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## SUBSECTION MICROBIOLOGY

### Studies on the Life Cycle of *Nocardia corallina*<sup>1</sup>

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The life cycle of *Nocardia corallina* on solid media has been reported by Webb and Clark (1957) and Clark and Frady (1957). However, little work has been done to elucidate the reasons for the changes in cultural morphology which occur as this organism goes through its typical cyclic development. This is a preliminary report on an initial approach to a study of the causes of the developmental cycle.

Initial experiments were performed to determine if the same life cycle occurred in broth cultures as was found on solid media. On solid media,

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fragmentation of the hyphal growth was more or less synchronous, terminated hyphal development and resulted in uniform appearing bacillary cells. In broth culture, fragmentation did not terminate hyphal development, was non-synchronous and resulted in the formation of additional hyphae of various lengths.

A re-appraisal of the life cycle suggested that the morphological changes could be attributed to the manner of cross septum formation in dividing cells. Germination of coccoidal cells and hyphal development involve nuclear division without cross septum formation, while fragmentation and coccoidal division involve nuclear division accompanied with cross septum formation.

Previous experiments by Webb (1956) indicated that the failure of coccoidal cells to germinate, unless they were transferred to fresh substrate, was due to the accumulation of an anti-germination factor in the old culture. Such a factor could conceivably induce cross septum formation. Therefore, media from broth cultures of various ages was centrifuged and then re-inoculated to test their ability to prevent germination. The results indicated that from only a narrow range of physiological age was the stale medium active in preventing coccoidal germination. At all other ages of the culture, the stale medium would support germination of a small inoculum of coccoidal cells, even though the inoculum was actively forming cross septa. These experiments give support to the view that an anti-germination factor may exist, but indicate that the factor may not accumulate in concentrations much above a critical or threshold level. Such a factor may be metabolized as it is formed and not accumulate in detectable amounts except at short, specific times of the developmental cycle.

Since cross septa are formed in old cultures, it appeared that there might be some correlation between total number of cells and the germination process. It was found that if the number of cells in an old, non-germinating coccoidal cell suspension was reduced by centrifugation, the remaining cells would germinate. Dilution of such a culture with sterile saline produced identical results and the percentage of cells germinating was found to increase with increasing dilution.

These results suggest that the factor involved, when germination occurs upon inoculation of a fresh medium, is not attributable so much to some component of the substrate as it is to reduction in cell numbers. If an inoculum size larger than one-half of the maximum cell population reached in an old culture was used, a significant decrease in germination in fresh medium was observed. Larger inocula were found to produce septate hyphae which quickly fragmented to form chains of bacillary and coccoidal cells which resembled old broth cultures.

These preliminary experiments indicate that (1) a possible anti-germination factor does exist which induces cross septum formation, (2) this factor seldom exceeds a threshold level and thus is not usually extractable from the culture filtrate, and (3) germination of coccoidal cells in a fresh medium can be attributed primarily to cell dilution.

#### LITERATURE CITED

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