NOTE

DNA–DNA relatedness between *Saccharomonospora* species: '*Saccharomonospora caesia*' as a synonym of *Saccharomonospora azurea*

Jung-Hoon Yoon,^{1,2} Sam-Bong Kim,¹ Sung Taik Lee,² and Yong-Ha Park¹

Author for correspondence: Yong-Ha Park. Tel: +82 42 860 4620. Fax: +82 42 860 4625.
e-mail: yhpark@kribb4680.kribb.re.kr

¹ Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience and Biotechnology (KRIIB), PO Box 115, Yusong, Taejeon 305-600, Korea

² Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejeon, Korea

Saccharomonospora species were subjected to DNA–DNA relatedness determination to investigate their genetic relationships based on genomic DNA. The levels of DNA–DNA relatedness among the type strains of the four validly described *Saccharomonospora* species ranged from 18 to 46%. *Saccharomonospora viridis* KCTC 9156, which was described as '*Saccharomonospora internatus*' but later reclassified as a member of *S. viridis*, showed a similarity level of 90% to *S. viridis* KCTC 9115^T. *Saccharomonospora* sp. strain K180 exhibited levels of DNA–DNA relatedness that are indicative of a new species. *Saccharomonospora azurea* exhibited levels of DNA–DNA homology of 84–95% to '*Saccharomonospora caesia*' strains including the type strain. Therefore, on the basis of DNA–DNA relatedness data and taxonomic data described previously, it is proposed that '*S. caesia*' should be considered as a synonym of *S. azurea*.

Keywords: *Saccharomonospora* species, DNA–DNA relatedness, taxonomic revision

The genus *Saccharomonospora* was proposed by Nonomura & Ohara (1971) for monosporic actinomycetes which contain *meso*-diaminopimelic acid, arabinose and galactose in the cell-wall peptidoglycan [wall chemotype IV *sensu* Lechevalier & Lechevalier (1970)]. The additional phenotypic characteristics and phylogenetic analysis of this genus have been described elsewhere (Kim *et al.*, 1995; Yoon *et al.*, 1996, 1997). There are currently four validly described *Saccharomonospora* species, namely, *Saccharomonospora azurea* (Runmao, 1987), *Saccharomonospora cyanea* (Runmao *et al.*, 1988), *Saccharomonospora glauca* (Greiner-Mai *et al.*, 1988) and *Saccharomonospora viridis* (Nonomura & Ohara, 1971), the type species of the genus. '*Saccharomonospora caesia*' was previously classified as *Micropolyspora caesia* (Kalakoutskii, 1964). However, Kurup (1981) considered *M. caesia* to be a member of the genus *Saccharomonospora*, and this was confirmed by Greiner-Mai *et al.* (1987). The name of '*S. caesia*' was not included on the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) and has not been validly published on subsequent Approved Lists or Validation Lists. '*Saccharomonospora internatus*', was proposed (Kurup, 1981; Greiner-Mai *et al.*, 1987)

for a strain previously classified as *Micropolyspora internatus* (Agre *et al.*, 1974), but this strain was regarded as a synonym of *S. viridis* (Greiner-Mai *et al.*, 1988). Another invalidly described *Saccharomonospora* strain, '*S. yunnanensis*' was reported by Chenglin & Lihua, 1985) but was not available for our study.

From 16S rDNA sequence analysis, the four validly described *Saccharomonospora* species exhibited high levels of 16S rDNA similarity (Kim *et al.*, 1995). The 16S rDNA sequences of most *Saccharomonospora* species were more than 98% similar; the only exception was *S. viridis* whose 16S rDNA showed levels similar to other *Saccharomonospora* species. In particular, *S. azurea* and '*S. caesia*' strains had identical 16S rDNA sequences (Kim *et al.*, 1995). *S. azurea* has also been shown to be closely related to '*S. caesia*' strains from several other taxonomic studies. *S. azurea* and '*S. caesia*' have identical sequences in 5S rRNA (unpublished), in 16S–23S rDNA ITS (internally transcribed spacer) region (Yoon *et al.*, 1997) and in approximately 340 bp of RNase P RNA gene (Cho *et al.*, 1998). The 23S–5S ITS sequences of *S. azurea*, except for one rRNA allele which has two inserted

nucleotides, also were identical to 23S–5S ITS sequences of '*S. caesia*' strains. In a previous ribotyping study, *S. azurea* and '*S. caesia*' produced identical ribotype patterns when the product of *Bam*HI, *Sal*I and *Pvu*II digests were treated with two types of rDNA probes (Yoon *et al.*, 1996). The result of recent numerical phenetic studies also showed that *S. azurea* and '*S. caesia*' are closely related to each other (Kim, 1995). The result of DNA–DNA relatedness is the most important and final criterion in confirming the relationship between *S. azurea* and '*S. caesia*', since the phylogenetic definition of a species in current bacteriology states that strains with approximately 70% or greater DNA–DNA relatedness are members of the same species (Wayne *et al.*, 1987). Therefore, the aims of the present study were to investigate the genetic relationships among existing *Saccharomonospora* species and to elucidate the genetic relationship between *S. azurea* and '*S. caesia*' based on results of DNA–DNA relatedness.

The isolation of chromosomal DNA was previously described (Yoon *et al.*, 1996). The DNAs were purified once more for this study. DNA–DNA hybridization was performed by the dot-blot hybridization method. Labelling of probe and visualization of hybridized DNA were performed by using a non-radioactive ECL direct nucleic acid labelling and detection system (Amersham International). Resultant quantitative DNA–DNA relatedness was determined using a Bio-Rad densitometer.

Saccharomonospora strains used for this study are as follows: *S. azurea* KCTC 9475^T (NA 128^T), '*S. caesia*' strains KCTC 9152^T (INMI 19125^T), KCTC 9153 (DSM 43068) and Ko18 (Greiner-Mai *et al.*, 1988), *S. cyanea* KCTC 9478^T (NA 134^T), *S. glauca* KCTC 9479^T (DSM 43769^T), *S. viridis* strains KCTC 9115^T and KCTC 9156 and *Saccharomonospora* sp. strain K180. Their sources have been described elsewhere (Kim *et al.*, 1995; Yoon *et al.*, 1996). The levels of DNA–DNA relatedness among the type strains of the four validly described *Saccharomonospora* species ranged from 18 to 46%, confirming their status as distinct species. *S. viridis* KCTC 9156, which was previously described as '*S. internatus*' (Greiner-Mai *et al.*, 1987) but reclassified as a member of *S. viridis* later (Greiner-Mai *et al.*, 1988), showed a similarity level of 90% to *S. viridis* KCTC 9115^T. This indicates that the reclassification of '*S. internatus*' as *S. viridis* is correct. *Saccharomonospora* sp. strain K180 exhibited levels of DNA–DNA relatedness of 32, 37, 23 and 15% to *S. azurea* KCTC 9475^T, *S. cyanea* KCTC 9478^T, *S. glauca* KCTC 9479^T and *S. viridis* KCTC 9115^T, respectively. The high possibility of strain K180 being a new species of the genus *Saccharomonospora* has already been shown (Kim *et al.*, 1995; Yoon *et al.*, 1997) and its description as a new species will be published elsewhere. *S. azurea* exhibited levels of DNA–DNA homology of 84–95% to '*S. caesia*' strains including the type strain, demonstrating that *S. azurea* and

'*S. caesia*' are the same species. Therefore, on the basis of DNA–DNA relatedness together with other taxonomic data described recently and according to the priority of validly described we propose that '*S. caesia*' should be considered as a synonym of *S. azurea*.

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