Luteimonas composti sp. nov., a moderately thermophilic bacterium isolated from food waste

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A yellow-pigmented, Gram-negative, rod-shaped bacterium (strain CC-YY255^T) was isolated from compost generated from food waste collected from Kinmen County, Taiwan. 16S rRNA gene sequence analysis indicated that the strain formed a monophyletic branch at the periphery of the evolutionary radiation occupied by the genus *Luteimonas*; its closest neighbour was the type strain of *Luteimonas mephitis* (94.4 % sequence similarity). The isolate was distinguished from *Luteimonas mephitis* on the basis of several phenotypic properties. The organism utilized glucose, maltose, gentiobiose, melibiose and turanose and only a few organic acids (acetate, propionate) and amino acids (L-alanyl glycine, glycyl L-aspartic acid and glycyl L-glutamic acid) as substrates. The fatty acid profile was slightly different from that reported for *Luteimonas mephitis*. It is evident from the genotypic, chemotaxonomic and physiological data presented that strain CC-YY255^T represents a novel species of the genus *Luteimonas*, for which the name *Luteimonas composti* sp. nov. is proposed. The type strain is CC-YY255^T (=CCUG 53595^T=CIP 109311^T=BCRC 17598^T).

During the characterization of micro-organisms from compost generated from food waste of Kinmen County located in Taiwan, strain CC-YY255^T was isolated and maintained on nutrient agar after incubating at 32 °C for 3 days. Subcultivation was performed on nutrient agar (Oxoid) at 30 °C for between 48 and 72 h. On this medium, strain CC-YY255^T was able to grow at 20–45 °C, but not at 10 or 50 °C. The organism was able to grow on nutrient agar and tryptone soy (TS) agar (Oxoid). Gram-staining was performed as described by Gerhardt *et al.* (1994). Poly- β -hydroxybutyrate granule accumulation was observed by light microscopy after staining cells with Sudan black. Phenotypic characteristics, biochemical tests, carbon source utilization (Biolog GN2) and API ZYM, API 20E and API 20NE (all bioMérieux) profiles were investigated. Additionally, antibiotic susceptibility testing was carried out by using ATB STAPH 5 strips (bioMérieux) according to the manufacturer's recommendations. Tests for fluorescence were made after plating on to King's B medium for 48 h. For

G+C content determinations, DNA was prepared and degraded enzymically into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture obtained was then separated by HPLC. The G+C content of strain CC-YY255^T was 68.1 mol%.

Cell morphology was observed under a Zeiss light microscope at $\times 1000$ magnification, using cells that had been grown for 3 days at 30 °C on nutrient agar (Oxoid). Details of cell morphology are given in the species description below. The pH range for growth was determined by measuring the OD₅₉₅ of the culture grown in nutrient broth (Difco), which was adjusted prior to sterilization to various pH values (pH 3–11 at intervals of 0.5 pH units) by using appropriate biological buffers (Chung *et al.*, 1995). Growth at various temperatures (15–70 °C) was measured on nutrient broth. Growth under anaerobic conditions was determined after incubation in an Oxoid AnaeroGen system on nutrient medium. Growth was recorded by measuring the OD₅₉₅ of the culture with respect to time.

The 16S rRNA gene was analysed as described previously (Young *et al.*, 2005). Analysis of the sequence data was performed by using the software package MEGA, version 2.1 (Kumar *et al.*, 2001), after multiple alignments of the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC-YY255^T is DQ846687.

Detailed results for strain CC-YY255^T with the Biolog GN2 system are available as supplementary material in IJSEM Online.

data by CLUSTAL_X (Thompson *et al.*, 1997). A distance matrix method (distance options according to the Kimura two-parameter model), including clustering by neighbour-joining (Fig. 1), and a discrete character-based maximum-parsimony method were used. In each case bootstrap values were calculated based on 1000 replications. The 16S rRNA gene sequence of strain CC-YY255^T was a continuous stretch of 1501 bp. Sequence similarity calculations (over 1355 bp) indicated that strain CC-YY255^T was related most closely to *Luteimonas mephitis* DSM 12574^T (94.4 % 16S rRNA gene sequence similarity), *Lysobacter antibioticus* DSM 2044^T (94.2 %), *Stenotrophomonas rhizophila* DSM 14405^T (94.0 %) (GenBank accession no. AJ293463), *Thermomonas brevis* DSM 15422^T (93.6 %) (GenBank accession no. AJ519985) and *Xanthomonas oryzae* LMG 5047^T (93.8 %) (GenBank accession no. X95921). Lower sequence similarities (<93.5 %) were found with representative members of the other genera shown in Fig. 1.

Fatty acid methyl esters were prepared, separated and identified according to the instructions of the Microbial Identification System (MIDI; Microbial ID). *Luteimonas mephitis* DSM 12574^T was tested for comparative purposes. The fatty acid profile of strain CC-YY255^T (Table 1) was similar to that given for *Luteimonas mephitis* (Finkmann *et al.*, 2000), but showed some significant differences (Table 1).

Isoprenoid quinone analysis was performed by reversed-phase TLC according to Collins (1985). Analysis of quinones revealed a spot that corresponded to ubiquinone-8 (Q-8). This quinone system is a characteristic feature of the *Beta-proteobacteria* (Collins & Jones, 1981; Yokota *et al.*, 1992; Busse *et al.*, 2002).

Results of the physiological characterization tests are given in the species description below and in Table 2. *Luteimonas mephitis* DSM 12574^T was tested for comparison. Strain

CC-YY255^T was non-fluorescent and was capable of producing acid from various carbohydrates. However, carbon substrate utilization tests with organic acids as substrates showed few positive results. In API 20E tests, strain CC-YY255^T was positive for β -galactosidase and gelatinase and oxidation of glucose, melibiose and arabinose; in API 20NE tests, strain CC-YY255^T was positive for the reduction of nitrate to nitrite, aesculin hydrolysis, gelatinase, β -glucosidase and assimilation of glucose, arabinose, *N*-acetylglucosamine and maltose. In API ZYM enzyme profiling, CC-YY255^T was positive for alkaline and acid phosphatase, butyrate esterase, caprylate esterase (C8), leucine arylamidase, valine arylamidase, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, α -glucosidase and *N*-acetyl- β -glucosaminidase.

Although strain CC-YY255^T showed low 16S rRNA gene sequence similarities (<95 %) to members of all other phylogenetically related genera (*Lysobacter*, *Thermomonas* etc.), including *Luteimonas*, a clear phenotypic differentiation from *Luteimonas mephitis* that might justify the proposal of a new genus was not found. We therefore consider that strain CC-YY255^T represents a second, novel member of the genus *Luteimonas*, for which the name *Luteimonas composti* sp. nov. is proposed.

Description of *Luteimonas composti* sp. nov.

Luteimonas composti (com.pos'ti. N.L. gen. n. *composti* of compost).

Cells are Gram-negative, aerobic, non-motile short rods, 0.5 μ m in length and about 1.0–1.5 μ m in width. Good growth occurs after 48 h incubation on TS agar and nutrient agar at 30 °C. Colonies on complex standard media at 37 °C are yellow, circular, smooth, shiny and convex with an entire edge, 1.0–2.0 mm in diameter and non-fluorescent. The optimal temperature for growth is 30 °C. Growth occurs at 20 and 45 °C, but not at 50 °C. The optimal pH for growth is

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses) constructed after multiple alignments of the data by using CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were determined by using the software package MEGA, version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are listed as percentages at branch points. Bar, 0.02 substitutions per nucleotide position.

Table 1. Fatty acid compositions (%) of strain CC-YY255^T and *Luteimonas mephitis*

Data for individual type strains are from this study. Comparative data for 15 strains of *Luteimonas mephitis* are from Finkmann *et al.* (2000). The type strains were grown on trypticase soy broth agar at 28 °C for 48 h prior to fatty acid analysis. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. *cis* isomers are indicated by the suffix *c*.

Fatty acid	Strain CC-YY255 ^T	<i>Luteimonas mephitis</i>	
		DSM 12574 ^T	15 strains
Saturated (straight-chain):			
C _{10:0}		0.1	
C _{14:0}		0.7	0.3–0.5
C _{16:0}	4.7	1.6	0.9–2.1
Saturated (branched):			
iso-C _{10:0}	0.4	0.1	
iso-C _{11:0}	6.5	5.2	3.8–5.1
anteiso-C _{11:0}	0.3	0.2	0.2–0.2
iso-C _{13:0}		0.5	0.2–0.6
iso-C _{14:0}	1.9	1.5	0.5–1.3
iso-C _{15:1} 7	1.9	3.0	0.8–4.1
iso-C _{15:0}	25.5	47.4	38.1–57.3
anteiso-C _{15:0}	2.7	3.0	2.0–3.8
iso-C _{16:0}	13.8	5.6	1.6–10.6
iso-C _{17:0}	5.5	5.5	3.7–11.0
Unsaturated:			
C _{16:1} ω 9 <i>c</i>			0.7–1.2
iso-C _{17:1} ω 9 <i>c</i>	25.5	18.7	18.2–26.4
Hydroxy:			
iso-C _{11:0} 3-OH	5.5	5.3	2.0–2.7
Summed feature 3*	5.5	0.9	
Cyclopropane acids:			
C _{17:0} cyclo		0.2	

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 comprised C_{16:1} ω 9*c* and/or iso-C_{15:0} 2-OH.

7.0; growth occurs at pH 7 and 10, but not at pH 6.0 or 11. Growth occurs in the presence of 0–6 % (w/v) NaCl; optimal growth occurs in the presence of 0–0.5 % (w/v) NaCl. No anaerobic growth on plain NA or TSA supplemented with nitrate. Poly- β -hydroxybutyrate granule accumulation is observed via microscopy. Shows aerobic metabolism. Positive for oxidase, catalase, gelatinase and aesculin. Nitrate is reduced to nitrite. Ubiquinone Q-8 is the predominant quinone. The fatty acid profile of strain CC-YY255^T is detailed in Table 1. The following carbon sources are utilized (positive with the Biolog GN2 system): gentiobiose, α -D-glucose, maltose, D-melibiose, turanose, pyruvic acid methyl ester, L-alaninamide, L-alanyl glycine, glycyl L-aspartic acid, glycyl L-glutamic acid and inosine. The remaining substrates of the Biolog GN2 system are not utilized (see Supplementary Table S1). Positive reactions

Table 2. Selected physiological characteristics that separate strain CC-YY255^T from *Luteimonas mephitis*

Data are from this study unless indicated. w, Weak.

Characteristic	Strain CC-YY255 ^T	<i>Luteimonas mephitis</i> DSM 12574 ^T
Growth at 37 °C	+	–
Nitrate reduction	+	–*
Utilization of:		
D-Glucose	+	w
D-Galactose	+	–
D-Gluconate	–	+
Acetate	+	–
Propionate	+	–
DL-3-Hydroxybutyrate	+	–

*Data from Finkmann *et al.* (2000).

(API 20E and 20NE systems) are seen for β -galactosidase, gelatinase, oxidation of glucose, melibiose and arabinose, reduction of nitrate to nitrite, β -glucosidase, aesculin hydrolysis, glucose assimilation, arabinose assimilation, *N*-acetylglucosamine and maltose assimilation, but negative reactions for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H₂S production, urease, tryptophan deaminase, indole production, acetoin, oxidation of mannitol, inositol, sorbitol, rhamnose, sucrose and amygdalin, reduction of nitrates to nitrogen, glucose fermentation, urease and assimilation of mannose, mannitol, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Positive in API ZYM enzyme reactions for alkaline phosphatase, butyrate esterase (C4), caprylate esterase (C8), leucine arylamidase, valine arylamidase, α -chymotrypsin, acid phosphatase, naphthol-AS-B-1-phosphohydrolase, β -galactosidase, α -glucosidase, β -glucosidase and *N*-acetyl- β -glucosaminidase, but negative for myristate lipase (C14), cystine arylamidase, trypsin, α -galactosidase, β -glucuronidase, α -mannosidase and α -fucosidase. Sensitive to gentamicin, tetracyclines, minocycline, nor/quinolones 2G and levofloxacin, but resistant to penicillin, cotrimoxazole, erythromycin, clindamycin, teicoplanin, nitrofurantoin, quinupristine-dalflo, coag-oxacillin and oxacillin. Intermediate resistance is observed for vancomycin, rifampicin and fusidic acid. Further physiological characteristics are given in Table 1. The DNA G + C content is 68.1 %.

The type strain, CC-YY255^T (=CCUG 53595^T=CIP 109311^T=BCRC 17598^T), was isolated from compost generated from food waste.

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