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Identification of the *Streptomyces* strain KS3-5

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Abstract. An actinomycete, designated strain KS3-5, was isolated from a soil sample collected from Kaohsiung, Taiwan, ROC. This organism is capable of producing a series of antibiotics that strongly inhibit the growth of Gram-positive and Gram-negative bacteria and yeast-like fungi. The spore morphology and cell wall chemotype suggest that strain KS3-5 is a streptomycete. Further cultural and physiological characterization and the DNA homology suggest that strain KS3-5 is identical to *Streptomyces toxytricini*.

Keywords: *Streptomyces*; *Streptomyces toxytricini*.

Introduction

The 'strike-back' of pathogens has revitalized the search for new drugs (Lemonick, 1994; Jaroff, 1994). Novel antibiotics are required to counter drug-resistant bacteria, fungi, and viruses. Only about 10% of the estimated total number of microbial species are known—there is an extensive and diverse resource that can be tapped for useful products, such as antibiotics, and processes, such as novel mechanisms of action (Bull et al., 1992). In this respect, natural antibiotics (particularly those from the genus *Actinomyces*, the most abundant microbial source of antimicrobial compounds; Miyadoh, 1993) are as important as those, such as the β-lactam antibiotics, which are derived from chemical modification of existing antibiotics.

In our screening program for bioactive compounds, an actinomycete (which we designated strain KS3-5) was isolated from a soil sample collected from southern Taiwan. This actinomycete is capable of producing antibiotics that strongly inhibit the growth of Gram-positive and -negative bacteria, but appear to be non-toxic to experimental mice and tomato seedlings. These data suggest a use for these KS3-5 antibiotics in the treatment of animal and plant diseases. We present the identification of strain KS3-5 through a study of its biological properties.

Materials and Methods

Microorganisms and Culture Conditions

Strain KS3-5 was isolated from a soil sample collected at Tapehu, Kaohsiung, Taiwan. *Streptomyces toxytricini* ATCC 19813 was purchased from the American Type Culture Collection for comparison. Except where otherwise specified, both strains were cultured on tryptone-yeast extract-glucose (TYG) agar medium containing 10 g glucose, 3 g yeast extract, 5 g peptone, 1 g KH_2PO_4, 1 g K_2HPO_4, and 20 g agar in 1,000 ml of distilled water, and incubated at 28°C.

Cultural and Morphological Characterization

Cultural characteristics of strain KS3-5 were compared on the basis of observations made after 7, 14, and 21 days incubation on Czapek-Dox agar, nutrient agar, Sabouraud agar, and ISP media (Shirling and Gottlieb, 1966). Morphology was examined by light microscopy and scanning electron microscopy (Zeiss DSM model 950).

Physiological Characterization

Utilization of carbohydrates was investigated with a basal carbon nutrient medium (Pridham and Gottlieb, 1948; Waksman, 1967). Methods and media used for physiological tests were as described by Luedemann and Brodsky (1964), Luedemann (1971), Neyra et al. (1977) and Waksman (1967). All cultures were incubated at 28°C for 10 days, except for the gelatin liquefaction (15°C, 21 days). The assay for enzymatic activity was performed according to Hopwood (1967) and Hopwood and Wright (1973). The cultural broth was tested for its antimicrobial activity using the cup or the paper disc diffusion methods (Wu, 1984).

Cell Chemistry

Determination of the cell-wall composition, including A_2pm (diaminopimelic acid) isomers, sugars, phospholipids, fatty acids and menaquinones was based upon the methods of Becker et al. (1965), Boone and Pine (1968), Kawamoto et al. (1981), Lechevalier and Lechevalier (1970, 1980), and Pine and Boone (1967).

DNA-DNA Homology Study

The DNA-DNA relatedness was determined by the method of Ezaki et al. (1989).

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Table 1. Cultural characteristics of strain KS3-5.

Medium	Characteristics				
	Growth	Vegetative mycelia	Aerial mycelia	Spore	Soluble pigment
Czapek-Dox agar	moderate	moderate, light brown	poor, white	poor, white	light-pink
Glycerol tyrosine agar	moderate	moderate, brown	moderate, light brown	poor, white	brown
Peptone yeast agar	moderate	moderate, brown	moderate, white	moderate, white	brown
Oatmeal agar	well, spreading	abundant, brown	abundant, white	abundant, white	pink
Glycerol asparagine agar	well, spreading	moderate, light brown	abundant, light brown	abundant, powdery	pink
Yeast-malt extract agar	well	abundant, light brown	abundant, grayish white	abundant, white, powdery	pink
Starch agar	well, elevated	moderate, light brown	abundant, brown	abundant, white, powdery	pink
Minimal actinomycetes medium	moderate	moderate, light brown	poor, white	poor, white	pink
Peptone agar	moderate	moderate, brown	moderate, white	poor, white	pink
Tryptone yeast glucose agar	well, elevated	well, brown	abundant, white	abundant, white, powdery	brown
Nutrient agar	moderate	moderate, brown	poor, white	poor, white	pink
Potato plug	moderate	moderate, brown	moderate, white	moderate, white	brown
Carrot plug	moderate	moderate, brown	poor	poor	brown

Results and Discussion

Cultural Characteristics

The cultural characteristics of strain KS3-5 on various media are presented in Table 1. Strain KS3-5 grew well on most of the organic and synthetic media tested. Typically, the colonies were elevated, spreading, and covered with white aerial mycelia and spores. Diffused melanoid pigments were sometimes observed.

Morphological Characteristics

The scanning electron micrograph of strain KS3-5 revealed that aerial mycelia were monopodially branched with compact spirals of sporophore. Each spore chain consisted of 10 to 50 grayish white, oblong (0.8–1.0 μm by 1.2–1.5 μm), smooth-surfaced spores formed on a short (1.0 μm in diameter, 2.0 μm in length) conidiophore developed on the terminal of an aerial mycelium (Figure 1). Such smooth spore containing spirals are common to most *Streptomyces* sp. (Tresner et al., 1961).

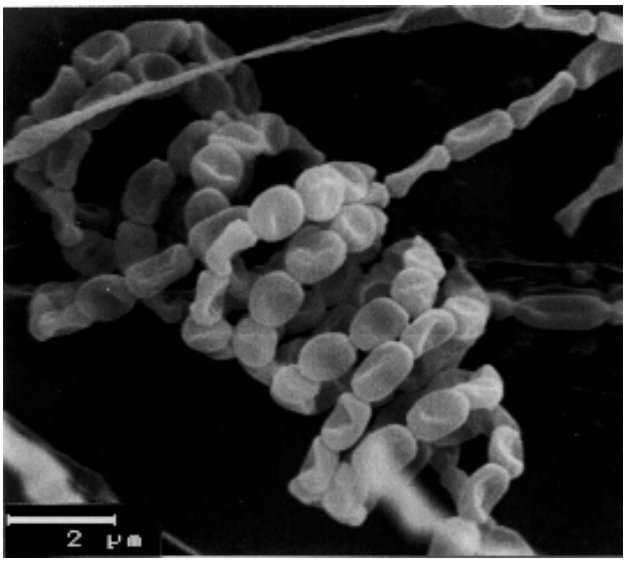

Figure 1. Scanning electron micrograph (×10,000) of a spore chain of strain KS 3-5.

Physiological Characteristics

Table 2 lists the physiological properties of strain KS3-5. It is capable of milk coagulation and peptonization, suggesting a proteolytic ability. The negative hemolysis result agreed with the lack of toxicity found in preliminary tests on mice (data not shown). Strain KS3-5 can also produce moderately active enzymes, e.g. α-amylase, gelatinase, protease and agarase (Table 3).

Table 2. Physiological characteristics of strain KS3-5.

Reaction	Medium	Response
Gelatin liquefaction	gelatin medium	–
Starch hydrolysis	starch agar	+
Milk coagulation	litmus milk	+++
Milk peptonization	litmus milk	+++
Nitrate reduction	nitrate broth	–
Tyrosinase reaction	tyrosine agar	+
H2S production	peptone iron agar	+
NaCl tolerance	TYG slant with NaCl	3%
Growth temperature	TYG slant	4–37°C
Melanin formation	PY slant	+
Growth on potato plug	potato plug	+
Growth on carrot plug	carrot plug	+
Blood hemolysis	5% blood agar	–
pH range	TYG agar slant	pH 6-11

+: positive; –: negative.

Carbohydrate Utilization

The utilization of various carbohydrates by strain KS3-5 suggests a very narrow pattern of carbon source assimilation (Table 4). Glucose and fructose were utilized well; rhamnose, sucrose, and xylose were poorly utilized, and other carbohydrates were not utilized.

Table 3. Enzyme production of strain KS 3-5 and *Streptomyces toxytricini.*

Enzymes	Reaction	
	Strain KS3-5	*S. toxytricini*
α-Amylase	+	++
Gelatinase	+	+
Lipase	–	–
Pectinase	–	–
Protease	+/–	+
Agarase	++	++

Cultures were grown on minimal agar medium (Hopwood, 1967; Hopwood and Wright, 1973) at 28°C for 3 weeks.

Table 4. Utilization of carbon sources by KS3-5 and *Streptomyces toxytricini.*

Carbon source	Response	
	Strain KS3-5	*S. toxytricini*
No carbon source	–	–
Fructose	+	+
D-glucose	+	NA
L-glucose	+	NA
Raffinose	–	–
L-rhamnose	+/–	–
Salicin	–	+/–
Sucrose	+/–	+/–
D-xylose	+/–	–

+: well utilized; +/–: poorly utilized; –: not utilized; NA: not available.
L-Arabinose, Cellulose, Dulcitol, D-Galactose, Glycerol, i-Inositol, Inulin, Lactose, D-Mannose, D-Maltose, D-Mannitol, Melibiose, Raffinose, S-Sorbitol, and Starch were not used by both KS 3-5 and *Streptomyces toxytricini*.

Antimicrobial Activities

Strain KS3-5 showed a broad antimicrobial spectrum against Gram (+) and (-) bacteria and yeast (Table 5). Many such broad-spectrum antibiotics have been produced by *Streptomyces* sp. (Korzybski et al., 1967).

Chemotaxonomy

Analysis of the whole-cell hydrolysate of strain KS3-5 showed the presence of a chemotype I cell wall characterized by LL-A_2pm. No diagnostic sugars were found. The main components of fatty acids were anteios-(*anteios*-C_{15}:0), iso-(iso-C_{16}:0) and normal (*n*-C_{16}:0) acids. The menaquinones were MK-9 (H_8) and MK-9 (H_{10}). The phospholipid pattern was PII type containing phosphatidylethanolamine. Integration of this information suggests that strain KS3-5 belongs to the genus *Streptomyces* (Lechevalier, 1989).

Identification

A computerized database was used to compare the biological properties of strain KS3-5 with those of other *Streptomyces* sp. The results suggest that strain KS3-5 is a streptomycete strongly related to *S. toxytricini* (M. Tseng, personal communication). Subsequently, some biological properties of *S. toxytricini* were tested for comparison. *Streptomyces toxytricini* was distinct from strain KS3-5 in having more-open spirals of spore chains and a red to gray spore mass (Figure 2). Both strains showed similar patterns of carbohydrate utilization and enzyme production (Tables 3 and 4), but *S. toxytricini* demonstrated a narrower antimicrobial spectrum than did strain KS3-5 (Table 5). The fluorometric, DNA-DNA hybridization experiment indicated a near-100% DNA similarity between strain KS3-5 and *S. toxytricini*, suggesting a significant genomic relatednesss. Consequently, strain KS3-5 belongs to the *Streptomyces* sp. and is identical to *S. toxytricini*.

Table 5. Antimicrobial activities of antibiotics KS3-5 and *Streptomyces toxytricini.*

Indicator[a]	Inhibition zone (mm dia.)[b]	
	Strain KS3-5	*S. toxytricini*
Staphyloccus aureus ATCC 25923	+	–
Sarcina lutea ATCC 9341	+(9.6)	–
Escherichia coli ATCC 10536	+	–
Escherichia coli NIHJ	+	–
Enterobacter coloacae ATCC 23355	+	–
Salmonella typhimurium ATCC 14028	+	–
Klebsiella pneumoniae ATCC 13883	+	–
Bacillus subtilis PCI 219	+(8.9)	+(26.5)
Bacillus subtilis ATCC 6633	+	+
Bacillus cerius ATCC 11778	+(7.8)	–
Pseudomonas aeruginosa	–	–
Candida albcans ATCC 10231	–	+/–
Saccharomyces cervisiae ATCC 9763	+(7.4)	–
Aspergillus niger	–	–
Gibberella fujikuroi	–	–
Penicillium digitatum	–	–

[a] Bacteria were incubated on antibiotic medium 1 (Difco) at 37°C for 24 hours. Fungi were incubated on Czapek-Dox agar, and yeast on sabouraud agar at 28°C for 5 days.
[b] +: positive; –: negative.

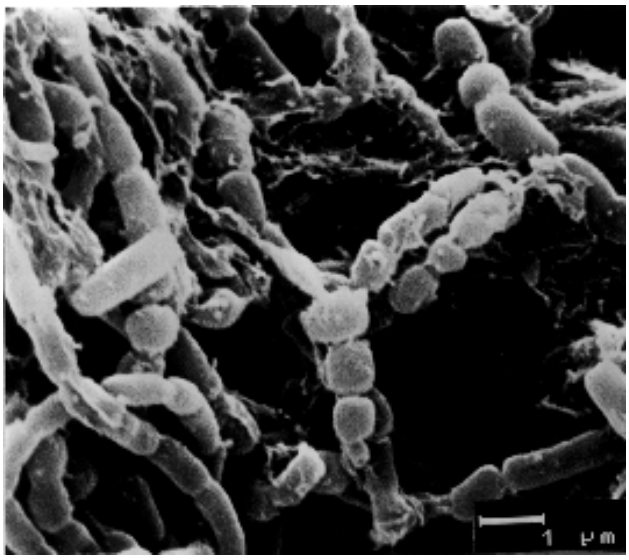

Figure 2. Scanning electron micrograph (×10,000) of aerial mycelia and spore chains of S. *toxytricini.*

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鏈黴菌 KS3-5 之鑑定

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放線菌 KS3-5 是篩選新抗生素產生菌過程中，自台灣高雄土壤中分離出之一株抗生素產生菌。所產生的抗生素能明顯抑制革蘭氏陽性及陰性的細菌和酵母菌。由其形態特徵和 whole cell chemotype 之試驗結果推斷，KS3-5 菌應屬於鏈黴菌屬，進一步進行培養特性，生理特性，醣類分解特性，DNA homology 之試驗顯示，KS3-5 菌應爲 *Streptomyces toxytricini* 。

關鍵詞： *Streptomyces*; *Streptomyces toxytricini* 。