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STUDIES ON THE *STREPTOMYCES* SC_4

II. Taxonomic and Biological Characteristics of *Streptomyces* Strain SC_4[1,2]

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Abstract

Streptomyces SC_4 was isolated from a soil sample collected at Kaohsiung, Taiwan. A comparison of the characteristics of strain SC_4 with related *streptomyces* indicates that strain SC_4 is a new streptothricin antibiotics producing organism.

Introduction

Our previous study demonstrated that *Streptomyces* SC_4 produces four kinds of basic and water soluble antibiotics (Wu *et al.*, 1983). A new streptothricin antibiotic designated as SC_4-X was isolated from the fermentation broth of *Streptomyces* SC_4 culture. SC_4-X is active against Gram-positive and Gram-negative bacteria and fungi. Structurally, SC_4-X contains three molecules of β-lysine, one molecule of aminosugar and one molecule of streptolidine. It differs from other known streptothricin antibiotics in that it has different modification of aminosugar moiety (Wu *et al.*, 1983). This report described the taxonomic and biological characteristics of strain *Streptomyces* SC_4.

Materials and Methods

Isolation and Purification of ***Streptomyces*** *SC_4*

Streptomyces strain SC_4 was originally isolated from the soil sample collected at Kaohsiung, Taiwan. The organism was kept in the lyophilized form. Stock

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slant cultures were maintained on trypton-yeast-glucose (TYG) agar slants. These slants and subsequent cultures used in this study were incubated at 28°C.

Microscopic Observations

Microscopic observations were made on cultures that were grown for 10 to 15 days on TYG agar and TYG soft agar. Media, such as oatmeal agar, glycerol asparagin agar, glycerol tyrosine agar and peptone yeast extract agar were also used for morphological studies. Aerial and vegetative mycelia were observed on undisturbed plates or slide cultures. Ion coating technique was used to obtain specimen for scanning electron micrograpic observation on a Hitachi S450 scanning electron microscope. For studying growth characteristics, *Streptomyces* SC_4 was inoculated on 17 different kinds of media: Czapek-Dox agar, glycerol tyrosine agar, peptone yeast agar, oatmeal agar CM-1, No. 3, potato peptone glycerol agar, glycerol asparagin agar, yeast malt extract agar, starch agar, minimal actinomycetes medium, peptone agar, tryptone yeast glucose agar, nutrient agar, potato plug, carrot plug and cellulose agar. The composition of each medium is shown in Table 1. Cultures were incubated at the following temperatures: 16°C, 22°C, 25°C, 28°C, 30°C, 37°C and 40°C. After 10–15 days of cultivation, shapes, colors and sizes of colonies, mycelia colors, cell size, spore shapes and development of melanoid pigment were examined microscopically.

Carbohydrates Utilization Test

The carbon assimilation patterns of SC_4 were determined according to the methods of Waksman (1947) and Krasil'nikov (1966). A total of 20 kinds of pure carbon compounds were used, namely L-arabinose, D-glucose, D-xylose, L-rhamnose, D-fractose, D-galactose, raffinose, D-mannitol, i-inositol, salicin, sucrose, sorbitol, trehalose, D-maltose, lactose, mannose, inulin, sodium succinate, sodium citrate and sodium acetate. The carbon sources were sterilized by intermittent sterilization method. The basal medium was carbon nutrition medium (Pridham and Gottlieb, 1948), adjusted pH between 6.8 and 7.0, tubed to 9.0 ml amounts and autoclaved. After cooling to about 45°C, sterile aqueous solutions of carbon compounds were added. Free carbohydrate was added to the basic medium to make a concentration of 1% while sodium salts were used the concentration was adjusted to 1.5%. After the addition of carbon sources, the tubes were slanted. Inocula were prepared by growing *Streptomyces* SC_4 on TYG broth at 28°C for 1–2 weeks. SC_4 cells were harvested by centrifugation at 3,000×g for 15 minutes and then washed three times with 0.85% NaCl solution. The washed cells were inoculated on agar slants. Growth of SC_4 and carbohydrate utilizations were measured 10 days after incubation at 28°C.

Production of H_2S

Difco peptone iron agar stabs supplemented with 0.1% yeast extract were

Table 1. *Composition of media (g/liter) used*

Component	Media													
	OA*	YME	CD	PPG	GA	SA	PA	PY	GT	CM-1	TYG	MAM	No. 3	NA
Potato				100										
Oatmeal	65													
Yeast extract		4					1	3		2	3			
Malt extract		10												
Soluble starch						10								
Glucose		4								10	10	20	10	
Sucrose			30											
Glycerol (ml)				5	35				15					
Asparagine			3.5						1			1		
Peptone				2			1	5		2				5
Tryptone											5			
Tyrosine									0.5					
Casein										2				
Beef extract														3
Molasses													10	
Corn starch													10	
Soy bean oil													2	
K_2HPO_4			1	0.5	2.5	0.3			0.5		1	3.48		
KH_2PO_4											1	5		
$MgSO_4$			0.5		0.3					0.5		0.5		
KCl			0.5											
KNO_3														1
$FeSO_4 \cdot 7H_2O$			0.01	0.01					0.01					
$NaNO_3$			3			1								
$MgCO_3$						1								
$(NH_4)_2SO_4$													6	
NaCl			5	0.55		0.5	8.5		0.5				5	5
NH_4NO_3												2		
$CaCO_3$													8	
$CaCl_2$					0.1									
CH_3CONH_4					6.5									
Agar-agar	20	20	20	20	20	20	20	20	20	20	20	20	20	20

* Abbreviations: OA=oatmeal agar; YME=yeast malt extract agar; CD=Czapek-Dox agar; PPG=potato peptone glycerol agar; GA=glycerol asparagine agar; SA=starch agar; PA=peptone agar; PY=peptone yeast agar; GT=glycerol tyrosine agar; CM-1=CM-1 agar; TYG=tryptone yeast glucose agar; MAM=minimal actinomycetes medium; No. 3=No. 3 agar; NA=nutrient agar.

ultilized and results were recorded after 4 days of incubation at 28°C. H_2S production could be identified as a brown to black spot along the line of inoculation.

Nitrate Reduction

Nitrate reduction study was made on complex and synthetic nitrate broth (Sanchez-Marroquin, 1962). Results were taken at the end of 15 days of incubation at 28°C. By adding 1.0 ml of sulfanilic acid and 0.5 ml of dimethyl-α-naphthylamine to 3 ml of the culture medium. The presence of nitrite was shown by a red to deep pink color. Ammonia production was determined by Nessler's reagent.

Physiological Studies

Media used for these studies were prepared according to the procedures of Waksman (1957), Gottlieb (1948) and Kawamra (1976). Mature spores and mycelia on TYG agar were used for inoculation. All cultures were incubated at 28°C for 2 weeks except for gelatin liquefaction which was incubated at 15°C for 20 days.

Determination of the Antimicrobial Spectrum

Bacteria were cultured on Difco antibiotics medium 1. Human pathogenic fungi were cultured on the Sabouraud medium. The Czapek-Dox medium was used for plant pathogenic fungi. Serial two-fold dilutions were employed to prepare the antibiotic test solutions. Antibacterial and antifungial activities of the SC_4-X antibiotic were measured by using the paper disc method. Aliquots of 5 μl of the test solutions were applied on paper discs (0.6 cm in diameter) which were then placed on agar plates seeded with appropriate organisms. *Bacillus subtilis* PCI 219 was used as the indicator bacterium.

Soft Agar Overlay Test

The *Streptomyces* SC_4 spores were cultured on TYG agar plates and incubated at 28°C for 5 days. After the formation of the single colony of the *Streptomyces*-SC_4, the test organism suspended in a soft-agar medium was overlaid on the colony. The sizes of inhibition zone were measured after 24 h of incubation at 37°C or 30°C.

Fermentative Production of Antibiotics SC_4-X

The sporulative medium consisted of 15 ml glycerol, 10 g asparagine, 5 g tyrosine, 0.5 g_2 K_2HPO_4, 0.5 g $MgSO_4$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.5 g NaCl, and 15 g agar-agar in 1,000 ml of deionized water. The seed medium contained 5 g tryptone, 3 g yeast extract, 10 g glucose, 1 g K_2HPO_4, 1 g KH_2PO_4 and 15 g agar-agar in 1,000 ml of deionized water. The composition of the fermentation medium was 15 ml glycerol, 1 g asparagine, 0.5 g tyrosine, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$, 0.01 g $FeSO_4 \cdot 7H_2O$ and 0.5 g NaCl in 1,000 ml of deionized water. Each sporulative slant was inoculated in 5 bottles of the seed medium, 30 ml in a 250-ml Erlenmeyer flask. After 3 days on

rotary shaker at 28°C, the inoculum, 10% by volume, was transferred to 2,000-ml Erlenmeyer flasks containing 1,000 ml of seed medium. After 3 days on rotary shaker at 28°C the inoculum, 10% by volume, was transferred to a 30-liter jar fermenter containing 20 liters of the medium. Fermentation was carried out at 28°C for 7 days under aeration at 20 liters/min and agitation at 200 rpm. During the fermentation period, 50 ml of the cultured fluid was withdrawn daily from the jar for bioassay. The antibiotic activity was measured by the paper disc method with *Bacillus subtilis* PCI 219 as the test organism.

Isolation and Purification of Antibiotics SC_4-X

The procedures for isolation and purification of antibiotics SC_4-X from culture broth were described by Wu *et al.* (1983).

Results

Cultural Characteristics

Strain SC_4 grew well on various media such as yeast malt extract agar, tryptone yeast glucose agar, potato peptone glycerol agar, No. 3 medium, oatmeal agar, Czapek-Dox agar and glycerol tyrosine agar. The cultural characteristics of strain SC_4 on various media are summarized in Table 2. As shown in Fig. 1, the colonies on TYG agar were about 5–10 mm in diameter, convex with indent edges and covered with white aerial mycelia. Spores are covered all over the surface of growth. As shown in Fig. 2, the colonies of SC_4 on peptone yeast extract agar were about 3–5 mm in diameter, convex with rough surface covered with white aerial mycelia and spores. Usually after 15–20 days cultivation the aerial mycelia and spores changed their color to velvety grayish-white. As shown in Fig. 3, the colonies on Czapek-Dox agar were light brown at the early stage of growth, and spores turned grayish-white later. However, only a few small colonies were observed when SC_4 was cultured on glycerol asparagin agar and cellulose agar. Melanin was released into medium when the culture medium contained peptone. Results also indicated that the optimal temperature for colony development was at 28°C.

Morphological Characteristics

Streptomyces SC_4 is easy to proliferate on TYG agar and GT agar with velvety aerial mycelia and powdery spores. The well developed vegetative mycelia penetrated into the agar and became network-like branches (Fig. 4). Aerial mycelia were long, filamentous and branched. No spiral mycelia were observed. The aerial mycelia were flexible and grew together in clusters (Fig. 5). The terminal filaments developed into conidiophores having straight spore chain. The dimension of conidiophores was 0.8 to 1.0 by 1.5 to 2.0 μ (Fig. 6). The shape of spore was oblong

Table 2. *Cultural characteristics of* ***Streptomyces*** *strain* SC_4

Plates were incubated at 28°C for 2 weeks

Medium	Characteristics				
	Growth	Vegetative mycelia	Aerial mycelia	Spore	Soluble pigment
Czapek-Dox agar	Good	Good White-brown	Moderate White	Moderate White	None
Glycerol tyrosine agar	Good	Abundant Brown	Abundant Velvety, White	Abundant White	Brown
Peptone yeast agar	Moderate	Moderate Brown	Moderate White	Moderate White	Brown
Oatmeal agar	Good	Abundant Brown	Abundant Brown	Abundant White	None
CM-1	Moderate	Moderate Brown	Moderate White	Moderate White	Brown
No. 3	Good	Abundant Brown	Abundant Grayish-White	Abundant White	None
Potato peptone glycerol agar	Good	Abundant Brown	Abundant Grayish-White	Abundant White	Brown
Glycerol asparagin agar	Poor	Scant Lighe brown	Scant	Scant	None
Yeast malt extract agar	Good	Abundant Light-brown	Abundant Grayish-White	Moderate White	None
Starch agar	Moderate	Moderate Light-brown	Moderate Scant	Poor White	None
Minimal actinomycetes medium	Moderate	Moderate Light-brown	Scant	Scant	None
Peptone agar	Moderate	Moderate Brown	Moderate Brown	Moderate White	Brown
Tryptone yeast glucose agar	Good	Good Brown	Abundant White	Abundant White	Brown
Nutrient agar	Moderate	Moderate Brown	Moderate White	Moderate White	Brown
Potato plug	Moderate	Moderate Brown	Moderate White	Moderate White	Brown
Carrot plug	Moderate	Moderate Brown	Scant	Poor	None
Cellulose agar	Poor	Scant White	Scant	None	None

to short cylindrical, averaging between 0.5 to 0.8 by 1.0 to 1.2 μ with smooth surface (Fig. 7). In general, there are 5–20 spores in a chain. No sporangia, flagellated spores or ball-like bodies were observed. Table 3 summarizes the spore morphology of the strain SC_4.

Carbon Utilization

Table 4 summarizes the utilization of carbon sourses. The strain SC_4 readily utilizes D-glucose, L-arabinose, sucrose, trehalose, D-maltose and mannose. Strain SC_4 exhibited a rather narrow carbohydrates utilization pattern.

Table 3. *Spore morphology of* ***Streptomyces*** *strain* SC_4 *as demonstrated by scanning electron microscopy*

Spore surface	Smooth
Spore shape	Oblong
Spore chain	Rectums flexible
Spore size	0.5-0.8 μ in width, 1.0-1.2 μ in length
Average spore number in a chain	5-20
Conidiophore	Develop on the terminal of the aerial mycelium 0.9-1.0 μ in width, 1.5-2.0 μ in length knobbed head

Table 4. *Carbohydrates utilization of* ***Streptomyces*** *strain* SC_4, ***Streptomyces lavendulae, Streptomyces lavendulae subsp. avireus and Streptomyces lavendulae subsp. brasilicus***

Carbohydrate	Response			
	SC_4	**S. lavendulae*	**S. lavendulae subsp. avireus*	**S. lavendulae subsp. brasilicus*
Negative control (no carbon)	−	−	−	−
D-Glucose	+	+	+	+
D-Xylose	−	−	−	−
L-Arabinose	+	−	−	−
L-Rhamnose	−	−	−	−
D-Fractose	−	−	−	−
D-Galactose	−	+	+	+
Raffinose	±	+	−	−
D-Mannitol	−	−	−	−
i-Inositol	−	−	−	−
Salicin	−	+	+	−
Sucrose	+	−	−	−
Sorbitol	−			
Trehalose	+			
D-Maltose	+			
Lactose	−			
Mannose	+			
Inulin	−			
Sodium succinate	−			
Sodium citrate	−			
Sodium acetate	−			

Basal medium: Minimal Actinomycetes medium.
+: Carbohydrate utilized.
±: Very slight utilization.
−: Not utilized.
* Data were taken from Bergey's Mannual of Determinative Bacteriology (Buchanan *et al.*, 1975).

Physiological Properties

Results obtained from physiological examinations of strain SC_4 are shown in Table 5. Low proteolytic activities were demonstrated by weak responses in gelatin liquefaction and milk peptonization. Strain SC_4 did not liquefy Löeffer's coagulated serum. Starch was hydrolyzed, and nitrate was reduced to nitrite. The capability of tyrosinase formation was very low, but the rate of H_2S formation was very rapid. Strain SC_4 tolerated NaCl up to a concentration of 2% in TYG medium.

Table 5. *Physiological characteristics of* ***Streptomyces*** *strain* SC_4

Reaction	Medium	Response
Gelatin liquefaction	Gelatin medium	Positive (weak)
Milk coagulation	Litmus milk	Positive
Milk peptonization	Litmus milk	Positive (weak)
Starch hydrolysis	Nutrient agar with 0.5% soluble starch	Positive
Growth on cellulose	0.5% cellulose agar	Poor
Nitrate reduction	Nitrate broth	Weak
Tyrosinase formation	Tyrosin agar	Weak
H_2S production	Peptone iron agar	Very rapid
Serum liquefaction	Löeffer's coagulated serum	Negative
NaCl tolerance	TYG with 0.5, 2, 4, 8, or 10% NaCl	2% NaCl
Growth on potato plug	Potato plug	Positive
Growth on carrot plug	Carrot plug	Positive
Temperatrue range	10°C, 20°C, 28°C, 35°C and 40°C	No growth at 10°C and 40°C

Antimicrobial Activities

As shown in Table 6, SC_4-antibiotics exhibited potent and broad spectrum of antibacterial activity against Gram-postive and negative bacteria and fungi including *Mycobacterium phlei*, and *Penicillum italicum* Wehmer.

Discussion

Streptothricin is produced by an aerobic, conidia forming species of *Streptomyces lavendulae* (Waksman *et al.*, 1942) which was identified on the basis of its pigmentation and certain cultural characteristics. According to the Bergey's Mannual of Determinative Bacteriology, 8th Edition, *Streptomyces lavendulae* is capable of producing the streptothricin complex, grows poorly on Czapek-Dox agar, and

Table 6. *Antimicrobial activities of antibiotics* SC_4

Test organisms	MIC (μl/ml)
Salmonella paratyphi B	2.5
Shigella flexneri 3b	20.0
Corynebacterium xeloses	2.5
Staphylococcus aureus 209 p	10.0
Sarcina lutea	20.0
Proteus vulgaris	80.0
Escherichia coli NIHJ	10.0
Mycobacterium phlei	20.0
Mycobacterium pseudotuberculosis 607	80.0
Pseudomonas aeruginosa	80.0
Bacillus subtilis PCI 617	10.0
Bacillus subtilis PCI 219	5.0
Bacillus sereus	10.0
Xanthomonas citri	2.5
Xanthomonas oryzae	80.0
Curvularia sp.	2.5
Gibberella fujikuroi	40.0
Alternaria sp.	5.0
Helminthosporium oryzae	40.0
Penicillium italicum Wehmer	10.0
Aspergilus niger	80.0
Candida albicans	20.0

Method: paper disc method.

their growth is inhibited by streptomycin. The spore chains are phalangiform, NaCl tolerance $\geqq 4\%$ but $<7\%$. Strain SC_4 grows well on Cazpek-Dox agar, and tolerates only 2% NaCl. As shown in Table 4, the utilization patterns of carbon compounds are much different between *Streptomyces lavendulae* and strain SC_4. Strain SC_4 utilizes L-arabinose, and sucrose efficiently but *Streptomyces lavendulae* does not. *Streptomyces lavendulae* utilizes D-galactose and salicin but strain SC_4 does not. According to the classification systems on *Actinomycetes* by Waksman (1967), Sykes *et al.* (1973), Tresner *et al.* (1961), Waksman (1943), Sanchez-Marroquin (1962) and Krasil'nikov (1966), strain SC_4 belongs to the chromogenic type of *Streptomyces*, because deep brown diffusible pigment is produced on organic media. SC_4 is categorized in the gray color series with smooth surface and rectums flexibile spore-chain morphology. Although physiological properties of SC_4 are very similar to those of *Streptomyces lavendulae*, there are some differences in cultural characteristics between them and they produce different antibiotics. Strain SC_4 grows

well on Czapek-Dox agar and produces the new streptothricin antibiotics designated SC_4-X (Wu *et al.* 1983).

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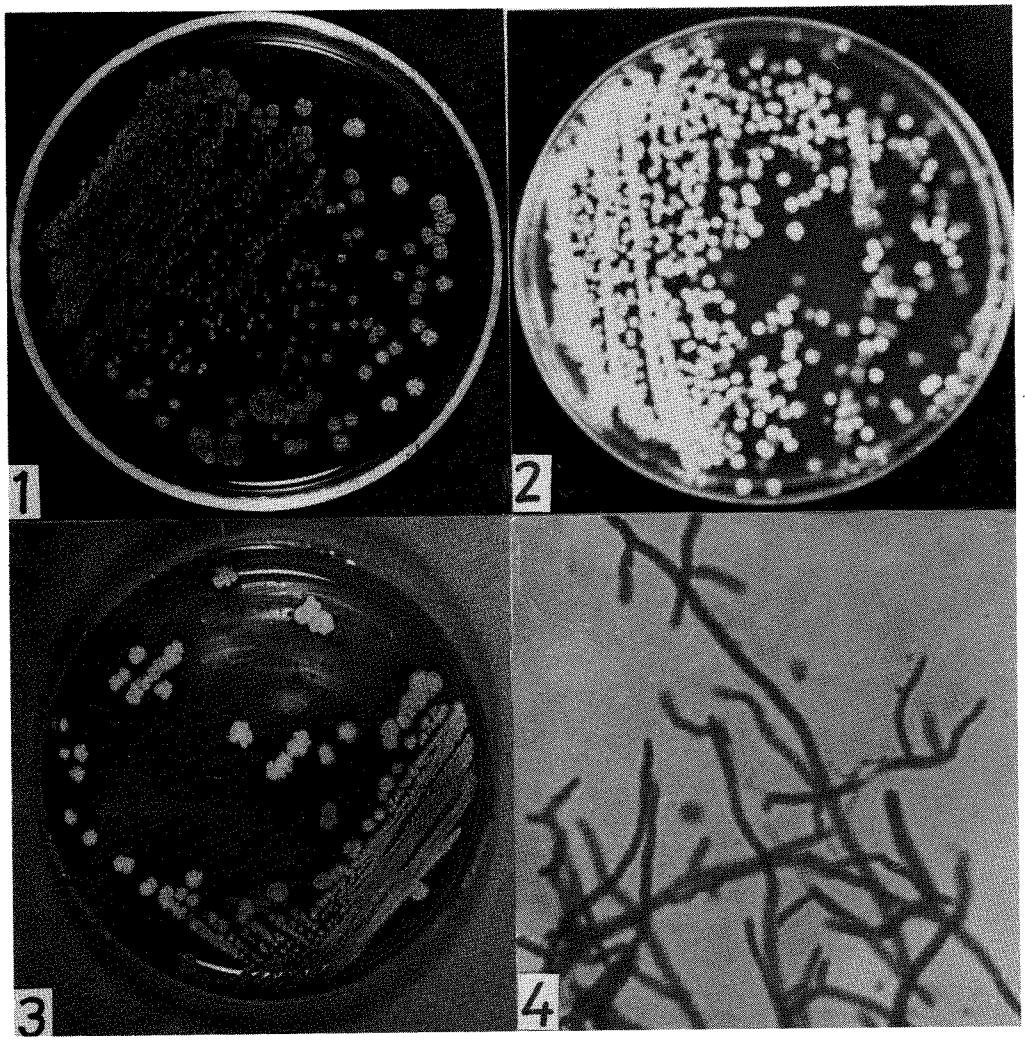

Fig. 1. Colonies of *Streptomyces* SC_4. *Streptomyces* SC_4 was cultivated on TYG agar plate at 28°C for 7-10 days. Colonies are convex with irregular edge, 5-10 mm in diameter. The medium turned to brown color after 3 days of incubation due to the melanin pigment excreted from the cells.

Fig. 2. Colonies of *Streptomyces* SC_4. *Streptomyces* SC_4 was cultivated on PY agar plate at 28°C for 7-10 days. Colonies are convex with rough surface, 3-5 mm in diameter.

Fig. 3. Colonies of *Streptomyces* SC_4 on Czapek-Dox agar plate at 28°C for 7-10 days.

Fig. 4. The light-micrograph of vegetative mycelia of *Streptomyces* SC_4. Spores were cultivated on TYG soft-agar by hanging drop method for 3 days at 28°C. The vegetative mycelia were examined under light microscope.

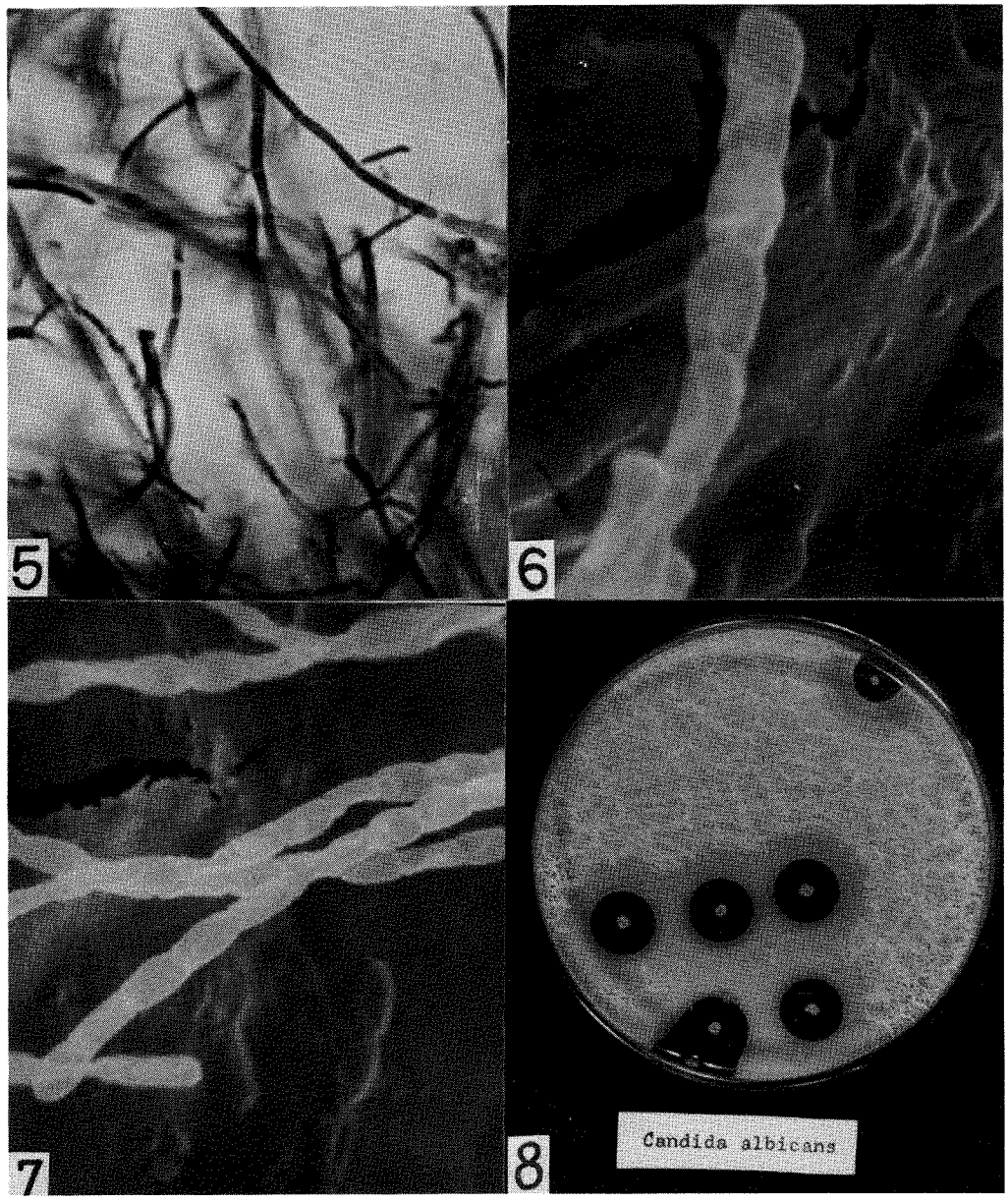

Fig. 5. The light-micrograph of the aerial mycelia of *Streptomyces* SC_4. The aerial mycelia are flexible and terminates in clusters.

Fig. 6. The scanning electron micrograph of the conidiophores and spores of *Streptomyces* SC_4.

Fig. 7. The scanning electron micrograph of the spore of *Streptomyces* SC_4. Each spore chain contains 5-20 spores which are in oblong shape and rectums flexible.

Fig. 8. Antifungial activity of *Streptomyces* SC_4 on *Candida albicans*. *Streptomyces* SC_4 was inoculated in TYG agar for 5 days, then overlaid with Sabouraud agar containing *Candida albicans* and incubated at 37°C for 5 days.

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鏈黴菌 SC_4 之研究

II. 鏈黴菌 SC_4 之特性及分類研究

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鏈黴菌 SC_4 爲從高雄土壤中分離而得之抗生素產生菌。SC_4 菌與有關之鏈黴菌相比較，找不出與 SC_4 菌之特性完全相同之菌。因此 SC_4 菌可能是一種能產生 Streptothricin 類抗生素之新抗生素產生菌。