Roseomonas pecuniae sp. nov., isolated from the surface of a copper-alloy coin

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Strain N75^T was isolated from the surface of a copper-alloy 50 Euro cent coin collected from general circulation. Phylogenetic analysis of the 16S rRNA gene sequence revealed that strain N75^T formed a distinct branch within the genus *Roseomonas* and placed it in the *Alphaproteobacteria*. Strain N75^T showed 16S rRNA gene sequence similarities of 92.4–97.1 % to type strains of species of the genus *Roseomonas*. Strain N75^T was a Gram-negative, non-spore-forming, non-motile coccoid, with an optimum growth temperature of about 30 °C; the strain did not grow at 5 or 37 °C. Strain N75^T did not grow in medium containing NaCl. The major respiratory quinone was ubiquinone 10 (Q-10). The major fatty acids were unsaturated C_{16:1}ω7c/C_{16:1}ω6c and C_{18:1}ω7c (around 70 % of the total fatty acids); the third most abundant fatty acid was the hydroxylated C_{18:1} 2-OH. The major polar lipids were phosphatidylcholine, phosphatidylethanolamine and an unknown aminolipid. The DNA G+C content was 72.8 mol%. On the basis of the phylogenetic analysis and physiological and biochemical characteristics, we conclude that strain N75^T represents a novel species of the genus *Roseomonas*, for which we propose the name *Roseomonas pecuniae* sp. nov. (type strain N75^T = LMG 25481^T = CIP 110074^T).

Coins are known to carry a transient microbial community, although they are usually made from copper alloys that reduce bacterial contamination. During a survey of the bacterial community of copper-alloy coins from general circulation, a copper-resistant coccoid, strain N75^T, was recovered among a large number of other isolates and was most closely related phylogenetically to species of the genera *Muricoccus* and *Teichococcus*. The genera *Teichococcus* and *Muricoccus* each contain a single species (Kämpfer *et al.*, 2003), but these species were recently reclassified in the genus *Roseomonas*, based on their phenotypic and phylogenetic relatedness, as *Roseomonas ludipueritiae* and *R. rosea*, respectively (Sánchez-Porro *et al.*, 2009). The alphaproteobacterial genus *Roseomonas* includes a number of species which are characterized by their typical pink pigmentation, oxidative metabolism and coccoid rod shape, appearing in pairs or short chains (Rihs *et al.*, 1993). At the time of writing, the genus comprised 11 species with validly published names: *Roseomonas gilardii* (the type species, with the subspecies *R. gilardii* subsp. *gilardii* and *R. gilardii* subsp. *rosea*) (Rihs *et al.*, 1993, 1998;

Han *et al.*, 2003), *R. aerilata* (Yoo *et al.*, 2008), *R. aquatica* (Gallego *et al.*, 2006), *R. cervicalis* (Rihs *et al.*, 1993, 1998), *R. fauriae* (Rihs *et al.*, 1993) (now considered a later heterotypic synonym of *Azospirillum brasilense*; Helsel *et al.*, 2006), *R. vinacea* (Zhang *et al.*, 2008), *R. lacus* (Jiang *et al.*, 2006), *R. mucosa* (Han *et al.*, 2003) and *R. terrae* (Yoon *et al.*, 2007), in addition to *R. rosea* and *R. ludipueritiae* (Sánchez-Porro *et al.*, 2009).

Species of the genus *Roseomonas* are characterized by having ubiquinone Q-10 as predominant quinone and polar lipid profiles exhibiting major to moderate amounts of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine. The fatty acid profiles contain alphaproteobacteria-characteristic compounds [C_{18:1}ω7c as the predominant fatty acid in addition to C_{16:0} and C_{16:1}ω7c/C_{16:1}ω6c (summed feature 3)] and the rarely found acid C_{18:1} 2-OH. The DNA G+C content is 65–71 mol% (Sánchez-Porro *et al.*, 2009). On the basis of the data presented here, we propose that strain N75^T represents a novel species of the genus *Roseomonas*.

Strain N75^T was isolated from the surface of a 50 Euro cent copper-alloy coin. The coin was stamped on R2A medium and incubated aerobically at 30 °C for 5 days. Isolates were

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain N75^T is GU168019.

purified by streaking on R2A medium and preserved at -80°C in nutrient broth (NB) containing 15% (v/v) glycerol. Strain N75^T, showing pink-red colonies, was revealed at the time of isolation to be related phylogenetically to the genus *Muricoccus* and was chosen for further characterization. *Muricoccus roseus* (= *R. rosea*) DSM 14916^T, *R. aerilata* DSM 19363^T and *R. vinacea* DSM 19362^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) for comparative studies.

The organisms were grown under an aerobic atmosphere and under anaerobic conditions (Genebox) on R2A medium at 30 and 37 °C for 5 days. The temperature range and optimum temperature for growth were examined in R2A incubated at 5, 10, 15, 22, 26, 30, 35, 37 and 40 °C; growth was examined after 5 days. The ability to grow in the presence of NaCl was examined in R2A supplemented with NaCl at final concentrations of 1 and 2% (w/v) at 30 °C. Cell morphology and motility were examined by phase-contrast microscopy after growth in NB at 30 °C for 24 h. The Gram reaction and the presence of cytochrome oxidase and catalase were determined after 24 h of incubation as described by Smibert & Krieg (1981). Enzymic profiles were determined using API ZYM and API 20NE test strips (bioMérieux), according to the manufacturer's instructions. Single-carbon-source assimilation was determined using API 50 CH test strips (bioMérieux) with 0.2 M phosphate buffer, pH 7.2, supplemented with 0.3% (w/v) agar (Difco), 0.05% yeast nitrogen base (Difco) and 0.7% yeast extract (Difco), as described by Morais *et al.* (2004). Biolog GN2 MicroPlates were also used for characterization of single-carbon-source assimilation, according to the manufacturer's instructions; plates were incubated at 26 °C and read after 48 and 96 h. The pH range for growth was examined at 30 °C in R2A medium with the pH adjusted by the addition of 50 mM MES (pH 5, 6, 7), TAPS (pH 8, 9) or CAPSO (pH 10) (Branco *et al.*, 2004).

Cultures used for polar lipid analysis were grown on TSA at 30 °C for 48 h. Cultures were harvested and lyophilized and the polar lipid fraction was separated by two-dimensional TLC on silica gel G plates (Merck; 10 × 10 cm, 0.25 mm thickness) using chloroform/methanol/water (65:25:4, by vol.) in the first direction and chloroform/acetic acid/methanol/water (80:15:12:4, by vol.) in the second direction (Collins *et al.*, 1980). Lipoquinones were extracted from freeze-dried cells, purified by TLC and identified by HPLC (Tindall, 1989). Cultures for fatty acid analysis were grown on TSA and in R2A, in sealed plastic plates, for 48 h (Morais *et al.*, 2004), and fatty acids were analysed using the standard MIS Library Generation Software (Microbial ID).

The G + C content of the genomic DNA was determined by HPLC as described by Mesbah *et al.* (1989). The 16S rRNA gene was sequenced as described by Morais *et al.* (2004). The quality of 16S rRNA gene sequences was checked

manually using the BioEdit editor (Hall, 1999) and sequences were aligned against representative reference sequences of the most closely related organisms, obtained from the Ribosomal Database Project (Cole *et al.*, 2003) and EMBL databases, using the multiple-alignment CLUSTAL_X software package (Thompson *et al.*, 1997). Evolutionary distances were calculated (Jukes & Cantor, 1969), phylogenetic dendrograms were constructed using the neighbour-joining method (Saitou & Nei, 1987) and tree topologies were evaluated by performing bootstrap analysis of 1000 datasets (Felsenstein, 1985) by using the MEGA4 package (Tamura *et al.*, 2007).

Strain N75^T formed Gram-negative, non-spore-forming, non-motile coccoid cells. After 2 days of incubation in R2A, colonies were small and pinkish red. Strain N75^T had a wide growth temperature range (10–35 °C), with an optimum at about 30 °C (Table 1). Strain N75^T was able to grow at pH 6.5–8 with an optimum at pH 7, which is a narrower range in comparison with *R. rosea* (= *M. roseus*) DSM 14916^T, which grew at pH 6–9. Strain N75^T grew aerobically but did not grow anaerobically. Strain N75^T did not grow in medium with added NaCl, whereas *R. rosea* DSM 14916^T was able to grow in medium with 1% NaCl and *R. vinacea* DSM 19362^T grew in medium with 2% NaCl. With the methods used, activities of the enzymes leucine arylamidase, α -chymotrypsin, urease and cytochrome oxidase were not detected. Urea hydrolysis was observed in *R. rosea* DSM 14916^T but not in N75^T, *R. vinacea* DSM 19362^T or *R. aerilata* DSM 19363^T.

Assimilation of single carbon sources was not observed using the API 50 CH strip; in fact, only maltose, malic acid and adipic acid gave weakly positive fermentation reactions using API NE (Table 1). When using Biolog GN2 MicroPlates, strain N75^T was able to assimilate five carbon sources, while *R. rosea* DSM 14916^T assimilated 57 of the 95 carbon sources in the test (Table 1). Like the other species of the genus *Roseomonas*, strain N75^T has C_{18:1}ω7c as the predominant fatty acid in addition to C_{16:0} and summed feature 3 (one or more of C_{16:1}ω7c, C_{16:1}ω6c and iso-C_{15:0} 2-OH) (Table 2). All tested strains also showed the production of the hydroxylated fatty acid C_{18:1} 2-OH. The fatty acid C_{18:0} 3-OH was produced by strain N75^T and also by the other closely related strains (*R. rosea* DSM 14916^T, *R. aerilata* DSM 19363^T and *R. vinacea* DSM 19362^T) and was a characteristic that differentiated these species from the other species of *Roseomonas* (Kämpfer *et al.* 2003; Sánchez-Porro *et al.* 2009). It is also interesting to note that C_{19:0} cyclo ω8c was not present in the fatty acid profiles of strain N75^T, *R. vinacea* DSM 19362^T and *R. aerilata* DSM 19363^T, whereas it was found in *R. rosea* DSM 14916^T and was reported in varying amounts in most type strains of other *Roseomonas* species (Sánchez-Porro *et al.*, 2009). The fatty acids C_{17:1}ω7c and 10-methyl C_{19:0} are present in small amounts in *R. rosea* DSM 14916^T but not in strain N75^T grown on TSA. Additionally, in R2A medium, the fatty acids C_{17:0} cyclo and C_{19:0} cyclo ω8c were found in the profile of *R. rosea* DSM 14916^T but not

Table 1. Phenotypic characteristics that differentiate strain N75^T from the type strains of the most closely related *Roseomonas* species

Strains: 1, strain N75^T; 2, *R. rosea* DSM 14916^T (originally isolated from non-water-damaged building material); 3, *R. vinacea* DSM 19362^T (soil sample); 4, *R. aerilata* DSM 19363^T (air). All tests were performed in this study unless indicated. +, Positive; –, negative; w, weak. All strains contain Q-10 as the predominant ubiquinone.

Characteristic	1	2	3	4
Growth temperature (°C)	10–35	10–40	4–40	5–37
Growth at:				
1 % NaCl	–	+	+	–
2 % NaCl	–	–	+	–
Pigmentation	Pink	Pink	Vinaceous	Pink
Morphology	Cocci	Cocci	Coccoid rods	Cocci
DNA G + C content (mol%)	72.8	71	67.3 ^{a*}	72.1 ^b
Reduction of nitrate to nitrite	+	+	+	–
Assimilation of:				
Malic acid	w	+	–	–
Adipic acid	w	+	–	–
Potassium gluconate	–	w	–	–
Hydrolysis of urea	–	+	–	–
Presence of:				
Esterase (C4)	w	w	w	+
Esterase lipase (C8)	+	+	w	+
Leucine arylamidase	–	+	–	+
Valine arylamidase	–	–	–	+
Cystine arylamidase	–	w	–	–
Trypsin	–	–	w	+
α-Chymotrypsin	–	+	–	–
Naphthol-AS-BI-phosphohydrolase	+	+	–	–
Utilization of:				
α-Cyclodextrin	–	–	–	+
Dextrin	+	w	+	+
Tweens 40 and 80	–	+	–	–
N-Acetyl-D-glucosamine	–	+	–	–
Adonitol	–	+	–	–
D-Arabitol	–	+	–	+
Cellobiose	–	–	+	–
Erythritol	–	+	–	–
D-Fructose	–	+	–	–
D-Galactose	–	+	–	–
Gentiobiose	–	–	–	+
α-D-Glucose	–	+	–	–
myo-Inositol	–	+	–	–
Maltose	–	–	+	+
D-Mannitol	–	+	–	+
D-Mannose	–	+	–	–
Melibiose	–	–	+	+
L-Rhamnose	+	+	–	–
Sucrose	+	–	+	+
Trehalose	–	+	–	–
Xylitol	–	+	–	–
Pyruvic acid methyl ester	–	+	–	–
Succinic acid monomethyl ester	–	+	–	–

Table 1. cont.

Characteristic	1	2	3	4
cis-Aconitic acid	–	+	–	–
Citric acid	–	+	–	–
D-Galactonic acid lactone	–	+	–	–
D-Gluconic acid	–	+	–	–
D-Glucosaminic acid	–	+	–	–
D-Glucuronic acid	–	–	–	+
α-Hydroxybutyric acid	–	w	–	–
β-Hydroxybutyric acid	–	+	–	–
γ-Hydroxybutyric acid	–	–	–	+
p-Hydroxyphenylacetic acid	–	+	–	–
Itaconic acid	–	+	+	–
α-Ketobutyric acid	–	w	–	–
α-Ketoglutaric acid	–	+	–	–
DL-Lactic acid	–	+	–	–
Malonic acid	–	+	–	–
Propionic acid	+	–	–	–
Quinic acid	+	+	–	–
Sebacic acid	–	+	–	–
Succinic acid	–	+	–	+
Bromosuccinic acid	–	+	+	+
Succinamic acid	–	+	–	–
Glucuronamide	–	–	+	+
L-Alaninamide	–	+	–	–
D-Alanine	–	+	–	–
L-Alanine	–	+	–	+
L-Alanyl glycine	–	+	–	–
L-Asparagine	–	+	–	–
L-Aspartic acid	–	+	–	–
L-Glutamic acid	–	+	–	–
Glycyl L-glutamic acid	–	+	–	–
L-Histidine	–	+	–	–
Hydroxy-L-proline	–	+	–	–
L-Leucine	–	+	–	–
L-Ornithine	–	+	–	+
L-Phenylalanine	–	–	–	+
L-Proline	–	+	–	+
L-Pyrogutamic acid	–	+	–	–
L-Serine	–	+	+	–
DL-Carnitine	–	+	–	–
γ-Aminobutyric acid	–	+	–	+
Urocanic acid	–	+	–	–
Inosine	–	+	+	–
Uridine	–	+	–	–
Putrescine	–	+	–	–
2-Aminoethanol	–	+	–	–
Glycerol	–	+	–	+
DL-α-Glycerol phosphate	–	+	–	+

*Data obtained from: a, Zhang *et al.* (2008); b, Yoo *et al.* (2008).

that of N75^T. The polar lipid pattern of strain N75^T on two-dimensional TLC revealed the presence of a small number of different lipids in comparison with *R. rosea* DSM 14916^T and the type species of the genus, *R. gilardii* (Sánchez-Porro *et al.*, 2009). Phosphatidylethanolamine,

Table 2. Fatty acid compositions of strain N75^T and related type strains

Strains: 1, strain N75^T; 2, *R. rosea* DSM 14916^T; 3, *R. vinacea* DSM 19362^T; 4, *R. aerilata* DSM 19363^T (does not grow on TSA). Data were obtained in this study. All strains were grown at 30 °C for 48 h. Values are percentages of total fatty acids; –, not detected or present at less than 0.5 %. Major components for the genus *Roseomonas* are highlighted in bold.

Fatty acid	1		2		3		4
	R2A	TSA	R2A	TSA	R2A	TSA	R2A
C _{12:0}	6.6	5.4	13.4	6.2	2.3	1.2	1.2
C _{14:0}	0.6	–	1.1	–	0.5	0.5	0.7
Summed feature 2*	0.7	0.8	2.3	1.1	0.8	0.5	1.0
Summed feature 3*	25.5	23.2	16.6	21.0	40.8	34.8	28.8
C _{16:0}	6.6	7.3	15.2	7.9	9.8	10.0	17.4
C _{16:0} 2-OH	–	–	0.8	–	–	–	–
C _{16:0} 3-OH	–	–	2.4	0.7	–	–	–
C _{16:1} ω5c	0.8	0.9	–	0.7	0.5	0.5	0.6
C _{17:0} cyclo	–	–	10.3	–	1.5	–	10.4
C _{17:1} ω6c	0.9	4.0	–	4.0	–	–	–
C _{17:1} ω7c	–	–	–	2.2	–	–	–
C _{18:0}	0.7	0.9	–	0.9	5.7	8.1	4.7
C _{18:1} ω5c	0.6	0.7	–	–	0.5	0.7	–
C _{18:1} ω7c	46.5	46.3	22.0	42.1	28.1	28.6	26.9
C _{18:1} 2-OH	8.7	9.3	3.2	7.4	6.8	11.6	2.8
C _{18:0} 3-OH	1.8	1.6	6.4	2.7	2.7	2.6	2.4
C _{19:0} cyclo ω8c	–	–	6.5	0.8	–	–	–
10-Methyl C _{19:0}	–	–	–	1.1	–	–	–

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 2 contains C_{14:0} 3-OH and/or iso-C_{16:1}; summed feature 3 contains one or more of C_{16:1}ω7c, C_{16:1}ω6c and iso-C_{15:0} 2-OH.

an unknown aminolipid and phosphatidylcholine were identified as major polar lipids of N75^T and were also present in the profile of *R. rosea* DSM 14916^T (Fig. 1). Unlike *R. rosea* DSM 14916^T, strain N75^T did not contain the aminolipid AL1. Other differences were the presence in *R. rosea* DSM 14916^T of several minor aminolipids, found close to the origin, indicating their relatively highly hydrophilic nature, and also detected in other species of the genus *Roseomonas* (Sánchez-Porro *et al.*, 2009). Ubiquinone Q-10 was found in all strains. The DNA

G+C content of strain N75^T was 72.5 mol% (Table 1), which is the highest G+C molar percentage reported for this group.

The almost-complete 16S rRNA gene sequence was determined for strain N75^T (1441 bp). The 16S rRNA gene sequence was aligned with those of representative members of the genus *Roseomonas*, including the type strains of the former *Teichococcus ludipueritiae* and *Muricoccus roseus*, both presently placed in the genus

Fig. 1. Two-dimensional TLC of polar lipids of *R. rosea* DSM 14916^T (a) and strain N75^T (b). DPG, Diphasphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL1–6, unknown aminolipids; PL, unknown phospholipid.

Fig. 2. Phylogenetic dendrogram based on 16S rRNA gene sequences showing the relationships of strain N75^T and the closest related taxa. The dendrogram was reconstructed from evolutionary distances by using the neighbour-joining method and the topology was evaluated by performing bootstrap analysis of 1000 datasets; bootstrap values are given as percentages. Bar, 1 inferred substitution per 100 nucleotide positions.

Roseomonas (Sánchez-Porro *et al.*, 2009). The sequence of strain N75^T clustered with a sequence deposited in GenBank under accession number EF633683 (reported as *Muricoccus* sp. PC IW 03; A. Ruckmani and T. Chakrabarti, unpublished) at 99.7 % similarity (not shown). Strain N75^T shared 16S rRNA gene sequence similarity of 97.1, 96.9 and 96.8 % with *R. rosea* 173/96^T, *R. aerilata* 5420S-30^T and *R. vinacea* CPCC 100056^T, respectively (Fig. 2). The type strains of *R. mucosa* and the two subspecies of *R. gilardii* clustered with N75^T at 95 % similarity, and *R. ludipueritiae* 170/96^T clustered with N75^T at less than 94 % similarity. Different phylogenetic tree reconstructions always grouped strain N75^T with *R. rosea* 173/96^T, showing a stable phylogenetic relationship. Moreover, the phylogenetic analysis supported the suggestion made by Helsel *et al.* (2006) that the strains of *R. fauriae* should be transferred to *A. brasiliense*, with the name *Roseomonas fauriae* being a later heterotypic synonym of *Azospirillum brasiliense*.

The phenotypic traits that separate N75^T from its closest phylogenetic relative, *R. rosea* DSM 14916^T, are the higher G+C content, the low diversity in lipid composition, the fatty acid profile, the absence of activity of leucine arylamidase, α -chymotrypsin, urease and cytochrome oxidase, the weak ability to assimilate maltose and the lack of assimilation of capric acid and adipic acid when API 50 CH was used and the ability to assimilate different substrates when the Biolog system GN2 was used. We propose that strain N75^T represents a novel species of the genus *Roseomonas*, which we name *Roseomonas pecuniae* sp. nov.

Description of *Roseomonas pecuniae* sp. nov.

Roseomonas pecuniae (pe.cu'ni.æ. L. gen. n. *pecuniae* of/ from money or a coin, referring to the isolation of the type strain).

Colonies are small, smooth, convex, circular and pinkish red after 2 days of incubation on R2A. Similar colony morphology is observed on TSA. Growth occurs only in

media without added NaCl. Optimal growth at 30 °C, with a temperature range of 10–35 °C; does not grow at 37 °C. Optimal growth at pH 7.0. Does not grow anaerobically. Gelatin is hydrolysed. Leucine arylamidase, α -chymotrypsin, urease and cytochrome oxidase activities are not detected. Esterase lipase and naphthol-AS-BI-phosphohydrolase activities are detected. Weak acid production is obtained from maltose, malic acid and adipic acid using the API NE test strip; no other substrates are fermented or assimilated using API 50 CH. With the Biolog GN2 system, dextrin, L-rhamnose, sucrose, propionic acid and quinic acid are assimilated (Table 1). Nitrate is reduced to nitrite. Major fatty acids are C_{18:1} ω 7c as the predominant fatty acid and C_{16:0}, summed feature 3 (one or more of C_{16:1} ω 7c, C_{16:1} ω 6c and iso-C_{15:0} 2-OH) and C_{12:0}. The fatty acid C_{18:0} 3-OH is present, but C_{19:0} cyclo ω 8c is not. The major respiratory quinone is ubiquinone 10 (Q-10). The DNA G+C content of the type strain is 72.5 mol% (determined by HPLC). The major polar lipids are phosphatidylcholine, phosphatidylethanolamine and an unknown aminolipid.

The type strain, N75^T (=LMG 25481^T =CIP 110074^T), was isolated from the surface of a 50 Euro cent copper-alloy coin.

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