Pyralomicins, Novel Antibiotics from *Microtetraspora spiralis*

I. Taxonomy and Production

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(Received for publication May 10, 1996)

During our screening program for new antibiotics, we have isolated new type of antibiotics, pyralomicins 1a ~ 1d, 2a ~ 2c[1], which were produced by *Microtetraspora spiralis* MI178-34F18. In this paper, the taxonomy of the producing organism and the production of pyralomicins are described.

The producing organism, strain MI178-34F18 was formerly called *Actinomadura spiralis* MI178-34F18[1] and according to a new classification[2] it was renamed.

The strain was identified as *Microtetraspora spiralis* based on the following observations. Strain MI178-34F18 formed branched vegetative mycelia. The strain formed aerial hyphae which bore hook or spirals (1 ~ 5 turns). The mature spore chain consisted of 6 to 25 spores. The spores were elliptical (0.6 ~ 0.9 × 1.0 ~ 1.4 μm) and not motile and their surfaces were rugose (Fig. 1). No synnemata or sporangia was observed.

The cultural characteristics of strain MI178-34F18 on various agar media are shown in Table 1. The physiological characteristics are shown in Table 2.

Whole-cell hydrolysates of the strain contained *meso*-diaminopimelic acid, glucose, galactose, madurose, mannose and ribose, hence cell wall type of this strain was classified as type III[3] and whole-cell sugar pattern was type B[3]. The phospholipid type was type P IV[4]; phosphatidyl-ethanolamine and unknown glucosamine-containing phospholipids were detected but neither phosphatidylcholine nor phosphatidylglycerol was found. The major menaquinone was MK-9(H4)[5]. On the basis of these characteristics, the strain was closely related to *Microtetraspora spiralis*[6~8] and *Micro-*

Fig. 1. Scanning electron micrograph of spore chains of strain MI178-34F18.

Sucrose-nitrate agar medium. Bar represents 1.0 μm.

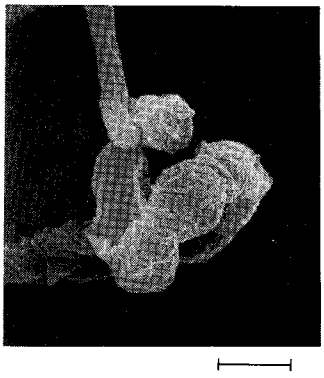


Table 1. Cultural characteristics of strain MI178-34F18.

Medium	Growth	Aerial mycelium	Soluble pigment
Sucrose - nitrate agar	Pale yellow [2gc, bamboo]	Brownish white [4ca, pearl pink]	None
Glucose - asparagine agar	Poor, Pale brown [3ic, lt amber]	None	None
Glycerol - asparagine agar (ISP-5)	Colorless-pale yellow	None	None
Inorganic salts - starch agar (ISP-4)	Pale yellowish brown-Yellowish brown [3ng, yellow maple-3pi, golden brown]	None	None
Tyrosine agar (ISP-7)	Pale yellow-Pale yellowish brown [2le, mustard]	Poor, white	None
Nutrient agar	Pale yellowish brown [2pg, mustard gold]	None	None
Yeast extract - malt extract agar (ISP-2)	Pale orange-Pale reddish brown [4gc, nude tan-4le, maple]	Brownish white	Partially, Pale purple
Oatmeal agar (ISP-3)	Pale yellowish brown [3ne, topaz]	Poor,white	Partially, Pale purple
Glycerol - nitrate agar	Colorless-Pale yellow or Dull orange [3lc, amber]	None	None
Starch agar	Pale yellowish brown-Yellowish brown [2pg, mustard gold-3ng, yellow maple]	None	None
Calcium - malate agar	Pale yellow-Pale yellowish brown [2pg, mustard gold]	White	None

Observation after incubation at 27°C for 21 days.

Table 2. Physiological characteristics of strain MI178-34F18.

Temperature range for growth (°C)	20-37	Utilization of	
Optimum temperature for growth (°C)	30	L-Arabinose	+
Formation of melanoid pigment		D-Fructose	+
ISP -1	−	D-Glucose	+
ISP -6	−	Inositol	±
ISP -7	−	Lactose	±
Liquefaction of gelatin	−	D-Mannitol	+
Coagulation of milk	+	Raffinose	+
Peptonization of milk	+	Rhamnose	+
Hydrolysis of starch	+	Sucrose	±
Nitrate reduction	+	D-Xylose	+

+: positive; ±: doubtful; ±: negative

Table 3. Comparison of taxonomic characterization of strain MI178-34F18 with *Microtetraspora spiralis* and *Microtetraspora salmonea*.

		MI178-34F18	*Microtetraspora spiralis* IMC A-0117 (IFO 14097^T)	*Microtetraspora salmonea* IMC A-0150 (JCM 3324^T)
Spore chain morphology		Hooks and Spirals (1 ~ 5 turns)	Hooks and Spirals (1 ~ 4 turns)	Hooks and Spirals (1 ~ 3 turns)
Spore surface		Rugose	Rugose	Rugose (rather deep)
Aerial mass		White~Brownish white	White	White
Color of growth		Pale yellow~Pale yellowish brown or Pale reddish brown	Pale yellow~Pale yellowish brown	Pale yellow~Pale yellowish brown or Dull reddish orange
Soluble pigment		Partially, Pale purple (ISP-2, ISP-3)	Partially, Pale purple (ISP-2)	Partially, Pale Red purple (ISP-2)
Formation of melanoid pigment		−	−	−
Liquefaction of gelatin		−	−	−
Coagulation of milk		+	+	+
Peptonization of milk		+	+	+
Reduction of nitrate		+	+	−
Hydrolysis of	starch	+	−	−
	casein	+	+	+
	hypoxanthine	−	−	−
	tyrosine	+	+	+
Utilization of [a]	L-Arabinose	+	+	(+)
	D-Fructose	+	(+)	(+)
	D-Glucose	+	+	+
	Inositol	±	(+)	(+)
	Lactose	±	(+)	(+)
	D-Mannitol	+	+	+
	Raffinose	+	(+)	(+)
	Rhamnose	+	+	(+)
	Sucrose	±	(−)	(+)
	D-Xylose	+	(+)	+

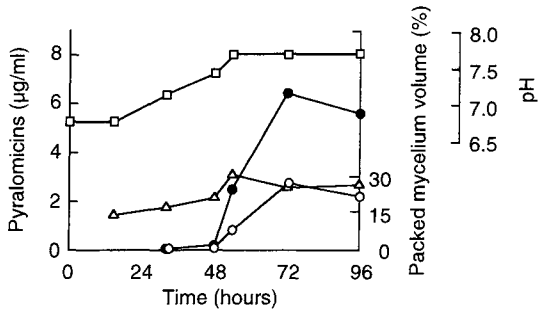
[a] +: utilized; (+): probably utilized; ±: doubtful; (−): probably not utilized; −: not utilized.

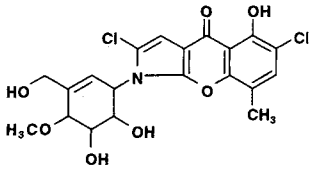
tetraspora salmonea[6,7,9] as shown in Table 3. Strain MI178-34F18 was further similar to the former species on the production of nitrate reductase and the shape of the spore surface. Therefore, strain MI178-34F18 was identified as *Microtetraspora spiralis* MI178-34F18. This strain was deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ibaragi, Japan with an accession number FERM P-10631.

The production of pyralomicins was studied by carrying out the fermentation using 500-ml Erlenmeyer flasks each containing 110 ml of the production medium on a rotary shaker. A typical time course of the fermentation is shown in Fig. 2. The pH gradually increased from the start of the fermentation, reached to pH 7.7 at 48 hours, and it was maintained until 96 hours. Packed mycelium volume was gradually accumulated from the beginning of the fermentation to 96 hours. The appearance of pyralomicins 1a and 2a was analyzed by means of HPLC. They were major components of two series of pyralomicins having cyclitol or sugar moiety as a substituent. The production of pyralomicins 1a and 2a

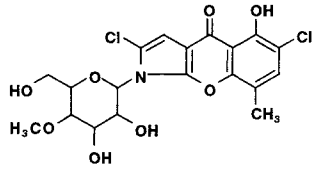
Fig. 2. Production of pyralomicins 1a and 2a.

● Pyralomicin 1a, ○ pyralomicin 2a, △ mycelium, □ pH.





Pyralomicin 1a



Pyralomicin 2a

began at 48 hours and the maximum was observed at 72 hours. The production of pyralomicins 1a and 2a proceeded in parallel.

Experimental

Microorganism

Strain MI178-34F18 was compared taxonomically with *Microtetraspora spiralis*[7,8] IMC A-0117 (IFO 14097^T) and *Microtetraspora salmonea*[7,9] IMC A-0150 (JCM 3324^T).

Taxonomic Studies

Cultural and physiological characteristics were determined by the methods of SHIRLING and GOTTLIEB[10] and by the methods of WAKSMAN[11]. Carbohydrate utilization was investigated using the procedure of PRIDHAM and GOTTLIEB[12]. The substrate and aerial mass color including soluble pigments were assigned by the Color Harmony Manual, 1958 (Container Corporation of America, Chicago). Characteristics of the spores and mycelia were observed with a scanning electron microscope (Hitachi S-570). Diaminopimelic acid and sugars in whole cell hydrolysate were analyzed according to the methods of BECKER *et al.*[13] and YOKOTA and HASEGAWA[14], respectively. Phospholipids and menaquinones were analyzed according to the method of LECHEVALIER *et al.*[4] and COLLINS *et al.*[5], respectively.

Fermentation

The producing strain was maintained on asparagine-glucose agar slant medium (asparagine 0.05%, K_2HPO_4 0.05%, glucose 1.0% and agar 2.0%). A slant culture of the strain MI178-34F18 was inoculated into a 500-ml Erlenmeyer flask containing 110 ml of medium (galactose 2.0%, dextrin 2.0%, Bacto-soytone (Difco) 1.0%, corn steep liquor (Iwaki) 0.5%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.2% and a drop of silicone oil (Shin-etsu Chemical Industry), adjusted to pH 7.4 before sterilization) and cultured at 27°C for 72 hours on a rotary shaker (180 rpm). This seed culture (2 ml) was transferred into 500-ml Erlenmeyer flasks each containing 110 ml of a producing medium (starch 3.0%, Toast soya (Nissin) 3%, corn steep liquor (Iwaki) 0.5%, yeast extract 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, NaCl 0.3%, $CoCl_2 \cdot 6H_2O$ 0.001%, $CaCO_3$ 0.3% and a small amount of silicone oil (Shin-etsu Chemical Industry), adjusted to pH 7.2 before sterilization). The fermentation was carried out at 27°C for 96 hours on a rotary shaker (180 rpm).

HPLC Analysis

The conditions for HPLC analysis were as follows; Nucleosil $5C_{18}$ (Senshu Pak, 4.6×250 mm), 55% methanol as the mobile phase at a flow rate of 0.7 ml/minute and UV detector at 350 nm. Under these conditions, pyralomicins 1a and 2a eluted at 22.7 and 17.8 minutes, respectively.

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