Aspromonas composti gen. nov., sp. nov., a novel member of the family *Xanthomonadaceae*

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Two novel bacteria, strains TR7-09^T and P2-12-1, were isolated from samples of compost and river sediment, respectively. The strains comprised Gram-negative, motile, non-spore-forming rods, produced creamy white colonies on R2A agar, contained Q-8 as the predominant ubiquinone, contained iso-15:0, iso-17:0 ω 9c and iso-11:0 3-OH as the major fatty acids, and had polar lipid profiles consisting of phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol and an unknown phospholipid. Phylogenetic analysis based on 16S rRNA gene sequences showed that the strains were most closely related to *Thermomonas haemolytica* DSM 13605^T, *Silanimonas lenta* KCTC 12236^T and *Xanthomonas campestris* LMG 568^T (with 92.5, 92.0 and 92.0 % sequence similarity, respectively) and formed a separate lineage within the family *Xanthomonadaceae*. The combined genotypic and phenotypic data supported the conclusion that the strains represent a novel genus and species, for which the name *Aspromonas composti* gen. nov., sp. nov. is proposed. The type strain is TR7-09^T (=KCTC 12666^T=DSM 18010^T).

The family *Xanthomonadaceae*, which belongs to the class *Gammaproteobacteria*, was described recently (Saddler & Bradbury, 2005) and contains 18 recognized genera (type genus, *Xanthomonas*). However, according to Rule 51b(1) of the *Bacteriological Code* (1990 Revision; Lapage *et al.*, 1992), the name of this family is illegitimate because it contains the genus *Lysobacter*, which is the type genus for the family *Lysobacteraceae*. Thirteen genera in the family *Xanthomonadaceae* (except the genera *Lysobacter*, *Pseudoxanthomonas*, *Stenotrophomonas*, *Thermomonas* and *Xanthomonas*) consist of one or two species. The genera including *Aquimonas* (Saha *et al.*, 2005), *Dokdonella* (Yoon *et al.*, 2006), *Dyella* (Xie & Yokota, 2005), *Luteibacter* (Johansen *et al.*, 2005) and *Silanimonas* (Lee *et al.*, 2005) have been described recently. Representative strains belonging to this family have been isolated from a variety of environmental sources including soil, water, biofilm, sludge, plants, fly larvae and human clinical specimens as if they reflected the common habitats of proteobacteria (Saddler & Bradbury, 2005).

During an investigation of the bacterial community present in compost from a farm in Daejeon, Korea, strain TR7-09^T

was isolated. Strain P2-12-1 was isolated during the screening of phenol-degrading bacteria from river sediment near the Gumi industrial complex in Korea. A compost sample was collected and diluted serially in 0.85 % saline solution. Aliquots of each serial dilution were spread on R2A agar (Difco) and incubated at 30 °C for 7 days. A creamy white colony, designated strain TR7-09^T, was isolated. A sediment sample from Nakdong River was initially stimulated with 100 p.p.m. (1.06 mM) phenol and then the stimulated culture was diluted serially in 0.85 % saline solution. Aliquots of each serial dilution were spread on R2A agar and incubated at 25 °C for 7 days. A creamy white colony, designated P2-12-1, was isolated. Both of the two isolates were subcultured on R2A agar at 30 °C for 48 h. On the basis of 16S rRNA gene sequence comparisons, the two strains were shown to belong to the family *Xanthomonadaceae* of the *Gammaproteobacteria*, but they showed low levels of 16S rRNA gene sequence similarity with respect to representative species of the genera within this family. For further classification, these strains were subjected to a polyphasic investigation.

For most experiments, strains were cultivated on R2A agar or broth (Difco) at 30 °C for 48 h. For the analysis of fatty acids, strains were cultivated on tryptic soy agar (BBL) at 30 °C for 48 h. *Thermomonas haemolytica* DSM 13605^T, *Silanimonas lenta* KCTC 12236^T and *Xanthomonas campestris* DSM 3586^T were used as reference strains under the same conditions.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TR7-09^T is AM229324.

A two-dimensional thin-layer chromatogram of polar lipids from strain TR7-09^T and a table showing the fatty acids of strains TR7-09^T and P2-12-1 are available with the online version of this paper.

The Gram reaction was performed as described by Gerhardt *et al.* (1994). Cell morphology and motility were observed under a phase-contrast microscope (Optiphot; Nikon), at $\times 1000$ magnification, using cells grown on R2A agar for 1–7 days. The presence of flagella was determined by using transmission electron microscopy (JEM-1011; JEOL) after negative staining of the cells with 2% (w/v) uranyl acetate. Oxidase activity was tested using 1% tetramethyl-*p*-phenylenediamine (Tarrand & Groschel, 1982) and catalase activity was tested using 3% H_2O_2 . Growth was investigated on R2A agar at different temperatures (5, 10, 15, 20, 25, 30, 37, 42 and 45 °C), at different NaCl concentrations (1, 2, 3 and 5%) and at pH 5–10 (using increments of 1 pH unit). For the pH experiments, the appropriate biological buffers were used, as follows: $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer was used for pH 5–7, and $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer was used for pH 8–10 (Bates & Bower, 1956; Gomori, 1955). Growth on nutrient agar (Difco) was investigated. Degradation of DNA was investigated, using DNA agar (Difco) supplemented with 0.01% toluidine blue (Merck). Degradation of casein, chitin and starch (Atlas, 1993), degradation of lipid (Kouker & Jaeger, 1987) and degradation of cellulose and xylan (Ten *et al.*, 2004) were also investigated. Duplicate antibiotic-susceptibility tests were performed using filter-paper discs containing the following: ampicillin (10 µg), erythromycin (30 µg), kanamycin (30 µg), neomycin (30 µg), penicillin G (10 IU) and streptomycin (10 µg) (Sigma). Tests of carbon-source utilization, tests for acid production and tests for additional physiological features were performed using API 20NE, API 50CH and API ZYM galleries according to the instructions of the manufacturer (bioMérieux).

Fatty acid methyl esters were prepared and analysed as described previously (Klatte *et al.*, 1994) using the standard Microbial Identification System (MIDI) for automated gas chromatographic analysis (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Isoprenoid quinones were extracted and purified as described previously (Tindall, 1990), dried preparations were dissolved in 200 µl 2-propanol and 1–10 µl samples were separated by using HPLC without further purification. Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1977).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA genes and sequencing of purified PCR products were carried out according to Rainey *et al.* (1996). The 16S rRNA gene sequences were aligned with published sequences, retrieved from EMBL, using CLUSTAL_X (Thompson *et al.*, 1997) and edited by using BioEdit (Hall, 1999). The phylogenetic tree was constructed on the basis of the neighbour-joining method (Saitou & Nei, 1987); distances were estimated by using the method of Jukes & Cantor (1969) with MEGA, version 2.1 (Kumar *et al.*, 2001). The resultant neighbour-joining tree topology was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resampled datasets. DNA G+C contents were

determined by using HPLC after hydrolysis, as described by Tamaoka & Komagata (1984), and non-methylated λ DNA (Sigma) was used as a standard. To determine genomic relatedness, DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using DNA probes labelled with photobiotin (A1935; Sigma) and microdilution wells (96-well microplate; Greiner Bio-one).

Strains TR7-09^T and P2-12-1 formed visible colonies (about 1–2 mm in diameter) within 48 h on R2A agar incubated at 30 °C. Growth occurred at temperatures ranging from 20 to 42 °C, but no growth was observed at 45 °C or at temperatures below 15 °C. Growth occurred at pH 6–9, but no growth was observed at pH 5 or 10. The colonies were creamy white, translucent, convex and circular with entire edges. The cells were Gram-negative, catalase-negative, oxidase-positive, motile rods with single polar flagellum. Detailed physiological and biochemical characteristics are summarized in Table 1 and in the species description.

The only respiratory quinone present was ubiquinone Q-8. The fatty acids iso-15:0, iso-17:0 ω 9c and iso-11:0 3-OH were predominant. The complete fatty acid profiles of strains TR7-09^T and P2-12-1 are presented in Supplementary Table S1 (available in IJSEM Online). The polar lipids comprised phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol and an unknown phospholipid; a two-dimensional thin-layer chromatogram of the polar lipids from strain TR7-09^T is shown in Supplementary Fig. S1, available in IJSEM Online.

The almost-complete 16S rRNA gene sequences (approx. 1500 nt) of strains TR7-09^T and P2-12-1 were determined and compared with those of representative species within the family *Xanthomonadaceae*. Strains TR7-09^T and P2-12-1, which shared 99.9% sequence similarity, showed the highest levels of sequence similarity with *T. haemolytica* DSM 13605^T, *S. lenta* KCTC 12236^T and *X. campestris* LMG 568^T, with values of 92.5, 92.0 and 92.0%, respectively, which are below the threshold level that is generally used to define a novel genus (Ludwig *et al.*, 1998). The levels of sequence similarity with respect to other representative species within the family *Xanthomonadaceae* were in the range 83.2–91.5%. In the phylogenetic tree (Fig. 1), strains TR7-09^T and P2-12-1 formed a separate lineage within the family *Xanthomonadaceae*. The DNA–DNA hybridization level between strains TR7-09^T and P2-12-1 was 92%, which confirmed that they belonged to the same genomic species (Wayne *et al.*, 1987).

In addition, strains TR7-09^T and P2-12-1 showed several significant phenotypic differences with respect to their closest phylogenetic neighbours, i.e. *T. haemolytica*, *S. lenta* and *X. campestris* (Table 1). The strains were mesophilic, whereas *T. haemolytica* and *S. lenta* were slightly thermophilic. The strains did not show catalase activity, whereas *T. haemolytica* and *S. lenta* did. When fatty acid

Table 1. Phenotypic characteristics differentiating *Aspromonas composti* from representatives of related genera

Species: 1, *A. composti*; 2, *T. haemolytica*; 3, *S. lenta*; 4, *X. campestris*. Data for *A. composti* were generated in this study; all other data were taken from previous studies (Bradbury, 1984; Busse *et al.*, 2002; Finkmann *et al.*, 2000; Lee *et al.*, 2005; Vauterin *et al.*, 1995, 1996). All taxa showed oxidase activity and polar flagellation and all had ubiquinone Q-8 as the major quinone. +, Positive; –, negative.

Characteristic	1	2	3	4
Temperature optimum (°C)	30	37–50	47	25–30
Catalase	–	+	+	–
Urease	+	+	–	–
Major fatty acids*	iso-15:0, iso-17:1	iso-15:0, iso-16:0	iso-15:0, iso-16:0	iso-15:0, anteiso-15:0, 16:1
Major hydroxyl fatty acid*	iso-11:0 3-OH	iso-11:0 3-OH	iso-11:0 3-OH	iso-13:0 3-OH
Major polar lipid(s)†	PG, PE, PME	DPG, PE, PG	DPG, PE, PG, PL	DPG, PE, PG, PME, PL
DNA G + C content (mol%)	70.8–71.1	67.1–68.7	50.7	65.8–66.6

*Fatty acid composition was analysed with cells grown at the optimal temperature.

†DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipid; PME, phosphatidylmethyl-ethanolamine.

compositions were analysed using cells grown at optimal temperatures, the strains had fatty acid profiles that differed from those of *T. haemolytica*, *S. lenta* and *X. campestris*, i.e. TR7-09^T and P2-12-1 contained iso-17:1 as one of the major fatty acids. Although the fatty acid compositions were analysed using cells grown at the temperature of 30 °C, the isolates showed profiles that differed from those of their phylogenetic neighbours, e.g. strains TR7-09^T and P2-12-1 lacked 14:0 and 15:1 iso F (see Supplementary Table S1). The strains showed different polar lipid profiles, i.e. the absence of diphosphatidylglycerol, with respect to

those of *T. haemolytica*, *S. lenta* and *X. campestris*. The DNA G + C contents of the strains were much higher than that of *S. lenta*, but the range was similar to those of *T. haemolytica* and *X. campestris*.

On the basis of the 16S rRNA gene dissimilarity with respect to related taxa, together with the phylogenetically distinct position, unique phenotypic characteristics and genomic relatedness, strains TR7-09^T and P2-12-1 represent a novel genus and species, for which the name *Aspromonas composti* gen. nov., sp. nov. is proposed.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strains TR7-09^T and P2-12-1 among representative species within the family Xanthomonadaceae. Numbers at branching points refer to bootstrap percentages (from 1000 resamplings; only values above 50% are shown). Bar, 5 substitutions per 100 nucleotide positions.

Description of *Aspromonas* gen. nov.

Aspromonas (As.pro.mo'nas. Gr. adj. *aspros* white; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Aspromonas* a white monad).

Cells are Gram-negative, non-spore-forming, motile rods with single polar flagellum. Cells are mesophilic and neutrophilic. Cells are oxidase-positive but catalase-negative. The major fatty acids are iso-15:0, iso-17:0 ω 9c and iso-11:0 3-OH. The polar lipids comprise phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol and an unknown phospholipid. The predominant respiratory quinone is ubiquinone Q-8. The type species is *Aspromonas composti*.

Description of *Aspromonas composti* sp. nov.

Aspromonas composti (com.pos'ti. N.L. n. *compostum* -i compost; N.L. gen. n. *composti* of compost).

Cells are Gram-negative, non-spore-forming, motile rods (0.4–0.5 $\times$ 1.0–1.8 μ m) with single polar flagellum. Good growth is observed on R2A agar, tryptic soy agar and nutrient agar. Growth occurs at 20–42 °C (optimum, 30 °C) and at pH 6–9 (optimum, pH 7). Growth occurs in the presence of 1 and 2 % NaCl, but not above 3 %. Colonies are creamy white, translucent, convex and circular with entire edges and produce a diffusible brownish decolorization of the medium after prolonged incubation (5–7 days). Oxidase-positive but catalase-negative. Indole is not produced. Arginine dihydrolase and urease are produced but β -galactosidase is not produced. Nitrate and nitrite are not reduced. Casein and DNA are hydrolysed, but aesculin, cellulose, chitin, gelatin, lipid, starch and xylan are not hydrolysed. According to the results from the API ZYM tests, 2-naphthyl phosphate (pH 8.5), 2-naphthyl butyrate, 2-naphthyl caprylate, L-leucyl 2-naphthylamide, 2-naphthyl phosphate (pH 5.4), N-glutaryl-phenylalanine 2-naphthylamide and naphthol-AS-BI-phosphate are hydrolysed, but 2-naphthyl myristate, L-valyl 2-naphthylamide, L-cystyl 2-naphthylamide, N-benzoyl-DL-arginine 2-naphthylamide, 6-bromo-2-naphthyl α -D-galactopyranoside, 2-naphthyl β -D-galactopyranoside, naphthol-AS-BI- β -D-glucuronide, 2-naphthyl α -D-glucopyranoside, 6-bromo-2-naphthyl β -D-glucopyranoside, 1-naphthyl N-acetyl- β -D-glucosaminide, 6-bromo-2-naphthyl α -D-mannopyranoside and 2-naphthyl α -L-fucopyranoside are not hydrolysed. Acid is produced from 5-ketogluconate, but not from glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β -D-xylose, galactose, glucose, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α -D-mannoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate or 2-ketogluconate. The following compounds are

utilized as sole carbon sources: glycogen, rhamnose, acetate, malonate, suberate, 3-hydroxybenzoate and L-serine. The following carbon sources are not utilized: D-glucose, N-acetylglucosamine, maltose, D-ribose, L-arabinose, mannose, adipate, gluconate, caprate, malate, valerate, mannitol, citrate, phenyl acetate, inositol, sucrose, itaconate, DL-lactate, L-alanine, 5-ketogluconate, salicin, D-melibiose, L-fucose, D-sorbitol, propionate, histidine, 2-ketogluconate, 3-hydroxybutyrate, 4-hydroxybenzoate and L-proline. Susceptible to kanamycin and neomycin. Resistant to ampicillin, erythromycin, penicillin G and streptomycin. The major respiratory quinone is ubiquinone Q-8. The predominant fatty acids are iso-15:0, iso-17:0 ω 9c and iso-11:0 3-OH. The polar lipids comprise phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol and an unknown phospholipid. The G + C content of the DNA is 70.8–71.1 mol% (70.8 mol% for the type strain).

The type strain, TR7-09^T (=KCTC 12666^T=DSM 18010^T), was isolated from compost.

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