JIETACINS A AND B, NEW NEMATOCIDAL ANTIBIOTICS FROM A *STREPTOMYCES* SP.

TAXONOMY, ISOLATION, AND PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES

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Jietacins A and B, new azoxy antibiotics, were isolated from the culture broth of a streptomycete. The antibiotics have the molecular formulae of $C_{18}H_{34}N_2O_2$ and $C_{19}H_{36}N_2O_2$, respectively. Both possess an azoxy group. They have potent activity against the pine wood nematode, *Bursaphelenchus lignicolus*, and are weakly active against some fungi.

In the course of our screening program for new nematocidal antibiotics from soil microorganisms, new antibiotics named jietacins A and B were isolated from the culture broth of an actinomycete strain KP-197 which was isolated from a soil sample collected at Jietai Temple in Beijing, Peoples Republic of China.

The present paper deals with taxonomy of the producing strain, fermentation, isolation, and physico-chemical and biological properties of jietacins A and B.

Taxonomy of the Producing Strain

Morphology

The aerial mycelia of strain KP-197 were poorly formed on inorganic salts - starch agar, tyrosine agar and glycerol - calcium malate agar, but were not formed on other media used (Table 1).

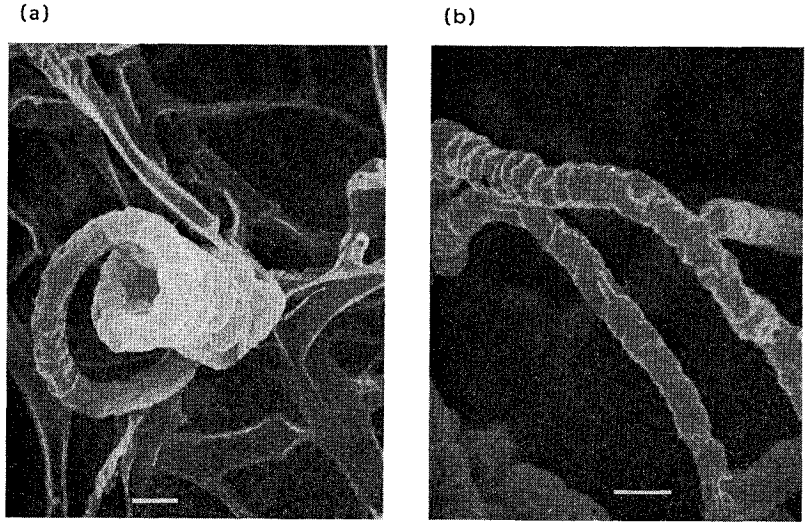
Morphological observation was made with a scanning electron microscope on colonies grown on tyrosine agar and glycerol - calcium malate agar at 27°C for 2 weeks.

The spore chains are of the *Spirales* and *Rectiflexibiles* type (Plates 1a and 1b) and have more than ten spores per chain. The spores are cylindrical in shape, $0.7 \sim 1.0 \times 0.7$ μm in size and have a smooth surface (Plate 1). Sporangia, sclerotic granules, zoospores and fragmentation of vegetative mycelia were not observed.

Chemical Compositions

The chemical analyses of sugar in whole cells and of diaminopimelic acid (DAP) in cell wall were carried out by the methods of LECHEVALIER and LECHEVALIER[1]. Strain KP-197 showed the presence of LL-DAP in the cell wall and no characteristic sugar pattern.

Plate 1. Scanning electron micrographs of aerial mycelia of *Streptomyces* sp. KP-197 on glycerol - calcium malate agar.
Bars represent 1 μm.



Cultural and Physiological Characteristics

The International Streptomyces Project (ISP) media recommended by SHIRLING and GOTTLIEB[2] and those recommended by WAKSMAN[3] were used. Cultures were observed after incubation at 27°C for 2 weeks. Color names and hue numbers indicated in Table 1 are those of the Color Harmony Manual (4th Ed.) published by Container Corporation of America. The utilization of carbon sources was tested by growth at 27°C on PRIDHAM and GOTTLIEB's medium containing a carbon source, 1%. The cultural and physiological characteristics and the utilization of carbon sources of strain KP-197 are shown in Tables 1, 2 and 3, respectively.

The vegetative mycelia of strain KP-197 grew abundantly on agar containing both synthetic and complex media and produced a yellowish brown color. The strain produced sparse white aerial mycelia on inorganic salts - starch agar and glycerol - calcium malate agar. Melanoid pigment was produced in tyrosine agar.

Strain KP-197 exhibits the following properties. Spore chain, more than ten spores per chain of the *Spirales* and *Rectiflexibiles* type; spore, cylindrical and smooth surface; color of vegetative mycelia, yellowish brown; color of aerial mycelia, white; melanoid pigment, positive; DAP in cell wall, LL-type.

Based on the taxonomic properties described above, strain KP-197 is considered to belong to the genus *Streptomyces*[4]. Strain KP-197 has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under the name *Streptomyces* sp. KP-197 with the accession No. FERM P-8889.

Fermentation and Isolation

A loopful of a well-sporulated culture of *Streptomyces* sp. KP-197 was inoculated into a 500-ml Sakaguchi flask containing 100 ml of a medium (pH 7.0) composed of glucose 0.1%, starch 2.4%, yeast extract 0.5%, peptone 0.3%, meat extract 0.3% and $CaCO_3$ 0.4%. The flask was incubated

Table 1. Cultural characteristics of strain KP-197.

Yeast extract - malt extract agar*	G:	Good, raised, light beige (3ec)
	R:	Light amber (3ic)
	AM:	None
	SP:	Luggage tan (4ne)
Oatmeal agar*	G:	Moderate, penetrant, light wheat (2ea)
	R:	Light wheat (2ea)
	AM:	None
	SP:	None
Inorganic salts - starch agar*	G:	Good, light amber (3ic)
	R:	Bamboo (2gc)
	AM:	Poor, white (a)
	SP:	Poor, pearl pink (3ca)
Glycerol - asparagine agar*	G:	Moderate, light beige (3ec)
	R:	Light beige (3ec)
	AM:	None
	SP:	Light tan (3gc)
Glucose - asparagine agar	G:	Poor, camel (3ie)
	R:	Camel (3ie)
	AM:	None
	SP:	None
Peptone - yeast extract - iron agar*	G:	Poor, beaver (3li)
	R:	Beaver (3li)
	AM:	None
	SP:	Beaver (3li)
Tyrosine agar*	G:	Moderate, camel (3li)
	R:	Camel (3li)
	AM:	Poor, white (a)
	SP:	Clove brown (3ni)
Sucrose - nitrate agar**	G:	Poor, light ivory (2ca)
	R:	Light ivory (2ca)
	AM:	None
	SP:	None
Glucose - nitrate agar**	G:	Moderate, yellow maple (3le)
	R:	Yellow maple (3le)
	AM:	None
	SP:	Yellow maple (3le)
Glycerol - calcium malate agar**	G:	Poor, bamboo (2gc) to golden brown (3pg)
	R:	Bamboo (2gc) to golden brown (3pg)
	AM:	Very poor, white (a)
	SP:	Bamboo (2gc) to golden brown (3pg)
Glucose - peptone agar**	G:	Moderate, raised and wrinkled, light beige (3ec)
	R:	Light spice brown (4g)
	AM:	None
	SP:	Maple (4e) to oak brown (4pi)
Nutrient agar**	G:	Poor, light ivory (2ca)
	R:	Light ivory (2ca)
	AM:	None
	SP:	None

* Medium recommended by ISP[2].

** Medium recommended by S. A. WAKSMAN[3].

Abbreviations: G; Growth of vegetative mycelium, R; reverse, AM; aerial mycelium, SP; soluble pigment.

Table 2. Physiclogical properties of strain KP-197.

Melanin formation	+
Tyrosinase reaction	+
H_2S production	−
Nitrate reduction	+
Liquefaction of gelatin	+
Peptonization of milk	+
Coagulation of milk	−
Cellulolytic activity	−
Hydrolysis of starch	+
Temperature range for growth	15~38°C

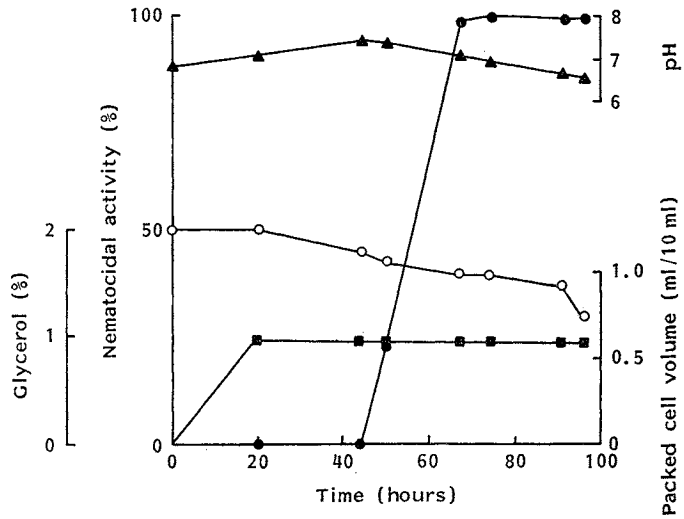
+: Active, −: not active.

Table 3. Utilization of carbon sources by strain KP-197.

D-Glucose	+
D-Fructose	+
L-Rhamnose	−
D-Mannitol	±
L-Arabinose	±
i-Inositol	−
Raffinose	−
D-Xylose	±
Sucrose	±
Melibiose	−
Salicin	−
Mannose	+

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

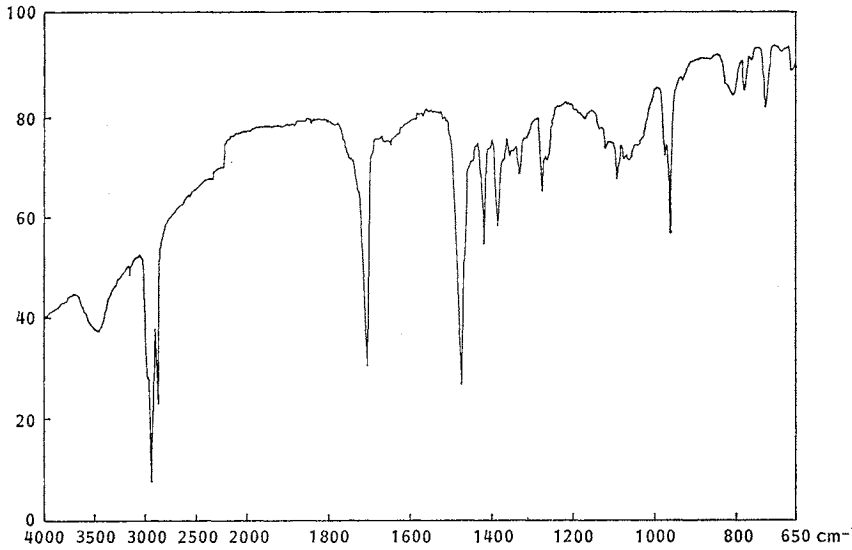
Fig. 1. A typical time course of jietacin production by *Streptomyces* sp. KP-197.
○ Glycerol, ● nematocidal activity, ■ packed cell volume, ▲ pH.



on a reciprocal shaker at 27°C for 2 days to give a seed culture. The seed culture (300 ml) was transferred into a 30-liter jar fermentor containing 15 liters of a medium (pH 7.0) composed of glycerol 2.0%, soybean meal 2.0%, NaCl 0.3%, citrulline 0.01% and $CoCl_2 \cdot 6H_2O$ 0.002%. After inoculation of the seed culture, the fermentation was carried out at 27°C for 5 days under aeration of 15 liters/minute and agitation of 250 rpm. The production of active compounds during fermentation was monitored by nematocidal activity according to the method of KIMURA *et al.*[5] using the pine wood nematode *Bursaphelenchus lignicolus* as test organism, except that the mortality was judged at 24 hours after incubation. A typical time course of fermentation in a 30-liter jar fermentor is shown in Fig. 1. Jietacins were produced after 40 hours of incubation.

The whole broth (*ca.* 30 liters) was adjusted at pH 3.0 with 6 N HCl and then centrifuged. The precipitate was extracted with aqueous acetone 50% (15 liters), and centrifuged. The supernatant fluid was concentrated *in vacuo*. The concentrate was re-extracted with an equal volume of EtOAc. The extract was evaporated *in vacuo* to give a crude oilish material. The active principle was trans-

Fig. 2. IR spectrum of jietacin A (KBr).



ferred into *n*-hexane from the oilish material. After concentration of the extract, it was chromatographed on a silica gel column with *n*-hexane - EtOAc (30 : 1). Further purification was carried out by preparative HPLC (CN column, elution using aqueous acetonitrile 60%) to give two active compounds, jietacins A (7.2 mg) and B (5.0 mg).

Physico-chemical Properties

Jietacins A and B are soluble in acetone, EtOAc, *n*-hexane and hot DMSO but insoluble in water and MeOH. IR and UV spectra of these compounds were superimposable (Figs. 2 and 3). In the field desorption mass spectra (FD-MS) of jietacins A and B, ion peaks at *m/z* 311 ($(M+H)^+$) and 325 ($(M+H)^+$) were observed respectively, while in the electron impact mass spectra (EI-MS) of them ion peaks at *m/z* 195 ($(M-OH)^+$-C_7H_{14}) and 293 ($(M-OH)^+$), and *m/z* 195 ($(M-OH)^+$-C_8H_{16}) and 307 ($(M-OH)^+$) were observed respectively. Through the high resolution EI-MS of the ion peak at *m/z* 293 (found 293.2592; calcd for $C_{18}H_{33}N_2O_2$ 293.2592) the molecular formulae of jietacins A and B were established as $C_{18}H_{34}N_2O_2$ and $C_{19}H_{36}N_2O_2$.

The UV absorption maximum at 228 nm (ε 3,650) and the characteristic band at 1475 cm^{-1} in the IR spectrum suggest the presence of an azoxy group.

Biological Properties

The nematocidal activities of jietacins A and B against *Bursaphelenchus lignicolus* were assayed as described above. As shown in Table 4, jietacins A and B exhibited 100% mortality at concentrations over 0.25 μg/ml. Avermectin B_{1a}, which is known to have a potent activity against various nematodes and which is used as a nematocidal agent in the veterinary field[6], exhibited 100% mortality at concentrations over 2.5 μg/ml. As a result, jietacins A and B have 10 times higher activities than that of avermectin B_{1a} against the organism.

The inhibitory activity of jietacin A against various bacteria and fungi was assayed by a conventional paper disc method using a ϕ 6 mm thin disk (Toyo Roshi Co., Tokyo). For bacteria, a medium (pH 7.0) containing peptone 0.5%, meat extract 0.5% and agar 0.8% was used and a diameter

Fig. 3. UV spectrum of jietacin A (cyclohexane).

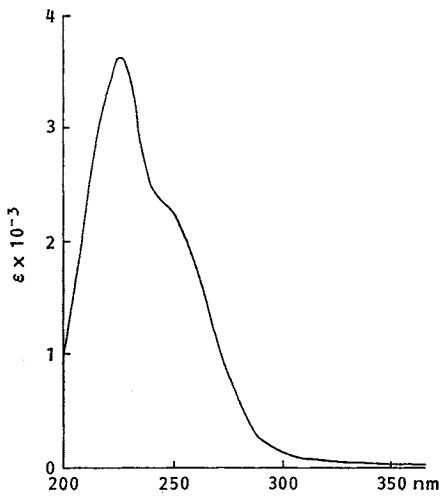


Table 4, Nematocidal activities of jietacins A and B and avermectin B_{1a} against *Bursaphelenchus lignicolus*.

The method is described in the text.

Compound	Concentration (μg/ml)	Mortality (%)
Jietacin A	0.5	100
	0.25	100
	0.125	99
	0.063	86
Jietacin B	0.5	100
	0.25	100
	0.125	93
	0.063	80
Avermectin B_{1a}	5	100
	2.5	100
	1.25	64
	0.63	58
	0.31	45

of a inhibitory zone was measured after incubation for 20 hours at 37°C. For fungi, a medium (pH 6.0) containing glucose 1.0%, yeast extract 0.5% and agar 0.8% was used and a diameter was measured after incubation for 44~48 hours at 27°C. Jietacin A was weakly active against some fungi, *e.g. Aspergillus niger* (diameter of inhibitory zone: 11 mm) and *Mucor racemosus* (7.5 mm) at the concentration of 1.0 mg/ml, but was not active against various Gram-positive and Gram-negative bacteria tested.

No acute toxicity was observed when jietacin A was administered at 10 mg/kg of body weight to *dd*Y mice by intraperitoneal injection.

Discussion

No compounds have been described which have the same physico-chemical characteristics as jietacins A and B. Therefore, the antibiotics are considered to be new ones. Some antibiotics with an azoxy group have been found; elaiomycin[7] is active against *Mycobacterium tuberculosis*, LL-BH872α[8] has antifungal activity, and valanimycin[9] is active against Gram-positive and Gram-negative bacteria and tumors. Jietacins A and B exhibit potent nematocidal activity. They are the first compounds with nematocidal activity among antibiotics with an azoxy group.

Acknowledgments

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