NOTE

Identification of Strain MF861-C4, a Kasugamycin-Producing Actinomycete, as *Streptomyces albulus***Naoko Kinoshita, Seiko Hattori, Souichi Ikeno¹, Makoto Hori¹ and Masa Hamada**

Institute of Microbial Chemistry, 14-23, Kamiosaki 3-chome, Shinagawa-ku, Tokyo 141, Japan and ¹Showa College of Pharmaceutical Sciences, 3-3165 Higashi Tamagawagakuen, Machida-shi, Tokyo 194, Japan

(Received Oct. 24, 1996 / Accepted Nov. 18, 1996)

We isolated a kasugamycin-producing actinomycete, strain MF861-C4, which had spiny spore surface. This strain was different from *Streptomyces kasugaensis*¹⁾ but rather close to *Streptomyces albulus* with regard to morphological, cultural, and physiological characteristics, DNA homology and partial base sequences of 16S ribosomal RNA. The strain MF861-C4 was identified as *S. albulus*.

We isolated several actinomycete strains producing kasugamycin, such as M338-M1, MB273-C4, and MF861-C4^{1,2)}. We previously reported that strains M338-M1 and MB273-C4 should belong together to a new species of the genus *Streptomyces* and officially proposed the name *Streptomyces kasugaensis*, the type strain being M338-M1 (= ATCC 15714)¹⁾. In the present paper we report that strain MF861-C4 is different from *S. kasugaensis* but rather close to *S. albulus* with regard to morphological, cultural, and physiological characteristics, and DNA homology.

The strain MF861-C4 was isolated from a soil sample collected at the ground of Tenmanguh shrine, Katsunuma-cho, Higashiyamanashi-gun, Yamanashi prefecture, Japan in 1977. Methods of taxonomic and genetic studies were described in the previous paper¹⁾.

Morphological characteristics

Strain MF861-C4 formed well-branched vegetative mycelia and formed long aerial hyphae which bore complete spirals of 5 or more turns. Mature spore chains consisted of 10 or more spores. The spores were oval (0.3 to 0.5 by 0.6 to 0.8 μm) and not motile and their surfaces were spiny (Fig. 1). No synnemata,

sclerotia or sporangia were observed.

Fig. 1. Scanning electron micrograph of strain MF861-C4 grown on sucrose-nitrate agar at 27°C for 4 days.

Table 1. Cultural characteristics of strain MF861-C4

Medium	Growth	Aerial mycelium	Soluble pigment
Sucrose-nitrate agar	Colorless	Thin, Natural [2 dc]	None
Yeast extract-malt extract agar (ISP No.2)	Bamboo [2 gc] ~ Lt Tan [3 gc]	White	None
Oatmeal agar (ISP No.3)	Cream [1 1/2 ca] ~ Lt Ivory [2 ca]	Silver Gray [3 fe] ~ Rose Taupe[5 ig]	None
Inorganic salts-starch agar (ISP No.4)	Bamboo [2 gc] ~ Lt Tan [3 gc]	White ~ Covert Gray [2 fe] ~ Silver Gray [3 fe]	None
Glycerol-asparagine agar (ISP No.5)	Mustard [2 le] ~ Lt Tan [3 gc]	Covert Gray [2 fe] ~ Silver Gray [3 fe]	Faint, brownish
Tyrosine agar (ISP No.7)	Lt Amber [3 ic] ~ Cinnamon [3 le]	White ~ Covert Gray [2 fe]	Faint, brownish
Glucose-asparagine agar	Lt Wheat [2 ea]	White ~ Silver Gray [3 fe] ~ Ashes [5 fe]	Faint, yellowish
Nutrient agar	Lt Wheat [2 ea]	Thin, white	None
Starch agar	Lt Yellow [1 1/2 ea]	White	Faint, yellowish
Calcium-malate agar	Colorless	Thin, white	None

Observation after incubation at 27 °C for 21 days.

Color names and numbers from Color Harmony Manual, Container Corporation of America.⁸⁾

For methods, see reference 1.

Cultural and physiological characteristics

The cultural characteristics of strain MF861-C4 on various agar media are shown in Table 1. The aerial mycelia were white to light gray. The vegetative mycelia were pale yellow to pale yellowish brown. This strain produced faintly yellowish to brownish soluble pigments on several media. Physiological characteristics and carbohydrate utilization are shown in Table 2. Permissive temperatures for growth ranged from 20°C to 37°C, with the optimal temperatures between 27°C and 30°C.

Chemotaxonomic characteristics

Whole-cell hydrolysates contained LL-diaminopimelic acid, but no detectable arabinose, galactose, or *meso*-diaminopimelic acid. Phospholipid pattern was type PII; phosphatidylinositol and phosphatidylethanolamine were present but none of phosphatidylmethylethanolamine, phosphatidylcholine, or unknown glucosamine-containing phospholipid was found. Mycolic acids were absent. Major menaquinones were MK-9(H₄) and MK-9(H₆). Cellular fatty acids consisted of iso-branched 14-meth-

Table 2. Physiological characteristics of strain MF861-C4

Temperature range for growth(°C)	20 ~37
Optimum temperature(°C)	27 ~30
Formation of melanoid pigment	
ISP No.1	Negative
ISP No.6	Negative
ISP No.7	Negative
Liquefaction of	
gelatin	Positive
glucose peptone gelatin	Positive
Coagulation of milk	Positive
Peptonization of milk	Positive
Hydrolysis of starch	Positive
Reduction of nitrate	Variable
Utilization of	
L-Arabinose	—
D-Xylose	—
D-Glucose	+
D-Fructose	+
Sucrose	—
Inositol	+
Rhamnose	—
Raffinose	—
D-Mannitol	+

+ : Utilization, — : No utilization

For method, see reference 1.

ylpentadecanoic acid (*i*-16:0), anteiso-branched 12-methyltetradecanoic acid (*ai*-15:0), iso-branched 13-methyltetradecanoic acid (*i*-15:0), anteiso-branched 14-methylhexadecanoic acid (*ai*-17:0), and other minor components. The G+C contents of DNA was 70.9 mol%.

Identity of strain MF861-C4

The taxonomic properties given above

suggested that strain MF861-C4 belonged to the genus *Streptomyces*. We searched the taxonomic data of known *Streptomyces* species in the ISP descriptions by Shirling and Gottlieb³⁻⁶) and in the descriptions of *Streptomyces* species in the International Journal of Systematic Bacteriology since 1980. The search showed that strain MF861-C4 was most closely related to *S. albulus*⁶). *S. albulus* is listed as an allied species of *Streptomyces lydicus* in the Bergey's Manual of Systematic Bacteriology⁷). Strain MF861-C4 was therefore compared with *S. albulus* IMC A-0802^T (ISP 5492) and *S. lydicus* IMC S-0321^T (ISP 5461), both of which had been maintained at the Institute of Microbial Chemistry. As shown in Table 3, the morphological, cultural and physiological characteristics of strain MF861-C4 were similar to those of *S. albulus* except that the latter did not produced kasugamycin. On the other hand, strain MF861-C4 was different from *S. lydicus* in that the latter had smooth spore surface and utilized L-arabinose and raffinose. Major menaquinones and fatty acid compositions of strain MF861-C4 were quantitatively different from those of *S. albulus*. DNA homology between strain MF861-C4 and *S. albulus* was relatively high (50~79%) while that between strain MF861-C4 and *S. lydicus* was low (26%). Our results of these taxonomic studies and DNA homology, incidentally, suggested that *S. albulus* is not allied species of *S. lydicus*.

The base sequences between 121st and 240th (V₂ region, where the high base replacement frequency is observed) of 16S ribosomal RNA genes of strain MF861-C4 and *S. albulus* were identical to each other, while they were different from that of *S. kasugaensis* in 8 bases (Fig. 2). Some genetic instability of strain MF861-C4 was noticed²).

Taken together, we conclude that strain MF861-C4 is a member of *Streptomyces albulus* and propose name it *Streptomyces albulus*

Table 3. Comparison of strain MF861-C4, *Streptomyces albulus* and *S. lydicus*

	Strain MF861-C4	<i>Streptomyces albulus</i> IMC S-0802 ^T (ISP 5492)	<i>S. lydicus</i> IMC S-0321 ^T (ISP 5461)
Spore chain	Spirales	Spirales	Spirales
Spore surface	Spiny	Spiny	Smooth
Aerial mycelium	White ~ light gray	Light gray	White ~ light gray
Substrate mycelium	Pale yellow ~ pale yellowish brown	Pale yellow	Colorless ~ pale yellow
Soluble pigment	None	None	None
Formation of melanoid pigment	Negative	Negative	Negative
Liquefaction of gelatin	Positive	Positive	Positive
Coagulation of milk	Positive	Positive	Positive
Peptonization of milk	Positive	Positive	Positive
Hydrolysis of starch	Positive	Positive	Positive
Reduction of nitrate	Variable	Positive	Negative
Utilization of			
D-Glucose	+	+	+
L-Arabinose	—	—	+
D-Xylose	—	—	±
D-Fructose	+	+	+
Sucrose*	—	—	—
Inositol	+	+	+
Rhamnose	—	—	—
Raffinose	—	—	+
D-Mannitol	+	+	+
Lactose	—	—	+
Production of kasugamycin	Positive	Negative	Negative

For method, see reference 1

	5'	120	140	160			
(1)		AATCTGCOCT	TCACTCTGGG	ACAAGCOCTG	GAAACGGGGT	CTAATACCGG	ATATGACAAG
(2)							
(3)							G•A

	180	200	220		3'		
(1)	GGGTGCGCATG	ATCTCGTGT	GGAAAGCTOC	GGGGTGAAG	GATGAGCCCG	CGGCTATCA	
(2)							
(3)	C•AC•.....	G•••GT•••					

Fig. 2. Nucleotide sequences of 16S rRNA gene.

These nucleotide sequences of the 16S rRNA gene correspond to the V₂ region (120 bp).

(1) *S. sp.* MF-861-C4 (2) *S. albulus* IMC S-0802 (ISP 5492) (3) *S. kasugaensis* M338-M1

MF861-C4. A subculture of strain MF861-C4 was submitted to the National Institute of Bio-science and Human-Technology, the Agency of Industrial Science and Technology, Tsukuba, Japan, under the accession No. FERM P-15912.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Tsuyoshi Tamamura, Central Research Laboratories, Hokko Chemical Industries Co. Ltd. for the analyses of fatty acids.

REFERENCES

- 1) Hamada, M.; N. Kinoshita, S. Hattori, A. Yoshida, Y. Okami, K. Higashide, N. Sakata & M. Hori: *Streptomyces kasugaensis* sp. nov.: A new species of genus *Streptomyces*. *Actinomycetol.* 9: 27-36, 1995.
- 2) Ikeno, S.; K. Higashide, M. Hori, N. Kinoshita & M. Hamada: Correlation between the presence of *kac*, kasugamycin acetyltransferase gene, and the productivity of kasugamycin in *Streptomyces*. *Actinomycetol.* 10: 73-79, 1996.
- 3) Shirling, E. B. & D. Gottlieb: Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. *Int. J. Syst. Bacteriol.* 18: 69-189, 1968.
- 4) Shirling, E. B. & D. Gottlieb: Cooperative description of type cultures of *Streptomyces*. III. Additional species descriptions from first and second studies. *Int. J. Syst. Bacteriol.* 18: 279-392, 1968.
- 5) Shirling, E. B. & D. Gottlieb: Cooperative description of type cultures of *Streptomyces*. IV. Species descriptions from the second, third and fourth studies. *Int. J. Syst. Bacteriol.* 19: 391-512, 1969.
- 6) Shirling, E. B. & D. Gottlieb: Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. *Int. J. Syst. Bacteriol.* 22: 265-394, 1972.
- 7) Williams, S. T.; M. Goodfellow & G. Alderson: Genus *Streptomyces* Waksman and Henrici 1943, 339AL, In "Bergey's manual of systematic bacteriology, vol. 4" (ed. S. T. Williams, M. E. Sharpe & J. G. Holt) pp.2452-2492, The Williams & Wilkins Co., Baltimore, 1989.
- 8) Jacobson, E.; W. C. Granville & C. E. Foss: Color harmony manual, 4th ed., Container Corporation of America, Chicago, 1958.