MATLYSTATINS, NEW INHIBITORS OF TYPEIV COLLAGENASES FROM *Actinomadura atramentaria*

I. TAXONOMY, FERMENTATION, ISOLATION, AND PHYSICO-CHEMICAL PROPERTIES OF MATLYSTATIN-GROUP COMPOUNDS

TAKESHI OGITA, AKIRA SATO, RYUZO ENOKITA†, KEIKO SUZUKI, MIYUKI ISHII, TERUAKI NEGISHI, TAKAO OKAZAKI†, KAZUHIKO TAMAKI†† and KAZUHIKO TANZAWA

Fermentation Research Laboratories,
††Bioscience Research Laboratories, Sankyo Co., Ltd.,
1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140, Japan
†Tsukuba Research Laboratories, Sankyo Co., Ltd.,
Miyukigaoka 33, Tsukuba-shi, Ibaraki 305, Japan

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During the course of a screening for inhibitors of typeIV collagenases, new metabolites, designated matlystatins, have been isolated from an actinomycete strain, which was identified as a strain of *Actinomadura atramentaria*. Matlystatins were composed of five congeners, which were separated and purified by *n*-butanol extraction and chromatography.

Matrix metalloproteinases are enzymes of current interest because of their role in pathogenesis such as angiogenesis, rheumatoid arthritis, and tumor invasion.[1~3] Among them, typeIV collagenases are responsible for the degradation of typeIV collagen, the major component of basement membranes. An accumulating body of evidence indicates that the degradation of basement membranes depends on a net increase in typeIV collagenase activity, which is determined by the balance between the amounts of activated enzymes and TIMPs.[4] Therefore, specific inhibitors of these enzymes will be of potential clinical value in the treatment of diseases that are accompanied by abnormal lysis of basement membranes. We have screened for low molecular inhibitors in fermentation extracts of microorganisms, and found a group of compounds, designated matlystatins (MTSs). In this paper, the taxonomy of the producing organism, fermentation, isolation, and physico-chemical properties of matlystatin-group of compounds are reported.

Experimental

General Procedures

Melting points were determined by a Yanagimoto micro apparatus and were uncorrected. The ^1H NMR and ^{13}C NMR spectra were measured in CD_3OD or $CDCl_3$ by a Jeol JNM GSX-400 or GX-500 spectrometer. Mass spectra were taken by an Jeol-HX 100 mass spectrometer and IR spectra were recorded by a JASCO FTIR 8300 spectrometer.

Discovery Screening

Culture filtrates of various microorganisms were extracted with *n*-BuOH at a neutral pH. The resulting organic phase was evaporated to dryness. The residual material was suspended into distilled water containing 1 mM diisopropyl fluorophosphate, 1 mM phenylmethyl sulfonyl fluoride, and 10 mM *N*-ethylmaleimide to inactivate the proteinases derived from the microbial culture. This solution was added to a typeIV collagenase assay.[5]

Taxonomy of the Producing Organism

Actinomycete Strain

Strain SANK 61488 was isolated from a soil sample collected at Derby, West Australia, in 1985. One drop of a water suspension of the soil sample was plated on the surface of humic acid agar medium, consisting of $Na_2HPO_4 \cdot 12H_2O$ 0.02%, KCl 0.01%, $FeSO_4 \cdot 7H_2O$ 0.01%, $CaCO_3$ 0.5%, humic acid 0.2%, and agar 2.0%. The pH of the medium was adjusted to 7.4 before sterilization. The plate was incubated at 28°C for 10 days. The strain SANK 61488 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of International Streptomyces Project (ISP) medium 1 and grown for 3 days at 28°C on a rotary shaker. After harvesting by centrifugation, the culture was washed twice with sterile distilled water by centrifugation and then was used as inoculum for various studies. In addition to the ISP media, described by SHIRLING and GOTTLIEB,[6] several agar media recommended by WAKSMAN[7] were also used in this study.

Morphological Characterization

The spore chain morphology and the hyphae of the strain grown on various agar media at 28°C for 14~21 days were determined using a light microscope. A sample, treated with a critical point dryer (HCP-1, Hitachi Co., Ltd.) after stepwise dehydration by ethanol, was observed under a S-510 scanning electron microscope (Hitachi Co., Ltd.).

Chemotaxonomy

Purified cell wall and whole-cell hydrolysates were analyzed by the methods of HASEGAWA *et al.*[8] and LECHEVALIER and LECHEVALIER.[9] Phospholipid, menaquinones, and mycolic acid components were investigated by the methods of LECHEVALIER and LECHEVALIER,[10] COLLINS *et al.*[11], and HECHT and CAUSEY,[12] respectively.

Cultural Characterization

Observation of the growth on various agar media was made after incubation at 28°C for 14 days unless otherwise mentioned. The mass colors of the growth were assigned in common terminology. Exact colors were determined by comparing the mycelial color with color chips from the "Guide to Color Standard" (Nippon Shikisai Kenkyusho, Tokyo, Japan).

Physiological Tests

Physiological tests were carried out with each medium as follows: ISP media 1, 6, and 7 for melanin formation; ISP medium 4 for starch hydrolysis; gelatin stab for gelatin liquefaction; dehydrated skim milk (Difco) for milk coagulation and peptonization; and nitrate broth (Difco) for nitrate reduction. The media used for determination of casein, tyrosine, and xanthine decomposition were respectively prepared by dissolving 10 g of dehydrated skim milk in 100 ml of distilled water, and by suspending 0.5 g of tyrosine or 0.4 g of xanthine in 100 ml of nutrient agar. The cultures on all of the media tested were incubated at 28°C for 14 days, except on milk (37°C, 10 days) and gelatin (26°C, 21 days) media. Carbohydrate utilization was studied by the procedure described by SHIRLING and GOTTLIEB. The effect of temperature on growth was determined on ISP medium 2 using a temperature gradient incubator TN-3F (Advantec Toyo Co., Ltd.). The tolerance against sodium chloride of the culture was investigated by streaking the inoculum onto the same medium as used for the temperature study, except that it contained sodium chloride at 1.0, 2.0, 3.0, 5.0, 7.0, 10.0, or 14.0%, and incubating at 28°C for 21 days. Resistance to lysozyme (Sigma Chemical Co., U.S.A.) was investigated by using the procedure of GORDON *et al.*[13] Susceptibility to antibiotics was determined by paper disc method using Showa Disc (Showa Yakuhin Kako Co., Ltd, Tokyo, Japan).

Fermentation

Fermentation for production of MTSs A, D, E, and F was achieved as follows. One loopful of a culture from an agar slant of *Actinomadura atramentaria* SANK 61488 was inoculated into a baffled 500-ml Erlenmeyer flask containing 100 ml of seed medium composed of glucose 1.0%, glycerol 1.0%, sucrose 1.0%, pressed yeast 1.0%, yeast extract 0.3%, soy bean meal 2%, oat meal 0.5%, casamino acid 0.5%, $CaCO_3$ 0.1%, and Nissan Disfoam CB442 0.02%. The flask was shaken on a rotary shaker for

6 days at 28°C. Twelve ml of the culture was inoculated into a 2-liter Erlenmeyer flask containing 600 ml of the seed medium. After 3 days, 600 ml of the culture was transferred into a 60-liter tank fermenter containing 30 liters of the seed medium. The seed fermentation was continued for 4 days. Six liters of the seed culture was then inoculated into 600-liter tank fermenters containing 300 liters of MBG3-7m medium consisting of glucose 3.0%, glycerol 7.0%, Polypepton 1.0%, soy bean meal 1.0%, corn steep liquor 1.0%, $MgSO_4$ 0.5%, $NaNO_3$ 0.5%, and Nissan Disform CB442 0.01%. Fermentation was carried out for 7 days at 28°C with an air-flow rate of 1.0 v/v/m. Dissolved oxygen of the medium was maintained around 5.0 ppm by controlling the agitation speed of the fermenter.

Production of MTS B was carried out in MP medium as follows. 300 ml of the seed culture fermented in essentially the same manner as described above was inoculated into 30-liter jar fermenters containing 15 liters of MP medium composed of glucose 0.5%, maltose 1.5%, Pharmamedia 1.0%, NaCl 0.3%, yeast extract 0.3%, and K_2HPO_4 0.2%. The fermentation was continued for 10 days at 28°C with an air-flow rate of 1 v/v/m. Dissolved oxygen of the medium was maintained around 5.0 ppm by controlling the agitation speed of the fermenter.

Isolation

The culture broth fermented in MBG3-7m medium was used for isolation of MTSs A, D, E, and F. A 620-liter culture was filtered by the aid of Celite to obtain 600 liters of culture filtrate. The filtrate was applied onto a column of Diaion HP-20 (200 liters, Mitsubishi Chemical Industries Ltd., Japan). After the column was washed with 500 liters of water and 600 liters of 20% aqueous acetone, the active principle was eluted with 600 liters of 50% aqueous acetone. Evaporation of the solvent yielded 540 g of crude material. This was dissolved in 30 liters of water and extracted twice with equal volumes of *n*-butanol. The organic layer was concentrated and lyophilized to afford 158 g of a dark green powder. This was then dissolved in 300 ml of 80% methanol in dilute trifluoroacetic acid, pH 3.5, and the insoluble material was filtered off. The resulting solution was applied in two portions onto a column of Sephadex LH-20 (5 liters) equilibrated and eluted with the same solvent. After the column was eluted with 1.8 liters of the solvent, the eluate was fractionated into 300-ml portions. Each fraction was assayed for enzyme inhibitory activity and the active effluent was combined, evaporated, and lyophilized to give 73 g of a brownish powder. About 25 g each of the crude powder was dissolved in 500 ml of 30% acetonitrile in 2% (v/v) triethylamine phosphate buffer, pH 3.0 (which will be abbreviated as Buffer A throughout the manuscript), and charged onto a preparative HPLC column (YMCPAK ODS 100 × 500 mm) equilibrated with the same solvent. The column was eluted by isocratic mode at a flow rate of 200 ml/minute, and absorption at 230 nm was detected. The effluent was fractionated with the guidance of UV peak, and each fraction was assayed for enzyme inhibitory activity. Peaks that eluted at around 24 minutes, 27 minutes, and 60 minutes were found to contain active principle. Each effluent containing active material was desalted by the following treatment: 1) Concentrated to remove acetonitrile, 2) charged onto a small column of HP-20, and 3) after the column was washed thoroughly with water, active material was eluted with 50% acetone. Each fraction was still a mixture and further purified as follows. MTSs A, D and F, and E were isolated from the 3rd, 2nd, and 1st fractions, respectively.

MTS A

The crude material (2.0 g) obtained from the 3rd fraction was further purified by a preparative ODS column (YMCPAK ODS 100 × 500 mm) eluted with 60% methanol in Buffer A at a flow rate of 200 ml/minute. UV 220 nm was monitored throughout the chromatogram and the peak that eluted at 66 minutes was found to contain active component. The effluent was desalted by the method described above and concentrated and lyophilized to give 550 mg of MTS A.

MTSs D and F

The crude material obtained from the 2nd peak (3.2 g) was loaded in 500-mg portions onto a preparative HPLC column (Senshupak, ODS-5301, 20 × 300 mm) equilibrated with 55% methanol in Buffer A, and was eluted with the same solvent at a flow rate of 6 ml/minute. The chromatogram was monitored by a refractive index detector and the fractions eluted at about 40 minutes and 44 minutes were collected. The former fraction contains MTS D and the latter MTS F. These samples were still mixtures

of the compounds and they were further purified by HPLC (Senshupak, ODS-5301) eluted with 25% acetonitrile in Buffer A at a flow rate of 6 ml/minute, in which MTS D eluted at 36 minutes and MTS F eluted at 38 minutes. Solutions containing MTSs D and MTS E were desalted by HP-20 followed by concentration and lyophilization, to give 24 mg of MTS D and 12 mg of MTS F, respectively.

MTS E

The crude powder from the 1st fraction was chromatographed on an ODS column (Senshupak, ODS-5301), which was eluted with 20% acetonitrile in Buffer A at a flow rate of 6 ml/minute. The chromatogram was monitored by a refractive index detector, and the peak eluted at 47 minutes was collected. The solution was desalted by the method described above and the resulting aqueous acetone solution was evaporated and lyophilized to give 8 mg of powder. The powder was further purified with another ODS column (Senshupak, ODS-5301), which was eluted with 50% methanol in Buffer A at a flow rate of 6 ml/minute. The fraction eluted at 24 minutes was collected, desalted, evaporated, and lyophilized to give 6 mg of MTS E.

MTS B

MTS B was obtained from the culture in MP medium. The culture broth (30 liters) was treated by the same sequence as above, including the treatment of HP-20, *n*-BuOH extraction, and Sephadex LH-20 to get 4.4 g of a black powder. This was loaded in about 1-g portions onto a Lobar column (E. Merck, RP-8, Size C) equilibrated with 30% methanol. The column was eluted with 2-column volume of each of 50%, 60%, and 70% methanol. Enzyme inhibitory activity was found in the 70% methanol fraction, which was evaporated and lyophilized to afford 1.1 g of a brownish powder. The powder was applied onto a column of Sephadex G-25, which was equilibrated with 50% methanol. The column was eluted with the same solvent and eluate was fractionated. Each fraction was assayed for enzyme inhibitory activity and the active fraction was combined, evaporated, and lyophilized to get 360 mg of powder. Finally, the powder was dissolved in 38% acetonitrile in Buffer A and loaded onto an HPLC column (Senshupak, ODS-4251N, 10 × 250 mm) and eluted with the same solvent at a flow rate of 5 ml/minute. The active eluate, which gave a peak at 23 minutes, was collected, desalted with HP-20, evaporated, and lyophilized, to give 25 mg of MTS B.

Results and Discussion

Producing Strain

Morphological Characterization

Strain SANK 61488 grew relatively well on various media. The aerial mycelium was well developed on oatmeal agar and BENNETT's agar, and might occasionally be developed on most of agar media employed except for peptone-yeast extract-iron agar (ISP 6) medium. The substrate mycelia were 0.4~0.6 μm in diameter and the aerial mycelia were 0.6~0.9 mm. Short spore chains of two to five spores were produced longitudinally on

Fig. 1. Scanning electron micrograph of strain SANK 61488 on potato extract-carrot extract agar at 28°C for 7 days.

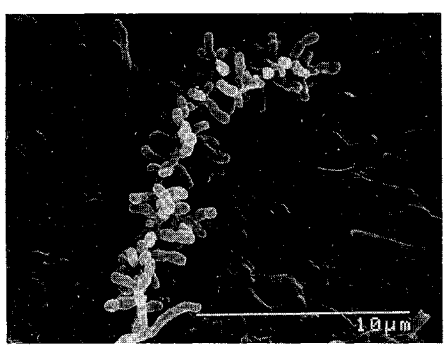


Table 1. Chemotaxonomic characteristics of strain SANK 61488 and *Actinomadura atramentaria* JCM 6250.

	SANK 61488	JCM 6250
Cell wall type	III	III
Whole-cell sugar pattern	B	B
Mycolic acid	None	None
Predominant menaquinone	MK-9 (H_6)	MK-9 (H_6)

Table 2. Cultural characteristics of strain SANK 61488 and *Actinomadura atramentaria* JCM 6250.

Medium		SANK 61488	*A. atramentaria* JCM 6250
Yeast extract - malt extract agar (ISP 2)	G:	Abundant, flat to raised, yellowish brown	Abundant, flat to raised, dark olive
	AM:	Poor, powdery, white	Poor, velvety, grayish white
	R:	Dark yellowish brown	Dark olive gray
	SP:	Yellowish brown.	Dark brown
Oatmeal agar (ISP 3)	G:	Abundant, flat, grayish white	Good, flat, grayish white
	AM:	Good, powdery, white	Poor, powdery, white
	R:	Pale yellowish orange	Yellowish gray
	SP:	None	None
Inorganic salts - starch agar (ISP 4)	G:	Good, flat to raised, grayish white	Good, flat, grayish white
	AM:	Moderate, powdery, white	Poor, powdery, white
	R:	Pale yellowish orange	Yellowish gray
	SP:	None	None
Glycerol - asparagine agar (ISP 5)	G:	Abundant, flat to raised, olive gray	Abundant, flat to raised, olive
	AM:	Moderate, cottony, white	Moderate, velvety, white
	R:	Brownish gray	Olive black
	SP:	Dark yellowish brown	Grayish olive
Peptone - yeast extract - iron agar (ISP 6)	G:	Good, raised, pale olive	Good, raised, wrinkled, yellowish gray
	AM:	None	None
	R:	Grayish yellow brown	Pale yellowish brown
	SP:	Pale yellowish brown	None
Tyrosine agar (ISP 7)	G:	Abundant, flat to raised, olive	Abundant, flat to raised, brownish gray
	AM:	Moderate, cottony, white	Poor, velvety, white
	R:	Brownish gray	Dark brownish gray
	SP:	Dark brown	Dark yellowish brown
Sucrose - nitrate agar	G:	Good, flat, grayish white	Good, flat, grayish white
	AM:	Moderate, powdery, white	Poor, powdery, white
	R:	Yellowish gray	Grayish white
	SP:	None	None
Glucose - asparagine agar	G:	Good, flat to raised, grayish olive	Abundant, flat to raised, light olive gray
	AM:	Poor, powdery, white	Poor, powdery, white
	R:	Grayish olive	Grayish olive
	SP:	None	None
Nutrient agar (Difco)	G:	Good, flat, yellowish gray	Good, flat to raised, grayish white
	AM:	Poor, powdery, white	Poor, powdery, white
	R:	Yellowish gray	Yellowish gray
	SP:	None	None
Potato extract - carrot extract agar	G:	Good, flat, grayish white	Good, flat, grayish white
	AM:	Poor, powdery, white	Poor, cottony, white
	R:	Yellowish gray	Yellowish gray
	SP:	None	None
Water agar	G:	Moderate, flat, grayish white	Moderate, flat, grayish white
	AM:	Poor, powdery, white	Poor, powdery, white
	R:	Yellowish gray	Yellowish gray
	SP:	None	None
BENNETT's agar	G:	Abundant, raised, wrinkled, dark olive gray	Abundant, flat to raised, dark olive gray
	AM:	Good, cottony, white	Good, powdery, grayish white
	R:	Olive black	Dark brownish gray
	SP:	Yellowish brown	Yellowish brown

G: Growth, AM: aerial mycelium, R: reverse, SP: soluble pigment.

the densely aggregated aerial mycelium only. The spores were oval to ellipsoidal with a smooth surface, and were $0.4 \sim 0.8 \times 0.6 \sim 1.7\,\mu m$ in size as revealed by scanning electron microscopy (Fig. 1). Protrusions at connections between individual longitudinal spores were observed. Development of whirls, sporangia, sclerotia, or other special structures was not observed.

Chemotaxonomy

As shown in Table 1, *meso*-diaminopimelic acid, glucose, galactose, mannose, ribose, and a small amount of madurose were found to be present in the cell walls, which were thus of type III, and the whole-cell sugar pattern was B. Mycolic acid was not detected. Major menaquinone was MK-9 (H_6).

Cultural Characteristics

The substrate mycelium colors of strain SANK 61488 were grayish white to olive. Aerial mycelia were well produced on oatmeal agar and BENNETT's agar, but is often poor on other media tested. A distinctive dark brown soluble pigment was produced in tyrosine agar (ISP 7) and it changed to dark violet with the addition of 0.05 N NaOH. Table 2 shows the results obtained after cultivation for 14 days at 28°C on various culture media.

Physiological Properties

The physiological properties of strain SANK 61488 are shown in Table 3. Starch hydrolysis, gelatin liquefaction, milk coagulation, and peptonization could not be demonstrated, however nitrate reduction and melanin formation were detected. Growth in 2% NaCl was observed but not in 3%. The temperature range for growth was 18~41°C. The strain was resistant to penicillin, polymyxin B, fusidic acid and nalidixic acid.

Table 3. Physiological properties of strain SANK 61488 and *Actinomadura atramentaria* JCM 6250.

	SANK 61488	JCM 6250		SANK 61488	JCM 6250
Starch hydrolysis	−	−	Susceptibility to antibiotics:		
Gelatin liquefaction	−	−	Chloramphenicol	S	S
Nitrate reduction	+	+	Penicillin	R	R
Milk coagulation	−	−	Streptomycin	S	S
Milk peptonization	−	−	Tetracycline	S	S
Melanin formation:			Erythromycin	S	S
ISP 1	+	+	Polymyxin B	R	R
ISP 6	+	+	Novobiocin	S	S
ISP 7	+	+	Fusidic acid	R	R
Decomposition:			Nalidixic acid	R	R
Casein	+	+	Carbohydrate utilization:		
Tyrosine	−	−	D-Glucose	+	+
Xanthine	−	−	L-Arabinose	−	−
Sodium chloride torelance	2%	2%	D-Xylose	−	−
Growth temperature	18~41°C	19~41°C	Inositol	−	−
(Optimum temperature)	25~34°C	24~34°C	D-Mannitol	−	−
Resistance to lysozyme	S	S	D-Fructose	−	−
			L-Rhamnose	−	−
			Sucrose	−	−
			Raffinose	−	−

+; Positive, −; negative, R; resistant, S; sensitive.

Identification and Classification

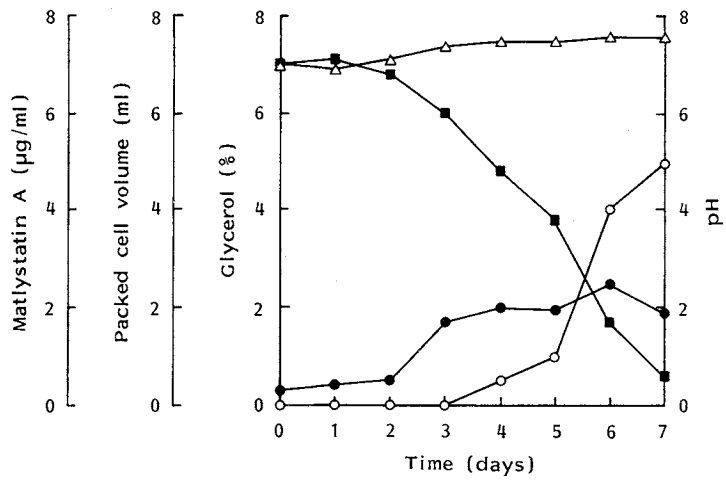
A comparison of the description of strain SANK 61488 was made with that of actinomycetes listed in the Approved Lists of Bacterial Names,[14] BERGEY's Manual of Systematic Bacteriology,[15] and recent taxonomic literature, and it was indicated that this organism belongs to genus *Actinomadura* based on morphological and chemotaxonomical characteristics. Among known species, *A. atramentaria*[16] was selected as the most closely related species from its sporulation manner, cultural characteristics and physiological properties. A direct comparison of strain SANK and the type strain of *A. atramentaria* JCM 6250 showed that they were very closely related to each other. Strain SANK 61488 differed from the type strain in some cultural characteristics, such as reverse side of colony and color of soluble pigment as follows. The reverse side of colony of strain SANK 61488 varied from yellowish gray, pale yellowish orange, dark yellowish brown to olive black, whereas *A. atramentaria* did from grayish white, yellowish gray, dark brownish gray to olive black in ISP media 2, 3, 4, 5, 6, 7, sucrose-nitrate agar, and BENNETT's agar. Strain SANK 61488 produced pale yellowish brown to dark brown soluble pigment, while *A. atramentaria* did grayish olive, dark yellowish brown and dark brown one. However, those differences noted above are not enough to conclude these two strains as different species, and the strain SANK 61488 is identified as *Actinomadura atramentaria*. The strain of *A. atramentaria* SANK 61488 has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Ibaraki Prefecture, Japan, with the accession number of FERM BP-3327.

Fermentation

In our early studies on the fermentation of matlystatins, MP medium (for composition, see Experimental section) were used for the fermentation medium, and MTSs A and B were produced in relatively low amounts (less than 2 μg/ml for both of the compounds). In order to increase the production of MTS A, much effort was expended screening different production media and doing monocolony selections of the producing strain. As a result, we found MBG3-7m medium, in which MTS A was produced at more than 10 μg/ml. Using this medium, MTSs D, E, and F were isolated, but MTS B was

Fig. 2. Fermentation profile of *Actinomadura atramentaria* SANK 61488.

○ Matlystatin A (μg/ml), ● packed cell volume (ml), ■ glycerol (%), △ pH.



Productivity of matlystatin A and glycerol concentration were calibrated using HPLC.

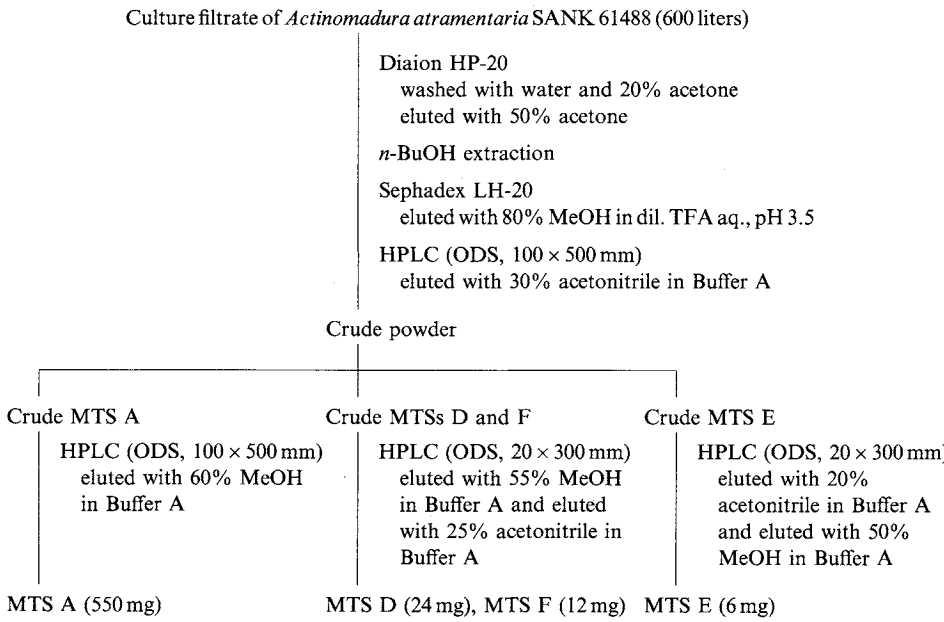
not produced.

A representative time course of the fermentation in a 600-liter tank fermenter is shown in Fig. 2. The production of the compounds reached a maximum at 7 days after consumption of most of the glycerol in the medium. The maximum titer of MTS A was around 5 μg/ml in the fermentation in 600-liter tank, however it exceeded 10 μg/ml in fermentation in a 30-liter jar fermenter (data not shown).

Isolation

As mentioned above, MTSs A, D, E, and F were produced in MBG3-7m medium, and MTS B was

Fig. 3. Isolation procedure of matlystatins (MTSs).

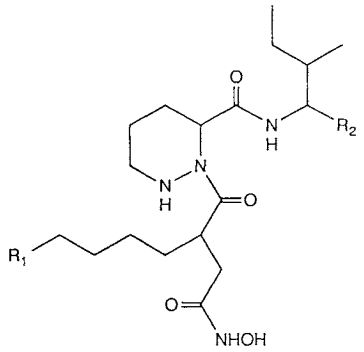


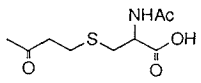
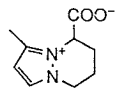
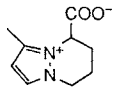
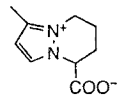
Matlystatin B was isolated by an alternative method described in experimental section.

Table 4. Physico-chemical properties of matlystatins.

	A	B	D	E	F
Nature	White hygroscopic powder	White hygroscopic powder	White hygroscopic powder	White hygroscopic powder	White hygroscopic powder
MP	113~115°C	69~72°C	122~126°C	89~94°C	69~75°C
Molecular formula	$C_{27}H_{47}N_5O_8S$	$C_{22}H_{40}N_4O_5$	$C_{27}H_{44}N_6O_6$	$C_{26}H_{42}N_6O_6$	$C_{27}H_{44}N_6O_6$
MW	601	440	548	534	548
HRFAB-MS $(M+H)^+$					
Found:	602.3198	441.3082	549.3385	535.3229	549.3388
Calcd:	602.3224	441.3078	549.3400	535.3243	549.3400
$[\alpha]_D^{20}$	−38.9° (*c* 1.06, MeOH)	−30.7° (*c* 1.0, EtOH)	−20.26° (*c* 1.14, MeOH)	−21.95° (*c* 0.41, MeOH)	+1.43° (*c* 0.14, MeOH)
UV λ_{max} (ε) in MeOH	End	End	228~230 nm (sh, 7,690)	228~230 nm (sh, 8,770)	228~230 nm (sh, 7,030)
IR ν_{max} (KBr) cm^{-1}	3300 (br), 1710, 1638	3303, 1713, 1667, 1626	3260, 1637, 1545	3260, 1638, 1544	3250, 1638, 1544

Fig. 4. Structures of matlystatins.



	R_1	R_2
A	CH_3	
B	CH_3	$COCH_2CH_3$
D	CH_3	
E	H	
F	CH_3	

obtained by the fermentation in MP medium. The isolation procedures for these compounds, however, were basically the same as described in Experimental section. Isolation scheme for MTSs A, D, E, and F is shown in Fig. 3. From 620 liters of culture broth from two 600-liter tank fermenters, 530 mg of MTS A, 25 mg of MTS B, 24 mg of MTS D, 6 mg of MTS E, and 12 mg of MTS F were obtained. Details of the isolation procedure are described in the Experimental section.

Physico-chemical Properties

Physico-chemical properties of matlystatins are summarized in Table 4. They are all very hygroscopic, white powders and the molecular formulae were confirmed by HRFAB-MS. IR absorption due to amide carbonyl, as well as ^1H and ^{13}C NMR data suggest that they are peptide-related compounds. However, amino acid analysis of acid hydrolysate (6 N HCl, 105°C, 16 hours) of MTS A did not give a known amino acid. In an ether extract of the hydrolysate, 2-pentyl succinic acid was identified. The structures of matlystatins were elucidated mainly by the spectroscopic method as depicted in Fig. 4. Details of the structure determination are described in a separate paper.[17]

Addendum in Proof

Matlystatin B was found to be identical with a compound designated as SF 2197 which was recently isolated as an antibiotic especially active against anaerobic bacteria.[18]

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