Transfer of *Teichococcus ludipueritiae* and *Muricoccus roseus* to the genus *Roseomonas*, as *Roseomonas ludipueritiae* comb. nov. and *Roseomonas rosea* comb. nov., respectively, and emended description of the genus *Roseomonas*

Cristina Sánchez-Porro,¹ Virginia Gallego,¹ Hans-Jürgen Busse,² Peter Kämpfer³ and Antonio Ventosa¹

Correspondence
Antonio Ventosa
ventosa@us.es

¹Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, E-41012 Sevilla, Spain

²Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

³Institute for Applied Microbiology, Justus-Liebig-Universität Giessen, IFZ, Heinrich-Buff-Ring 26–32, D-35392 Giessen, Germany

Phylogenetic analyses based on 16S rRNA gene sequences revealed that *Teichococcus ludipueritiae* and *Muricoccus roseus* are closely related to the species of the genus *Roseomonas*. The type strain of *Teichococcus ludipueritiae*, 170/96^T, exhibited 16S rRNA gene sequence similarity levels of 96.4 % to *Roseomonas cervicalis* ATCC 49957^T, 95.0 % to *Roseomonas aquatica* TR53^T, 94.5 % to *Muricoccus roseus* 173/96^T, 93.4 % to *Roseomonas mucosa* ATCC BAA-692^T and 93.5 % to *Roseomonas gilardii* subsp. *gilardii* ATCC 49956^T, while *Muricoccus roseus* 173/96^T showed 16S rRNA gene sequence similarity values of 95.7 % to *R. mucosa* ATCC BAA-692^T, 95.7 % to *R. aquatica* TR53^T and 95.3 % to *R. gilardii* subsp. *gilardii* ATCC 49956^T and *R. gilardii* subsp. *rosea* ATCC BAA-691^T. Different phylogenetic analysis methods (neighbour-joining, maximum-likelihood and maximum-parsimony) confirmed that both species are within the *Roseomonas* branch. Neither polyamine patterns (spermidine predominant) nor major characteristics in the polar lipid profiles distinguished the two species from representatives of the genus *Roseomonas*. The fatty acid composition of the two species exhibited alphaproteobacterial characteristics but, like *Roseomonas* species, they also showed considerable amounts of the rarely encountered C_{18:1} 2-OH. On the other hand, they showed some phenotypic differences, but their features are compatible with the transfer of these two species to the genus *Roseomonas*. We propose the reclassification of *Teichococcus ludipueritiae* and *Muricoccus roseus* as *Roseomonas ludipueritiae* comb. nov. (type strain 170/96^T = CIP 107418^T = DSM 14915^T) and *Roseomonas rosea* comb. nov. (type strain 173/96^T = CIP 107419^T = DSM 14916^T), respectively. Emended descriptions of the genus *Roseomonas* and the species *Roseomonas gilardii* (and its subspecies *Roseomonas gilardii* subsp. *gilardii* and *Roseomonas gilardii* subsp. *rosea*), *Roseomonas aquatica*, *Roseomonas cervicalis*, *Roseomonas mucosa* and *Roseomonas lacus* are provided.

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,

16S rRNA gene sequence-based maximum-parsimony and maximum-likelihood trees, fatty acid profiles of various strains and 2D TLC of polar lipids of *R. gilardii* subsp. *gilardii* CIP 104026^T are available as supplementary material with the online version of this paper.

1998). At the time of writing, the genus comprised six recognized species: *Roseomonas gilardii* (type species, with two subspecies, *R. gilardii* subsp. *gilardii* and *R. gilardii* subsp. *rosea*) (Rihs *et al.*, 1993, 1998; Han *et al.*, 2003), *Roseomonas cervicalis* (Rihs *et al.*, 1993, 1998), *Roseomonas mucosa* (Han *et al.*, 2003), *Roseomonas lacus* (Jiang *et al.*, 2006), *Roseomonas aquatica* (Gallego *et al.*, 2006) and *Roseomonas terrae* (Yoon *et al.*, 2007). Another species

originally described as *Roseomonas fauriae* (Rihs *et al.*, 1993, 1998) has been proposed to be a later heterotypic synonym of *Azospirillum brasilense* (Helsel *et al.*, 2006). The genera *Teichococcus* and *Muricoccus* were proposed by Kämpfer *et al.* (2003) and each include a single species: *Teichococcus ludipueritiae* and *Muricoccus roseus*. These species are short, Gram-negative rods, strictly aerobic and able to use sugars, organic acids and amino acids as carbon sources. They are characterized by the presence of straight-chain fatty acids with C_{18:1} 2-OH (for *Teichococcus*) and C_{18:1} 2-OH and C_{18:0} 3-OH (for *Muricoccus*) (Kämpfer *et al.* 2003).

During the course of a previous study (Gallego *et al.*, 2006), we determined that the species *T. ludipueritiae* and *M. roseus* were phylogenetically closely related to the species of the genus *Roseomonas*. In this study, we have compared these two species with six *Roseomonas* species. Phenotypic and phylogenetic data suggest that *T. ludipueritiae* and *M. roseus* should be placed within the genus *Roseomonas*. We also amend the description of the genus and five species of *Roseomonas*, including new data on their nutritional capabilities, polyamines and polar lipid composition.

The following strains were used in this study: *T. ludipueritiae* DSM 14915^T, *M. roseus* DSM 14916^T, *R. gilardii* subsp. *gilardii* CIP 104026^T, *R. gilardii* subsp. *rosea* CIP 108267^T, *R. aquatica* TR53^T, *R. cervicalis* CIP 104027^T, *R. lacus* CIP 109168^T and *R. mucosa* CIP 108268^T. They were routinely cultured on tryptic soy agar (TSA) at 30 °C for 48 h.

Tests for acid production from carbohydrates and enzymes were carried out using API 20 NE, API ID 32E and API ZYM strips (bioMérieux) inoculated according to the manufacturer's instructions and incubated at 30 °C. The utilization of sugars and other compounds was determined on API 50CH strips (bioMérieux) inoculated as described by Kersters *et al.* (1984). Table 1 shows the results of these tests.

The 16S rRNA gene sequences of *T. ludipueritiae* 170/96^T and *M. roseus* 173/96^T were determined previously (Kämpfer *et al.*, 2003); the identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Sequence analysis was carried out using the ARB program package (Ludwig *et al.*, 2004). Following the recommendations of Ludwig *et al.* (1998), alternative treeing methods (neighbour-joining, maximum-parsimony and maximum-likelihood) were used. A comparison using 16S rRNA gene sequences from databases revealed that the 16S rRNA gene sequences of *T. ludipueritiae* 170/96^T and *M. roseus* 173/96^T display a high degree of similarity to certain *Roseomonas* species. The closest relatives of *T. ludipueritiae* 170/96^T are *R. cervicalis* ATCC 49957^T, *R. aquatica* TR53^T, *M. roseus* 173/96^T, *R. mucosa* ATCC BAA-692^T and *R. gilardii* subsp. *gilardii* ATCC 49956^T, with 96.4, 95.0, 94.5, 93.4 and 93.5 % sequence similarity, respectively. The closest relatives of *M. roseus* 173/96^T are *R. mucosa* ATCC BAA-692^T, *R. aquatica* TR53^T, *R. gilardii* subsp. *gilardii* ATCC

49956^T and *R. gilardii* subsp. *rosea* ATCC BAA-691^T, with 95.7, 95.7, 95.3 and 95.3 % sequence similarity, respectively. The phylogenetic tree obtained by the neighbour-joining method shows that *T. ludipueritiae* 170/96^T and *M. roseus* 173/96^T fall within a cluster constituted by the species of *Roseomonas* (Fig. 1). The tree topology was similar when two other algorithms (maximum-parsimony and maximum-likelihood) were used (Supplementary Fig. S1, available in IJSEM Online).

Fatty acids were analysed by gas chromatography as described in detail by Kämpfer & Kroppenstedt (1996). Cells were cultured on TSA at pH 7.0, 28 °C for 24 h. Fatty acid profiles for the strains studied are shown in Supplementary Table S1. The predominant fatty acids were C_{18:1}ω7c, in addition to C_{16:0} and C_{16:1}ω7c/iso-C_{15:0} 2-OH (summed feature 3). The data were in congruence with those published previously (Kämpfer *et al.*, 2003; Gallego *et al.*, 2006), with the exception of the fatty acid profile published for *R. lacus* by Jiang *et al.* (2006), who found major amounts of iso- and anteiso-branched fatty acids, which are very atypical for alphaproteobacteria of the *Roseomonas* group and may have been reported in error. In our study, we found a profile typical of *Roseomonas* for *R. lacus* CIP 109168^T (Supplementary Table S1). All type strains shown in Supplementary Table S1 also showed the production of the hydroxylated fatty acid C_{18:1} 2-OH. The fatty acid C_{18:0} 3-OH was produced only by *M. roseus*. This was already demonstrated in the study of Kämpfer *et al.* (2003). It is also interesting to note that C_{19:0} cyclo ω8c was not shown for *M. roseus*, *R. aquatica* or *R. cervicalis*, whereas this fatty acid was found in different amounts in all other type strains of the tested species. Hence, the fatty acid profiles are very useful for differentiation at the species level.

For the determination of polyamine patterns and polar lipids, strains were grown on 3.3 × PYE medium (1.0 % peptone from casein, 1.0 % yeast extract, pH 7.2) at 28 °C with the exception of *R. aquatica* TR53^T, which was grown on PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) at 28 °C. Polyamines were extracted and analysed as described previously (Busse & Auling, 1988; Stolz *et al.*, 2007). Analysis of *T. ludipueritiae* DSM 14915^T, *M. roseus* DSM 14916^T, *R. gilardii* subsp. *gilardii* CIP 104026^T, *R. gilardii* subsp. *rosea* CIP 108267^T, *R. aquatica* TR53^T, *R. cervicalis* CIP 104027^T, *R. lacus* CIP 109168^T and *R. mucosa* CIP 108268^T revealed homogeneous polyamine patterns, with the predominant compound spermidine [28–45 μmol (g dry weight)⁻¹], moderate to minor amounts of putrescine [0.1–8.1 μmol (g dry weight)⁻¹] and minor amounts of spermine [0.1–2.5 μmol (g dry weight)⁻¹] and 1,3-diaminopropane [0.1–0.2 μmol (g dry weight)⁻¹].

The same set of strains exhibited polar lipid profiles consisting of the major compounds diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and an unidentified aminolipid

Table 1. Characteristics that differentiate *Roseomonas ludipueritiae* comb. nov. and *Roseomonas rosea* comb. nov. from other species of the genus *Roseomonas*

Strains: 1, *R. ludipueritiae* DSM 14915^T; 2, *R. rosea* DSM 14916^T; 3, *R. gilardii* subsp. *gilardii* CIP 104026^T; 4, *R. aquatica* TR53^T; 5, *R. cervicalis* CIP 104027^T; 6, *R. gilardii* subsp. *rosea* CIP 108267^T; 7, *R. lacus* CIP 109168^T; 8, *R. mucosa* CIP 108268^T. +, Positive; –, negative; ±, weak reaction, v, variable; ND, not determined. Data were obtained in this study unless indicated. All strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase, urease, naphthol-AS-BI-phosphohydrolase and L-aspartic acid arylamidase. All strains were negative for utilization of erythritol, dulcitol, methyl α -D-mannopyranoside, inulin, glycogen and D-tagatose, activities of valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, N-acetyl- β -glucosaminidase, α -mannosidase, α -fucosidase, β -galactosidase, β -glucuronidase, α -maltosidase and lysine decarboxylase, reduction of nitrate to nitrogen, indole production, fermentation of glucose and acid production from L-arabitol, galacturonate, 5-ketogluconate, mannitol, maltose, adonitol, palatinose, glucose, sucrose, D-arabitol, trehalose, inositol, cellobiose and sorbitol.

Characteristic	1	2	3	4	5	6	7	8
Motility*	–	–	ND	–	+	v	–	+
Optimum growth temperature (°C)*	30	30	35	28	35	35	30	35
Assimilation of (API 50CH):								
Glycerol	+	–	+	–	+	+	+	+
D-Arabinose	+	–	+	–	+	–	–	+
L-Arabinose	+	–	+	–	+	+	+	+
D-Ribose	+	+	–	–	+	–	–	+
D-Xylose	+	–	+	–	+	+	–	+
L-Xylose	+	–	–	–	–	–	–	–
D-Adonitol	+	–	–	–	–	–	–	+
Methyl β -D-xylopyranoside	+	–	–	–	–	–	–	–
D-Galactose	+	–	+	–	+	+	–	+
D-Glucose	+	–	–	–	+	+	–	+
D-Fructose	+	–	+	–	+	+	+	+
D-Mannose	+	+	+	–	+	–	–	+
L-Sorbose	+	–	–	–	–	–	–	–
L-Rhamnose	+	–	+	–	–	+	+	+
Inositol	+	–	–	–	–	–	–	+
D-Mannitol	+	–	+	–	–	+	±	+
D-Sorbitol	+	–	–	–	–	+	±	+
Methyl α -D-glucopyranoside	+	–	–	–	+	–	+	+
N-Acetylglucosamine	+	–	–	–	+	–	–	+
Amygdalin	+	+	+	–	+	+	+	+
Arbutin	+	–	–	–	+	+	+	+
Aesculin ferric citrate	+	+	+	–	+	+	+	+
Salicin	+	–	–	–	+	–	–	–
Cellobiose	+	–	+	–	+	+	+	+
Maltose	+	+	+	–	+	+	–	+
Lactose	+	–	–	–	+	±	–	+
Melibiose	+	+	–	–	+	–	–	+
Sucrose	+	–	–	–	+	+	–	+
Trehalose	+	+	+	–	+	+	+	+
Melezitose	+	–	–	–	+	–	–	+
Raffinose	+	–	–	–	+	+	–	+
Starch	+	+	–	–	+	–	–	+
Xylitol	+	–	–	–	±	–	–	+
Gentiobiose	–	–	–	–	+	–	–	+
Turanose	+	–	–	–	+	–	+	+
D-Lyxose	+	+	+	–	+	+	–	+
D-Fucose	+	–	+	–	–	+	+	+
L-Fucose	+	–	–	–	+	+	+	+
D- and L-Arabitol	+	–	–	–	–	+	–	+
Potassium gluconate	+	–	+	–	+	+	+	+
Potassium 2-ketogluconate	+	–	+	–	+	+	+	+
Potassium 5-ketogluconate	+	–	–	–	+	–	–	+
Enzyme activities (API ZYM)								
Leucine arylamidase	+	–	±	+	–	–	–	±

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8
Acid phosphatase	–	–	+	+	–	–	+	+
α -Galactosidase	–	–	+	–	–	+	+	+
α -Glucosidase	+	–	+	–	–	+	+	+
β -Glucosidase	–	–	±	–	–	–	–	–
Other tests (API 20NE and ID 32E)								
Reduction of nitrate to nitrite	–	+	–	+	–	–	+	–
β -Glucosidase (aesculin hydrolysis)	+	–	±	–	+	±	–	+
Protease (gelatin hydrolysis)	+	–	±	–	+	±	–	+
Ornithine decarboxylase	+	–	+	–	–	+	+	+
Arginine dihydrolase	+	+	–	–	–	–	+	–
Malonate utilization	–	–	+	–	–	–	+	–
L-Arabinose acidification	+	+	+	+	+	+	–	+
Rhamnose acidification	+	–	–	–	+	–	–	–
DNA G + C content (mol%)*	65.8	68.9	ND	68.6	70.4	67.6	71.9	ND

*Data from Kämpfer *et al.* (2003), Jiang *et al.* (2006), Han *et al.* (2003), Rihs *et al.* (1993) and Gallego *et al.* (2006).

(AL2) and moderate amounts of another unknown aminolipid (AL1). As an example, the polar lipid profile of *R. gilardii* subsp. *gilardii* CIP 104026^T is shown in Supplementary Fig. S2. The most striking difference was detected in the polar lipid profile of *R. lacus* CIP 109168^T, which exhibited moderate amounts of phosphatidylmono-methylethanolamine, which was also detected in trace amounts in *R. mucosa* CIP 108268^T and *R. cervicalis* CIP 104027^T (not shown). Differences among the strains were observed in the presence of several minor spots found close to the origin of spotting, indicating their relatively hydrophilic nature. Since only a single strain of each

species was subjected to analysis, the importance of this finding for species identification is so far unclear. It is worth mentioning here that the chromatographic behaviour of the unknown aminolipid AL2 is identical to that of an unknown aminolipid found in *Brucella* and *Ochrobactrum* species (Scholz *et al.*, 2008; Kämpfer *et al.*, 2007).

Sequence similarities below 95.0 % of the 16S rRNA gene of *T. ludipueritiae* 170/96^T with *R. gilardii* subsp. *gilardii* CIP 104026^T, *R. gilardii* subsp. *rosea* CIP 108267^T and *R. mucosa* CIP 108268^T, the core of the genus, since it

Fig. 1. Phylogenetic tree, based on neighbour-joining of the 16S rRNA gene sequence, showing the positions of *Teichococcus ludipueritiae*, *Muricoccus roseus*, species of *Roseomonas* and other closely related bacterial species. Sequence accession numbers used are shown in parentheses. Bootstrap values higher than 50 % are indicated at branch points. *Rhodovibrio salinarum* ATCC 35394^T was used as the outgroup. Bar, 1 % sequence divergence.

contains the type strain of the type species, suggest that it should not be placed in the genus *Roseomonas* and that *R. cervicalis* CIP 104027^T, its nearest relative, should be reclassified in the genus *Teichococcus*. However, we could not detect any significant phenotypic traits that would support this reclassification. In order to create a phylogeny reflecting taxonomy and considering homogeneity in phenotypic traits, especially in respect of more conserved characteristics such as major polar lipids and polyamine patterns, we propose the transfer of the species *Teichococcus ludipueritiae* to the genus *Roseomonas* as *Roseomonas ludipueritiae* comb. nov. and *Muricoccus roseus* as *Roseomonas rosea* comb. nov. Table 1 shows some features that permit the differentiation of these two species from other *Roseomonas* species.

Emended description of the genus *Roseomonas* Rihs *et al.* 1998

In addition to the characteristics listed by Rihs *et al.* (1993), members of the genus exhibit a polar lipid profile exhibiting major to moderate amounts of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and two unidentified aminolipids (AL1 and AL2). Fatty acid profiles contain alphaproteobacterial characteristic compounds and the rarely encountered fatty acid C_{18:1} 2-OH. The polyamine pattern is characterized by the predominant compound spermidine. The type species is *Roseomonas gilardii*.

Description of *Roseomonas ludipueritiae* comb. nov.

Roseomonas ludipueritiae [lu.di.pue.ri'ti.ae. L. n. *ludus* garden (sic); L. n. *pueritia* boyhood, childhood, youth; N.L. gen. n. *ludipueritiae* intended to mean of a kindergarten].

Basonym: *Teichococcus ludipueritiae* Kämpfer *et al.* 2003.

The description is the same as that given for *Teichococcus ludipueritiae* by Kämpfer *et al.* (2003). The species exhibits the characteristics listed in the emended description of the genus *Roseomonas*. Other phenotypic features are described in Table 1. The type strain is 170/96^T (=CIP 107418^T =DSM 14915^T).

Description of *Roseomonas rosea* comb. nov.

Roseomonas rosea (ro'se.a. L. fem. adj. *rosea* rose-coloured, pink).

Basonym: *Muricoccus roseus* Kämpfer *et al.* 2003.

The description is the same as that given for *Muricoccus roseus* by Kämpfer *et al.* (2003). The species exhibits the characteristics listed in the emended description of the genus *Roseomonas*. Other phenotypic features are described in Table 1. The type strain is 173/96^T (=CIP 107419^T =DSM 14916^T).

Emended description of *Roseomonas gilardii* Rihs *et al.* 1998

In addition to the characteristics listed by Rihs *et al.* (1993), the species exhibits the characteristics listed in the emended genus description. The type strain is 5424^T (=ATCC 49956^T =CCUG 33005^T =CIP 104026^T).

Emended description of *Roseomonas gilardii* subsp. *gilardii* Rihs *et al.* 1998

In addition to the characteristics listed by Rihs *et al.* (1993) and Han *et al.* (2003), the subspecies exhibits the characteristics listed in the emended genus description. The type strain is 5424^T (=ATCC 49956^T =CCUG 33005^T =CIP 104026^T).

Emended description of *Roseomonas gilardii* subsp. *rosea* Han *et al.* 2003

In addition to the characteristics listed by Han *et al.* (2003), the subspecies exhibits the characteristics listed in the emended genus description. The type strain is strain MDA5605^T (=ATCC BAA-691^T =NCTC 13290^T).

Emended description of *Roseomonas aquatica* Gallego *et al.* 2006

In addition to the characteristics listed by Gallego *et al.* (2006), the species exhibits the characteristics listed in the emended genus description. The type strain is TR53^T (=CECT 7131^T =JCM 13556^T).

Emended description of *Roseomonas cervicalis* Rihs *et al.* 1998

In addition to the characteristics listed by Rihs *et al.* (1993) the species exhibits the characteristics listed in the emended genus description. The type strain is E7107^T (=ATCC 49957^T =CIP 104027^T).

Emended description of *Roseomonas mucosa* Han *et al.* 2003

In addition to the characteristics listed by Han *et al.* (2003), the species exhibits the characteristics listed in the emended genus description. The type strain is MDA5527^T (=ATCC BAA-692^T =CCUG 48654^T =NCTC 13291^T).

Emended description of *Roseomonas lacus* Jiang *et al.* 2006

In addition to the characteristics listed by Jiang *et al.* (2006), the species exhibits the characteristics listed in the emended genus description. The type strain is TH-G33^T (=CGMCC 1.3617^T =JCM 13283^T).

Acknowledgements

This study was supported by grants from the Spanish Ministerio de Educación y Ciencia (project BIO2006-006927) and from the Junta de Andalucía (project P06-CVI-01829).

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