Reclassification of the species Kocuria erythromyxa (Brooks and Murray 1981) as Kocuria rosea (Flügge 1886)

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Comparison of phenotypic, chemotaxonomic and genomic properties of the type strains of Kocuria rosea and Kocuria erythromyxa indicates that these taxa are members of the same species. The conclusion is based upon 16S rDNA similarity of 99.9% [Rainey, F. A., et al. (1997). Int J Syst Bacteriol 47, 510-514], DNA-DNA reassociation of 95%, identical fatty acid patterns and almost identical physiological reactions against substrates provided by the BIOLOG plate. According to Rule 42 of the International Code of Nomenclature of Bacteria, which requires that the oldest legitimate epithet be retained when taxa of equal rank are united, Kocuria (Micrococcus) rosea (Flügge 1886) has priority over Kocuria (Deinococcus) erythromyxa (Brooks and Murray 1981).

Keywords: Kocuria erythromyxa, Kocuria rosea, reclassification

In their classification of members of genus Deinococcus based on lipid and cell wall amino acid composition, Embley et al. (1987) described the history of Deinococcus erythromyxa as chequered. The organism was described as 'Micrococcus erythromyxa' by Zopf (1891) and as 'Sarcina erythromyxa' by Chester (1901). Other combinations were 'Bacterium erythromyxa' (Migula, 1900), 'Bacillus erythromyxa' (Matzuschita, 1902) and 'Mycobacterium erythromyxa' (Krasil'nikov, 1941). The original culture was deposited in the Král Collection (Prague, now Czech Republic) and later deposited in other collections, such as the American Type Culture Collection (ATCC 187), Czech Collection of Microorganisms, Brno, Czech Republic (CCM 706), and the strain collection of the Department of Microbiology and Immunology of the University of Western Ontario (UWO 1045). The species was not included in the Approved Lists of Bacterial Names (Skerman et al., 1980). The strain 'Sarcina erythromyxa' CCM 706 was included in the study on the revised characterization of Micrococcus roseus (Kocur & Pálová, 1970) and its affiliation to this species was confirmed.

Based upon morphological properties and resistance to gamma radiation, M. roseus UWO 1045 was redescribed as the type strain of Deinococcus erythromyxa (Brooks & Murray, 1981). However, because of differences in cell wall structure and amino acid composition of peptidoglycan the inclusion of this species in the genus Deinococcus was considered tentative. Results of lipid and cell wall analysis obtained for the type strains of all validly described Deinococcus species together with significant differences between the G+C content of the DNA of D. erythromyxa strain UWO 1045T and the other type strains of Deinococcus, led Embley et al. (1987) to reconfirm the doubtful classification of D. erythromyxa as a species of Deinococcus. Analysis of the 16S rDNA (Rainey et al., 1997) of strain ATCC 187T demonstrated the close relatedness between this strain and members of Kocuria, a genus described in 1995 to embrace the species M. roseus, Micrococcus varians and Micrococcus sedentarius (Stackebrandt et al., 1995). While the primary structure of the 16S rDNA of strain ATCC 187T was 99.9% similar to that of Kocuria rosea DSM 20447T (= ATCC 186T = CCM 679T = UWO 1057T), the low binary DNA-DNA hybridization value of 21%, determined for these two type strains (Brooks et al., 1980), was taken as evidence of the separate species status of D. erythromyxa, which was subsequently transferred to Kocuria as Kocuria erythromyxa (Rainey et al., 1997). In this communication we will provide evidence that, on the basis of identical fatty acid profiles, a high DNA-DNA reassociation value, almost identical primary structures...
of 16S rDNA and almost identical physiological properties, *K. erythromyxa* must be considered to be synonymous with *K. rosea*.

The description of *Micrococcus roseus* UWO 1045 as the type strain of *Deinococcus erythromyxa* [species *incertae sedis* according to Brooks & Murray (1981)] was based mainly on two findings:

Firstly, strain UWO 1045 was found to be moderately resistant to gamma radiation ($D_{10} = 0.127-0.254$ Mrad), although the $D_{10}$ values were significantly lower than those reported for authentic *Deinococcus* species (resistant to 1.5 Mrad) and the $D_{10}$ value overlapped with that described for *M. roseus* UWO 1057T ($D_{10} = 0.075-0.157$ Mrad) (unpublished data of R. G. E. Murray, D. G. Storey & J. L. Whitby, as cited in Brooks & Murray, 1981).

Secondly, strain UWO 1045 was found to be unrelated to the type strain of *Micrococcus roseus* UWO 1057T by fatty acid analysis and by a low DNA–DNA reassociation value. Most emphasis was placed on the fatty acid profile; that described for *Micrococcus roseus* contained significant amounts of ai-C$_{15}$:0, while straight chain and monounsaturated acids dominated in the pattern of strain UWO 1045. Reinvestigation of the fatty acid composition of *Deinococcus erythromyxa* UWO 1045T by Embley et al. (1987) contradicted the previously published data in that the pattern was found to be very similar to that described by Brooks et al. (1980) for *Micrococcus roseus* UWO 1057T. As pointed out by Embley and co-workers, differences in the type of gas-chromatographic columns used for resolving positional isomers of fatty acids may have been the cause of the discrepancies. The fatty acid composition reported for strain *Deinococcus erythromyxa* UWO 1045T by Embley et al. (1987) was later verified by Rainey et al. (1997) for *Deinococcus erythromyxa* ATCC 1877T. Among other properties, the high similarity in the fatty acid composition between *Deinococcus erythromyxa* and *Kocuria* species was the basis for the transfer of *D. erythromyxa* to the genus *Kocuria* as *Kocuria erythromyxa* (Rainey et al., 1997).

The almost complete sequence similarity of 99.9% shared between the 16S rDNAs of *K. rosea* DSM 20447T (EMBL accession no. X87756) and *K. erythromyxa* ATCC1877T (EMBL accession no. Y11330; Koch et al., 1994) and the overall similarity in chemotaxonomic properties such as the composition of fatty acids and menaquinones (Rainey et al., 1997), amino acid composition of peptidoglycan and polar lipids (Embley et al., 1987), as well as the base composition of DNA (Brooks et al., 1980) contradicted the low DNA–DNA reassociation value of 21% found for these two organisms by Brooks et al. (1980). To check this parameter, which is crucial in the polyphasic approach to species description, the DNA–DNA reassociation value for the type strains of the two species was reinvestigated.

DNA was isolated as described by Cashion et al. (1977). DNA hybridization was carried out as described by Hulst et al. (1983) and Escara & Hutton (1980) using a Gilford System 2600 spectrophotometer equipped with a Gilford 2527-R thermoprogrammer and plotter. Renaturation rates were computed by the program TRANSFER.BAS (Jahnke, 1992). Two independent measurements of the reassociation gave values of 96 and 94%, which are about 70% higher than those described previously for the same pair of type strains. The similarity value of about 95% clearly indicates that the two strains belong to the same species. Similarities in physiological properties were tested using the BIOLOG GP-plate, System Release 3.50 (1993). Only two of the reactions tested were different: *K. rosea* DSM 20447T did not utilize propionic acid as a carbon source and did not degrade Tween 80, whereas *K. erythromyxa* CCM 706T did.

The placement of *K. rosea* and *K. erythromyxa* in the same species clearly contradicts the results of Brooks et al. (1980) and the conclusions of Rainey et al. (1997) and raises the question of whether the same strains were used in the different studies. The fatty acid

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**Table 1. Fatty acid composition of type strains of *Kocuria rosea* and *Kocuria erythromyxa* deposited in different culture collections**

<table>
<thead>
<tr>
<th>Species</th>
<th>Type strain</th>
<th>i-C$_{15}$:0</th>
<th>i-C$_{16}$:0</th>
<th>i-C$_{16}$:1</th>
<th>ai-C$_{15}$:0</th>
<th>ai-C$_{16}$:0</th>
<th>ai-C$_{16}$:1</th>
<th>ai-C$_{17}$:0</th>
<th>ai-C$_{17}$:1</th>
<th>ai-C$_{18}$:0</th>
<th>ai-C$_{18}$:1</th>
<th>ai-C$_{19}$:0</th>
<th>ai-C$_{20}$:0</th>
<th>ai-C$_{20}$:1</th>
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<tr>
<td><em>K. rosea</em></td>
<td>UWO 1057</td>
<td>1.6</td>
<td>1.7</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Brooks et al. (1980)</td>
</tr>
<tr>
<td></td>
<td>DSM 20447</td>
<td>1.0</td>
<td>1.45</td>
<td>1.01</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CCM 706</td>
<td>1.0</td>
<td>1.2</td>
<td>0.93</td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td>This study</td>
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<tr>
<td><em>K. erythromyxa</em></td>
<td>UWO 1045</td>
<td>1.9</td>
<td>1.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Embley et al. (1987)</td>
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<tr>
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<td>ATCC 187</td>
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<td>1.8</td>
<td>1.41</td>
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<td></td>
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<td></td>
<td></td>
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<td>Summarized by Rainey et al. (1997)</td>
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<tr>
<td></td>
<td>ATCC 187</td>
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<td>2.7</td>
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<td>CCM 706</td>
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</table>

Values less than 0.5% are not shown.

*Listed as 15:0iBr.*
composition of *Kocuria (Micrococcus) rosea* UWO 1057^T^, determined by Brooks *et al.* (1980), was verified in strain DSM 20447^T^ (Stackebrandt *et al.*, 1995). This, together with the results of phylogenetic analysis and other chemotaxonomic properties, led to the reclassification of *Micrococcus roseus* as *Kocuria rosea*. The present study confirms the fatty acid composition of *K. rosea* by investigation of strain CCM 679^T^.

Comparison of the 16S rDNA oligonucleotide catalogue of strain UWO 1057^T^ (Brooks *et al.*, 1980) with the oligonucleotide catalogue of *K. rosea* DSM 20447^T^, which was extracted from the almost complete sequence (Koch *et al.*, 1994), gave an $S_{AB}$ value of 0.93. This value can be extrapolated to about 99.0% sequence similarity, using the correlation graph of Woese (1987). The reason for not obtaining 100% similarity is due to the lack of oligonucleotides in the terminal regions of 16S rDNA for which no information is available. Consequently, there is no doubt that the identity of *Kocuria (Micrococcus) rosea* has been confirmed.

To confirm the identity of *Kocuria (Deinococcus) erythromyxa* the type strain of this species, CCM 706^T^ was investigated with respect to 16S rDNA sequence analysis and fatty acid composition. Results of 16S rDNA analysis of the almost complete sequence (1500 nucleotides) gave 100% identity with the previously analysed strain ATCC 187^T^ (Rainey *et al.*, 1997) and 99.9% similarity with *K. rosea* DSM 20447^T^. The fatty acid spectrum was very similar to that determined previously for strain UWO 1045^T^ (Embley *et al.*, 1987) and strain ATCC 187^T^ (Rainey *et al.*, 1997). When analysed in the same laboratory at the DSMZ, the spectra obtained for *K. rosea* and *K. erythromyxa* were qualitatively very similar and differed only slightly in the quantity of a few fatty acids (i.e. i-C_{15:0} and ai-C_{17:1}O) (Table 1).

Consequently, we have no doubt that strains deposited as the type strain of *Kocuria erythromyxa* in the ATCC, CCM and the strain collection of the Department of Microbiology and Immunology of the University of Western Ontario (UWO), are identical. The type strain of this species shows such a high morphological, chemotaxonomic, genomic and physiological similarity to the type strain of *K. rosea*, that we propose the transfer of members of *K. erythromyxa* (Brooks & Murray, 1981; Rainey *et al.*, 1997) to *Kocuria rosea* (Stackebrandt *et al.*, 1995), which, on the basis of Rule 42 of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992) has priority in the union of species of the same rank. As a result of the union, the species description of *K. rosea* does not need to be emended.

The fate of the species `'Kocuria erythromyxa'" is a good example of the role of genomic and epigenetic properties in taxonomic changes over the past 30 years. It also emphasizes the necessity to carefully recheck published data which play an important role in current species definition.

### Acknowledgements

The authors wish to thank Helmut Prauser, Jena, for giving us advice on the history of strains investigated in this study.

### References


