Phylogenetic analysis of the genera *Rhodococcus* and *Nocardia* and evidence for the evolutionary origin of the genus *Nocardia* from within the radiation of *Rhodococcus* species

Frederick A. Rainey, Jutta Burghardt, Reiner M. Kroppenstedt, Stefan Klatte and Erko Stackebrandt

Author for correspondence: Erko Stackebrandt. Tel: +49 531 2616 100. Fax: +49 531 2616 418.
e-mail: Stackebrandt@venus.gbf-braunschweig.d400.de

DSM–Deutsche Sammlung
von Mikroorganismen und
Zellkulturen GmbH,
Mascheroder Weg 1b, 38124
Braunschweig, Germany

16S rDNA analysis was performed on 32 strains of 26 species of the genera *Rhodococcus* and *Nocardia* in order to investigate the phylogenetic structure of these genera within the radiation of other mycolic-acid-containing genera such as *Corynebacterium*, *Dietzia*, *Gordona*, *Mycobacterium* and *Tsukamurella*. The genus *Rhodococcus* shows a complex structure, consisting of six phylogenetically equidistant lineages. The genus *Nocardia* does not appear to be a sister taxon of *Rhodococcus* but branches off from within the radiation of *Rhodococcus*; thus its species can be considered to be derived from a *Rhodococcus* ancestor. The main known phenotypic feature that separates *Nocardia* species from those of *Rhodococcus* appears to be the presence of a cyclic component in menaquinone of the MK-8(H₄) type.

Keywords: 16S rDNA sequences, phylogeny, *Nocardia*, *Rhodococcus*, CMN group of *Actinomycetales*

INTRODUCTION

In the chapter ‘The taxonomy of the genus *Nocardia*: some light at the end of the tunnel?’, Lechevalier (1976) provides an excellent summary of the history of the genus *Nocardia*, from its original description (Trevisan, 1889) to the advent of numerical taxonomy and chemotaxonomy. After reading of the many attempts to define an authentic *Nocardia* species, actinomycologists who entered taxonomy in the 1980s realized how difficult it must have been to delineate taxa when emphasis was placed mainly on morphological criteria. The differentiation of species today recognized as being clearly different from *Nocardia asteroides* and its relatives took about ten years and extensive numerical phenetic studies, only some of which should be mentioned here (Goodfellow, 1971; Goodfellow *et al.*, 1974; Kurup & Schmitt, 1973; Tsukamura, 1969, 1974). As a result of these studies, it was agreed that (i) *N. asteroides* as defined by Gordon & Mihm (1962) was heterogeneous, (ii) *N. asteroides* clusters closely with *N. brasiliensis* and ‘*N. caviae*’, (iii) the exact position of the rhodochrous complex remained unclear, but constitutes a heterogeneous collection of organisms distinct from nocardiae and mycobacteria, and (iv) *N.*

madurae (and *N. pelletieri* when tested) clustered separately from the other strains tested.

The taxonomic status of the rhodochrous complex has been a matter of controversy, in that these organisms were classified as *Nocardia* (Lechevalier *et al.*, 1971), *Gordona* (Tsukamura, 1971) and *Proactinomyces* (Bradley & Bond, 1974). A thorough co-operative numerical phenetic study, supplemented with an analysis of precipitinogenic properties and types of mycolic acids, of 78 rhodochrous cultures and additional marker cultures from *Mycobacterium*, *Nocardia* and *Actinomadura* (Goodfellow & Orchard, 1974; Minnikin *et al.*, 1975) confirmed the distinctness of the rhodochrous complex from these neighbouring taxa. Possible membership of the rhodochrous strains to *Mycobacterium*, *Nocardia*, *Actinomadura* and *Oerskovia* could be excluded (Goodfellow, 1971; Goodfellow *et al.*, 1974; Bousfield & Goodfellow, 1976). This was supported by the results of antibiotic sensitivity (Goodfellow & Orchard, 1974), chemical analysis (Ratledge & Patel, 1974), DNA hybridization (Bradley & Mordarski, 1976; Mordarski *et al.*, 1977) and serology (Ridell & Norlin, 1973) studies. Interestingly, Bradley (1971) found cultures from the rhodochrous complex to be more closely related

to *Nocardia* than to *Mycobacterium*. Based on the results of these studies, and on a comprehensive numerical taxonomic classification study, the generic name *Rhodococcus* was proposed for the 'rhodochrous' taxon (Goodfellow & Alderson, 1977), thus giving *Rhodococcus* Zopf 1891 priority over *Proactinomyces* (Jensen, 1953), *Jensenia* (Bisset & Moore, 1949) and *Gordona* (Tsukamura, 1971). *R. rhodochrous* was designated the type species and eight additional species were described: *R. bronchialis*, *R. coprophilus*, *R. corallinus*, *R. erythropolis*, *R. equi*, *R. rhodnii*, *R. ruber*, *R. rubropertinctus* and *R. terrae*.

More than 20 species have been allocated to the genus *Rhodococcus*, on the basis of the combination of morphological and chemotaxonomic characteristics. Nevertheless, the delineation of *Rhodococcus* from *Nocardia* remains problematic, as hardly a single character of diagnostic value has been found to distinguish members of the two genera (Goodfellow, 1992). Only recently, by the finding of a cyclic component in the menaquinone fraction of the MK-8(H₄) type exclusively in members of *Nocardia*, has a character of differentiating value been described (Howard *et al.*, 1986). Molecular studies applying sequence analysis of 5S rRNA, 16S rRNA cataloguing or reverse transcriptase of 16S rRNA, were performed on only a few species of each of the two genera (Collins *et al.*, 1989; Stackebrandt *et al.*, 1988; Luehrsen *et al.*, 1989). However, it became apparent that the genus *Rhodococcus* contained several species that did not group phylogenetically with *R. fascians* and *R. erythropolis* (the type species *R. rhodochrous* has not been included in phylogenetic analyses so far). As the phylogeny of the individual strains was supported by chemotaxonomic properties, two new genera were described, *Gordona* for *R. bronchialis*, *R. rubropertinctus* and *R. terrae* (Stackebrandt *et al.*, 1988), and *Tsukamurella paurometabola* for *R. aurantiacus* and *Corynebacterium paurometabolum* (Collins *et al.*, 1988). In order to investigate the coherency of *Rhodococcus* and its phylogenetic relatedness to members of the genus *Nocardia*, in this study the majority of *Rhodococcus* species and several species of *Nocardia* were investigated by 16S rDNA sequence analysis and the sequences compared to other genera of the *Corynebacterium-Mycobacterium-Nocardia* group (enlarged CMN group) of mycolic acid-containing actinomycetes.

METHODS

Bacterial strains and cultivation. Strains analysed, with their 16S rDNA EMBL accession numbers, are listed in Table 1. All strains were cultivated as indicated in the DSM Catalogue of Strains (1993).

16S rDNA sequencing. Extraction of genomic DNA and amplification of the 16S rDNA was carried out as described previously (Rainey *et al.*, 1992). PCR products were sequenced directly using the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's protocol. The sequence reactions were electrophoresed using the Applied Biosystems 373A DNA sequencer.

Sequences were manually aligned with published sequences from representatives of the main actinomycete sublines of descent included in the Ribosomal Database Project (Larsen

Table 1. Strains analysed, with their 16S rDNA accession numbers

Organism	Strain*	EMBL accession no.
<i>Gordona</i>		
<i>G. bronchialis</i>	DSM 43247 ^T	X79287
<i>G. rubropertincta</i>	DSM 43197 ^T	X80632
<i>G. sputi</i>	DSM 43896 ^T	X80634
<i>G. terrae</i>	DSM 43249 ^T	X79286
<i>Nocardia</i>		
<i>N. amarae</i> †	DSM 43392 ^T	X80635
<i>N. asteroides</i>	DSM 43757 ^T	X80606
<i>N. brasiliensis</i>	DSM 43758 ^T	X80608
<i>N. calcarum</i>	DSM 43188 ^T	X80618
<i>N. carnea</i>	DSM 43397 ^T	X80607
<i>N. corynebacteroides</i>	DSM 20151 ^T	X80615
<i>N. farcinica</i>	DSM 43665 ^T	X80610
<i>N. otitidiscalearum</i>	DSM 43242 ^T	X80611
<i>N. restricta</i>	DSM 43199 ^T	X80613
<i>N. transvalensis</i>	DSM 43405 ^T	X80609
<i>Rhodococcus</i>		
<i>R. aichiensis</i> ‡	DSM 43978 ^T	X80633
<i>R. chlorophenolicus</i> ‡	DSM 43826 ^T	X79292
<i>R. chubuensis</i> §	DSM 44019 ^T	X80627
<i>R. coprophilus</i>	DSM 43347 ^T	X80626
<i>R. equi</i>	DSM 20307 ^T	X80614
<i>R. erythropolis</i>	DSM 43066 ^T	X79289
<i>R. fascians</i>	DSM 20669 ^T	X79186
<i>R. globerulus</i>	DSM 43954 ^T	X80619
<i>R. globerulus</i>	DSM 43953	X80620
<i>R. luteus</i>	DSM 43673 ^T	X79187
<i>R. marinonascens</i>	DSM 43752 ^T	X80617
<i>R. maris</i>	DSM 43672 ^T	X79290
<i>R. maris</i>	DSM 46102	X79291
<i>R. opacus</i>	DSM 43205 ^T	X80630
<i>R. opacus</i>	DSM 43206	X80631
<i>R. rhodnii</i>	DSM 43336 ^T	X80621
<i>R. rhodnii</i>	DSM 43337	X80622
<i>R. rhodnii</i>	DSM 43959	X80623
<i>R. rhodochrous</i>	DSM 43241 ^T	X79288
<i>R. roseus</i>	DSM 43274 ^T	X80624
<i>R. ruber</i>	DSM 43338 ^T	X80625
<i>Rhodococcus</i> sp.	DSM 43943	X80616
<i>Tsukamurella</i>		
<i>T. paurometabola</i>	DSM 20162 ^T	X80628
<i>Corynebacterium</i>		
<i>C. glutamicum</i>	DSM 20300 ^T	X80629

* A superscript T denotes a type strain.

† *N. amarae* and *R. aichiensis* were recently renamed *Gordona amarae* and *Gordona aichiense* (Klatte *et al.*, 1994a).

‡ Recently renamed *Mycobacterium chlorophenolicum* (Häggblom *et al.*, 1994).

§ Recently renamed *Gordona sputi* (Riegel *et al.*, 1994).

et al., 1993) and from our own entries. Pairwise evolutionary distances were computed using the correction of Jukes & Cantor (1969). Phylogenetic analyses were carried out by neighbour-joining analysis (Saitou & Nei, 1987). Bootstrap values, based on the analysis of 1000 trees of 814 polymorphic sites, were calculated using the programs NJFIND and NJBOOT.

DNA isolation and characterization. Isolation of DNA and DNA–DNA hybridization followed described procedures (Escara & Hutton, 1980; Huß *et al.*, 1983).

RESULTS AND DISCUSSION

The almost complete 16S rDNA sequence was determined for 10 *Nocardia* species and 22 strains of 16 *Rhodococcus* species. In order to compare sequences of similar length and the same quality, sequences were redetermined for several members of the genera *Nocardia*, *Rhodococcus*, *Gordona*, *Tsukamurella* and *Corynebacterium*, sequences of which have been published previously (compiled by Larsen *et al.*, 1993) (Table 1). The length of the sequences comprised 95% of the *E. coli* 16S rDNA sequence (Brosius *et al.*, 1978).

The phylogenetic relationships between the strains sequenced in this study and four reference strains each of the genera *Mycobacterium* and *Corynebacterium* are shown in Fig. 1. The corresponding 16S rDNA similarity values are available from the authors on request. As shown before, the genera *Corynebacterium* (Collins *et al.*, 1989), which forms the deepest-branching lineage within the group of mycolic-acid-containing organisms, thus the root (Stackebrandt & Smida, 1988), *Mycobacterium* (Stackebrandt & Smida, 1988), *Tsukamurella* (Collins *et al.*, 1988) and *Gordona* (Stackebrandt *et al.*, 1988) represent phylogenetically well-defined taxa. Previous studies, which included only a few *Rhodococcus* and *Nocardia* species, could not provide an insight into the inter- and intra-generic relationships of these two genera (Collins *et al.*, 1988; Stackebrandt *et al.*, 1988). The data obtained with an almost complete set of validly described *Rhodococcus* species (*R. zopfii* has been published only recently and was not included in this study) reveal a surprising phylogenetic diversity. With the exception of *R. chlorophenolicus*, which has been recently reclassified as a *Mycobacterium* species (Häggblom *et al.*, 1994), the remaining 14 species are found to represent, or to be members of, seven lines of descent.

The genus *Nocardia*

The majority of *Nocardia* species are related to the type species *N. asteroides*. However, *N. restricta* and *N. calcarea* group closely with *R. equi* and *R. erythropolis* respectively, while *N. corynebacteroides* forms an individual branch within *Rhodococcus*, and *N. amarae* clusters with *Gordona*. The transfer of this species into *Gordona* as *G. amarae* has recently been proposed (Klatte *et al.*, 1994a). The taxonomic conclusion of Goodfellow (1986) that *N. restricta* is a synonym of *R. equi*, and that *N. calcarea* is a synonym of *R. erythropolis*, is confirmed at the phylogenetic level (see below) and by results of DNA–DNA reassociation, which

show these pairs of organisms to be genomically almost indistinguishable (96 and 93% DNA similarity, respectively). The exclusion of *N. corynebacteroides* from the genus *Nocardia* and its transfer to *Rhodococcus* as a synonym of *R. globerulus* has been proposed previously (Goodfellow, 1986). We confirm the conclusion that *N. corynebacteroides* is not a member of *Nocardia* but rather of *Rhodococcus*, but the phylogenetic data exclude the possibility that the type strain of this species is a strain of *R. globerulus* (96.7–96.8% sequence similarity). Except for the type strains of *N. farcinica* and *N. otitidiscaviarum*, the other authentic members of *Nocardia* are phylogenetically well separated and their species character is confirmed. *N. asteroides* appears to be closely related to *N. carnea*, while *N. brasiliensis* and *N. transvalensis* are another pair of closely related species. The high degree of relatedness of the two pathogenic and soil-dwelling organisms (type strains) of *N. farcinica* and *N. otitidiscaviarum* is unexpected. DNA–DNA similarity values of 85% indicate that, based on priority of the epithet '*farcinica*', *N. otitidiscaviarum* could be considered a synonym of *N. farcinica*.

The genus *Rhodococcus*

rDNA group I. The type species of *Rhodococcus*, *R. rhodochrous*, together with *R. roseus*, *R. ruber* and *R. coprophilus* form a cluster (95.7–99.2% sequence similarity). The high degree of relatedness between *R. rhodochrous* and *R. roseus* (99.2%) is supported by DNA–DNA reassociation values of 80% and similar chemotaxonomic properties (Rainey *et al.*, 1995b), indicating that *R. roseus* could be considered a strain of *R. rhodochrous*. The lack of high 16S rDNA relationship between these two organisms and *R. ruber* is supported by DNA reassociation values of less than about 40%.

rDNA group II. This distinct lineage is composed of three strains of *R. rhodnii* (100% sequence similarity).

rDNA group III. This group contains two yellow-pigmented *Rhodococcus* species, *R. fascians* and *R. luteus*, showing 99.9% sequence similarity. Based on high DNA–DNA similarity values of 79% and chemotaxonomic evidence, *R. luteus* has recently been proposed as a later subjective synonym of *R. fascians* (Klatte *et al.*, 1994a).

rDNA group IV. Group IV is the largest *Rhodococcus* cluster, containing four phylogenetically well-separated species (97.2–98.5% similarity), an undescribed *Rhodococcus* isolate and *Nocardia calcarea*. *R. opacus* has recently been described for a group of organisms shown to be nutritionally versatile (Klatte *et al.*, 1994c). The classification of *N. calcarea* as a synonym of *R. erythropolis* (Goodfellow, 1986) is confirmed by 16S rDNA analysis and DNA–DNA hybridization. The close phylogenetic position of the phage-propagating strain DSM 43943, isolated from forest soil, next to the obligate marine and psychrotrophic species *R. marinonascens* (98.9% sequence similarity) is surprising. As no physiological and chemotaxonomic data are as yet available on strain DSM 43943, taxonomic conclusions cannot be drawn.

Fig. 1. Phylogenetic dendrogram based on the comparison of 16S rDNA sequences of *Rhodococcus* and *Nocardia* species and their relatives. The branching pattern was generated by the neighbour-joining method (Saitou & Nei, 1987). The bar represents five inferred substitutions per 100 nucleotides.

rDNA group V. This single-species group is composed of *Nocardia corynebacteroides*, clustering within the radiation of *Rhodococcus* species. Thus we confirm the transfer of this species into *Rhodococcus* as proposed by Goodfellow (1986). However, we cannot confirm the conclusion that *N. corynebacteroides* constitutes a strain of *R. globerulus* (96.7% sequence similarity), a member of rDNA group IV. *N. corynebacteroides* lacks the *Nocardia*-specific signature of menaquinone of the MK-8(H₄cyelic) type, thus substantiating its removal from the genus *Nocardia*.

rDNA group VI. The two species of this subline of descent, *N. restricta* and *R. equi*, considered to be synonyms by Goodfellow (1986), also share high sequence similarity of 100% and a DNA reassociation value of 96%. It appears from the phylogenetic tree that the *R. equi* line of descent and members of *Nocardia* share a common

ancestry, but, as mentioned above, the statistical significance for this branching is not high.

The genus *Gordona*

This genus was established as a home for misclassified *Rhodococcus* species, which clustered separately in a phylogenetic study and showed significant chemotaxonomic differences from the majority of *Rhodococcus* species (Stackebrandt *et al.*, 1988). Recently *R. aichiensis* and *N. amarae* have been transferred to the genus *Gordona* (Klatte *et al.*, 1994b). Here we report that *R. chubuensis* is also a member of *Gordona*, showing a high 16S rDNA similarity value of 99.8%. The transfer of *R. chubuensis* into *G. sputi* has to await further genomic and comparative phenotypic studies.

The genus *Dietzia*

This genus has recently been proposed for two strains formerly classified as *Rhodococcus maris*. The branching point of these strains adjacent to members of *Corynebacterium* and *Tsukamurella paurometabola*, together with the presence of characters distinguishing *R. maris* from all other mycolic-acid-containing taxa, led to the description of a new genus *Dietzia*, as *Dietzia maris* (Rainey *et al.*, 1995a).

Conclusion

The study presented here indicates the phylogenetic position of the majority of *Rhodococcus* species and a substantial number of *Nocardia* species. The results add to the ongoing attempts to improve the general taxonomic status of *Rhodococcus* by excluding misclassified strains and identifying overclassified species. The number of validly described *Rhodococcus* species has been reduced by application of phylogenetic and chemotaxonomic techniques from 23 to 10 over the last six years. Former *Rhodococcus* strains are found in the genera *Dietzia*, *Gordona*, *Mycobacterium* and *Tsukamurella*, or have lost their species status through synonymy with other species. The generally accepted genotypic and phenotypic diversity of morphologically similar organisms may have been the problem of interpreting the results of earlier numerical phenetic studies (e.g. Tsukamura, 1969, 1974; Goodfellow, 1971; Goodfellow *et al.*, 1974; Goodfellow & Alderson, 1977), which are not suitable to reliably determine relatedness at the genus level.

The phylogenetic depth of the authentic genus *Rhodococcus* is comparable to that of *Corynebacterium*, *Mycobacterium* and *Gordona*, consisting of several individual sublines of descent. The most interesting result from an evolutionary aspect is the finding that the genus *Nocardia* does not appear to be a sister taxon of *Rhodococcus* or any other genus of the enlarged CMN group, but can be considered a branch developing from within the radiation of *Rhodococcus* species. The bootstrap value determined for the branching point that separates the genera *Nocardia* and *Rhodococcus*, is 71 %, which can be considered significant. Thus, it seems unlikely that the position of the authentic *Nocardia* species radiating from within *Rhodococcus* will change to an extent that it would represent an individual line, equivalent to those found for *Rhodococcus*, *Gordona* or other genera depicted in Fig. 1. The bootstrap value determined for the branching point of the *Nocardia*/*Rhodococcus* cluster from the other genera shown in Fig. 1 is as high as 59 %. On the other hand, the individual sublines defined by *Rhodococcus* species and misclassified *Nocardia* species (clusters I–VI) are defined by low bootstrap values (often below 16 %) and hence the branching order of these sublines may change to some extent with the number of reference and outgroup species included in the analysis. The inclusion of an outgroup organism such as *Arthrobacter globiformis* shows the *Corynebacterium* lineage to represent the position of the root in Fig. 1, thus excluding the possibility that the root could separate the genus *Nocardia* from the genus

Rhodococcus. As compared to properties defining *Rhodococcus* species it is not just the morphology but mainly the presence of a unique menaquinone type that has been identified as a *Nocardia*-specific character. It could be speculated that these features evolved from ancestral states present in *Rhodococcus* species of hitherto unknown affiliation. In order to obtain a more complete picture of the diversity of the genus *Nocardia*, it appears necessary to analyse the remaining species using both phylogenetic and chemotaxonomic approaches.

REFERENCES

- Bisset, K. A. & Moore, F. W. (1949). The relationship of certain branched bacterial genera. *J Gen Microbiol* **3**, 387–391.
- Bousfield, I. J. & Goodfellow, M. (1976). The 'rhodochrous' complex and its relationships with allied taxa. In *The Biology of Nocardiae*, pp. 39–65. Edited by M. Goodfellow, G. H. Brownell & J. A. Serrano. London: Academic Press.
- Bradley, S. G. (1971). Criteria for definition of *Mycobacterium*, *Nocardia* and the rhodochrous complex. *Adv Front Plant Sci* **28**, 349–362.
- Bradley, S. G. & Bond, J. S. (1974). Taxonomic criteria for mycobacteria and nocardia. *Adv Appl Microbiol* **18**, 131–190.
- Bradley, S. G. & Mordarski, M. (1976). Association of poly-deoxyribonucleotides of deoxyribonucleic acids from nocardioform bacteria. In *The Biology of Nocardiae*, pp. 310–336. Edited by M. Goodfellow, G. H. Brownell & J. A. Serrano. London: Academic Press.
- Brosius, J., Palmer, J. J., Kennedy, J. P. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal gene from *Escherichia coli*. *Proc Natl Acad Sci USA* **75**, 4801–4805.
- Collins, M. D., Smida, J., Dorsch, M. & Stackebrandt, E. (1988). *Tsukamurella* gen. nov., harboring *Corynebacterium paurometabolum* and *Rhodococcus aurantiacus*. *Int J Syst Bacteriol* **38**, 385–391.
- Collins, M. D., Smida, J. & Stackebrandt, E. (1989). Phylogenetic evidence for the transfer of *Caseobacter polymorphus* (Crombach) to the genus *Corynebacterium*. *Int J Syst Bacteriol* **39**, 7–9.
- Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethylsulphoxide solutions: acceleration of renaturation rate. *Biopolymers* **19**, 1315–1327.
- Goodfellow, M. (1971). Numerical taxonomy of some nocardioform bacteria. *J Gen Microbiol* **69**, 33–80.
- Goodfellow, M. (1986). Genus *Rhodococcus*. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1472–1481. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Goodfellow, M. (1992). The family Nocardiaceae. In *The Prokaryotes*, pp. 1188–1212. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Goodfellow, M. & Alderson, G. (1977). The actinomycete genus *Rhodococcus*: a home for the 'rhodochrous' complex. *J Gen Microbiol* **100**, 99–122.
- Goodfellow, M. & Orchard, V. A. (1974). Antibiotic sensitivity of some nocardioform bacteria and its value as a criterion for taxonomy. *J Gen Microbiol* **83**, 375–387.
- Goodfellow, M., Lind, A., Mordarska, H., Pattyn, S. & Tsukamura, M. (1974). A co-operative numerical analysis of cultures considered to belong to the 'rhodochrous' taxon. *J Gen Microbiol* **85**, 291–302.
- Gordon, R. E. & Mihm, J. M. (1957). A comparative study of some strains received as nocardiae. *J. Bacteriol* **73**, 15–27.

- Häggblom, M. M., Liesa, L. J., Nohynek, N., Palleroni, N. J., Kronqvist, K., Nurmiäho-Lassila, E.-L., Salkinoja-Salonen, M. S., Klatte, S. & Kroppenstedt, R. M. (1994). Transfer of polychlorophenol-degrading *Rhodococcus chlorophenolicus* (Apajalahti *et al.*, 1986) to the genus *Mycobacterium* as *Mycobacterium chlorophenolicum* comb. nov. *Int J Syst Bacteriol* **44**, 485–493.
- Howard, O. W., Grund, E., Kroppenstedt, R. M. & Collins, M. D. (1986). Structural determination of a new naturally occurring cyclic vitamin K. *Biochem Biophys Res Commun* **140**, 916–923.
- Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Jensen, H. L. (1953). The genus *Nocardia* (or *Proactinomyces*) and its separation from other actinomycetales, with some reflections on the phylogeny of the actinomycetes. In: *Actinomycetales, Morphology, Biology and Systematics* (Symposium VI International Congress of Microbiology, Rome), pp. 69–75.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Klatte, S., Jahnke, K.-D., Kroppenstedt, R. M., Rainey, F. & Stackebrandt, E. (1994a). *Rhodococcus luteus* is a later subjective synonym of *Rhodococcus fascians*. *Int J Syst Bacteriol* **44**, 627–630.
- Klatte, S., Rainey, F. A. & Kroppenstedt, R. M. (1994b). Transfer of *Rhodococcus aichiensis* Tsukamura 1982 and *Nocardia amarae* Lechevalier and Lechevalier 1974 to the genus *Gordona* as *Gordona aichiense* comb. nov. and *Gordona amarae* comb. nov. *Int J Syst Bacteriol* **44**, 769–773.
- Klatte, S., Kroppenstedt, R. M. & Rainey, F. A. (1994c). *Rhodococcus opacus* sp. nov., and unusual nutritionally versatile *Rhodococcus* species. *Syst Appl Microbiol* (in press).
- Kurup, P. V. & Schmitt, J. A. (1973). Numerical taxonomy of *Nocardia*. *Can J Microbiol* **19**, 1035–1048.
- Larsen, N., Olsen, G. J., Maidak, B. L., McCaughey, M., Overbeek, J. R., Macke, T. J., Marsh, T. L. & Woese, C. R. (1993). The ribosomal database project. *Nucleic Acids Res* **21**, 3021–3023.
- Lechevalier, M. P. (1976). The taxonomy of the genus *Nocardia*: some light at the end of the tunnel? In *The Biology of the Nocardiae*, pp. 1–38. Edited by M. Goodfellow, G. H. Brownell & J. A. Serrano. London: Academic Press.
- Lechevalier, M. P., Horan, A. C. & Lechevalier, H. A. (1971). Lipid composition in the classification of nocardiae and mycobacteria. *J Bacteriol* **105**, 313–318.
- Luehrsén, K., Woese, C. R., Wolters, J. & Stackebrandt, E. (1989). Nucleotide sequence of the 5S rRNA from *Rhodococcus fascians*. *Nucleic Acids Res* **17**, 7378.
- Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolsates. *J Gen Microbiol* **88**, 200–204.
- Mordarski, M., Goodfellow, M., Szyba, K., Pulverer, G. & Tkacz, A. (1977). Classification of the 'rhodochrous' complex and allied taxa based upon deoxyribonucleic acid reassociation. *Int J Syst Bacteriol* **27**, 31–37.
- Rainey, F. A., Dorsch, M., Morgan, H. W. & Stackebrandt, E. (1992). 16S rDNA analysis of *Spirochaeta thermophila*: its phylogenetic position and implications for the systematics of the order *Spirochaetales*. *Syst Appl Microbiol* **15**, 197–202.
- Rainey, F. A., Klatte, S., Kroppenstedt, R. M. & Stackebrandt, E. (1995a). *Dietzia*, a new genus including *Dietzia maris* comb. nov. formerly *Rhodococcus maris*. *Int J Syst Bacteriol* **45** (in press).
- Rainey, F. A., Burghardt, J., Klatte, S. & Stackebrandt, E. (1995b). Polyphasic evidence for the transfer of *Rhodococcus roseus* to *Rhodococcus rhodochrous*. *Int J Syst Bacteriol* **45** (in press).
- Ratledge, C. & Patel, P. V. (1974). Lipid-soluble, iron-binding compounds in *Nocardia*, 'M'. *rhodochrous*, and *Mycobacterium*. *Proceedings of the First International Conference on the Biology of Nocardiae*, Merida, Venezuela, pp. 58–59.
- Riddel, M. & Norlin, M. (1973). Serological study of *Nocardia* by using mycobacterial precipitation reference systems. *J Bacteriol* **113**, 1–7.
- Riegel, P., Kamme-Fotso, M. V., DeBriel, D., Prévost, G., Jehl, F., Piémont, Y. & Monteil, H. (1994). *Rhodococcus chubuensis* Tsukamura 1982 is a later subjective synonym of *Gordona sputi* (Tsukamura 1978) Stackebrandt 1989 comb. nov. *Int J Syst Bacteriol* **44**, 764–768.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Stackebrandt, E. & Smida, J. (1988). The phylogeny of the genus *Mycobacterium* and development of rDNA probes for rapid identification of slow growing mycobacteria. In *The Biology of the Actinomycetes*, pp. 244–250. Edited by Y. Okami, T. Beppu & H. Ogawara. Tokyo: Japan Scientific Societies Press.
- Stackebrandt, E., Smida, J. & Collins, M. D. (1988). Evidence of phylogenetic heterogeneity within the genus *Rhodococcus*: revival of the genus *Gordona* (Tsukamura). *J Appl Microbiol* **34**, 341–348.
- Trevisan, V. (1889). *I Generi e le Specie delle Batteriacee*, p. 9. Milan: Zanaboni & Gabuzzi.
- Tsukamura, M. (1969). Numerical analysis of the genus *Nocardia*. *J Gen Microbiol* **56**, 265–287.
- Tsukamura, M. (1971). Proposal of a new genus, *Gordona*, for slightly acid-fast organisms occurring in sputa of patients with pulmonary disease and in soil. *J Gen Microbiol* **68**, 15–26.
- Tsukamura, M. (1974). A numerical taxonomic study of the relationship between *Mycobacterium*, the rhodochrous group and *Nocardia*. *Proceedings of the First International Conference on the Biology of Nocardiae*, Merida, Venezuela, pp. 10–17.

Received 3 August 1994; revised 6 October 1994; accepted 24 October 1994.