A NEW SPECIES OF ACTINOMADURA PRODUCING A POLYETHER ANTIBIOTIC, CATIONOMYCIN

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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., *Streptovericillium*1), *Dactylosporangium*2), *Nocardia*3), and *Actinomadura*4,5). As already reported6,7), 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 GOTTLIEB8) and LECHEVALIER and LECHEVALIER9).

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.](image-url)
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. 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*.

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

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

Table 2. Physiological properties of *Actinomadura azurea*.

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

Cell Wall Composition

The method described by LECHEVALIER and LECHEVALIER⁹ 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*.

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Utilization*</th>
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<th>Utilization*</th>
<th>Carbon source</th>
<th>Utilization*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>++</td>
<td>L(+)Sorbose</td>
<td>+</td>
<td>Dulcitol</td>
<td>–</td>
</tr>
<tr>
<td>L(+)-Arabinose</td>
<td>++</td>
<td>Sucrose</td>
<td>++</td>
<td>Inositol</td>
<td>++</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>++</td>
<td>Lactose</td>
<td>++</td>
<td>D-Mannitol</td>
<td>++</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>++</td>
<td>Cellobiose</td>
<td>++</td>
<td>D-Solubitol</td>
<td>–</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>++</td>
<td>Melibiose</td>
<td>+</td>
<td>Salicin</td>
<td>–</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>++</td>
<td>Trehalose</td>
<td>++</td>
<td>Cellulose</td>
<td>–</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>++</td>
<td>Raffinose</td>
<td>++</td>
<td>Chitin</td>
<td>–</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>++</td>
<td>D(+)-Melezitose</td>
<td>++</td>
<td>Keratin</td>
<td>++</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>++</td>
<td>Soluble starch</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* –, 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.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>3</td>
</tr>
<tr>
<td>Oatmeal</td>
<td>3</td>
</tr>
<tr>
<td>Dried yeast</td>
<td>0.5</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>0.5</td>
</tr>
<tr>
<td>Na2HPO4·12H2O</td>
<td>0.5</td>
</tr>
<tr>
<td>MgCl2·6H2O</td>
<td>0.1</td>
</tr>
</tbody>
</table>

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 LECHEVALIER10). Five saprophytic species of *Actinomadura* were proposed by NONOMURA and OHARA in 197111). An identification key for the species of *Actinomadura* which includes 21 species of the genus was described by PREOBRAZHENSKAYA et al. in 197712). In addition, 11 species of *Actinomadura* were reported recently12-21. 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

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References


