A Preliminary Study of Nocardia asteroides

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Identification of the species of the genus *Nocardia*, and the actinomycetes in general, is based on color, biochemical reactions and type of growth on standard media. Many irregular forms of actinomycetes, such as found in clinical specimens, are difficult to identify by the present system.

This is a preliminary report on a study of the growth cycle of *Nocardia asteroides* to determine if some phase of the cycle exists that might serve as a stable characteristic for identification.

Three cultures of *N. asteroides* have been studied to date; ATCC 9970, ATCC 3306, and a culture from an udder infection in an Hawaiian herd, which will be referred to as S-655. These cultures were grown on nutrient agar with 0.5 per cent fructose, at 37C., for a minimum of 14 days, then were transferred to a fresh medium for growth cycle studies. Cellular changes occurring during the cycle were shown by the Chance

crystal violet nuclear stain (Chance, 1952) and the Webb cell wall stain (Webb, 1955).

Fourteen day old cultures of *N. asteroides* consisted mainly of uninucleate coccoid type cells. Marked differences were seen in the coccoid cells within the three cultures. Cultures 3308 and 9970 contained elongate coccoid cells whereas the cells of S-655 were spherical. These coccoid cells germinated, giving rise to hyphae, which in the early phase were coenocytic. The nuclear material of the coenocytic hyphae varied considerably. It sometimes appeared as "bars" or in irregular circular form, while in culture 9970 the aging hyphae appeared granular with the nuclear stain. Within eight hours after germination the hyphae formed cross septa. The nuclear material within the newly formed segments of the hyphae appeared in varous forms ranging from rod-like to spherical.

In 24 to 36 hours the hyphae fragmented. The hyphae of culture 9970 broke down cell by cell, usually from the end toward the center. Fragmentation in the other two cultures consisted of a breakdown of the long hyphae into shorter components and subsequent breakdown of these into bacillary cells. Fragmentation was complete within sixty hours.

Fragmentation products of the three cultures studied varied extensively. A majority of the cells resulting from fragmentation were elongate and uninucleate. The cells changed considerably in the following 12 to 24 hours of growth.

In groups 3308 and S-655 the cells, after fragmentation, appeared to germinate. There was a definite protrusion of the cell which appeared to be a rudimentary germ tube, however, no hypha was formed. Aparently a cross wall was formed and the "protrusion" became a new cell. The resultant cells from this type division were very irregular in shape and size. During this phase of growth some chaining and clumping of cells occurred. At approximately 20 hours after fragmentation a majority of the cells were apparently in a state of division. Many cells were binucleate and remained so for several hours, some indefinitely. These binucleate cells eventually changed to the uninucleate forms seen in old cultures.

Fragmentation products of culture 9970 formed few, if any, binucleate cells, as described above. They apparently remained in the uninucleate stage after fragmentation.

The three organisms studied follow a similar overall pattern; however, upon detailed study several outstanding differences were observed. Perhaps with further study these differences, along with physiological and serological studies, will aid in the identification of the *Nocardias* and other actinomycetes.

Literature Cited

Chance, H. L. 1952. Crystal violet as a nuclear stain for *Gaffkya tetragena* and other bacteria. Stain Technol., 27: 253-258.

Webb, R. B. 1954. A useful bacterial cell wall stain. J. Bacteriol., 67: 252-253.