**NOTE**

The genus *Rothia* was proposed by Georg & Brown (1967); *Rothia dentocariosa* is the type species. Historically, this taxon was classified in the family *Actinomycetaceae* because of its morphological characteristics. Based on phylogenetic analysis, Stackebrandt *et al.* (1997) transferred it to the family *Micrococcaceae*. The phenotypic heterogeneity within the species has long been known (Lesher *et al.*, 1974; Schofield & Schaal, 1981; Fotos *et al.*, 1984) and the existence of a second genomovar was described by Kronvall *et al.* (1998). *R. dentocariosa* was recognized as the only species of this genus until Collins *et al.* (2000) validly described a novel species (*Rothia nasimurium*) and reclassified *Stomatococcus mucilaginosus* as *Rothia mucilaginosa*. All these *Rothia* species are Gram-positive organisms, isolated from humans or animals, that form non-motile coccoid cells, produce catalase but not urease, reduce nitrate and hydrolyse aesculin (Georg & Brown, 1967; Lesher *et al.*, 1974; Bergan & Kocur, 1982; Ruoff, 1999; Collins *et al.*, 2000). It is not known whether the organisms occur in natural habitats, especially soil and water. Recently, during the course of strengthening environmental protection, an unknown Gram-positive coccus, which was capable of deodorizing dung and sewage, was isolated from a sludge sample taken from a foul water sewer. The present study was undertaken to determine the taxonomic position of this strain. Polyphasic taxonomic data show that this unknown bacterium represents a novel species of the genus *Rothia*, for which the name *Rothia amarae* sp. nov. is proposed.

Strain J18T was isolated on an LB plate (tryptone, 10 g; yeast extract, 5 g; NaCl, 10 g; distilled water, 1 l; pH 7.0) that had been seeded with a sludge suspension and incubated at 32 °C for 2–3 days. The sludge sample was collected from a foul water sewer in Shanghai Jiao Tong University, China. The isolate was maintained on blood agar or trypticase soy agar (BBL) slants at 4 °C and as a glycerol suspension (20%, v/v) at −20 °C. Biomass for chemical and molecular systematic studies was prepared as described previously (Huang *et al.*, 2002) with the modification that the strain was grown at 37 °C for 3 days. Culture and morphological properties were examined both by eye and microscopically (Williams *et al.*, 1983) after growth on blood agar for 2–10 days at 37 °C. The degradation tests and acid production from sugars were examined according to the procedures of Brown *et al.* (1969) and Gordon *et al.* (1974). Growth and enzyme tests were carried out according to Goodfellow *et al.* (1997). Cell wall composition, menaquinone profile and polar lipids were determined as described by Schleifer & Kandler (1972), Collins (1985), Wu *et al.* (1989) and Minnikin *et al.* (1984). The strain was

**Rothia amarae sp. nov., from sludge of a foul water sewer**

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A Gram-positive bacterium, strain J18T, isolated from sludge of a foul water sewer, was subjected to a polyphasic taxonomic study. Phylogenetic analysis of the bacterium based on its 16S rDNA sequence showed that it belongs to the genus *Rothia* and forms a distinct phyletic clade with the type strain of *Rothia nasimurium*. Morphological, physiological and chemotaxonomic characteristics supported the assignment of this organism to the genus *Rothia* and distinguished it from the type strains of all validly described *Rothia* species. Therefore, it is proposed that this bacterium be classified in the genus *Rothia* as *Rothia amarae* sp. nov. The type strain is strain J18T (= AS 4.1721T = JCM 11375T).

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processed using the Sherlock Microbial Identification System (MIDI) to acquire whole-cell fatty acid methyl esters for subsequent GC analysis.

Genomic DNA preparation and PCR amplification of 16S rDNA were performed using the method of Chun & Goodfellow (1995). The amplified product was sequenced as described previously (Huang et al., 2001). The nucleotide sequence was obtained automatically processed using the Applied Biosystems DNA sequencer (model 377) and software provided by the manufacturer. The 16S rDNA sequence of strain J18\textsuperscript{T} was aligned manually against corresponding sequences retrieved from the GenBank database using the program CLUSTAL W 1.8 (Thompson et al., 1997). Phylogenetic trees were inferred using the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) treeing algorithms. Evolutionary distance matrices were generated as described by Kimura (1980). The resultant unrooted tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings. The PHYLIP package (Felsenstein, 1993) was used for all phylogenetic analyses. The G+C content of the DNA was determined using the thermal denaturation ($T_m$) method (Marmur & Doty, 1962) as described previously (Huang et al., 2001).

Strain J18\textsuperscript{T} showed morphology consistent with members of the family Micrococcaceae. It was Gram-positive and non-motile and cells were ovoid to spherical, 0.6–0.9 μm in diameter, occurring singly or in pairs, tetrads or packets. The phenotypic properties of strain J18\textsuperscript{T} were very similar to those of the genus Rothia. It was facultatively anaerobic with good aerobic growth and poor anaerobic growth, producing creamy, mucoid colonies with a smooth convex surface, a convex convoluted surface or a rough surface in young, middle and old age, respectively, similarly to some strains of \textit{R. dentocariosa} (Brown et al., 1969). The organism was catalase-positive, but trypsin-, phosphatase- and urease-negative. It reduced nitrate, hydrolysed aesculin and gelatin and produced acid from glucose, sucrose, glycerol, maltose, mannose, salicin and trehalose but not from mannitol, raffinose or sorbitol. However, strain J18\textsuperscript{T} could be distinguished from the validly described \textit{Rothia} species by a combination of physiological features: production of acid from ribose but not lactose and weakly positive reactions for ester lipase C-8 and valine arylamidase (Table 1). To clarify the taxonomic position of this unknown bacterium further, its almost complete 16S rDNA sequence (1467 nt) was determined and compared with those of representatives of the genera \textit{Rothia}, \textit{Kocuria}, \textit{Micrococcus} and \textit{Arthrobacter}. Results showed that, phylogenetically, strain J18\textsuperscript{T} was a member of the genus \textit{Rothia}; this was supported by a high bootstrap value (94%) recorded using the neighbour-joining method (Fig. 1). The phylogenetic tree also showed that the organism formed a distinct phyletic clade with \textit{R. nasimurium}, but this relationship was supported by a lower bootstrap value (51%). The 16S rDNA sequence similarities between strain J18\textsuperscript{T} and its nearest neighbours, namely \textit{R. nasimurium}, \textit{R. nasimurium}, \textit{R. nasimurium}, \textit{R. nasimurium}, \textit{R. nasimurium}, and \textit{R. nasimurium}.
mucilaginosa, Kocuria rosea and R. dentocariosa genovar I, were respectively 97.3, 96.7, 96.6 and 96.0%. Similarity values to other members of the family Micrococccaceae were below 96.0%. Such a low 16S rDNA sequence similarity (95.4%) indicated that strain J18T should not be confused with the previously reported R. dentocariosa genovar II. Strain J18T also shared chemotaxonomic characteristics with the genus Rothia. The cell wall contained peptidoglycan amino acids Lys and Ala, variation A32 (Schleifer & Kandler, 1972). The predominant polar lipids were phosphatidylglycerol and diphasphatidylglycerol, with phosphatidylglycerol as a minor component. The detectable isoprenoid quinones contained MK-7, but also MK-6(H2) [peak area ratio of MK-7: MK-6(H2), 47:52]. The major cellular fatty acid was the anteiso methyl-branched type ai-C15:0 as observed in other Rothia species, but it was present in a higher proportion (72.3%) in strain J18T than in other Rothia species (Table 1). The G+C content of the DNA was 54.5 mol% (Tm method).

On the basis of phenotypic, chemotaxonomic and phylogenetic data, the unknown organism, strain J18T, merits novel species status in the genus Rothia. Therefore, the name Rothia amarae sp. nov. is proposed.

**Description of Rothia amarae sp. nov.**

Rothia amarae (a’ma.rae. L. adj. amara, -ae of a trench, conduit, referring to fact that the bacterium was isolated from a sewage duct).

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References


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