Designation of Neotype Strains for *Micromonospora coerulea* Jensen 1932 and *Micromonospora chalcea* (Foulerton 1905) Ørskov 1923

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ATCC 27008 and ATCC 12452 are designated as neotype strains for *Micromonospora coerulea* Jensen 1932 and *Micromonospora chalcea* (Foulerton 1905) Ørskov 1923, respectively. The data of earlier workers who studied these organisms are discussed, and additional characteristics considered useful for the identification of these species are presented.

Neotype strains are designated for *Micromonospora coerulea* Jensen and *Micromonospora chalcea* (Foulerton) Ørskov because none of the strains on which the original descriptions of these organisms were based appears to be extant.

MATERIALS AND METHODS

The strains studied are identified in the discussion and description which follow. Methods used were previously described (14).

DISCUSSION

Micromonospora coerulea Jensen 1932. Jensen (7) described three new species of *Micromonospora* from a study of 67 micromonosporae which he had isolated from Australian soils. The species were based primarily on color of the vegetative mycelium (*M. coerulea*), diffusible pigment (*M. fusca*, see reference 14), and sparse to poor growth (*M. parva*). Kriss (10) did not consider these criteria to be relevant for speciation, based on his own work with a small number of micromonosporae isolated from soil. I would normally agree with the opinion of Kriss concerning the use of pigment production and strain vigor as criteria for speciation. However, in the case of *M. coerulea*, the dusky blue-green color of Ridgway (18) is quite unusual for a micro-organism, and, in studies of many soil micromonosporae, only a small number of these atypically pigmented organisms have been encountered. I have detected only three soil isolates of this color, and their morphological and physiological characters agree with most of those noted by Jensen (7), who isolated only two of these blue-green strains.

Micromonospora coerulea was listed as a synonym of *Micromonospora* (*Thermoactinomyces*) *vulgaris* by Waksman et al. (21); fortunately, this synonymy was not perpetuated by Waksman (*Bergey's Manual of Determinative Bacteriology*, 7th ed., p. 744-825).

Sveshnikova et al. (19) described several new species of *Micromonospora* and cited what appear to be reference strains for a number of other micromonosporae. Among the latter are *M. chalcea* and *M. coerulea*. It is not clear what these authors intended when they listed under *M. chalcea* "type strain 12452 ATCC" and under *M. coerulea* "type strain 510 INA" [see definition of type strain (3, 4, 11)], for no evidence is presented to show that either of these strains was used in preparing the original descriptions of these organisms. In fact, from the information available, both of these strains appear to have been isolated subsequent to the publication of the original descriptions of these organisms.

Description of the neotype strain (no. 36; ATCC 27008) of *Micromonospora coerulea* Jensen 1932. Colonies in shaken broth culture are usually composed of long, loosely woven hyphae (open web type) with spores often borne singly on short or long sporophores (Fig. 1, a-f). The mycelium is 0.4 to 0.6 μ m in diameter. The spores are spherical, smooth walled by phase-contrast microscopy, and are 0.8 to 1.5 μ m in diameter. Mycelium rarely fragments except in old cultures. Growth on glucose asparagine agar or starch casein agar is fair to poor. Colonies are of a granular texture, small, and often glossy. The color of the colonies is dark jade green [g21ni (2, 20)]. According to the Inter-Society Color Council-National Bureau of Standards (8), this color is

Fig. 1. *Micromonospora coerulea*. a and c, Strain no. 36 (= ATCC 27008), the designated neotype for *M. coerulea*. b and d, Strain no. 981 (= ATCC 27082), a strain of *M. coerulea*. e, Enlarged photographic reproduction of strain 176 IV by Jensen (6), 18-day filter paper asparagine solution culture; considered by Jensen (7) to be a strain of *M. coerulea*. f, Enlarged photographic reproduction of *M. coerulea* by Jensen (7); simple monopodial branching system. a-d. The marker represents 10 μ m.

equivalent to the designation dark bluish green 165, which is equivalent to the Ridgway 1912 designation dusky green blue, the color originally designated for *M. coerulea* by Jensen (7). No diffusible pigment or aerial mycelium is produced. Growth on 1% yeast extract-1% glucose agar is good; the texture is granular, and the color of the periphery of the colony is amber (g3lc), whereas the center of the colony is dark green (g21pl). Growth on Bennett's agar is good, plicate, and dark olive green (g24 ½ pn). Growth on Emerson's agar is fair, granular, and dark green (g24pn). Growth on agar media is often slow, requiring three to five weeks to develop. Growth is initially a pale yellow orange turning to a yellow green and finally a dark blue green or greenish black. The blue-green mycelial pigment is water-soluble and is an acid-base indicator, being green in the basic range and a blue gray and precipitated in the acid range. Carbohydrate utilization patterns are given in Table 1. Maximum sodium-chloride tolerance, 1.5%. Grows well on potato plug without the addition of CaCO₃. Hydrolyzes starch. Hydrolysis of gelatin is poor. Growth on milk agar is fair; hydrolysis is restricted, requiring three to five weeks before a small zone of clearing occurs around the colony. Cellulose strips are decomposed very slowly, if at all; colonies above the liquid level of the cellulose are blue-green, whereas below the liquid level the colonies are a pale yellow orange. Nitrate not reduced to nitrite. Source: isolated by the author from a soil sample from

the top of Mt. Haleakala, Island of Maui, Hawaiian Islands.

Two other strains of this species have been isolated which produce a greenish blue pigment similar to that produced by the neotype strain. Strain 37 (= ATCC 27081) was isolated from a soil sample from Israel and grows poorly on most organic media, perhaps a little better on inorganic nitrogen salts media; it bears a resemblance to the description of *Micromonospora bicolor* Krasil'nikov 1941. Strain 981 (= ATCC 27082) was isolated from a soil sample from Mexico. This strain grows well on most media and differs in its carbohydrate utilization pattern from that of strain 36 (Table 1). Strain 981 might be considered as belonging to a subspecies or a biotype of *M. coerulea*. "Carbohydrate patterns should be treated with a degree of flexibility in order to attain an understanding of strain latitude and overall grouping value of the method. Strains of a species that have evolved in different geographic localities may of necessity have had to develop a different 'bag' of enzymes in order to utilize locally available substrates; what remains of a common utilization pattern among geographically diverse strains of a species should have species group recognition value" (13).

Micromonospora chalcea (Foulerton 1905) Ørskov 1923. The description by Foulerton of *Streptothrix chalcea* (5) consists of a report made before the Pathological Society of London and recorded in *The Lancet* in one

TABLE 1. Carbohydrate utilization by strains of *Micromonospora coerulea*

		Control																
		D-Glucose	Sucrose	D-Xylose	D-Levulose	D-Galactose	β-Lactose	L-Arabinose	α-Melibiose	D-Mannitol	Raffinose	L-Rhamnose	D-Ribose	Glycerol	i-Inositol	Melezitose	D-Arabinose	Dulcitol
#36	f	0/8	8/8	8/8	8/8	8/8	8/8	5/8	0/8	8/8	8/8	8/8	0/8	1/8	0/8	0/8	0/8	0/8
	i	%	96	84	88	88	50			92	92	84		12				
#37	f	0/4	4/4	3/4	4/4	4/4	4/4	2/4	1/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
	i	%	50	50	67	42	50	17	8	50	67	33						
#981	f	0/5	5/5	5/5	5/5	5/5	5/5	0/5	4/5	4/5	5/5	1/5	5/5	0/5	5/5	0/5	0/5	0/5
	i	%	100	100	100	100	100		80	74	100	20	100		100			

f = No. of Trials

i = Intensity of Response

Strain	Control	D-Glucose	Sucrose	D-Xylose	D-Levulose	D-Galactose	β -Lactose	L-Arabinose	α -Melibiose	D-Mannitol	Raffinose	L-Rhamnose	D-Ribose	Glycerol	i-Inositol	Melezitose	D-Arabinose	Dulcitol
ATCC 12452																		
f	0/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
i	%	100	89	100	67	89	78	89	89		78							
NRRL B-944																		
f	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
i	%	100	100	67	67	67	67	67	100		67							
M. sp. Went CBS																		
f	0/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
i	%	84	84	100	84	84	84	67	84		67							
NRRL 3222																		
f	0/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
i	%	84	84	67	34	84	50	67	67		67							

f = No. of Trials i = Intensity of Response

Fig. 2. *Micromonospora chalicea*. a, *M. chalicea* NRRL B-944 (= ATCC 27083), showing a monopodial branching system. b, *M. chalicea* ATCC 12452, the neotype strain, showing sessile spores. c, Enlarged photographic reproduction of strain 176 XVII by Jensen (6), showing monopodial and pseudodichotomous branching systems; considered by Jensen (7) to be a strain of *M. chalicea*. d, Enlarged photographic reproduction of *M. chalicea* by Jensen (7), showing a simple monopodial branching system; grown in glucose asparagine solution for 6 days at 32 °C. a and b, The marker represents 10 µm.

stronger in this than in the other species of this genus. Optimum temperature for growth, 30–35° C. Thermal death point of mycelium, 70° C in 2–5 minutes. Spores resist 80° C for 1–5 minutes."

Description of the neotype strain (ATCC 12452) of *Micromonospora chalicea* (Foulerton 1905) Ørskov 1923. The strain here designated as the neotype of *M. chalicea* is ATCC 12452, which is cited in U.S. Patent 2,809,919. 1957. This strain has been chosen because it produces a pigment closely resembling the copper to deep reddish brown colony color mentioned by Foulerton (5) and the deep cinnabar red of Ørskov (17). The color of this strain also appears to agree with the brownish black colony color produced early on water agar as noted by Ørskov (17). The physiological properties of this strain agree with those

described by Foulerton (5). Previously, descriptive data were given on this strain by Luedemann and Brodsky (15, 16).

Other strains studied and believed to be identifiable with *M. chalicea* are NRRL B-944 (= ATCC 27083), NRRL 3222 (= ATCC 27084) identified as *M. chalicea* subsp. *flavida* Weinstein et al. (U.S. Patent 3,454,696. 1968), and the *Micromonospora* sp. deposited by Went at the CBS.

Micromonospora sp. ATCC 10026 has also been identified as *M. chalicea* (U.S. Patent 2,809,919. 1957); however, I believe this strain has a greater affinity to *M. narashinoensis* (12, 13, 15).

Data useful in recognizing strains of *M. chalicea* include: carbohydrate-utilization patterns (Table 2); maximum sodium chloride tolerance, 4 to 5%; good growth on acid or

CaCO₃-neutralized potato plugs; and strong proteolytic action on milk agar. Sporulating hyphae of strains identified as *M. chalicea* may be compared in Fig. 2. Colonies in shaken broth cultures are of the open web type (13).

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