Communications to the editor

ANTIBIOTIC No. 5879, A NEW WATER-SOLUBLE ANTIBIOTIC AGAINST GRAM-NEGATIVE BACTERIA

Sir:

In the course of our search for new antibiotics produced by *Streptomyces* sp., a water-soluble antibiotic, effective against gram-negative bacteria, was obtained from the filtrate of a culture broth of *Streptomyces* No. 5879 isolated from a soil sample collected in Aizu area (Fukushima prefecture). Antibiotic No. 5879 appears to be a novel one from its physico-chemical and biological properties, and strain No. 5879 is considered to be a new species in the genus *Streptomyces* and is designated *Streptomyces aizunensis* nov. sp.

The taxonomic characteristics of *Streptomyces* No. 5879 are as follows: Aerial hyphae are well branched, 1~1.25 μ in diameter. Straight and wavy sporophores are observed. Conidia are elliptical to spherical in shape and 0.6~0.8 by 1.3 μ in size. The surface appearance of conidia are smooth. The growth characteristics were studied on 26 media. In general, the organism made good growth with white to yellow color. The aerial mycelia on most of the media tested were white to yellow or pale pinkish gray in color and the reverse side of the colonies were white to yellow or brown. A pale yellow soluble pigment was produced when grown on most of the media tested, but pale brown color was formed when grown on peptone-yeast extract iron agar and tyrosine agar. The utilization of carbon sources was tested with the basal medium described by PRIDHAM and GOTTLIEB. Glucose, fructose, xylose, arabinose, starch and mannose were utilized, but saccharose, rhamnose, mannitol and inositol were not. Nitrate reduction was positive. Starch and milk were hydrolyzed, but cellulolytic activity, gelatin liquefaction were negative.

Our strain No. 5879 resembles *Streptomyces griseolus* and *Streptomyces flavogriseus*. However, these organisms differ from strain No. 5879 in the following characters: *Streptomyces griseolus* produces generally soluble brown pigment on various media, grows spreadingly with brown color on bouillon agar, liquefies gelatin and gives alkaline reaction on litmus-milk media.

Streptomyces flavogriseus bears spherical conidia, grows excellently with abundant aerial hyphae on potato plug, poorly on starch agar and forms white aerial hyphae and does not produce soluble pigment on bouillon agar. Differences in taxonomic properties given above and those of recognized species in the genus *Streptomyces* are considered to be significant enough to propose the new species *Streptomyces aizunensis* nov. sp. for the strain No. 5879.

Fermentations were conducted under submerged culture conditions for 60~65 hours at 30°C in a medium consisting of 2% glucose, 2% soybean meal, 0.2% peptone, 0.2% $NaNO_3$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, and 0.0001% $FeSO_4 \cdot 7H_2O$. The pH was adjusted to 7 prior to sterilization. The fermented broth was acidified to pH 2, filtered to remove the precipitate, and then the acidic filtrate was adjusted to pH 8 with anion-exchange resin (Daiaion A-6) under stirring. After removing the anion-exchange resin by filtration, the filtrate was passed through a column of IRC-50 (H^+). The column was washed with water and 0.01 N hydrochloric acid, and the antibiotic was eluted with 0.5 N hydrochloric acid. Fractions containing strong activity were pooled, neutralized to pH 7 by mixing with anion-exchange resin (A-6) and then filtered. The filtrate was concentrated under reduced pressure, and the concentrated solution was then freeze-dried. This powder was extracted with approximately 5 volumes of 50% aqueous ethanol, the aqueous ethanol was filtered to separate the residues. After concentration of the filtrate, the resulting solution was adjusted to pH 8 with dilute aqueous sodium hydroxide and passed through a column of activated charcoal (Wako Pure Chem. Ind. Co.). The column was washed

with water, and the antibiotic was eluted with 80% aqueous ethanol. The crude antibiotic was isolated as off-white powder by the lyophilization of combined fractions. Further purification was effected by chromatography on cellulose powder (Whatman CF 11) using water-saturated *n*-butanol for elution. The material recovered from the active fraction was further purified by means of preparative thin-layer chromatography on silica gel (B-5, Wako Pure Chem. Ind. Co.) in the *n*-butanol - methanol system (9 : 1). The antibiotic-containing portion of the absorbant was scraped off from the glass plate, and the antibiotic adsorbed on the silica gel was eluted by repeated swirling in 50% aqueous methanol and centrifuging. The aqueous methanol containing the antibiotic was evaporated *in vacuo* to dryness. The resulting purified powder was dissolved

Fig. 1. Ultraviolet absorption spectrum (water).

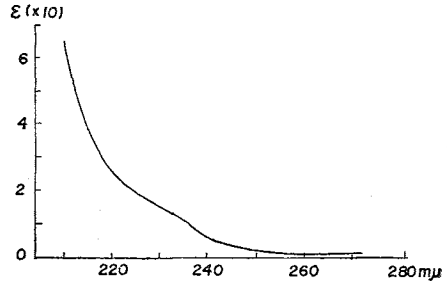

Fig. 2. Infrared absorption spectrum (KBr).

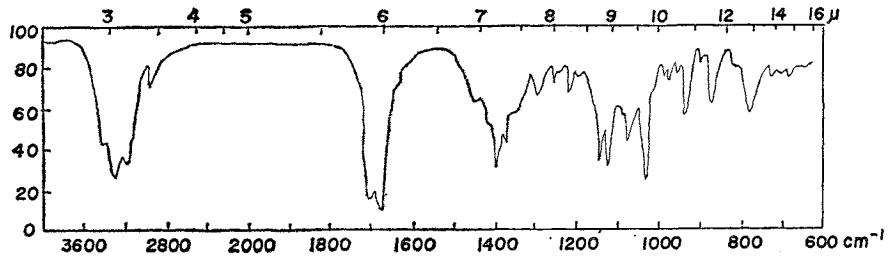

Fig. 3. NMR spectrum (100 MHz, D_2O).

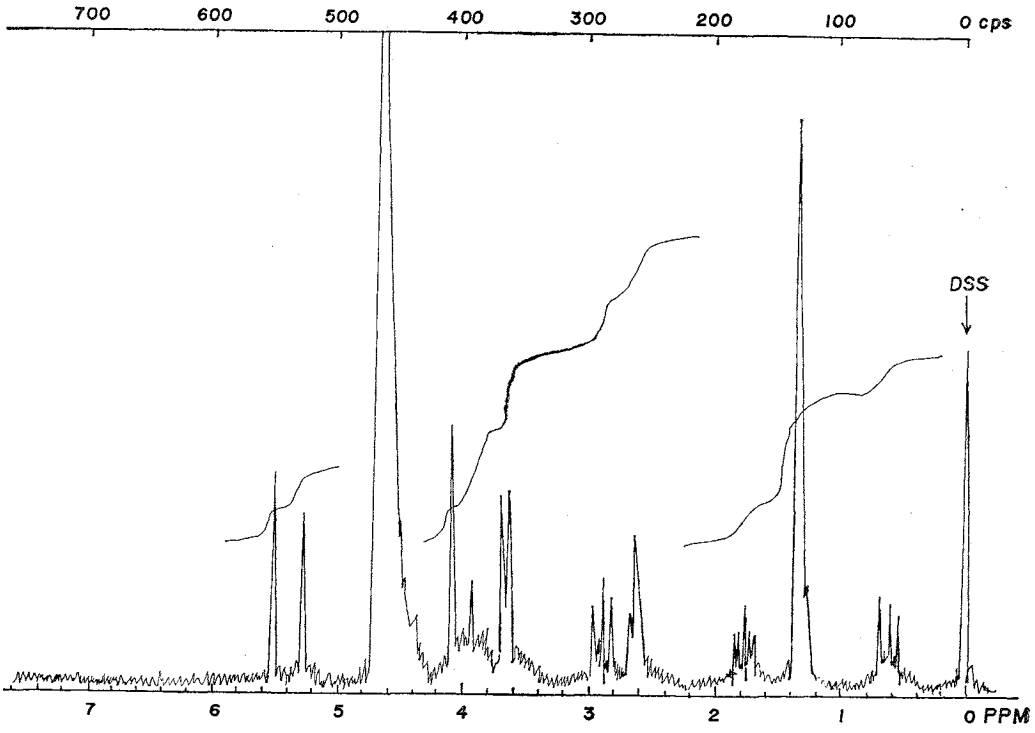

Table 1. Antibacterial spectrum of Antibiotic No. 5879

Test organism*	Minimal inhibitory concentration (mcg/ml)
Staphylococcus aureus FDA 209P	500
Sarcina lutea PCI 1001	62.5
Bacillus anthracis 1	>500
Bacillus subtilis ATCC 6633	>500
Pseudomonas aeruginosa 35	>500
Klebsiella pneumoniae S	15.6
Salmonella typhosa 376	15.6
Salmonella enteritidis NG 567	7.8
Escherichia coli B	31.2
Escherichia coli K-12	31.2
Shigella flexneri 3a 3196	15.6
Shigella sonnei R-1	15.6
Brucella melitensis K-3	0.9
Vibrio comma 384	3.9
Proteus vulgaris X-19	>500
Serratia marcescens 2	>500
Mycobacterium phlei 607	>500
Morganella 3	>500
Rettgerella 15	>500

* Heart infusion agar, 37℃, 24 hours.

in a small volume of methanol. This solution was mixed with approximately 10 volumes of acetone, concentrated under reduced pressure until crystallization occurred, and the mixture was then refrigirated overnight. The crude crystalline antibiotic was recrystallized from a mixture of methanol and acetone to yield colorless prisms.

Antibiotic No. 5879 forms colorless prism-shaped crystals, melts at 170~171°C (decomp.), and gives $[\alpha]_D^{31}$ +82.6° (*c* 0.6366, H_2O). It is easily soluble in water, soluble in methanol and scarcely soluble in ethanol. The molecular weight was estimated to be 302 by isothermal distillation and the elementary analysis suggested to be $C_{12}H_{18}N_2O_7$ for its molecular formula.

Analysis: Calcd. for $C_{12}H_{18}N_2O_7$:
C 47.76, H 6.01, N 9.27
Found: C 47.86, H 6.05, N 9.17

The ultraviolet absorption spectrum exhibits only end absorption (Fig. 1). The infrared spectrum measured in KBr tablet and the NMR spectrum are shown in Figs. 2 and 3, respectively. The mass spectrum could not be obtained because of pyrolysis of this antibiotic during the measurement. Ninhydrin test is only positive after degrading the antibiotic with 0.1 N aqueous potassium hydroxide at room temperature overnight. Ferric hydroximate and *vic*-diol splitting test with periodic acid are positive.

The antibiotic spectrum obtained by the agar dilution method is shown in Table 1. Antibiotic No. 5879 is especially effective against gram-negative bacteria. The intraperitoneal injection of 400 mg/kg to mice did not exhibit any toxicity.

Acknowledgement

The authors wish to express their thanks to Prof. TAMARI and Mr. UMEZU, Tohoku University, for elementary analysis and mass spectrum. We are also grateful to Mr. SAISU, the Analytical Center of Niigata University, for the physical analysis.

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(Received August 23, 1972)