

ISOLATION OF L-2-(1-METHYLCYCLOPROPYL)GLYCINE FROM
MICROMONOSPORA MIYAKONENSIS SP. NOV.

I. TAXONOMIC STUDIES ON THE PRODUCING MICROORGANISM

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An actinomycete, strain PA-4046, was found to produce a novel amino acid L-2-(1-methylcyclopropyl)glycine in the fermentation broth. Based on the results of taxonomic studies, the strain was identified as a new species of *Micromonospora* and the name *Micromonospora miyakonensis* sp. nov. is proposed.

In the course of a screening program for micromonosporae which exhibit antimicrobial activity against *Escherichia coli* on a synthetic medium, a strain numbered PA-4046 was found to produce several active substances including a novel amino acid L-2-(1-methylcyclopropyl)glycine. This paper deals with the taxonomic studies on the strain PA-4046.

Materials and Methods

(1) The strain PA-4046 was isolated from a soil sample collected in Miyako Island, Okinawa Prefecture, Japan.

(2) Morphology: The morphology of the strain was studied on cultures grown on BENNETT's agar at 28°C for 21 days.

(3) Cultural characteristics: Spores of the strain were collected from the 21-day-old cultures grown on BENNETT's agar and suspended in sterile water. One drop of the suspension was inoculated on the various media according to the description of SHIRLING and GOTTLIEB¹⁾ and was incubated at 28°C. The cultural characteristics were observed after 21 days.

(4) Utilization of carbon sources was investigated by the method of PRIDHAM and GOTTLIEB²⁾ and that of LUEDEMANN and BRODSKY³⁾.

Results and Discussion

Morphological Characteristics

Vegetative mycelium develops well and is branched, but no septum is observed. Fair growth occurs on various media, but aerial mycelium is not formed. A single spore is formed at the tip of the sporophore branching from the vegetative hypha. Each spore is spherical or ellipsoidal. Under electron microscopic observation, its surface appears smooth or with only minor irregularities. Sporangium and flagellated spores are not observed. Also, fragmentation and sclerotia in vegetative mycelium are not observed.

Cultural Characteristics

The cultural characteristics of strain PA-4046 grown on various media are shown in Table 1. The growth is slower than that of *Streptomyces* in general. The vegetative mycelium is pale brown

Table 1. Cultural characteristics of *Micromonospora* sp. strain No. PA-4046.

Media	Growth	Sporulation	Color of colony	Reverse color	Soluble pigment
Sucrose nitrate agar	Fair	Fair	Brownish black	Pale brown	None
Glucose asparagine agar	Poor	No	Pale brown	Pale brown	None
Glycerol asparagine agar	Poor	No	Pale brown	Pale brown	None
Inorganic salts starch agar	Good	No	Pale brown	Pale brown	None
Tyrosine agar	Fair	Fair	Brownish black	Pale brown	None
Nutrient agar	Good	No	Pale yellowish brown	Pale yellowish brown	None
Yeast ex.-malt ex. agar	Good	Good	Black	Pale brown	None
Oatmeal agar	Fair	No	Brown	Brown	None
BENNETT'S agar	Good	Good	Black	Pale brown	None

to brown, later becoming brownish black to black with moistened to glossy surface. No soluble pigment is produced on almost all media.

Physiological properties of the strain are shown in Table 2. Hydrolysis of starch, peptonization and coagulation of milk are positive, but production of melanoid pigment, tyrosinase reaction and liquefaction of gelatin are negative. The temperature range for growth is rather narrow: good growth occurs at 28°C, but no growth at 10°C and 37°C or higher.

Table 2. Physiological properties of *Micromonospora* sp. strain No. PA-4046.

Growth temperature	10°C: No growth 28°C: Good growth and good sporulation 37°C: No growth 45°C: No growth
Production of melanoid pigment	Negative
Tyrosinase reaction	Negative
Milk coagulation	Positive
Milk peptonization	Positive
Starch hydrolysis	Positive
Gelatin liquefaction	Negative

Table 3. Utilization of carbon sources by *Micromonospora* sp. strain No. PA-4046.

Carbon source	Growth	
	A*	B*
L-Arabinose	—	+
D-Xylose	‡	‡
D-Glucose	‡	‡
D-Fructose	—	‡
Sucrose	‡	‡
Inositol	—	±
L-Rhamnose	‡	‡
Raffinose	—	±
D-Mannitol	—	±
Melibiose	—	±
None (Control)	—	±

A*: The method of PRIDHAM & GOTTLIEB.

B*: The method of LUEDEMANN & BRODSKY.

‡: Abundant growth, +: Moderate growth,

±: Poor growth, —: No growth.

Utilization of carbon sources by strain PA-4046 is shown in Table 3. According to the method of PRIDHAM and GOTTLIEB²⁾, D-xylose, D-glucose, sucrose and L-rhamnose are utilized for growth, but L-arabinose, D-fructose, inositol, raffinose, D-mannitol and melibiose are not. In contrast, according to the method of LUEDEMANN³⁾, L-arabinose and D-fructose are also utilized.

Comparison of Strain PA-4046 with Other Known *Micromonospora* Species

From the results of morphological and cultural characteristics described above, the strain PA-4046 is considered to belong to the genus *Micromonospora* ØRSKOV. According to the taxonomic criteria⁴⁻⁶⁾ of the genus *Micromonospora*, strain PA-4046 resembles *Micromonospora chalcea* (FOULERTON) ØRSKOV and *Micromonospora carbonacea* LUEDEMANN and BRODSKY⁷⁾. However, strain PA-4046 differs from

M. chalcea in liquefaction of gelatin, utilization of L-rhamnose, melibiose and growth temperature range. Strain PA-4046 differs from *M. carbonacea* in liquefaction of gelatin, utilization of L-rhamnose, melibiose and growth temperature range. One of the characteristics of strain PA-4046 is its utilization of L-rhamnose which is not utilized by most micromonosporae. *M. echinospora* is known to utilize L-rhamnose, but differs from strain PA-4046 in spore surface morphology, color of vegetative mycelium and liquefaction of gelatin.

From these results, strain PA-4046 was considered to be a new species of *Micromonospora* and the name *Micromonospora miyakonensis* is proposed. Strain PA-4046 has been deposited in Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under the accession number FERM P-4934.

References

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