Luteibacter anthropi sp. nov., isolated from human blood, and reclassification of *Dyella yeojuensis* Kim *et al.* 2006 as *Luteibacter yeojuensis* comb. nov.

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A yellow-pigmented bacterium was isolated from blood of a 3-year-old boy in Göteborg, Sweden. Comparative analysis of 16S rRNA gene sequences showed that this bacterium is most closely related to *Luteibacter rhizovicius* LJ96^T and *Dyella yeojuensis* R2A16-10^T, with sequence similarity of 98.3% to both. Strain CCUG 25036^T is clearly different from *L. rhizovicius* CCUG 50033^T and also from *D. yeojuensis* DSM 17673^T in that DNA–DNA hybridization revealed relatedness of 33.7 and 29.8%, respectively. Strain CCUG 25036^T is further distinguished from *L. rhizovicius* CCUG 50033^T and *D. yeojuensis* DSM 17673^T by being able to use D-mannose, D-mannitol and gluconate as sole carbon sources and by lacking the ability to use maltose or sucrose as sole carbon sources. The name *Luteibacter anthropi* sp. nov. is proposed for this novel species, with the type strain CCUG 25036^T (=CCM 7598^T). The proposal is also made to reclassify *Dyella yeojuensis* as *Luteibacter yeojuensis* comb. nov., with the type strain R2A16-10^T (=DSM 17673^T =KACC 11405^T).

Since the description of *Luteibacter rhizovicius* (Johansen *et al.*, 2005), no additional members of the genus *Luteibacter* have been described. Members of the genus are characterized as Gram-negative rods, showing aerobic growth and producing yellow-pigmented colonies, with membranes showing a fatty acid composition consisting largely of branched compounds (mainly iso-C_{15:0}, iso-C_{17:1ω9c} and iso-C_{17:0}) (Johansen *et al.*, 2005). Here, we describe strain CCUG 25036^T, a new member of this genus. Furthermore, we propose the reclassification of *Dyella yeojuensis* Kim *et al.* 2006 into the genus *Luteibacter*.

Strain CCUG 25036^T was isolated from the blood of a 3-year-old boy in Göteborg, Sweden. Colonies of CCUG 25036^T grown on tryptone soy agar (TSA; Oxoid) are yellow, circular and low-convex with entire margins and a smooth appearance. Phenotypic properties of strain CCUG 25036^T compared with *L. rhizovicius* CCUG 50033^T are shown in Table 1. Cell morphology was observed under a Zeiss light microscope at ×1000, with cells grown for 3 days at 28 °C on TSA. Cells of CCUG 25036^T were thin rods, 3–4 µm long and 1–2 µm wide. Cells were motile. The ranges of temperature and pH for growth of strain CCUG 25036^T were determined by examining growth on TSA. To determine the temperature range, TSA plates were

incubated at 8, 15, 20, 25, 28, 36, 37, 38, 45 and 55 °C. Growth of *L. rhizovicius* CCUG 50033^T was tested in parallel with strain CCUG 25036^T. Growth was observed from 20 to 38 °C, but not at or below 15 °C or at 45 °C or above. In contrast, growth of *L. rhizovicius* CCUG 50033^T was observed only at 15, 20, 25 and 28 °C, and not at 8 or 36 °C or above. To determine the pH range, tryptone soy broth was buffered to various pH values and growth at each pH was monitored spectrophotometrically at 600 nm. Growth was observed within a pH range of 4.5 to 9.5, but not at pH 10.5.

The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). DNA extraction was carried out using the GenElute Plant Genomic DNA kit (Sigma-Aldrich) according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using the primer pair 27F (5'-GAGTTTGATCMTGGCTCAG-3') and MR 1492R (5'-ACGGYTACCTTGTTACGACTT-3') (Lane, 1991) and the following cycle conditions: 95 °C for 3 min, 28 cycles of 94 °C for 1 min, 57.3 °C for 45 s and 72 °C for 2 min and a final step at 72 °C for 15 min. The PCR product was purified with the QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions and was sequenced by a sequencing service (Institute of Microbiology and Molecular Biology, Justus-Liebig-Universität Giessen, Giessen, Germany) with standard sequencing primers for the 16S rRNA gene. Phylogenetic

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CCUG 25036^T is FM212561.

Table 1. Comparison of characteristics of the type strains of *Luteibacter* species

Strains: 1, CCUG 25036^T; 2, *L. rhizovicinus* CCUG 50033^T; 3, *L. (Dyella) yeojuensis* DSM 17673^T. All three strains are positive for utilization of D-glucose and hydrolysis of aesculin, *o*-nitrophenyl β-D-galactopyranoside, *bis*-*p*-nitrophenyl (pNP) phosphate and pNP α-D-glucopyranoside. All three strains are negative for utilization of L-arabinose, D-gluconate, adipate, citrate, lactate and phenylacetate. Results for the utilization of L-arabinose, D-gluconate, adipate, citrate, *N*-acetyl-D-glucosamine, D-mannitol, maltose, DL-malate and phenylacetate and for acid formation from D-glucose were confirmed with the API 20 NE system. On the basis of the tests of the API 20 NE system, strain CCUG 25036^T and *L. rhizovicinus* CCUG 50033^T are negative for utilization of caprate, tryptophan deaminase, arginine dihydrolase, urease and reduction of nitrate. All three strains grow on MacConkey agar and on TSA at 28 °C. +, Positive; (+), weakly positive; –, negative.

Characteristic	1	2	3
Carbon sources utilized			
<i>N</i> -Acetyl-D-glucosamine	+	–	+
Trehalose	–	+	+
D-Mannitol	+	–	–
Maltose	+	–	+
Sucrose	–	+	+
D-Mannose	+	–	+
DL-Malate	+	–	–
Acid formation from D-glucose	(+)	–	–

analysis was performed using the ARB software package (version December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSURF 95 database (version July 2008; Pruesse *et al.*, 2007). Trees were reconstructed using the maximum-likelihood method with fastDNAMl (Olsen *et al.*, 1994) and 50 % as well as 30 % conservation filters (only alignment columns in which the frequency of the most abundant nucleotide is equal to or more than 50 and 30 %, respectively, are included in the calculation). Tree topology was further tested without filters. No significant differences could be detected between these trees. Trees reconstructed with the neighbour-joining and maximum-parsimony methods showed similar results (data not shown).

The almost-complete 16S rRNA gene sequence (1419 bp) of the strain was compared by sequence similarity calculations. The results of these calculations indicated that the closest relatives of strain CCUG 25036^T were *L. rhizovicinus* LG96^T (98.3 % similarity), *D. yeojuensis* R2A16-10^T (98.3 %) and *Dyella koreensis* BB4^T (96.4 %). A maximum-likelihood tree is shown in Fig. 1. The strong relationship with *D. yeojuensis* is obvious, and the branching pattern of CCUG 25036^T and its nearest relatives, including the type strain of *D. yeojuensis*, was confirmed by neighbour-joining and maximum-parsimony analyses, with *L. rhizovicinus* as the closest relative (data not shown). For this reason, we included *D. yeojuensis* DSM 17673^T in our further comparative studies.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses). The phylogenetic tree was constructed using the ARB software package (version December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSURF 95 database (version July 2008; Pruesse *et al.*, 2007). Tree building was performed using the maximum-likelihood method with fastDNAMl (Olsen *et al.*, 1994) and no conservation filter. Bar, 0.10 substitutions per nucleotide position.

The other members of the genus *Dyella*, including the most recently described species *Dyella ginsengisoli* (Jung *et al.*, 2009), *D. marensis* (Lee & Lee, 2009), *D. soli* and *D. terrae* (Weon *et al.*, 2009), and the members of the genera *Luteibacter* and *Frateriuria* are also very similar in regard to phenotypic properties, and the combination of these genera may be justified on the basis of a more comprehensive dataset.

Carbon source utilization by strain CCUG 25036^T was tested in comparison with *L. rhizovicius* CCUG 50033^T and *D. yeojuensis* DSM 17673^T by inoculating the strains into microtitre plate wells as described by Kämpfer *et al.* (1991). In addition, strain CCUG 25036^T and *L. rhizovicius* CCUG 50033^T were tested with the API 20 NE system (bioMérieux), according to the instructions of the manufacturer. Compared with *L. rhizovicius* CCUG 50033^T, strain CCUG 25036^T differed in its abilities to use several sugars only after 5 days of incubation. Differences from *D. yeojuensis* DSM 17673^T are also obvious, as detailed in Table 1.

Whole-cell fatty acid analysis was carried out using the MIDI protocol as described previously (Kämpfer & Kroppenstedt, 1996). Cells were grown on TSA at 28 °C prior to fatty acid extraction according to the MIDI protocol. *L. rhizovicius* CCUG 50033^T and *D. yeojuensis* DSM 17673^T were studied concurrently. Like *L. rhizovicius* CCUG 50033^T, strain CCUG 25036^T has large proportions of branched compounds (mainly iso-C_{15:0}, iso-C_{17:1ω9c} and iso-C_{17:0}), which is in congruence with the findings of Johansen *et al.* (2005). The fatty acid profile of *D. yeojuensis* DSM 17673^T was also very similar to those of the other two strains; however, some (mainly quantitative) differences could be observed. The fatty acid patterns of the three strains are shown in Table 2.

To further clarify the relationship between these three strains, DNA–DNA hybridization experiments were performed using genomic DNA as described by Ziemke *et al.* (1998), except that, for nick translation, 2 µg DNA was labelled during a 3 h incubation at 15 °C. With this method, the reassociation value between strain CCUG 25036^T and *L. rhizovicius* CCUG 50033^T was 33.7 % (repetition 23.7 %). The reassociation value between strain CCUG 25036^T and *D. yeojuensis* DSM 17673^T was 29.8 % (repetition 16.1 %). These values are well below the recommended threshold that is used to delineate species, confirming that strain CCUG 25036^T represents a novel species.

On the basis of clear differences in the 16S rRNA gene sequence and the phenotype, we propose here a novel species of the genus *Luteibacter*, *Luteibacter anthropi* sp. nov. In addition, we propose the reclassification of *Dyella yeojuensis* as *Luteibacter yeojuensis* comb. nov. on the basis of our results.

Description of *Luteibacter anthropi* sp. nov.

Luteibacter anthropi (an.thro'pi. Gr. n. *anthropos* a human being; N.L. gen. n. *anthropi* of a human being, since the type strain was recovered from a human clinical specimen).

Table 2. Fatty acid profiles of the type strains of *Luteibacter* species

Strains: 1, CCUG 25036^T; 2, *L. rhizovicius* CCUG 50033^T; 3, *L. yeojuensis* DSM 17673^T. Values are percentages of total fatty acids; –, not detected/not reported.

Fatty acid	1	2	3	
			This study	Kim <i>et al.</i> (2006)
iso-C _{11:0}	3.6	4.0	3.8	3.7
Unknown 11.543	–	0.2	–	–
Unknown 11.799	0.8	2.2	1.9	2.3
iso-C _{11:0} 3-OH	2.9	3.9	4.0	4.2
C _{12:0}	–	0.1	–	–
iso-C _{12:0}	–	–	0.3	–
iso-C _{13:0}	0.4	0.5	–	–
Unknown 13.565	1.8	–	0.4	–
C _{14:0}	0.5	0.4	–	–
iso-C _{14:0}	–	–	0.5	1.1
iso-C _{12:0} 3-OH	–	–	1.0	1.0
iso-C _{13:0} 3-OH	1.2	2.7	2.3	2.4
iso-C _{15:0}	21.7	17.0	9.2	14.5
anteiso-C _{15:0}	2.4	4.0	3.0	6.9
iso-C _{16:0}	0.5	0.8	18.6	21.3
Summed feature 3*	6.5	9.2	4.3	5.2
C _{16:0}	5.6	6.5	1.4	1.8
iso-C _{16:0} 3-OH	–	–	0.2	–
iso-C _{17:1ω9c}	23.8	24.4	26.5	21.5
iso-C _{17:0}	27.0	22.4	14.9	7.9
anteiso-C _{17:0}	0.9	0.6	1.6	1.6
C _{18:0}	0.5	–	–	–
C _{17:1ω6c}	–	–	1.8	–
C _{18:1ω7c}	–	0.6	–	–
iso-C _{18:0}	–	–	2.2	1.2
iso-C _{17:0} 3-OH	–	0.5	0.8	–

*Summed feature 3 contains C_{16:1ω7c} and/or iso-C_{15:0} 2-OH.

Cells are Gram-negative, motile rods, 2–4 µm long. On TSA, colonies are yellow, circular and convex with a smooth appearance and entire margins. Growth occurs between 15 and 37 °C; no growth is observed below 10 °C or above 38 °C. The optimum temperature is 28 °C. Growth occurs at pH 4.5–9.5, but not at pH 10.5. Optimum growth at pH 7.0. Aerobic and positive for oxidase, but negative for catalase. The major membrane fatty acids are iso-C_{15:0}, iso-C_{17:1ω9c} and iso-C_{17:0}. Minor fatty acids are C_{16:0}, iso-C_{11:0} 3-OH, iso-C_{13:0} 3-OH, anteiso-C_{15:0}, anteiso-C_{17:0} and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH). Uses the following as sole carbon sources: D-glucose, N-acetyl-D-glucosamine, D-mannitol, D-mannose, gluconate and DL-malate. Cannot use the following carbon sources: D-ribose, D-fructose, sucrose, L-glutamate, L-cysteine, DL-aspartate, citrate, pyruvate, acetate, N-acetylgalactosamine, cellobiose,

DL-lactate, salicin and D-xylose. Not able to hydrolyse starch or gelatin.

The type and only strain, CCUG 25036^T (=CCM 7598^T), was isolated from the blood of a 3-year-old boy in Göteborg, Sweden.

Description of *Luteibacter yejuensis* comb. nov.

Luteibacter yejuensis (yeo.ju.en'sis. N.L. masc. adj. *yeojuensis* pertaining to Yeosu, a city in Korea, from where the type strain originated).

Basonym: *Dyella yejuensis* Kim *et al.* 2006.

The description is as given for *Dyella yejuensis* by Kim *et al.* (2006). In addition, the following carbon sources can be utilized: D-glucose, N-acetyl-D-glucosamine, arbutin, cellobiose, D-fructose, D-galactose, maltose, D-mannose, D-ribose, sucrose (weak), trehalose, D-xylose, acetate and pyruvate. Cannot use the following carbon sources: gluconate, DL-malate, L-glutamate, L-cysteine, DL-aspartate, citrate, pyruvate, N-acetylgalactosamine, DL-lactate and salicin.

The type strain is R2A16-10^T (=KACC 11405^T =DSM 17673^T).

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