Taxonomic Revision of the Genus *Saccharomonospora* and Description of *Saccharomonospora glauca* sp. nov.

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Representatives of *Saccharomonospora viridis* (type strain DSM 43017), *Saccharomonospora caesia* (type strain DSM 43044), and *Saccharomonospora internatus* (type strain DSM 43671), together with 52 *Saccharomonospora* isolates from compost, manure, hay, and soil, were characterized by determining morphological, biochemical, and physiological properties, phage sensitivity, antibiotic activity, enzyme, and protein patterns (polyacrylamide gel electrophoresis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis), and restriction patterns of chromosomal deoxyribonucleic acids. The type strain of *S. internatus* proved to be identical to *S. viridis*, and thus *S. internatus* should be regarded as a synonym of *S. viridis*. A total of 16 strains differed significantly from *S. viridis* and *S. caesia* and are proposed as members of a new species, *Saccharomonospora glauca* (type strain, DSM 43769). Since the three species differ in phage sensitivity, production of antibiotic substances, and enzyme, protein, and deoxyribonucleic acid restriction patterns, these properties can be used for reliable species identification.

The genus *Saccharomonospora* was created by Nonomura and Ohara (24) for monosporic actinomycetes with cell wall type IV (21) (i.e., meso-diaminopimelic acid in the peptidoglycan and arabinose and galactose in cell wall type IV actinomycete genera which do not contain mycolic acids (4)). Kroppenstedt (17) extended the range of biochemical characteristics for this genus as follows: fatty acid type 2a plus 2-hydroxy fatty acids (mainly iso-branched and 2-hydroxy branched fatty acids with 16 carbon atoms), phospholipid type 2 (mainly hydroxy- and lyso-phosphatidylethanolamine), and menaquinone type 2 [MK-8(H₄), MK-9(H₂)]. Furthermore, the combination of a green to bluish green color of the aerial and substrate mycelia and monosporic morphology appears to be a good indication that an isolate belongs to the genus *Saccharomonospora*.

Whereas the genus *Saccharomonospora* is clearly distinguished from other genera of the Actinomycetales, the taxonomy of this group at the species level appears to be less certain. Goodfellow and Pirouz (6), McCarthy and Cross (22), and Greiner-Mai et al. (10) observed heterogeneity among strains labeled *Saccharomonospora viridis* with regard to color of aerial mycelia (the mycelia of some isolates are violet instead of green), physiological properties, and phage sensitivity. However, the data available at the time that these studies were done did not allow partition of *Saccharomonospora viridis* into separate species. Kurup (18) considered *Micro polyspora caesia* (13) and *Micro polyspora internatus* (1) to be members of the genus *Saccharomonospora*. This was confirmed by Greiner-Mai et al. (10), who distinguished three species, *Saccharomonospora viridis*, *Saccharomonospora caesia*, and *Saccharomonospora internatus*. More recently, two new species have been described, *Saccharomonospora azurea* (27) and *Saccharomonospora yunnanensis* (3). Since the type cultures of *Saccharomonospora azurea* and *Saccharomonospora yunnanensis* were not available for our studies and the data presented previously are rather limited, it is difficult to discuss the relationship between these two species and the three species included in this study. More reliable properties are required for species differentiation in the genus *Saccharomonospora*, and this was the primary objective of this study.

**MATERIALS AND METHODS**

**Strains.** The 58 strains which we studied are listed in Table 1.

**Cultivation and maintenance of strains.** The methods of Greiner-Mai et al. (10) were used for cultivation and maintenance. The media used for sporulation were YM agar, PM agar (10), and GC agar (0.5% glycerol, 0.5% cornsteep powder, 0.3% yeast extract, 0.1% beef extract, 0.3% peptone from casein, 0.3% NaCl, 1.2% agar, pH 7.2).

**Morphology.** The methods used to determine morphology have been described in detail by Greiner-Mai et al. (10).

**Biochemical criteria.** The diamino-pimelic acid isomers, sugar identities, and fatty acid, phospholipid, and menaquinone profiles were determined as described by Kroppenstedt (17).

**Physiological properties.** The temperature range for growth was determined first; the other tests were then carried out at the optimum temperature (37 or 30°C). Resistance to lysyme and NaCl, formation of melanin, hemolysis, and degradation of adenine, xanthine, hypoxanthine, tyrosine, starch, egg yolk, esculin, and arbutin were determined as described by Greiner-Mai et al. (10). Degradation of allantoin, urea, and uric acid and utilization of organic acids as sole carbon sources were determined as described by Kutzner (19). Degradation of triglycerols was tested on tributyrin agar (E. Merck) in tubes, and degradation of casein was tested on calcium caseinate agar (Merck) in petri dishes; formation of clear zones indicated positive reactions. Collagenase activity was determined by the method described below. Collapur (1% solution of native collagen in 36 mM lactic acid, pH 3.5; Freudenberg) was neutralized with 0.1 M tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 10.4) and then poured onto a well-dried base layer containing 25 g of standard II nutrient agar (Merck) per liter and 2 mM CaCl₂ dissolved in 0.1 M tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 7.6). Collagen membranes formed at 37°C within 1 h. The plates were then inoculated and incubated at 37°C, since the collagen disintegrates at higher temperatures. Collagenase activity resulted...
in disappearance of the membrane around the colonies. Utilization of carbohydrates for growth was determined on the mineral salts medium of Shirling and Gottlieb (30), which was supplemented with 0.5% Casamino Acids (Difco Laboratories), 20 mg of tryptophan per liter, 0.1 μg of biotin per ml, and 0.1 μg of thiamine per ml. The following ether-sterilized carbohydrates were added after autoclaving to give final concentrations of 10 g/liter: D-glucose, L-arabinose, D-xylose, D-fructose, L-rhamnose, lactose, sucrose, raffinose, mannitol, inositol, and dextrin.

**Antibiotic activity.** Antibiotic activities were determined and antibiotics were characterized by using the methods of Greiner-Mai et al. (9).

**Phage typing.** The methods used for isolation and propagation of phages, as well as phage typing, were described by Korn et al. (16). Phage Tm1 was supplied by H. Prauser (Jena, German Democratic Republic); the other eight phages (Llg, Llv, MK5g, MK5s, MK22, Mplg, Mplv, R14, R16, R17, R18, R19, R20, R21, R22, R25, R26g, R27, AA8, E13, K018, K027, K033, ZA7, ZA8, ZA13) were isolated in our laboratory from plant compost, waste compost, and soils from the Federal Republic of Germany, Argentina, and Tenerife.

**Analysis of enzymes and total proteins.** (i) Preparation of whole-cell protein extracts. Strains were grown in GPYB broth (10) for 1 to 3 days. Approximately 0.4 g of washed mycelium was suspended in 3 ml of 0.1 M sodium phosphate buffer (pH 7.2) and disrupted by ultrasonication for 30 s at 0°C (Branson Sonifier). Mycelium debris was removed by centrifugation at 45,000 × g for 30 min. The resulting soluble protein fraction was divided into 1.0-ml volumes and stored at −20°C.

(ii) **Analysis of enzymes.** Polyacrylamide gel electrophoresis was performed in horizontal gels (T = 5%; C = 3%; size, 125 by 250 by 2 mm) in 0.1 M sodium phosphate buffer (pH 7.2) (25). Samples (10 μl) were applied directly into gel slots (1 by 10 mm); bromophenol blue (0.25%) was used as the marker dye. Electrophoresis was carried out at 10°C at a constant current of 200 mA for approximately 3.5 h.

(iii) **Esterase stain.** A 40-mg portion of l-naphthylacetate, dissolved in 5 ml of acetone, was added to a solution containing 100 mg of diazonium salt (Fast Blue RR salt [Sigma Chemical Co.] or Fast Red TR salt [Boehringer GmbH]) in 100 ml of 0.2 M sodium phosphate buffer (pH 7.2). The gel was flooded with the staining mixture and incubated at 37°C. Dark or reddish bands developed within 1 to 3 h, which indicated esterase activity.

(iv) **Nicotinamide adenine dinucleotide-dependent malate dehydrogenase stain.** The substrate solution contained 700 mg of sodium l-malate and 100 mg of nicotinamide adenine dinucleotide in 4 ml of 4 mM Na2CO3. This solution was mixed with a dye solution containing 75 mg of Nitro Blue Tetrazolium chloride (Serva) and 4 mg of phenazinemethosulfate (Serva) in 100 ml of 0.1 M tris(hydroxymethyl)aminomethane buffer (pH 8.0) and poured onto the gel. Dark blue bands of malate dehydrogenase activity appeared after 2 to 6 h of incubation at 37°C.

(v) **Analysis of total proteins.** Sodium dodecyl sulfate disc gel electrophoresis was performed in vertical gels (110 by 140 by 2 mm) by using the method of Laemmli (20). For Coomassie blue staining the method of Fehrmann and Moberg (5) was used, and for silver staining the method of Merril et al. (23) was used.

**Restriction analysis of chromosomal DNA.** For restriction analysis of chromosomal Deoxyribonuclease acid (DNA), organisms were grown for 24 to 48 h in modified YEME broth (12) (10.3% sucrose, 1% glucose, 0.3% yeast extract, 0.3% malt extract, 0.5% peptone from casein, 0 to 0.2% glycine, pH 7.2). Then 2 ml of a 2.5 M MgCl2 solution per liter was added after autoclaving. The mycelium was harvested by centrifugation, washed with W1 solution [10 mM tris(hydroxymethyl)aminomethane, 1 mM ethylene diamine tetraacetate, sucrose 15%], and stored at −20°C. DNA was isolated by using the rapid method of Hopwood et al. (12) with the following two modifications: (i) we added only 1 mg of lysozyme (grade I; Sigma) per ml; and (ii) we used very short incubation times in the lysozyme solution at 37°C (the times varied from 30 s to 10 min depending on the strain investigated). For restriction of the chromosomal DNA, the following enzymes were used: BamHI, BglII, BstEII,
EcoRI, and PsiI (Stehelin). Electrophoresis was performed in 0.8% agarose gels (type I; Sigma) in TBE buffer (pH 8.0) (12). Submarine gels (size 1406 mm, 160 mm) were run at a constant voltage (35 V) for 15 h.

RESULTS

All of the results obtained in this study distinguished three homogeneous groups of strains (Table 2). One group contained the type cultures of Saccharomonospora viridis and Saccharomonospora internatus, and a second group contained the type strain of Saccharomonospora caesia. The third group of 16 strains differed consistently from the previously described species and therefore was regarded as belonging to a new species, for which we propose the name Saccharomonospora glauca.

Morphology. Six strains formed light to dark violet aerial mycelia and soluble pigments, and all of the other strains had the typical macroscopic appearance of Saccharomonospora. (i.e., light to dark green aerial mycelia with shades of gray or blue and a dark green or sometimes grayish brown colony reverse). All strains produced single, tightly packed spores on aerial hyphae (Fig. 1). Light microscopy occasionally gave the impression of the presence of short spore chains; however, electron microscopy always revealed the formation of single spores on short, unbranched sporophores.

Biochemical criteria. All of the strains examined in this study exhibited the following biochemical markers which are diagnostic for the genus Saccharomonospora (4, 10, 17): meso-diaminopimelic acid in the peptidoglycan, arabinose and galactose in whole-cell hydrolysates (type A), phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, and lyso-phosphatidylethanolamine as main phospholipids, fatty acid pattern 2a (mainly saturated fatty acids, iso-branched fatty acids with 16 and 18 carbon atoms, and 2-hydroxydecanoic acid), and menaquinones MK-9(H4) (60%) and MK-8(H4) (20 to 30%). The combination of the tetrahydro-multiprenyl menaquinones MK-9(H4) and MK-8(H4) is characteristic for the genus Saccharomonospora. Mentioning is the observation that five isolates and the type strain of Saccharomonospora caesia contained up to 90% MK-9(H4).

Physiological properties. Two temperature ranges for growth were found among the strains tested (Table 3). Most strains grew in the range from 28 to 60°C with an optimum of 50°C. The type strain of Saccharomonospora caesia and five other strains grew only in the range from 28 to 45°C with an optimum of 37°C; the five other strains were later identified as members of Saccharomonospora internatus.

However, the other physiological properties were of little diagnostic value. All strains either behaved identically in the tests used (sensitivity to lysozyme, resistance to NaCl, failure to produce melanin, ability to degrade starch, triglycerides, and casein, no degradation of adenine, xanthine, hypoxanthine, or arbutin), or the properties of the type strains and the 52 isolates did not lead to any consistent groupings. Tests for degradation of allantoin, urea, and uric acid and utilization of organic acids as sole carbon sources gave false-positive reactions (change of the pH indicator) at incubation temperatures of 50°C. Degradation of the β-glycosides esculin and arbutin was difficult to recognize, because the dark green or dark brown soluble pigment of the organisms could not always be discriminated from the dark Fe3⁺-dye complex indicating a positive reaction. The carbohydrate utilization tests did not always give unequivocal results because many strains showed moderate growth with the negative control (Table 3).

Antibiotic activity. A selection of 20 strains of the three Saccharomonospora spp. were tested for their antibiotic properties. With the exception of the type strains of Saccharomonospora viridis and Saccharomonospora internatus and one other strain, all of the strains were active against the gram-positive bacteria Bacillus subtilis, Staphylococcus aureus, and Brevibacterium divaricatum. In addition, Saccharomonospora caesia was active against Streptomyces coelicolor Müller. The separated antibiotic substances exhibited differences in Rf values when they were subjected to thin-layer chromatography. Furthermore, the active bands of some Saccharomonospora viridis strains showed a typical blue fluorescence at 366 nm. However, our data did not allow identification of the three substances as any of the known antibiotics from Saccharomonospora spp. (28, 33).

Phage typing. All of the strains were tested against nine phages. Seven phages exhibited the same host range; thus, three phages were useful for species differentiation (Table 2). None of the phages lysed members of other actinomycete genera (Actinomadura, Faenia, Pseudonocardia, Streptomyces, Thermomonospora, and Thermoactinomyces). A total of 27 strains, including the type strain of Saccharomonospora viridis, were sensitive to all of the phages. Eight strains of this species and Saccharomonospora internatus showed increased resistance to phages φL1g and φ771. Lysis occurred only with high-titer phage lysates (>10⁸ phage per ml). Saccharomonospora caesia strains were lysed by phage φL1g. In addition, four strains showed weak reactions with three phages of the Tm group and φ771. All strains of Saccharomonospora glauca sp. nov. were sensitive to phage φ771, and six strains were also sensitive to high-titer lysates of phage φL1g (>10¹⁰ phage per ml).

Enzyme patterns. The analysis of esterases revealed three basic patterns and led to clear-cut grouping of the type strains, cultures from the Deutsche Sammlung von Mikroorganismen, and isolates (Fig. 2 and 3a). Group I (36 strains, including the type strains of Saccharomonospora viridis and Saccharomonospora internatus) was characterized by main bands at Rf values of 0.2, 0.35, 0.45, and 0.68. Group II strains (six strains, including the type strain of Saccharomo-
**FIG. 1.** Scanning electron micrographs of *Saccharomonospora glauca* AA10 (a) and *Saccharomonospora caesia* Ko20 (b). Cultures were grown on GC agar for 4 days at the optimal temperature. Magnification, ×9,000.

*Saccharomonospora caesia* showed bands at Rf values of 0.28 (only type strain DSM 43044), 0.39, 0.54, 0.66, 0.76, and 0.94. Group III strain (16 isolates) showed bands at Rf values of 0.28, 0.43, 0.56, and 0.72. The bands at Rf values of 0.28 and 0.39 of groups II and III were identical.

Figure 3b shows the malate dehydrogenase bands obtained after electrophoresis and staining. Although the Rf values did not vary to a great extent, the differences between the strains turned out to be significant. All 58 strains fell into three groups which were identical to those obtained on the basis of the esterase patterns. Group I strains showed one band at an Rf value of 0.46, group II strains showed one band at an Rf value of 0.52, and group III strains showed one band at an Rf value of 0.50.

**Analysis of total proteins.** Our analysis of total proteins also revealed three patterns among the strains tested, confirming the groups obtained by enzyme analysis (Fig. 4). The patterns of individual strains varied only slightly within one group.

**Restriction analysis of genome DNA.** Three different restriction patterns of chromosomal DNA were found (Fig. 5). The grouping of strains again was consistent with the grouping established by enzyme and protein analyses. The strains of each group showed identical patterns.

**DISCUSSION**

The genus *Saccharomonospora*, which was created by Nonomura and Ohara (24), is now well characterized by biochemical criteria (4, 10, 17). For almost 15 years *Saccharomonospora viridis* was the only species, until *Micropolyspora caesia* and *Micropolyspora internatus* were trans-
TABLE 3. Physiological properties of Saccharomonospora strains

<table>
<thead>
<tr>
<th>Property</th>
<th>Saccharomonospora viridis</th>
<th>Saccharomonospora caesia</th>
<th>Saccharomonospora glauca</th>
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<td></td>
<td>Strain DSM 43017&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Strain DSM 43044&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Strain DSM 43769&lt;sup&gt;T&lt;/sup&gt; + 15 other strains</td>
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<tr>
<td>Growth temp range (°C)</td>
<td>37–60</td>
<td>28–50</td>
<td>37–60</td>
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<tr>
<td>Growth temp optimum (°C)</td>
<td>50</td>
<td>37</td>
<td>50</td>
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<tr>
<td>Aerial mycelium color&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Degradation of:</td>
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<td>Tyrosine</td>
<td>+&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+</td>
<td>+&lt;sup&gt;(+)&lt;/sup&gt;</td>
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<tr>
<td>Egg yolk&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Erythrocytes</td>
<td>–</td>
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<td>Collagen</td>
<td>+</td>
<td>–</td>
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<td>Esulin</td>
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<td>v&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>Utilization of carbohydrates&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>D-Glucose</td>
<td>+</td>
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<td>D- and L-Arabinose</td>
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<tr>
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<td>+</td>
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<sup>a</sup> Strains B1, L1v, MK5v, Mplv, R24, and R25.
<sup>b</sup> Type strain of the former species Saccharomonospora internatus.
<sup>c</sup> Green aerial mycelium with shades of blue or gray.
<sup>d</sup> +, Positive reaction; –, negative reaction; (+), weak reaction; v, variable reaction.
<sup>e</sup> None of the strains grew on egg yolk agar; nevertheless, enzyme activities could be detected.
<sup>f</sup> Strains did not grow with inositol, lactose, raffinose, and L-rhamnose.

ferred to Saccharomonospora (10, 18). More recently, two new species of this genus have been described (3, 27). It has become evident that the taxonomy of this genus at the species level is not satisfactory. In addition, studies by Goodfellow and Pirouz (6), McCarthy and Cross (22), and Greiner-Mai et al. (10) revealed heterogeneity within the type species, Saccharomonospora viridis. Thus, it appeared desirable to develop an unequivocal system for species identification in Saccharomonospora. Therefore, we performed a thorough study of the available cultures of the species Saccharomonospora viridis, Saccharomonospora caesia, and Saccharomonospora internatus together with 52 new isolates belonging to the genus.

The results of morphological and physiological tests revealed that these properties are of very little diagnostic value. No differences in morphology were found among the

FIG. 2. Esterase patterns of Saccharomonospora strains in a native polyacrylamide slab gel. Lane A, Saccharomonospora caesia DSM 43044<sup>T</sup>; lanes B and D, Saccharomonospora glauca strains (lane D, strain DSM 43769<sup>T</sup>); lane C, Saccharomonospora viridis DSM 43017<sup>T</sup>.

FIG. 3. Enzyme patterns of Saccharomonospora species. (a) Esterase patterns. (b) Malate dehydrogenase patterns. The bands in parentheses are not common to all strains or are not always detectable, depending on the age of the culture.
strains tested; furthermore, the violet color of some isolates turned out to be a variant of the green pigment of *Saccharomonospora viridis*. In physiology, the type strains and other isolates either behaved identically or did not show striking differences. Sometimes it seemed that the type cultures had lost some physiological properties, presumably because of prolonged cultivation and maintenance under laboratory conditions. In addition, some tests did not work properly at a temperature of 50°C because of the instability of substrates (e.g., urea, uric acid, collagen, blood cells) and the drying of agar media at 50°C, which limited the incubation time.

Therefore, new methods for species differentiation were tried, and we were successful in using (i) phage typing, (ii) protein patterns, (iii) analysis of enzymes, and (iv) restriction analysis of chromosomal DNA. Sensitivity to phages has proved to be an excellent aid for identification of many genera of the *Actinomycetales* (16, 26). The phages used in our study were also useful for species differentiation. Sodium dodecyl sulfate gel electrophoresis of soluble proteins is widely used and has been established as a taxonomic tool for species identification of bacteria (2, 7, 14). Enzyme patterns have been used for species differentiation for many years, especially for pathogenic bacteria (8, 11, 29, 32); this property was recently used for thermophilic actinomycetes (31; E. Greiner-Mai, A. Kempf, F. Korn-Wendisch, and H. J. Kutzner, in D. Behrens, ed., *DECHEMA Biotechnology Conferences*, Vol. 1, p. 491–496, 1988). Although enzyme patterns yield fast and unequivocal results for species identification of unknown strains, these methods are not yet included in routine tests. Our study revealed the existence of basic esterase patterns for each species, which did not show variations. Several additional bands occurred depending on the strains or on the age of the mycelium (Fig. 3a). However, these bands did not influence the reliability of this taxonomic tool. The restriction analysis of chromosomal DNA, like the protein pattern analysis, gave a characteristic fingerprint for each species tested (15). Our results also revealed that the strains of one species exhibited the same restriction pattern, which clearly separated them from strains of the other two species. In addition, each species produced an antibiotic substance which differed in (i) inhibition spectrum toward gram-positive bacteria and (ii) Rf values on thin-layer chromatograms.

The combination of all of these methods led to rapid and reliable species identification of strains of *Saccharomonospora*. Regarding the value of the criteria tested it should be mentioned that in this study the esterase pattern gave the first indication of the existence of three species, one of which has not been described previously. This grouping was supported by DNA restriction patterns, protein patterns, phage typing, and synthesis of antibiotics. In conclusion, the following three species can be distinguished: *Saccharomonospora viridis* (including the type strain of *Saccharomonospora internata*), *Saccharomonospora caesia*, and *Saccharomonospora glauca* sp. nov.

**Description of Saccharomonospora glauca** sp. nov. *Saccharomonospora glauca* (glau ca. L. fem. adj. glauca, bluish, greenish, grayish blue, referring to the color of the aerial mycelium).

(i) **Morphology.** Branching, nonfragmenting aerial and substrate mycelia. Single spores are produced tightly packed on aerial hyphae. Spores are smooth or slightly roughened, round to ovate, and 0.8 to 1.0 μm in diameter.

(ii) **Cultural characteristics.** Colonies produce light green to bluish green (turquoise) aerial mycelium, dark green...
substrate mycelium, and soluble pigment on GC agar and GYM agar.

(iii) Biochemical characteristics. *meso*-Diaminopimelic acid, arabinose, and galactose (sugar type A) are found in cell wall hydrosylates. The main fatty acids are iso-branched fatty acids with 16 carbon atoms and 2-hydroxy-branched fatty acids with 16 carbon atoms (type 2a); the diagnostic phospholipids are phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, and lyso-phosphatidylethanolamine; the main menaquinones are MK-8(H4) and MK-9(H2).

(iv) Physiological characteristics. The temperature range for growth is 37 to 60°C, and the optimum temperature is 50°C. The strains are sensitive to lysozyme (200 U/ml) and resists 7.5% NaCl. No melanin is produced. Tyrosine, starch, triglycerides, blood cells (hemolysis), casein, collagen, and esculin are degraded. D-Glucose, mannitol, dextrin, and arabinose are used as carbon sources. Antibiotic activity against gram-positive bacteria occurs; the *R* of the antibiotic is 0.28.

(v) Phage sensitivity. All strains of *Saccharomonospora glauca* are sensitive to φ771; in addition, several strains are sensitive to φ1Lg. All strains are resistant to the Tm, family of phages, which is specific for *Saccharomonospora viridis*.

(vi) Analysis of proteins and genome DNA. All strains show identical total protein and DNA restriction patterns; esterase pattern III (four main bands) is found. The *R* of malate dehydrogenase is 0.50.

(vii) Type strain. The type strain is strain K62 (= DSM 43769).

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LITERATURE CITED


