A NEW SPECIES OF *ACTINOMADURA* PRODUCING A POLYETHER ANTIBIOTIC, CATIONOMYCIN

GOTO NAKAMURA and KIYOSHI ISONO*

Antibiotics Laboratory, The Institute of Physical and Chemical Research, Wako-shi, Saitama 351, Japan

(Received for publication June 7, 1983)

Taxonomic studies on the new species, *Actinomadura azurea* are presented. A significant property of this species is the production of a new polyether antibiotic, cationomycin.

Polyether antibiotics are a diverse group of ionophores, most of which are produced by the genus *Streptomyces*. Recently several new polyether antibiotics have been reported, which are produced by actinomycetes other than *Streptomyces*, *e.g.*, *Streptoverticillium*[1], *Dactylosporangium*[2], *Nocardia*[3], and *Actinomadura*[4,5]. As already reported[6,7], cationomycin is structurally unique, having an aromatic side chain (Fig. 1), and is produced by a soil actinomycete, strain 76-11 belonging to the genus *Actinomadura*. Taxonomic studies described herein have led to the conclusion that it is a new species and the name *Actinomadura azurea* sp. nov. Nakamura *et* Isono is proposed for the strain because of the characteristic blue color of the substrate mycelium. This strain was isolated from a soil sample collected in Masuda-shi, Shimane-ken, Japan. The taxonomic studies were carried out in accordance with the procedures described by SHIRLING and GOTTLIEB[8] and LECHEVALIER and LECHEVALIER[9].

Microscopic Characteristics

The substrate mycelium is 0.3~0.6 μm in width and is well developed and branched. Terminal and intercalary swellings were observed, which were globose, oval, or broadly elliptical to elongated. Submerged mycelium is well developed after 5 days in glucose - yeast medium (glucose 1%, dry yeast 1%, oatmeal 0.1%) at 28°C on a rotary shaker (Fig. 2a). However, it breaks up into coccoid form after 7 days (Fig. 2b). A thin and rudimental aerial mycelium was observed on oatmeal agar or starch agar enriched with a vitamin B mixture. Figs. 3 and 4 show scanning electron micrographs of the aerial mycelia of a culture cultivated on starch - vitamin B agar. Spore-like swellings (0.6~1.0 μm in diameter) or curls were observed on termini of sporophores (Fig. 3). In some cases a spore-like chain was also observed (Fig. 4). No selerotia, zoospores, sporangia or pseudosporangia were observed.

Fig. 1. Structure of cationomycin.

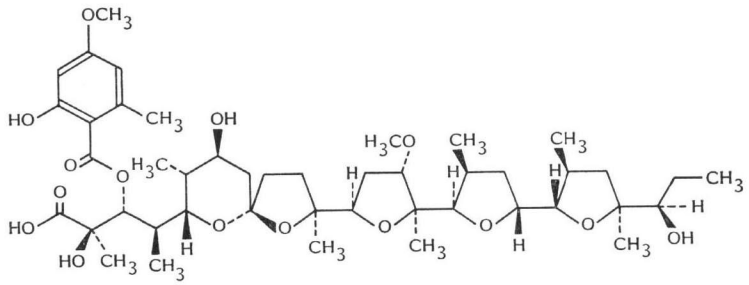


Fig. 2. Scanning electron micrograph of a submerged culture of *Actinomadura azurea* (a, 28°C 5 days; b, 28°C 7 days).

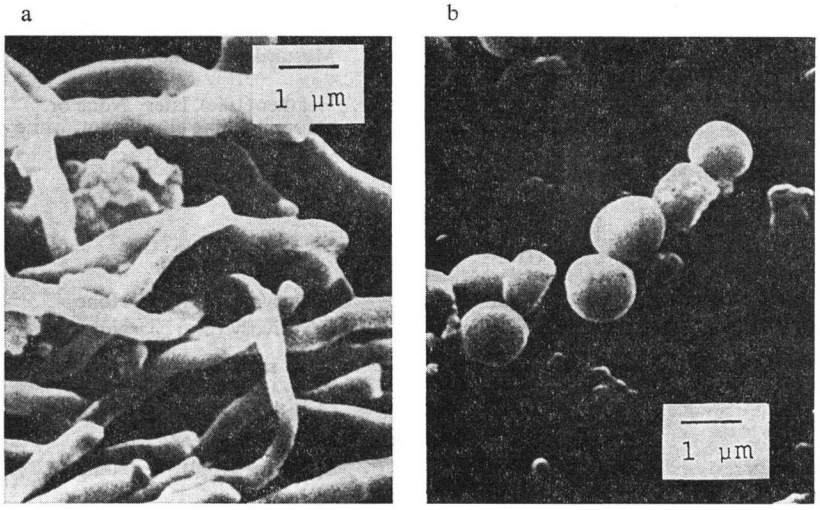


Fig. 3. Scanning electron micrograph of aerial mycelia of *Actinomadura azurea* (starch - vitamin B agar).

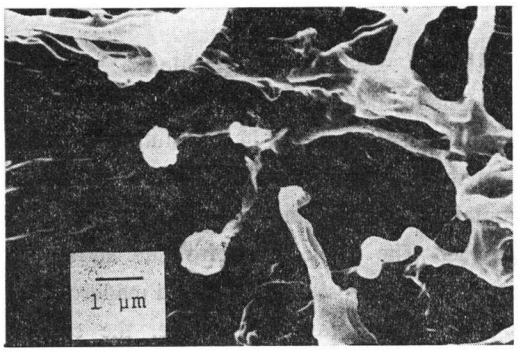
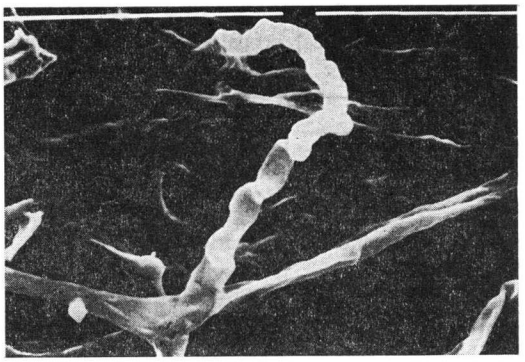


Fig. 4. Scanning electron micrograph of a spore-like chain of *Actinomadura azurea* (starch - vitamin B agar).



Cultural and Physiological Characteristics

The organism was cultivated on various media at 27°C. Cultural characteristics were observed after 7, 14 and 21 days incubation. Results are summarized in Table 1. The substrate mycelium shows a characteristic dark blue color after three weeks on oatmeal agar and glucose - yeast extract agar. No aerial mycelium was formed except on oatmeal agar.

The physiological properties were examined according to the method described by SHIRLING and GOTTLIEB[8]. The results are summarized in Table 2.

Utilization of Carbon Sources

Utilization of carbon sources was examined on PRIDHAM and GOTTLIEB's inorganic medium, supplemented with yeast extract (0.1%). Without yeast extract almost no growth was observed with any of the carbon sources. The growth was observed after 30 days incubation at 30°C. As shown in Table 3, pentoses and hexoses are generally well utilized by the organism.

Table 1. Cultural characteristics of *Actinomadura azurea*.

Medium	Growth	Reverse color*	Aerial mycelium	Soluble pigment
Yeast extract - malt extract agar (ISP No. 2)	Good, wrinkled	Mustard gold (2ne)	None	None
Oatmeal agar (ISP No. 3)	Good, smooth	Heather (10ie), later becoming dark blue (12 1/2 pg)	None or scant, white	None or faint blue
Inorganic salt - starch agar (ISP No. 4)	Poor	Camel (3ie)	None	None
Glycerol - asparagine agar (ISP No. 5)	None			
Peptone - yeast extract - iron agar (ISP No. 6)	None			
Tyrosine agar (ISP No. 7)	Poor	Light ivory (2ca)	None	None
Sucrose - nitrate agar	Poor	Light ivory (2ca)	None	None
Glucose - asparagine agar	None			
Ca-malate agar	None			
Nutrient agar	Poor	Light ivory (2ca)	None	None
Bennett agar	Good, wrinkled	Shell (3ca)	None	None
Hickey & Tresner agar	Good	Egg plant (10pl)	None	None
Glucose - yeast extract agar	Good, wrinkled	Light wheat (2ea), becoming dark blue	None	None
Potato - glucose agar	Poor	Light wheat (2ea)	None	None
Potato plug	Poor	Light wheat (2ea)	None	None

* Color number designation was taken from Color Harmony Manual, 4th edition, Container Corporation of America, 1958.

Table 2. Physiological properties of *Actinomadura azurea*.

Test	Result
Temperature requirement	Good growth at 27°C, 30°C and 37°C, poor growth at 23°C and 45°C, no growth at 15°C.
Nitrite from nitrate	Negative
Action on milk	Coagulation; faint Peptonization; faint
Gelatin liquefaction	Positive
Melanin production	None
Hydrolysis on starch	Weak
Tyrosine decomposition	Negative
Xanthine decomposition	Negative
NaCl tolerance	1~3%

Cell Wall Composition

The method described by LECHEVALIER and LECHEVALIER[9] was used in this study. The hydrolysate of the cell wall fraction of the strain contains *meso*-diaminopimelic acid but lacks glycine. The whole cell hydrolysate shows the presence of glucose, galactose, and madurose (3-*O*-methyl-D-galactose). Therefore, the culture can be considered to have a cell wall type IIIB which strongly supports that it belongs to the actinomycete genus *Actinomadura*[10].

Table 3. Utilization of carbon source by *Actinomadura azurea*.

Carbon source	Utilization*	Carbon source	Utilization*	Carbon source	Utilization*
Glycerol	++	L(−)-Sorbose	+	Dulcitol	−
L(+)-Arabinose	++	Sucrose	++	Inositol	++
D-Xylose	++	Lactose	++	D-Mannitol	++
D-Ribose	++	Cellobiose	++	D-Solibitol	−
L-Rhamnose	++	Melibiose	+	Salicin	−
D-Glucose	++	Trehalose	++	Cellulose	−
D-Galactose	++	Raffinose	++	Chitin	−
D-Fructose	++	D(+)-Melezitose	++	Keratin	++
D-Mannose	++	Soluble starch	+		

* −, no growth; +, moderate growth; ++, good growth.

Fermentation

The organism needs a relatively long period of fermentation and requires a specific medium composition for the production of cationomycin. Cultures grown on oatmeal agar for 21 days at 33°C were used to inoculate the seed medium (70 ml in 500-ml flasks). This was incubated at 28°C on a rotary shaker. After 9 to 11 days, the seed culture (140 ml) was transferred to 18 liters of the main fermentation medium in a 30-liter jar fermenter. Fermentation was carried out at 30°C for 9 days. The composition of the seed medium and the production medium is the same as is shown in Table 4. If soybean meal, wheat embryo, or meat extract was used in place of oatmeal, good growth was obtained; however, the production of cationomycin decreased. Similarly, if glucose or starch was used instead of glycerol as a carbon source, no cationomycin was produced although mycelial growth was good.

Table 4. Medium used for production of cationomycin.

Composition	Concentration (%)
Glycerol	3
Oatmeal	3
Dried yeast	0.5
KH_2PO_4	0.5
$Na_2HPO_4 \cdot 12H_2O$	0.5
$MgCl_2 \cdot 6H_2O$	0.1

Discussion

The strain 76-11 forms short rudimentary aerial mycelium. Occasionally spore-like elements are formed on oatmeal agar or starch - vitamin B agar. The color of the substrate mycelium is blue but neither melanin nor any other soluble pigment is produced. The cell wall type is IIIB. On the basis of the morphological and physiological characteristics as well as the chemical composition of the cell wall, it was concluded that the strain belonged to the genus *Actinomadura* proposed by LECHEVALIER and LECHEVALIER[10]. Five saprophytic species of *Actinomadura* were proposed by NONOMURA and OHARA in 1971[11]. An identification key for the species of *Actinomadura* which includes 21 species of the genus was described by PREOBRAZHENSKAYA *et al.* in 1977[12]. In addition, 11 species of *Actinomadura* were reported recently[13~24]. Among these species, *Actinomadura macra*[17] has some resemblance to the strain 76-11 in morphological and in some cultural characteristics. However, considerable differences were noted in characteristics on ISP 2 and 3 media. Moreover, although the utilization of carbohydrates is very limited in *Actinomadura macra*, the strain 76-11 utilizes a variety of carbon sources. *Actinomadura spadix*[11] was also different from the strain 76-11 in cultural characteristics on ISP 2 and 3 media and some physiological properties such as nitrate reduction, gelatin liquefaction and milk peptonization. In addition, none of the known species of the genus *Actinomadura* was reported to produce a blue pigment in substrate mycelium. Thus, the strain 76-11 is considered to be a new species of genus *Actinomadura* for which the name *Actinomadura azurea* sp. nov. is proposed. Strain 76-11 is the type strain of *Actinomadura azurea*; a culture of this strain was deposited in Japan Collection of Microorganisms at the Institute of Physical and Chemical Research under the number JCM 2033.

Acknowledgment

The authors wish to thank Dr. A. SEINO of Japan Collection of Microorganisms at this Institute for his valuable advice and for the electron micrographs.

References

1) KITAME, F.; K. UTSUSHIKAWA, T. KOHAMA, T. SAITO, M. KIKUCHI & N. ISHIDA: Laidlomycin, a new antimycoplasmal polyether antibiotic. J. Antibiotics 27: 884~888, 1974
2) TONE, J.; R. SHIBAKAWA, H. MAEDA, K. INOUE, S. NISHIYAMA, M. ISHIGURO, W. P. CULLEN, J. B. ROUTIEN, L. R. CHAPPEL, C. E. MOPPETT, M. T. JEFFERSON & W. D. CELMER: CP-44,161, a new species of *Dactylosporangium*. Abstract No. 171, 18th Intersci. Conf. on Antimicrob. Agents Chemother., Atlanta, 1978

3) LIU, C-M.; T. E. HERMANN, A. DOWNEY, B. LA T. PROSSER, E. SCHILDKNECHT, N. J. PALLERONI, J. W. WESTLEY & P. A. MILLER: Novel polyether antibiotics X-14868A, B, C and D produced by a *Nocardia*. Discovery, fermentation, biological as well as ionophore properties and taxonomy of the producing culture. J. Antibiotics 36: 343~350, 1983
4) TONE, J.; R. SHIBAKAWA, H. MAEDA, K. INOUE, Y. YAMAUCHI, K. TSUKUDA, M. YAMADA, W. P. CULLEN, L. R. CHAPPEL, C. E. MOPPETT, J. R. OSCARSON, C. J. LAPLANTE, L. H. HUANG & W. D. CELMER: CP-47,433 and CP-47,434, new polycyclic ether antibiotics produced by a new species of *Actinomadura*. Abstract No. 1030, 19th Intersci. Conf. Antimicrob. Agents Chemother., Boston, 1979
5) CELMER, W. D.; W. P. CULLEN, J. R. OSCARSON, L. H. HUANG, R. SHIBAKAWA & J. TONE: New polycyclic ether antibiotic. U. S. Patent 4,195,079, Mar. 25, 1980
6) NAKAMURA, G.; K. KOBAYASHI, T. SAKURAI & K. ISONO: Cationomycin, a new polyether ionophore antibiotic produced by *Actinomadura* nov. sp. J. Antibiotics 34: 1513~1514, 1981
7) SAKURAI, T.; K. KOBAYASHI, G. NAKAMURA & K. ISONO: Structure of the thallium salt of cationomycin. Acta Cryst. B38: 2471~2473, 1982
8) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Intl. J. Syst. Bact. 16: 313~340, 1966
9) LECHEVALIER, M. P. & H. A. LECHEVALIER: Chemical methods as criteria for the separation of *Nocardia* from other actinomycetes. The Biology of the Actinomycetes and Related Organisms 11: 78~92, 1976
10) LECHIVALIER, H. A. & M. P. LECHEVALIER: A critical evaluation of the genera of aerobic actinomycetes. *In* The Actinomycetales, *Ed.*, H. PROUSER, pp. 393~405, Jena, Gustav Fischer Verlag, 1970
11) NONOMURA, H. & Y. OHARA: Distribution of actinomycetes in soil. XI. Some new species of the genus *Actinomadura* Lechevalier *et al.* J. Ferment. Technol. 49: 904~912, 1971
12) PREOBRAZHENSKAYA, T. P.; M. A. SVESHNIKOVA & L. P. TEREKHOVA: Key for identification of the species of the genus *Actinomadura*. The Biology of the Actinomycetes and Related Organisms 12: 30~38, 1977
13) TAMURA, A. & A. TANAKA: Antibiotic AB-85 and its production. Japan Kokai 78-28,101, Mar. 16, 1978
14) TAMURA, A. & A. TANAKA: Antibiotic AB-97 and its production. Japan Kokai 78-101,301, Sept. 4, 1978
15) MATSUYAMA, K.; Y. TAKAHASHI, M. YAMASHITA, A. HIRANO & S. ŌMURA: 2′-Amino-2′-deoxyadenosine produced by a strain of *Actinomadura*. J. Antibiotics 32: 1367~1369, 1979
16) ŌMURA, S.; Y. IMAI, Y. TAKAHASHI & J. HIRANO: New antibiotic, hugamycin and its production. Japan Kokai 80-94,391, July 17, 1980
17) HUANG, L. H.: *Actinomadura macra* nov. sp., the producer of antibiotics CP-47433 and CP-47434. Intl. J. Syst. Bact. 33: 565~568, 1980
18) TOMITA, K.; Y. HOSHINO, T. SASAHIRA & H. KAWAGUCHI: BBM-928, a new antitumor antibiotic complex. II. Taxonomic studies on the producing organism. J. Antibiotics 33: 1098~1102, 1980
19) OGAWA, Y.; H. SUGI, N. FUJIKAWA & H. MORI: Rubeomycin, a new anthracycline antibiotic complex. J. Antibiotics 34: 938~951, 1981
20) MORI, H.; Y. OGAWA, H. SUGI, N. FUJIKAWA & K. TAMAI: New antibiotics FA-1180 A, B, C and their production. Japan Kokai 81-43,293, Apr. 21, 1981
21) OSHIMA, M.; T. KITAHARA, N. ISHIZAKI & Y. MARUMOTO: Antibiotics DF-4466 A and DF-4466 B. Japan Kokai 81-90,098, July 21, 1981
22) SUGAWARA, H.; S. MIYAZAKI & A. SEINO: New antibiotic and its production. Japan Kokai 81-113,791, Sept. 7, 1981
23) OBA, O.; K. SHOMURA, M. SEZAKI, T. NIWA, M. KOZIMA & J. ITOH: New antibiotic SF-2140 and its production. Japan Kokai 82-85,397, May 28, 1982
24) WAITZ, J. A.; A. C. HORON, M. K. KALYANPUR, B. K. LEE, D. LOEBENBERG, J. A. MARQUEX, G. MILLER & M. G. PATEL: Kijanimicin (Sch 25663), a novel antibiotic produced by *Actinomadura kijaniata* SCC 1256. Fermentation, isolation, characterization and biological properties. J. Antibiotics 34: 1101~1106, 1982