Pseudofulvimonas gallinarii gen. nov., sp. nov., a new member of the family *Xanthomonadaceae*

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A Gram-stain-negative, rod-shaped, oxidase-positive, non-spore-forming bacterium (Sa15^T) was isolated from the air in a duck barn. On the basis of 16S rRNA gene sequence similarity studies, the organism was grouped into the class gammaproteobacteria in the neighbourhood of the genus *Fulvimonas*. The quinone system consisted exclusively of ubiquinone Q-8. The polar lipid profile was mainly composed of the major lipids diphosphatidylglycerol, phosphatidylmonomethylethanolamine and phosphatidylcholine, and moderate amounts of phosphatidylglycerol and an unidentified lipid. This profile was substantially different from that of *Fulvimonas soli* LMG 19981^T examined concurrently. The polyamine pattern showed the predominant amine spermidine. Major fatty acids (iso-C_{15:0}, iso-C_{17:1ω9c} and iso-C_{17:0}) were in agreement with its phylogenetic affiliation in the vicinity of *Fulvimonas*; however, differences in the polar lipid and fatty acid patterns and the polyamine profiles could be observed as well. On the basis of DNA–DNA pairing results, chemotaxonomic data and physiological and biochemical data, the strain can be clearly differentiated from *Fulvimonas soli*. It is evident that this organism represents a novel genus, for which the name *Pseudofulvimonas gallinarii* gen. nov., sp. nov. is proposed, with the type strain Sa15^T (=DSM 21944^T =CCM 7599^T).

The genus *Fulvimonas* was described by Mergaert *et al.* (2002), with a single species, *Fulvimonas soli*, isolated from soil after enrichment on plasticized acetylated starch granules as a source of carbon. On the basis of 16S rRNA gene sequence data, *Fulvimonas soli* was grouped into the neighbourhood of the genera *Rhodanobacter* and *Frateriella*. The genus was characterized by a unique fatty acid pattern, consisting almost exclusively of branched fatty acids.

Strain Sa15^T was isolated from the air sampled in a duck barn located close to Belzig, Brandenburg, Germany. Subcultivation was done on tryptone soy agar (TSA) at 28 °C for 48 h. On this agar, the organism was able to grow at 15–45 °C, but not at 10 or 50 °C. Growth at 30 °C was also observed on nutrient agar and R2A, but not on MacConkey agar (all from Oxoid).

Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PME, phosphatidylmonomethylethanolamine; pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Sa15^T is FN298515.

Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at ×1000, with cells grown for 3 days at 28 °C on TSA. The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). Phylogenetic analysis was performed using the ARB software package (Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 95 database (version July 2008; Pruesse *et al.*, 2007) as well as the software package MEGA version 4.0 (Tamura *et al.*, 2007) after multiple alignment of data by CLUSTAL X (Thompson *et al.*, 1997). The sequenced length of the 16S rRNA gene was 1387 bp for strain Sa15^T. Nucleotide sequence distances were calculated (distance options according to the Kimura-2 model) using MEGA version 4.0.

Nucleotide sequence similarities were below 93.5 % with all species of the genera *Rhodanobacter*, *Frateriella* and *Dyella* with validly published names. The highest similarity (93.1 %) was observed to the sequence of *Fulvimonas soli* (AJ311653). The phylogenetic tree shown in Fig. 1 results from a maximum-likelihood reconstruction with 50 % conservatory filter (only alignment columns in which the

Fig. 1. Phylogenetic analysis of species most closely related to strain Sa15^T based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory database (accession numbers are given in parentheses). The 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 fastDNAmI (Olsen *et al.*, 1994) and 50 % conservatory filter. Bar, 0.10 nucleotide substitutions per nucleotide position.

frequency of the most abundant nucleotide is equal to or above 50 % are included in the calculation). Maximum-likelihood reconstructions with 30 % conservatory filter and without filter resulted in identical (30 % filter) and nearly identical (without filter) trees (data not shown).

Quinones and polar lipids were extracted from biomass grown on PYE (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) by the integrated method and analysed as described previously (Tindall, 1990a, b; Altenburger *et al.*, 1996). For HPLC analysis, the equipment described by Stolz *et al.* (2007) was employed. The quinone system of Sa15^T consisted exclusively of ubiquinone Q-8, which is in accordance with the quinone system detected in other members of the family *Xanthomonadaceae* (Finkmann *et al.*, 2000; Busse *et al.*, 2002; Young *et al.*, 2007). The polar lipid profile was composed of the major lipids diphosphatidylglycerol (DPG), phosphatidylmonomethylethanolamine (PME) and phosphatidylcholine, moderate amounts of phosphatidylglycerol (PG) and an unidentified lipid (L1), and minor amounts of two unidentified aminolipids (AL1, AL2), one phospholipid (PL1) and three polar lipids (L1, L2, L3) not staining with any of the specific staining reagents (Supplementary Fig. S1, available in IJSEM Online). The unidentified aminolipid AL1 exhibited the chromatographic behaviour expected for phosphatidylethanolamine (PE), but staining with molybdenum blue for detecting phosphate groups was definitely negative. This profile was substantially different from that of *Fulvimonas*

soli LMG 19981^T examined concurrently. This was composed of the major compounds DPG and PE, moderate amounts of PG, an unidentified phospholipid (PL2) exhibiting highly hydrophobic chromatographic behaviour, an unidentified aminolipid (AL3) and an unidentified aminophospholipid (APL1), and some minor unidentified lipids (AL4, AL5, AL6, L4). In addition, a yellow pigment was detected directly after the chromatography as a very weak yellow spot, but not after staining with molybdato-phosphoric acid.

Polyamines were extracted from late exponentially growing cells in PYE medium according to Busse & Auling (1988) and analysed by HPLC using the apparatus described by Stolz *et al.* (2007). The polyamine pattern of strain Sa15^T contained the major compound spermidine [11.7 µmol (g dry weight)⁻¹] plus spermine [0.2 µmol (g dry weight)⁻¹] and putrescine [0.1 µmol (g dry weight)⁻¹]. *Fulvimonas soli* exhibited a similar polyamine pattern consisting of the major compound spermidine [96.3 µmol (g dry weight)⁻¹] plus putrescine [2.7 µmol (g dry weight)⁻¹], spermine [2.0 µmol (g dry weight)⁻¹] and cadaverine [1.7 µmol (g dry weight)⁻¹]. The most remarkable difference in the polyamine pattern was the huge divergence in the overall content of the major compound spermidine. However, at this time the differences should not be used as a differentiating trait until they are confirmed by analysis of additional strains of both species or closely related species. These polyamine patterns were quite similar to those

of other members of the family *Xanthomonadaceae* including *Xanthomonas*, *Pseudoxanthomonas*, *Thermomonas*, *Rhodanobacter*, *Frateriia*, *Arenimonas* and *Aquimonas* but could be distinguished from *Stenotrophomonas* and *Dyella* (Auling *et al.*, 1991; Hamana & Matsuzaki, 1993; Yang *et al.*, 1993; Busse *et al.*, 2002; Hamana *et al.*, 2007; Young *et al.*, 2007).

Fatty acids were extracted and analysed as reported by Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain Sa15^T was mainly composed of iso-C_{15:0}, iso-C_{17:1}ω9c and iso-C_{17:0}. The detailed fatty acid profile is given in Table 1. Similar to *Fulvimonas soli*, the fatty acid pattern consisted almost exclusively of branched fatty acids. The absence of iso-C_{10:0} and anteiso-C_{17:1}ω9c, and the presence of traces of iso-C_{12:0}, appear to be useful for differentiation of Sa15^T from *Fulvimonas soli* (Table 1).

Results of the physiological characterization are given in the species description and in Table 2. Methods used have been described previously (Kämpfer *et al.*, 1991). Strain Sa15^T and the type strain of *Fulvimonas soli* can be clearly differentiated on the basis of several tests.

Isolation of DNA for estimation of the G + C content was done according to Cashion *et al.* (1977), and a DNA G + C content of 68.6 % was estimated according to Mesbah *et al.* (1989). DNA–DNA hybridization experiments (two repetitions) were performed with Sa15^T and the type strain *Fulvimonas soli* DSM 14263^T on the basis of the method described by Ziemke *et al.* (1998), except that, for nick translation, 2 µg of DNA was labelled during a 3 h incubation at 15 °C. Strain Sa15^T showed relatively low DNA–DNA similarity to *Fulvimonas soli* DSM 14263^T (18 ± 6 %).

From the results of 16S rRNA gene sequencing and from the chemotaxonomic and physiological/biochemical characteristics it is evident that strain Sa15^T represents a new genus with one new species: *Pseudofulvimonas gallinarii* gen. nov., sp. nov.

Description of *Pseudofulvimonas* gen. nov.

Pseudofulvimonas (pseu.do.ful.vi.mo'nas. Gr. adj. *pseudes* false; N.L. fem. n. *Fulvimonas* generic name of a bacterium; N.L. fem. n. *Pseudofulvimonas* a false *Fulvimonas*).

Cells are non-motile, non-spore-forming rods (approx. 2 µm in length). Gram-stain-negative, oxidase-positive, showing an oxidative metabolism. Polyamine patterns comprise the predominant amine spermidine. The quinone system is ubiquinone Q-8. The polar lipid profile is composed of the major lipids PME, DPG and phosphatidylcholine, and moderate amounts of PG and an unidentified lipid. The fatty acid profile contains high amounts of iso-C_{15:0}, iso-C_{17:1}ω9c, iso-C_{16:0} and iso-C_{17:0}; iso-C_{10:0} and anteiso-C_{17:1}ω9c are not detected.

The DNA G + C content is 68.6 mol% (for strain Sa15^T). The type species is *Pseudofulvimonas gallinarii*.

Table 1. Fatty acid profiles of strain Sa15^T and *Fulvimonas soli* (percentages of fatty acids are given)

1, Sa15^T; 2, *Fulvimonas soli* DSM 14263^T (data obtained with the same methods as in this study); 3, *Fulvimonas soli* (six strains, data from Mergaert *et al.*, 2002). Summed feature 3, C_{16:1}ω7c/iso-C_{15:0} 2-OH.

Fatty acid	1	2	3
iso-C _{10:0}		3.9	<1.0
iso-C _{11:0}	5.7	3.9	4.0–5.6
Unknown 11.543			
Unknown 11.799		0.7	2.6–3.5
C _{10:0} 2-OH		0.3	
iso-C _{11:0} 3-OH	4.1	3.6	5.6–7.4
iso-C _{12:0} 3-OH	0.5	0.2	0.6–1.4
C _{12:0} 3-OH			<1.0
iso-C _{12:0}	0.3		<1.0
iso-C _{13:0}			<1.0
Unknown 13.565		2.3	<1.0
C _{14:0}	0.2		<1.0
iso-C _{14:0}	0.3	0.5	<1.0
iso-C _{13:0} 3-OH	1.9	1.6	3.8–4.3
iso-C _{15:1} F	0.5	0.2	<1.0
iso-C _{15:0}	28.8	28.2	25.5–31.2
anteiso-C _{15:0}	0.2	4.7	2.8–4.0
C _{15:0}	0.2		
iso-C _{16:0}	9.0	8.3	3.7–13.2
Summed feature 3*	0.9	0.7	2.0–2.8
C _{16:0}	2.5	0.8	0.4–2.2
iso-C _{17:1} ω9c	30.8	20.4	15.8–21.7
anteiso-C _{17:1} ω9c		0.5	0.6–1.1
iso-C _{17:0}	13.6	17.2	10.9–15.0
anteiso-C _{17:0}	0.2	1.4	0.6–1.1
C _{17:0} cyclo		0.3	<1.0
C _{17:1} ω6c			0.3–1.5
C _{18:1} ω7c	0.2		
iso-C _{18:0}		0.3	<1.0

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

Description of *Pseudofulvimonas gallinarii* sp. nov.

Pseudofulvimonas gallinarii (gal.li.na'ri.i. L. gen. n. *gallinarium* a hen house; L. gen. n. *gallinarii* of a hen house).

Shares all characteristics listed in the genus description. Good growth occurs on R2A agar, TSA and nutrient agar at 25–30 °C. Yellow, translucent and shiny colonies with entire edges form within 24 h, with a diameter of approximately 2 mm. L-Alanine *p*-nitroanilide (pNA) and L-glutamate-γ-3-carboxy-pNA are hydrolysed on the basis of the method described by Kämpfer *et al.* (1991). The following compounds are not hydrolysed: *p*-nitrophenyl (pNP) β-D-galactopyranoside, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, pNP β-D-xylopyranoside, bis-pNP phosphate, bis-pNP phenylphosphonate, bis-pNP phosphorylcholine and L-proline pNA. The following compounds are used as sole sources of

Table 2. Comparison of characteristics of strain Sa15^T and *Fulvimonas soli*

Both strains are positive for utilization of D-fructose and cellobiose, and hydrolysis of bis-pNP-phosphate, L-alanine-pNA and L-glutamate-γ-3-carboxy-pNA. Both strains were negative for utilization of N-acetyl-D-glucosamine*, p-arbutin, D-gluconate*, L-rhamnose, D-mannose, α-melibiose, D-ribose, sucrose, adipate*, citrate*, malate* and phenylacetate*. APL1, aminophospholipid; PL2, unknown phospholipid.

Characteristic	Sa15 ^T	<i>Fulvimonas soli</i> DSM 14263 ^T
Carbon sources utilized		
L-arabinose*, D-galactose	+	—
Glucose*, maltose*, trehalose, acetate, propionate, fumarate	—	+
Polar lipid profile		
PME	+	—
PE	—	+
APL1	—	+
PL2	—	+

*Results were identical to those reported by Mergaert *et al.* (2002) for the type strain of *Fulvimonas soli*. Only those test results marked by an asterisk were compared.

carbon: L-arabinose, cellobiose, D-fructose, D-galactose, and DL-3-hydroxybutyrate (all weak). The following compounds are not used as sole sources of carbon: acetate, propionate, *cis*-aconitate, *trans*-aconitate, 4-aminobutyrate, citrate, fumarate, glutarate, itaconate, DL-lactate, malate, mesaconate, 2-oxoglutarate, pyruvate, L-alanine, β-alanine, L-aspartate, L-leucine, L-ornithine, L-proline, L-serine, N-acetylglucosamine, N-acetyl-D-glucosamine, L-arbutin, D-glucose, D-gluconate, maltose, D-mannose, α-melibiose, L-rhamnose, D-ribose, sucrose, salicin, trehalose, D-xylose, adonitol, *myo*-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, adipate, azelate, suberate, L-histidine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate and phenylacetate. No acids are produced from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl-D-glucoside, erythritol, melibiose, D-arabitol or D-mannose. In addition to the lipids listed in the genus description, minor amounts of two unidentified aminolipids, one phospholipid and two polar lipids are present.

The type strain is Sa15^T (=DSM 21944^T =CCM 7599^T), isolated from the air in a duck barn in Germany.

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