SLIME AS A POSSIBLE FACTOR IN CELL CLUMPING IN Nocardia corallina

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Work on the nuclear cytology and life cycle of Nocardia corallina has previously been reported (Webb et al., 1954; Webb and Clark, 1957; Clark and Frady, 1957). Extensive studies on the radiation response, physiology, and genetics of this organism have also been carried out in this laboratory. Throughout this work, one of the main problems involved in the experimental approach has been the elimination of clumps of cells, since N. corallina forms large, tenacious clumps of cells under all environmental conditions.

The presence of large numbers of cell aggregates interfered with many experimental approaches, and this investigation was undertaken to determine if clumping could be minimized.

MATERIALS AND METHODS

Nocardia corallina (ATCC4273) was grown on nutrient agar containing 1 per cent fructose and incubated at 29 C. Microscopic examination of living cells was done with an American Optical dark contrast, medium phase objective. Electron microscope studies were done with an RCA model EMU-2 electron microscope with the cells suspended on a formvar screen. Cells were either unfixed for electron microscope studies or fixed in osmium tetroxide vapors (Clark, 1955), or in Bouin’s, Schaudinn’s, F.A.A. (Tellyesniczky’s Formalin-aceto-alcohol), or Carnoy’s solution.

RESULTS AND DISCUSSION

Phase and electron microscopic examination of clumps of coccoidal cells of N. corallina revealed a random orientation of the cells (figure 1), which indicated that incomplete cell cleavage was not the cause of clumping. Earlier work (Webb and Clark, 1957) showed that fragmenta-

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tion of the hyphal growth by cross septum formation was usually completed during the formation of the bacillary cells. The coccoidal cells which result after nuclear fusion in the bacillary cells were always unicellular and completely cleaved from adjoining cells.

The possibility that surface charge might be a factor in clumping was investigated by suspending the cells in buffers at pH values from 2 to 9. Even after being shaken for 1 hr in the buffers, equal clumping was observed at all pH values. Therefore, it was concluded that surface charge was not a major factor in the formation of cell clumps.

A study of cells under the electron microscope revealed a structure between many of the cells. This structure resembled a plasmodesm (figure 2) and was found in all stages of division, including very old coccoidal cultures. However, it was not found to occur uniformly in the plane of division of the cells (figure 3) and also occurred between hyphae in a position that could not have been caused by cell division (figures 4 and 5), so this structure could not be a plasmodesm.

The plasmodesm-like structure was not an artifact due to fixation since it appeared equally in unfixed preparations and preparations fixed for varying times in osmium tetroxide vapors, Bouin’s solution, F.A.A., Schaudinn’s, or Carnoy’s solutions. It is probable that shrinkage of cells in the electron microscope accentuated the appearance of the structure, since it appeared only between cells which were close together and which had probably, at some time during preparation of the specimens, been in contact.

The structural material appearing between the cells could be removed by washing in distilled water. It was not removed by washing the cells in various salt solutions, dilute acids or bases, or organic solvents.

Similar structures were found in cultures of Nocardia blackwelli (Oklahoma University culture...
collection), Nocardia caprae (McClung no. 88), Nocardia sp. (McClung no. 14) and Proactinomycetes polychromogenes (McClung no. 6). They were found to some extent in Corynebacterium pseudodiphtheriticum (Oklahoma University culture collection). The structure was not found in cultures of Staphylococcus aureus, Gaffkya tetragena, Bacillus megaterium, Escherichia coli strain K-12, E. coli strain B, or E. coli strain B/r (all from Oklahoma University culture collection).

This structure could be attributed to a sticky nature of the cell wall, or to the slime layer of the organism. Since the sticky property of the cells can be reduced by washing with distilled water, it would appear that this is not a property of the cell wall per se, but instead is due to a secretion at the cell wall. Such secretion would form the slime layer of the cell. Therefore it is believed that this structure is made up of portions of the slime layer which normally adhere tightly to the cell and thus are not detectable by the microscope. When two cells come in contact and are then separated slightly, the slime layer material connects the cells and can be seen with the electron microscope. The fiber-like structure of this material is not resolved by the light microscope. It is believed that this slime layer is the primary cause of clumping in N. corallina.

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SUMMARY

An electron dense, plasmodesm-like structure was found connecting cells of Nocardia corallina. This structure was not a fixation artifact and was not a plasmodesm since it did not occur
uniformly in the plane of division. The general appearance and behavior of the structure suggests that it is derived from slime layer. This is believed to be the major cause of the clumping of cells of this organism.

REFERENCES


