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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Survival of bacteria; Death of bacteria; Cysts; Dormancy; Pseudomonas aeruginosa; Azotobacter; Chemotaxis; Micrococcus luteus; Predation; Predators; Lysis of bacteria; Pyocyanin; Streptomyces venezuelae; Genetic instability; Ensifer adhaerens; Myxobacteria; Conidia; Desiccation of soil; pH of soil;

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A new, non-obligate system of bacterial predation on bacteria in soil was discovered. The predators comprising this system were 1) a genetically-unstable strain of Streptomyces venezuelae, 2) a new kind of budding bacterium (now designated as Ensifer adhaerens, gen. n., sp. n.), and 3) a myxobacterium. The natural population of these organisms in soil is attracted to and rapidly destroys (lyses) added Micrococcus luteus cells. There is also attack of predator on predator in this system.

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19. Key Words Continued

Microcysts; Arthrobacter; Polymorphic growth; Myceloid growth; Manganese; Bacteriophage; Poly-β-hydroxybutyrate; Hypoxanthine.

20. Abstract Continued

The transitory myceloid stage that occurs in the pleomorphic growth cycle of various Arthrobacter species was expanded (by manganese removal) so that it became the main form of growth. This stage was found to be more sensitive to bacteriophage attack than were the other growth stages. A new procedure for isolating Arthrobacter sp. bacteriophage from soil was developed for these studies.

It was found that the encystment of Azotobacter vinelandii does not require prior intracellular production of poly-β-hydroxybutyrate, and that it is triggered by hypoxanthine.

The human pathogen, Pseudomonas aeruginosa, was found to survive well in soil. It did not utilize pyocyanin production for this but, instead, went into a cell dormancy state with some of the characteristics of a cyst.

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SURVIVAL OF MICROORGANISMS IN NATURE

Final Report

L. E. Casida, Jr.

April 7, 1982

U. S. Army Research Office

DAAG29-79-G-0043

The Pennsylvania State University

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STATEMENT OF THE PROBLEM STUDIED

Many different microorganisms reside in soil. Others, in one way or another, find their way into soil. In either case, the survival of the organisms depends on their ability to contend with the chemical, including nutritional, and physical conditions imposed on them by the soil habitat. For example, they must have a physiology compatible with this environment. In addition, the individual organism must compete with other organisms for nutrients and space, and must in some manner survive attack by predators. Lastly, the organisms must be able to survive, in at least limited numbers, the drying down of soil, which is the most likely killing event associated with their habitat. Each of these factors plays a role in the organism's survival, but it is not clear as to the relative importance of these factors, or how the factors interact. Our approach was to study several model systems that pertained to at least some of these factors and their interactions. The model systems allowed evaluation of the following (as a partial list) in relation to microbial survival: inorganic and organic nutrition, chemotaxis, natural physiology of the organism, pleomorphic growth cycles, genetic stability, soil pH, oxygen availability, production of antibiotics and other inhibitors or lytic agents, dormancy, encystment, conidia involvement, bacteriophage attack, predation, and soil desiccation and water availability. Where possible, these were studied in relation to the organisms as they occurred in situ in natural (unsterilized) soil. Studies were also conducted using pure or mixed cultures in sterile soil and on laboratory media.

SUMMARY OF IMPORTANT RESULTS

Micrococcus luteus was shown to survive only poorly in soil (Casida, 1980a). This was because it was relatively quickly destroyed by two previously-unknown, non-obligate, bacterial predators of bacteria (Casida, 1980b). These predators were isolated and studied. One was a strain of Streptomyces venezuelae (see later). It produced mycelium that, through some form of chemotaxis, sought out the M. luteus cells, surrounded them, and lysed them. The other predator was an unusual new kind of bacterium that reproduced by budding and was highly motile. It is now designated (Casida, 1982) by the new genus and specific epithet names, Ensifer adhaerens; it was originally designated merely as Strain A. It attaches to the M. luteus cells, and to the above S. venezuelae strain, and lyses both of them.

The mycelium-producing predator (S. venezuelae mentioned above) was originally thought to be a Streptoverticillium species. Further studies, however, showed that actually it was a genetically-unstable S. venezuelae strain that continuously produced a white variant, but also produced a second white variant whose relation to the parent strain is still unclear. Although this organism was attacked by E. adhaerens in soil, it survived relatively well. This apparently was because its conidia (as opposed to mycelium) were not subject to attack by E. adhaerens. Also, any decrease in the moisture content of the soil decreased the activity of E. adhaerens.

A third bacterial predator of M. luteus multiplied in soil after E. adhaerens and the S. venezuelae strain were well along in their attack on M. luteus. This predator could lyse both of the other predators, in addition to lysing M. luteus. It was isolated and found to be a myxo-bacterium, possibly a Myxococcus species. It was shown to survive in soil by making microcysts. In neutral pH soil, but not in acidic soil, these cysts germinated in response to added M. luteus cells.

The common soil bacterium, Arthrobacter globiformis, produces a transitory myceloid stage in some media when it initiates its pleomorphic growth cycle. We found, however, that this myceloid growth, as it applies to A. globiformis and other Arthrobacter species, becomes the main growth form when chelating agents are present to complex manganese, or when manganese has been completely removed from the medium (Germida and Casida, 1980). This phenomenon perhaps affects the survival of these bacteria in nature, because replication of both the lytic and temperate bacteriophages that attack these bacteria was found to be more extensive during myceloid growth. To perform these bacteriophage studies, we developed a new procedure (Germida and Casida, 1981) for the isolation of Arthrobacter species bacteriophage from soil. This procedure, however, seems to also work for bacteriophages for other bacteria and bacterial predators in soil. Based on this, we are developing a procedure for following the activities of bacterial hosts and predators in situ in soil by following the multiplication and decline of the bacteriophages that attack these bacteria. So far, this procedure looks quite promising.

Azotobacter vinelandii survives adverse environmental conditions in soil by encysting. We showed that, contrary to statements in the literature, the encystment process does not require prior poly-β-hydroxybutyrate formation in the cell. We also showed that hypoxanthine directly triggers the encystment process. Other workers have shown that hypoxanthine is produced by Azotobacter cells during periods of decreasing adenylate energy charge: i.e., during starvation. Thus, it appears that encystment in Azotobacter is starvation related and is regulated through purine metabolism.

We have not yet evaluated the possible ability of hypoxanthine to bring on encystment in other bacteria that are not known to encyst.

Pseudomonas aeruginosa is a human pathogen that is not normally considered to be an inhabitant of soil. We found, however, that it survives very well in soil if the soil does not dry out or become anaerobic. It does not make use of its antibiotic-producing ability (pyocyanin) in this survival. Instead, it produces a previously unknown dormant form (Zechman and Casida, 1982) which in several characteristics resembles a cyst.

I would like to point out that, based on the foregoing studies and results, non-obligate predation of bacteria on other bacteria may be playing a much greater role than previously thought in microbial survival in soil. We have reported on the discovery of one new predation system, but have initial evidence (not reported here) of other major, interlocking, predation systems operating in soil.

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