNA–DNA relatedness between strain RS-15^T and *Frondihabitans australicus* DSM 17894^T was 17.2 % (17.1 %, duplicate measurements). On the basis of the physiological, chemotaxonomic and DNA–DNA hybridization data, strain RS-15^T is considered to represent a novel species of the genus *Frondihabitans*, for which the name *Frondihabitans peucedani* sp. nov. is proposed. The type strain is RS-15^T (=KCTC 13435^T =DSM 22180^T). An emended description of the genus *Frondihabitans* is also presented.

The genus *Frondihabitans* was recently established in the family *Microbacteriaceae* by Greene *et al.* (2009) to replace the illegitimate name '*Frondicola*' (Zhang *et al.*, 2007). The type strain of the only known species, *Frondihabitans australicus*, was isolated from decaying leaf litter of a pine forest. Members of the genus *Frondihabitans* possess a type B2 β peptidoglycan with ornithine as the diagnostic diamino acid, fatty acids C_{18:1} ω 7c and C_{14:0} 2-OH, menaquinones MK-8 and MK-7 and polar lipid profiles that include several unknown glycolipids. The genus is phylogenetically related to the genus *Frigoribacterium* of the family *Microbacteriaceae*. In this paper, I describe the isolation and classification of a *Frondihabitans* strain from rhizosphere soil of a plant (*Peucedanum japonicum* Thunb.).

During a culture-dependent study on rhizosphere bacteria, strain RS-15^T was isolated from rhizosphere soil of a cliff-associated plant (*Peucedanum japonicum* Thunb.) on Mara

Island, Jeju, Republic of Korea. The procedure and medium for bacterial isolation were described previously (Lee, 2009). The isolate was maintained on International *Streptomyces* Project (ISP) 2 medium (Shirling & Gottlieb, 1966) and as 20 % (v/v) glycerol suspensions at –20 and –80 °C. For phenotypic comparison and DNA–DNA hybridization, *Frondihabitans australicus* DSM 17894^T was grown on trypticase soy agar (TSA; Difco).

Cell morphology and motility were observed by using phase-contrast and transmission electron microscopy. Colony morphology and pigmentation were observed with cells grown on ISP 2 for 3 days at 30 °C. Growth was examined on ISP 2 at 4–42 °C and at pH 4.1–12.1, at intervals of 1 pH unit, adjusted with 6 M HCl or 10 M NaOH before sterilization. NaCl tolerance for growth was tested on ISP 2 with 1–9 % (w/v) NaCl, at intervals of 1 %. Gram staining and catalase and oxidase activities were tested as described previously (Lee, 2007). Decomposition of hypoxanthine, DL-tyrosine and xanthine was tested according to the method of Gordon *et al.* (1974). Hydrolysis of casein and cellulose was determined on ISP 2 medium amended with 1 % (w/v) skimmed milk and 0.5 % (w/v) cellulose, respectively. Hydrolysis of starch and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RS-15^T is FM998017.

A comparison of fatty acid profiles is available as supplementary material with the online version of this paper.

DNA was determined by using starch agar (Difco) and DNase test agar (Difco), respectively. Utilization of substrates was tested using GN2 MicroPlates (Biolog) for 95 substrates, inoculated with cells of strain RS-15^T grown on ISP 2 for 3 days at 30 °C and suspended in sterilized distilled water, following the manufacturer's instructions, and incubated for 48 h at 30 °C. Other physiological and biochemical properties were determined using API 20NE and API ZYM strips (bioMérieux), following the instructions of the manufacturer.

Strain RS-15^T grew well on ISP 2, TSA and nutrient agar (Difco), with extensive production of extracellular polysaccharides on ISP 2. Cells of strain RS-15^T were Gram-stain-positive, aerobic, non-motile, irregular, short rods (0.3–0.5 × 0.9–1.0 µm). Colonies were circular, smooth, convex and bright yellow with entire margins after incubation on ISP 2 for 3 days at 30 °C. The results for other physiological and biochemical properties are given in Table 1 and the species description.

Table 1. Differential characteristics between strain RS-15^T and *Frontrahabitsans australicus* E1HC-02^T

Strains: 1, *Frontrahabitsans peucedani* sp. nov. RS-15^T; 2, *Frontrahabitsans australicus* E1HC-02^T (unless indicated, data from Zhang *et al.*, 2007). +, Positive; w, weak; –, negative.

Characteristic	1	2
Cell size (µm)	0.9–1.0 × 0.3–0.5	0.5–1.0 × 0.2–0.4
Temperature for growth (°C)		
Range	4–37	15–37
Optimum	20–30	30
pH for growth		
Range	5.1–12.1	6.0–9.5
Optimum	8.1–11.1	9.1
Enzyme activities (API ZYM)*		
Acid phosphatase	+	w
Esterase (C4)	–	w
α-Galactosidase	–	+
β-Glucosidase	w	+
N-Acetyl-β-glucosaminidase	+	–
α-Mannosidase	–	+
Naphthol-AS-BI-phosphohydrolase	+	–
Cystine arylamidase	–	w
Valine arylamidase	–	+
Assimilation of (API 20NE):*		
D-Glucose	–	+
D-Mannose	–	+
D-Mannitol	–	+
N-Acetyl-D-glucosamine	–	+
Polar lipids†	PG, PL, GL, L	DPG, PG, PL, GL
DNA G + C content (mol%)	68.3	71 ± 1

*Data obtained in this study with strain DSM 17894^T.

†DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; GL, unknown glycolipids; PL, unknown phospholipids; L, unknown lipids.

Extraction of genomic DNA and amplification of the 16S rRNA gene by PCR were performed as described by Lee (2007). The resultant PCR product was purified and sequenced directly using an automatic DNA sequencer (model 3730xl) with a BigDye Terminator cycle sequencing kit (both from Applied Biosystems). The CLUSTAL X program (Thompson *et al.*, 1997) was used for multiple alignment of the 16S rRNA gene sequence of strain RS-15^T determined in this study and corresponding sequences retrieved from public databases. Phylogenetic analyses were performed using several programs of the PHYLIP software package (Felsenstein, 1993). A neighbour-joining tree (Saitou & Nei, 1987) was constructed using an evolutionary distance matrix generated by the model of Jukes & Cantor (1969). Bootstrap analysis (Felsenstein, 1985) was performed with 1000 neighbour-joining datasets.

A partial 16S rRNA gene sequence (1429 nt) of strain RS-15^T was compared with those of members of the family *Microbacteriaceae*. In total, 1376 unambiguously aligned nucleotides were used for phylogenetic analyses. A 16S rRNA gene sequence tree (Fig. 1) showed that strain RS-15^T belongs to the family *Microbacteriaceae* and forms a tight phylogenetic cluster with *Frontrahabitsans australicus* E1HC-02^T (98.5% 16S rRNA gene sequence similarity). The relationship was supported by a high bootstrap value (96%) and was also found in the trees constructed by the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Other closely related strains were *Frigoribacterium faeni* 801^T (97.9% sequence similarity), *Labedella gwakjiensis* KSW2-17^T (96.8%) and *Frigoribacterium mesophilum* MSL-08^T (96.5%). Strain RS-15^T showed low levels of 16S rRNA gene sequence similarity (<95.7%) to other representatives of the family *Microbacteriaceae*.

To determine the amino acid composition of the peptidoglycan, purified cell walls were obtained as described by Hancock (1994). Hydrolysis of the purified cell walls was carried out by using 6 M HCl at 120 °C for 16 h. Quantitative analysis of amino acids in the purified peptidoglycan was performed by HPLC as described previously (Lee, 2007). The acyl type of the cell wall was analysed according to the method of Uchida & Aida (1984). Menaquinones were analysed by HPLC as described by Kroppenstedt (1985). Analyses of polar lipids and mycolic acids were performed by TLC as described by Minnikin *et al.* (1975) and Minnikin *et al.* (1980), respectively. The G + C content of the DNA was determined by HPLC (Mesbah *et al.*, 1989). The DNA–DNA relatedness between strain RS-15^T and *Frontrahabitsans australicus* DSM 17894^T was determined in duplicate by the initial renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983). The cellular fatty acids of strain RS-15^T and *Frontrahabitsans australicus* DSM 17894^T were determined by using cells grown on TSA for 3 days at 30 °C. Methyl esters of cellular fatty acids were prepared and analysed according to the instructions of the Sherlock Microbial Identification System (version 6; MIDI). For assessing

Fig. 1. Neighbour-joining tree showing the phylogenetic relationships between strain RS-15^T and representatives of the family Microbacteriaceae based on 1376 unambiguously aligned nucleotides of the 16S rRNA gene. Bootstrap support values (>50%) based on 1000 replications are shown at branch nodes. Asterisks indicate that the corresponding nodes were recovered in trees obtained by the maximum-likelihood and maximum-parsimony methods. Bar, 0.01 substitutions per nucleotide position.

reproducibility, methyl esters of cellular fatty acids were analysed using two independent cultures obtained 30 days apart.

The cell-wall peptidoglycan of strain RS-15^T consisted of alanine, glutamic acid, ornithine, glycine and homoserine in the ratio 1.0:1.0:0.7:3.0:1.0. Aspartate, serine and threonine were not detected in the cell wall. Based on these results, the structure of the linkage may be Orn–Ala, suggesting that strain RS-15^T possessed peptidoglycan of the type B2β [{Gly}[L-Hsr]D-Glu–D-Orn] (Schleifer & Kandler, 1972). The murein was found to be N-acetylated. The menaquinones comprised MK-8, MK-9 and MK-7 in the ratio 75:15:10. Among these, MK-9 was reported to be absent from cells of *Frondihabitans australicus* DSM 17894^T (Zhang *et al.*, 2007). The polar lipids were phosphatidylglycerol, five unknown glycolipids, of which two were present as major components, three unknown phospholipids and two unknown lipids. Diphosphatidylglycerol, which was detected in cells of *Frondihabitans australicus* DSM 17894^T (Zhang *et al.*, 2007), was not found in the extract of strain RS-15^T. Mycolic acids were absent. The cellular fatty acid profiles of strain RS-15^T and *Frondihabitans australicus* DSM 17894^T were reproducible in two independent experiments (Supplementary Table S1, available in IJSEM Online). The cellular fatty acid profile of strain RS-15^T was represented by considerable amounts of unsaturated, anteiso-methyl branched and 2-hydroxy components. The predominant fatty acids were summed feature 7 (one or more of C_{18:1}ω9c, C_{18:1}ω12t and C_{18:1}ω7c; 31.5–35.0%), anteiso-C_{15:0} (28.4–30.9%) and C_{14:0} 2-OH (16.7–18.7%). On the other hand, *Frondihabitans australicus* DSM 17894^T contained a large amount of C_{18:1} (70.0–71.1%) together with moderate amounts of C_{14:0} 2-OH (6.5–9.7%), as also reported by

Zhang *et al.* (2007). The exact position of the double bond in the C_{18:1} acid was not determined in this analysis. Strain RS-15^T differed significantly from *Frondihabitans australicus* DSM 17894^T in the type and relative amounts of major fatty acids, particularly anteiso-C_{15:0} (Supplementary Table S1). The DNA G + C content of strain RS-15^T was 68.3 mol%.

Duplicate DNA–DNA relatedness values between strain RS-15^T and *Frondihabitans australicus* DSM 17894^T were 17.2 and 17.1%, showing that strain RS-15^T belongs to a different species (Wayne *et al.*, 1987). Strain RS-15^T is readily differentiated from *Frondihabitans australicus* DSM 17894^T by phenotypic features such as temperature and pH ranges for growth, assimilation of carbon sources and enzyme activities (Table 1).

On the basis of the phenotypic and DNA–DNA hybridization data, strain RS-15^T is considered to be a novel species of the genus *Frondihabitans*, for which the name *Frondihabitans peucedani* sp. nov. is proposed.

Emended description of the genus *Frondihabitans* Greene *et al.* 2009

The description of the genus *Frondihabitans* is as given by Zhang *et al.* (2007) and Greene *et al.* (2009) with the following amendments. Cells are aerobic, Gram-stain-positive, non-endospore-forming, non-motile, irregular-shaped rods. Mesophilic. No mycelium is produced. A range of carbohydrates, organic compounds and amino acids are metabolized. The cell-wall peptidoglycan type is B2β with ornithine as the diagnostic diamino acid. The major menaquinone is MK-8. The presence and relative amounts of MK-7 and MK-9 are variable according to the species. The predominant fatty acids are C_{18:1} (with the

exact position of the double bond being uncertain) and C_{14:0} 2-OH or C_{18:1}, anteiso-C_{15:0} and C_{14:0} 2-OH depending on the species. The polar lipid profile comprises a common core of phosphatidylglycerol, glycolipids and unidentified phospholipids, with the presence of diphosphatidylglycerol being variable. The DNA G + C content is 68.3–71 mol%.

Description of *Fron dihabitans peucedani* sp. nov.

Fron dihabitans peucedani [peu.ce.da'ni. L. n. *peucedanum* the plant hog's-fennel or sulphurwort and also a botanical genus name; L. gen. n. *peucedani* of *Peucedanum*, isolated from *Peucedanum japonicum* Thunb.].

Cells are Gram-stain-positive, aerobic, oxidase-negative, catalase-positive, non-motile, irregular, short rods (0.3–0.5 × 0.9–1.0 µm). Colonies are circular, smooth and convex with entire margins and bright yellow after incubation on ISP 2 at 30 °C for 3 days. Grows at 4–37 °C (optimum 20–30 °C), at pH 5.1–12.1 (optimum pH 8.1–11.1) and with 4.0 % (w/v) NaCl, but not with 5.0 % (w/v) NaCl. Hydrolyses DNA, but not casein, cellulose or starch. Does not decompose hypoxanthine, DL-tyrosine or xanthine. H₂S production is not observed. Positive for aesculin degradation but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis and assimilation of (API 20NE) D-arabinose, potassium gluconate, N-acetyl-D-glucosamine, D-glucose, maltose, D-mannose, D-mannitol, adipic acid, capric acid, DL-malic acid, trisodium citrate and phenylacetic acid. With API ZYM, positive for acid phosphatase, β-galactosidase, α-glucosidase, N-acetyl-β-glucosaminidase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase, weakly positive for esterase lipase (C8) and β-glucosidase and negative for alkaline phosphatase, esterase (C4), lipase (C14), α-chymotrypsin, α-fucosidase, α-galactosidase, β-glucuronidase, α-mannosidase, trypsin, cystine arylamidase and valine arylamidase. With Biolog GN2, utilizes L-arabinose, D-arabitol, dextrin, D-fructose, D-galactose, D-gluconic acid, lactulose, maltose, melibiose, D-psicose, D-sorbitol and sucrose and, in contrast to the results obtained with API 20NE, α-D-glucose, D-mannitol and D-mannose, but does not utilize α-cyclodextrin, glycogen, Tweens 40 or 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, cellobiose, i-erythritol, L-fucose, gentiobiose, myo-inositol, lactose, methyl β-D-glucoside, raffinose, L-rhamnose, trehalose, turanose, xylitol, methyl pyruvate, monomethyl succinate, acetic acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-, β or γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic

acid, glycyl L-glutamic acid, L-histidine, hydroxy L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglytamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, α-D-glucose 1-phosphate or D-glucose 6-phosphate. The cell-wall peptidoglycan type is B2β with ornithine as the diagnostic diamino acid. The major menaquinone is MK-8; small amounts of MK-9 and MK-7 are also present. The polar lipid profile comprises phosphatidylglycerol, five unknown glycolipids, three unknown phospholipids and two unknown lipids. Mycolic acids are not present. The predominant cellular fatty acids are C_{18:1}, anteiso-C_{15:0} and C_{14:0} 2-OH. The DNA G + C content of the type strain is 68.3 mol%.

The type strain, RS-15^T (=KCTC 13435^T =DSM 22180^T), was isolated from rhizosphere soil of a plant (*Peucedanum japonicum* Thunb.) on Mara Island, Jeju, Republic of Korea.

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science & Technology, Republic of Korea. The author is thankful to Dr R Pukall for providing *Fron dihabitans australicus* DSM 17894^T and Seong Hae Seo for analysing amino acids in the cell-wall peptidoglycan.

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