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

As related in earlier reports we are concerned with the following with regard to trace elements and bacterial sporulation and germination: a) the manganese sporulation requirement, b) trace elements and germination in a highly purified system, c) germination of synchronously grown spores, particularly low Mn spores, and d) temperature induced sporulation mutants. This report will be concerned primarily with the Mn requirement.

It has been difficult to relate the established Mn sporulation requirement to the activation of a particular enzyme. Our evidence to date for the activation of a protease has been largely circumstantial based on studies with crude extracts and our attempts to isolate a specific protease have been unsuccessful to date.

The previous studies on the Mn requirement, based on the additions of Mn to Mn deficient cells at various times of the growth cycle, are being extended by measuring the Mn levels of the various cell stages.

We hope to compare Mn uptake patterns with known enzymatic patterns established with our synchronous system. We are currently measuring the Mn levels using the atomic absorption spectrophotometer (AAS) and presently we hope to extend this approach to other metals as well.

In anticipation of experiments on the germination of Mn-low spores and on the location and fate of the important metals of spores, we have been seeking a simple reproducible AAS method for spore metal analysis. Parallel assays by means of the AAS were done using spores of B. megaterium treated by: a) acid digestion, b) dry ashing at 600C, and c) suspension of untreated spores in deionized distilled water. Comparison of the results indicate a high degree of correlation and reproducibility. Several different liquid phases have been tried to make a homogenous spore suspension for analysis. Organic solvents tend to enhance the degree of absorption and appear to minimize the tendency for spore aggregation. An acidic pH enhances the absorption. These phenomena are currently under investigation.

The preliminary results with the use of the AAS to examine spore metal content show much promise. By using a suitable suspension of clean spores in the proper liquid phase, we may be able to analyze directly the content of such biologically important metals as Ca, Mg, Mn, Zn and Fe in the 0.1 to 10 PPM range. Subsequently, with this type approach we may be able to assess the importance of these metals in maintaining the high dormancy found in bacterial spores.

II. Publications during the Period of the Report

Imanaka, H. and Slepecky, R.A. Enzyme synthesis during sporulation of Bacillus megaterium in a synchronous growth system. IX International Congress for Microbiology. Abstracts of Papers 1966:151.

Northrop, J. and Slepecky, R.A. Sporulation mutations induced by heat in Bacillus subtilis. Science, (1967) (IN PRESS).

Publications in preparation:

The following papers have been submitted for presentation at the 1967 meeting of the American Society for Microbiology:

Imanaka, H. and Slepecky, R.A. Patterns of enzyme synthesis during sporulation of Bacillus megaterium.

Ellar, D.J., Lundgren, D.G. and Slepecky, R.A. Fine structure of the mesosome and its involvement in cell wall synthesis and sporulation of synchronously dividing Bacillus megaterium.

The following paper has been submitted to the Seventh International Congress of Biochemistry, August 1967, Tokyo:

Imanaka, H. and Slepecky, R. The inhibition of the sporulation of Bacillus megaterium by L -cysteine.

The following manuscript has been submitted to the Journal of Bacteriology:

Imanaka, H., Gillis, J. and Slepecky, R.A. Synchronous growth and sporulation of Bacillus megaterium.

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

Mr. Robert Longsworth, Research Assistant