Phytohabitans suffuscus gen. nov., sp. nov.,
an actinomycete of the family Micromonosporaceae
isolated from plant roots

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An actinomycete strain, K07-0523T, was isolated from the roots of an orchid collected in Okinawa prefecture, Japan. 16S rRNA gene sequence analysis indicated that the new strain belonged to the family Micromonosporaceae and the similarity values between strain K07-0523T and the type species of 24 genera in the family Micromonosporaceae were 93.3–97.7 %. Strain K07-0523T contained d-glutamic acid, glycine, d-alanine, meso-diaminopimelic acid, hydroxydiaminopimelic acid and l-lysin in the cell wall. The major menaquinones were MK-9(H6), MK-10(H4) and MK-10(H8). Galactose, glucose, mannose, ribose and xylose were present in the whole-cell sugars. The acyl type of the peptidoglycan was glycolyl. Major fatty acids were anteiso-C17 : 0, iso-C17 : 0, iso-C16 : 0 and iso-C15 : 0. Phosphatidylethanolamine was detected as the major phospholipid and corresponded to phospholipid type II. The G+C content of the genomic DNA was 73 mol%. On the basis of phylogenetic and chemotaxonomic data, the new strain represents a member of a new genus and novel species, namely Phytohabitans suffuscus gen. nov., sp. nov., in the family Micromonosporaceae. The type strain of the type species is K07-0523T (=DSM 45306T =NBRC 105367T).

It is well known that the majority of actinomycete strains isolated from soil samples belong to the genus Streptomyces (Lechevalier & Lechevalier, 1967). Okazaki et al. (1995) and Matsumoto et al. (1998) reported that the microbial flora in plant matter was different from that in soil samples and that the majority of species in such samples were rare actinomycetes. We tried to isolate actinomycete strains from plants in order to discover new microbial resources for screening for potential novel natural products. As part of these studies, we have discovered many rare or novel actinomycete strains. One of these strains, K07-0523T, was identified as being a member of the family Micromonosporaceae. The family Micromonosporaceae was first described by Krasil’nikov (1938), emended by Koch et al. (1996) on the basis of chemotaxonomic data and further emended by Stackebrandt et al. (1997) and Zhi et al. (2009) on the basis of 16S rRNA gene sequence analysis. At the time of writing, the family Micromonosporaceae comprised 24 genera: Micromonospora (Orskov, 1923), Actinoplanes (Couch, 1950), Pseudonocardia (Kane, 1966), Dactylosporangium (Thiemann et al., 1967), Actinomadura (Asano & Kudo, 1986), Catenuloplanes (Yokota et al., 1993), Couchioplanes (Tamura et al., 1994), Spirilliplanes (Tamura et al., 1997), Verrucosispora (Rehms et al., 1998), Virgiosporangium (Tamura et al., 2001), Asanoa (Lee & Hah, 2002), Longispora (Matsumoto et al., 2003), Salinispora (Maldonado et al., 2005), Actinocatenispora (Thawai et al., 2006), Polyphoromspora (Tamura et al., 2006), Luedemannella (Ara & Kudo, 2007a), Krasilnikovia (Ara & Kudo, 2007b), Planosporangium (Wiese et al., 2008), Pseudosporangium (Ara et al., 2008a), Catelloglobospora (Ara et al., 2008b), Hamadacae (Ara et al., 2008b), Plantactinospora (Qin et al., 2009), Rugosimonospora (Monciardini et al., 2009) and Actinorurispora (Thawai et al., 2010). The characterization and classification of strain K07-0523T is described in this paper.

Strain K07-0523T was isolated from the roots of a variety of orchid collected in Okinawa prefecture, Japan, in March

Abbreviation: SEM, scanning electron microscope.
The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain K07-0523T is AB490769.
Additional phylogenetic trees based on maximum-likelihood and maximum-parsimony analyses of 16S rRNA gene sequences are available with the online version of this paper.
Phytohabitans suffuscus gen. nov., sp. nov.

2007. The roots were soaked in 70% ethanol for 30 s and then soaked in 1% sodium hypochlorite for 30 s. The roots were then rinsed in sterilized water. A 0.5 g root sample was dried in a chamber with silica gel and then ground with a mortar and pestle in 5 ml extraction solution [0.38% K2HPO4, 0.12% KH2PO4, 0.51% MgSO4.7H2O, 0.25% NaCl, 0.005% Fe3(SO4)2.nH2O (Wako Pure Chemical Industries, Ltd.), 0.005% MnSO4.5H2O]. A 0.2 ml aliquot of the resultant liquid was mixed into cellobiose asparagine agar (1.0% cellobiose, 0.1% l-asparagine, 0.1% K2HPO4, 0.0001% FeSO4.7H2O, 0.0001% MnCl2.4H2O, 0.0001% ZnSO4.7H2O, 1.5% agar, pH 7.0) and colonies were picked up after incubation for 5 weeks at 27 °C. After culturing on ISP 3 (Nihon Pharmaceutical) and nutrient agar (Difco) for 2–4 weeks at 27 °C, morphology was observed using a scanning electron microscope (SEM; model JSM-5600, JEOL). For SEM inspection, the cultures were fixed with 4% osmium tetroxide vapour in situ for 16 h at room temperature and then dried at room temperature. Cultural characteristics were examined on ISP 3, ISP 4 (Difco), ISP 5 (Nihon Pharmaceutical), nutrient agar and HV agar (Nihon Pharmaceutical) was used for H2S production and coagulation and peptonization of milk, ISP 6 (Nihon Pharmaceutical) was used to test for gelatin liquefaction, 10% skimmed milk (Difco) was used for peptone, 20% gelatin, pH 7.0) was used to test for casein hydrolysis.

Production of acid was evaluated by using the media of carbohydrates as sole carbon sources was tested using ISP 8 (0.5% peptone, 0.3% beef extract, 0.1% KNO3, pH 7.0) was used for nitrate reduction, ISP 9 (Nihon Pharmaceutical) as the basal medium followed by 2 min final extension at 72 °C. The PCR products were sequenced on a DNA sequencer (Applied Biosystems) according to the manufacturer’s instructions. The CLUSTAL_X program version 1.83 (Thompson et al., 1997) was used for multiple alignments with selected sequences for calculating evolutionary distances (Kimura, 1980). Alignments were manually verified and a phylogenetic tree was constructed based on the neighbour-joining method (Saitou & Nei, 1987). Data were resampled with 1000 bootstrap replications (Felsenstein, 1985). For the creation of phylogenetic trees by the maximum-likelihood (Felsenstein, 1981) and the maximum-parsimony methods (KLUGE & FARRIS, 1969), SeaView version 4.2 was used and data were resampled with 100 bootstrap replications. The values for sequence similarities among the most closely related strains were determined using the EzTaxon server (Chun et al., 2007).

Strain K07-0523T showed moderate growth and produced long chains of more than 10 spores on ISP 3, ISP 4, ISP 5, according to the method of Minnikin et al. (1977). The presence of mycolic acids was examined by TLC following Tomiyasu (1982). Whole-cell sugar composition was analysed according to the methods of Becker et al. (1965). Methyl esters of cellular fatty acids were prepared by direct transmethylation with methanolic hydrochloride using cells grown in nutrient broth (Oxoid) for 10 days at 27 °C and analysed on a GLC system (HP 6890; Hewlett Packard). Identification and quantification of the fatty acid methyl esters, as well as the numerical analysis of the fatty acid profiles, were performed according to the instructions for the Microbial Identification System (MIDI).

Chromosomal DNA was prepared following the procedure of Saito & Miura (1963) and the DNA G+C content was determined by HPLC according to Tamaoka & Komagata (1984). The 16S rRNA gene was amplified using the primers described by Takahashi et al. (2002). Amplifications were performed in a TaKaRa thermal cycler (Takara) with an initial incubation of 1 min at 95 °C followed by 30 cycles of 1 min at 95 °C, 1 min at 50 °C and 1.5 min at 72 °C, followed by 2 min final extension at 72 °C. The PCR products were sequenced on a DNA sequencer (Applied Biosystems 3130 Genetic Analyzer) using a BigDye Terminator v3.1 cycle Sequencing kit (Applied Biosystems), according to the manufacturer’s instructions. The CLUSTAL_X program version 1.83 (Thompson et al., 1997) was used for multiple alignments with selected sequences for calculating evolutionary distances (Kimura, 1980). Alignments were manually verified and a phylogenetic tree was constructed based on the neighbour-joining method (Saitou & Nei, 1987).

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nutrient agar and HV agar. Vegetative mycelia were branched, but not fragmented. The surface of the spores was smooth and the spores were 0.8 × 1.0 μm in size (Fig. 1). Spores were non-motile. Sporangia were not observed. The temperature and pH range for growth were 22–37 °C and pH 7–11, respectively. Good growth occurred at 26–35 °C and pH 7–10. Strain K07-0523T did not grow on 3% (w/v) NaCl medium. Additional physiological characteristics are given in the species description.

The cell-wall peptidoglycan contained d-glutamic acid, glycine, d-alanine, meso-diaminopimelic acid, hydroxydiaminopimelic acid and l-lysine. Galactose, glucose, mannose, ribose and xylose were detected as whole-cell sugars. The N-acyl type of the muramic acid was glycolyl. Phosphatidylethanolamine and five unknown phospholipids were detected but phospholipids containing glucosamine, phosphatidylcholine and phosphatidylglycerol were absent. This polar lipid profile corresponded to phospholipid type II of Lechevalier.

**Fig. 2.** Neighbour-joining tree based on 16S rRNA gene sequences showing relationships between strain K07-0523T and members of family *Micromonosporaceae*. Only bootstrap values >50% (1000 replications) are indicated. The solid circles and asterisks indicate that the corresponding nodes were also recovered in the maximum-likelihood tree and maximum-parsimony trees, respectively. Bar, 0.01 nucleotide substitutions per site.
Table 1. Morphological features and chemotaxonomic characteristics of the genus *Phytohabitans* gen. nov. and other genera in the family *Micromonosporaceae*


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<td>Diamino acid(s)</td>
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<td>m-DAP</td>
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<td>9(H4), 9(H6,8)</td>
<td>10(H4,6), 10(H6,8)</td>
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<td>DNA G+C content (mol%)</td>
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et al. (1977). Mycolic acids were not detected. The predominant menaquinones were MK-9(H₄) (16 %), MK-10(H₄) (21 %) and MK-10(H₆) (33 %) and the minor menaquinones were MK-9(H₄) (6 %), MK-9(H₆) (11 %) and MK-10(H₆) (13 %). The cellular fatty acids representing >1 % of total fatty acids were anteiso-C₁₇:₀ (28.6 %), iso-C₁₇:₀ (19.5 %), iso-C₁₆:₀ (12.4 %), iso-C₁₅:₀ (10.2 %), 9-cis-C₁₈:₁ (5.0 %), 2-OH-C₁₆:₁ (4.9 %), C₁₈:₀ (4.5 %), 9-cis-C₁₇:₁ (2.6 %), anteiso-C₁₅:₀ (2.2 %), 10-methyl-C₁₆:₀ (2.2 %), anteiso-C₁₇:₁ (1.6 %), C₁₇:₀ (1.5 %), C₁₆:₀ (1.3 %), 9-cis-C₁₆:₁ (1.3 %) and iso-C₁₈:₀ (1.2 %). This profile corresponded to fatty acid type 2d of Kropfenstedt (1985). The G+C content of the genomic DNA was 73 mol %.

The 16S rRNA gene sequence of strain K07-0523T showed a close relationship with members of family Micromonosporaceae. Similarity values between strain K07-0523T and the type species of all genera belonging to the family Micromonosporaceae were 93.3–97.7 %. The species showing the highest similarity values to strain K07-0523T were Micromonospora pattaloonensis (97.7 %), Pseudosporangium fergusineum (97.7 %), Micromonospora auratinigra (97.6 %) and Catenuloplanes crispus (97.6 %). The phylogenetic analysis based on the 16S rRNA gene sequences also indicated that the new isolate fell within the lineage of the family Micromonosporaceae and formed a cluster within the genera Catenuloplanes and Asanoa (Fig. 2) (see Supplementary Figures S1 and S2 in IJSEM Online). Signature nucleotides of the 16S rRNA gene sequence of strain K07-0523T were shared in all positions with genera of the family Micromonosporaceae (Zhi et al., 2009).

Strain K07-0523T could be classified as a member of the family Micromonosporaceae on the basis of both the phylogenetic analysis and the fact that the N-acyl type of muramic acid was glycolyl. However, strain K07-0523T could be distinguished from all genera of the family Micromonosporaceae by the unique characteristic that the cell-wall peptidoglycan contained both meso-diaminopimelic acid and L-lysine as the diamino acids (Table 1). Furthermore, strain K07-0523T was clearly different from members of the genera Micromonospora, Pseudosporangium and Catenuloplanes, with which it showed high 16S rRNA gene sequence similarity values, on the basis of their morphology, as these genera form only single spores, pseudosporangia or motile spores, respectively. The genera Catenuloplanes and Asanoa, which clustered with strain K07-0523T on the phylogenetic tree, could be distinguished from strain K07-0523T by the major menaquinones, the characteristic whole-cell sugars, the fatty acid profile and the phospholipid profile (Table 1). On the basis of the above results, it is proposed that strain K07-0523T represents a novel genus and species in the family Micromonosporaceae, namely Phytohabitans suffuscus gen. nov., sp. nov.

**Description of Phytohabitans suffuscus sp. nov.**

*Phytohabitans* suffuscus (suff.us'cus. L. masc. adj. suffuscus brownish, named after the brownish colour of the vegetative mycelium).

Morphological, chemotaxonomic and cultural characteristics are as given in the genus description above. Good growth occurs at 26–35 °C and at pH 7–10. No growth on 3 % (w/v) NaCl. Exhibits moderate growth and forms long chains, of more than 10 spores, on ISP 3, ISP 4, ISP 5, nutrient agar and HV agar. The surface of the spores is smooth and spore size is 0.8×1.0 μm. The vegetative mycelia are pale orange to pale brown in colour. Soluble pigment is not produced. Major fatty acids are anteiso-C₁₇:₀, iso-C₁₇:₀, iso-C₁₆:₀, iso-C₁₅:₀. Degrades casein and weakly hydrolyses gelatin. Does not hydrolyse starch. Peptonizes and coagulates milk. Acid is produced from fructose, arabinose, mannitol, rhamnose, galactose and glucose. No acid is produced from raffinose. Nitrate is not reduced to nitrite. H₂S is not produced. Utilizes D-glucose, D-mannitol, sucrose, D-fructose, D-galactose, L-rhamnose, D-xyllose, D-mannose, raffinose, L-arabinose. Does not utilize maltose, dulcitol, D-sorbitol, methyl α-D-glucopyranoside, β-lactose, trehalose, adonitol, melibiose, myo-inositol or meso-erythritol.

The type strain, K07-0523T (=DSM 45306T=NBRC 105367T), was isolated from the roots of an orchid.

**Acknowledgements**

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**References**

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