NOTE

***Pelczaria aurantia* ATCC 49321^T (= DSM 12801^T) is a strain of *Kocuria rosea* (Flügge 1886) Stackebrandt *et al.* 1995**

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Phylogenetic and chemotaxonomic analyses of *Pelczaria aurantia* ATCC 49321^T (= DSM 12801^T) indicate that this species is very closely related to *Kocuria rosea*. The DNA–DNA reassociation value of 87.1% determined for the type strains of the two species supports this finding. The results of phylogenetic analysis of the 16S rDNA of a subculture of the original strain of *Pelczaria aurantia*, deposited at the National Institutes of Health, Bethesda, MD, USA, as '*Neisseria aurantia*', are identical to those for strain ATCC 49321^T and indicate that *Pelczaria aurantia* ATCC 49321^T is an authentic subculture of the original culture described by Poston (1993). On the basis of these findings it is concluded that *P. aurantia* ATCC 49321^T and *K. rosea* DSM 20447^T are members of the same taxon. The taxonomic consequences of this union are discussed.

Keywords: *Pelczaria aurantia*, *Kocuria rosea*, *Actinobacteria*, *Micrococcineae*

Pelczaria aurantia ATCC 49321^T was proposed as a new species within a new genus (Poston, 1993) on the basis of the fact that it was a Gram-positive coccus, often appearing as a doublet or tetrad, exhibiting histidine in the cell wall and a DNA G + C content of 59 mol%. Polar lipid analysis indicated that the phospholipids phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine were present and the major carotenoid pigment was tentatively identified as rhodopin. On the basis of the fact that it shared only very low DNA similarities with Gram-positive and Gram-negative cocci as determined by DNA reassociation studies (< 20%), in addition to the fact that there was also no significant RNA–DNA hybridization (< 5%) to the same range of taxa, the taxonomic distinctness of this organism was indicated. Subsequently, this organism has been included in a chemotaxonomic survey which showed that the type strain (the only strain included in the species) contained L-lysine as the diamino acid of the peptidoglycan (type A3 α , according to Schleifer & Kandler, 1972) and menaquinones of the MK-8(H₂) type (Miyadoh, 1997). Because this combination is typical for some Gram-positive cocci, *P. aurantia* was included in the *Atlas of Actinomycetes* (Miyadoh, 1997) in the list of chemotaxonomic characteristics of actinobacteria and related taxa. However, neither a strain designation nor the phylogenetic analysis was included in the *Atlas*.

During the course of the extension of the 16S rDNA database of type strains of actinobacterial species we analysed the 16S rDNA of *P. aurantia* DSM 12801^T (= ATCC 49321^T, kindly provided by the American Type Culture Collection). Extraction of DNA, PCR amplification and cycle sequencing analysis of the PCR product were performed according to described methods (Rainey *et al.*, 1996). The phylogenetic analysis of the 16S rDNA, based upon 1502 nucleotides, revealed that strain DSM 12801^T was 100% identical to the type strain of *Kocuria rosea* (DSM 20447^T), which agrees with the chemotaxonomic characterization of these strains as indicated in the *Atlas of Actinomycetes* (Miyadoh, 1997). However, this contradicts the data published by Poston (1993) and in order to confirm the presence of these properties in strain DSM 12801^T (= ATCC 49321^T) the most widely used chemotaxonomic characteristics were investigated for *P. aurantia* DSM 12801^T and *K. rosea* DSM 20447^T by using previously published methods (Groth *et al.*, 1996). The results of this chemotaxonomic investigation were compared to the data of Poston (1993) given in the original description for strain ATCC 49321^T and the data given in the *Atlas of Actinomycetes* (Miyadoh, 1997) (Table 1). Examination of the results obtained in this study and the results published by Poston (1993) needs further comment.

Table 1. Chemotaxonomic characteristics of *P. aurantia* (as reported in different studies) and of *K. rosea*

ND, Not determined; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid; GL, unknown glycolipid.

Property	<i>P. aurantia</i> ATCC 49321 ^{*†}	<i>P. aurantia</i> [†]	<i>P. aurantia</i> DSM 12801 ^{†‡}	<i>K. rosea</i> DSM 20447 ^{†§}
Petidoglycan:amino acid ratio	Glu:Ser:His:Lys:Ala (1:1:1:1:8)	ND	Glu:Lys:Ala (1:1:5:3:4)	ND
Peptidoglycan type	ND	A3 α , L-Lys	A3 α , Lys-Ala ₂₋₃	A3 α , Lys-Ala ₃₋₄
Polar lipids	PC, PE, PG	ND	PG, DPG, PL, GL	PG, DPG, (PI, PL, GL)
Menaquinones	ND	MK-8(H ₂)	MK-8(H ₂), MK-9(H ₂), MK-6(H ₂), MK-7(H ₂), MK-10(H ₂) (67:14:8:5:1)	MK-8(H ₂), MK-7(H ₂), MK-9(H ₂), (71:9:8)
DNA G+C content (mol %)	59 (HPLC), 60 (T _m)	59	72 (HPLC)	66–75 (T _m)

^{*} Poston (1993).

[†] Miyadoh (1997).

[‡] This study.

[§] Stackebrandt *et al.* (1995).

Table 2. Percentage cellular fatty acid composition of the type strains of *P. aurantia* and of *K. rosea*

Values less than 1 % are not shown.

Organism	C _{14:0}	C _{16:0}	C _{16:1}	i-C _{14:0}	i-C _{15:0}	i-C _{16:0}	i-C _{17:0}	ai-C _{15:0}	ai-C _{17:0}	ai-C _{17:1}	Total
<i>P. aurantia</i>	3.4	6.4	2.1	2.0	11.6	2.5	1.1	65.0	4.5		98.6
<i>K. rosea</i> [*]	1.5	1.6	5.9	1.8	7.6	1.6		70.9	4.3	3.0	98.2

^{*} Stackebrandt *et al.* (1995).

In our study on *P. aurantia* DSM 12801^T and *K. rosea* DSM 20447^T, using published methods for DNA isolation (Cashion *et al.*, 1977; Escara & Hutton, 1980) and the spectrophotometric measurement of DNA–DNA reassociation (Huß *et al.*, 1983; Jahnke, 1992), these two strains show 87.1 % DNA–DNA similarity, which would indicate that they are members of the same species. This is also supported by the fact that the two strains share 100 % 16S rDNA sequence similarity. This is in contrast to data published by Poston (1993), which indicated that there was no significant DNA–DNA hybridization or RNA–DNA hybridization between *P. aurantia* and *K. rosea* ATCC 186^T (= DSM 20447^T). Furthermore, the reported G+C values of 59 mol % and 60 mol % (HPLC and thermal melting point, respectively) (Poston, 1993) are significantly different from the value of 72 mol % (HPLC) obtained in this study.

The cellular fatty acid composition of *P. aurantia* was not reported either by Poston (1993) or by Miyadoh (1997). In our study, the cellular fatty acid profiles of *P. aurantia* DSM 12801^T and *Kocuria rosea* DSM 20447^T were found to be very similar and contained both 12-methyl tetradecanoic (ai-C_{15:0}) and 13-methyl tetradecanoic acid (i-C_{15:0}) as the predominating components (Table 2). The polar lipid composition reported for *P. aurantia* (Poston, 1993) is phosphatidylglycerol, phosphatidylethanolamine and phosphatidyl-

choline, which, like the DNA–DNA and RNA–DNA data, indicates that this organism is neither a member of the species *K. rosea*, nor a member of the genus *Kocuria*. In fact, this polar lipid pattern is not found in any of the taxa within the ‘*Arthrobacter–Micrococcus*’ line of descent, but is not uncommon in members of the α subclass of the *Proteobacteria* (see Ratledge & Wilkinson, 1988; B. J. Tindall, unpublished results). However, studies on *P. aurantia* DSM 12801^T and *K. rosea* DSM 20447^T indicated that the two had almost identical polar lipid patterns, both of which were (1) typical of members of the genus *Kocuria* and (2) typical of *K. rosea* in particular.

Although we did not examine the pigment composition of *P. aurantia* it should be noted that the methods used by Poston (1993), HPLC and photodiode array detection, were sufficient to tentatively identify the major pigment as rhodopin. Despite the reports that *K. rosea* (ATCC 516) produces canthaxanthin, together with a range of other bicyclic carotene derivatives (Cooney & Berry, 1981; Cooney *et al.*, 1966; Schwartzel & Cooney, 1970, 1972; Ungers & Cooney, 1968), Nelis & De Leenheer (1989) were unable to find these pigments in *K. rosea* CCM 839 or ATCC 516 (which are identical strains). They did, however, find canthaxanthin in a *Brevibacterium* species. It is significant that Poston (1993) used the methods of Nelis & De Leenheer (1989), which separate and spectroscopically detect

rhodopin, provides details of the separation and detection of canthaxanthin and showed that *K. rosea* CCM 839 or ATCC 516 had a distinctive pigment pattern that was not identical to *P. aurantia* (Poston, 1993). It should be noted that rhodopin is not uncommon in members of the anoxygenic phototrophic bacteria within the α subclass of the *Proteobacteria* (Schmidt, 1978).

The obvious discrepancy between the chemotaxonomic data given for strain ATCC 49321^T of Poston (1993), originally deposited in the American Type Culture Collection as '*Neisseria aurantia*', and the data for strain DSM 12801^T (= ATCC 49321^T) obtained from the ATCC in 1999 could be explained by the failure to maintain the original culture in any of the collections involved. To verify the authenticity of strain ATCC 49321^T (= DSM 12801^T), Michael Poston, formerly of the Laboratory of Biochemistry, National Institutes of Health, Bethesda, MD, USA, arranged to ship a subculture of his original culture (labelled '*N. aurantia*') to the DSMZ, where this strain was analysed with respect to the almost complete 16S rDNA sequence. The sequence thus obtained was 100% identical to those of *P. aurantia* DSM 12801^T (= ATCC 49321^T) and *K. rosea* DSM 20447^T, which confirms the authenticity of ATCC 49321^T and DSM 12801^T subsequently subcultured from the ATCC strain.

At the time of description of *P. aurantia* Poston 1994 (see Poston, 1993), members of the genus *Kocuria* were classified as members of the genus *Micrococcus*, i.e. *K. rosea* was classified as *Micrococcus roseus*. *M. roseus* ATCC 186^T was included in the DNA–DNA and RNA–DNA reassociation experiments between *P. aurantia* ATCC 49321^T and a variety of Gram-positive and Gram-negative bacteria (Poston, 1993), but no significant DNA–DNA reassociation was reported with any organism (12% for low stringency; < 5% for high stringency), nor could any close evolutionary relationship be detected by RNA–DNA hybridization. The lack of reassociation between any of the reference species was, among others, the main rationale for describing strain ATCC 49321^T as the type strain of a new species within a new genus. Histidine, the presence of which in cell wall preparations is the unique feature of *P. aurantia*, is probably not a constituent of the peptidoglycan, but a contaminating cellular protein.

On the basis of the 16S rDNA sequence data, DNA–DNA hybridization data, chemotaxonomic data and morphological data, we consider that DSM 12801^T (= ATCC 49321^T) (the type strain of *P. aurantia*) and *K. rosea* DSM 20447^T are strains of the same species. However, we consider that the data we have collected does not allow us to apply Rule 42 of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992) and simply unify the genera *Pelczaria* Poston 1994 and *Kocuria* Stackebrandt *et al.* 1995. In an accompanying Request for an Opinion (Tindall *et al.*, 2000), we propose that ATCC 49321^T (= DSM

12801^T) be placed in the species *K. rosea* (Flügge 1886) Stackebrandt *et al.* 1995 and that the generic name *Kocuria* Stackebrandt *et al.* 1995 be retained on the basis of the fact that there are significant differences between the circumscription of the taxon *P. aurantia* (Poston, 1994) and the properties of the strains currently in circulation, which are supposed to represent the type strain of the species.

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