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Streptomyces spitsbergensis Wieczorek et al. 1993 Is a Later Subjective Synonym of *Streptomyces baldaccii* (Farina and Locci 1966) Witt and Stackebrandt 1991

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Reexamination of the morphological, cultural, and physiological characteristics of *Streptomyces spitsbergensis* Wieczorek et al. 1993 revealed that this organism belongs to the whorl-forming streptomycetes, and DNA-DNA hybridization data confirmed that *S. spitsbergensis* is a later subjective synonym of *Streptomyces baldaccii* (Farina and Locci 1966) Witt and Stackebrandt 1991.

Streptomyces spitsbergensis, which was proposed as a new species of the genus *Streptomyces* by Wieczorek et al. (6), has been described as follows: the morphology of its spore chains is recti-flexibilis, its aerial mass color is pink to violet, its substrate mycelium is red to reddish brown, and it utilizes glucose, xylose, raffinose, and rhamnose. However, we unexpectedly found that the strain provided by the American Type Culture Collection (ATCC) forms whorls in its aerial mycelia. To reexamine the organism, we obtained the original strain from the Polish Collection of Microorganisms (PCM). The morphological, cultural, and physiological characteristics and patterns of utilization of carbon sources of the two strains studied were the same. In addition, the levels of DNA relatedness between the two strains were 92 to 97%, indicating that these organisms are identical genomically. In this study we characterized *S. spitsbergensis* IFO 15745^T (= ATCC 51269^T = PCM 2404^T) (T = type strain) by using the International *Streptomyces* Project (ISP) method (5) and identified this organism as *Streptomyces baldaccii* (Farina and Locci 1966) Witt and Stackebrandt 1991 (2, 7) on the basis of DNA-DNA hybridization data (1).

S. spitsbergensis IFO 15745^T (= ATCC 51269^T = PCM 2404^T), *S. baldaccii* IFO 14693^T, and whorl-forming *Streptomyces* species having red series aerial and substrate mycelia as (Table 1) were used. These strains were cultured in 5-ml portions of YG medium (1% glucose, 1% yeast extract; pH 7.0) in test tubes at 28°C for 2 days on a reciprocal shaker. The cultured mycelia were harvested and washed twice with sterile distilled water and then suspended in 5 ml of sterile water. Washed mycelia were inoculated onto four kinds of agar plates (ISP media 2, 3, 4, and 5) as recommended by Shirling and Gottlieb (5) and incubated at 28°C for 14 days. The morphological, cultural, and physiological characteristics of the organisms were observed and described by using the ISP method (5). DNAs were extracted from the organisms and purified by the method of Saito and Miura (4). DNA-DNA hybridization experiments were performed by the method of Ezaki et al. (1) at a temperature of 55°C in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0) containing 50% formamide. Each experiment was performed at least three times, and the

TABLE 1. Phenotypic characteristics of *S. spitsbergensis* IFO 15745^T and whorl-forming streptomycetes having red aerial mycelia

Species	Strain	Other designation	Aerial mass color[a]	Reverse color[a]	Melanin formation	Utilization of carbon sources								
						Glucose	Arabinose	Fructose	Inositol	Mannitol	Raffinose	Rhamnose	Sucrose	Xylose
Streptomyces spitsbergensis	IFO 15745^T		R	d-R	+[b]	++	−	−	++	−	−	−	−	−
Streptomyces baldaccii	IFO 14693^T		R	d-R	+	++	−	−	++	−	−	−	−	−
Streptomyces biverticillatus	IFO 12845^T	ISP 5272	R, V	R, or-R	+	++	−	+	+	−	−	−	−	−
Streptomyces distallicus	IFO 15815^T		R	d-y-Br, d-r-Br	+	++	−	++	++	−	−	−	−	−
Streptomyces fervens	IFO 13343^T	ISP 5086	R	R, d-R	+	++	−	−	++	−	−	−	−	−
Streptomyces flavopersicus	IFO 12769^T	ISP 5093	R	y-Br, Br	+	++	−	+	++	−	−	−	−	−
Streptomyces hiroshimensis	IFO 12785^T	ISP 5037	R	r-Y, r-y-Br	+	++	−	±	++	−	−	−	−	−
Streptomyces kentuckensis	IFO 12880^T	ISP 5052	R, W	y-Br, Br	+[c]	++	−	−	++	−	−	−	−	−
Streptomyces rectiverticillatus	IFO 13079^T	ISP 5436	R, W	y-Br, P	+	++	−	++	++	−	−	−	−	−
Streptomyces netropsis	IFO 12893^T	ISP 5259	R	y-Br, r-Br	+[c]	++	−	−	++	−	−	−	−	−
Streptomyces roseoverticillatus	IFO 12817^T	ISP 5039	R	r-Y	+	++	−	−	++	−	−	−	−	−
Streptoverticillium salmonis	IFO 15865^T		R, Y	d-R, r-V	+	++	−	±	++	−	−	−	−	−

[a] R and r, red; W, white; Y and y, yellow; V, violet; Br, brown; P, pink; or, orange; d, dark.
[b] +, positive; ++, strongly positive; ±, doubtful; −, negative.
[c] Melanin formation is doubtful as determined in this study.

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FIG. 1. Morphology of the spore chains in aerial mycelia of *S. spitsbergensis* IFO 15745^T. The organism was grown on ISP medium 2 for 10 days at 28°C.

level of DNA relatedness was expressed as a percentage of the homologous DNA binding value.

S. spitsbergensis IFO 15745^T formed abundant verticillate aerial mycelia on ISP media 2, 3, 4, and 5 (Fig. 1). Table 1 shows cultural and physiological characteristics of *S. spitsbergensis* IFO 15745^T and related strains. The aerial mass colors of strain IFO 15745^T are white, pink, and red to violet. Substrate mycelia are orange or red to dark reddish brown. Soluble pigments in media are not observed. Melanin is formed on ISP media 1 and 6, but not on ISP medium 7. Glucose and inositol are utilized as carbon sources for growth, but arabinose, fructose, mannitol, raffinose, rhamnose, sucrose, and xylose are not utilized. These morphological, cultural, and physiological characteristics resemble very closely the characteristics of *S. baldaccii* (Farina and Locci 1966) Witt and Stackebrandt 1991 among the known red series whorl-forming *Streptomyces* species, as shown in Table 1. The levels of DNA relatedness between *S. baldaccii* IFO 14693^T and *S. spitsbergensis* IFO 15745^T in mutual hybridization experiments were 86 to 93% (Table 2), indicating that the two strains belong to the same species.

We therefore propose that *S. spitsbergensis* Wieczorek et al. 1993 is a later subjective synonym of *S. baldaccii* (Farina and Locci 1966) Witt and Stackebrandt 1991.

TABLE 2. Levels of DNA relatedness between *S. baldaccii* IFO 14693^T and *S. spitsbergensis* IFO 15745^T

Strain	% DNA relatedness with labeled DNA from:	
	IFO 14693^T	IFO 15745^T
S. baldaccii IFO 14693^T	100	86 ± 2.6[a]
S. spitsbergensis IFO 15745^T	93 ± 3.0	100

[a] Mean ± standard deviation (n = 3).

Recently, Labeda reported that *Streptomyces roseoverticillatus* NRRL B-1993, *Streptomyces biverticillatus* ISP 5272, "*Streptomyces rubrochlorinus*" NRRL B-12558, and *Streptomyces fervens* NRRL 2755 form one species cluster along with *S. baldaccii* NRRL B-3500 as determined by the levels of DNA relatedness among whorl-forming *Streptomyces* species (3). We confirmed the finding of Labeda that the levels of DNA relatedness between these strains and *S. baldaccii* were more than 80%, indicating that these names are subjective synonyms of *S. baldaccii*. A detailed report will be presented in the near future.

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