Disease-Suppressive Soil and Root-Colonizing Bacteria

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Summary. Soils in many areas suppress certain plant diseases. Understanding the basis for this disease suppressiveness could lead to improved plant health in less favorable areas. Some forms of disease suppression may be caused by bacteria in the genus *Pseudomonas* which aggressively colonize root surfaces. Increased plant growth and yield are closely associated with the capacity of some of these bacteria to produce iron-binding compounds called siderophores. This article addresses the biological characteristics of these soil-borne root epiphytes, their contribution to plant health, and their potential use in biotechnology.

The health of flowering plants is determined by countless biotic and abiotic interactions in the soil and on root surfaces. This relatively unexplored aspect of plant biology is attracting particular attention today because of the opportunity to apply biotechnological developments in exploiting the key beneficial microorganisms that inhabit plant roots. Also, there is growing recognition of opportunities for successful entrepreneurial activity in this area. Initial success will depend on the selection of systems that readily lend themselves to cultural and genetic manipulation. Many plant scientists favor the emphasis of efforts to improve the effectiveness of such bacteria as *Azotobacter* spp. or *Rhizobium* spp. in the root nodulation process. However, the greatest possibility for increasing plant yields substantially and making a dramatic change in agricultural practices may involve bacteria that protect plant roots from the many deleterious microorganisms that occur in all agricultural soils.

The soil microorganisms that suppress plant diseases have evolved with plants and are a primary factor determining plant health. They influence selection of crops and crop varieties, rotation procedures, pesticide application, and land use. However, the importance of these bacteria has not been generally recognized, and hence they have not been a focus of interest for development and exploitation.

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Disease-Suppressive Soils

Agricultural or uncultivated soils in which the development of specific soil-borne diseases is impeded are called disease-suppressive. Two broad types of suppressiveness are recognized: natural and induced (*1*). Natural suppressiveness is associated with certain physical and chemical characteristics that affect the microbiology of the soil, and is usually unaffected by cropping sequences. This type of suppressiveness can often be predicted once the soil characters essential for suppressiveness are identified. In contrast, induced suppressiveness is usually independent of soil types and dependent on cropping or cultural practices. Unlike natural suppressiveness, which is expressed from the outset, induced suppressiveness is expressed after several crop generations. A history of monoculture with a susceptible crop is usually a prerequisite for the induction of suppressiveness.

Suppressiveness is a relative quality. Soils in which certain soil-borne diseases occur to a significant degree are called conducive. Yet, even in conducive soils, soil-borne pathogens usually do not express their full potential as disease incitants.

A precise monetary assessment of the value of disease-suppressive soil in affecting crop yield is not easily made because of the difficulty of controlling diverse biotic interactions. However, available data show that when disease suppressiveness occurs, it has impressive effects on yield. For example, take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* was assessed as causing a 33 percent decrease in grain yield, but with the onset of induced suppression, yield recovered to just 9 percent below the original maximum (*2*). An even more dramatic example of natural suppressiveness involves muskmelon culture in the Chateaurenard, Cavaillon, and Carpentras regions of France (*3*). Soils in these regions vary greatly in disease suppressiveness, which can be evaluated by infesting the soil with increasing amounts of inoculums, planting a susceptible host, and then comparing disease severity (Fig. 1). Muskmelons have been grown since antiquity in the Chateaurenard region with little trouble from *Fusarium* wilt (*Fusarium oxysporum* f.sp. *melonis*), even though the fungus is present. In the two nearby regions the disease is so severe that it sometimes causes abandonment of the crop.

Clearly, crop yields would be greatly increased if the factors in disease-suppressive soil were understood and applied to disease-conducive soils. A comparison of the yields from food plants grown in fumigated and nonfumigated soils further shows that the yield potential of plants is grossly unrealized because of the many low-grade disease-causing agents in soil. Strawberry yields average 19 to 22 tons per acre in fumigated soil and only 5 to 6 tons in untreated soil (*4*). This effect is attributed largely to the eradication of soil-borne plant pathogenic fungi in the fumigated soil.

It is now possible to identify at least a half-dozen forms of soil suppressiveness. Yet, while many suggestions are proposed, factors responsible for suppression are still unclear. Physical, chemical, and biological qualities of soils are tied to natural suppressiveness. Extensive studies of soil suppression of vascular wilt diseases have implicated the clay mineral fractions as contributing to the natural control of this disease (*5*). The *p*H of soils also plays a role in some diseases. A lowering of *p*H with a *Fusarium*-suppressive soil resulted in increased wilting of carnations (*6*), whereas an increase in *p*H led to greater infection of radishes by *Rhizoctonia solani* (*7*). The alteration of *p*H apparently affected the antagonistic activities of a *Pseudomonas* sp. and *Trichoderma harzianum* in the soils. More recently, soil salinity was proposed as a contributing factor in suppression of *Pythium ultimum* in California's San Joaquin Valley (*8*).

In each of these cases, biological com-

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ponents of the soil were also essential to suppressiveness: treatments that destroyed the native microflora of these soils rendered them conducive. Biological factors are also of major significance in induced suppressiveness. Soil components responsible for declines in take-all of wheat and potato scab are sensitive to heat and fumigation treatments. Soil suppression of *Phytophthora* root rot of avocados is also destroyed by heat (*9*). The hope is to identify the microbes responsible for suppressiveness and to harness them as biological control agents.

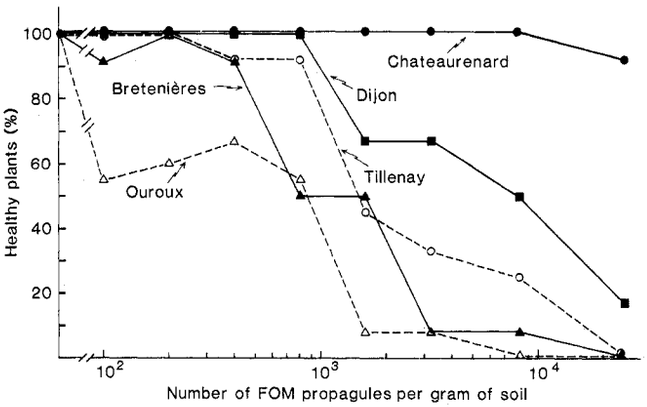

Fig. 1. Comparison of suppressiveness of five soils to *Fusarium oxysporum* f. sp. *melonis* (*FOM*), the cause of muskmelon wilt. [Adapted from Alabouvette *et al.* (*3*) with permission from Academic Press]

Microorganisms in Disease-Suppressive Soils

The striking phenomenon that conducive soils can be made suppressive by transferring into them a small volume of suppressive soil (*6*, *9*) has challenged investigators to find the biological factors responsible for suppression. However, it is unlikely that the occurrence of several key organisms in a soil could account for sustained disease suppressiveness. It seems logical that a disease-suppressive soil is suppressive because of the activity of an assortment of microorganisms whose populations are favored and sustained by interactions with the abiotic environment (physical and chemical factors) and with associative microorganisms. However, it should be possible to obtain the benefits of a disease-suppressive soil on a temporary basis, perhaps for several years, if some of the key microorganisms can be identified and used under conditions which favor their activities. There have been some successes in field tests with microorganisms from various environments, but these successes resulted from inoculating seeds and root systems with high concentrations of specific microorganisms (*10*, *11*), such as *Bacillus* spp., *Agrobacterium radiobacter* K-84, *Trichoderma* spp., *Pythium oligandrum*, *Laetisaria* sp., *Chaetomium* sp., and *Pseudomonas* spp., rather than from incorporating them into soil.

The demonstration that microorganisms from disease-suppressive soils and other environments (*3*, *10*, *12*) can effectively protect plants from certain deleterious components of the microflora may or may not be related to why a disease-suppressive soil is suppressive, but it does indicate the potential for using microorganisms in disease control or in mimicking disease-suppressive soils, at least on a short-term basis. With greater understanding of the physical and chemical factors that affect the ecology of antagonistic microflora, it should be possible to alter the abiotic factors in the soil to favor their activities, thus sustaining a long-term soil suppressiveness.

The approach of incorporating microorganisms directly into soil to control diseases would probably not be successful in commercial agriculture unless vast amounts of concentrated inoculums were applied regularly, a procedure that would be economically unfeasible. Survival time would probably be short because of antibiosis and competition from other soil microorganisms. It is difficult to establish culture-grown microorganisms in alien environments or biologically buffered communities at densities at which their biological activities can be realized over a significant length of time. In the complex ecosystem of the soil, where carbon nutrition is limiting, microbial residents are well-entrenched and not readily displaced by intruders.

The direct application of microorganisms to seeds or other plant parts gives them a competitive advantage over pathogens, which must compete for the same sites and nutrients prior to infection. Microorganisms tend to retain possession of their niches when confronted by other aggressive microorganisms. Most root-infecting pathogens are at a disadvantage in that they exist in soil as dormant propagules; colonization and infection can occur only if preceded by activation of the dormant propagules, generally through the exudation of nutrients from the invading root. Therefore, pathogens must compete with microorganisms that previously colonized the root surface or that were simultaneously activated.

Many groups of microorganisms have been cited as candidates for the biotic suppressive factor in disease-suppressive soil (*10*, *12*). *Pseudomonas* spp. were selected for experimentation because of their nutritional versatility and ability to grow under a variety of environmental conditions. Also, in initial studies (*13*), most strains that aggressively colonized root systems belonged to the *Pseudomonas* group. When inoculated onto seeds and other plant parts, some caused substantial increases in plant growth and yield. The terms rhizobacteria and plant growth–promoting rhizobacteria (PGPR) were coined (*14*, *15*) to describe these and other bacteria well-adapted as epiphytes on plant roots and to differentiate them from soil bacteria that do not colonize roots or cannot do so aggressively. Rhizobacteria, which are subdivided into beneficial, deleterious, and neutral groups, are distinguished from rhizoplane and rhizosphere bacteria because the latter two terms are commonly used to refer to bacteria that are isolated from that region with little consideration to function or population. Rhizosphere and rhizoplane bacteria may not be root colonizers and may well be transients.

Most of the beneficial *Pseudomonas* rhizobacteria fall into the *P. fluorescens* and *P. putida* groups and as such are oxidase-positive, fluorescent, and arginine dihydrolase–positive. Extensive testing of their phenotypic properties shows that they are very heterogeneous and that no one character is common to all members. Furthermore, most do not fit the biovar groupings of Stanier *et al.* (*16*) and are intermediary with many of these groups. Stanier's groups blur in distinctiveness in direct relation to the number of strains that are examined. These pseudomonads are found in both suppressive and conducive soils.

Plant Growth–Promoting Rhizobacteria

The full potential of rhizobacteria and other microorganisms to promote plant growth will be approached only when there is a better understanding of the factors that affect their ecology and establishment on roots. This necessitates

detailed studies of their physiology and biochemistry, determination of the mechanisms by which they promote plant growth, and strain improvement by classical and genetic engineering methods. Still, despite the fact that relatively little is known about rhizobacteria, their application to plant organs [principally seeds, seed pieces (Fig. 2), and roots] has resulted in surprisingly consistent increases in plant growth and yield ranging well over 100 percent—an indication of the potential of this group to improve plant health. Potato yields increased 5 to 33 percent in field plots in California and Idaho (*13, 17*). A 30 percent increase in yield was obtained in 1981 in Pennsylvania, in the first test run by a commercial company. The treatment of sugar beet seeds with various strains of *Pseudomonas* spp. resulted in yield increases of 4 to 8 tons per hectare in six of eight trials, with increases in sugar ranging from 955 to 1227 kilograms per hectare (*18*). As with potato rhizobacteria, however, the same strains were not effective in all soil types. The most striking results were obtained with radishes, a 30-day crop. Treatment with various strains led to increases in root weight ranging from 60 to 144 percent in all field trials (*15*).

Although the frequency of obtaining significant increases in yield has improved with better understanding of the ecology and physiology of the rhizobacteria and appropriate adjustment of methodology, there is great need for improvement in technology allowing larger populations of rhizobacteria to survive the inoculation and planting processes. Although several seed-coating procedures have been developed (*18, 19*), problems remain. Some soil bacteria colonize the coating mixtures, and the pelleting process now used by commercial companies for other purposes appears to reduce the viability of rhizobacteria.

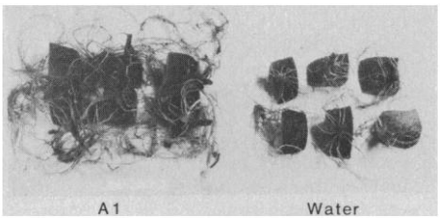

Fig. 2. Increased growth of roots from potato seed pieces (cultivar Centennial) resulting from seed inoculation with *Pseudomonas* sp. strain A1.

Root Colonization

Manipulation of the root environment to favor a particular organism is critical to all schemes that propose to increase plant growth by use of beneficial microorganisms. Few studies have addressed the competitive ability of specific soilborne bacteria on roots under natural systems. The prevailing opinion among plant scientists is that it would not be possible to easily alter the composition of root microflora to favor any one microbe. Studies with rhizobacteria, however, indicate that the root microflora can be manipulated if bacteria are found which have apparently adapted as epiphytes on roots and which have the potential to dominate that ecological niche (*20, 21*). Their identification is difficult, as only 2 to 5 percent of bacteria isolated from plant roots have the ability to aggressively colonize roots and to increase plant growth (*12*).

Specific strains of *Pseudomonas* rhizobacteria, when inoculated onto sugar beet seeds, colonized roots (Fig. 3) and persisted throughout the growing season, reaching populations as large as 10^5 colony-forming units per centimeter of root. The population of fluorescent *Pseudomonas* spp. on nontreated controls ranged from 90 to 600 colony-forming units per centimeter (*18*). Similar results were obtained with potato plants inoculated with other strains (*15*). Strains resistant to rifampicin and nalidixic acid colonized the entire root system of treated plants and developing daughter tubers. Populations as large as 9.6×10^5 colony-forming units per centimeter were seen 2 weeks after plant emergence, and averaged 10^3 colony-forming units throughout the season. These populations are much greater than those reported for other beneficial root bacteria such as *Azotobacter* (*22*). The best explanation for this is that *Azotobacter* and most other bacteria that have been tested do not colonize roots effectively.

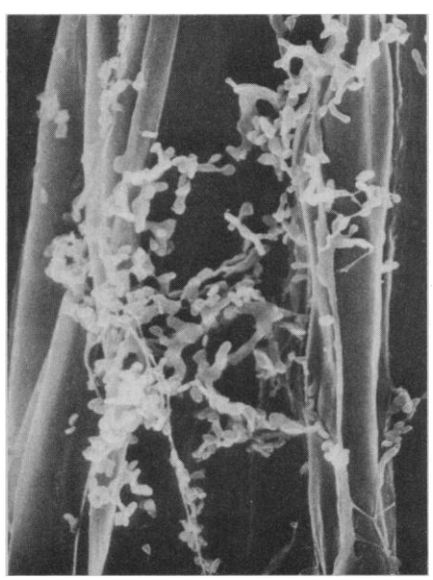

Fig. 3. Electron micrograph showing attachment of a PGPR (*Pseudomonas* sp. SH-5) to the root of a sugar beet seedling grown in nonsterile sand (×5000). The seeds were inoculated with the bacterium, which subsequently colonized the roots during plant growth.

Antagonism

The favored hypothesis on how rhizobacteria promote plant growth is that the aggressive colonization of the root system results in a displacement or exclusion of deleterious components of the microflora about roots. An alternative hypothesis involves the production of growth-promoting substances, such as auxins or kinetins (*23*). These hypotheses have been examined by comparing the growth of plants with and without rhizobacteria under gnotobiotic conditions or in nonsterilized soil. If growth-promoting substances were produced, the rhizobacteria would be expected to affect growth under gnotobiotic conditions. However, this did not occur (*24*), even though the bacteria colonized the roots. Growth differences were obtained only between plants, grown in nonsterilized soil, that were or were not inoculated with rhizobacteria.

A general screening of rhizobacteria for antibiotic activity shows that they inhibit a wide variety of microorganisms. The question of whether or not this is an important mechanism relating to plant growth promotion has been tested by using antibiosis-negative mutants (*20*). In field tests with potato plants, the mutants did not cause increases in stolon development or plant growth and wild-type strains did. However, the mutants still colonized roots at densities similar to those of wild-type strains. The effect of mutants and wild-type strains in causing quantitative and qualitative changes in microflora that colonize roots have also been evaluated. Wild-type strains caused reductions in fungi and Gram-positive bacteria on the rhizoplane ranging from 23 to 64 percent and 25 to 93 percent, respectively, whereas there were no differences between the general microflora on the rhizoplane of control plants and those inoculated with antibiosis-negative mutants.

Similar studies with sugar beets have extended these findings, showing that

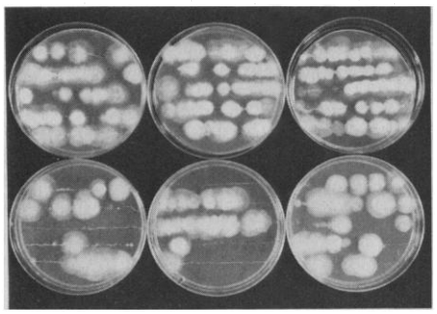

Fig. 4. Fungal colonization of roots from untreated sugar beets (upper three plates) and roots from sugar beets treated with *Pseudomonas* sp. SH-5 (lower three plates). Seeds were inoculated or not inoculated and grown in the field; roots were laid horizontally on agar medium to determine fungal colonization.

plant growth promotion is related to reduced infection and colonization by specific fungi and bacteria (Fig. 4) (*21, 25*). Sugar beet growth 8 weeks after seedling emergence increased 73 to 120 percent as a result of treating the seeds with PGPR. This increase in growth was associated with reductions in fungal root colonization up to 62 percent. The PGPR also cause shifts in the composition of fungi that colonize roots, as some fungi apparently are not affected by their activity and increase in number. In some cases, this proves disadvantageous to the plant; there may be increases in root infections by *Pythium* and *Fusarium* spp.

Although antibiosis is an important mechanism in plant growth promotion, at least with the strains that have been tested, this character should be considered as only one of the many important determinants. Less than 5 percent of the bacteria that exhibit antibiosis in vitro affect plant growth, probably because of the absence of other important characters, such as those related to root colonizing ability. Also, 8 to 15 percent antibiotic strains subsequently prove to be deleterious, causing stunting and root necrosis. Moreover, a few strains that promote plant growth do not show antibiotic activity in vitro. This is indicative of the complexity of the system. There is no one factor that is common to all PGPR.

The finding that roots are colonized by a variety of bacteria that deleteriously affect root growth was unexpected, since, with several exceptions (*26*), known bacterial pathogens penetrate plants through wounds and natural openings and then cause localized or systemic reactions. The vast majority of bacterial diseases cause damage to the aboveground parts of plants, where there are abundant natural openings. The deleterious rhizobacteria (DR) appear to be toxigenic (pathogenic but not parasitic), as there is no evidence of root invasions. They are a major component of the microflora of field-grown sugar beets (*21*). Tests with specific strains indicate that DR cause reduced seed germination, root distortion, root lesions, reduced root elongation, and increased root infection by certain fungi. They have been tentatively identified as belonging to *Pseudomonas, Enterobacter, Klebsiella, Citrobacter, Flavobacterium, Achromobacter,* and *Arthrobacter,* genera which, with the exception of *Pseudomonas,* do not include any of the hundreds of species of known bacterial plant pathogens.

It has been hypothesized that growth promotion by PGPR might in part be related to their ability to exclude DR from root systems. In greenhouse tests, various strains of PGPR caused a significant reduction in the density of DR populations on sugar beet roots. This was reflected by an increase in plant growth. Plant growth with PGPR was 50 to 97 percent greater than growth in plants treated only with DR (Fig. 5). We suggest that DR represent an important group of bacterial pathogens which has been overlooked because of the nonparasitic, relatively subtle nature of their attack on plants.

These findings show the importance of the composition of the microflora to plant health and indicate the potential for increasing crop yields by developing techniques to beneficially manipulate the root surface ecosystem.

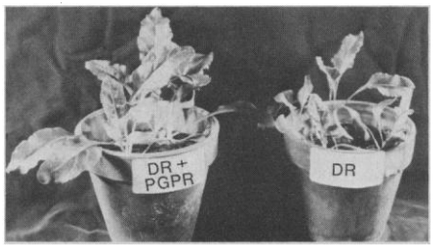

Fig. 5. Inhibition of DR by PGPR, resulting in increased growth of sugar beet seedlings. Seeds in the experiment were uniformly inoculated with 10^4 colony-forming units of DR, half of which (PGPR versus DR) were subsequently inoculated with 10^5 colony-forming units of PGPR.

Antibiosis

The fluorescent pseudomonads produce a range of secondary metabolites, many of which exhibit antibiotic or phytotoxic activity (*27*). Most antibiotics belong to the class of nitrogen-containing heterocycles, such as phenazines and pyrrolnitrin-type antibiotics. They also produce a number of unusual amino acids and peptides. The role of these and other secondary metabolites in the physiology and ecology of these organisms is largely unknown. The possibility that some PGPR elaborate siderophores [high-affinity iron (III) ion transport agents] has been investigated since iron is needed by perhaps all living organisms (*28*). Any event limiting iron availability would greatly affect the ecology of microorganisms. Thus, the production of siderophores by PGPR in the rhizosphere could efficiently complex iron, inhibiting the growth of certain components of the native microflora, including root pathogens. With many strains of PGPR, antibiosis in vitro appears related to the production of a fluorescent siderophore whose production is iron-regulated. Antibiosis did not occur in vitro nor was the yellow-green fluorescent pigment associated with the presence of the siderophore produced when King's medium B agar plates were amended with 1 μM $FeCl_3$ (*29*). Furthermore, PGPR exhibited antibiosis against mutant *Escherichia coli* K-12AN193, which does not produce its native siderophore, enterobactin, but not against its siderophore-producing parent, *E. coli* K-12AN194.

The possibility that the elaboration of siderophores by pseudomonads plays a major role in plant growth promotion has been investigated by adding iron to soil in which potato seed pieces treated with PGPR were planted. The addition of ethylenediaminetetraacetatoferrate [Fe(III)EDTA$^-$] to soil resulted in no significant plant growth promotion by PGPR, although the bacteria still colonized roots (*29*). In a related experiment, the siderophore of strain B-10 was purified and its biological activity as a plant growth promoter was tested in soil. The addition of the siderophore, named pseudobactin, to soil caused increases in plant growth and reductions in fungal colonization of the rhizosphere similar to those caused by inoculation of seed with PGPR. On the other hand, no increases in plant growth were obtained by the addition of ferric pseudobactin to soil. These and other results strongly indicate that PGPR produce siderophores which sequester Fe(III) in the rhizoplane, making it less available to certain rhizosphere microorganisms that cannot obtain sufficient iron for growth because they produce siderophores in insufficient quantities or with less affinity for iron than those from PGPR.

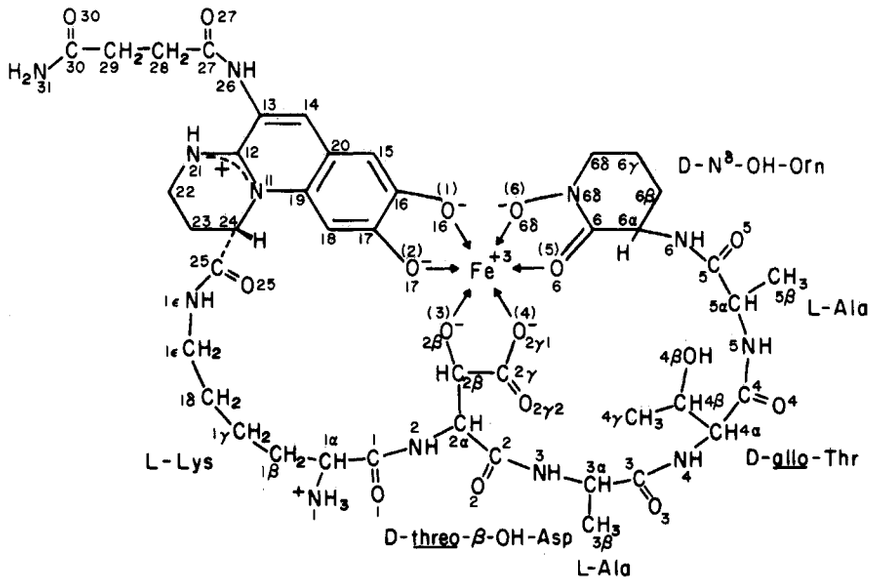

Fig. 6. Structure of ferric pseudobactin. [From Teintze *et al.* (*34*); courtesy of *Biochemistry*]

These findings have led to the investigation of the role of pseudomonads and their siderophores in a disease-suppressive soil. Suppressive soil has high populations of pseudomonads, and the proportion that exhibit antibiosis is greater than that found in conducive soils (*30, 31*). Should the suppressive nature of suppressive soil be caused in part by iron deprivation as a result of siderophore production by PGPR and other microorganisms, the addition of Fe(III)EDTA$^-$ to these soils should convert them to a conducive state. Iron in this form is available to the microorganisms. When Fe(III)EDTA$^-$ was incorporated into two suppressive soils, the survival of wheat seedlings affected by *Gaeumannomyces graminis* var. *tritici* and flax seedlings affected by *Fusarium oxysporum* f. sp. *lini* decreased from 83 to 27 percent and from 82 to 48 percent, respectively, making the soil conducive to disease (*32*). Conversely, conducive soils were made temporarily suppressive to both pathogens by inoculating seed with *Pseudomonas* strain B-10 or by adding pseudobactin to the soil. However, the addition of Fe(III)EDTA$^-$ with B-10 or the use of ferric pseudobactin did not induce such a conversion. Both *G. graminis* and *F. oxysporum* were inhibited by B-10 and pseudobactin in antibiosis assays.

In a related study, Scher and Baker (*6*) made a conducive soil suppressive to *Fusarium* wilt of radish and flax with a *Pseudomonas* sp. or by adding ethylenediamine di-(*o*-hydroxyphenylacetate) [Fe(III)EDDHA$^-$] to the soil. They suggested that the iron in Fe(III)EDDHA$^-$ is available to microorganisms which produce siderophores, and that consequently this favors the activity of antagonists like fluorescent pseudomonads. These and other results (*33*) indicate that certain fluorescent pseudomonads and other siderophore-producing microorganisms play an important role in contributing to disease suppressiveness in soil.

Ferric Pseudobactin

The yellow-green fluorescent siderophore from *Pseudomonas* B-10, designated pseudobactin (Fig. 6), consists of a linear hexapeptide in which N$^{\delta}$ of the N^{δ}-hydroxy ornithine is cyclized with the terminal carboxyl, and the α-amino group of the lysine is linked by an amide bond to a fluorescent quinoline derivative. The iron chelating groups consist of a hydroxamate group, α-hydroxy acid, and an *o*-dihydroxy aromatic group. None of the three chelating groups have been observed in previously described siderophores. As pointed out by Teintz *et al.* (*34*), the alternating L- and D-amino acids in the pseudobactin sequence are also unusual and could explain why pseudobactin is not affected by trypsin, chymotrypsin, pepsin, pronase, or leucine amino aminopeptidase. This characteristic could protect pseudobactin against degradation by other organisms in soil. Pseudobactin apparently is sufficiently chemically stable and resistant to degradation when incorporated into soil to suppress the fungi that colonize roots. This is in contrast to enterobactin, a siderophore elaborated by enteric organisms, which is inherently unstable, having a half-life in aqueous solution of a few hours (*28*). Teintz and Leong (*35*) have identified ferric pseudobactin A, a nonfluorescent siderophore, in strain B-10. It apparently is produced simultaneously with pseudobactin. It has antibiotic activity but differs chemically from pseudobactin in the saturation at carbons 3 and 4 of the quinoline derivative.

Since many microorganisms produce siderophores, competition for iron should be greatly affected by the stability of the association of the elaborated siderophore with Fe(III) and by the hydrogen ion concentration of the rhizoplane and its effect on the association of this complex. It is interesting to note that fungi, including representatives from groups that infect roots, synthesize siderophores only of the hydroxymate type. Among these are the ferrichromes, which have a modest affinity for Fe(III). Thus, bacteria that produce siderophores with a high affinity for Fe(III) should easily outcompete ferrichrome-producing fungi for the available iron.

Future Prospects

There is compelling evidence that exploitation of the microorganisms that protect plants from pathogenic microorganisms would increase plant growth and crop yields in the near and long term. Most crop plants are fettered by the many deleterious microorganisms that colonize and infect their root systems. Although manipulation of root microflora to favor beneficial microorganisms at the expense of deleterious ones has generally been considered impractical, it now has been demonstrated that the root microflora can be altered qualitatively and quantitatively and that this can lead to substantial increases in yield.

There are distinct advantages in working with root-colonizing rhizobacteria as opposed to attempting to establish on root systems bacteria whose natural habitats are elsewhere. It is questionable whether free-living heterotrophic nitrogen fixers, for example, have the competency to obtain the high population density required to significantly affect plant growth. With respect to genetic engineering, it seems unlikely that an organism whose natural habitat is not the root could easily be transformed into an aggressive root colonizer. The special characters that govern root colonization are not known. However, there are undoubtedly a number of genetic determinants governing this capacity, and it will take considerable research to identify them.

The development of efficacious micro-

bial products that are cost-effective and can be adapted to fit the technology of modern agriculture represents a significant challenge. Considerable work must be done in such areas as developing a highly concentrated inoculum with a relatively long shelf life and in a form that can be applied commercially. Also, health-related data for state and federal registration must be obtained depending on the claimed mode of action of the product. Ironically, a major nuisance at the marketplace may be how to distinguish an efficacious microbial product from the many dubious microbial elixirs that have been sold to farmers since the beginning of the century. None of the dozens of the microbial concoctions sold as soil catalysts, activators, and soil builders has proved effective, and the success that some have had in marketing products of no known value does not argue well that the truth will ultimately prevail.

The development of efficacious microbial products will depend on the cooperative efforts of bacterial ecologists, plant pathologists, physiologists, biochemists, and genetic engineers. Such a team could cooperate, for example, on the determination of the important key characters and systems which enable a microorganism to successfully compete in a particular ecological niche. With PGPR, once the specific metabolite in excluding a deleterious microorganism is known, research can begin on the regulatory mechanisms affecting its production, followed by genetic manipulation of key biochemical processes. As in industrial microbiology, it should be possible to obtain highly productive strains. The competitive ability of epiphytic bacteria to colonize roots and their capacity to exclude deleterious microorganisms from the root surface also could be greatly improved through genetic engineering to enable them to tolerate great moisture stress or to produce a wider array of metabolites that would affect a greater spectrum of deleterious microorganisms. Characters that allow rhizobacteria to proliferate on roots should be examined with particular care; they are the key to using other beneficial bacteria as root colonizers.

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Controlled Photoperiodic Environments for Food Animals

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In many areas of the world, climate limits the productivity as well as the survival of livestock. Consequently, man has provided shelter to animals in an effort to improve their productivity. Since the end of World War II, animals in the more developed countries have been housed in increasingly closer confinement and their environments have been more regulated. This shift toward more closely controlled environments has contributed to increased productivity. This in turn has reduced production costs and, in proportion to income, has lowered costs of food from animals.

Seasonal variation in the reproductive activity of several domestic species used for food has been known for centuries. Of the variables associated with the environment, it is now recognized that photoperiod is the primary cue regulating seasonal reproduction. Modern production methods often expose animals to photoperiods substantially different in intensity and duration from those of natural photoperiods. Manipulation of photoperiod has been practiced commercially for more than 60 years to control the onset of egg production and to stimulate egg laying and regulate body growth in chickens. Results of recent research suggest that photoperiod may be manipulated to stimulate reproduction and body growth, increase milk production and the efficiency of feed utilization, and hasten puberty in several domestic species. In this article we focus on the role of photoperiod in the regulation of these traits.

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