Angustibacter luteus gen. nov., sp. nov., isolated from subarctic forest soil

Tomohiko Tamura,¹ Yuumi Ishida,¹ Misa Otoguro,¹ Hideki Yamamura,² Masayuki Hayakawa² and Ken-ichiro Suzuki¹

Correspondence

Tomohiko Tamura
tamura-tomohiko@nite.go.jp

¹NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation, 2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan

²Faculty of Engineering, University of the Yamanashi, 4-3-11, Takeda, Kofu, Yamanashi 400-8511, Japan

An actinobacterial strain was isolated from subarctic forest soil, and subjected to polyphasic taxonomic characterization. The cell-wall peptidoglycan comprised *meso*-diaminopimelic acid, alanine and glutamic acid. MK-9(H₄) was the predominant isoprenoid quinone, and iso-C_{17:0}, iso-C_{15:0} and C_{16:0} were detected as the major cellular fatty acids. Phylogenetic analyses based on 16S rRNA gene sequences showed that this organism was related to members of the suborders *Kineosporiineae* and *Micrococcineae*. The phenotypic properties readily differentiated this organism from the phylogenetic neighbours. Based on the phenotypic and genotypic evidence, the strain is assigned to a novel species of a novel genus: *Angustibacter luteus* gen. nov., sp. nov. with type strain TT07R-79^T (=NBRC 105387^T =KACC 14249^T).

The family '*Kineosporiaceae*' (Garrrity *et al.*, 2007) was described as one of six families belonging to the suborder *Frankineae*, which accommodated the genera *Cryptosporangium* (Tamura *et al.*, 1998), *Kineococcus* (Yokota *et al.*, 1993) and *Kineosporia* (Pagani & Parenti, 1978). However, Carlsohn *et al.* (2008) reported that the family '*Kineosporiaceae*' did not emerge as a phylogenetically coherent family. Subsequently, Zhi *et al.* (2009) proposed the new suborder *Kineosporiineae* and the valid family *Kineosporiaceae* for accommodating the genera *Kineococcus*, *Kineosporia* and *Quadrisphaera* (Maszenan *et al.*, 2005), and the new family *Cryptosporangiaceae* in the suborder *Frankineae* for the genus *Cryptosporangium* based on 16S rRNA gene sequence analysis. The genus *Kineosporia* forms elongating, club-shaped sporangia containing motile cells, the genus *Kineococcus* forms motile cocci, and the genus *Quadrisphaera* forms non-motile cocci.

Strain TT07R-79^T was isolated from broad-leaf forest soil on Rishiri Island, Hokkaido, Japan, in 2007. Strain TT07R-79^T was isolated by using the yeast extract-SDS method (Hayakawa & Nonomura, 1989) on HV medium (Hayakawa & Nonomura, 1987) containing 20 mg nalidixic acid l⁻¹ and 50 mg cycloheximide l⁻¹.

PCR amplification and sequencing of the 16S rRNA gene were performed as described previously (Tamura &

Hatano, 2001). The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved by using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). The 16S rRNA gene sequence was aligned with published sequences retrieved from the DDBJ by using CLUSTAL X (Thompson *et al.*, 1997) and the alignment was manually edited by using BioEdit version 7.0.9 (Hall, 1999). Phylogenetic trees were reconstructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms using the MEGA 4.1 program (Tamura *et al.*, 2007). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 replications.

Sequence comparisons with 16S rRNA gene sequences from the EzTaxon database (Chun *et al.*, 2007) revealed that strain TT07R-79^T had highest sequence similarity with *Oryzihumus leptocrescens* KV-628^T (94.95 %), *Kineosporia rhamnosa* JCM 9954^T (94.76 %), *Ornithinicoccus hortensis* HKI 0125^T (94.66 %), *Knoellia sinensis* DSM 12331^T (94.45 %) and *Kineococcus xinjiangensis* S2-20^T (94.43 %), which belong to the family *Kineosporiaceae* in the suborder *Kineosporiineae* and the family *Intrasporangiaceae* in the suborder *Micrococcineae*. The relationship of strain TT07R-79^T to members of the suborders *Kineosporiineae* and *Micrococcineae* can be seen in the phylogenetic tree reconstructed by using representatives of families of the order *Actinomycetales* shown in Supplementary Figure S1 (available in IJSEM Online). The 16S rRNA gene sequence analysis with related taxa, including the type species of the families *Kineosporiaceae* and *Intrasporangiaceae*, revealed

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TT07R-79^T is AB512285.

A phylogenetic tree derived from 16S rRNA gene sequences of strain TT07R-79^T and representatives of the order *Actinomycetales* is available with the online version of this paper.

that strain TT07R-79^T formed a monophyletic clade with the members of the suborder *Kineosporiineae* in neighbour-joining and maximum-parsimony phylogenetic trees (Fig. 1), but this branch was only supported by a low bootstrap value (45 %). With all treeing methods, strain TT07R-79^T appeared as an individual line of descent (see Fig. 1 for the neighbour-joining dendrogram). In view of its phylogenetically deep branching point within the family *Kineosporiaceae*, strain TT07R-79^T should be considered to represent a novel genus.

Cell-wall amino acids, the acyl type of the peptidoglycan, the whole-cell sugar pattern, cellular fatty acids, mycolic acids, menaquinones and the DNA base composition were analysed as described previously (Tamura *et al.*, 1994). Freeze-dried cells for chemotaxonomic analyses were prepared from cultures grown in yeast extract-glucose (YG) broth (Hatano *et al.*, 2003) on a rotary shaker at 28 °C for 3 days. The predominant menaquinone was MK-9(H₄), with MK-9(H₆) as a minor component. Strain TT07R-79^T contained *meso*-diaminopimelic acid, alanine and glutamic acid in the cell wall, and galactose, glucose and ribose as whole-cell sugars. The fatty acid profile was characterized by a predominance of iso-C_{17:0} (19.5 %), iso-C_{15:0} (19.2 %), C_{16:0} (18.4 %) and the presence of smaller proportions of 9 methyl-C_{16:0} (9.8 %), anteiso-C_{17:0} (6.5 %), C_{18:1}ω9c (4.1 %), C_{17:0} (3.7 %), 10 methyl-C_{17:0} (3.5 %), C_{16:1} 2-OH (3.0 %), C_{17:1}ω9c (2.2 %), iso-C_{18:0} (2.1 %), C_{18:0} (2.0 %), 10 methyl-C_{18:0} (1.9 %), iso-C_{16:0} (1.7 %), iso-C_{14:0} (1.2 %) and anteiso-C_{15:0} (1.2 %).

Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannoside were detected. The DNA G+C content was 70.9 mol%. Mycolic acids were absent. The acyl type of the cell-wall muramic acid was the acetyl type.

Morphological characteristics were observed by using scanning electron microscopy as described previously (Tamura *et al.*, 1994). Strain TT07R-79^T formed spherical to rod-shaped cells, 0.3 to 0.5 µm in diameter and 0.3 to 2.0 µm long, sometimes up to 5 µm long (Fig. 2). Cell motility was not observed in distilled water, 0.04 % NaCl solution or 0.01 M phosphate buffer (pH 7.0) with 1/10 soil extract. Determination of the cultural and physiological characteristics was performed as described by Seino *et al.* (1985) and Shirling & Gottlieb (1966) and by using API ZYM, API 50CH and API coryne test strips (bioMérieux). Good growth was observed on yeast extract-malt extract agar (ISP-2), oatmeal agar (ISP-3) and yeast extract-starch agar containing, per litre distilled water (pH 7.2), 15.0 g soluble starch, 4.0 g yeast extract, 0.5 g K₂HPO₄, 0.5 g MgSO₄·7H₂O and 15.0 g agar. Colonies were circular, smooth, convex and orange in colour. Strain TT07R-79^T grew under both aerobic and anaerobic conditions. The pH range for growth was analysed by using filter-sterilized YG broth (pH adjusted using 5 M sulfuric acid). Strain TT07R-79^T grew optimally at pH 6 to 7. Detailed results of these analyses are provided in the species description.

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences of strain TT07R-79^T and members of the suborder *Kineosporiineae*. The tree was reconstructed by using the neighbour-joining method based on 1326 bp of the 16S rRNA gene sequence. The 16S rRNA gene sequence of *Streptomyces ambifaciens* (M27245) was used as the outgroup. Numbers on branches are confidence limits (expressed as percentages) estimated using bootstrap analysis with 1000 replicates; only values >50 % are provided. Solid circles indicate that the corresponding nodes (groupings) were also recovered in the maximum-parsimony tree. Bar, 0.01 nt substitutions per site.

Fig. 2. Scanning electron micrograph of strain TT07R-79^T grown on ISP-2 at 28 °C for 5 days. Bar, 5 µm.

The colony colour of strain TT07R-79^T was orange, similar to those of *Kineosporia* and *Kineococcus*. However, strain TT07R-79^T was clearly different from those organisms in cell shape and motility. Strain TT07R-79^T was distinguishable from the related members of the suborder *Kineosporiineae* by some properties listed in Table 1. Therefore, based on morphological, physiological and chemotaxonomic data and phylogenetic distinctiveness, strain TT07R-79^T should be assigned to a novel species of a new genus. The name *Angustibacter luteus* gen. nov., sp. nov. for strain TT07R-79^T (=NBRC 105387^T =KACC 14249^T) is proposed.

Description of *Angustibacter* gen. nov.

Angustibacter (An.gu.sti.bac'ter. L. adj. *angustus* narrow; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Angustibacter* narrow bacterium).

Cells are Gram-positive, non-motile, non-spore-forming, facultatively anaerobic, catalase-positive cocci to rods. The peptidoglycan is of the A type (A1γ *sensu*; Schleifer & Kandler, 1972) and contains *meso*-diaminopimelic acid, alanine and glutamic acid. Cell-wall sugars are galactose, glucose and ribose. Mycolic acids are absent. The major menaquinone is MK-9(H₄). The phospholipids comprise diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannoside. The cellular fatty acids are a complex mixture of straight-chain saturated, monounsaturated and iso- and anteiso-methyl-branched acids and consist mainly of iso-methyl-branched saturated and straight-chain saturated acids, with iso-C_{17:0}, iso-C_{15:0} and C_{16:0} predominating. The type species is *Angustibacter luteus*.

Description of *Angustibacter luteus* sp. nov.

Angustibacter luteus (lu'te.us. L. masc. adj. *luteus* orange-coloured).

Displays the properties of the genus with the following additions. Cells are usually 0.3–0.5 µm wide and 0.3–2.0 µm long, and rarely up to 5 µm long. Colonies are orange in colour. Growth occurs between 5 and 30 °C. The pH range for growth is 6 to 8 (optimum 6 to 7). Growth

Table 1. Differential characteristics of strain TT07R-79^T and related genera

Data from Groth *et al.* (1999, 2002), Itoh *et al.* (1989), Kageyama *et al.* (2005), Kudo *et al.* (1998), Maszenan *et al.* (2005), Tamura *et al.* (1998), Weon *et al.* (2007), Yokota *et al.* (1993) and this study. A₂pm, diaminopimelic acid; Orn, ornithine; A, anteiso-methyl-branched; I, iso-methyl-branched; M, 9- or 10-methyl-branched; S, straight-chain saturated; U, monounsaturated; DPG, diphosphatidylglycerol; GL, unknown glycolipid(s); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unknown phospholipid(s); PS, phosphatidylserine; ND, no data.

Characteristic	Strain TT07R-79 ^T	<i>Kineococcus</i>	<i>Kineosporia</i>	<i>Quadrifphaera</i>	<i>Oryzihumus</i>	<i>Ornithinicoccus</i>	<i>Knoellia</i>
Cell morphology	Irregular rods and cocci	Cocci in tetrad arrangements	Single spores borne at the tips of substrate hyphae and spore clusters on a sporophore	Cocci in tetrad arrangements	Irregular rods	Cocci	Irregular rods and cocci
Motility	Non-motile	Motile	Motile	Non-motile	Non-motile	Non-motile	Non-motile
Wall diamino acid(s)	<i>meso</i> -A ₂ pm	<i>meso</i> -A ₂ pm	<i>meso</i> - and LL-A ₂ pm	<i>meso</i> -A ₂ pm	<i>meso</i> -A ₂ pm	L-Orn	<i>meso</i> -A ₂ pm
Fatty acid type	S, I, A, U, M	S, I, A	S, U, M	S, I, A, U	S, I, A	S, I, A	S, I, A
Predominant menaquinone	MK-9(H ₄)	MK-9(H ₂)	MK-9(H ₄), MK-8(H ₄), MK-9(H ₆)	MK-8(H ₂)	MK-8(H ₄)	MK-(8H ₄)	MK-(8H ₄)
Polar lipids	DPG, PG, PI, PIM	DPG, PG, GL	PC, DPG, PI, PIM	DPG, PG, PI	ND	PI, PG, DPG, PS	PE, PI, DPG, PG, PL
DNA G+C content (mol%)	71	73–77	69–71	75	72–73	70	68–69

does not occur in the presence of 1 % (w/v) NaCl on yeast extract-starch agar. Negative for nitrate reduction. Arbutin, cellobiose, aesculin, ferric citrate, D-fructose, gentiobiose, D-glucose, glycerol, lactose, D-lyxose, maltose, D-mannose, melibiose, D-raffinose, L-rhamnose, D-ribose, salicin, starch, sucrose, trehalose, turanose and D-xylose are used as sole carbon source, but D-adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, L-fucose, D-galactose, 5-keto-glucuronate, methyl α -D-glucopyranoside, N-acetylglucosamine, glycogen, inositol, inulin, D-mannitol, D-melezitose, methyl α -D-mannopyranoside, D-sorbitol, L-sorbose, D-tagatose, methyl β -D-xylopyranoside, xylitol and L-xylose are not assimilated. β -Galactosidase, α -glucosidase, aesculin hydrolysis, gelatin hydrolysis, esterase lipase (C-8), leucine aminopeptidase, cystine aminopeptidase, acid phosphatase and α -galactosidase are detected in the API ZYM enzyme assay; tests for pyrazinamidase, pyrrolidonyl arylamidase, β -glucuronidase, N-acetyl- β -glucosaminidase, urea hydrolysis, alkaline phosphatase, esterase (C-4), valine aminopeptidase, trypsin, chymotrypsin, phosphohydrolase, α -mannosidase and α -fucosidase are negative. The DNA G+C content of the type strain is 70.9 mol%.

The type strain is strain TT07R-79^T (=NBRC 105387^T =KACC 14249^T).

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