Reclassification of ATCC 9341 from *Micrococcus luteus* to *Kocuria rhizophila*

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Strain ATCC 9341, currently known as *Micrococcus luteus*, has been designated as a quality-control strain in a number of applications. It is also cited as the standard culture in several official methods and manuals, as well as the Code of Federal Regulations. Over the years, it has become apparent that ATCC 9341 does not resemble other *M. luteus* strains; however, its phenotypic characteristics alone were ambiguous. Recently, a polyphasic study was performed in which molecular data were combined with cytochemical properties and physiological characteristics. The results clearly indicate that ATCC 9341 is a member of the genus *Kocuria*. Thus, it is proposed to reclassify ATCC 9341 as *Kocuria rhizophila* and to alert users worldwide of this name change.

Strain ATCC 9341, currently known as *Micrococcus luteus* (FDA strain PCI 1001), has been designated as a quality-control strain in a number of applications. The Code of Federal Regulations and the US Pharmacopeia list it as a control strain for sterility testing (US National Archives and Records Administration, 1997c; US Pharmacopeial Convention, 1995b). The Association of Official Analytical Chemists (AOAC) Official Methods, the US Pharmacopeia and the European Pharmacopoeia use this culture to assay a variety of antibiotics and fungicide residues (AACC, 1983; AOAC, 1995; British Pharmacopoeia Commission, 1993; EDQM, 1997; Huang & English, 1966; Ragheb & Smallidge, 2000a–h; US National Archives and Records Administration, 1997a; US Pharmacopeial Convention, 1995a, c). Furthermore, the Code of Federal Regulations also includes this strain for doxycycline, tetracycline and chloramphenicol susceptibility disc testing (US National Archives and Records Administration, 1997b).

Strain ATCC 9341 was deposited as *Sarcina lutea* by the US Food and Drug Administration over 40 years ago. Although the name was changed to *M. luteus* in 1977, based on the 8th edition of *Bergey's Manual of Determinative Bacteriology* (Baird-Parker, 1974), its characteristics were not identical to those of typical *M. luteus* strains. Table 1 shows some characteristics of ATCC 9341 when compared with ATCC 4698^T, the type strain of *M. luteus*.

During the past few years, users have alerted us that this culture was identified as *Micrococcus varians* (now *Kocuria varians*) by using the bioMérieux ID 32 Staph kit as well as the Biolog GP MicroPlate. We ran this strain along with the type strains of *M. luteus* (ATCC 4698^T) and *K. varians*

(ATCC 15306^T) on the RiboPrinter (Qualicon) to analyse their DNA fingerprinting patterns. This automated molecular biology workstation performs a Southern hybridization using the bacterial rRNA genes as probes after the genomic DNA has been digested with *EcoRI*. The riboprint from ATCC 9341 did not match either of the fingerprints from *M. luteus* or *K. varians* (Fig. 1).

To assess the relationship between the strains in question, we compared the gene sequences of their 16S rRNA. Amplification of the 16S rRNA genes was accomplished by using primers L27F and L1492R (Lane, 1991) and the PCR products were purified and sequenced. The 16S rRNA gene sequence fragments for each strain were assembled by using Sequencher (Gene Codes Corp.) and the consensus sequence for each strain was determined. The consensus sequence of each strain had at least twofold coverage in each direction and was used to perform a BLAST search of GenBank. The 16S rRNA gene sequences from closely related genera were downloaded and aligned using CLUSTAL X (Thompson *et al.*, 1997).

Table 1. Phenotypic and chemotaxonomic comparison between strains ATCC 9341 and *M. luteus* ATCC 4698^T

Character	ATCC 9341	ATCC 4698 ^T
Oxidase	–	+
Simmons' citrate	+	–
Acid production from:		
Glucose	+	–
Fructose	+	–
Major fatty acids	ai-C _{17:0} , ai-C _{15:0} , i-C _{15:0}	ai-C _{15:0} , i-C _{15:0}

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain ATCC 9341 is AF542072.

Fig. 1. Riboprint pattern of ATCC 9341 compared with those of *M. luteus* ATCC 4698^T, *K. varians* ATCC 15306^T and *K. rhizophila* ATCC BAA-50^T.

Parsimony analyses were conducted using PAUP 4.0b8a (Swofford, 2001) and the data matrix consisted of 1343 base pairs with 120 parsimony-informative characters. Bootstrap analysis was performed using 100 replicates and the bootstrap values are indicated in Fig. 2. Results indicate that ATCC 9341 and the type strain of *Kocuria rhizophila* (ATCC BAA-50^T) were clearly in the same clade and were quite distinct from the *M. luteus* clade (strains ATCC 4698^T, D7 and Ballarat). This observation was supported by high bootstrap values, and the SSU rRNA gene sequence from *K. rhizophila* was 99 % similar to that of ATCC 9341 for the region aligned. This high similarity also helped in excluding it from *K. varians*. Furthermore, several species of

Fig. 2. Maximum-likelihood tree with bootstrap values based on 16S rDNA sequences, showing the position of strain ATCC 9341 relative to related strains.

Arthrobacter separated the *Micrococcus* clade from the *Kocuria* clade, indicating that these two clades are indeed separate genera.

In addition to 16S rRNA sequencing, DNA–DNA hybridization experiments were conducted between ATCC 9341 and the type strains of *M. luteus* (ATCC 4698^T), *K. varians* (ATCC 15306^T) and *K. rhizophila* (BAA-50^T). The results showed 42.3, 54.7 and 67.6 % similarity with each respective type strain. Thus, it further confirmed that ATCC 9341 does not belong to the genus *Micrococcus*. Although the similarity to *K. rhizophila* did not quite reach the threshold of 70 % recommended by Wayne *et al.* (1987), it definitely indicated close affiliation with this species. The DNA fingerprinting pattern of ATCC 9341 did not match exactly those generated by ATCC BAA-50^T, but there was definitely more similarity to *K. rhizophila* than to the type strain of *M. luteus* (Fig. 1).

Finally, a comparison of phenotypic traits, fatty acid methyl ester analysis and riboprint patterns between ATCC 9341 and *K. rhizophila* ATCC BAA-50^T also showed agreement with the sequencing results. Both strains grew on Simmons' citrate and produced acid from glucose and fructose. The major fatty acids produced by ATCC 9341 were ai-C_{17:0}, ai-C_{15:0} and i-C_{15:0}, which are indicative of the genus *Kocuria* (Kovács *et al.*, 1999). Furthermore, ATCC 9341 has type A11.6 as the peptidoglycan structure, which is identical to that of the type strain of *K. rhizophila* (Schleifer & Kandler, 1970; Kovács *et al.*, 1999). Thus, we can conclude that ATCC 9341 is indeed a strain of *K. rhizophila*.

In conclusion, we propose to reclassify ATCC 9341 as *K. rhizophila*. Since this culture is cited in many applications as an official standard strain, we hope to alert users by publishing the name change in this journal. Manuals, procedures and documents need to be updated to reflect this change.

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