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Synonymy of *Micropolyspora internatus* and *Saccharomonospora viridis* and Emended Description of *Saccharomonospora viridis* S

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Transfer of *Micropolyspora internatus* into the genus *Saccharomonospora* as "*Saccharomonospora internatus* comb. nov." was proposed by Kurup and Greiner-Mai, but the nomenclatural change has not been validly published. Although the inclusion of *M. internatus* in the genus *Saccharomonospora* has not being established, the synonymy of "*Saccharomonospora internatus*" and *S. viridis* was proposed by Greiner-Mai. A number of recent publications regarded *M. internatus* as a synonym of *S. viridis*, but the name *M. internatus* is still used in some cases instead of *S. viridis*. This is because of the complicated history of *M. internatus* and *S. viridis*, but it is different from the generally accepted view of prokaryotic taxonomy. To clearly verify the synonymy of *M. internatus* and *S. viridis*, a literature review and experimental verification were conducted in this study. Based on the genomic and phenotypic characteristics obtained in this study, the synonymy of the two species was obvious. The emended description of *S. viridis* is given.

Keywords: *Micropolyspora internatus*, *Saccharomonospora viridis*, whole genome sequencing

Introduction

The genus *Saccharomonospora* belongs to the family Pseudonocardiasea established by Nonomura and Ohara [1]. *Saccharomonospora* strains usually produce single spores on aerial hyphae, and their cell wall (chemotype IV) contains *meso*-diaminopimelic acid together with the sugars arabinose and galactose [2]. At the time of writing, the genus *Saccharomonospora* comprised 11 recognized species, with *Saccharomonospora viridis* being the type strain [1, 3, 4].

The genus *Micropolyspora*, with the type species *Micropolyspora brevicatena* [5], was proposed to include actinomycetes producing short chains of spores, both on substrates and on aerial mycelia. According to the Approved List of Bacterial Names [3], the genus contained five species: *M. brevicatena* [5], *M. angiospora* [6], *M. faeni* [7], *M. internatus* [8], and *M. rectivirgula* [9, 10]. In 1982, the type species of the genus, *M. brevicatena*, was transferred to the genus *Nocardia* as *Nocardia brevicatena* [11] and the name *Micropolyspora* became illegitimate. In the following year, *M. faeni* and *M. rectivirgula* were combined and reclassified as *Saccharopolyspora rectivirgula* [12]. One of the remaining species, *M. angiospora*, was reclassified as *Nonomuraea angiospora* [13].

The only species that remained in the genus *Micropolyspora* was *M. internatus*. In 1981, Kurup [11] intended to transfer *M. internatus* to the genus *Saccharomonospora* and suggested the new combination '*Saccharomonospora internatus*' for *M. internatus*. However, this nomenclatural change was not clearly stated or indicated in the publication. Thus, Greiner-Mai *et al.* [14] proposed '*S. internatus* comb. nov.' in 1987, but the corresponding publication has not been validated.

Although the new combination '*S. internatus* comb. nov.' was not validly published, the synonymy of '*S. internatus*' and *S. viridis* was suggested by Greiner-Mai *et al.* [15] in 1988. The main problem of this publication was insufficient data to support the synonymy of '*S. internatus*' and *S. viridis*. They did not provide DNA-DNA relatedness, which was

suggested as fundamental data to draw clear lines of species demarcation [16]. In addition, doubts on the type strain's authenticity remain. According to DSMZ, the type strain used in this publication (DSMZ 43671) is not *M. internatus* but *Nonomuraea salmonea.*

During the study on the taxonomic relationship between '*S, caesia*' and *S. azurea* in 1999, Yoon *et al.* [17] additionally reported that the level of DNA-DNA hybridization between '*S. internatus* KCTC 9156^T' and *S. viridis* KCTC 9115^T is higher than 90%, supporting the reclassification of *M. internatus* to *S. viridis*. Hereafter, '*S. internatus*' has been considered as a synonym of *S. viridis.*

Because of the long and confusing taxonomic history of *M. internatus* and *S. viridis*, the name *M. internatus* is still in use, but this is not consistent with widely accepted and published current taxonomic opinion. In this study, we aimed to clearly verify the synonymy of *M. internatus* and *S. viridis* by using genomic and phenotypic taxonomic data.

Materials and Methods

Strains

The type strains *M. internatus* JCM 3315^T and *S. viridis* JCM 3036^T were obtained from the Japan Collection of Microorganisms. Both strains were cultivated on ISP2 medium (Difco, USA) at 45°C.

Phenotype Analyses

Growth characteristics were assessed using ISP2 medium at different temperatures (20°C, 26°C, 30°C, 37°C, 42°C, 50°C, 55°C, 60°C, and 65°C), pH (4–11 with 1 pH unit increments), and NaCl concentrations (0–9% with 1% increments). The pH was adjusted using the following buffering systems: citric acid/sodium citrate (pH 4–5), KH_2PO_4/K_2HPO_4 (pH 6–8), and $NaHCO_3/Na_2CO_3$ (pH 9–11). Results were recorded for up to 4 weeks. Carbon source utilization and culture characteristics were determined as described previously [18]. Enzymatic activities were examined using the API CORYNE and API ZYM identification systems (France) according to the manufacturer's instructions. Whole cell fatty acid methyl esters (FAMEs) were analyzed by gas chromatography (model 7890B; Agilent Technologies, USA) following instructions from the Microbial Identification System (TSBA6; MIDI, ver. 6.2). Cells were grown on TSA medium at 37°C for 2 days. The other morphological, physiological, biochemical, and chemotaxonomic analyses were performed as described previously [19] except that the basal medium was ISP2 and the growth temperature was 45°C.

Genome Analyses

Since the complete genome sequence of *S. viridis* was publicly available [20], only *M. internatus* JCM 3315^T was subjected to whole genome sequencing. Genomic DNA extraction and sequencing by the MiSeq system (Illumina, USA) were performed as previously described [19]. De novo genome assembly was done using CLC Genomics Workbench 6.5.1 and gene annotation was done using the NCBI Prokaryotic Genome Annotation Pipeline. Average nucleotide identity (ANI) between the two test genomes was calculated using the OrthoANI program [21]. To compare the gene content similarity, orthologous genes were determined using reciprocal BLAST searches using an e-value threshold of 10^{-5}. To analyze gene synteny, whole genome sequences from two strains were aligned by Murasaki 1.68.6 using a seed pattern of weight 28 and length 36. The aligned result was visualized using a dot plot drawn with the GMV genome map viewer 1e-93 build 991.

Phylogenetic Analyses

The genome-derived 16S rRNA gene sequences were aligned with those from other members of the *Saccharomonospora* genus using EzEditor [22]. Phylogenetic analyses were performed using MEGA 6.06 [23]. Phylogenetic trees were inferred based on the neighbor-joining [24] and maximum-likelihood [25] methods. The tree topologies were evaluated by bootstrap analyses [26].

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the genome sequence of *Micropolyspora internatus* JCM 3315^T are KU179035 and JRZE00000000, respectively.

Results and Discussion

Phenotypic Characteristics

S. viridis is an unusual organism because it has a gram-negative reaction but has a mycelium morphology typical of gram-positive actinomycetes [1]. Thus, the gram reaction was re-evaluated by both Gram staining and the KOH method. Both tests confirmed the gram-negative reaction of *S. viridis* cells, in accordance with previous reports.

Cell growth was observed in yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salt-starch agar (ISP 4), and glycerol-asparagine agar (ISP 5). The aerial mycelium was green and the substrate mycelium was yellowish on ISP2 agar. No striking phenotypic differences were observed between the two strains. They could only be distinguished in terms of *N*-acetyl-β-glucosaminidase activity, for which strain DSM 43017^T was positive whereas strain JCM 3315^T was negative or only weakly positive. Both strains showed similar fatty acid profiles (Table 1) with a predominance of iso-$C_{16:0}$ (43.4–44.6%), $C_{17:1}$ ω6*c* (10.2–11.3%), $C_{16:1}$ ω6*c* and/or $C_{16:1}$ ω7*c* (8.2–7.4), $C_{16:0}$ (6.9–7.3%), anteiso-$C_{17:0}$ (4.9–4.5%), and iso-$C_{16:0}$H (3.3–3.6%). The results of the phenotypic tests are presented in the emended species description and in Table S1.

Table 1. Cellular fatty acid composition.

Fatty acids	1	2
Saturated		
$C_{16:0}$	6.7	7.3
$C_{17:0}$	1.8	2.9
Unsaturated		
$C_{15:1}$ ω6*c*	2.0	2.2
Iso-$C_{16:1}$ H	3.3	3.6
$C_{17:1}$ ω6*c*	11.3	10.2
$C_{17:1}$ ω8*c*	3.0	3.7
Branched		
Iso-$C_{14:0}$	1.4	1.6
Iso-$C_{15:0}$	2.0	1.6
Iso-$C_{16:0}$	44.6	43.4
Iso-$C_{17:0}$	2.3	4.2
Anteiso-$C_{17:0}$	4.9	4.5
Iso-$C_{18:0}$	1.7	1.6
Hydroxy		
Iso-$C_{17:0}$ 3-OH	1.9	ND
Methyl		
10-methyl $C_{17:0}$	1.2	1.2
Summed feature[a]		
3	8.2	7.4
9	1.8	2.7

[a]Summed feature 3 comprises $C_{16:1}$ ω6*c* and/or $C_{16:1}$ ω7*c*. Summed feature 9 comprises iso-$C_{17:1}$ and/or 10-meyhyl $C_{16:0}$.

Strains: 1, *M. internatus* JCM 3315^T; 2, *S. viridis* JCM 3036^T. Values represent percentages of total fatty acids, as obtained in this study. Fatty acids with >0.5% abundance are shown. ND, not detected.

Genomic Characteristics

The sequencing reads produced were assembled into 13 contigs (>1 kb long; N50 = 1,283,693) with an average coverage of 737×. The genome size of *M. internatus* JCM 3315^T was 4,304,348 bp with a G+C content of 67.4 mol% and 3,897 genes. This was in agreement with the values for *S. viridis* DSM 43017^T (CP001683; 4,308,349 bp; G+C content 67.3 mol%; 3,890 genes). The gene contents of the two genomes were very similar by sharing 3,714 orthologous genes (96% of both genomes). The high similarity of the two genomes was also demonstrated by the gene synteny (Fig. 1). Comparison of the genome maps revealed conserved synteny and gene order between the two chromosomes. Exception was observed in the block containing the replication origin, but this is because the genome of *M. internatus* JCM 3315^T is incomplete. Even in this exceptional block, the micro-synteny was evident. Overall, a significant pattern of

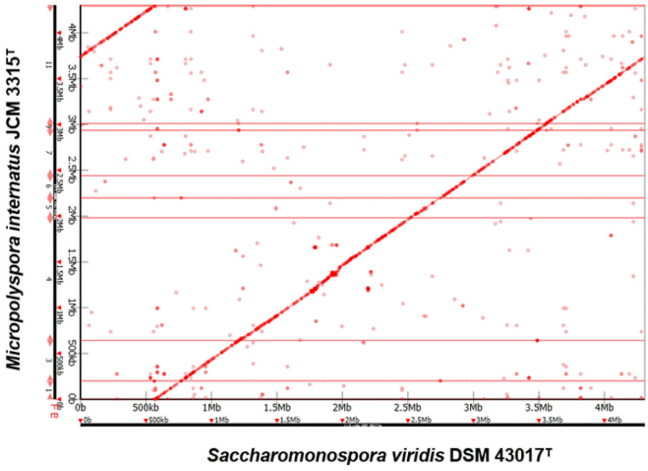

Fig. 1. Syntenic dot plot between the genomes of *S. viridis* DSM 43017^T and *M. internatus* JCM 3315^T.

Dots represent putative homologous gene pairs, and syntenic gene pairs are plotted with solid red circles.

synteny was obvious, demonstrating the low divergence of the two genomes.

Taxonomic Relationship

The average nucleotide identity between the two test strains was calculated as 99.5%. Such value is clearly above the suggested ANI score (95–96%) for demarcating genomic species [27–31]. In contrast, ANI values were 75.2–80.7% when *M. internatus* JCM 3315^T was compared with other *Saccharomonospora* species (Table 2). The genome sequence-based digital DNA-DNA hybridization (dDDH) value [32] was calculated using the Genome-to-Genome Distance Calculator ver. 2, with identities/HSP length option (http://ggdc.dsmz.de). The dDDH value between the two strains was 96.3%, which was higher than the 70% DDH criterion for bacterial species affiliation [16, 33]. Comparison with other species yielded dDDH values of 20.1–23.6% (Table 2), clearly demonstrating that the two strains belong to the same species.

The genome-derived 16S rRNA gene sequences from the two type species (NR_074713.1 and KU179035) shared 100% sequence similarity. The close relatedness of the two strains was supported by the phylogenetic trees, as indicated by a solid monophyletic clade within the cluster enclosed by the genus *Saccharomonospora* (Fig. 2).

On the basis of high genomic relatedness (99.6% ANI and 96.3% dDDH), identical 16S rRNA gene sequences, and indistinguishable chemotaxonomic and phenotypic characteristics, it is clear that *M. internatus* is a heterotypic synonym of *S. viridis*. Based on the genomic and phenotypic

Table 2. ANI and dDDH values of *M. internatus*, *S. viridis*, and other *Saccharomonospora* genomes.

dDDH \ ANI	1	2	3	4	5	6	7	8	9	10
1	-	**99.6**	80.7	80.5	77.6	79.7	78.8	77.6	75.2	76.8
2	**96.3**	-	80.8	80.5	77.7	79.7	78.9	77.6	75.3	76.8
3	23.6	23.7	-	87.9	78.1	84.4	83.9	78.2	75.9	77.5
4	23.4	23.4	35.0	-	78.2	83.4	82.4	78.2	75.7	77.4
5	22.1	22.2	22.9	22.4	-	78.3	77.7	82.1	77.7	81.5
6	22.6	22.6	28.5	26.8	22.2	-	81.2	78.2	75.8	77.1
7	22.1	22.1	27.4	25.5	22.0	24.6	-	77.7	75.4	76.6
8	22.3	22.4	23.0	22.6	26.1	22.7	22.2	-	77.7	93.6
9	20.1	20.2	20.4	20.3	22.4	20.3	20.3	22.8	-	76.9
10	21.6	21.6	22.3	22.1	25.5	21.9	21.6	54.9	22.1	-

Strains (genomes): 1, *M. internatus* JCM 3315^T (JRZE00000000.1); 2, *S. viridis* DSM 43017^T (CP001683.1); 3, *S. cyanea* NA-134^T (CM001440.1); 4, *S. glauca* K62^T (CM001484.1); 5, *S. saliphila* YIM 90502^T (KB912588.1); 6, *S. azurea* NA-128^T (CM001466.1); 7, *S. xinjiangensis* XJ-54^T (JH636048.1); 8, *S. halophila* 8^T (KB912472.1); 9, *S. marina* XMU15^T (CM001439.1); and 10, *S. paurometabolica* YIM 90007^T (KI912184.1).

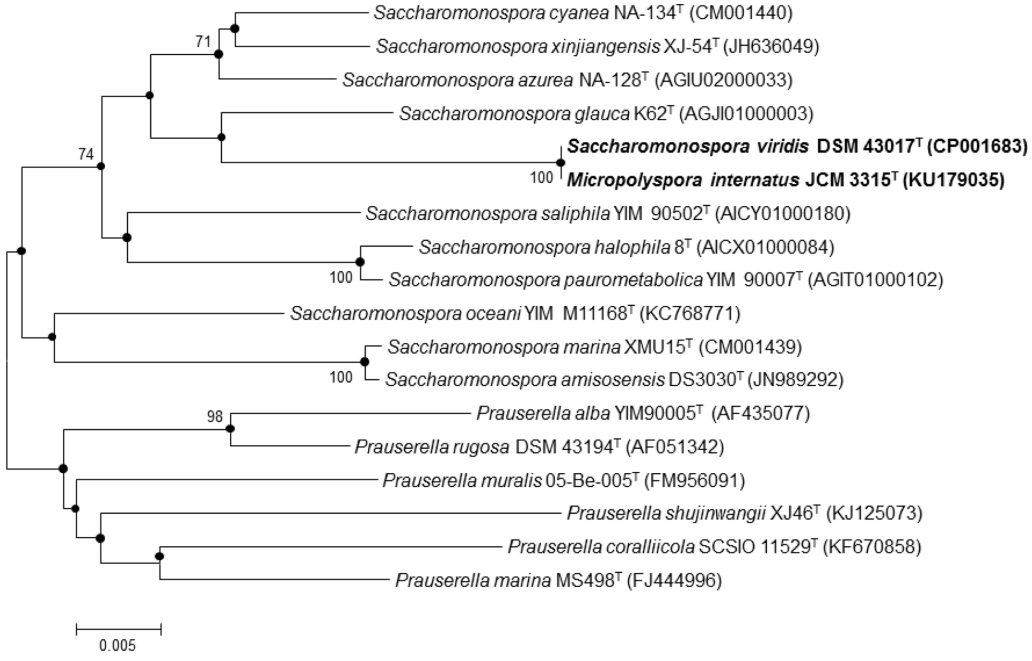

Fig. 2. Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship between *Micropolyspora internatus*, *Saccharomonospora viridis*, and other related species.

The numbers at the nodes are given as percentages and represent the levels of bootstrap support (>70%) based on 1,000 resampled data sets. The circles indicate that the corresponding nodes (groupings) were also recovered in the maximum likelihood tree. Scale bar, 0.005 nt substitutions per position.

characteristics determined in this study, the emended description of *S. viridis* is given as follows.

Emended Description of *Saccharomonospora viridis* (Schuurmans *et al.* 1956) Nonomura and Ohara 1971

The description coincides with that given by Nonomura and Ohara (1971), with the following amendments. Gram-reaction-negative. Catalase- and oxidase-positive. Cells are aerobic and nonmotile. Produces green aerial mycelium and yellow substrate mycelium on ISP2 agar. Grows at pH 6–10, at 37–60°C, and in 0–6.5% (w/v) NaCl. Uses D-glucose, starch, sucrose, and D-xylose as sole carbon

source, but not L-arabinose, lactose, mannitol, L-rhamnose, or D-sorbitol. Does not reduce nitrate to nitrite or nitrogen. Hydrolyzes esculin and gelatin. Possesses the following enzymatic activities: alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, α-glucosidase, β-glucosidase, and pyrazinamidase; but not urease, lipase, α-chymotrypsin, α-galactosidase, β-galactosidase, α-mannosidase, or α-fucosidase. *N*-Acetyl-β-glucosaminidase activity is strain-dependent. The major cellular fatty acids are iso-$C_{16:0}$, $C_{17:1}$ ω6*c*, $C_{16:1}$ ω6*c* and/or $C_{16:1}$ ω7*c*, $C_{16:0}$, anteiso-$C_{17:0}$, and iso-$C_{16:0}$H. The DNA G+C content is 67.3 mol%. The type strain is P10^T (= ATCC 15386^T = CCUG 5913^T = DSM 43017^T = NBRC 12207^T = JCM 3036^T = NRRL B-3044^T = VKM Ac-681^T). The GenBank/DDBJ/EMBL accession number for the complete genome sequence of the type strain is CP001683.

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