TETRAZOMINE, A NEW ANTIBIOTIC PRODUCED BY AN ACTINOMYCETE STRAIN

TAXONOMY, FERMENTATION, ISOLATION AND CHARACTERIZATION

KENICHI SUZUKI, TSUTOMU SATO, MOTO MORIOKA, KOJI NAGAI, KENJI ABE, HIROSHI YAMAGUCHI and TAKESHI SAITO

Biomedical Research Laboratories I, Central Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd., 1-1-8 Azusawa, Itabashi-ku, Tokyo 174, Japan

YUKIO OHMI and KIYOSHI SUSAKI

Medical Research Laboratories III, Central Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd., 1-1-8 Azusawa, Itabashi-ku, Tokyo 174, Japan

(Received for publication December 21, 1990)

A new antibacterial antibiotic tetrazomine was found from the fermentation broth of an actinomycete strain which was isolated from beach sand collected at Chichijima, Ogasawara Islands, Tokyo, Japan. The strain Y-09194L, was identified as *Saccharothrix mutabilis* subsp. *chichijimaensis* subsp. nov. The antibiotic exhibited broad antimicrobial activity against Gram-positive and Gram-negative bacteria *in vitro*. It also exhibited strong cytotoxic activity against P388 leukemia cells and showed antitumor activity against P388 leukemia. The apparent molecular formula of tetrazomine was determined as $C_{24}H_{34}N_4O_5$. It has a rare structure which consists of six rings including piperidine, piperadine, oxazole, and pyrrolidine.

In the course of screening program for new antibiotic from so-called rare actinomycetes, we isolated a strain which produced a new antibiotic, tetrazomine. It exhibited antimicrobial activity against Gram-positive and Gram-negative bacteria. It also exhibited strong cytotoxicity against P388 leukemia cells and showed antitumor activity against P388 leukemia. In this paper, we describe the taxonomic studies of the producing organism, the fermentation, isolation and characterization of the antibiotic.

Materials and Methods

Isolation of Producing Organisms

Strain Y-09194L was isolated from beach sand collected at Chichijima, Ogasawara Islands, Tokyo, Japan. Isolation method was as follows. Soil samples were dried at room temperature overnight. Then, air dried samples were heated at 100°C for 30 minutes. The samples were inoculated in the isolation medium (MG 3) by the dilution-plate method. The isolation medium consisted of copra-meal 0.5%, L-asparagin 0.1%, K_2HPO_4 0.05% and soil extract solution[1], pH 8.0 before sterilization. 100 μg/ml of cefotetan (Yamatetan: Yamanouchi Pharmaceutical Co., Ltd.), 100 μg/ml of Penicillin G Potassium (Wako Pure Chemical Industries, Ltd.), and 100 μg/ml of ampicillin (Pentrex: Banyu Pharmaceutical Co., Ltd.) were added for the selection of microorganisms with resistance to β-lactam antibiotics. Nystatine and actidione (50 μg/ml, respectively) were also added to inhibit the growth of fungi.

The plates were incubated at 32°C for 1 month and colonies grown on the medium were classified preliminarily and picked up on YS-agar slants (yeast extract 0.2%, potato starch 1.0%, agar 1.5%, pH 7.6 before sterilization).

Taxonomic Examination

For taxonomic studies, most cultures were grown in accordance with the methods adopted by the International Streptomyces Project[2]. Morphological characterization was carried out under light and electron microscopic observation. Jeol T220 scanning electron microscope was used for ultrastructural evaluation. Cultures of the test strain were fixed with 2% glutalaldehyde, dried at critical point and coated with gold by sputter. For experiments on cultural properties, all cultures were incubated at 28°C and were observed for 14~21 days. The color was described according to the "Guide to Color Standard"[3]. Physiological properties including utilization of carbon sources were examined by the method of PRIDHAM and GOTTLIEB[4]. Chemotaxonomical analysis were performed based on the methods in "Actinomycete Taxonomy"[5] and the methods by BECKER *et al.*[6].

Fermentation

Stock cultures of the producing organisms were inoculated into a 500-ml flask containing 60 ml of the seed medium consisting of glucose 0.5%, dextrin 2.0%, Polypeptone (Daigo Eiyo Kagaku Co., Ltd.) 0.5%, yeast extract 0.5%, meat extract 0.3%, Brain heart infusion broth (Eiken Kagaku Co., Ltd.) 0.52% and $CaCO_3$ 0.2% (pH 8.0). The flasks were incubated at 28°C for 3 days on a reciprocal shaker (100 stroke/minute; 25-cm stroke).

The seed culture was transferred to 500 ml flasks (second seed) containing 60 ml of the same medium. The inoculated flask was shaken at 28°C for 3 days. Then, 600 ml of the second seed were inoculated into 20 liters of the same medium in a 30-liter jar fermenter (third seed). After cultivating at 28°C for 3 days, 3% inoculum of the third seed was transferred to a 300-liter fermenter containing 200 liters of the production medium consisting of fructose 2.0%, defatted soybean flour 1.0%, yeast extract 0.2%, antifoam (Adecanol, Asahi Denka Co., Ltd.) 0.03% (pH 7.0).

Isolation

The culture broth was adjusted to pH 2.5 and filtered with the aid of Celite. The filtrate was readjusted to pH 6.5 and passed through 5 liters of CM-Sephadex C-25 (Na). After being washed with 10 liters of water, the column was eluted with 15 liters of 5% NaCl solution. Then the eluate was passed through 2 liters of Diaion HP-20. The column was washed with 4 liters of water and eluted with 4 liters of 40% MeOH solution. The eluate was concentrated *in vacuo* and passed through 3 liters of Dowex 1 (Cl). The effluent was adjusted to pH 7 and absorbed on 900 ml of CM-Sephadex C-25 (Na). The column was eluted by a gradient of 0 to 5% NaCl solution, and the eluate was fractionated and assayed against *Bacillus subtilis* ATCC 6633. Active fractions were collected and adjusted to pH 7.0 and absorbed with 250 ml of Diaion CHP-20P. The elution was carried out by a gradient of 0 to 20% MeOH solution and fractionated. The active fractions were collected and concentrated *in vacuo*. Further purification was done with HPLC (YMC-ODS, 20 i.d. × 250 mm, 11 ml/minute, 210 nm, solvent was 0.1 M sodium sulfate 100 ml, acetic acid 0.05 ml, sodium 1-pentanesulfonate 0.02 g, acetonitrile 3.1 ml). The peak at 9 minutes was collected, concentrated *in vacuo* and lyophilized. MeOH extract from the residual powder was dried and dissolved in water. Then it was passed through Dowex 1 (Cl), and lyophilized.

Antimicrobial Activity

The antimicrobial spectrum of tetrazomine was determined by a conventional agar dilution method using Müller-Hinton medium for Gram-positive and Gram-negative organisms. MICs were expressed in μg/ml after overnight incubation at 37°C.

Cytotoxicity and Antitumor Activity

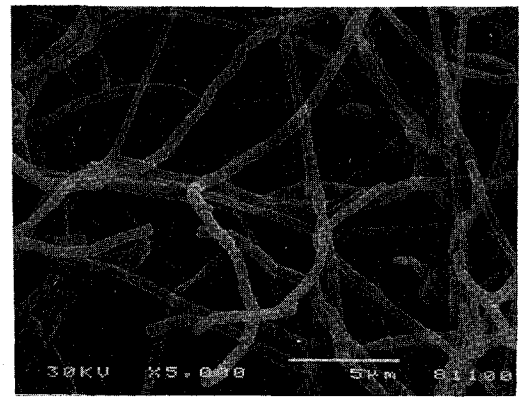
L1210 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS); P388 mouse leukemic cells were maintained in the peritoneal cavity of CDF_1 mice. To determine the cytotoxicity of tetrazomine, L1210 or P388 cells (1×10^4) in 1 ml of medium (RPMI-1640 + 10% FBS) containing various concentrations of the antibiotic were placed in a tissue culture plate (Falcon, 24-cell) and incubated for 72 hours at 37°C in a 5% CO_2-95% air atmosphere. At the end of the incubation period, the cells were counted by a hemacytometer. Concentration of the antibiotic required for 50% inhibition of cell growth (IC_{50} μg/ml) was examined by plotting the logarithms of the treated cells.

The antitumor activity of tetrazomine was determined in experimental tumor system in mice against P388 cells. P388 leukemia was implanted intraperitoneally into *dd*Y-SLC mice (female, 8 weeks old, purchased from Shizuoka Laboratory Animal Center, Japan) at inoculum size of 1×10^6 cells per mouse. Drug treatments were given intraperitoneally once daily from day 1 to 5 (qd $1 \sim 5$) starting 24 hours after the tumor implantation. Ten mice were used in each test group. The injection volume was 0.2 ml in all experiments. Control animals received intraperitoneal dose of physiological saline solution. Tetrazomine was dissolved in physiological saline solution. Mitomycin C (Kyowa Hakko Kogyo Co., Ltd., Japan) was suspended in phisiological saline solution and comparatively tested simultaneously as a reference compound. Death or survival of the treated and non-treated animals was recorded daily during the observation period of 30 days after the tumor implantation, and the median survival time (MST) was calculated for each of the test and control groups. Antitumor activity was evaluated by the MST of group of mice and also expressed as T/C % value (MST of treated group/MST of non-treated group, $\times 100$).

Physico-chemical Properties

MW was determined (*m/z* 459) by FAB-MS and molecular formula was determined by HRFAB-MS and ^{13}C NMR spectrum. MP was measured using MP-500D apparatus and is uncorrected. UV spectra were recorded on Shimadzu UV-240 spectrophotometer.

Fig. 1. Scanning electron micrograph of spore chain of strain Y-09194L.



Results and Discussion

Taxonomic Studies of the Producing Strain

Aerial mycelia of the strain Y-09194L were well developed, long, straight to wavy and monopodially branched on yeast extract-malt extract agar (ISP

Table 1. Cultural characteristics of strain Y-09194L on various media.

Yeast extract-malt extract agar (ISP-2)	G:	Good
	R:	Pale yellowish orange
	A:	Abundant, white
	P:	None
Oatmeal agar (ISP-3)	G:	Good
	R:	Pale yellowish orange
	A:	Abundant, grayish white
	P:	None
Inorganic salts-starch agar (ISP-4)	G:	Good
	R:	Pale yellow
	A:	Abundant, grayish white
	P:	None
Glycerol-asparagin agar (ISP-5)	G:	Moderate
	R:	Pale yellow
	A:	Poor, white—grayish white
	P:	None
Peptone-yeast extract-iron agar (ISP-6)	G:	Moderate
	R:	Pale yellow
	A:	Moderate, white—light gray
	P:	None
Tyrosine agar (ISP-7)	G:	Moderate
	R:	Pale yellow
	A:	Moderate, white—light gray
	P:	None
Sucrose-nitrate agar	G:	Moderate
	R:	Pale yellowish orange
	A:	Moderate, white—grayish white
	P:	None
Nutrient agar	G:	Poor
	R:	Yellowish gray
	A:	Poor, white—light gray
	P:	None
BENNETT agar	G:	Good
	R:	Pale yellowish gray
	A:	Abundant, white—light gray
	P:	None

Abbreviations: G, growth of vegetative mycelium; R, reverse side; A, aerial mycelium; P, soluble pigment.

medium 2), oatmeal agar (ISP medium 3), inorganic salts-starch agar (ISP medium 4) and BENNETT agar. They then divided into many spores, giving an appearance of total sporulation on various media described above. Spores were non-motile, $0.4 \sim 0.6 \times 0.6 \sim 1.5\ \mu m$ in size, oval to cylindrical in shape and with smooth surfaces (Fig. 1). Aerial mycelia sometimes showed a zig-zag appearance at early stages of spore formation.

Fragmentation of substrate mycelia was observed at early stages of liquid culture. The mycelia grown on various agar media were aerobic, Gram-positive, non-motile, non-acid fast.

Cultural properties of strain YL-09194L are shown in Table 1. Aerial mass color was in the white series when grown on yeast extract-malt extract agar, oatmeal agar, inorganic salts-starch agar, and BENNET agar.

Physiological properties of strain Y-09194L are shown in Table 2 and the pattern of carbohydrate utilization in Table 3. Temperature range for growth was from 15 to 37°C with an optimum at 24 to 32°C. Gelatin liquefaction and production of melanoid pigment were nagative. Milk coagulation and peptonization were positive. Nitrate reduction and starch hydrolysis were positive.

Chemical analysis of cell wall preparation showed the presence of *meso*-diaminopimelic acid, alanine, glutamic acid and small amount of galactose. But glycine and arabinose were not detected. Whole cell

Table 2. Physiological properties of strain Y-09194L.

Melanin formation	−
Tyrosinase reaction	−
H_2S production	−
Liquefaction of gelatin (21°C)	−
Peptonization of milk (37°C)	+
Coagulation of milk (37°C)	+
Cellulolytic activity	−
Hydrolysis of starch	+
Temperature range for growth	15~37°C
Optimum temperature for growth	24~32°C

Table 3. Utilization of carbohydrates by strain Y-09194L.

D-Arabinose	+	Mannose	+
D-Xylose	+	Melibiose	+
D-Glucose	+	D-Galactose	+
D-Fructose	+	Maltose	+
D-Mannitol	+	Salicin	±
Sucrose	+	Trehalose	+
i-Inositol	+	Glycerol	+
Raffinose	+	Dextrin	+
L-Rhamnose	+	Xanthin	−
Starch	+		

+: Utilized, ±: weakly utilized, −: not utilized.

Table 4. Comparison of Y-09194L with *Saccharothrix mutabilis*.

	Y-09194L	*S. mutabilis* IFO 14310
Spore shape	Cylindrical	Cylindrical
Spore surface	Smooth	Smooth
Aerial mass color	White—light gray	White—light gray
Reverse side color	Pale yellow	Pale yellow
Fragmentation of substrate mycelium	+	+
Soluble pigment	None	None
Temperature range for growth (°C)	5~37	15~42
Tyrosinase reaction	−	−
Starch hydrolysis	+	+
Peptonization of milk	+	+
Coagulation of milk	+	+
Liquefaction of gelatin	−	−
Utilization of carbon source:		
Mannitol	+	−
Raffinose	+	−
Salicin	±	−
Antibiotic produced	Tetrazomine	None

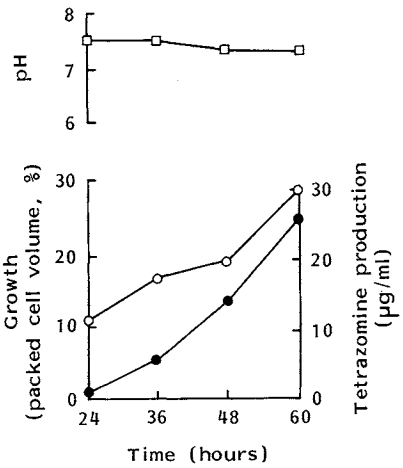
+: Positive, ±: weakly positive, −: negative.

acid hydrolysate contained galactose, glucose, rhamnose and ribose, but not arabinose, madurose and xylose. This indicates that the cell wall of this strain belongs to type III and whole cell sugar pattern to type C. The types of phospholipids and menaquinone were II and MK9 (H_4) with additional minor amounts MK-8 (H_4), respectively. Nocardiomycolic acid was not detected.

On the basis of the above features, strain Y-09194L belongs to the genus *Saccharothrix*[7~10] on morphological properties, cultural properties and chemotaxonomic characteristics. Furthermore, strain Y-09194L belongs to *Saccharothrix mutabilis*. Therefore, we carried out the direct comparison of strain Y-09194L with *S. mutabilis* IFO 14310 and the results are shown in Table 4. Strain Y-09194L differed from *S. mutabilis* as follows: (1) the growth temperature (*S. mutabilis* was 15~42°C, whereas strain Y-09194L at 15~37°C), (2) carbon utilization (*S. mutabilis* did not utilize mannitol, raffinose and salicin as sole carbon sources while strain Y-09194L utilized these sugars and (3) antibiotic (tetrazomine) was produced by strain Y-09194L. But, otherwise, strain Y-09194L resembled *S. mutabilis*. Therefore, the strain is considered to be a new subspecies of *S. mutabilis* and the name *S. mutabilis* subsp. *chichijimaensis* subsp. nov. is proposed. The specific epithet is described from the source of the soil in which the organism was isolated. Strain Y-09194L has been deposited in the Fermentation Research

Fig. 2. Time course of tetrazomine production.

○ Growth, ● tetrazomine production, □ pH.



Packed volume was determined by centrifugation at 2,000×*g* for 10 minutes.

Total activity was determined by a paper disk method using *Escherichia coli* K-12 as the test organism.

Fig. 3. Purification procedure.

Filtrate
↓ pH 6.5
CM-Sephadex C-25 (Na)
↓ 5% NaCl
Diaion HP-20
↓ 40% MeOH
Dowex 1 (Cl)
↓
Effluent
↓
CM-Sephadex C-25 (Na)
↓ 0~5% NaCL
Diaion CHP-20P
↓ 0~20% MeOH
HPLC
↓ YMC-ODS[a]
MeOH extract
↓
Dowex 1 (Cl)
↓
Effluent
(Tetrazomine: HCl)

[a] 0.1 M Na_2SO_4 100 ml, acetic acid 0.05 ml, $CH_3(CH_2)_4SO_3Na$ 0.02 g, acetonitrile 3.1 ml.

Table 5. Antimicrobial spectrum of tetrazomine.

Test organisms	MIC (μg/ml)
Bacillus subtilis ATCC 6633	6.25
Staphylococcus aureus FDA 209P JC-1	6.25
S. epidermidis IID 866	25
Streptococcus pyogenes Cook	0.78
Enterococcus faecalis IID 682	6.25
E. faecium CAY 09-1	3.13
Mycobacterium smegmatis ATCC 607	12.5
Escherichia coli NIHJ	1.56
Citrobacter freundii CAY 17-1	0.78
Klebsiella pneumoniae ATCC 10031	3.13
Proteus vulgaris OXK US	3.13
Pseudomonas aeruginosa NCTC 10490	6.25
P. aeruginosa ATCC 8689	50

Table 6. Antitumor activitiy of tetrazomine against P388 leukemia.

Antibiotic	Dose (mg/kg/day)	MST (days)	T/C (%)	Survival (40 days)
Tetrazomine	0.0125 × 7 ip	11.0	100	0/8
	0.025	14.0	127	0/8
	0.05	19.0	173	0/8
	0.1	9.0	82	0/8
Mitomycin C	0.5 × 5 ip	27.0	245	2/8
	1.0	24.5	223	2/8
Control	—	11.0	100	0/24

Fig. 4. Chemical structure of tetrazomine.

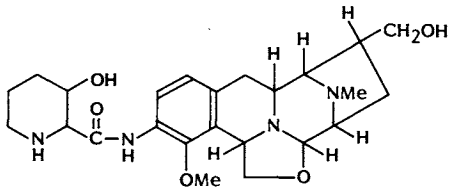


Table 7. Physico-chemical properties of tetrazomine.

Molecular formula	$C_{24}H_{34}N_4O_5$
FAB-MS (*m/z*)	459, 243, 100
HRFAB-MS (*m/z*)	459.26711
MP	190°C (dec)
$[\alpha]_D$	−62° (*c* 1, MeOH)
IR KBr (cm^{-1})	3360, 1680, 1540, 1460, 1300, 1050
UV λ_{max}^{MeOH} nm	207, 224
Rf value[a] (I)	0.29
(II)	0
Solubility	Soluble in H_2O, MeOH, EtOH
Color reaction	Ninhydrin, sulfuric acid

[a] Silica gel TLC, solvent (I) $CHCl_3$ - MeOH - NH_4OH (15 : 15 : 0.1), (II) Pro - H_2O (4 : 1).

Institute, Agency of Industrial Science and Technology, Japan, with the accession No. FERM P-10461.

Production and Isolation

The fermentation of tetrazomine was carried out as described in Materials and Methods. Fermentation was maintained at 28°C for 65 hours with an airflow rate of 35 liters/minute and agitation at 250 rpm. The progress of fermentation was monitored by determination of the growth measured as packed mycelial volume (PMV, %), pH and potency of tetrazomine by paper disk diffusion assay using *Escherichia coli* K-12 (Fig. 2). The production of tetrazomine was maximum at 60 hours after inoculation, reaching 27 μg/ml. From the culture filtrate (200 liters) the antibiotic was isolated as shown in Fig. 3. The total yield of tetrazomine was 3 g.

Biological Properties

Antimicrobial Activity

The antimicrobial activity of tetrazomine is shown in Table 5. The antibiotic showed broad activity against Gram-positive and Gram-negative organisms with MIC ranging from 0.78 to 25 μg/ml and from 0.78 to 50 μg/ml, respectively. Compared with structurally related quinocarcin[11,12], tetrazomine exhibited stronger activity, especially against Gram-negative bacteria. MIC values of tetrazomine against *E. coli* and *Klebsiella pneumoniae* were 1.56 and 3.13 μg/ml, respectively. So we are interested in making more active compounds by chemical modification.

Antitumor Activity

Tetrazomine possessed cytotoxic activity against lymphoid leukemia L1210 and leukemia P388 with IC_{50} value of 0.0427 and 0.0140 μg/ml, respectively. Additionally, the antitumor activity of the antibiotic was determined in experimental tumor system described in Materials and Methods. The results are shown in Table 6. Tetrazomine exhibited antitumor activity against P388 leukemia.

Physico-chemical Properties

The physico-chemical properties of tetrazomine are summarized in Table 7. As tetrazomine free base is unstable, the physico-chemical data were obtained in tetrazomine hydrochloride. MW was determined as *m/z* 459 by FAB-MS and molecular formula was determined as $C_{24}H_{34}N_4O_5$ by HRFAB-MS and ^{13}C NMR spectrum. In elementary analysis the obtained tetrazomine hydrochloride was determined as $C_{24}H_{34}N_4O_5 \cdot 2HCl \cdot 3.8H_2O$: Obsd: C 48.01, H 6.87, N 9.04, Cl 11.43, Calcd: C 48.05, H 7.33, N 9.34, Cl 11.82. Tetrazomine is soluble in water, methanol and ethanol, but insoluble in ethyl acetate, chloroform and *n*-hexane. Studies in our laboratories have shown that tetrazomine has the chemical structure shown in Fig. 4. Structurally the antibiotic is rare and does not belong to any group of the known antibiotics. Tetrazomine shares the same partial structure as quinocarcin, SF-1739 HP[13], saframycins[14,15] and naphthyridinomycin A[16]. However, the tetrazomine producing strain did not produce any of them. Details of structure elucidation will be reported in a separate paper.

Acknowledgments

The authors are grateful to the co-workers of the Biomedical I. Laboratories, Central Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd., for their helpful assistance in soil sampling, isolation of microorganisms, fermentation, biological examination and chemical analysis. We also thank to Dr. INUKAI, Director of our laboratories, for his instructive advice and helpful discussions.

References

1) SUZUKI, K.; K. NAGAI, S. MIYAZAKI & T. SAITO: Distribution of *Saccharopolyspora* in soils of the southwest islands in Okinawa Prefecture, Japan. Actinomycetologia 2: 99~107, 1988
2) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
3) Guide to Color Standard. Nihon Shikisai Sha, 1954
4) PRIDHAM, T. G. & D. GOTTLIEB: The utilization of carbon compounds by some *Actinomycetales* as an aid for species determination. J. Bacteriol. 56: 107~114, 1948
5) DIETZ, A. & D. W. THAYER (*Ed.*): Actinomycete Taxonomy. SIM special publication No. 6. Society for Industrial Microbiology, 1980
6) BECKER, B.; M. P. LECHEVALIER & H. A. LECHEVALIER: Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. Appl. Microbiol. 13: 236~243, 1965
7) GRUND, E. & R. M. KROPPENSTEDT: Chemotaxonomy and numerical taxonomy of the genus *Nocardiopsis* Meyer 1976. Int. J. Syst. Bacteriol. 40: 5~11, 1990
8) LABEDA, D. P.; R. T. TESTA, M. P. LECHEVALIER & H. A. LECHEVALIER: *Saccharothrix*: a new genus of the *Actinomycetales* related to *Nocardiopsis*. Int. J. Syst. Bacteriol. 34: 426~431, 1984
9) SHEARER, M. C.; P. M. COLMAN & C. H. NASH, III: *Nocardiopsis mutabilis*, a new species of nocardioform bacteria isolated from soil. Int. J. Syst. Bacteriol. 33: 369~374, 1983
10) LABEDA, D. P. & M. P. LECHEVALIER: Amendment of the genus *Saccharothrix* Labeda *et al.* 1984 and descriptions of *Saccharothrix espanensis* sp. nov., *Saccharothrix cryophilis* sp. nov., and *Saccharothrix mutabilis* comb. nov. Int. J. Syst. Bacteriol. 39: 420~423, 1989
11) TOMITA, F.; K. TAKAHASHI & K. SHIMIZU: DC-52, a novel antitumor antibiotic. 1. Taxonomy, fermentation and biological activity. J. Antibiotics 36: 463~467, 1983
12) TAKAHASHI, K. & F. TOMITA: DC-52, a novel antitumor antibiotic. 2. Isolation, physico-chemical characteristics and structure determination. J. Antibiotics 36: 468~470, 1983
13) ITOH, J.; S. OMOTO, S. INOUYE, Y. KODAMA, T. HISAMATSU, T. NIIDA & Y. OGAWA: New semisynthetic antitumor antibiotics, SF-1739 HP and naphthocyanidine. J. Antibiotics 35: 642~644, 1982
14) ARAI, T.; K. TAKAHASHI, K. ISHIGURO & K. YAZAWA: Increased production of saframycin A and isolation of saframycin S. J. Antibiotics 33: 951~960, 1980
15) ARAI, T.; K. TAKAHASHI, A. KUBO, S. NAKAHARA, S. SATO, K. AIBA & C. TAMURA: The structures of novel antibiotics, saframycin B and C. Tetrahedron Lett. 1979: 2355~2358, 1979
16) KLUEPFEL, D.; H. A. BAKER, G. PIATTONI, S. N. SEHGAL, A. SIDOROWICZ, K. SINGH & C. VÉZINA: Naphthyridinomycin, a new broad-spectrum antibiotic. J. Antibiotics 28: 497~502, 1975