Saccharothrix tangerinus sp. nov., the Producer of the New Antibiotic Formamycin: Taxonomic Studies

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The taxonomy of a soil isolate, strain MK27-91F2 which produced a new antifungal antibiotics formamycin, was studied. We propose that the strain should belong to a new species of the genus *Saccharothrix* with the new name *Saccharothrix tangerinus* sp. nov., the type strain being MK27-91F2 (= JCM 10302 = IFO 16184 = FERM P-16053). This new species was characterized by a type III cell wall, galactose and rhamnose as whole-cell sugars, type PII phospholipids, MK-9(H₂) menaquinone, fatty acid components of *i*-16:0, *i*-14:0, *i*-15:0, 16:0, 16:1, 17:1 and *i*-16:1, but lacking mycolic acids, a G+C content in DNA of 74 mol %, and a base sequence of 16S ribosomal RNA gene.

In the course of screening for new antimicrobial substances from microorganism origin, we found an actinomycete strain MK27-91F2 to produce formamycin^{1,2)}, a new 16-membered macrolide belonging to the bafilomycin-concanamycin family. Formamycin showed a strong antifungal activity against phytopathogenic fungi. In the previous paper¹⁾, we described briefly the taxonomic characteristics of strain MK27-91F2. In the present paper, we report further taxonomic studies of strain MK27-91F2 and propose the name *Saccharothrix tangerinus* for the strain as a new species.

MATERIALS AND METHODS

Organism: Strain MK27-91F2 was isolated from a soil sample collected at Setagaya-ku, Tokyo, Japan. This strain was compared taxonomically with *Saccharothrix aelocolonigenes* subsp. *aelocolonigenes* IMC A-0180 (ISP 5034^T) (T= Type strain), *S. aelocolonigenes* subsp. *staurosporea* IMC A-0194 (JCM 9734^T), *S. australiensis* IMC A-0202 (JCM 3370^T), *S. coeruleofusca* IMC A-0203 (JCM 3313^T), *S. coeruleoviolacea* IMC A-0207 (JCM 9110^T), *S. cryophilis* IMC A-0208 (JCM 9111^T), *S.*

espanaensis IMC A-0209 (JCM 9112^T), *S. flava* IMC A-0195 (JCM 3296^T), *S. longispora* IMC A-0204 (JCM 3314^T), *S. mutabilis* subsp. *mutabilis* IMC A-0196 (JCM 3380^T), *S. mutabilis* subsp. *capreolus* IMC A-0183 (ISP 5225^T), *S. syringae* IMC A-0205 (JCM 6844^T), *S. texasensis* IMC A-0206 (JCM 9113^T), *S. waywayandensis* IMC A-0197 (JCM 9114^T) and *Lentzea albidocapillata* IMC A-0201 (JCM 9732^T).

Morphological characterization:

Morphological observations under a light microscope and a scanning electron microscope (model S-570, Hitachi) were made on cultures grown on sucrose-nitrate agar and glucose-asparagine agar, incubated at 27°C for 4 to 21 days. For the scanning electron microscope samples, the cultures were fixed in 2% osmium tetroxide vapor in situ at 4°C for 18 hours, frozen in liquid nitrogen, and then freeze-dried. Each specimen was coated with gold by using an ion sputter instrument (model SC-701 quick coater; Sanyu Denshi). The motility of spores was observed by the method of Hasegawa et.al.³⁾

Cultural and physiological

characterization: Cultural and physiological characteristics, and carbohydrate utilization

patterns were determined using methods of Shirling and Gottlieb⁴⁾, Waksman⁵⁾, and Gordon et al.⁶⁾. Permissive temperatures for growth were determined on glucose-asparagine agar.

Chemotaxonomic characterization:

Cells used for chemotaxonomic analysis were obtained by incubating the organism at 27°C for 5 days in yeast extract-glucose broth (1.0% yeast extract, 1.0% glucose, pH 7.2) on a rotary shaker. Cell walls were prepared according to the method by Kawamoto et al.⁷⁾ except that the precipitates were not treated with an enzyme. Cell wall hydrolysates were analyzed using thin layer chromatography (TLC) according to the method of Stanek and Roberts⁸⁾ for diaminopimelic acid isomers. Cell wall sugars and whole-cell sugars were prepared by the method of Lechevalier and Lechevalier⁹⁾ and analyzed using high performance liquid chromatography (HPLC) according to the method of Mikami and Ishida¹⁰⁾. The cell wall acyl type was determined by the method of Uchida¹¹⁾. Phospholipids and mycolic acids were analyzed by the methods of Minnikin et al.¹²⁾ and Minnikin et al.¹³⁾, respectively. Menaquinones were extracted by the method of Collins et al.¹⁴⁾, and analyzed using a LC-MS (model M-1200H, Hitachi) with a CAPCELL PAK C18 column (150mm by 4.6mm, Shiseido) using methanol-isopropanol (2:1, v/v) as a mobile phase. Methyl esters of cellular fatty acids were prepared by the method of Suzuki and Komagata¹⁵⁾ and analyzed using a set of gas chromatography (model GC-17A, Shimadzu) consisting of a Megabore DB-1 column (15m by 0.35 mm, film 1.5 µm, J & W Scientific), a flame ionization detector, and a SHIMADZU CHROMATOPAK C-R6A.

DNA analysis: The preparation of total DNAs was described in a previous paper¹⁶⁾. The G+C contents of DNAs were determined using HPLC according to the method of Tamaoka and Komagata¹⁷⁾. Genome homologies were examined by fluorometric DNA-DNA hybridization in microdilution wells according to the method of Ezaki et al.¹⁸⁾. The 16S ribosomal RNA gene was amplified by PCR using genomic DNA of each

strain as template, and 5'-TCACGGAGA-GTTTGATCCTG (sense, 1~20, the positioning number was based on that of the 16S rRNA gene of *Streptomyces violaceoruber*^{19,20)}, 5'-GTTT-TAGCCTTGCGGCCGTACTCC (antisense, 860 ~ 884), 5'-ATGCGCAGATATCAGGAGGAA-CA (sense, 668 ~ 690) and 5'-AGAAAGGA-GGTGATCCAGCCGC (antisense, 1507 ~ 1528) as primers. PCR was performed using a MiniCycler™ (MJ Research). Each reaction mixture (100 µl) contained 50 ~ 150 ng genomic DNA, 50 µM each dNTPs, 1XPCR buffer (20 mM Tris-HCl pH 8.3, 1.5 mM MgCl₂, 25 mM KCl, 0.05 % Tween 20), 20 pmol each of the two primers and water to volume. After addition of 2 units Vent (exo-) polymerase (New England BioLabs, 257S), the DNA template was denatured at 100°C for 3 min. Amplification was carried out by 30 cycles of annealing (1 min. at 50°C), extension (1 min. at 72°C) and denaturation (30 sec. at 97°C). The resulting PCR products were sequenced with an automated laser fluorescence sequencer (ALFred™ DNA Sequencer, Amersham Pharmacia Biotech). Sequencing reactions were done using the ALFexpress™ AutoCycle™ Sequencing Kit (Amersham Pharmacia Biotech, 27-2693-02) according to the supplier's instructions. The following Cy5 labeled oligonucleotides were used as the sequencing primer: 5'-ACGAACGCTGG-CGGCGTGCTTAACA (sense, 28~52), 5'-TCA-CGGAGAGTTTGATCCTGGCTCA (sense, 1~25), 5'-ACTGAGACACGGCCCAGACTCC-TAC (sense, 313 ~ 337), 5'-ATGCGCAGATA-TCAGGAGGAACA (sense, 668 ~ 690), 5'-CTCAAAGGAATGACGGGGG (sense, 884 ~ 903), 5'-CGACGTCAAGTCATCATGCCCT-TATG (sense, 1169 ~ 1195), 5'-GTAGGAGTC-TGGGCCGTGTCTCAGT (antisense, 313~337), 5'-ATTACCGCGGCTGCTGGCACGTAG (anti-sense, 483 ~ 506), 5'-GTTTTAGCCTTGC-GGCCGTACTCCC (antisense, 860 ~ 884), 5'-TCGTCCCCACCTTCCTCCGAGTTGA (anti-sense, 1147~1171), 5'-AGAAAGGAGGTGAT-CCAGCCGCA (antisense, 1507~1528).

The sequences of the 16S rDNA of

Saccharothrix coeruleofusca DSM 43679^T (X76963), *S. australiensis* ATCC 31947^T (X53193), *S. longispora* DSM 43749^T (X76964), *S. mutabilis* subsp. *mutabilis* DSM 43853^T (X76966), *S. mutabilis* subsp. *capreolus* DSM 40225^T (X76965), *Actinosynnema mirum* IFO 14064^T (D85475), *Lentzea albidocapillata* DSM 44073^T (X84321), *Saccharomonospora cyanea* K161^T (Z38018) and *Streptomyces anbofaciens* ATCC 23877^T (M27245) were obtained from EMBL, GenBank, and DDBJ (DNA Data Base of Japan, Mishima, Japan) for comparison. Phylogenetic tree was constructed by the neighbor joining (NJ) method²¹⁾.

The sequences of the 16S rDNA determined in this study are available from DDBJ under the

following accession numbers: *Saccharothrix aerocolonigenes* subsp. *aerocolonigenes* IMC A-0180 (ISP 5034^T), AB020030; *S. aerocolonigenes* subsp. *staurosporea* IMC A-0194 (JCM 9734^T), AB024287; *S. waywayandensis* IMC A-0197 (JCM 9114^T), AB020029; *S. tangerinus* MK27-91F2 (= JCM 10302 = IFO 16184), AB020031.

RESULTS AND DISCUSSION

Morphological characteristics: The substrate mycelia of strain MK27-91F2 were well branched and fragmented. The aerial mycelia of this strain were straight or flexuous, and were fragmented into cylindrical and ellipsoidal spore-

(A)

(B)

Fig. 1. Scanning electron micrographs of strain MK27-91F2 grown on sucrose-nitrate agar at 27°C for 10 days. (A) Aerial hyphae, (B) Pseudosporangium-like structure.

Table 1. Cultural characteristics of strain MK27-91F2.

Medium	Growth	Aerial mycelium	Soluble pigment
Sucrose-nitrate agar	Colorless	White	None
Yeast extract-malt extract agar (ISP No.2)	Pale yellow orange [3 ga, Melon Yellow] ~ pale yellowish brown [3 ic, Lt Amber]	White ~ brownish white [3 ba, Pearl]	None
Oatmeal agar (ISP No.3)	Pale yellow [2 gc, Bamboo]	White	None
Inorganic salts-starch agar (ISP No.4)	Pale yellow [2 gc, Bamboo ~ 2 ic, Honey Gold]	White	None
Glycerol-asparagine agar (ISP No.5)	Pale yellow orange [3 ga, Melon Yellow] ~ pale yellowish brown [2 ne, Mustard Gold]	White	None
Tyrosine agar (ISP No.7)	Pale yellow orange [3 ga, Melon Yellow] ~ pale yellowish brown [2 pg, Mustard Gold]	White	Faint, brownish

Observation after incubation at 27°C for 21 days.

Color names and numbers from Color Harmony Manual, Container Corporation of America.³³⁾

like elements (0.3 to 0.5 by 1.1 to 1.9 μm). The surface of spore-like elements was smooth. Some aerial mycelia formed pseudosporangia (Fig.1). None of synnemata, sclerotia, sporangia and motile spores was observed.

Cultural and physiological characteristics: The cultural characteristics of strain MK27-91F2 are shown in Table 1. The aerial mycelia were white to brownish white, and substrate mycelia were pale yellow to pale yellow orange to pale yellowish brown depending on different agar media. The soluble pigments were faint brown or not produced. Physiological characteristics and the carbohydrate utilization pattern are shown in Table 2. Permissive temperatures for growth ranged from 20°C to 30°C, with the optimal temperature at 30°C.

Chemotaxonomic characteristics: Cell wall hydrolysates of strain MK27-91F2 contained *meso*-2,6-diaminopimelic acid and galactose but not arabinose. Whole-cell hydrolysates contained galactose and rhamnose but not arabinose. These data indicate that the strain has a type III cell wall and a type E whole-cell sugar pattern²²⁾. N-acetyl type of muramic acid in cell wall was acetyl type. Phospholipid pattern was type PII; phosphatidylethanolamine was present but none of

phosphatidylcholine or unknown glucosamine-containing phospholipids was found. Mycolic acids were absent. Major menaquinones were MK-9(H₄) and a small amount of MK-10(H₄).

Table 2. Physiological characteristics of strain MK27-91F2.

Temperature range for growth(°C)	20 ~ 30
Optimum temperature(°C)	30
Formation of melanoid pigment	
ISP No.1	Negative
ISP No.6	Negative
ISP No.7	Negative
Hydrolysis of starch	Positive
Reduction of nitrate	Negative
Utilization of	
L-Arabinose	+
D-Xylose	+
D-Glucose	+
D-Fructose	+
Sucrose	-
Inositol	+
Rhamnose	d
Raffinose	-
D-Mannitol	d

* + : utilization, d : doubtful, - : no utilization.

Table 3. Taxonomic comparison of strain MK27-91F2 with family *Pseudonocardiaceae*

Genus	Formation of sporangia	Fragmentation of SM ^a	Cell wall type	Whole-cell sugar type ^b	Phospholipid type	Major menaquinone
Strain MK27-91F2	-	+	III	C(E)	PII	MK-9(H ₄)
<i>Saccharothrix</i>	-	+	III	C(E)	PII or PIV	MK-9(H ₄), MK-10(H ₄)
<i>Lentzea</i>	-	ND	III	C	PII	MK-9
<i>Actinosynnema</i>	-	+/-	III	C	PII	MK-9(H ₄), MK-10(H ₄)
<i>Streptoalloteichus</i>	-	-	III	C(E)	PII	MK-9(H ₆), MK-10(H ₆)
<i>Kutzneria</i>	+	ND	III	C(E)	PII	MK-9(H ₄)
<i>Thermocrispum</i>	-	ND	III	C(A)	PII	MK-9(H ₄)
<i>Amycolatopsis</i>	-	+	IV	A	PII	MK-9(H ₄ , H ₂)
<i>Saccharomonospora</i>	-	-	IV	A	PII	MK-9(H ₄), MK-8(H ₄)
<i>Pseudonocardia</i>	-	+/-	IV	A	PIII	MK-8(H ₄)
<i>Actinopolyspora</i>	-	+	IV	A	PIII	MK-9(H ₄ , H ₆)
<i>Saccharopolyspora</i>	-	+	IV	A	PIII	MK-9(H ₄ , H ₂)
<i>Kibdelosporangium</i>	-	+	IV	A(B)	PII	MK-9(H ₄)

^a SM: substrate mycelium, ND: no data.

^b A: arabinose and galactose are present, C: no characteristic sugar, C(E): galactose and rhamnose are present, C(A): traces of galactose are present in some strain, A(B): traces of madurose are present in some strains in addition to arabinose and galactose.

Cellular fatty acids consisted of *i*-16:0 as the major component and *i*-14:0, *i*-15:0, 16:0, 16:1, 17:1 and *i*-16:1 as minor components. The G+C contents of DNA was 74 mol%.

Identity of strain MK27-91F2: The strain MK27-91F2 was compared with related genera based on literatures, as shown in Table 3. The results of the comparison suggested that strain MK27-91F2 belonged to the genus *Saccharothrix*²³, because strain MK27-91F2 was different from genus *Lentzea*²⁴ in the composition of whole-cell sugar and the major menaquinone; different from genus *Actinosynnema*²⁵ in the formation of synnemata and motile spores and in the composition of whole-cell sugar; different from genus *Streptoalloteichus*²⁶ in the fragmentation of substrate mycelia and the formation of motile spores and in the major menaquinone; and different from genus *Kutzneria*²⁷ in the formation of sporangia.

Strain MK27-91F2 was directly compared with twelve species and two subspecies of the genus *Saccharothrix*^{23, 28-32} and one species of the genus *Lentzea*, as shown in Tables 4 and 5. Although strain MK27-91F2 was similar to *S.*

aerocolonigenes subsp. *staurosporea* in the cultural and physiological characteristics, the former was unable to grow at 37°C and produced formamycin, while the reverse was true with the latter. Furthermore, strain MK27-91F2 shared low homology of total DNA's with the latter and with all the other strains of genus *Saccharothrix* tested, as shown in Table 6.

Fig. 2 shows the phylogenetic tree of 16S rRNA genes of strain MK27-91F2 and several other strains. *Lentzea albidocapillata* and *Actinosynnema mirum* formed one cluster with strain MK27-91F2 and several strains of genus *Saccharothrix*. Although our data disagreed with the description of literature²⁴, *L. albidocapillata* should be closely related to genus *Saccharothrix*, since *L. albidocapillata* had similar morphological characteristics and contained galactose and rhamnose as whole-cell sugars and MK-9(H₄) as major menaquinone. *L. albidocapillata* showed a high genomic DNA homology value with *S. aerocolonigenes* subsp. *staurosporea* and *S. waywayandensis*, whereas, *A. mirum* was different from genus *Saccharothrix* in the morphological characteristics. We should study

Table 4. Comparison of strain MK27-91F2, genus *Saccharothrix* species and *Lentzea albidocapillata*.

	MK27-91F2	<i>S. aer.</i> subsp. <i>aerocol.</i> ISP 5034 ^T	<i>S. aer.</i> subsp. <i>staurop.</i> JCM 9734 ^T	<i>S. wayway-</i> <i>andensis</i> JCM 9114 ^T	<i>Lentzea</i> <i>albidocapillata</i> JCM 9732 ^T	<i>S. flava</i> JCM 3296 ^T	<i>S. mut.</i> subsp. <i>mutabilis</i> JCM 3380 ^T
Decomposition of							
Adenine	-	-	-	-	-	-	-
Casein	+	+	+	+	+	+	+
Hippurate	v	v	+	+	+	-	+
Hypoxanthine	+	+	+	+	+	+	+
Potato starch	+	+	+	+	+	+	+
Tyrosine	+	+	+	+	+	+	+
Urea	-	+	-	+	+	-	-
Acid production from							
Adonitol	-	-	-	+	+	d	+
L-Arabinose	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+
Dextrin	+	+	+	+	+	+	+
<i>meso</i> -Erythritol	-	-	-	-*	d	-	-
Glucose	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+
Inositol	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Melibiose	+	+	+	+	+	+	+
Raffinose	+	-*	d	d	d	d	-*
L-Rhamnose	+	+	+	+	+	+	-
Salicin	d	+	d	d	+	d	+
D-Sorbitol	+	-	+	-	-	-	-
Sucrose	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+
Methyl α -D-glucoside	d	-	-	-	d	-	+
Utilization of							
Citrate	+	+	+	+	+	+	+
Oxalate	+	-*	+	+	+	+	-
DL-Tartrate	-	-*	-	-	-	-	-
Growth in the presence of							
4%NaCl	+	+	+	+	+	+	+
5%NaCl	d	d	+	+	+	d	d
Growth at							
37°C	-	+	+	d	+	+	+
45°C	-	-	-	-	-	-	d
Production of							
Nitrate reductase	-	+	-	-	-	-	+
Phosphatase	+	+	+	+	+	d	+
Formamycin	+	-	-	-	-	-	-

v: variable, d: doubtful.

*: Our results were different from reference description.

<i>S. mut.</i> subsp. <i>capreolus</i> ISP 5225 ^T	<i>S. coeruleo-</i> <i>fusca</i> JCM 3313 ^T	<i>S. texas-</i> <i>ensis</i> JCM 9113 ^T	<i>S. syringae</i> JCM 6844 ^T	<i>S. australi-</i> <i>ensis</i> JCM 3370 ^T	<i>S. espana-</i> <i>ensis</i> JCM 9112 ^T	<i>S. longi-</i> <i>spora</i> JCM 3314 ^T	<i>S. cryo-</i> <i>philis</i> JCM 9111 ^T	<i>S. coeruleo-</i> <i>violacea</i> JCM 9110 ^T
-	-	-*	-	-	-	-*	-	-
+	+	+	+	+	+	+	+	+
+	-	+	+	-	-*	-	-	-
+	-	d	+	-	+	-	-	-
+	+	+	+	-	-	+	d	-
+	-	+	+	+	+	+	+	-
-	-	-	-	-	-	+	+	-
+	d	-	-	-	-	-	-	+
+	+	+	+	-	-	+	-	+
+	+	+	+	-*	+	+	+	+
+	+	+	+	-*	-	+	+	+
-	-	-	-	d	-	-	-	d
+	+	+	+	+	+	+	+	+
+	+	+	-	d	+	+	+	-
+	-	-*	-	-	-	d	+	-
+	+	d	+	-	-	-	+	+
+	+	+	-	-	-	-	+	+
d	+	-	d	-	-	-	+	+
-	-	+	+	-	-	+	-	+
+	+	d	d	-	-	d	-	-
-	d	-	-	-*	-	-	-	-
d	+	+	+	-	+	+	-	-
+	+	+	+	-	-	+	+	+
-	+	-*	-	-	-	-	-	d
+	-	-	+	-	-	-*	-	+
-	-	-	-	-	-	-	+	+
-	-	-	-	-	-	-	-	-
d	+	+	-	+	+	+	+	-
-	-	+	-	d	-	-	-	-
+	+	+	+	+	+	+	-	+
+	+	-	+	+	-	-	-	+
-	-	+	+	+	-	+	+	+
+	+	+	+	-	+	+	+	d
-	-	-	-	-	-	-	-	-

Table 5. Cultural characteristics of MK27-91F2, genus *Saccharothrix* and *Lentzea albidocapillata*.

	Aerial mycelium	Color of growth	Soluble pigment
Strain MK27-91F2	White	Pale yellow ~ pale yellow orange ~ pale yellowish brown	None
<i>S. aelocolonigenes</i> subsp. <i>aerocolonigenes</i> ISP 5034 ^T	None	Pale yellow ~ pale yellowish brown	None
<i>S. aelocolonigenes</i> subsp. <i>staurosporea</i> JCM 9734 ^T	White	Pale yellow orange ~ pale yellowish brown	None
<i>S. waywayandensis</i> JCM 9114 ^T	None	Pale yellow ~ pale yellow orange ~ dull yellow	None
<i>Lentzea albidocapillata</i> JCM 9732 ^T	White	Pale yellow ~ pale yellow orange	None
<i>S. flava</i> JCM 3296 ^T	None	Pale yellow orange ~ dull yellow ~ reddish yellow	Yellowish
<i>S. mutabilis</i> subsp. <i>mutabilis</i> JCM 3380 ^T	White	Reddish yellow ~ light brown	None ~ pale orange
<i>S. mutabilis</i> subsp. <i>capreolus</i> ISP 5225 ^T	Yellowish white	Pale yellow ~ light brown	None
<i>S. coeruleofusca</i> JCM 3313 ^T	Bluish gray	Reddish yellow	Yellowish
<i>S. texasensis</i> JCM 9113 ^T	White	Pale yellow ~ pale yellowish brown	Brownish
<i>S. syringae</i> JCM 6844 ^T	Brownish white	Pale brown ~ dark brown	None ~ brownish
<i>S. australiensis</i> JCM 3370 ^T	Yellowish white ~ yellowish gray	Pale brown ~ yellowish brown	Brownish
<i>S. espanaensis</i> JCM 9112 ^T	None ~ white	Grayish yellow ~ yellowish brown	Yellow ~ brown
<i>S. longispora</i> JCM 3314 ^T	Yellowish gray ~ bluish white	Pale yellow orange ~ dull orange	None
<i>S. cryophilis</i> JCM 9111 ^T	White ~ yellowish white	Pale yellow ~ pale yellowish brown	None
<i>S. coeruleoviolacea</i> JCM 9110 ^T	White ~ bluish white	Pale purple ~ grayish blue green	None ~ purplish

the taxonomical positions between genus *Saccharothrix*, genus *Lentzea* and genus *Actinosynnema* more carefully.

Judging from the results described above, we concluded that strain MK27-91F2 was a new species of genus *Saccharothrix*. We propose, therefore, the name *Saccharothrix tangerinus* to

the strain MK27-91F2 as a new species in the genus *Saccharothrix*.

Description of *Saccharothrix tangerinus* sp. nov. : *Saccharothrix tangerinus* (tan.ge.ri.nus L. adj. *tangerinus* is tangerine-colored, due to the growth color).

The substrate mycelia are fragmented. The

Table 6. DNA hybridization of strain MK27-91F2, genus *Saccharothrix* species and *Lentzea albidocapillata*.

Plate-bound DNA		% Hybridization with photobiotin labeled DNA																		
MK27-91F2		100	14	14	14	13	19	13	14	21	17	15	15	15	14	9	9	9	9	9
<i>S. aerocol.</i> subsp. <i>aerocol.</i> ISP 5034 ^T		8	100	45	27	28	47	8	9	15	12	8	10	8	9	6	5	5	5	5
<i>S. aerocol.</i> subsp. <i>staurop.</i> JCM 9734 ^T		11	39	100	42	46	38	9	10	15	14	9	11	10	10	6	5	5	5	5
<i>S. waywayandensis</i> JCM 9114 ^T		14	50	69	100	46	48	11	14	16	16	11	14	15	14	9	8	8	8	8
<i>Lentzea albidocapillata</i> JCM 9732 ^T		13	55	81	52	100	52	11	13	20	16	13	15	13	12	7	5	5	5	5
<i>S. flava</i> JCM 3296 ^T		17	43	33	33	29	100	9	15	14	12	12	11	13	10	7	7	7	7	7
<i>S. mut.</i> subsp. <i>mutabilis</i> JCM 3380 ^T		20	17	18	17	16	24	100	83	39	30	25	32	31	30	12	12	12	12	12
<i>S. mut.</i> subsp. <i>capreolus</i> ISP 5225 ^T		18	17	15	16	16	17	72	100	28	30	25	29	28	25	11	11	11	11	11
<i>S. coelureofusca</i> JCM 3313 ^T		21	16	13	11	11	17	20	26	100	27	25	27	25	23	10	12	12	12	12
<i>S. texasensis</i> JCM 9113 ^T		19	17	13	14	15	18	22	22	31	100	28	35	24	24	9	11	11	11	11
<i>S. syringae</i> JCM 6844 ^T		20	17	7	14	14	18	22	25	35	36	100	36	25	25	14	15	15	15	15
<i>S. longispora</i> JCM 3314 ^T		17	13	14	13	14	11	19	20	22	30	25	100	22	22	8	10	10	10	10
<i>S. australiensis</i> JCM 3370 ^T		19	16	20	15	15	20	24	27	34	28	22	28	100	32	12	13	13	13	13
<i>S. espanaensis</i> JCM 9112 ^T		16	14	16	14	15	15	21	24	25	27	20	24	27	100	9	10	10	10	10
<i>S. cryophilis</i> JCM 9111 ^T		9	9	10	11	10	13	10	12	18	15	14	11	11	11	100	10	10	10	10
<i>S. coeruleoviolacea</i> JCM 9110 ^T		10	9	8	9	9	12	9	11	16	15	13	11	11	11	10	100	100	100	100

Fig. 2 Phylogenetic tree derived from 16S rRNA sequences of strain MK27-91F2 and related actinomycetes. Scale bar = 0.01 Knucl in nucleotide sequences.

aerial mycelia are straight or flexuous, and are fragmented into cylindrical and ellipsoidal spore. The surface of spore is smooth. Some aerial mycelia form pseudosporangia. The substrate mycelia are pale yellow, pale yellow orange to pale yellowish brown. The aerial mycelia are white to brownish white. The soluble pigments are faint brown or not produced. Melanoid pigments are not produced. L-Arabinose, D-xylose, D-glucose, D-fructose and inositol are utilized. Casein, hypoxanthine, starch and tyrosine are hydrolyzed, but not adenine or urea. Acid is produced from L-arabinose, cellobiose, dextrin, D-glucose, glycerol, inositol, lactose, melibiose, raffinose, L-rhamnose, D-sorbitol, sucrose and D-xylose; no acid is produced from adonitol and *meso*-erythritol. Citrate and oxalate are assimilated, but not DL-tartrate. The strain grows in the presence of 4% NaCl. The temperature range for growth is 20 to 30°C. Nitrate is not reduced to nitrite. Phosphatase is produced. Formamycin is produced. The G+C content of DNA is 74 mol %. It is isolated from soil. The type strain of *Saccharothrix tangerinus* is the strain MK27-91F2 (= JCM 10302 = IFO 16184 = FERM P-16053). Data on the analysis of DNA sequence homology are included.

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