STUDIES ON T-2636 ANTIBIOTICS. I

TAXONOMY OF *STREPTOMYCES ROCHEI* VAR. *VOLUBILIS* VAR. NOV. AND PRODUCTION OF THE ANTIBIOTICS AND AN ESTERASE

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The characteristics of streptomycete strain No. T-2636 producing antibiotics T-2636 A, B, C, D, E, F and M are given and *Streptomyces rochei* var. *volubilis* is proposed as the name for this new taxon. The antibiotics T-2636 A, B, C and D show antibacterial activity against Gram-positive bacteria and antibiotic T-2636 M shows antifungal activity. The production ratio of components A, B, C and D depends on cultural conditions. An esterase produced by the organism hydrolyzes the acetyl group of antibiotic T-2636 A converting it into antibiotic T-2636 C.

In the course of screening for a new antibiotic which would be effective by oral administration and would have no cross-resistance to known antibiotics, streptomycete strain No. T-2636 was found to produce an antibiotic mixture active against Gram-positive bacteria and fungi. The mixture is made up of seven components, A, B, C, D, E, F and M[1] (hereafter refered to as **A**, **B**, **C**, **D**, **E**, **F** and **M**). Among these antibiotics, **A** and **C** were found to be identical[1] with bundlin B[2,18] and lankacidin[3], respectively. Lankacidin is reported to be identical with bundlin A[18]. **B**, **D**, **E**[4] and **F**[5] were found to be new antibiotics and **M** is an unidentified pentaene antifungal.

It also was found that strain No. T-2636 produced an enzyme, T-2636 esterase, which hydrolyzed **A**.

This paper deals with the taxonomic characteristics of *Streptomyces rochei* var. *volubilis*, the production and the antimicrobial activities of **A**, **B**, **C**, **D** and **M**, and the production and properties of T-2636 esterase.

Materials and Methods

Characterization of strain No. T-2636

1. *Streptomyces* sp., strain No. T-2636: Isolated from a soil sample collected in Nukata, Osaka Prefecture, Japan.

2. Morphological characteristics: Strain No. T-2636 was grown on glucose-asparagine agar or BENNETT's agar at 28°C for 14 days. The culture was observed with a light microscope and a JEM-SS electron-microscope (Japan Electron Optics Co., Ltd., Tokyo, Japan).

3. Cultural characteristics: The organism was grown on glucose-asparagine agar and

the spores were suspended in sterilized water. The spore suspension was used to inoculate various media described by WAKSMAN[6]. All cultures were incubated at 28°C for 14 days except gelatin, litmus milk, egg and LOEFFLER's medium. Color determinations of the cultures were made with reference to RIDGWAY[7].

4. Utilization of carbon sources: The method of PRIDHAM and GOTTLIEB[8] was used.

Antibiotic studies

1. Shake flask fermentations: Fermentations were carried out with 30 ml of medium in 200-ml Erlenmyer flasks. Spores from a slant culture were inoculated into seed culture medium containing 1 % soluble starch, 2 % soy bean flour and 1 % calcium carbonate. Inoculum was grown at 28°C for 24 hours on a rotary shaker (220 rpm). The resultant culture was inoculated with 10 % inoculum rate into a medium containing 2 % glucose, 3 % soluble starch, 1 % corn steep liquor, 1 % soy bean flour, 0.3 % peptone and 0.5 % calcium carbonate (adjusted to pH 7.0). Fermentations were conducted at 28°C for 66 hours on a rotary shaker.

2. Tank fermentations: The organism was inoculated into the seed culture medium and incubated at 28°C for 24 hours on a reciprocal shaker (120 rpm). One liter of the resultant culture served as inoculum for a seed tank (30 liters of the seed culture medium in 50-liter fermentor) which was maintained at 28°C for 24 hours with stirring (280 rpm) and under aeration (30 liters/min.). Ten liters of the resulant seed culture were used to inoculate a large culture tank (90 liters of production medium in 200-liter fermentor). Fermentations were carried out at 28°C for 42 hours to 90 hours with stirring (200 rpm) and under aeration (100 liters/min.).

Antibiotic assays

1. Cup method: The test organisms used for the study of antibacterial activity are given in Table 3. Most of the bacteria were grown on nutrient agar at 37°C for 18 hours. The acid-fast bacteria were grown on glycerol nutrient agar for 40 hours. The fungi and the yeasts were grown on glucose nutrient agar at 28°C for 40 hours. Diameters of the inhibition zones were determined as a measure of antimicrobial activity.

2. Agar dilution method: The serial agar dilution method was applied in this study. The test organisms used for the study are given in Tables 3 and 4. The assay media used were the same as for the cup method.

3. Paper disk method: *Sarcina lutea* PCI 1001 was used as the test organism. It was grown on Trypto-Soy Agar (Nihon Eiyo Kagaku Co.) with added 0.25 % K_2HPO_4 (adjusted to pH 6.5). Cultures were incubated at 37°C for 18 hours after placement of disks. **C** (lankacidin, bundlin A) was used as a standard for comparative purposes.

4. Differential assay method for **A** and **C**: In connection with studies on the production of **A** and **C**, particular attention was given to a differential assay method for **A** and **C**. Samples (fermented broth or extracts) were developed on silica-gel thin-layer plates (Spotfilm, Tokyo Kasei Co.) with the solvent system ethyl acetate - diethyl ether (1 : 3). Antibiotic activities were detected bioautographically with *Sarcina lutea* PCI 1001. Potencies were determined by measuring the diameters of the inhibition zones and comparing with those obtained with standard samples (**A** and **C**).

Enzyme studies

Enzymes with esterase activity produced by *Aspergillus sojae*[9], *Aspergillus niger* and *Streptomyces*, strain No. T-2636, and Lipase MY (Meito Sangyo Co.) from *Candida* sp. were used. Esterase activity was determined with the following methods:

1. Titration method: The method of FIORE and NORD[10] was used to determine hydrolytic activities of the enzymes.

2. Colorimetric method: Esterase activities were determined by the method of HUGGINS and LAPIDES[11] using *p*-nitrophenol acetate as the substrate.

3. Antibacterial activity method: The substrate **A** was incubated with enzyme in 0.1 M Tris buffer (pH 7.2) at 34°C for 30 minutes. The enzymatic reaction was stopped

with ethyl alcohol and the amount of **C** produced by the reaction was determined by the paper disk method based on the fact that **C** is very active by this method, whereas **A** exhibits very weak activity. Using **A** as the substrate, the enzyme activity which hydrolyzed the substrate to give 1 mcg of **C** was designated as 1 unit (1 u).

Preparation of T-2636 esterase

The antibiotic T-2636 mixture was removed from 90 liters of the fermented broth of *Streptomyces*, strain No. T-2636 by solvent extraction. The aqueous layer was concentrated to 15 liters at 40°C under reduced pressure. Ethyl alcohol (90 liters) was added to the concentrate at 0°C to 5°C to give crude enzyme I (2 kg). Crude enzyme I (300 g) was dissolved in 1 liter of 0.1 M Tris buffer (pH 7.2) and the undissolved precipitate removed by centrifugation at 0°C for 20 minutes under 10,000 G. The supernatant was fractionally precipitated with ethyl alcohol. The precipitate forming at a concentration of 30 % ethyl alcohol was removed by centrifugation. The enzyme, precipitated at a concentration of 60 % ethyl alcohol, was dissolved in 100 ml of 0.1 M Tris buffer (pH 7.2) and dialyzed against distilled water for 20 hours in the cold. The material within the dialysis bag was lyophilized to give crude enzyme II (4.4 g). Crude enzyme II was dissolved in 20 ml of 0.001 M Tris buffer (pH 7.2) and subjected to column chromatography. Chromatography was carried out on a DEAE cellulose column (3 cm×7 cm) with gradient elution using Tris buffer (pH 7.2) and 1 M NaCl solution below 5°C. Active fractions were collected and dialyzed against distilled water for 20 hours. The material within the dialysis bag was lyophilized to give 1.3 g of purified enzyme.

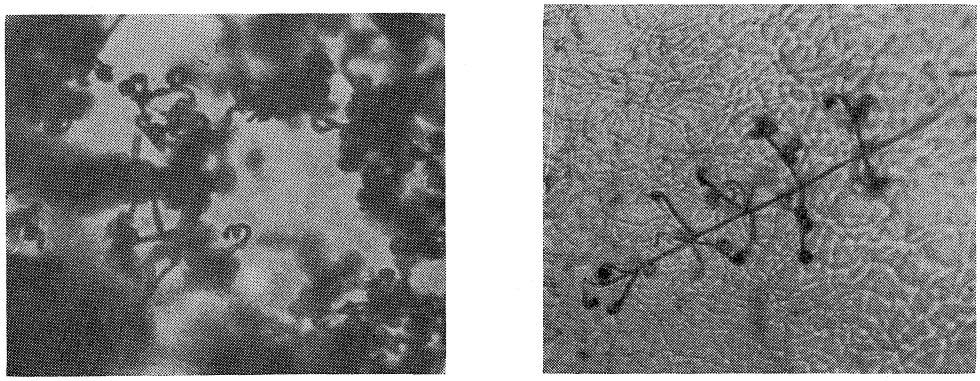
Results and Discussion

Streptomyces rochei BERGER *et al.*, *in* WAKSMAN, *in* WAKSMAN and LECHEVALIER, 1953, var. *volubilis** HIGASHIDE and SHIBATA var. nov.

The aerial mycelium is branching and whorl-like, the chains of spores form loops or coils (Fig. 1) and the spores are oval or ellipsoidal (0.5 μ~1.0 μ×0.9 μ~1.5 μ) with smooth surfaces (Fig. 2). The verticillate-like sporophore of strain No. T-2636 differ from the typical verticillate sporophores of *Streptomyces reticuli* (WAKSMAN and CURTIS, 1916) WAKSMAN and HENRICI, 1948, and *Streptomyces netropsis* FINLAY and SOBIN, 1952. Sporophores of the strain are considered to be pseudoverticillate[12,13] as

Fig. 1. Photomicrographs of sporophores and spore chains of *Streptomyces rochei* var. *volubilis*.

(×2,000 ×1/1.5) (×2,000 ×1/1.5)



* The name of this taxon has appeared in several previous publications in none of which it is validly published.[1,20,21] In two of these publications (1, 21) the name is incorrectly cited as *Streptomyces rochei* var. *bolubilis* (sic).

Table 1. Cultural characteristics of *Streptomyces rochei* var. *volubilis*, strain No. T-2636

Medium	Growth	Aerial mycelium	Reverse	Soluble pigment	
CZAPEK's agar	Thin, colorless	Poor, powdery, white to Light Drab (Rdg. XLVI, 17″″-b)	Colorless	None	
Glucose CZAPEK's agar	Thin, spreading, colorless	Same as on CZAPEK's agar	Same as on CZAPEK's agar	Same as on CZAPEK's agar	
Glycerol CZAPEK's agar	Thin, spreading, colorless	Poor, powdery, white to Light Cinnamon-Drab (Rdg. XLVI, 13″″-b) to Light Drab (Rdg. XLVI, 17″″-b)	Colorless	None	
Glucose asparagine agar	Moderate, spreading, colorless	Moderately abundant, powdery, Light Drab (Rdg. XLVI, 17″″-b) to Drab (Rdg. XLVI, 17″″) or Drab Gray (Rdg. XLVI, 17″″-d)	Colorless to pale yellow	None	
Nutrient agar	Moderately abundant, colorless to pale yellow	Poor, white or sometimes Light Drab (Rdg. XLVI, 17″″-b)	Colorless to faint yellow	None	
Glucose nutrient agar	Abundant, colorless to pale yellow	Poor, white or Drab Gray (Rdg. XLVI, 17″″-d)	Faint brown	None or sometimes faint brown	
Glycerol nutrient agar	Abundant, folded, colorless to pale yellowish brown	Moderate, white	Faint brown with dark brown patches	None or pale yellow	
Nutrient broth	Pellicle or ring, later sediment	None or poor, white		None	
Starch agar	Poor, colorless	None or scant, white	Colorless	None	
Yeast extract agar	Abundant, wrinkled, colorless to pale brown	Abundant, white to Drab Gray (Rdg. XLVI, 17″″-d) or Mouse Gray (Rdg. LI, 15″″)	Yellowish brown	None	
Egg (37°C)	Moderate, colorless or black patches later becoming black	Abundant, white to Pale Olive Gray (Rdg. LI, 23″″-f) to Mouse Gray (Rdg. LI, 15″″) or Drab Gray (Rdg. XLVI, 17″″-d)			
Potato plug	Abundant, lichenoid, colorless	Abundant, white to Drab Gray (Rdg. XLVI, 17″″-d) or Mouse Gray (Rdg. LI, 15″″)		None	
Carrot plug	Abundant, linchenoid, colorless	Abundant, powdery, white Light Drab (Rdg. XLVI, 17″″-b)		None	
Calcium malate agar	Moderate, colorless or sometimes poor, thin	Moderate, powdery, white to Light Drab (Rdg. XLVI, 17″″-b)	Colorless or pale yellow	None	
Tyrosine agar	Moderate, colorless to Cream Buff (Rdg. XXX, 19″-d)	Poor or moderate, white	Colorless to faint yellow	None	
Cellulose	Scant, colorless or none	Scant, Drab Gray (Rdg. XLVI, 17″″-d) or none		None	
Peptone agar	Poor, colorless	Poor, white	Colorless	None	
Gelatin (24°C)	Poor, colorless ring	Poor, white to Pale Drab Gray (Rdg. XLVI, 17″″-f)		None	No liquefaction or very slow liquefaction
Litmus milk (37°C)	Ring, colorless to pale brown	None or poor, white		None	Peptonization without coagulation

(to be continued)

(*continued*)

Medium	Growth	Aerial mycelium	Reverse	Soluble pigment	
LOEFFLER'S serum medium (37°C)	Moderate, wrinkled, colorless	None or scant, Drab Gray (Rdg. XLVI, 17″″-d)		None	No liquefaction or weak liquefaction
Nitrate	No reduction in CZAPEK's solution, but reduction to nitrite in peptone solution.				
Starch hydrolysis	Enzymatic zone/Growth zone: 26~30 mm/7~10 mm				

Rdg. : R. RIDGWAY, Color Standards and Color Nomenclature. Published by the author, Washington, D.C., 1912

are those of *Streptomyces tendae* ETTLINGER *et al.* 1958 and *Streptomyces rimosus* SOBIN *et al.* 1950. Also, the chains of spores of the strain are assumed to form coils or spirals representative of Sections Retinaculum-Apertum or Spira of PRIDHAM *et al.*[14] The aerial mass color is in the Red color series or in the Cinnamoneus group of ETTLINGER *et al.*[15]

The cultural characteristics of strain No. T-2636 are shown in Table 1. On most media colorless vegetative mycelia develop moderately and the aerial mass color is grayish brown. No soluble pigment forms on chemically-defined media, or on glucose agar, peptone agar and tyrosine agar. Accordingly, strain No. T-2636 is considered to be non-chromogenic. The utilization of carbon sources by strain No. T-2636 is shown in Table 2. D-Glucose, D-xylose, D-fructose, L-arabinose, saccharose, raffinose and mannitol are utilized for growth of the organism.

Fig. 2. Electron micrograph of spores of *Streptomyces rochei* var. *volubilis*. (×12,000 ×1/1.5)

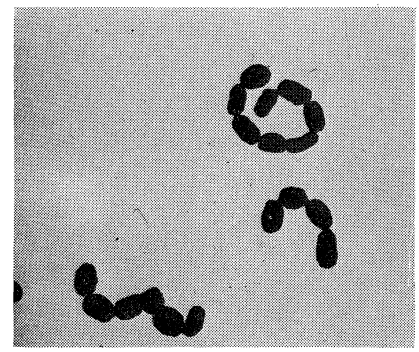


Table 2. The utilization of carbon sources by *Streptomyces rochei* var. *volubilis*, strain No. T-2636

Carbon source	Growth	Carbon source	Growth
Erythritol	±	Saccharose	+++
Adonitol	±	Rhamnose	±
D-Sorbitol	±	Raffinose	++
D-Mannitol	+++	Trehalose	+++
i-Inositol	±	Melibiose	±
Dulcitol	±	Salicin	+++
D-Xylose	+++	Esculin	±
L-Arabinose	+++	Inulin	±
L-Sorbose	±	Dextran	+++
D-Galactose	+++	Starch	+++
D-Glucose	+++	Glycerol	+++
D-Fructose	+++	Na-Acetate	+++
D-Mannose	+++	Na-Succinate	+++
D-Maltose	+++	Na-Citrate	+++
D-Lactose	+++	Control	±

+++ : Abundant growth. ++ : Moderate growth.
+ : Growth. ± : Scant growth.

Among known species of *Streptomyces*, *Streptomyces violaceoniger* (WAKSMAN and CURTIS, 1916) WAKSMAN and HENRICI, 1948, (strain NRRL 2834), *Streptomyces griseofuscus* SAKAMOTO *et al.*, 1962, and *Streptomyces rochei* BERGER *et al.*, in WAKSMAN in WAKSMAN and LECHEVALIER, 1953 were considered to be similar to strain No. T-2636; *Streptomyces violaceoniger*, strain NRRL 2834, is different from strain No. T-2636 in characteristics of the vegetative mycelia on CZAPEK's agar and glucose-asparagine agar. *Streptomyces griseofuscus* differs from the strain in cultural characteristics on CZAPEK's glucose agar, starch agar, and potato plug. Recently, *Streptomyces spinichromogenes* var. *kujimyceticus* NAMIKI *et al.*, 1969[16]

Table 3. Comparison of properties of *Streptomyces rochei* var. *volubilis* with those of similar streptomycetes

		Streptomyces violaceoniger NRRL 2834	*Streptomyces griseofuscus*	*Streptomyces spinichromogenes* var. *kujimyceticus*	*Streptomyces rochei*	*Streptomyces rochei* var. *volubilis*, strain No. T-2636
Sporsphores		Closed spiral	Claster, loops or spiral	Closed spiral	Straight or open spiral	Loops or open spiral pseudo-verticillate
Spores		Smooth	—	Spiny	Smooth to warty	Smooth
CZAPEK's agar	G	Pale yellow	Colorless	Yellowish brown to blackish brown	Colorless	Colorless
	A		Grayish red to reddish ocher	White to grayish white	Sandy lavender to dark gray	White to brownish gray
	S		None	None	None	None
Glucose CZAPEK's agar	G		Scant, colorless	Reddish brown		Colorless
	A		None	White to gray		White to brownish gray
	S		None	None		None
Glucose asparagine agar	G		Colorless	Pale yellow to colorless		Colorless
	A		White to grayish red	White		Brownish gray
	S		Pale yellow	None		None
Starch agar	G	Pale yellow	Colorless to yellow		Brownish	Colorless
	A	None	Grayish to reddish ocher		Mouse gray	White
	S	None	None			None
	R				Slight purple	Colorless
Potato plug	G	Brownish yellow	Yellow		Cream color	Colorless
	A	White to pale brown	White		White to gray	White to gray
	S		Brown		Reddish tan	None
Gelatin	G	Pale yellow to brownish yellow	Pale yellow	Cream to yellowish brown	Cream color	Colorless
	A		White	White	White	White to pale gray
	S			Dark brown	Faint yellow	None
Liquefaction		±	+	+	+	−～±
Nutrient agar	G		Colorless to pale yellow	Blackish gray to brownish gray	Cream color	Colorless to pale yellow
	A		None	None	White	White to pale gray
	S		None	Dark brown	None	None
Carbon utilization						
D-Xylose			+	±	+	+
i-Inositol			±	+	+	±
L-Arabinose			±		+	+
D-Glucose			−	+	+	+
Saccharose			+	+	−	+
Rhamnose			+	−	+	±
Raffinose			±	+	−	+

(*to be continued*)

(*continued*)

	Streptomyces violaceoniger NRRL 2834	*Streptomyces griseofuseus*	*Streptomyces spinichromogenes* var. *kujimyceticus*	*Streptomyces rochei*	*Streptomyces rochei* var. *volubilis*, strain No. T-2636
Products	Lankacidin	Bundlin A			T-2636C (=lankacidin)
		Bundlin B			T-2636A (=bundlin B)
	Lankamycin		Kujimycin B (=lankamycin)		
			Kujimycin A (similar to lankamycin)		T-2636B (Similar to lankamycin)
					T-2636D, E, F
	Pentaene	Moldcidin A			T-2636M (pentaene)
		Moldcidin B (=pentamycin)			
				Borrelidin	
					T-2636 esterase

was reported as a lankamycin producer[17]. The spores of this organism have echinulate surfaces. Strain No. T-2636 has no such morphological characteristics. No taxonomic data have been reported on the lankacidin producer, *Sreptomyces* sp. No. 6642 G.[18] On the other hand, strain No. T-2636 resembles *Streptomyces rochei* in cultural and morphological characteristics and is considered to belong to that species, although it differs from *Streptomyces rochei* in the liquefaction of gelatin, in formation of brownish growth on starch agar and in its products. The spore chains of *Streptomyces rochei* are straight or coiled[6]. Strain No. T-2636 forms predominantly loops or coils and rarely forms straight chains or spores. The comparison is shown in Table 3. Therefore strain No. T-2636 is considered a new variety of *Streptomyces rochei* and the name *Streptomyces rochei* var. *volubilis* is proposed with reference to the morphology of the chains of spores. A culture of the new taxon has been deposited in the Institute for Fermentation, Osaka, Japan where it has been assigned accession number IFO 12507.

Table 4. Antimicrobial properties of the fermented broth of *Streptomyces rochei* var. *volubilis*, strain No. T-2636

Test organism	pH of the assay medium	Inhibition diameter (mm)
Escherichia coli	7	0
Staphylococcus aureus	6	21
〃 〃	7	19
〃 〃	8	18
〃 〃 OE-R	7	16
〃 〃 CTC-R	7	19
〃 〃 CM-R	7	18
〃 〃 CP-R	7	19
〃 〃 GM-R	7	19
〃 〃 NV-R	7	18
〃 〃 XM-R	7	20
Bacillus subtilis	7	0
Bacillus cereus	7	18
Mycobacterium avium	7	15
〃 〃 SM-R	7	15
〃 〃 NM-R	7	15
Piricularia oryzae	6	22
Candida albicans	6	19.5

R : Resistant strain
OE : Oleandomycin-erythromycin
CTC : Chlortetracycline
CM : Chromomycin
CP : Chloramphenicol
GM : Glumamycin
NV : Novobiocin
XM : Xanthomycin
SM : Streptomycin
NM : Neomycin

Production of Antibiotic T-2636 Mixture

The antimicrobial activity of shaken-culture broths was determined by the cup

method and is shown in Table 4. The antibiotic mixture produced by the organism is active against Gram-positive bacteria and fungi, and is physiologically acidic antibiotic[19]. Activity of the mixture against an oleandomycin-erythromycin resistant strain was weaker than that against sensitive strains.

Antibiotic T-2636 is a mixture of seven components. The cultural conditions for production of the individual components were investigated by assaying the antibacterial activity of culture broths and isolating the active components. As shown in Fig. 3, the antibacterial properties of culture broths varied with the cultural conditions. Under conditions illustrated in Fig. 3 (a) the production of **A** and **C** was predominant. Under conditions illustrated in Fig. 3 (b) the production of **B** and **D** was predominant. Production of **E** and **F** was minimal under these conditions. The production of **A** and **C** was further investigated by the paper disk and differential assay methods. As shown in Fig. 4, **C** was the main product. Production of **A** was maximum at 47 hours and could not be detected at 51 hours.

Fig. 3. Fermentative production of antibiotics by *Streptomyces rochei* var. *volubilis* under various conditions.

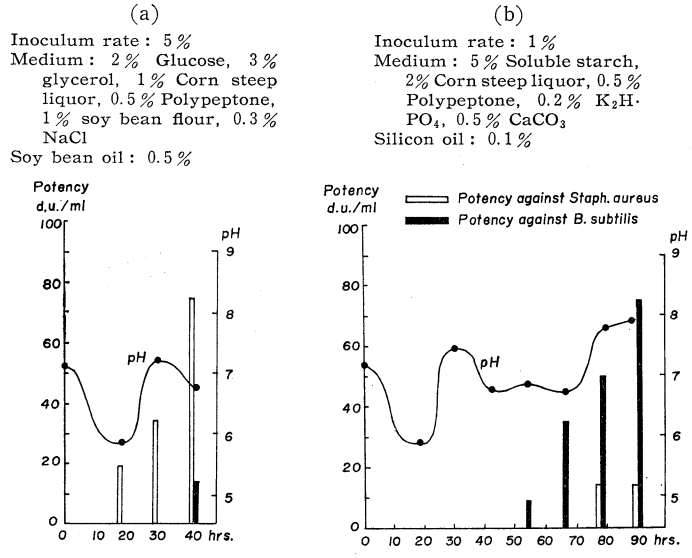


Table 5. Antimicrobial spectra of antibiotics T-2636 A, B, C, D and M.

Test organisms	Minimum inhibitory concentration (mcg/ml)				
	A**	**B**	**C****	**D**	**M**
Escherichia coli	>100	>100	50	>100	>100
Proteus vulgaris	>100	>100	50	>100	>100
Staphylococcus aureus	10~20	50	0.75	>100	>100
〃 (OE-R)*	20~50	100	1~2	>100	>100
Bacillus subtilis	100	10	50	>100	>100
Bacillus cereus	100	50	20	>100	>100
Bacillus brevis	50~100	20	5	>100	>100
Sarcina lutea	<2.0	1.0	0.02	5~10	50
Micrococcus flavus	<2.0	2.0	0.2	50	100
Mycobacterium avium	100	50	100	>100	20
Mycobacterium phlei	>100	20	50	>100	20
Mycobacterium sp. 607	>100	>100	100	>100	2.0
Piricularia oryzae	>100	>100	100	>100	1~2
Penicillium chrysogenum	>100	>100	>100	>100	2
Aspergillus niger	>100	>100	>100	>100	2
Saccharomyces cerevisiae	>100	>100	>100	>100	2
Candida albicans	>100	>100	>100	>100	2
Xanthomonas oryzae	0.5		0.05	5.0	

* OE-R : Oleandomycin-erythromycin resistant strain.
** A=Bundlin B. C=Bundlin=A=lankacidin.

Antimicrobial Activities of T-2636 Antibiotics

The antimicrobial spectra of **A**, **B**, **C**, **D** and **M** as determined by the serial agar dilution method are shown in Table 5. All, except **M**, are active against Gram-positive bacteria. Component **C** is the most active. Component **A** (14-acetyl C[4]) has

one-twentieth activity of **C**. Component **D** (2′-dihydro **A**[4]) is less active than **A**. Component **B** exhibits an antibacterial spectrum different from those of **A**, **C** and **D**. Synergistic activities of **B** with the other components are shown in Table 6. The synergistic activities were two to ten times those of the individual antibiotics.

Enzyme of *Streptomyces rochei* var. *volubilis*

As shown in Table 7, the purified enzyme was 100 times as potent as the starting material. Crude enzyme I exhibited both esterase and dehydrogenase activities, but the purified enzyme exhibited only esterase

Fig. 4. The production of antibiotic T-2636 A and C and T-2636 esterase.

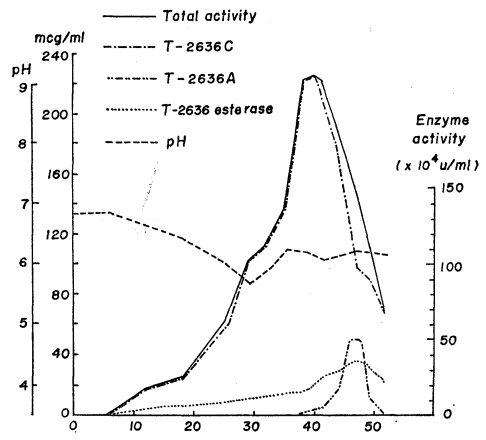


Table 6. Synergistic activity of antibiotics of the T-2636 mixture

Antibiotics	MIC (mcg/ml)	
	Staphylococcus aureus	*Bacillus subtilis*
T-2636 **A**	20.0	100.0
B	50.0	10.0
C	0.75	50.0
D	250.0	50.0
A-**B** (1 : 1)	2.0	10.0
B-**C** (1 : 1)	0.5	5.0
B-**D** (1 : 1)	20.0	13～20

Table 7. Summary of T-2636 esterase purification

Stage No. & sample	Volume	Total activity (u) (×107)	Specific activity (u/mg of protein)	Yield (%)	Purification
1 Fermented broth	90 liters	162	60	100	1
2 Concentrate	15 liters	118	84	73	1.4
3 Crude enzyme I	2 kg	34.01	572	21	9.5
4 Crude enzyme II	29.5 g	19.6	2310	12.1	38.5
5 Purified enzyme	12.7 g	5.83	6356	3.6	105.9

Activities were determined by the antibacterial activity method.

Table 8. Specific activity of esterase from *Streptomyces rochei* var. *volubilis* and *Aspergillus sojae*

Substrate	Specific activity μM/mg of protein	
	T-2636 esterase	*Asp. sojae* esterase
Ethyl formate	3.2	—
Ethyl acetate	5.8	1.0
Ethyl propionate	2.0	—
Ethyl butyrate	1.4	8.0
Monoacetin	11.0	14.0
Triacetin	8.6	—
Tributyrin	—	17.0
Triolein	0	—
Di-*n*-butyrin	0	—
Methyl laurate	0	3.5
Olive oil	0	0
Antibiotic T-2636 A	6.0	0

Activities were determined by the titration method.

Table 9. Effect of inorganic metal salts on T-2636 esterase activity

Inorganic metal salts	Concentration of the salts and activity (%)	
	10^{-2} M	10^{-3} M
Control	100	100
$CuSO_4 \cdot 5H_2O$	100	100
$BaCl_2 \cdot 2H_2O$	100	100
$Pb(CH_3COO)_2$	100	100
$FeSO_4 \cdot 7H_2O$	100	100
$NiSO_4 \cdot 7H_2O$	100	100
$ZnSO_4 \cdot 7H_2O$	100	100
$CaCl_2$	117.6	100
$CoCl_2 \cdot 6H_2O$	110.7	100
$MnSO_4 \cdot 4H_2O$	100	100
$HgCl_2$	20	60

Activities were determined by the antibacterial activity method.

Fig. 5. The effect of pH on T-2636 esterase activity.

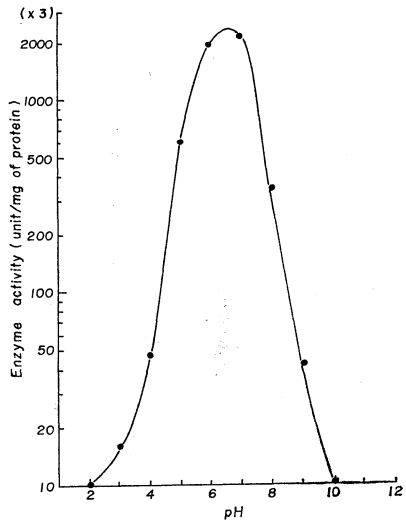


Fig. 6. The effect of the temperature on T-2636 esterase activity.

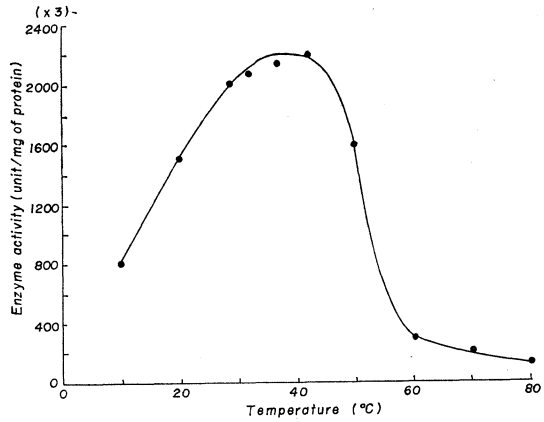


Fig. 7. The effect of pH on stability of T-2636 esterase (33°C, 30 min.)

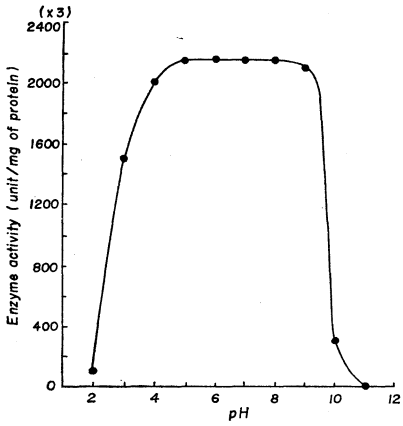
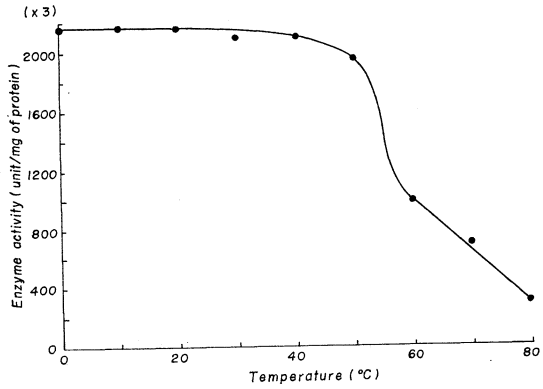


Fig. 8. The effect of temperature on stability of T-2636 esterase (pH 7.0, 10 min.)



activity. The enzyme has the special affinity for the acetyl group on C-14 of **A** and hydrolyzes **A** to give **C**. The effect of pH and temperature on enzyme activity and stability are shown in Figs. 5, 6, 7 and 8. The specificity of the enzyme for various substrates is shown in Table 8. The enzyme effectively hydrolyzes esters of lower aliphatic acids. Among alcohol esters, glycerol esters were more readily hydrolyzed than primary alcohol esters. **A** was hydrolyzed as readily as ethyl acetate. In Table 9, the

Table 10. Comparison of esterase activity of T-2636 esterase with those of other enzymes

Enzymes	Specific activity μM/mg of protein	
	p-Nitrophenol acetate[1]	Antibiotic T-2636 A[2]
T-2636 esterase	1.2	1.65
Aspergillus sojae esterase	3.75	0.011
Candida enzyme	3.23	0
Aspergillus niger enzyme	2.14	0.008

1) Activities were determined by the method of HUGGINS *et al.*
2) Activities were determined by antibacterial activity method.

effect of inorganic salts on enzyme activity is presented. Enzyme activity was not affected by salts except $HgCl_2$.

Comparison of esterase activity of the enzyme with those of other enzymes shown in Table 10 reveals the marked speceficity of T-2636 esterase for substrate **A**.

The productions of **A**, **C** and T-2636 esterase at various stages of the fermentation are summarized in Fig. 4.

From the above results and from a previous report[20], it is concluded that certain enzymes along with T-2636 esterase of strain No. T-2636 play an important role in the biosynthesis of the T-2636 antibiotics.

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