FINAL REPORT

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TITLE: FLUORESCENT ANTIBODY DETECTION OF MICROORGANISMS IN TERRESTRIAL ENVIRONMENTS

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Summary of Research and Results

The study of microorganisms directly in complex natural environments has always been severely hampered by inherent limitations in techniques. Limitations are most obvious in attempts to study that most complex of microbial habitats, the soil. Soil is too complex to be simulated in any but superficial detail; the microorganisms that exist and function in this environment are morphologically nondescript and of microscopic dimension. Necessarily, most studies have been indirect: the microorganisms that could be isolated are removed from the soil, examined in artificial laboratory culture, and the results are extrapolated to the soil situation. Alternatively it has been possible to a limited extent to examine the soil directly with the microscope and to observe microorganisms in this habitat. The direct microscopic approach has been useful, but it has a crippling drawback in that the microorganisms that can be seen, cannot be recognized, since most simply look alike.

We have centered our attention on the fluorescent antibody technique because of its promise to provide an entirely new dimension to direct microscopic examination of soil. The great promise of the fluorescent antibody lies in possibility of both observing and recognizing a microorganism in the soil at one and the same time. It appears now that much of this promise can be fulfilled. Some soil isolate of special interest is used as an antigen, and antiserum to this is labeled with a fluorochrome dye. The labeled antiserum is used as a stain of soil material, and if the organism of interest (the antigen) is present the highly specific antigen-antibody reaction results where, and only where, the antigen microorganism occurs. Thus when examined by fluorescent microscopy the microorganism of primary interest should be readily distinguished from all others present by virtue of its characteristic fluorescence.

The research program for which support was requested was comprised of two related problems involving the interaction of soil microorganisms with plant roots to form symbiotic structures. One was concerned with the developmental ecology and biology of the root nodule of alder (Alnus) and the second was concerned with the ectotrophic mycorrhizal structure on forest trees, especially pines (Pinus). In both, the fluorescent antibody detection of the microbial symbiont both as a free living form in soil, and as a root inhabiting form in the higher plant was emphasized. A third aspect of the research involved the detection of autotrophic ammonia oxidizing microorganisms in soil. Chemosynthetic autotrophic bacteria such as the ammonia oxidizers can

derive energy and nutrients from completely inorganic substances, and techniques for their detection may be of particular relevance to the examination of extra-terrestrial environments.

Alnus Root Nodule Endophyte

Work on the mature of the endophyte involved in the formation of nitrogen-fixing root modules of Alnus has not reached a publication stage. Among numerous isolates obtained from surface sterilized nodules, two were promising as possible endophytes. Both are actinomycete bacteria and both show some morphological features in culture, reminiscent of those seen internally in cross sections of the intact nodule. A fluorescent antibody was prepared for the more rapidly growing isolate, but staining of nodules was unsatisfactory. It is not yet clear whether the staining problems are due to non-homologous endophytes in the nodule or whether the negative reactions are due to technical problems in handling the plant tissues.

Mycorrhizae

Studies with the ectomycorrhizæ of pine were generally very successful and established clearly the possibilities for the direct microscopic identification of mycorrhizal fungi in roots and in soil. Fluorescent antibody was prepared for two mycorrhizal symbionts, Thelephora terrestris (Tt) and Pisolithus tinctorius (Pt); both gave highly satisfactory homologous reactions. A low but detectable degree of cross reactivity was removed by absorbing Pt - FA with Tt and Tt - FA with Pt. The two antibody stains were checked on contact slide preparations of 12 mycorrhizal isolates, 14 cultures of common phycomycetes, ascomycetes, basidiomycetes, and fungi imperfecti, and 16 cultures of wood rotting Thelephoraceae. The Tt - FA correctly identified all 7 mycorrhizal isolates of T. terrestris and cross reacted with only 1 of 35 other test fungi. Pt - FA stained each of 2 P. tinctorius mycorrhizal isolates at the 4+ level, and gave 2+ to 3+ cross reacting fluorescence with 7 of the 40 other test fungi. Sections of P. tinctorius ectomycorrhizal of red pine stained with Pt - FA demonstrated 3+ to 4+ specific fluorescence in both mantle and Hartig net. A manuscript describing this work is in preparation.

Chemoautotrophic Nitrifying Bacteria

Preliminary studies with the genus Nitrosomonas established that fluorescent antibodies prepared against that organism were satisfactory as to intensity of staining and specificity. This work was expanded for the development of FA preparations for the specific staining and visualization of Nitrobacter species in nature. N. winogradskyi and N. agilis were used to immunize rabbits. The antibodies derived from each species gave high agglutionation titers, and each FA gave specific 4+ homologous staining. Low level cross reactions between the two species were removed by absorptions. All 7 Nitrobacter isolates obtained from Minnesota soils proved to be N. winogradskyi. Extensive testing of both Nitrobacter FA preparations against randomly selected heterotrophic bacteria from soil and sewage gave no evidence of staining due to cross reactions. The ability to visualize Nitrobacter in direct microscopic preparations of soil, water, and sewage environments by immunofluorescence techniques was demonstrated. The details of this aspect of the study are being prepared in manuscript form.

Publications:

Fliermans C. B., B. B. Bohlool, and E. L. Schmidt. Autocotogical Study of the Autotroph Nitrobacter by Immunofluorescence. (in preparation)

Schmidt, E. L., J. A. Biesbrock, B. B. Bohlool and D. H. Marx. Immunofluorescence for the Study of Mycorrhizae. (in preparation)

Theses:
None

Inventions or Discoveries:
None

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Comments:
NASA Grant NGR 24-005-198 was not renewed in fiscal year 1973.

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