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In general, both predator and prey bacterial species in soil are dormant much of the time. This seems to be a means of avoiding starvation, desiccation, and predation. For many species of soil bacteria, the breaking of dormancy is controlled by small amounts of either magnesium or copper. The cells need the metal to allow breaking of dormancy. However, there usually isn't enough of the respective metal that is readily available in the environment. Therefore, the cells produce a specific peptide growth initiation factor (GIF) which chelates the respective metal (scavenges it from the environment) to make it available to the cells. The cells then break dormancy and start growth. The cells do not need the GIF for their growth, however: only to initiate growth.

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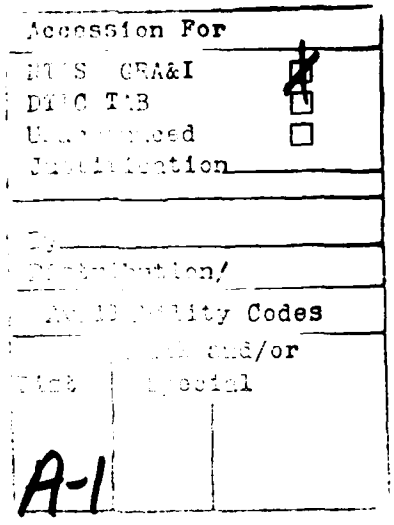
# Survival of Microorganisms and Bacterial Predation and Competition in Nature


Final Report

L. E. Casida, Jr.

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FINAL REPORT

This final report covers our studies on the non-obligate bacterial predators of bacteria in soil. We discovered these predators in 1980 (1,2), and the Literature Cited section of this report shows the publications on the subject since 1980. All of these studies were funded by grants from the U.S. Army Research Office: the original was DAAG29-79-G-0043, and the present one, starting in 1985, is DAAG29-85-K-0084. You will note that a "Minireview" (22) on the subject was published in 1988.

The studies from 1980 to 1985 showed that, if a bacterium such as Micrococcus luteus is added to soil, it is rapidly attacked by non-obligate predator bacteria. There are several kinds of these predator bacteria. However, they all attack and destroy prey bacterial cells in soil, but do so only if the supply of soluble nutrients in the environment is relatively low. At higher soluble nutrient levels they are saprophytes. The initial attack on M. luteus in soil is by a Streptomyces species that puts out feeler mycelium to locate the prey cells. Once located, the prey cells are surrounded by mycelium and lysed. This Streptomyces species and the M. luteus cells, however, are in turn almost immediately attacked by a new kind of bacterium which we have named Ensifer adhaerens (5). This bacterium attaches endwise to its host cells and causes them to lyse. About a day later, a myxobacterium (2,9) multiplies in the vicinity. It uses an extracellular enzyme to lyse some of the nearby cells, but usually doesn't do much harm.

In general, both predator and prey bacterial species in soil are dormant much of the time (3,6,9,12,14,15,24). This seems to be a means of avoiding starvation, desiccation, and predation. For many species of soil bacteria, the breaking of dormancy is controlled by small amounts of either magnesium

(11) or copper (20,21,23). The cells need the metal to allow breaking of dormancy. However, there usually isn't enough of the respective metal that is readily available in the environment. Therefore, the cells produce a specific peptide growth initiation factor (GIF) which chelates the respective metal (scavenges it from the environment) to make it available to the cells. The cells then break dormancy and start growth. The cells do not need the GIF for their growth, however: only to initiate growth. Some predator bacteria, such as Agromyces ramosus (8,13,17,18) and Actinomyces humiferus (11), are so efficient at making their own particular magnesium-GIF that other micro-organisms literally may become starved for magnesium because they can't compete for it. These magnesium-GIF predator bacteria are not at the top of the predatory hierarchy, however. This exalted position seems to be held by the bacteria that use the copper-chelating GIF for breaking dormancy (10,16,17,20,21,23,24). They are very resistant to copper. An example of these bacterial types is the new bacterium we discovered and named Cupriavidus necator (20,21).

A. ramosus has no qualms about attacking C. necator, although it usually must be goaded into doing so (13). C. necator does not use Mg-GIF. Nevertheless, it makes some and elaborates it into the environment. This causes A. ramosus to initiate growth. A. ramosus mycelium then advances toward and kills about 1/3 of the C. necator cells. The mycelium then fragments into rod-shaped dormant cells. After a short delay during the initial attack, C. necator initiates growth and counter attacks A. ramosus to obtain nutrients(13). The Cu-GIF that C. necator produces is very toxic to the A. ramosus mycelium (20) but not to its dormant rod-shaped cells. Thus, in the attack-counter attack process, and through attack of these predator bacteria on other prey cells in the vicinity, both organisms have had some of their

cells destroyed, but have made enough new ones before going dormant that they have actually increased their numbers.

C. necator does not encounter a counterattack in most other situations when it attacks prey cells. Nevertheless, we now know (unpublished) that there is a yet more powerful Cu-GIF utilizing predator bacterium in soil. It is a Pseudomonas (apparently a new species), and it can control most of the other Cu-GIF predators, including C. necator. We have designated this bacterium as strain 679-2. It is such a powerful predator because it produces a very toxic extracellular toxin in addition to its CuGIF and its ability to attach to host cells in its attack. So far, we have found this bacterium in only one soil. Nevertheless, it is highly competitive. With as few as 3 cells added to a g of moist soil (any soil), it will multiply extensively in 24 hours. If 0.8 mg of glutamic acid is also added to the soil (as a substrate for making CuGIF and the inhibitor) strain 679-2 will multiply to become a major component of the soil microbial population within 24 h. Once it has multiplied in the soil, it dies back only very slowly, unless the soil dries out. Strain 679-2 is quite sensitive to desiccation.

The activities and numbers of the various predator bacteria in soil can be increased by making various additions to the soil. The soil protozoa do not stop this increase (25). We are able to follow these increases by using some techniques we developed. E. adhaerens numbers in soil can be counted by plating dilutions of the soil over the surface, without breaking the surface, of a pregrown lawn of M. luteus cells (2,5). E. adhaerens and most other predator bacteria, and even some of their prey, can be followed in soil by use of our indirect phage analysis technique (4,7,17). Very low numbers can be detected and enumerated using our special MPN procedure (19). The Cu-GIF predators can be counted easily by plating soil dilutions on a medium contain-

ing toxic levels of copper (23). Strain 679-2 is detected and counted on this medium because it has strange-looking, easily-recognized colonies.

The numbers of the various predator bacteria can be greatly increased by adding suitable prey bacteria to the soil (17,23). M. luteus is almost a universal prey species for this. Alternatively, soluble nutrients can be added in very small amounts: just enough for making the respective GIF or other inhibitor compounds. Larger amounts cause the predators to become saprophytes. As noted previously, glutamic acid was found to be the best addition for causing multiplication of the copper-GIF predators, and particularly of strain 679-2. Glutamic acid works in both low and high fertility soils. Most other possible additions work only in low fertility soils, if they work at all.

Strain 679-2 in initial trials looks like it might be valuable in the treatment or prevention of plant diseases. There also is the obvious possibility of adding glutamic acid or some other compound to soil to cause the copper-GIF predators to take control of the soil population to handle a bacterial challenge. Furthermore, the fact that these predators die back only slowly means that a soil can be pre-treated with glutamic acid, or with glutamic acid plus strain 679-2 cells. Thereafter, the soil will contain a large population of the respective predator bacteria to handle a bacterial challenge. An example of such a challenge would be if bacteria were inadvertently or purposely added to the soil, or if chemical or other additions to the soil might cause growth of unwanted or harmful bacteria in the soil.

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