Spore formation in *Actinomadura dassonvillei* (Brocq-Rousseu)
Lechevalier and Lechevalier

By S. T. WILLIAMS, G. P. SHARPLES AND R. M. BRADSHAW

Department of Botany, University of Liverpool, Liverpool L69 3BX

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The genus *Actinomadura* (Lechevalier & Lechevalier, 1970a) contains aerobic actinomycetes with a branching substrate mycelium which sometimes gives rise to aerial mycelium-bearing chains of arthrospores. The walls contain meso-diaminopimelic acid but usually lack arabinose and galactose, the pattern designated as type III by Becker, Lechevalier & Lechevalier (1965). In some strains, traces of L-diaminopimelic acid and arabinose are present (Mordarska, Mordarski & Goodfellow, 1972). Lechevalier & Lechevalier (1970a) transferred *Nocardia madurae* (Gordon, 1966), *N. pelletieri* (Gordon, 1966) and *N. dassonvillei* (Gordon & Horan, 1968) to the genus *Actinomadura*. Several new species have since been described (Nonomura & Ohara, 1971).

*Actinomadura dassonvillei* differs from *A. madurae* and *A. pelletieri* in several ways. While the substrate hyphae of the latter species are generally stable, those of *A. dassonvillei* fragment in a manner very similar to hyphae of Nocardia species (Lechevalier & Lechevalier, 1970a; Guzeva, Agre & Sokolov, 1972). Its macroscopic appearance and some physiological characters are similar to those of *Streptomyces griseus* (Gordon & Horan, 1968). On most media it forms abundant aerial hyphae with chains of spores, unlike many strains of *A. madurae* and *A. pelletieri* which form little aerial growth and lack spores. Whole cell hydrolysates of *A. madurae* and *A. pelletieri* contain the sugar madurose, but this is absent from *A. dassonvillei* (Lechevalier & Lechevalier, 1970a, b). In a numerical taxonomic study of nocardioform organisms, Goodfellow (1971) found that *A. dassonvillei* strains formed a minor cluster, separate from other Actinomadura species and all other clusters.

In the present study, the process of spore formation by the aerial hyphae was studied by electron microscopy. It was hoped to obtain further information on the characteristics and relationships of this species.

**METHODS**

*Actinomadura dassonvillei* strains NCTC10488 and NCTC10489 were studied. They were grown on oatmeal agar at 25 °C for 7 to 14 days.

Material for preparation of ultra-thin sections was obtained by growing the organisms on a sheet of cellophane overlying the medium. Growth was scraped off and fixed in buffered 1 % (w/v) osmium tetroxide for 16 h at room temperature. It was then washed with 0·5 % (w/v) uranyl acetate for 3 h. Specimens were dehydrated with ethanol, embedded in an epoxy resin (Spurr, 1969), sectioned, and stained with uranyl acetate and lead citrate.

Carbon replicas of spores were prepared using growth on coverslips inserted into the medium at an angle of about 45°. Coverslips with growth on them were removed from the medium, air-dried and coated with carbon under vacuum. Small squares (2 × 2mm) were cut
from the carbon film and floated off in distilled water. After digestion overnight in 5 N-
sodium hydroxide, they were washed six times with distilled water and picked up on
Formvar-coated grids. They were shadowed with gold–palladium before examination. The
replicas and sections were examined with an EM 6B electron microscope (A.E.I.) operated
at 60 kV.

Scanning electron microscopy was carried out with agar blocks (5 × 5 × 5 mm) cut from
the colonies. These were quenched in isopentane at about −150 °C and freeze-dried in an
Edwards-Pearse tissue dryer for 18 h. They were coated with gold–palladium under vacuum
and examined with a Stereoscan electron microscope (Cambridge Scientific Instruments)
operated at 20 kV.

RESULTS AND DISCUSSION

Both strains of *A. dassonvillei* produced abundant aerial hyphae and spore chains. Examina-
tion by scanning electron microscopy showed that chains were long, with up to about 50
spores. In mature cultures most of the chains were straight, but in younger cultures they
were often twisted. Examination at higher magnification showed that this was due to the
zig-zag arrangement of developing spores in these chains (Fig. 1a). Not all chains in any
one culture had this arrangement, which seemed to occur at an intermediate stage in spore
formation.

Carbon replication of spores showed that they were covered by a sheath with a distinct
pattern (Fig. 1b). This closely resembled the patterns observed in *Streptomyces griseus* and
other Streptomyces species, which reflect the fibrillar nature of the sheath (Wildermuth,
Wehrli & Horne, 1971; Wildermuth, 1972a, b; Williams, Bradshaw, Costerton & Forge,
1972).

Spore formation was initiated by a single ingrowth of the hyphal wall (Fig. 1c) to produce
a cross-wall of the kind designated type I by Williams, Sharples & Bradshaw (1973). The
narrow electron-dense mid-line of the ingrowth persisted throughout spore delimitation.
The order of formation of cross-walls in a chain was irregular. The first elements delimited
were often long (up to 2 μm) and were sometimes subdivided by further cross-wall formation
(Fig. 1c). The completed cross-walls increased considerably in thickness, reaching 200 to
250 nm. At the same time the lateral walls of the spores increased in thickness to 30 to 60 nm
(Fig. 1e). These developments occurred within the prominent sheath. Spores of various
lengths were finally delimited by cleavage of the thickened end-walls along their electron-
dense mid-line (Fig. 1d). At this stage, disruption of the sheath between the spores was
apparent. From these observations it seems likely that the zig-zag arrangement of developing
spores chains was caused by lateral displacement of spores within the sheath as their
adjoining end-walls thickened. Young spores contained nuclear material, membranous ele-
ments and polyphosphate granules (Fig. 1c). As their walls thickened, cytoplasmic contents
were less easily discerned and many electron-light areas were present (Fig. 1d, e).

Spore production by *A. dassonvillei* is therefore by fragmentation of a hypha within its
sheath, a process common to many actinomycetes (Williams *et al.* 1973). While spores in
chains may be delimited almost simultaneously (as in *Streptomyces*) or basipetally (as in
*Micropolyspora rectivirgula*), in *A. dassonvillei* the cross-walls form in a relatively unco-
ordinated manner and result in spores of various lengths. In this respect, the spore formation
process in *A. dassonvillei* resembles the fragmentation of Nocardia substrate hyphae into
irregularly sized units and indeed the fragmentation of its own substrate hyphae. The zig-zag
form of the sporulating hyphae has also been compared with that of Nocardia substrate
hyphae before fragmentation (Lechevalier & Lechevalier, 1970a). However, our results
Fig. 1. (a) Spore chains showing variation in arrangement of spores (scanning electron micrograph). (b) Carbon replica of spore showing fibrillar pattern on sheath. (c) Initiation of cross-wall (arrowed) to subdivide long element. Note electron-dense polyphosphate granule. (d) Cleavage of thickened end walls and sheath disruption. (e) Part of spore chain with thickened end walls and lateral walls, with prominent sheath.
suggest that this may be a superficial resemblance. The fibrillar pattern of the sheath, which resembles that in streptomycetes, also occurs on sporing aerial hyphae of *Nocardia asteroides* (Bradshaw, unpublished). The presence of polyphosphate granules in *A. dassonvillei* is another characteristic shared with *Nocardia* species (Kawata & Inoue, 1965; Williams et al. 1973).

The most prominent feature of *A. dassonvillei* spores was the development of very thickened end-walls and somewhat less thickened lateral walls. Very similar developments were observed in *Micropolyspora rectivirgula*, where spore walls reached a diameter of 100 nm (Dorokhova, Agre, Kalakoutskii & Krassilnikov, 1969, 1970). The spores were formed within a prominent sheath and, when mature, varied in size and shape. *Streptomyces megasporus* also produces spores with very thickened end walls and moderately thickened lateral walls (Dorokhova, Agre & Krassilnikov, 1971).

*Actinomadura dassonvillei* therefore shares a number of morphological characteristics with species of *Nocardia*, *Micropolyspora* and *Streptomyces*. It is difficult to place this species in any one genus using morphological criteria. Other evidence is also contradictory. The absence of arabinose, galactose and lipid LCN-A from its cell hydrolysates distinguish it from *Nocardia* and *Micropolyspora* species (Lechevalier & Lechevalier, 1970b; Mordarska et al. 1972). It differs from *A. madurae* and *A. pelletieri* in many morphological and physiological characters (Goodfellow, 1971), and in its lack of madurose (Lechevalier & Lechevalier, 1970b). Its correct taxonomic position may best be found by determination of its overall similarity to all recognized species of *Actinomadura*, *Micropolyspora* and *Nocardia*.

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**REFERENCES**


