AN ANTITUMOR ANTIBIOTIC, No. 4601 FROM *STREPTOMYCES*, IDENTICAL WITH YC 73 OF *PSEUDOMONAS* ORIGIN

Sir:

In the course of our systematic screening of antitumor substances produced by *Streptomyces*, an antitumor substance No. 4601 was isolated as grayish green needles from the culture filtrate of *Streptomyces* No. 4601. The antitumor activity of this antibiotic was assayed by MIYAMURA's method, *i.e.* the paper-disc plate method with HeLa cells.

The taxonomic characteristics of *Streptomyces* No. 4601 are as follows: Aerial hyphae are well branched, 0.8~1.0 μ in diameter. Straight and curved sporophores are observed. Numerous, small and open spirals (2~7 coils) are formed on the terminal of branches. Conidia are oval to spherical in shape and 1.0~1.5 by 1.3~1.7 μ in size. The surface appearance of conidia are granular. The growth characteristics were studied on 26 media. In general, the organism made good growth with white to pale yellow or brown colors. The vegetative mycelia were white and the reverse side of the colonies were pale yellow to brown. The aerial mycelia on most of the media tested were white to gray in color. A pale brown soluble pigment was produced when grown on several synthetic media.

The utilization of carbon sources was tested with the basal medium described by PRIDHAM and GOTTLIEB. Glucose, rhamnose, mannitol, mannose, lactose, xylose, arabinose, and starch were utilized, but raffinose, cellulose, inositol, and salicin were not. Nitrate reduction was positive. Starch, milk, and cellulose were digested, but hydrogen sulfide production and gelatin liquefaction were negative.

Our strain No. 4601 resembles *Streptomyces anulatus*, *Streptomyces fasciculatus*, *Streptomyces cinnamonensis*, *Streptomyces marinus*, and *Streptomyces olivaceus*. Although strain No. 4601 is most similar to *S. marinus*, these culture differ in several growth characteristics.

Fermentations were conducted under submerged culture conditions for 85 hours at 30°C in a medium containing 2 % glucose, 2 % soybean meal, 0.2 % yeast powder, 0.2 % meat extract, 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 7.0 prior to sterilization. The filtered broth was extracted at pH 8.0~8.5 with *n*-butyl alcohol. The butanol extract was washed with distilled water and then concentrated under reduced pressure. This concentrated material was diluted 20-fold with distilled water, and the aqueous

Fig. 1. Ultraviolet spectrum of No. 4601 substance in methanol.

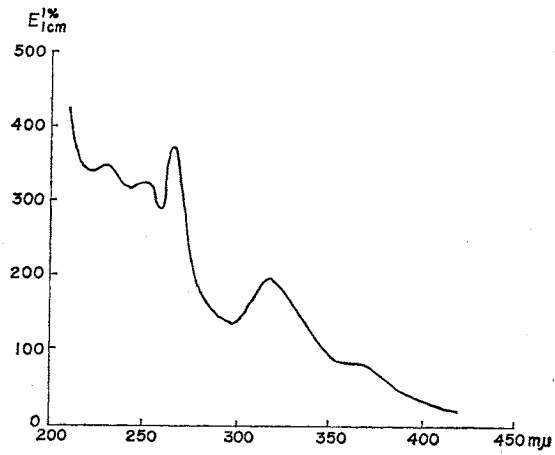
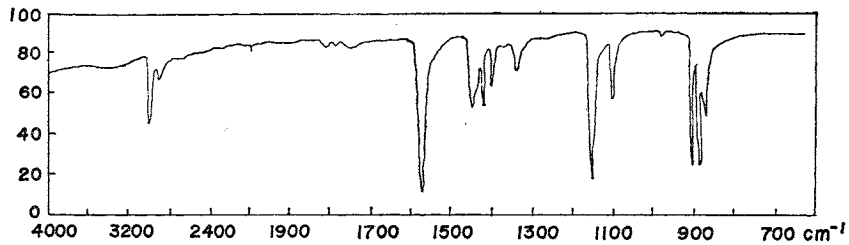


Fig. 2. Infrared spectrum of No. 4601 substance (KBr).



solution was adjusted to pH 6.2 and passed through a column of IRC 50 (H^+). This column was washed with distilled water, and the active substance was eluted with 50 % methyl alcohol containing 2.88 % ammonia. The fraction of the eluate showing strong activity was concentrated under reduced pressure, and then the concentrated solution was lyophilized. This powder was extracted with 10 volumes of acetone and an equal volume of *n*-hexane was gradually added to the filtered acetone extract. This solution was passed through a silica gel column which was developed and eluted with the mixture of acetone and *n*-hexane (1 : 1). The active substance was developed as greenish brown band. The colored fractions of the eluate were collected and concentrated to dryness under reduced pressure. The resultant crystalline product was recrystallized from 50 % methyl alcohol to yield grayish green needles.

The crystalline substance decomposed at 165~170°C. It was soluble in acetone, and slightly soluble in water, methanol, ethanol, and ethylacetate. The ultraviolet and the infrared absorption spectra of the antitumor substance are shown in Figs. 1 and 2, respectively. Elementary analysis, mass spectrometry and atomic absorption analysis are consistent with the molecular formula $C_4H_8N_2O_2Cu$, and are the same as those reported for YC 73[2] and fluopsin C[3] produced by *Pseudomonas* sp.

The HeLa cell had to be used to follow fermentation and isolation because the culture broth did not exhibit antibacterial activity. After purification, the substance was also effective against bacteria and fungi. The *in vitro* susceptibility of HeLa cell and various microorganisms to the crystalline No. 4601 substance is shown in Table 1. The acute intraperitoneal toxicity (LD_{50}) in mice is 4.5 mg/kg.

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Table 1. *In vitro* susceptibility of HeLa cell and microorganisms to No. 4601 substance

Test organism	Minimal inhibitory concentration (mcg/ml)
HeLa cell	0.195
Staphylococcus aureus FDA 209P	0.78
Sarcina lutea PCI 1001	0.78
Streptococcus haemolyticus 693	1.56
Streptococcus faecalis 5	3.12
Diplococcus pneumoniae 1	1.56
Neisseria gonorrhoeae T	0.195
Bacillus anthracis 2	0.39
Bacillus subtilis ATCC 6633	1.56
Corynebacterium diphtheriae 90	0.195
Mycobacterium phlei 607	0.78
Bordetella pertussis T	0.195
Pseudomonas aeruginosa 35	50
Klebsiella pneumoniae K-1	3.12
Salmonella typhosa 378	1.56
Shigella flexneri 3a 3196	1.56
Escherichia coli K-12	3.12
Escherichia coli B	1.56
Proteus vulgaris X-19	3.12
Aspergillus niger N-1	6.25
Candida albicans YU-1200	3.12

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