

STREPTOMYCES SCLEROGANULATUS SP. NOV.,
THE PRODUCER OF SCLEROTHRICIN

AKIRA SHIMAZU, TETSURŌ HIDAKA, SHINJI OTSUKA*,
MIYOKO NISHIYAMA and HIROSHI YONEHARA

Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan

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Six strains of *Streptomyces*, all producing sclerothricin and forming sclerotic granules on their vegetative mycelium, have been isolated from soil samples of Japan. A comparison of the characteristics of these strains with related *Streptomyces* indicates that these should belong to a new species, named by us as *S. sclerogranulatus* SHIMAZU et YONEHARA sp. nov. with No. 7672-MC₄ as the type strain.

A new basic antibiotic sclerothricin¹⁾ was isolated from the culture broth of *Streptomyces* strain No. 7672-MC₄. The strain was characterized by the formation of sclerotic granules on the vegetative mycelium and by velvety to cottony and almost white aerial mycelium on several agar media. Strain No. 8898-CC₁, a strain isolated afterwards, formed abundant sclerotic granules and had powdery and reddish-yellow aerial mycelium on the same agar media, but, after successive transfers on agar slants, the characteristics of this strain became similar to those of No. 7672-MC₄. The same was true of the other four strains. On the basis of comparative morphological and cultural characteristics observed on various media, it is possible to group all the six sclerothricin-producing strains as belonging to the same species with No. 7672-MC₄ as the representative strain.

Materials and Methods

Strains: Table 1 gives the preisolation information regarding the six strains under study. *Streptomyces candidus* KCC STM-0195 (NRRL B-1517) and *Chainia poonensis* KCC A-0071 (RIA 569) used for comparative studies with above strains were gifts from Dr. AKIO SEINO, Kaken Chemical Co., Ltd., Tokyo.

Media: Media used for morphological, cultural and physiological studies were prepared according to WAKSMAN²⁾ except the glucose-casein digest-yeast-beef agar; N-Z-amine A of WAKSMAN was replaced by "Polypepton" Daigo (pancreatic digest of casein). Nutrient agar, LOEFFLER's blood serum and the medium for nitrate reduction were prepared from Difco's dried media.

Inocula: Suspensions of washed mycelia obtained from shake cultures at 27°C in a starch-casein digest-molasses-meat extract medium (10:10:10:10, g/liter) were used as inocula.

Observation: Cultural characteristics were seen after 3, 7, 14 and 21 days of incubation at 27°C. The color terms described are according to "Guide to Color Standard"³⁾.

* Present address: Odawara Research Laboratories, Kumiai Chemical Industry Co., Ltd., Odawara, Kanagawa, Japan.

Table 1. The information of preisolation regarding the sclerothricin-producing strains

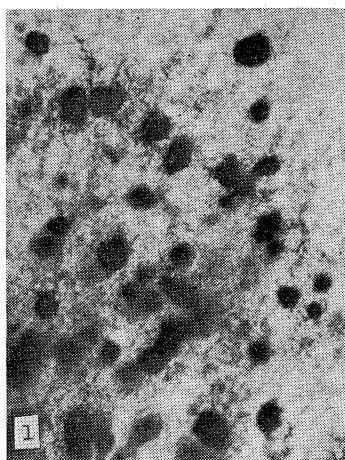
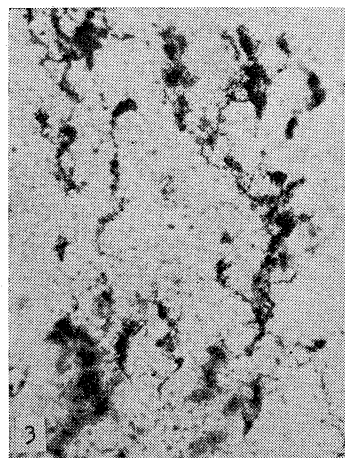
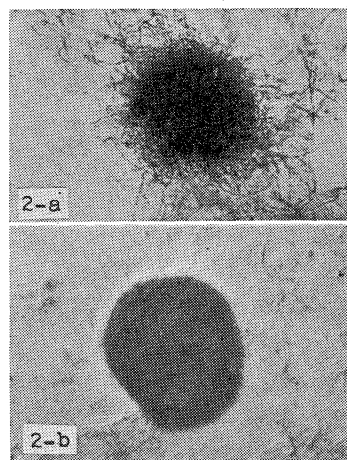
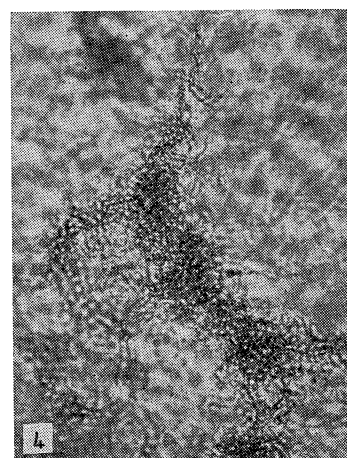
Strain No.	Place	Habitat	Date of isolation
7672-MC ₄	Kawauchi-chyo, Ehime Pref.	Orange-orchard	'64 10 16
8898-CC ₁	Nakayama-machi, Ehime Pref.	Orange-orchard	'67 8 12
9139-S ₈	Naganuma-machi, Fukushima Pref.	Pine-forest	'67 11 8
9140-S ₈	Naganuma-machi, Fukushima Pref.	Kitchen-garden	'67 11 8
9143-S ₁	Naganuma-machi, Fukushima Pref.	Mulberry-field	'67 11 8
9244-S ₁₁	Wakayama city, Wakayama Pref.	Orange-orchard	'67 3 15

Morphology was studied by direct microscopy of the culture surface in Petri dishes on sucrose-nitrate, glucose-asparagine and starch agar media as well as by electron micrographs without fixing or shadowing the materials.

Results

Morphological Description

The sclerothricin-producing strains formed sclerotic granules on vegetative mycelium as do the *Chainia* species (Fig. 1). When freshly isolated from soil, strains produced abundant round-shaped sclerotic granules 20~150 μ in diameter which consisted of darkly pigmented masses of hyphae with spore-like cells (Fig. 2). The sclerotic granules on the agar surface had a short flexuous crowded aerial mycelium. After several transfers on agar slants, the sclerotic granules gradually assumed irregular-forms, consisting of darkly pigmented masses of spore-like cells along with the main hyphae, and looked like the cluster sporophores of *Micromonospora* species (Figs. 3, 4). Aerial my-

Plate A. Sclerotic granules of strain No. 7672-MC₄ on glucose-asparagine agar.Fig. 1. Sclerotia formation on the vegetative mycelium, 14-day culture (110 \times).Fig. 3. Sclerotia of irregular-forms, 14-day culture (110 \times).Fig. 2. (a) Young sclerotum after 7-day culture (550 \times), (b) Mature sclerotum after 21-day culture (275 \times).Fig. 4. Part of irregular-forms sclerotia, 14-day culture (1,100 \times).

celium was observed as monopodial branches in cluster. Aerial hyphae touched on agar surface became zigzag and irregularly-branched short aerial hyphae, and then produced newly actinomorphic substrate mycelia at the branching parts (Fig. 5). Sporophores were straight (Fig. 6) and sometimes produced masses of spores as observed in *Streptomyces massasporeus* SHINOBU *et* KAWATO⁶⁾ (Fig. 7) and loop-like structures similar to "unidentified bodies" of *S. luteocolor* FURUMAI *et* OKUDA⁶⁾. The spores had a cylindrical shape (0.5μ by $1.3\sim 1.7 \mu$), a smooth surface and phalangoform⁷⁾ (Fig. 8).

Cultural and Physiological Characteristics

These characteristics are summarized in Tables 2 and 3. Growth colors of the strains were dull yellow to reddish yellow

and colors of the aerial mycelium were white to pale yellow on most of the media. When repeatedly transferred, texture and colors of aerial mycelium changed to velvety or cottony and almost white from powdery and pale yellow to reddish yellow on glucose-casein digest-yeast-beef agar, glucose-asparagine agar and glycerol-nitrate agar. Soluble pigments were absent or very slight on most of the synthetic media and were of pale yellow to dull yellow shade on some organic media. These strains were mesophilic and nonchromogenic organisms having positive diastatic and proteolytic properties. Sucrose was utilized as a carbon source for growth only by strain No. 8898-CC₁.

Plate B. Aerial mycelium and spore chain of strain No. 7672-MC₄.

Fig. 5. Aerial hyphae touched on agar surface. 14-day culture on glucose-casein digest-yeast-beef agar (1,100 \times).

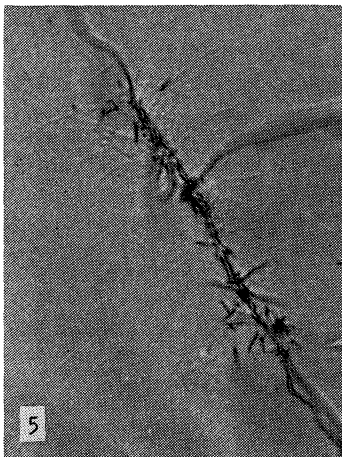


Fig. 6. Aerial mycelia after 14-day culture on sucrose-nitrate agar (110 \times).

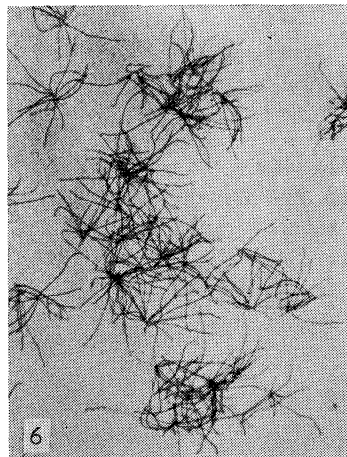


Fig. 7. Aerial mycelium producing masses of spores, 14-day culture on starch agar (550 \times).

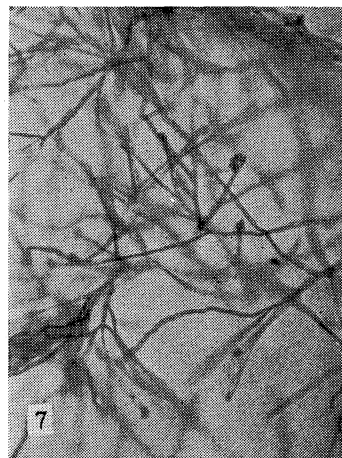


Fig. 8. Electron micrograph of spore chain from 14-day culture on starch agar (6,700 \times).

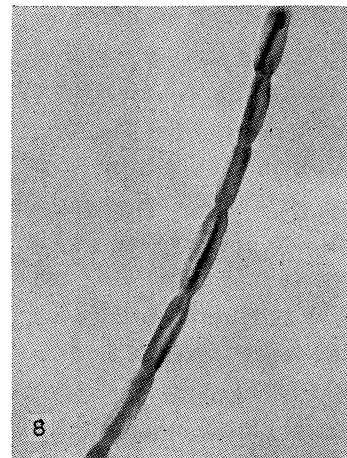


Table 2. Cultural characteristics of the sclerothricin-producing strains

Medium	Cultural characteristics
Sucrose-nitrate agar	G : thin, colorless to yellowish gray; some strains moderate, dull yellow AM : abundant, velvety to cottony, white to white with yellowish tinge SP : none or very slight, yellowish
Glycerol-nitrate agar	G : thick and wrinkled, dull yellow to light reddish yellow AM : abundant, velvety, white with yellowish tinge SP : yellowish gray to pale orange
Glucose-asparagine agar	G : moderate, yellow to reddish yellow AM : abundant, velvety, white with yellowish tinge; some strains, powdery pale yellow orange SP : none or very slight, yellowish
Glycerol-calcium malate agar	G : moderate, yellow to light reddish yellow AM : abundant, velvety, pale yellow SP : none
Starch agar	G : moderate, yellow to dull yellow AM : abundant, velvety, white with yellowish tinge SP : none or very slight, yellowish
Glycerol-urea agar	G : thick and wrinkled, slightly peeled off, light reddish yellow to dull yellow AM : poor, powdery, white with yellowish tinge SP : none
Nutrient agar	G : thick and wrinkled, pale yellow to dull yellow AM : poor, powdery, white SP : none or slight, pale yellowish brown
Glucose-peptone-beef extract agar	G : thick and wrinkled, dull yellow AM : moderate, powdery, white SP : dull yellow
Glucose-peptone agar	G : thick and wrinkled, reddish yellow; later becoming to dark yellow AM : abundant, velvety, white SP : dull yellow
Glucose-casein digest-yeast-beef agar	G : thick and wrinkled, light yellow orange to dark yellow orange AM : abundant, velvety, white to white with orange tinge SP : pale yellowish brown
Oatmeal agar	G : moderate, yellow to light reddish yellow AM : abundant, powdery to velvety, white with orange tinge SP : none or very slight, orange tinge
Potato plug	G : thick and wrinkled, dull yellow to light yellow orange AM : abundant, velvety, white with yellowish tinge SP : pale yellow
LOEFFLER'S blood serum	G : moderate, pale yellowish brown AM : poor, powdery, white SP : pale yellowish brown
Gelatin	G : at surface, moderate, yellow AM : none SP : slight, pale yellow
Milk	G : surface pellicle, light yellow orange AM : none SP : pale orange

G : Growth, AM : Aerial mycelium, SP : Soluble pigments.

Discussion

From morphological considerations, strain No. 7672-MC₄ and the others used in this study belong to the streptomycete which produces sclerotic granules. The main characteristic of *Chainia*, proposed as a separate genus by THIRUMALACHER⁴⁾ in 1955, was the formation of sclerotic granules as large aggregate masses of the vegetative mycelium. This raised quite a controversy and GATTANI⁸⁾ and WAKSMAN^{9,10)} did not accept *Chainia* as a distinct genus, since the formation of sclerotic granules was frequently observed under some conditions amongst *Streptomyces* species as well. THIRUMALACHER and SUKAPURE¹¹⁾, however, maintained that sclerotic granules

were produced on all media, no special conditions were needed and this dominant character of the vegetative mycelium formed a stronger basis for generic differentiation than characters like septation, fragmentation of mycelium and thermophilic habitat. KRASSILNIKOV¹²⁾, KALAKOUTSKII and KRASSILNIKOV¹³⁾, and KUZNETSOV¹⁴⁾ supported the genus *Chainia* since sclerotia appeared to be a characteristic external diagnostic criterion and could be considered towards differentiation of species and perhaps even of genus. WAKSMAN's viewpoint was supported by BECKER *et al.*¹⁵⁾ and LECHEVALIER and LECHEVALIER^{16,17)} based on the chemical composition of the cell wall; *Chainia* had a *Streptomyces*-form cell wall. MAJOROVA¹⁸⁾ however found fucose as a characteristic constituent of *Chainia* polysaccharides and not in a strain of *Actinomyces* (*Streptomyces*).

Recently several taxonomic systems for actinomycetes have been proposed considering the morphological characteristics only or both morphological characteristics and cell wall compositions. According to these systems, *Chainia* was included in the genus *Streptomyces* by LECHEVALIER and LECHEVALIER¹⁶⁾, HÜTTER¹⁹⁾ and WILLIAMS, DAVIES and CROSS²⁰⁾ as a sclerotic granule-forming streptomycetes and by BALDACCII and LOCCI²¹⁾ as a subgenus. However, *Chainia* continues to be admitted as a separate genus by KRASSILNIKOV²²⁾.

We have included the sclerotic granule-forming streptomycetes in genus *Streptomyces* because of the following considerations. According to THIRUMALACHER and SUKAPURE¹¹⁾, the character of the *Chainia* species generally changed to that of the *Streptomyces* species in which the aerial mycelium and sporophores development predominated and the extent of sclerotic development diminished by repeated transfers and maintenance over long periods as artificial cultures. And they consider this phenomenon is similar to the loss of sporulation character in streptomycetes on repeated transfers. The chainiae-form characteristics of sclerothricin producing strains changed to those of the *Streptomyces*-form by relatively fewer transfers than are needed by the typical chainiae, like *Chainia antibioticus* and *C. poonensis*. This would suggest that these strains are a kind of link (intermediate) between the typical species of *Streptomyces* and those of *Chainia*. Taxonomically the loss or diminution of sclerotic granules could not be compared with the loss or lack of sporulation in *Streptomyces*, since formation of sclerotia is only one of the morphological characteristics for separation of the consecutive group streptomycetes-chainiae. In fact the ability to form

Table 3. Physiological characteristics of the sclerothricin-producing strains

Growth temperature	
optimum growth	25~30°C
limited growth	37°C
no growth	above 42°C
Chromogenicity	
deep brown pigment on organic media	—
melanine formation from tyrosine	—
Starch hydrolysis	+
Nitrate reduction	+
Cellulose decomposition	—
Acid formation from glucose	+
Proteolytic activities	
gelatin liquefaction	+
milk peptonization	+
blood serum liquefaction	+
Carbon utilization	
Glucose, fructose, mannose, raffinose, mannitol, inositol, sucrose (some strains)	++
Xylose, lactose, salicin	+
Arabinose, rhamnose, sucrose (some strains)	—

Table 4. Comparison of taxonomical characteristics of strain No. 7672-MC₄ and *Streptomyces luteocolor*

	Strain No. 7672-MC ₄	<i>S. luteocolor</i>
Melanine formation	negative	negative
Spore surface	smooth	smooth
Sporophores morphology	straight	straight
Sclerotic granules	formation	not recorded
Synthetic agar		
Growth	pale yellow to reddish yellow	cream to gold
Aerial mycelium	white to pale yellow	cream to light ivory or pearl pink
Soluble pigment	absent or slight	absent
Organic agar		
Growth	pale yellow to dark yellow	bright yellow to amber
Aerial mycelium	white	cream to light ivory
Soluble pigment	dull yellow to straw	absent
Nitrate reduction	positive	negative
Diastatic activity	positive	positive
Proteolytic activity	positive	positive

sclerotia appears to be quite widespread among actinomycetes and is not restricted to chainiae alone^{8,9,17,21,23}). Also, it is not exactly known whether the mechanism of sclerotic granule formation or their role in the life cycle is of consequence to the taxonomical evaluation of *Actinomycetales*.

In view of the above, we have classified our sclerothricin-producing strains in the genus *Streptomyces* WAKSMAN *et* HENRICI after comparison of their characteristics with *Streptomyces* species described by WAKSMAN²⁴), SHIRLING and GOTTLIEB^{25,26}) and others, as well as of *Chainia* by KUZUNETSOV¹⁴) and THIRUMALACHER and coworkers^{11,27,28}). The strains showed some resemblance of characteristics to *Chainia* (*Streptomyces*) *poonensis* THIRUMALACHER in KALAKOUTSKII *et* KRASSILNIKOV (1960)¹³), *Streptomyces candidus* (KRASSILNIKOV) WAKSMAN *et* HENRICI (1953)²⁴)—a variant of which produced the streptothricin-like antibiotic LL-AB 664²⁹) and *S. luteocolor* FURUMAI *et* OKUDA (1968)—the producer of the basic water-soluble antibiotic BD-12³⁰). *C. poonensis* KCC A-0071, *S. candidus* KCC STM-0195 and our strain No. 7672-MC₄ were compared for characteristics under the same experimental conditions. *S. candidus* KCC STM-0195 differed from our No. 7672-MC₄ in that (a) its aerial mycelium was of powdery texture and yellowish gray with olive tinge on most media, (b) its growth color was colorless to pale yellow and (c) it did not form sclerotic granules on the experimental media. *C. poonensis* KCC A-0071 was very similar to our No. 7672-MC₄ in respect to sporophore morphology, sclerotic granule formation and growth color, but it gave little or no aerial mycelium on most media, utilized arabinose and rhamnose but not raffinose, did not reduce nitrate and acquired little or no change in characteristics with respect to the *Streptomyces*-form after several transfers. *S. luteocolor* was also compared with our No. 7672-MC₄ and the results are summarized in Table 4. This strain was relatively the most similar to No. 7672-MC₄ except that (a) the color of its aerial mycelium on glucose-asparagine agar was pearl-pink (belonging to Red Series according to TRESNER and BAKUS³⁰)), it did not produce soluble pigments on any media except whole egg medium and it did not reduce nitrate. The formation of sclerotic granules, however, was not recorded.

The considerations outlined above lead us to conclude that the six sclerothricin-producing strains are a new species of *Streptomyces* and are accordingly named as *Streptomyces sclerogranulatus* SHIMAZU *et* YONEHARA sp. nov. because of their capacity to form sclerotic granules, with strain No. 7672-MC₄ as the type strain.

Acknowledgement

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