CAPURAMYCIN, A NEW NUCLEOSIDE ANTIBIOTIC

TAXONOMY, FERMENTATION, ISOLATION AND CHARACTERIZATION†

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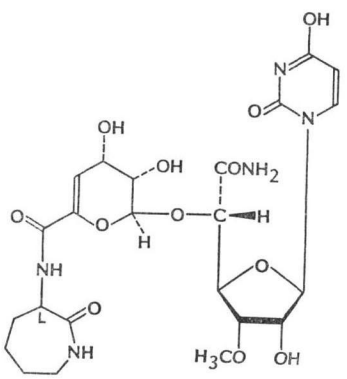
A new antibiotic capuramycin was isolated from the culture filtrate of *Streptomyces griseus* 446-S3 by adsorption and partition column chromatography. Based on its chemical structure being an uracil nucleoside with a caprolactam substituent, this substance was named as capuramycin. This paper describes the taxonomy of a producing organism, fermentation, isolation, characterization and biological properties of capuramycin.

In the course of our screening program for new antibiotics, a streptomycete, strain 446-S3 was found to produce a new antibiotic. Based on it's chemical structure[1] consisting of an uracil nucleoside and a caprolactam substituent, the antibiotic was designated as capuramycin (Fig. 1). This compound was previously called 446-S3-1 substance[2]. Capuramycin was active against *Streptococcus pneumoniae* and *Mycobacterium smegmatis* ATCC 607.

Fig. 1. Structure of capuramycin.



This paper deals with the taxonomy of the producing organism, fermentation, isolation, physico-chemical and biological properties of capuramycin.

Taxonomy of the Capuramycin Producer

The capuramycin producing strain 446-S3 was isolated from a soil sample collected at Yamadera, Yamagata-shi, Yamagata prefecture, Japan, in August, 1982.

The strain has been deposited at The Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, where it has been assigned accession number FERM-P No. 7416.

Characterization of the strain was performed by the methods of the International Streptomyces

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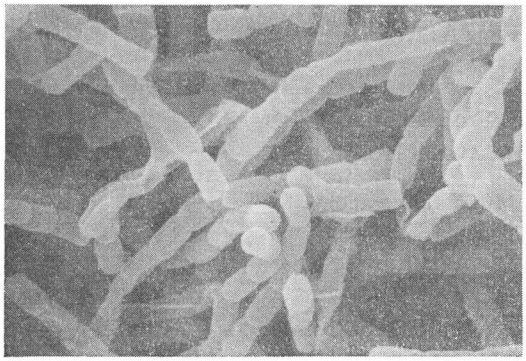
Fig. 2. Scanning electron micrograph of capuramycin-producing strain on yeast extract - malt extract agar.



Project (ISP)[3] and of WAKSMAN[4]. The color names used in this study were based on Jacol Color Cards 220 (Japan Color Research Institute).

Morphological Properties

After cultivation on inorganic salts - starch agar and yeast extract - malt extract agar at 27°C for 14 days, strain 446-S3 showed the following morphological properties.

The vegetative mycelium developed well without fragmentation. The aerial mycelium branched monopodially with sporophores forming spore chains with more than ten spores per

Table 1. Cultural properties of strain 446-S3.

Medium	Growth	Aerial mycelium	Substrate mycelium (reverse side)	Soluble pigment
Sucrose - nitrate agar	Good	Poor, white to pale greenish yellow	Light yellow	Bright greenish yellow
Glucose - asparagine agar	Poor	Poor, pale greenish yellow	Pale greenish yellow	Pale greenish yellow
Glycerol - asparagine agar	Good	Moderate, pale reddish yellow to pale yellow	Grayish yellow	Light greenish yellow
Inorganic salts - starch agar	Good	Abundant, pale yellow green	Dull yellow	Light greenish yellow
Tyrosine agar	Abundant	Abundant, grayish white to pale yellow green	Yellowish brown to dark yellowish brown	Pale yellow
Nutrient agar	Poor	Scant, white	Pale yellow	None
Yeast extract - malt extract agar	Abundant	Abundant, pale greenish yellow	Dull yellow	Bright yellow
Oatmeal agar	Moderate or poor	Poor, pale greenish yellow	Pale yellow to grayish yellow to gold	Pale yellow

Table 2. Physiological properties of strain 446-S3.

Temperature	
For growth	15~37°C
Optimum	27°C
Production of melanoid pigments	
Tyrosine agar	Negative
Peptone - yeast extract iron agar	Negative
Tryptone - yeast extract broth	Negative
Hydrolysis of starch	Positive
Liquefaction of gelatin	Positive
Peptonization of milk	Positive
Coagulation of milk	Negative
Utilization of carbon sources (ISP No. 9)	
Positive (+)	D-Xylose, D-glucose, D-fructose, D-mannitol, D-galactose, salicin
Negative (−)	L-Arabinose, L-rhamnose, raffinose, cellulose
Doubtful (±)	Sucrose, *i*-inositol

chain. The morphology of aerial mycelium and spore chains are *Rectus-Flexibilis*, and tufts are also observed. The spores are cylindrical ($0.9 \sim 1.8 \times 0.5 \sim 1.0$ μm) with smooth surface under an electron microscope (Fig. 2). Sporangia, flagellated spores, sclerotia, and other special morphology are not observed.

Cultural and Physiological Properties

The cultural properties of strain 446-S3 grown on various media at 27°C for 2 weeks and the physiological properties of the strain are shown in Tables 1 and 2, respectively.

Analysis of cell wall diaminopimeric acid isomers performed by the method of HASEGAWA *et al.*[5] showed that LL-diaminopimeric acid was present.

Comparison with Other Related Species

On the basis of its characteristics, strain 446-S3 seemed to belong to the yellow series of the genus *Streptomyces*. Among the species of yellow series of *Streptomyces* described in the 8th edition of BERGEY's manual[6] and SHIRLING's ISP reports[7~10], and the other species listed on "Approved lists of bacterial names"[11], strain 446-S3 closely resembled to *Streptomyces griseus*. The properties of the strain were compared with those of *S. griseus*, and good agreements were obtained except that strain 446-S3 produced a new antibiotic, capuramycin. Therefore, strain 446-S3 was identified as a strain of *S. griseus* and was designated as *S. griseus* 446-S3.

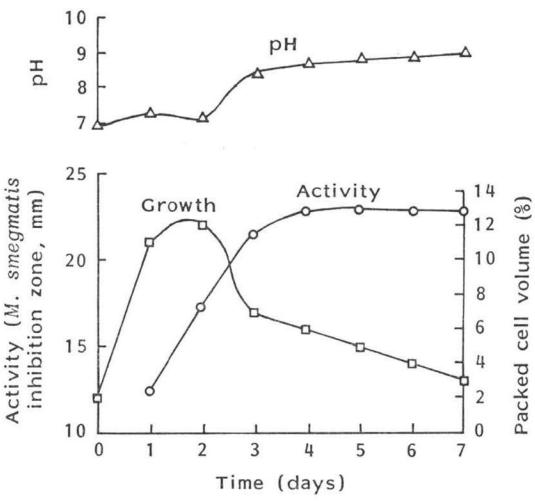
Fermentation

Erlenmeyer flasks (500-ml) containing 100 ml of a medium consisting of glycerol 1.8%, Polypeptone 0.6%, meat extract 0.5% and NaCl 0.3% were inoculated with spores from a slant culture of the producing strain and incubated at 28°C on a rotary shaker with 5 cm-radius at 200 rpm for 2 days to prepare seed cultures.

Fifty Erlenmeyer flasks (500-ml) containing 100 ml of the same medium were inoculated with 3% volume of the seed culture and incubated for 6 days under the same condition. The potency was assayed by the paper disc-agar diffusion method using *M. smegmatis* ATCC 607 as a test organism.

A typical time course of the fermentation is presented in Fig. 3.

Fig. 3. Time course of the production of capuramycin.



Isolation

The culture broth (5 liters) was adjusted to pH 7.0 with 2 N HCl and centrifuged. The supernatant (4.4 liters) was applied to a column of Diaion HP-20, and the adsorbed antibiotic was eluted with 50% aqueous acetone. The eluate was concentrated *in vacuo* to remove the acetone and freeze dried. The resulting brown powder (6.0 g) was dissolved in methanol and was subjected to column chromatography using Toyopearl HW-40F. The active fraction (300 ml)

eluted with methanol was concentrated *in vacuo* to give a brown syrup which was then applied to a silica gel column and eluted with chloroform - methanol gradient (5: 1 ~ 2: 1). The active fraction was concentrated *in vacuo* to yield a crude powder which was rechromatographed on a silica gel column and eluted with chloroform - methanol gradient (10: 1 ~ 5: 1). Concentration of appropriate fractions afforded a white powder of capuramycin (0.2 g).

The flow diagram for the isolation of capuramycin is presented in Fig. 4.

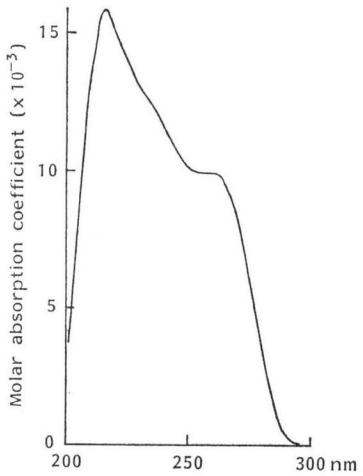
Fig. 4. Flow diagram of the isolation of capuramycin.

Culture broth (5 liters)
↓ adjusted to pH 7.0 with 2 N HCl
↓ centrifuged
Supernatant (4.4 liters)
↓ column chromatography on Diaion HP-20
eluted with 50% aq Me_2CO
Active fraction (500 ml)
↓ concd *in vacuo* and freeze dried
Crude powder (6.0 g)
↓ dissolved in MeOH
↓ column chromatography on Toyopearl HW-40F
eluted with MeOH
Active fraction (300 ml)
↓ concd *in vacuo*
↓ column chromatography on silica gel
eluted with $CHCl_3$ - MeOH gradient (5: 1 ~ 2: 1)
Active fraction
↓ concd *in vacuo* to dryness
Crude powder
↓ column chromatography on silica gel
eluted with $CHCl_3$ - MeOH gradient (10: 1 ~ 5: 1)
Active fraction
↓ concd *in vacuo* to dryness
White powder of capuramycin (0.2 g)

Table 3. Physico-chemical properties of capuramycin.

Appearance	White amorphous powder
MP	173 ~ 176°C
Specific rotation $[\alpha]_D^{25}$	+99° (*c* 0.5, H_2O)
MW	569
SI-MS (*m/z*)	570 $(M+H)^+$, 592 $(M+Na)^+$
Molecular formula	$C_{23}H_{31}O_{12}N_5$
Elemental analysis	C H O N
Calcd	48.50 5.49 33.71 12.30
Found	48.59 5.79 33.27 12.37
UV λ_{max}^{MeOH} nm (ε)	214 (16,200), 257 (sh, 9,800)
IR ν_{max}^{KBr} cm^{-1}	3400, 2930, 1680, 1515, 1270, 1100
Rf values on silica gel TLC	0.43 ($CHCl_3$ - MeOH, 2: 1), 0.21 (benzene - MeOH, 2: 1), 0.43 (BuOH - AcOH - H_2O, 3: 1: 1)
Color reaction: Positive	$KMnO_4$, Molisch
Negative	Ninhydrin, anthrone, $FeCl_3$, Sakaguchi

Fig. 5. UV spectrum of capuramycin.



Physico-chemical Properties

Physico-chemical properties of capuramycin are listed in Table 3. The UV, IR and ^{1}H NMR spectra are presented in Figs. 5, 6 and 7, respectively. The ^{13}C NMR spectral data are summarized in Table 4.

Capuramycin is soluble in methanol and water; slightly soluble in ethanol and acetone; insoluble in ethyl acetate, chloroform, benzene and *n*-hexane. The *pKa* in water is 9.1.

Biological Properties

The minimum inhibitory concentrations of capuramycin, assayed by the agar dilution

Fig. 6. IR spectrum of capuramycin.

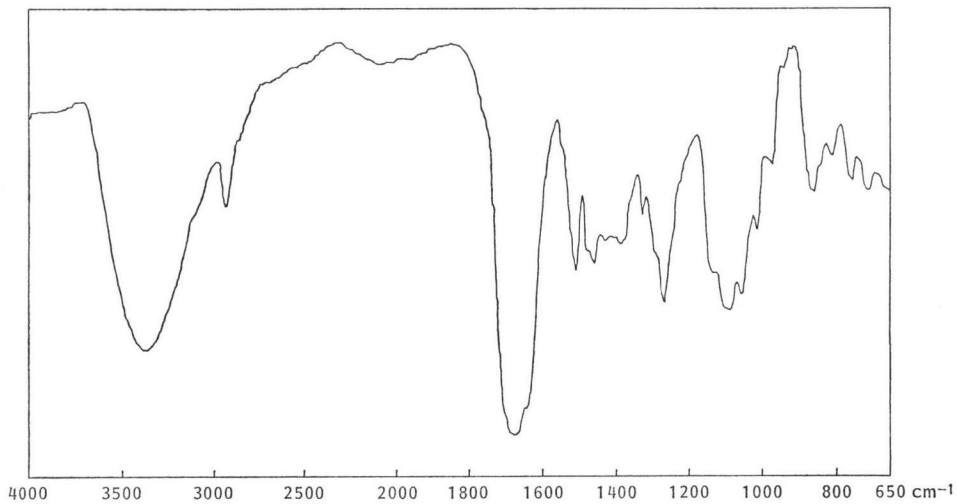


Table 4. ^{13}C NMR spectrum of capuramycin (in D_2O, 100 MHz).

Carbon No.	Chemical shift (ppm)	Multiplicity*	Carbon No.	Chemical shift (ppm)	Multiplicity*
2	151.6	s	1″	100.0	d
4	166.3	s	2″	65.7	d
5	102.5	d	3″	62.7	d
6	141.5	d	4″	109.9	d
1′	90.7	d	5″	142.1	s
2′	72.8	d	6″	161.7	s
3′	79.0	d	1‴	176.5	s
3′-OCH_3	58.7	q	2‴	53.1	d
4′	82.4	d	3‴	28.4†	t
5′	76.4	d	4‴	28.6†	t
6′	173.2	s	5‴	31.3	t
			6‴	42.3	t

* s: Singlet, d: doublet, t: triplet, q: quartet.
† Assignments may be exchanged.

Fig. 7. ^1H NMR spectrum of capuramycin (in D_2O, 400 MHz).

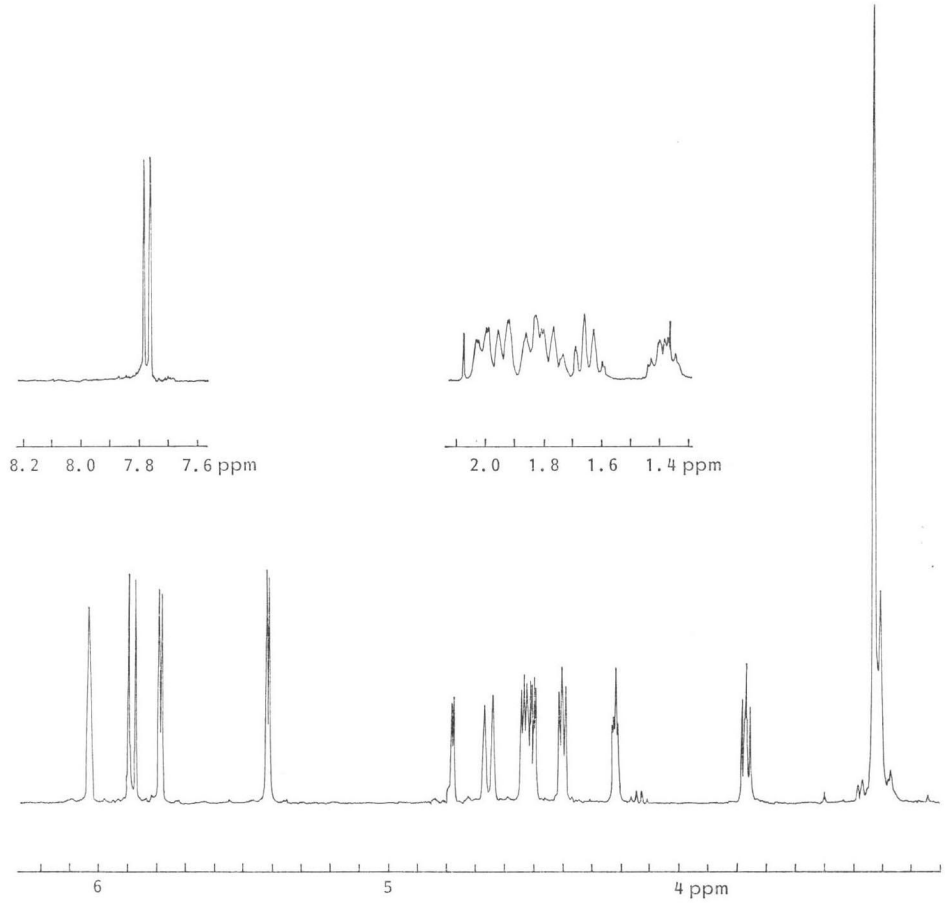


Table 5. Antimicrobial activities of capuramycin.

Test organisms	MIC (μg/ml)	Test organisms	MIC (μg/ml)
Bacillus subtilis ATCC 6633	>100	*Enterobacter aerogenes* ATCC 13048	>100
Micrococcus luteus ATCC 9341	>100	*E. cloacae* 963	>100
Staphylococcus aureus FDA 209P JC-1	>100	*Escherichia coli* NIHJ JC-2	>100
Streptococcus faecalis	>100	*Klebsiella pneumoniae* PCI-602	>100
S. pneumoniae IID 553	12.5	*Proteus vulgaris* HX-19	>100
S. pneumoniae IID 554	12.5	*Salmonella enteritidis* G14	>100
S. pyogenes Cook	100	*Serratia marcescens* IAM 1184	>100
S. pyogenes IID 715	100	*Mycobacterium smegmatis* ATCC 607	3.13
Neisseria gonorrhoeae IID 844	>100	*Aspergillus oryzae* IFO 5239	>100
N. meningitidis IID 854	>100	*Penicillium chrysogenum* ATCC 10002	>100
Haemophilus influenzae IID 985	>100	*Candida albicans* 3147	>100
Pseudomonas aeruginosa NCTC 10490	100	*Saccharomyces cerevisiae* IFO 0205	>100
Citrobacter freundii IID 976	>100		

method, are given in Table 5. Capuramycin showed activity against *S. pneumoniae* and *M. smegmatis* ATCC 607, but it was inactive against other microorganisms tested so far.

No toxic sign was observed when capuramycin was intravenously administered to *dd*Y mice up

to the dose of 1,000 mg/kg.

Acknowledgment

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