Existence of a Simple Configuration in the Wall Surface of *Nocardia* Mycelium

By K. TAKEO AND I. UESAKA

Department of Bacteriology, Chest Disease Research Institute,
Kyoto University, Kyoto, Japan

(Received 2 October 1974; revised 5 December 1974)

INTRODUCTION

In Actinomycetales the phylogenetical relationship can be traced from simpler forms to more complex ones. Jensen (1952) presumed that *Actinomycetales* originated as a unicellular organism, a coryneform bacillus; subsequently, this developed through hypha-producing organisms to true spore-bearing organisms.

Streptomyces is one of the most differentiated members of the Actinomycetales. It has both vegetative and aerial mycelia, and bears spores only on the aerial mycelium. In ordinary conditions the vegetative mycelium does not divide. Many researchers have studied the ultrastructure of this organism by means of freeze-etching, carbon replica, and negative staining (Vernon, 1955; Hopwood & Glauert, 1961; Wildermuth, Wehrli & Horne, 1971; Williams *et al.* 1972). They found beautiful arrays of 'rodlets' on the extracellular coat of the spore and aerial mycelium in some species.

The genus *Nocardia* also has aerial and vegetative mycelia, but the difference between them is not as distinct as is found in *Streptomyces* and some other Actinomycetales. Both forms of mycelia have the ability to fragment into bacillary or coccoid elements which serve to propagate. Thus, *Nocardia* seems to be located in an intermediate position, between true spore-bearing organisms and *Mycobacterium* or other unicellular organisms. Its ultrastructure has been studied by Kawata & Inoue (1965) and Beaman & Shankel (1969), using thin sectioning or freeze-etching, but they did not describe its surface structure. No carbon replica or negative staining studies have been reported.

This paper describes a linear configuration found in the wall surface of *Nocardia asteroides* and *Nocardia brasiliensis* by means of freeze-etching, and discusses its relationship to the rodlets of *Streptomyces*.

METHODS

The organisms used were stock cultures in this laboratory. They were grown either on glycerol nutrient agar (10 g glycerol, 10 g polypeptone, 10 g meat extract, 2 g NaCl and 20 g agar/l water) or in glycerol nutrient broth, with shaking, at 25 or 30 °C for 2 to 5 days. The growths were fixed for a few days at 4 °C with 2.5% glutaraldehyde containing 0.07 M-phosphate buffer pH 7.2, then cryoprotected against ice-crystal formation by immersing in 30% glycerol overnight at 4 °C. Freeze-etching was done as described previously (Takeo *et al.* 1973).

Fig. 1. (a) Freeze-etched mycelia of *N. brasiliensis* grown on a glycerol nutrient agar plate for 3 days at 25 °C. Note linear structures on the wall surfaces (arrows). (b) Freeze-etched mycelia of *N. asteroides* grown in glycerol nutrient broth, with shaking, for 2 days at 30 °C. Linear configurations are seen as in (a) (arrows).

RESULTS AND DISCUSSIONS

Fig. 1(a) shows *N. brasiliensis* grown on an agar plate. Mycelia of *Nocardia* almost always freeze-fractured along the cell surface. This characteristic is the same as found in *Mycobacterium* (M. Nishiura & K. Takeo, unpublished) and seems to offer further evidence for the relationship of the two genera, in addition to the well-known acid-fastness, major cell wall constituents, and mycolic acids in a broader sense (Lechevalier, Lechevalier & Gerber, 1971). However, *Nocardia* wall surface revealed an additional feature: linear structures were superimposed on the surface layer of the wall (Fig. 1(a)). They were about 20 nm wide – about the same size as the rodlets found earlier in the spore or the aerial mycelium of some species of *Streptomyces* (Wildermuth *et al.* 1971). However, the surface configuration of *Nocardia* is much simpler than that reported in *Streptomyces*. The rodlets of *Streptomyces* cover the whole surface of the spore and aerial mycelium with local regularity, and they are about the same length (200 nm). The linear structures of *Nocardia* were sometimes long enough to encircle the cell wall and were only sparsely distributed on the surface layer (Fig. 1(a)). This linear structure seems to be characteristic of *Nocardia*, as no other reports of it have been made. We shall refer to it as ‘*Nocardia* lines’ or ‘N-lines’. *Nocardia asteroides* grown on agar plate also showed clear N-lines on the surface of the wall. The size and abundance of the lines were about the same as those for *N. brasiliensis*.

Fig. 1(b) shows *N. asteroides* grown in submerged culture, with shaking. As seen in Fig. 1(b), almost all cells possessed N-lines; this was also true in *N. brasiliensis*. Since it may reasonably be assumed that most mycelia in submerged culture are vegetative, the vegetative as well as the aerial mycelia of *Nocardia* have N-lines. In *Streptomyces*, on the other hand, the spore and wall surfaces of aerial mycelia are entirely covered with the rodlets, while the vegetative mycelium shows no rodlets and does not freeze-fracture along the wall surface (Williams *et al.* 1972). Our results provide further evidence that in *Nocardia* the difference between the aerial and vegetative mycelia is only slight, if any.

Thermoactinomyces is also reported to have a surface configuration made by rodlets which resembles that of *Streptomyces* (McVittie, Wildermuth & Hopwood, 1972), and the same applies to the spores of many fungi (Hess, Sassen & Remsen, 1968; Hess & Stocks, 1969; our unpublished data). However, nobody has reported the existence of the surface configuration in non-spore-generating organisms. It may thus be said that the surface configuration made by the rodlets is specifically found in spore-bearing micro-organisms, and that the N-line is a forerunner of the complicated surface configuration. Of course, *Nocardia* itself cannot be regarded as a forerunner of *Streptomyces* or *Thermoactinomyces*, as the data of cell wall constituents show (Lechevalier *et al.* 1971). However, a forerunner of *Streptomyces* may reasonably be supposed to have an aerial mycelium less clearly differentiated from its vegetative mycelium than in *Streptomyces*, and to have a simple surface configuration such as that found in *Nocardia*. In this sense, we suggest that the N-line links the presence of the complicated surface configuration in *Streptomyces* on the one hand, and the absence of surface configuration in *Mycobacterium* and other unicellular micro-organisms on the other.

We thank Mr H. Yamada, Mrs E. Yamagishi, Mrs Y. Takaoki and Mr T. Nonaka for technical assistance.

REFERENCES

- BEAMAN, B. L. & SHANKEL, D. M. (1969). Ultrastructure of *Nocardia* cell growth and development on defined and complex agar media. *Journal of Bacteriology* **99**, 876–884.
- HESS, W. M., SASSEN, M. M. A. & REMSEN, C. C. (1968). Surface characteristics of *Penicillium* conidia. *Mycologia* **60**, 290–303.
- HESS, W. M. & STOCKS, D. L. (1969). Surface characteristics of *Aspergillus* conidia. *Mycologia* **61**, 560–571.
- HOPWOOD, D. A. & GLAUERT, A. M. (1961). Electron microscope observations on the surface structures of *Streptomyces violaceoruber*. *Journal of General Microbiology* **26**, 325–330.
- JENSEN, H. L. (1952). The coryneform bacteria. *Annual Review of Microbiology* **6**, 77–90.
- 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. & GERBER, N. N. (1971). Chemical composition as a criterion in the classification of actinomycetes. In *Advances of Applied Microbiology*, vol. 14, pp. 47–72. Edited by D. Perlman. New York and London: Academic Press.
- MCVITTIE, A., WILDERMUTH, H. & HOPWOOD, D. A. (1972). Fine structure and surface topography of endospores of *Thermoactinomyces vulgaris*. *Journal of General Microbiology* **71**, 367–381.
- TAKEO, K., UESAKA, I., UEHARA, K. & NISHIURA, M. (1973). Fine structure of *Cryptococcus neoformans* grown *in vitro* as observed by freeze-etching. *Journal of Bacteriology* **113**, 1442–1448.
- VERNON, T. R. (1955). Spore formation in the genus *Streptomyces*. *Nature, London* **176**, 935–936.
- WILDERMUTH, H., WEHRLI, E. & HORNE, R. W. (1971). The surface structure of spores and aerial mycelium in *Streptomyces coelicolor*. *Journal of Ultrastructure 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.