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

(Received 5 June 1974)

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 × 2 mm) 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 ($5 \times 5 \times 5$ 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. Examination 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. 1*a*). 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. 1*b*). 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, 1972*a, b*; Williams, Bradshaw, Costerton & Forge, 1972).

Spore formation was initiated by a single ingrowth of the hyphal wall (Fig. 1*c*) 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 $2\ \mu\text{m}$) and were sometimes subdivided by further cross-wall formation (Fig. 1*c*). 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. 1*e*). 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. 1*d*). 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 elements and polyphosphate granules (Fig. 1*c*). As their walls thickened, cytoplasmic contents were less easily discerned and many electron-light areas were present (Fig. 1*d, 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 uncoordinated 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, 1970*a*). 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 retivirgula*, 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*.

This investigation was supported by a grant from the Science Research Council.

REFERENCES

- BECKER, B., LECHEVALIER, M. P. & LECHEVALIER, H. A. (1965). Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. *Applied Microbiology* **13**, 236–243.
- DOROKHOVA, L. A., AGRE, N. S., KALAKOUTSKII, L. V. & KRASSILNIKOV, N. A. (1969). Fine structure of sporulating hyphae and spores in a thermophilic actinomycete, *Micropolyspora rectivirgula*. *Journal de Microscopie* **8**, 845–854.
- DOROKHOVA, L. A., AGRE, N. S., KALAKOUTSKII, L. V. & KRASSILNIKOV, N. A. (1970). A study of the morphology of two cultures belonging to the genus *Micropolyspora*. *Mikrobiologiya* **39**, 79–86.
- DOROKHOVA, L. A., AGRE, N. S. & KRASSILNIKOV, N. A. (1971). Ultrastructure of hyphae and spores in *Actinomyces megasporus*. *Mikrobiologiya* **40**, 450–455.
- GOODFELLOW, M. (1971). Numerical taxonomy of some nocardioform bacteria. *Journal of General Microbiology* **69**, 33–80.
- GORDON, R. E. (1966). Some criteria for the recognition of *Nocardia madurae* (Vincent) Blanchard. *Journal of General Microbiology* **45**, 355–364.
- GORDON, R. E. & HORAN, A. C. (1968). *Nocardia dassonvillei*, a macroscopic replica of *Streptomyces griseus*. *Journal of General Microbiology* **50**, 223–233.
- GUZEVA, L. N., AGRE, N. S. & SOKOLOV, A. A. (1972). Taxonomy of actinomycetes forming catenate spores. *Mikrobiologiya* **41**, 957–961.
- KAWATA, T. & INOUE, T. (1965). Ultrastructure of *Nocardia asteroides* as revealed by electron microscopy. *Japanese Journal of Microbiology* **9**, 101–114.
- LECHEVALIER, H. A. & LECHEVALIER, M. P. (1970a). A critical evaluation of the genera of aerobic actinomycetes. In *The Actinomycetales*, pp. 393–405. Edited by H. Prauser. Jena: Gustav Fischer.
- LECHEVALIER, M. P. & LECHEVALIER, H. A. (1970b). Chemical composition as a criterion in the classification of aerobic actinomycetes. *International Journal of Systematic Bacteriology* **20**, 435–444.
- MORDARSKA, H., MORDARSKI, M. & GOODFELLOW, M. (1972). Chemotaxonomic characters and classification of some nocardioform bacteria. *Journal of General Microbiology* **71**, 77–86.
- NONOMURA, H. & OHARA, Y. (1971). Distribution of actinomycetes in soil. XI. Some new species of the genus *Actinomadura* Lechevalier *et al.* *Journal of Fermentation Technology* **49**, 904–912.

- SPURR, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research* **26**, 31–43.
- WILDERMUTH, H. (1972*a*). The surface structure of spores and aerial hyphae in *Streptomyces viridochromogenes*. *Archiv für Mikrobiologie* **81**, 309–320.
- WILDERMUTH, H. (1972*b*). Morphological surface characteristics of *Streptomyces glaucescens* and *Streptomyces acrimycini*, two streptomycetes with 'hairy' spores. *Archiv für Mikrobiologie* **81**, 321–332.
- WILDERMUTH, H., WEHRLI, E. & HORNE, R. W. (1971). The surface structure of spores and aerial mycelium in *Streptomyces coelicolor*. *Journal of Ultrastructural Research* **35**, 168–180.
- WILLIAMS, S. T., BRADSHAW, R. M., COSTERTON, J. W. & FORGE, A. (1972). Fine structure of the spore sheath of some *Streptomyces* species. *Journal of General Microbiology* **72**, 249–258.
- WILLIAMS, S. T., SHARPLES, G. P. & BRADSHAW, R. M. (1973). The fine structure of the Actinomycetales. In *Actinomycetales: Characteristics and Practical Importance*, pp. 113–130. Edited by G. Sykes and F. A. Skinner. London and New York: Academic Press.