NOTE

Characterization of a *Rothia*-like organism from a mouse: description of *Rothia nasimurium* sp. nov. and reclassification of *Stomatococcus mucilaginosus* as *Rothia mucilaginosus* comb. nov.

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An unknown, Gram-positive, ovoid-shaped bacterium isolated from the nose of a mouse was subjected to a polyphasic taxonomic analysis. Comparative 16S rRNA gene sequencing demonstrated that the unknown organism was a member of the family Micrococcaceae and possessed a specific phylogenetic association with *Rothia dentocariosa* and *Stomatococcus mucilaginosus*. Phenotypically, the bacterium closely resembled *R. dentocariosa* and *S. mucilaginosus* but could be distinguished from these species by biochemical tests and electrophoretic analysis of whole-cell proteins. Based on both phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified in the genus *Rothia*, as *Rothia nasimurium* sp. nov. In addition, it is proposed that *S. mucilaginosus* be reclassified in the genus *Rothia*, as *Rothia mucilaginosus* comb. nov.

Keywords: *Rothia*, *Stomatococcus*, taxonomy, phylogeny, 16S rRNA

Rothia dentocariosa is found in the oral cavity and pharynx of man where it forms part of the normal microflora (Schaal, 1992). This species is also now recognized as an opportunistic pathogen causing septicaemia and endocarditis, as well as other serious infections (e.g. Schafer *et al.*, 1979; Pape *et al.*, 1979; Pers *et al.*, 1987; Minato & Abiko, 1984; Schiff & Kaplan, 1987). The taxonomic affinities of *R. dentocariosa* have always been controversial. Primarily because of its cellular morphology, the species has historically been associated with *Actinomyces* and related genera (Schaal, 1992). In recent years it has become apparent, however, that it is only remotely related to these taxa, and is phylogenetically a member of the family Micrococcaceae (Stackebrandt *et al.*, 1997), exhibiting a specific association with *Stomatococcus mucilaginosus*. Like *R. dentocariosa*, *S. mucilaginosus* is a resident of the human oral cavity and pharynx. *R. dentocariosa* is presently the only recognized species of the genus *Rothia*. There is, however, a considerable body of evidence indicating some bio-

chemical and serological heterogeneity within the species (e.g. Leshner *et al.*, 1974; Schofield & Schaal, 1981; Fotos *et al.*, 1984; Kronvall *et al.*, 1998). In addition, the existence of a second genomovar of *R. dentocariosa* has recently been described from humans (Kronvall *et al.*, 1998). It is currently not known whether *R. dentocariosa* or similar organisms occur in other animal species (Schaal, 1992). During the course of a study of unusual *Actinobacteria* from animals, we have characterized a novel Gram-positive, facultatively anaerobic coccus from the nose of a healthy mouse which phenotypically resembles the genus *Rothia*. Based on the results of a polyphasic taxonomic study, we propose a new species, *Rothia nasimurium*, for this bacterium.

Strain CCUG 35957^T was isolated from the nose of a healthy mouse. The unidentified organism was cultured on Columbia blood agar base supplemented with 5% defibrinated horse blood at 37 °C in air plus 5% CO₂. The strain was biochemically characterized by using the API rapid ID32 STREP, API CORYNE and API ZYM systems according to the manufacturer's instructions (API bioMérieux). Preparation of cellular protein extracts for PAGE analysis, densito-

The GenBank accession number for the 16S rRNA sequence of *Rothia nasimurium* CCUG 35957^T is AJ 131121.

Fig. 1. Dendrogram derived from the unweighted pair group average linkage of correlation coefficients (expressed as percentage values) between whole-cell protein patterns of *R. nasimurium* sp. nov. and some relatives. Duplicate cultures of strain CCUG 20499 were run to assess reproducibility.

metric analysis, normalization of the protein profiles and numerical analyses were performed as described by Pot *et al.* (1994) using the GelCompar 3.0 software package (Applied Maths). The similarity between all pairs of traces was expressed by the Pearson product moment correlation coefficient, converted for convenience to a percentage similarity value. Long-chain cellular fatty acids were examined using the MIDI system. Cells for fatty acid analysis were prepared on blood agar at 37 °C. The DNA G+C content was determined by thermal denaturation as described by Garvie (1978). The 16S rRNA genes of the isolate were

amplified by PCR and directly sequenced using a *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A, Applied Biosystems). The closest known relatives of the new isolate were determined by performing database searches. These sequences and those of other known related strains were retrieved from the GenBank or Ribosomal Database Project (RDP) libraries and aligned with the newly determined sequence using the program PILEUP (Devereux *et al.*, 1984). The resulting multiple sequence alignment was corrected manually and a distance matrix was calcu-

lated using the programs PRETTY and DNADIST (using the Kimura-2 correction parameter) (Felsenstein, 1989). A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR (Felsenstein, 1989). The stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989). The GenBank accession number of the 16S rRNA gene sequence of strain CCUG 35957^T is AJ 131121.

The unidentified isolate consisted of Gram-positive, non-motile, ovoid-shaped cells. The organism grew on blood agar producing a weak α -haemolytic reaction and was non-pigmented. It was facultatively anaerobic and catalase-positive. Using commercially available API systems, the isolate produced acid from glucose, lactose, maltose, methyl β -D-glucopyranoside, sucrose and trehalose, but failed to produce acid from L-arabinose, D-arabitol, cyclodextrin, glycogen, mannitol, melibiose, melezitose, N-acetylglucosamine, pullulan, raffinose, ribose, sorbitol, tagatose or D-xylose. Reactions for alanine phenylalanine proline arylamidase, cystine arylamidase, ester lipase C-8, β -galacturonidase, α -glucosidase, leucine arylamidase, valine arylamidase, pyrazinamidase and trypsin were positive, whereas those for alkaline phosphatase, arginine dihydrolase, esterase C-4, α -fucosidase, α -galactosidase, β -glucosidase, β -glucuronidase, lipase C14, α -mannosidase, β -mannosidase and urease were negative. The organism hydrolysed aesculin, reduced nitrate and was Voges-Proskauer-negative. In terms of its overall biochemical characteristics, the unidentified bacterium closely resembled *R. dentocariosa* and *S. mucilaginosus*. However, it differed from these species in producing acid from lactose and by its positive reactions for valine arylamidase, ester lipase C-8 (weak reaction) and trypsin (weak reaction). By contrast, *R. dentocariosa* (genomovar I and II) and *S. mucilaginosus* do not produce acid from lactose and are negative for these enzymes. An examination of the long-chain cellular fatty acids of the isolate revealed acids predominantly of the iso and anteiso methyl-branched types (composition: ai-C13, 2.0%; i-C14, 7.7%; C14:0, 1.3%; i-C15, 5.1%; ai-C15, 56.8%; C15:0, 0.5%; i-C16, 18.7%; C16:0, 2.5%; ai-C17, 5.4%). Determination of the fatty acid compositions of *R. dentocariosa* CCUG 35437^T (composition: i-C14, 4.0%; i-C15, 3.3%; ai-C15, 53.3%; C15:0, 2.2%; i-C16, 14.6%; C16:0, 6.0%; ai-C17, 16.9%), *R. dentocariosa* genomovar II CCUG 17835 (composition: i-C14, 3.5%; i-C15, 4.5%; ai-C15, 51.6%; i-C16, 17.3%; C16:0, 7.1%; ai-C17, 16.0%) and *S. mucilaginosus* CCUG 20962^T (composition: i-C14, 10.8%; i-C15, 5.9%; ai-C15, 45.7%; i-C16, 21.6%; C16:0, 9.6%; ai-C17, 6.4%) grown under similar conditions revealed very similar profiles to the unknown isolate, thereby reinforcing the affinity between these taxa. PAGE analysis of whole-cell protein patterns, however, further demonstrated that the unidentified organism was phenotypically distinct from both *R.*

dentocariosa and *S. mucilaginosus* (Fig. 1). To ascertain the phylogenetic position of the unknown isolate, its almost complete 16S rRNA gene sequence was determined and subjected to a comparative analysis. Sequence database searches revealed that the unknown bacterium was phylogenetically a member of the *Actinobacteria* and displayed a specific association with members of the family *Micrococcaceae* (Stackebrandt *et al.*, 1997). Highest sequence similarity was shown with *R. dentocariosa* (96.6%), *S. mucilaginosus* (96.6%), *Kocuria erythromyxa* (96.6%), *Kocuria rosea* (96.6%), *Kocuria kristinae* (95.3%), *Kocuria palustris* (95.5%), with other members of the *Micrococcaceae* displaying somewhat lower levels of relatedness (approx. 92–95%; data not shown). A tree constructed by the neighbour-joining method depicting the phylogenetic relationships of the unidentified organism is shown in Fig. 2 and unequivocally demonstrates that it represents a new species within the *Micrococcaceae*. The bacterium formed a distinct line branching from the base of a cluster which embraced *R. dentocariosa* (genomovar I and II) and *S. mucilaginosus*.

The polyphasic taxonomic analysis has shown that the unknown ovoid-shaped bacterium isolated from the nose of a mouse represents a hitherto unrecognized species within the *Micrococcaceae*. Phylogenetically, the bacterium forms a distinct subline and exhibits a specific association with the *Rothia-Stomatococcus* group of organisms. The clustering of the unknown mouse bacterium with this latter group was supported by a bootstrap resampling value of 100% (Fig. 2). The close relationship between the unknown organism and *Rothia-Stomatococcus* is supported by their close biochemical resemblance. Based on phylogenetic and phenotypic evidence, the unknown bacterium clearly merits classification as a new species, for which *Rothia* is the most appropriate genus. It has long been recognized that *R. dentocariosa* is phenotypically heterogeneous (Leshner *et al.*, 1974; Schofield & Schaal, 1981; Fotos *et al.*, 1984), raising the possibility of a second species within the genus *Rothia*. Indeed, a recent study using protein profiling and 16S rRNA gene sequencing has resulted in the recognition of a second species, designated *R. dentocariosa* genomovar II (Kronvall *et al.*, 1980). The latter genomovar has, however, not been formally named as it is not possible to biochemically distinguish it from authentic *R. dentocariosa*. Thus, given the distinct biochemical characteristics of the new murine isolate, the organism reported here should not be confused with earlier reports of variants within *R. dentocariosa*. In addition to biochemical differences, the murine bacterium also differs from *R. dentocariosa* strains (both genomovars I and II) in whole-cell protein profiles and by displaying >3% 16S rRNA sequence divergence. It is pertinent to note that *S. mucilaginosus* displays a closer phylogenetic affinity to *R. dentocariosa* than to the unknown bacterium. Hence the classification of the new mouse bacterium within the genus *Rothia* also

Fig. 2. Unrooted tree based on 16S rRNA analysis showing the phylogenetic relationships of *R. nasimurium* sp. nov. The bar represents 1% sequence divergence.

necessitates the assignment of *S. mucilaginosus* to this genus. We consider that the case for reclassifying *S. mucilaginosus* in the genus *Rothia* is very strong given their close biochemical similarity, and also on phylogenetic grounds (tree topology considerations and 16S rRNA sequence divergence values). Support for the close relationship between *Rothia* and *Stomatococcus* is also evident from PAGE analysis of whole-cell proteins. Indeed, based on whole-cell protein patterns, it is apparent that *S. mucilaginosus* phenotypically more closely resembles *R. dentocariosa* genomovar I (which includes the type strain of the species) than does *R. dentocariosa* genomovar II (Fig. 1; see also Kronvall *et al.*, 1998). Therefore, based on both phylogenetic and phenotypic evidence, we propose the unknown mouse bacterium be classified in the genus *Rothia* (Georg and Brown 1967), as *Rothia nasimurium* sp. nov. In addition, we propose the reclassification of *S. mucilaginosus* (Bergan and Kocur 1982) in the genus *Rothia*, as *Rothia mucilaginosus* comb. nov.

Description of *Rothia nasimurium* sp. nov.

Rothia nasimurium (na.si.mu'ri.um. M.L. pl. gen. n. *nasimurium* of the nose of mice).

Cells are Gram-positive, non-motile cocci. Produce a weak α -haemolysis on blood agar. Non-pigmented. Facultatively anaerobic and catalase-positive. Acid is produced from glucose, lactose, maltose, methyl β -D-glucopyranoside, sucrose and trehalose. Acid is not

produced from L-arabinose, D-arabitol, cyclodextrin, glycogen, mannitol, melibiose, melezitose, *N*-acetylglucosamine, pullulan, raffinose, ribose, sorbitol, tagatose or D-xylose. Alanine phenylalanine proline arylamidase, cystine arylamidase, ester lipase C-8, β -galacturonidase, α -glucosidase, leucine arylamidase, valine arylamidase, pyrazinamidase and trypsin are produced. Alkaline phosphatase, arginine dihydrolase, esterase C-4, α -fucosidase, α -galactosidase, β -glucosidase, β -glucuronidase, lipase C14, α -mannosidase, β -mannosidase, pyrrolydonyl arylamidase and urease are not produced. Acid phosphatase, chymotrypsin, pyroglutamic acid arylamidase, β -galactosidase, glycine tryptophan arylamidase may or may not be produced. Aesculin is hydrolysed but not hippurate. Nitrate is reduced. Voges-Proskauer-negative. Cellular fatty acids are mainly of the methyl-branched types with ai-C15 predominating. Isolated from the nose of a healthy mouse. Habitat unknown. The G + C content of the DNA is 56 mol%. The type strain is CCUG 35957^T.

Description of *Rothia mucilaginosus* (Bergan and Kocur 1982) comb. nov.

Rothia mucilaginosus (mu.ci.la.gi.no'sa. L. adj. *mucilaginosus* slimy).

The description of *Rothia mucilaginosus* is as given for *Stomatococcus mucilaginosus* by Bergan & Kocur (1982).

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