Amorphosporangium (Actinoplanaceae): Report of Motility and Additional Characters

By WILMA KANE HANTON
Department of Botany, University of North Carolina, Chapel Hill, North Carolina 27514, U.S.A.

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SUMMARY

Motility and flagellated sporangiospores are reported for the first time for the type culture (253) of Amorphosporangium auranticolor; microconidia on the substrate mycelia are also reported. Otherwise, the morphological description by Couch (1963) remains unchanged. Tests for utilization of 21 carbon sources, gelatin, starch, casein, tyrosine, and cellulose acetate, and for nitrate reduction have been made and the results are reported.

INTRODUCTION

The genus Amorphosporangium was described by Couch (1963) as having non-motile sporangiospores. In the present study of Amorphosporangium auranticolor (type culture 253), this member of the Actinoplanaceae has been found to have flagellated and motile spores. A more complete characterization of this culture, both physiologically and morphologically, is given.

METHODS

The stock culture of Amorphosporangium auranticolor 253, which has been in culture since 1955 on Czapek and peptone Czapek agars (both with 3%, w/v, sucrose), lost the ability to produce sporangia sometime before 1961 (Couch, 1963). Therefore a culture freeze-dried in 1958 was used.

Inoculum. Peptone Czapek agar plates were covered with a cellulose acetate strip and inoculated. After one week, the growth was scraped off, placed in 30 ml. of sterile distilled water, and blended in a Waring Blender semimicro cup until a uniform suspension of mycelial material was obtained.

Media. The carbon sources (Table 1) (all but the Na acetate and glycerol obtained from Nutritional Biochemicals Corp., Cleveland, Ohio) were prepared in a 1% (w/v) concentration in basal Czapek agar (NaNO₃, 3.0 g.; K₂HPO₄, 1.0 g.; MgSO₄·7H₂O, 0.5 g.; KCl, 0.5 g.; FeSO₄·7H₂O, 0.01 g.; Difco agar, 15.0 g.; 1000 ml. H₂O), the basal salts mixture prepared by Fisher Scientific special order no. 8548. These agars, nutrient gelatin (peptone, 5.0 g.; beef extract, 3.0 g.; gelatin, 100.0 g.; 1000 ml. H₂O), and nitrate broth (peptone, 10.0 g.; NaNO₃, 1.0 g.; 1000 ml. H₂O) in 8 ml. portions in Kimax screw-cap test tubes, autoclaved at 121° for 15 min. were sloped. Four tubes of each medium were inoculated with 0.4-0.5 ml. of blended inoculum by using a hypodermic syringe (no. 18 needle). The inoculum was spread over the surface. Pour plates of starch (soluble starch, 10.0 g.; yeast extract, 2.5 g.; tryptone, 5.0 g.; agar,
15.0 g.; 1000 ml. H₂O), casein (powdered skim milk, 40.0 g.; agar, 15.0 g.; 1000 ml. H₂O), and tyrosine (peptone, 5.0 g.; beef extract, 3.0 g.; L(-) tyrosine, 5.0 g.; agar, 15.0 g.; 1000 ml. H₂O) agars were prepared. Several drops of inoculum were placed on one side of the cooled agar and the plates tilted, allowing the inoculum to run across the centre of the plate in a line. Plates prepared as those for growing the inoculum material were used for the cellulose acetate breakdown. Three per cent sucrose Czapek agar plates were also inoculated to test the culture for sporangial production on this medium.

Table 1. *Amorphosporangium auranticolor* growth on various single carbon sources

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Relative growth</th>
<th>Carbon source</th>
<th>Relative growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>L(+) Arabinose</td>
<td>++ + +</td>
<td>d(+) Lactose</td>
<td>++ + +</td>
</tr>
<tr>
<td>d(+) Xylose</td>
<td>+</td>
<td>d(+) Maltose</td>
<td>++ + +</td>
</tr>
<tr>
<td>L(+) Rhamnose</td>
<td>++ + +</td>
<td>d(+) Celllobiose</td>
<td>++ + +</td>
</tr>
<tr>
<td>Salicin</td>
<td>++ +</td>
<td>d(+) Sucrose</td>
<td>++ + +</td>
</tr>
<tr>
<td>i-Inositol</td>
<td>++ +</td>
<td>d(+) Raffinose</td>
<td>++ + +</td>
</tr>
<tr>
<td>d(−) Glucose</td>
<td>++ + +</td>
<td>Amylopectin</td>
<td>++ + +</td>
</tr>
<tr>
<td>d(−) Mannitol</td>
<td>++ + +</td>
<td>Inulin</td>
<td>+</td>
</tr>
<tr>
<td>d(−) Levulose</td>
<td>++ + +</td>
<td>Na Succinate</td>
<td>+</td>
</tr>
<tr>
<td>d(+) Galactose</td>
<td>++ + +</td>
<td>Na Acetate</td>
<td>++ +</td>
</tr>
<tr>
<td>Dulcitol</td>
<td>++ + +</td>
<td>Glycerol</td>
<td>++ + +</td>
</tr>
<tr>
<td>d(+) Sorbitol</td>
<td>++ + +</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+++ = excellent growth; ++ = fair growth; + and + = poor growth.

All cultures were incubated at room temperature (25 to 27°) for one month before results were recorded.

Electron microscope preparations. A sporangial suspension was prepared from a 1-month-old sorbitol agar slope culture by adding 5 ml. sterile distilled water to the culture and scraping the surface to loosen and wet the sporangia. After 30 min. the spores were swimming. The preparation was mounted on 200-mesh copper grids by the agar block technique (Sharp, 1953) and shadowed with germanium. Pictures were taken on Kodak Fine Grain Positive 35 mm. film with a Phillips EM 75.

**RESULTS**

**Morphology**

Many sporangia on both the Czapek agar plates and the sorbitol agar slopes were irregular in shape (Pl. 1, fig. 1, 4) and similar to those reported by Couch (1963). An electron micrograph of an empty sporangium is shown in Pl. 1, fig. 1A, illustrating its irregular shape with four or five lobes. The sporangial wall and a portion of the upper sporangiophore had a rough-textured surface, whereas the vegetative hyphae (Pl. 1, fig. 1B) had smooth walls. Plate 1, fig. 4, illustrates a similar sporangium with a light micrograph. 'Microconidia', which Couch (1963) described on substrate mycelia of other Actinoplanaceae (*Ampullariella campanulata*, *Actinoplanes utahensis*), were observed (Pl. 1, fig. 5). Couch also illustrated these in his light microscope studies of the genus *Amorphosporangium* (unpublished data, personal communication). The branched coiled arrangement of the spores can be seen in the partially broken-open sporangial
Characters of Amorphosporangium

lobe in Pl. 1 fig. 6. Flagellated spores are present on the upper edge of the lobe where the sporangial wall is broken. Plate 1, fig. 2, shows two rod-shaped spores, the lower one displaying a polar tuft of flagella as reported for many other Actinoplanaceae (Higgins, Lechevalier & Lechevalier, 1967; Kane, 1966; Lechevalier & Holbert, 1965). The shape of the spores varied from spherical to rod shaped, and a few curved spores were seen (Pl. 1, fig. 3). The majority, however, were short rods.

Physiology

Table 1 gives the results of growth of *Amorphosporangium auranticolor* 253 on various carbon sources. Cultures with substrates which supported excellent growth (indicated by confluent surface growth which penetrated the agar approximately 1/8 of an inch) also had orange pigmentation. With glycerol, the culture also produced a brown pigment which diffused into the agar. Fair growth but no orange pigmentation was obtained with salicin, inositol, and Na acetate. There was poor growth (barely visible or visible only with a dissecting microscope) with xylose, inulin, and Na succinate. A few sporangia were produced on dulcitol, whereas the surface of the sorbitol agar was entirely covered with sporangia. These two were the only carbon sources in slopes on which sporangia were formed. Nitrate reduction and tyrosine utilization were negative. Gelatin liquefaction, starch and casein hydrolysis, and cellulose acetate breakdown were positive.

DISCUSSION

Although this work has shown *Amorphosporangium auranticolor* to have basically rod-shaped spores with a tuft of polar flagella, as has been reported for the genus *Ampullariella* (Higgins et al. 1967; Kane, 1966), it retains the distinction of having these rod-shaped spores arranged in coils within the irregularly lobed sporangium. It remains distinct from the genus *Actinoplanes*, which has round to ovoid spores usually arranged in coils within round or only slightly irregular sporangia.

It is understandable that Couch did not observe motility in Amorphosporangium. In many of the Actinoplanaceae, motility in the same culture will vary from one day to the next for no apparent reason. Also, even slight differences in cultural conditions such as osmotic pressure or age of the culture may affect motility (Higgins, 1967).

With the discovery of motility in *Amorphosporangium auranticolor* only the genus *Streptosporangium* Couch remains non-motile. The genus *Microellobosporia* has been tentatively transferred to the Streptomycetaceae on the basis of cell-wall composition (Lechevalier & Lechevalier, 1967).

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REFERENCES


EXPLANATION OF PLATE

*Amorphosporangium auranticolor*

Fig. 1 A. Empty sporangium with lobes (L) and showing texture of sporangial wall and sporangiophore (SP).

Fig. 1 B. Fragments of vegetative hyphae (VH) and the bases of two sporangiophores, showing the differences in wall textures.

Fig. 2. Two spores, the lower with a tuft of polar flagella.

Fig. 3. Curved spore with polar flagella.

Fig. 4. Light micrograph of lobed sporangium.

Fig. 5. Hypha with a ‘microconidium’ (MC).

Fig. 6. Lobe of sporangium with spores showing coiled and branching (B) pattern of spores. One micron marker for figs. 2, 3, 5 and 6.