STUDIES ON ANTIBIOTIC SF-733, A NEW ANTIBIOTIC. I
TAXONOMY, ISOLATION AND CHARACTERIZATION

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A new antibiotic SF-733 inhibiting both Gram-negative and Gram-positive bacteria was isolated from *Streptomyces ribosidificus* nov. sp., strain SF-733. Antibiotic SF-733 obtained as colorless needles has a molecular formula $C_{17}H_{34}N_4O_{10}$, and is concluded to be a new aminoglycosidic, water-soluble and basic antibiotic based on its physical and chemical properties.

In the course of a screening program for antibiotics active against Gram-negative bacteria, we found a new antibiotic, antibiotic SF-733, from the culture filtrate of a newly isolated streptomycete, strain SF-733 for which we propose the name *Streptomyces ribosidificus* nov. sp.

Antibiotic SF-733 is a new amino-sugar antibiotic with low toxicity, and exhibits antibacterial activity against both Gram-negative and Gram-positive bacteria. Taxonomic studies on antibiotic SF-733-producing organism, and isolation and characterization of antibiotic SF-733 are reported in this paper.

Taxonomic Studies

Strain SF-733, that produces antibiotic SF-733, was isolated from a soil collected at Tsu City of Mie Prefecture in Japan. It shows the following properties:

1. Morphological Properties

The vegetative mycelium develops well on most of the media used. On some media such

Fig. 1. Aerial mycelium of *Streptomyces ribosidificus*, strain SF-733.

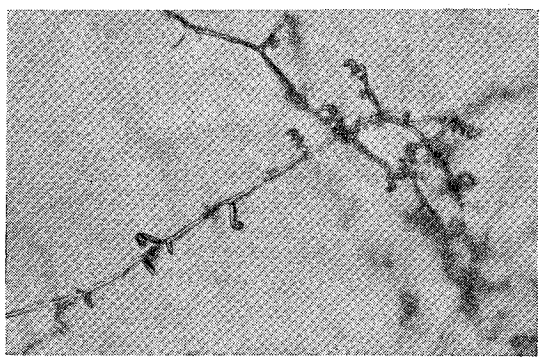


Fig. 2. Electron micrograph of spores of *Streptomyces ribosidificus*, strain SF-733.

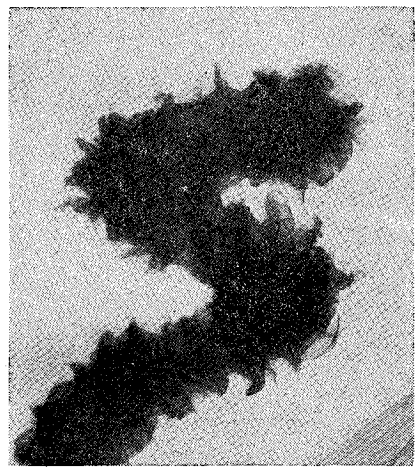


Table 1. Cultural properties of *Streptomyces ribosidificus* strain SF-733.

Medium	Growth	Aerial mycelium	Soluble pigment
Sucrose CZAPEK's agar (28°C)	Good, thick, wrinkled, penetrating into medium, yellowish cream, reverse color; light brown	Very scant, light yellowish white	None
Glycerine CZAPEK's agar (28°C)	Good, thick, wrinkled, penetrating into medium, light yellowish brown	Scant, white~cream	None
Glucose asparagine agar (KRAINSKY) (28°C)	Good, penetrating into medium, dark cream, reverse; brownish yellow	Scant, cream~light yellowish white	None
Glucose asparagine agar (USHINSKY) (28°C)	Good, penetrating into medium, brownish yellow with greenish tinge	Abundant, powdery, greenish yellow, later gray~grayish brown with greenish yellow patches	Faint yellow
Ca-malate agar (28°C)	Poor, light yellowish cream	Scant, white	None
Glycerine Ca-malate agar (28°C)	Poor, yellow	None, or scant, light yellow	None
Starch synthetic agar (28°C)	Good, penetrating into medium, yellow, at center of colony; greenish brown	Abundant, powdery, greenish yellow, later grayish brown	Faint yellow
Starch yeast extract agar (28°C)	Good, yellowish brown	Abundant, gray~brownish gray	None
Bouillon agar (28°C)	Penetrating into medium dark cream, reverse; brownish yellow	None, or scant light yellow	None
Glucose bouillon agar (28°C)	Wrinkled finely, yellowish brown	Scant, cream	None
Potato plug (28°C)	Elevated, wrinkled finely, light brownish cream	Scant, whitish gray	None
Carrot plug (28°C)	Dark cream	None	None
Tyrosine agar (28°C)	Dark cream	Gray	None
Egg (37°C)	Light brownish yellow	None	None
LÖFFLER's coagulated serum (37°C)	Dark brownish yellow	None	None
Bacto nitrate broth (28°C)	Bottom growth, colorless	None	None
Skimmed milk (37°C)	Ring and bottom growth, yellowish orange	None	Light brown~orange
Gelatin (20°C)	Colorless~cream	None	None
Cellulose medium (28°C)	No growth		

as starch synthetic agar and starch-yeast extract agar, it forms abundant aerial mycelia which develop into many open spirals (Fig. 1).

The spores are oval to ellipsoidal and 0.8~1.1 μ×1.1~1.4 μ in size. With electron microscope examination, surfaces of the spores (Fig. 2) are spine-like.

2. Cultural Properties

Cultural properties on different media are listed in Table 1.

3. Physiological Properties

Physiological properties, including utilization of carbon sources are summarized

Table 2. Physiological properties of *Streptomyces ribosidificus*, strain SF-733.

Production of H_2S	negative
Tyrosinase reaction	negative
Reduction of nitrate	positive
Coagulation of skimmed milk	nagative (28°C, 37°C)
Peptonization of skimmed milk	positive (37°C) negative (28°C)
Hydrolysis of starch	positive
Liquefaction of gelatin	negative
Liquefaction of LÖFFLER's coagulated serum	positive (weak)

Table 3. Carbon source utilization of *Streptomyces ribosidificus*, strain SF-733*

Positive utilization	arabinose, rhamnose, glucose, mannose, galactose, saccharose, lactose, inulin, raffinose, dextrin, starch, glycerol, sorbitol, mannitol, maltose
Doubtful utilization	inositol, Na-citrate
Negative utilization	xylose, fructose, dulcitol, salicin, cellulose, Na-acetate, Na-succinate

* Carbon utilization was investigated using the method of PRIDHAM and GOTTLIEB[2].

in Tables 2 and 3.

4. Growth Temperatures

Growth of strain SF-733 is observed at 17~45°C. The optimal temperature is between 35°C and 40°C when the organism is grown on starch-yeast extract agar medium.

5. Comparison of Strain SF-733 with Related *Streptomyces*

Strain SF-733 is non-chromogenic and, when cultured on synthetic media, aerial mycelium colored gray to grayish brown develops on the surface of yellowish vegetative growth. These properties of strain SF-733 allow its placement in WAKSMAN's *"flavus"* series of *Streptomyces*[1]. With respect to the properties mentioned above, strain SF-733 most closely resembles *Streptomyces flavus* (KRAINSKY, 1914) WAKSMAN and HENRICI, 1948, *Streptomyces flaveolus* (WAKSMAN, 1923) WAKSMAN and HENRICI, 1948 and *Streptomyces flavovirens* (WAKSMAN, 1919) WAKSMAN and HENRICI 1948.

These three species, however, principally differ from strain SF-733 as follows :

(1) *Streptomyces flavus* usually forms straight chains of spores (rarely open coils), does not reduce nitrate and grows optimally at 25°C. Strain SF-733 forms many open coils, reduces nitrate and grows optimally within a higher temperature ranges (35~40°C).

(2) *Streptomyces flaveolus* forms hairy spores, whereas strain SF-733 forms spine-like spores.

(3) *Streptomyces flavovirens* usually forms straight chains of spores (rarely open coils) and produces greenish-yellow diffusible pigment when grown on chemically-defined media. Strain SF-733 forms many open coils and produces only a faint yellow or generally no diffusible pigment when grown on chemically-defined media.

In view of the above, strain SF-733 was believed to be a new species and was named *Streptomyces ribosidificus** nov. sp. SHOMURA and NIIDA.

Antibiotic Production

Antibiotic SF-733 was produced in a medium composed of starch 2.0 %, soy bean meal 2.5 %, wheat embryo 1.0 % and sodium chloride 0.25 %, pH 7.0 before sterilization. The optimal temperature for 30-liter jar fermentor cultivation of *S. ribosidificus*,

* Strain SF-733 was first named *Streptomyces thermoflavus* nov. sp., and deposited in the American Type Culture Collection for the application for a patent and accessioned as ATCC 21294 on July 16, 1968. But a referee kindly let us know that *Actinomyces thermoflavus* had been listed in USSR in 1963 (Mikrobiologiya 32(4) : 623~631, 1963). Therefore, according to the referee's opinion, we changed its name to *Streptomyces ribosidificus* nov. sp. in this paper to avoid duplication.

strain SF-733 was 30~37°C, but the maximum titer of the antibiotic was reached at lower temperature (25~30°C).

Isolation and Purification

The isolation procedure for antibiotic SF-733 was comparable to that used for other similar, water-soluble, basic antibiotics as outlined in Fig. 3.

Antibiotic SF-733 in the broth filtrate was concentrated by adsorption on Amberlite IRC-50 (NH_4^+ type), followed by elution with dilute ammonium hydroxide. The active eluate was concentrated under reduced pressure, and adsorbed on Amberlite CG-50 (NH_4^+ type). Elution with dilute ammonium hydroxide and concentration of the active fractions gave a crude powder of antibiotic SF-733. The crude powder was chromatographed on a colum of Dowex 1X2 (OH^- type) using development with water. Active fractions detected by assay with *Bacillus subtilis* were collected, concentrated under reduced pressure and finally freeze-dried. The white powder thus obtained was dissolved in methanol. Upon standing overnight, antibiotic SF-733 crystallized out as a methanol solvate, which readily lost methanol on drying at

Fig. 3. Isolation and purification of antibiotic SF-733.

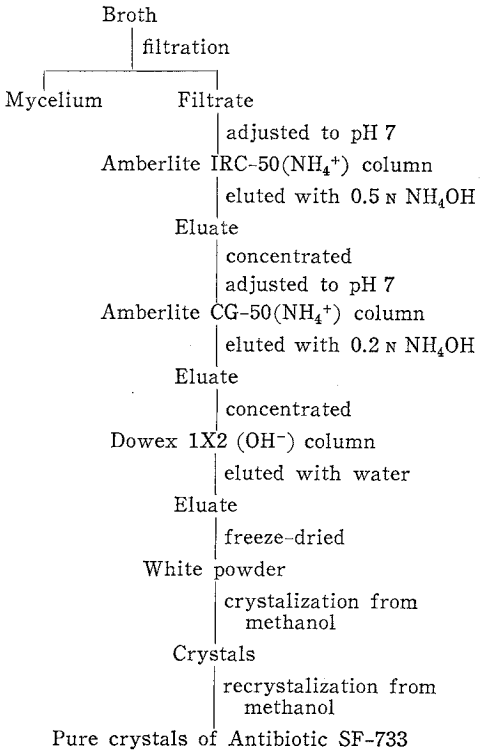


Table 4. Physical and chemical properties of free base of antibiotic SF-733.

Appearance	Colorless needle
Melting point (dec.)	192~195°C
Nature	Basic, pKa′ 7.70
Solubility	Soluble : water Slightly soluble : methanol Insoluble : acetone, *n*-butanel, ethyl acetate, benzene, hexane, ether, *etc.*
Optical activity	$[\alpha]_D^{28}$ +42° (*c* 1, water)
Color reaction	Positive : ninhydrin, MOLISCH, anthrone Negative : FEHLING, ferric chloride, maltol, biuret, TOLLENS, SAKAGUCHI
Molecular weight	475 (vapor pressure osmometry) 452 (titration)
Elemental analysis	Found : C 44.19, H 7.55, N 11.92, O 36.21 Calcd. for $C_{17}H_{34}N_4O_{10}$: C 44.92, H 7.54, N 12.33, O 35.20
Stability of aqueous solution	Stable : neutral, alkaline Slightly unstable : acidic

Table 5. Comparison of $[\alpha]_D$ values of dextrorotatory, water-soluble and basic antibiotics in water.

Antibiotics	$[\alpha]_D$	Antibiotics	$[\alpha]_D$
Antibiotic SF-733	+42	Gentamicin A	+146
Neomycin A	+123	〃 C_1	+158
〃 B	+58	〃 C_2	+160
〃 C	+82	Nebramycin 2	+159
Paromomycin I	+64	〃 4	+114
〃 II	+78	〃 5	+118
Kanamycin A	+121	〃 6	+127
〃 B	+135	Destomycin A	+7
〃 C	+126	〃 B	+6
Kasugamycin	+120	Hygromycin B	+19.2
Actinospectacin	+7.6	Capreomycin	+2.5

room temperature *in vacuo*. Recrystallization from methanol gave pure crystals of antibiotic SF-733.

Physical and Chemical Properties

Physical and chemical properties of antibiotic SF-733 are summarized in Table 4. Fig. 4 shows the infrared absorption spectrum using a KBr tablet of de-solvated crystalline SF-733. Fig. 5 shows the ultraviolet absorption spectrum in water. No maxima were observed up to 210 mμ. The nuclear magnetic resonance spectrum of antibiotic SF-733 in deuterium oxide is shown in Fig. 6.

Fig. 4. Infrared absorption spectrum of antibiotic SF-733 (KBr).

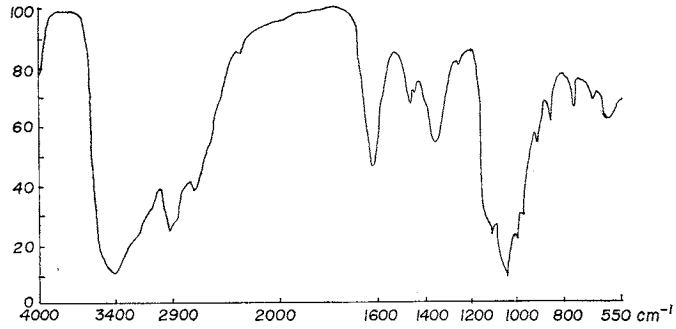
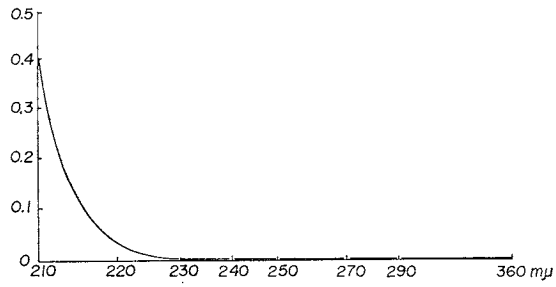


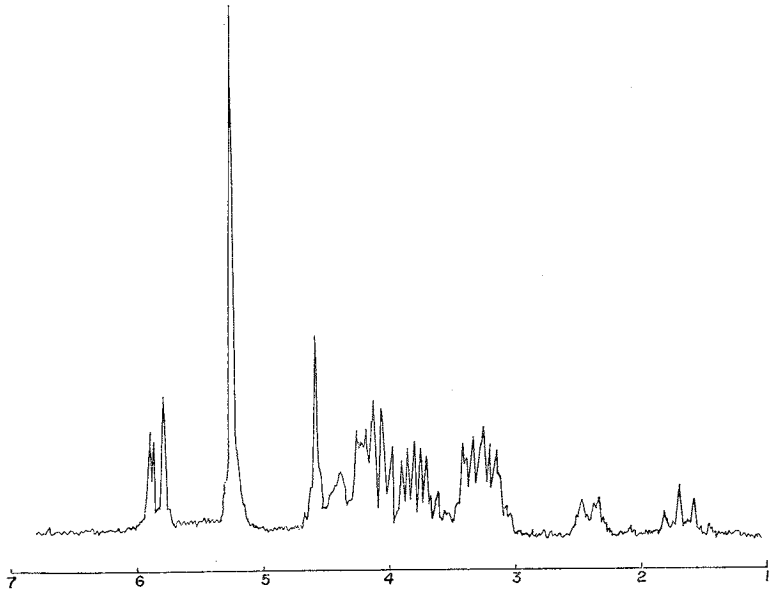
Fig. 5. Ultraviolet absorption of antibiotic SF-733 (in water).



Differentiation of Antibiotic SF-733 from Other Related Antibiotics

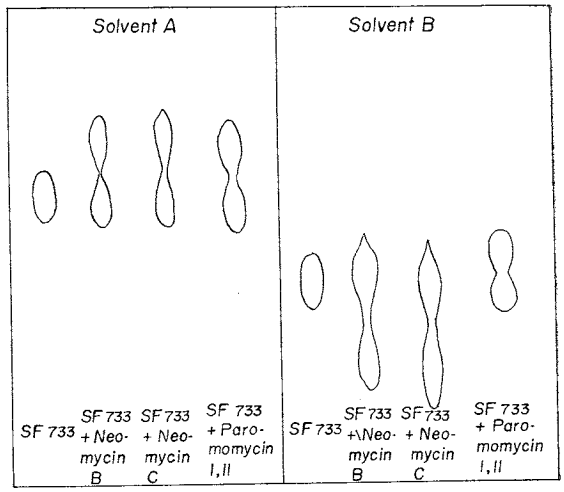
From the physical and chemical properties described above, it is apparent that antibiotic SF-733 belongs to a group of dextrorotatory, water-soluble and basic antibiotics which

Fig. 6. Nuclear magnetic resonance of antibiotic SF-733.



includes kanamycin, neomycin, paromomycin, *etc.* Table 5 shows the comparison of $[\alpha]_D$ values of antibiotic SF-733 with those of known antibiotics of this group. Based on $[\alpha]_D$ values, antibiotic SF-733 was differentiated from neomycin A, kanamycins A, B and C, gentamicins A, C_1 and C_2, kasugamycin, actinospectacin, destomycins A and B, hygromycin B, capreomycin and nebramycins 2, 4, 5 and 6[3]. Differentiation of antibiotic SF-733 from neomycins B and C and paromomycins I and II was accomplished with paperchromatography using two solvent systems as shown in Fig. 7.

Fig. 7. Comparison of antibiotic SF-733 with other related antibiotics on paparchromotogram detected by bioautography.
Solvent A : *n*-BuOH-pyridine-AcOH-H_2O (6 : 4 : 1 : 3) (descending, developed for 4 days)
Solvent B : 2 %-*p*-toluensulfonic acid in wet *n*-BuOH (descending, developed for 25 hours)



Thus, antibiotic SF-733 differs from all of the known antibiotics studied, and is believed to be a new antibiotic. This was confirmed by structural studies described in Part II of this series[4].

Biological Activity

Table 6 summarized the antimicrobial spectrum of antibiotic SF-733. Antibiotic SF-733 is active against a wide variety of Gram-negative and Gram-positive bacteria, but has no activity against yeasts or molds. The activity of antibiotic SF-733 against Gram-negative bacteria is comparable to that of kanamycin A.

The acute toxicity of antibiotic SF-733 was examined by intravenous injection into mice. The LD_{50} value for the sulfate of antibiotic SF-733 was approximately 225 mg/kg (mg=unit).

Table 6. Antimicrobial spectrum of antibiotic SF-733 by broth dilution method.

Test organisms	M. I. C. (mcg/ml)	Medium	Test organisms	M. I. C. (mcg/ml)	Medium
Bacillus subtilis ATCC 6633	0.39	1	*Salmonella typhosa*	3.125	1
Staphylococcus aureus 209P	3.125	1	*Shigella dysenteriae*	6.25	1
〃 〃 52-34	3.125	1	*Pseudomonas aeruginosa*	>100	1
〃 〃 193	3.125	1	*Xanthomonas oryzae*	0.78	1
〃 〃 Smith	0.39	1	*Mycobacterium phlei*	6.25	2
〃 〃 Terajima	0.19	1	〃 *smegmatis* 607	12.5	2
Streptococcus faecalis	3.125	1	〃 〃 streptomycin-R	12.5	2
Sarcina lutea	100	1	〃 〃 kanamycin-R	>100	2
Escherichia coli	12.5	1	*Candida albicans*	>100	3
〃 K-12	3.125	1	*Torula utilis*	>100	3
〃 chloramphenicol-R	1.56	1	*Aspergillus niger*	>100	3
Klebsiella pneumoniae	6.25	1	*Penicillium chrysogenum*	>100	3

Medium : 1=Bouillon, 2=Glycerine bouillon, 3=SABOURAUD'S broth

References

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