INFORMAL SEMI-ANNUAL REPORT ON RESEARCH GRANT NASA-NsG-693

"Studies on trace elements in the sporulation of bacteria and the germination of bacterial spores"

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I. Summary of Progress and Proposed Research

Earlier reports have indicated the areas of study and the various approaches that have been used in an attempt to understand the relationships between trace elements and the formation of spores and their subsequent dormancy and germination. The areas of study may be relegated to the following categories: a) the manganese sporulation requirement, b) trace elements and germination in a highly purified system, c) comparison of the germination of asynchronously grown with synchronously grown spores and d) temperature induced sporulation mutants.

The manganese sporulation requirement (Gruft, H., Buckman, J., and Slepecky, R.A., Bact. Proc. 1965:37 (an abstract)) has been covered in the 1 June 1965 report. It can be stated that a sporeformer needs manganese at a proper concentration before growth ceases in order to sporulate. There appears to be three levels of manganese required for the morphogenesis: one for growth; one for forespore formation; and one for spore formation. The third level can be replaced by amino acids suggesting that it is required for

a protease; the current hypothesis for the need for the manganese at other times also revolves around the activation of particular enzymes. These previous studies were concerned with various additions of manganese to manganese deficient cells at various times of the growth cycle. At present, we are trying to correlate these results with data obtained by measuring the manganese levels of the various cell stages using atomic absorption spectrophotometry. Concurrently, other studies in our lab are concerned with the pattern of formation of various enzymes, many of which are suspected to be manganese activated, during the cycle.

With regard to the phase concerned with trace elements and germination, it appears that molar ionic effects, in either a potassium phosphate buffered system or a sodium chloride unbuffered system, supplemented with the physiological germinants, L-alanine and inosine, were solely caused by potassium or sodium, and that any extraneous heavy metal or alkaline earth metal contamination displayed either inert or inhibitory activity in the germination process. The validity of the purification procedures was confirmed by atomic absorption spectrophotometry. We are checking these results further since such a finding strengthens the ionic germination ideas of Rode and Foster (Arch. Mikrobiol. 43:201 (1962) and Nature 194:1300 (1962)) and may simplify further studies of ion involvement. For example, we can ask the question, without being concerned with other ions, whether the sodium and potassium are acting at the surface or internally.

Our recent studies have shown that spores formed in a synchronous growth system exhibited a faster rate of germination than those grown in an asynchronous system. We have examined this phenomenon further by comparing the germination of both types of spores at various times after

formation. Asynchronous spores did not exhibit large differences in germinative ability, and the variations were not consistent. From this, it was concluded that the effect of age on germinative ability could not be observed clearly using the asynchronous system. The most probable reason for the small and inconsistent variation was the heterogeneity of ages in any sample from an asynchronous system. On the other hand, the results of the experiments using synchronous systems appeared to be intrinsically similar, if not parallel. Synchronously formed sporangia germinated most rapidly and most completely. As the sporangia became older and free spores began to be released, germinative ability decreased. These studies are preliminary but are being continued for they may lead to more knowledge on dormancy and the breaking of that unusual state.

Lastly, we have been concerned with temperature induced sporulation mutants in Bacillus subtilis. This work was related in the 1 January 1966 report and an abstract has been published (Northrop, J., and Slepecky, R.A., Bact. Proc. 16 (1966)). As indicated in the previous report these studies not only support the hypothesis of cytoplasmic genetic determinants for sporulation (possibly episomes) previously presented from this laboratory (Rogolsky, M., and Slepecky, R. A., BBRC 16:204-208 (1964)) but they are germane to the problem of dormancy and the elimination of spores by high temperatures, a problem of much concern to NASA in its planetary quarantine program. We are examining, critically, whether such treatments may induce mutants with increased heat resistance, a heretofore unconsidered possibility.

II. Publications during the Period of the Report

Remsen, C. C., Lundgren, D. G., and Slepecky, R. A. Inhibition of spore septum and forespore membrane development in Bacillus cereus by beta-phenethyl alcohol. J. Bacteriol. 91:324-331 (1966).

Slepecky, R. A. The use of combined sonication-germicide treatment in surgical instrument cleaning. Hospital Topics 44:133-134 (1966).

Northrop, J., and Slepecky, R. A. Temperature-induced sporulation mutations in Bacillus subtilis. Bact. Proc. 16 (1966) (an abstract).

Imanaka, H., and Slepecky, R. A. Enzyme synthesis during sporulation of Bacillus megaterium in a synchronous growth system. (Submitted to the International Congress for Microbiology, Moscow, July 1966 and publication as an abstract in the proceedings of that meeting).

III. List of Personnel Engaged in the Project during the Period of the Report

Dr. Ralph A. Slepecky, Principal Investigator

Miss Zita Celkis, Technician

Mr. Wayne Crosby, Research Assistant

Mr. Jere Northrop, Research Fellow