

RHIZOSPHERE BACTERIA AND THEIR USE TO INCREASE PLANT PRODUCTIVITY: A REVIEW

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ABSTRACT

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Rhizosphere bacteria influence plant growth through several mechanisms. Beneficial interactions are often difficult to identify and isolate for study, and therefore favorable effects on plant productivity are not easily described in quantitative terms. Major beneficial activities of soil bacteria include solubilization of minerals, fixation of nitrogen, production of growth-promoting hormones and competitive suppression of pathogens. Most recent research to improve crop responses has emphasized the study of nitrogen-fixing bacteria indigenous to rhizospheres of cereal crops and other grasses. The amount of nitrogen available to crops from fixation by these organisms is significant under some circumstances, but efforts to control and increase fixation activity have not been consistently successful. Despite considerable research, inoculation experiments frequently fail to improve rates of nitrogen fixation. However, substantial progress has been achieved in understanding mechanisms involved in plant-bacterial interactions, and in defining conditions which control them. Also, recent studies show clearly that bacterial inoculation can be highly beneficial even when nitrogen fixation is not affected. Further research is needed to develop new and effective methods for suppressing growth of competing microorganisms, and promoting dominance of those most useful to plants. Environmental manipulation may not be sufficient to achieve this objective. Genetic development of both plant and bacterial lines which enhance desired interactions is likely to be the most productive research effort for the future.

INTRODUCTION

Plants growing under natural conditions interact continuously with a broad array of prokaryotic and eukaryotic microorganisms. Many of these are injurious, but various soil-dwelling organisms are beneficial to plants. The mechanisms are obvious in some cases. These include symbioses with *Rhizobium* species and the associations which involve mycorrhizal fungi.

They are well known, have been extensively studied, and will not be discussed here. This paper will describe less well defined associations in which soil-dwelling bacteria are distinctly beneficial to growing plants.

Our purpose is to call attention to the variety of ways in which root-associated bacteria interact with plants to improve their growth, to review and evaluate the practicality of deliberate use of bacterial inoculants for this purpose, and to assess the probable value of future research aimed at further understanding and exploiting the interactions described. In an earlier publication, we applied the term "cryptic" to plant-bacterial interactions which are not well understood (Hubbell and Gaskins, 1980).

HABITATS

Microorganisms can form close associations with roots within the root tissue itself, on the root surface (rhizoplane), and within the soil immediately adjacent to the root (rhizosphere). Inhabitants of these sites rely heavily for their energy supply on organic substances provided by the roots, and their growth is therefore related intimately to the metabolic activity of the plants involved. Among these microorganisms, ability to metabolize the relatively simple compounds exuded by living roots is universal. A more limited segment of the population is capable of decomposing proteins, structural polymers and other complex products of plant metabolism. The energy yield from degradation of these materials is relatively low, and the reactions often proceed slowly. Frequently, species other than root-associated microorganisms are involved. Various elements of the complex metabolic interactions between plants and soil microorganisms have been reviewed (Starkey, 1959; Lynch, 1976; Rovira, 1976).

The root

The cap which protects the tip of an elongating root consists of cells which are constantly renewed. Decomposition of the cast-off root cap cells and active secretion of materials by the living cells (Barlow, 1975) provide organic substrates which are used by microorganisms in the immediate vicinity of the root cap. The underlying root tip, where cell division occurs, as well as the region of cell elongation proximal to it, probably contribute less to the substrate pool than either the cap itself or the section where cell maturation occurs. In this latter region, root hairs mature and function, and cause a rapid increase in the surface area and absorptive capacity of this part of the root (Itoh and Barber, 1983). However, since absorptive capacity declines rapidly with age, constant root growth is normal on actively growing plants, to maintain the capability of absorbing nutrients at the rate required by the growing plant. As root tissue matures, root hairs and other cortical cells slough from the surface. The cytoplasm of these cells serves as an important source of nutrients for rhizosphere micro-

organisms, and rapid multiplication of bacteria is normally observed on maturing root tissue where such cellular degradation takes place (Old and Nicholson, 1978; Schippers and van Wurde, 1978).

Deposition of organic material in the soil by roots is not confined to passive loss from senescing tissue. Active exudation of many organic substances has been documented (Bokhari et al., 1979; Matsumoto et al., 1979). Exudation rates vary with age of the root tissue (Hamlen et al., 1972), and have been correlated with development of root hairs (Frenzel, 1960) and emergence of lateral roots (McDougall, 1970). Physical or chemical injury can increase loss of organic substances from roots (Ayres and Thornton, 1968; Barber 1978), and the abrasions that occur normally as roots push their way through the soil have been shown to be sources of some organic compounds found in the rhizosphere (Hale et al., 1971). The constant deposition in the soil of cast-off, or sloughed, debris from the root cap and epidermal surface, coupled with secretion from living cells, provides a substantial quantity of reduced carbon compounds which are readily used as energy sources by microorganisms of the rhizosphere. Hale and co-workers have discussed in detail the various materials deposited by roots and factors influencing rates of deposition (Hale et al., 1971, 1978; Hale and Moore, 1979).

The rhizoplane

This term suggests a precision in definition of the root surface which can be misleading. Root surfaces are not at all planar. Root hairs, which in some cases extend 1 mm or more from their points of origin, create a highly complex interface. This complexity is increased by the presence of living and dead microorganisms, sloughed plant tissue, root exudates, and various degradation products of both plant and microbial origin (Old and Nicholson, 1978; Schippers and van Wurde, 1978). The surface polysaccharides of root tissue are believed to be involved in the capability of symbiotic partners to achieve selective recognition (Albersheim and Anderson-Prouty, 1975). The mechanism and level of specificity involved are often subjects of disagreement.

The physical and chemical nature of the root surface changes substantially with age, and this characteristic, in addition to the complexity noted above, must be considered when the rhizoplane is defined.

The rhizosphere

The region in which growing roots modify their habitat by absorbing water and nutrients and by depositing organic substances is termed the rhizosphere. Significant physical, chemical and biological changes in the soil result. In many instances, intense microbiological activity is made possible by the presence of easily metabolized carbon substrates.

The rhizosphere is generally defined as the volume of soil that is within the zone of influence of living roots. Attempts have been made to define or model the rhizosphere mathematically (Leonard, 1980), but the term remains an imprecise one. Many variables such as age of the plant and of the root tissue involved, species and physiological state, and chemical and physical properties of the soil, influence its size or volume. The complex biological, chemical and physical relationships among rhizosphere components are the subject of several reviews (Starkey, 1959; Katznelson, 1965; Nicholas, 1965).

MECHANISMS WHICH AFFECT PLANT GROWTH AND DEVELOPMENT

Nutrient uptake

The extent to which microorganisms promote uptake of mineral nutrients is a topic of considerable disagreement. Microorganisms may alter nutrient uptake rates by direct effects on the roots, by effects on the environment which in turn modify root behavior, and by competing directly for nutrients.

Many investigators agree that rhizosphere organisms promote uptake of minerals by roots, but there is no generally accepted explanation for the process. Bacteria which reduce the soil pH by production of organic acids or other acidic materials have been studied extensively for their possible role in solubilizing phosphates and other minerals (Mishustin and Naumova, 1962; Bajpai and Sundara Rao, 1971). Although there is some evidence that some bacteria having this capability proliferate selectively in the vicinity of roots, this is not invariably the case. In a recent study, no relationship was identified between rhizosphere pH changes and numbers of acid-producing bacteria (Hedley et al., 1982). Reviews by Barber (1978), Katznelson (1965) and Nicholas (1965) discuss the effects of bacteria on mineral uptake by plants. It is evident from these that this extensively studied subject remains one about which a great deal of conflicting evidence has accumulated. Current research continues to produce conflicting results. Recently, it has been reported that inoculating plants with *Azospirillum* cultures stimulates accumulation of minerals from the soil (Okon, 1982; Lin et al., 1983), but in our own studies this effect has not been seen consistently (M.H. Gaskins and S.L. Albrecht, unpublished data, 1983). Conflicting results produced by so great a number of experiments suggest that the plant bacterial relationship is highly variable under different experimental conditions.

In these cases and others, it is assumed that the beneficial effects of the microorganisms are proportional to their numbers and/or growth rate in the rhizosphere. We are not aware of an instance in which this has been clearly shown for a single bacterial species, although it is generally accepted that bacterial populations are higher in fertile, productive soils than in poor soils. In a short-term solution culture experiment, bacterial numbers

were positively correlated with nitrogen fertilization levels through a considerable range (Lee and Gaskins, 1982). However, in another (Trolldenier, 1972), bacterial numbers were inversely related to levels of potassium fertilization. This effect was attributed to suppression of root exudation by high levels of potassium.

Nitrogen fixation

The highly evolved symbiotic association between leguminous plants and *Rhizobium* bacteria, of great importance in agriculture, is not a complete solution to soil nitrogen deficiencies. Legumes fail to grow well in many climatic conditions, and of course do not provide all needed agricultural commodities. Vast land areas, supporting substantial portions of the world's population, could produce much greater quantities of cereals, forages and other important food and feed crops if economically feasible means were at hand to supply them with additional nitrogen.

Root-associated bacteria capable of fixing nitrogen occur regularly in diverse soils which vary widely in nitrogen content. While it seems that the capability to metabolize dinitrogen should provide a strong competitive advantage in soils of low organic nitrogen content, these bacteria usually are nevertheless far less numerous than are species which are unable to do so. Possible reasons for this are discussed below.

Despite their limited numbers, non-symbiotic nitrogen-fixing bacteria are important in the natural environment. Long-term nitrogen-balance studies have shown them to be capable of significant rates of nitrogen accumulation (Day et al., 1975; Neyra and Döbereiner, 1977). Several studies indicate nitrogen fixation rates of about 30–40 kg ha⁻¹ year⁻¹ by associative bacteria. Much higher estimates have been proposed (von Bülow and Döbereiner, 1975), but these have been based on an assay method of questionable validity (van Berkum and Bohlool, 1980) and are not widely accepted. Various crop-yield studies support the more conservative estimates (Smith et al., 1978; Albrecht et al., 1981). These lower rates, although not sufficient to support maximum crop yields, represent significant nutrient inputs in production of animal-harvested forage crops, in rangeland production, and in diversified low-technology agriculture. A moderate improvement in the nitrogen supply to crops such as these is significant because of their importance in world agriculture.

Sprent (1979) presents an extensive list of species currently known to fix nitrogen. Common genera capable of fixing nitrogen include *Azospirillum*, *Azotobacter*, *Bacillus*, *Clostridium*, *Derxia* and *Klebsiella*. These are commonly designated "freeliving" bacteria, since they are able to exist in the soil and reduce nitrogen without entering into a symbiotic association with plants. While the capability of these organisms to fix nitrogen *in vitro* is easily demonstrated, efforts to determine rates of fixation under natural conditions have produced widely varying results. The acetylene reduction

assay is the most widely used method because of its simplicity and low cost. The procedure has been used as an *in situ* method (Dommergues et al., 1973), with intact root systems (Harris and Dart, 1973) and excised roots (Döbereiner and Day, 1975). The method has been used to demonstrate nitrogen fixation in the rhizosphere of a wide variety of grass species including most, if not all, of the more important cereal crops. A recent review discusses the acetylene reduction assay and its use in studies of associative nitrogen fixation, and points out precautions that must be used in interpretation of results (van Berkum and Bohlool, 1980). It is generally accepted that results based on the acetylene reduction method should be confirmed by ^{15}N incorporation techniques, and this has been done in several studies with nonsymbiotic nitrogen fixation systems (Ruschel et al., 1975; De-Polli et al., 1977; Rennie, 1980; Eskew et al., 1981).

Many crop-inoculation studies have been conducted with root-associated bacteria in the recent past to determine whether the bacteria supply significant amounts of nitrogen to cultivated plants. Crop yields have been increased in many locations (Smith et al., 1976; Kapulnik et al., 1981; Schank et al., 1981). However, results are often negative or inconsistent (Barber et al., 1976; Albrecht et al., 1977), and the significance of the nitrogen-fixation capability of the bacteria has been questioned (Gaskins et al., 1983).

Reasons for variations in crop responses have not been identified satisfactorily. Differences in crop responses may be associated with survival of the inoculant strains in the rhizosphere. There is substantial evidence that survival is often poor. Rapid decline in bacterial numbers, following inoculation into the rhizosphere, has been noted in both greenhouse experiments (Albrecht et al., 1983) and in the field (Smith and Schank, 1982). The apparently limited capability of these rhizosphere bacteria to establish themselves in soils of low nitrogen content, an environmental condition that should favor their survival, has received very little attention and is not understood. It is interesting to note that even when a favorable crop response is achieved, it may be impossible to recover the inoculant organism. It seems highly unlikely when significant crop growth responses occur that the strain fails completely to survive.

The high energy requirement for reduction of dinitrogen is well known. Through specialized morphological and metabolic relationships with host plants, symbiotic endophytes are able to use energy-rich plant metabolites in environments to which potential competitors have no access. In the case of rhizosphere organisms, this highly efficient relationship with a host plant is not present. There is evidence for specificity between host plants and certain non-symbiotic nitrogen-fixing bacteria, but it is not clear whether the efficiency of nitrogen fixation is increased by the interactions which occur. No mechanism has been identified which gives associative nitrogen-fixing organisms a competitive advantage analogous to that available to endophytes within nodules. Facing severe competition

for plant metabolites released by roots, associative organisms absorb at best only a small part of the recently synthesized plant products which might be available to them if competitors were not present. They grow in environments often rich in organic substances produced by earlier generations of plants, but most of these are polymers of limited value as growth substrates. The nitrogen-fixing bacteria grow well and fix nitrogen rapidly only in the presence of energy-rich, low-molecular-weight substances such as simple sugars. Most are not capable of degrading complex molecules such as cellulose. There is evidence that they obtain some energy substrates from activities of organisms having the capability to degrade resistant compounds, but these partnerships show very low efficiency in terms of energy substrate consumed per unit of nitrogen reduced (Jensen, 1965). An additional population of microorganisms must be supported, low energy yields are produced by some of the biochemical steps involved, and many of the potentially degradable polymers in plant residues are protected by highly resistant substances such as lignins.

Denitrification

Denitrification, which transforms reduced nitrogen compounds into gaseous substances, allows return of nitrogen to the atmosphere from the soil. The metabolic capability for denitrification, which is a multi-step process, is confined to a few bacterial genera.

The soil bacteria capable of denitrification are common types. The most frequently isolated denitrifying bacteria are *Alcaligenes*, *Bacillus* and *Pseudomonas* species (Gamble et al., 1977). Although these bacteria are frequently isolated from the soil and rhizospheres of plants, it is difficult accurately to determine the predominant or metabolically active bacterial populations, and which denitrifying organisms are the most important. With the exception of the *Thiobacillus* species, which utilize inorganic sulfur compounds, all oxidize organic substrates. Nitrate is the usual electron acceptor, although some *Alcaligenes* species start the process at nitrite. Dinitrogen is the usual end product, but in the case of some *Pseudomonas* species, the end product is nitrous oxide. The process is usually considered to be one which occurs only under anaerobic conditions, but since anaerobic microsites are common in many soils, this does not prevent denitrification in the rhizosphere of dry-land crops (Brar, 1972).

Denitrification can be increased by the presence of roots (Smith and Tiedje, 1979), particularly when soil nitrate levels are high. The removal of soil nitrogen by denitrifying bacteria is normally considered detrimental to crop production, because in most instances nitrogen is the element which most severely limits plant growth. However, these bacteria are useful in some instances since they prevent nitrogen compounds from accumulating to toxic levels, particularly in poorly drained areas. Also, denitrification activity beneath the root zone may be considered beneficial, since it will

reduce the nitrate load in ground water. Denitrification tends to maintain a balance between soil and atmospheric nitrogen, and thus avoid problems that would become serious if no mechanism were available to return nitrogen to the atmosphere. An extensive account of denitrification processes may be found in a recently published text (Payne, 1981).

Plant growth substances

The significance of plant growth substances in activation and regulation of plant growth and development is beyond the scope of this paper. More general information is available in various reviews and texts (Pilet, 1977; Thimann, 1977). We shall confine our discussion to growth substances produced by rhizosphere microorganisms and the effects of these on plants.

The favorable effects of treating seeds with bacterial suspensions have been recognized for decades. Methods long studied in Russia to improve plant growth by "bacterization", the term applied to coating of seeds with bacteria before planting, are described by Allison (1947) and Mishustin and Naumova (1962). The most widely used bacteria have been strains of *Azotobacter*, perhaps because of their prevalence in cold soils. Mishustin (1970), in discussing various bacterization experiments, noted that most of the Russian tests with *Azotobacter* and other bacterial species did not produce statistically significant yield increases, and indicated doubt that the beneficial effect was a result of nitrogen fixation. However, in some manured experiments, and others in which bacterization was used together with soil enrichment, substantial yield increases frequently occurred. Allison (1947) and Brown et al. (1964) also reported beneficial effects from inoculation experiments.

Brown and Burlingham (1968), after studying the results of *Azotobacter* inoculation, concluded that plant growth substances of bacterial origin accounted for the favorable effect on growth. Subsequently, Brown (1972) showed that various bacterial species from the rhizosphere of wheat produce substances with the properties of gibberellins and auxin, and that responses are similar in plant seedlings either inoculated with these bacteria or receiving authentic plant growth substances.

Production of plant growth substances by bacteria has been confirmed in other investigations (Katznelson and Cole, 1965; Phillips and Torrey, 1972; Tien et al., 1979). It is obvious that this property is common among rhizosphere organisms, although the reason for this is not clear. Nor is it clear whether normal plants need quantities of growth substances greater than they produce themselves. Favorable responses to treatment with plant growth regulating substances are achieved consistently in only a limited number of situations. More general use can be expected as the mechanisms through which these substances control growth are better understood. Further understanding of the mechanisms may permit extensive use of bacteria which supply growth regulators to control and increase plant growth.

Plant growth substances of bacterial origin may be of greatest significance to plants in early growth stages. Although quantities produced are small, the location of production, at the rhizoplane, and time of production, in synchrony with the development of new tissue, certainly allow efficient use if they are needed by the plant tissue. It has been shown (Wightman et al., 1980) that development of exised and intact roots can be accelerated by treatment with a variety of natural or synthetic auxins or cytokinins, and that auxin synthesized by bacteria is absorbed by roots (Libbert and Silhengst, 1970). Growth of small plants can be accelerated by adding plant growth substances to the soil or by adding either live or heat-killed bacteria (Jackson et al., 1964; Gaskins and Hubbell, 1979). These observations suggest that inadequate supplies of growth-promoting hormones may be a common problem in seedling development, and that these supplies can be augmented by rhizosphere bacteria.

Pathogenesis

Plant-microorganism interactions discussed here are concerned only with cryptic plant responses, and clearly identifiable plant disease reactions will not be discussed. A wide variety of substances excreted by microorganisms may injure plants if present in sufficient quantity. Thus their significance is frequently related to environmental conditions which control rates of production and removal. Ammonia, hydrogen sulfide, carbon dioxide, organic acids, growth-regulating hormones, amino acids and antibiotics are well-known metabolic products which, in sufficient concentration, may cause plant injury or death. Their effects, and instances of production in phytotoxic quantities by soil microorganisms, have been reviewed by McCalla and Haskins (1964) and Ponnampereuma (1972). Toxic concentrations of metabolites are often associated with abnormal soil conditions such as unusual temperatures or flooding. These conditions may reduce the capacity of roots to detoxify or remove high concentrations of bacterial products, and may also increase their production by affecting bacterial populations and growth rates.

Competitive interactions

Since the pool of nutrients provided by the rhizosphere must be shared by the roots and all microorganisms in the root zone, growth of any one of these will be significantly affected by the competitive ability of any other. The equilibrium of a system may be severely unbalanced by any disturbance. Indeed, the concept of a state of equilibrium may severely over-simplify the real-world conditions which apply to most environmental systems of agricultural significance. In farmed systems, the cyclical phenomena are obvious, since land preparation, cropping, fertilization, irrigation, crop harvest and other manipulations are evident. In rangelands and forests,

less disturbance results from management practices, but environmental effects such as soil and air temperatures and water availability nevertheless cause biological activity to fluctuate constantly.

In the case of managed agricultural systems, many cyclical phenomena may function at the same time. Moisture content of the soil, level of mineral nutrients, and rate of organic solute production by roots (as governed by plant size, growth rate, age, etc.) are subject to temporal cycles, but are also influenced by management practices. It is possible that purposeful manipulation of environmental conditions can be used as a method for increasing the competitive advantage, and thereby promoting growth, of beneficial rhizosphere organisms. Inoculation efforts in the past have not given specific attention to altering conditions in the soil to promote development of the inoculant organism. Various studies show that this procedure gives beneficial results in many instances.

Beresova and Naumova (1939), in an early report, discussed use of bacteria to suppress pathogenic fungi attacking flax and wheat. Chang and Kommedahl (1968) found that *Fusarium* could be suppressed in the rhizosphere of maize by coating the seeds before planting with *Bacillus* or *Chaetomium*. Merriman and co-workers (1974, 1975), in studying the effectiveness of *Streptomyces* and *Bacillus* isolates in controlling root disease on a variety of crop plants, observed that treatments in some cases caused growth rates to increase although there was no apparent suppression of pathogenic organisms. In those instances, beneficial effects were presumed to result from production by the bacteria of hormonal growth-promoting substances.

More recently, Schroth and Hancock (1982) have used isolates of *Pseudomonas* in attempts to suppress fungal pathogens of potatoes. In these and earlier studies, beneficial effects from inoculation were often seen and, although favorable results did not always occur, the experiments suggest that in some circumstances rhizosphere inoculants were strongly competitive against indigenous pathogenic organisms. Competitive interactions among rhizosphere microorganisms are discussed extensively in the text by Baker and Cook (1974) and in the volume edited by Bruehl (1975a).

MANIPULATION OF RHIZOSPHERE ORGANISMS

Most efforts to establish selected bacteria into the rhizosphere have not been successful. Experiments by Brown et al. (1962), Patel (1969), Patel and Brown (1969) and Ridge (1970) showed that *Azotobacter* inoculants did not survive well in soil. Rovira (1963) reported similar results with *Azotobacter*, but found, however, that *Clostridium*, *Bacillus* and *Pseudomonas* strains survived and multiplied after inoculation into root zones of various plants. Alexander (1981), in a review of microbial predator-parasite relationships, cites various studies to demonstrate that certain of the *Enterobacteraceae* survive in substantial numbers for months after

inoculation. *Azospirillum* strains are believed by some investigators to grow inside the root tissue of certain grass species as an internal symbiont (Döbereiner and Day, 1975; Patriquin and Döbereiner, 1978). While there is little question about their presence inside certain plant roots, it has not been shown conclusively that the internal bacterial cells fix nitrogen. It is evident, however, that the bacteria grow readily in the grass rhizosphere when competing organisms are excluded (Umali-Garcia et al., 1978). It has not been shown, however, that these or other potentially beneficial species do so when competing organisms are present. As pointed out by Bowen and Foster (1978), performance of microorganisms under artificial conditions may provide scant insight into their behavior under natural conditions, where their growth may be severely suppressed by competition from indigenous organisms or other growth-inhibiting environmental conditions. The assumption that introduced bacteria fail to survive in the soil is basic to standard methods of sewage disposal, and is supported by the fact that fecal coliform species in such effluents disappear rapidly when distributed on soil (Bell, 1976). As a result, sewage disposal in this manner is accepted as safe. Whether root-associated strains can be made to act differently in the rhizosphere remains to be demonstrated conclusively.

The major cause for the loss of introduced microorganisms is probably biotic. In addition to competing directly for available energy supplies, soil microorganisms regularly prey on one another. Myxobacteria kill and digest other bacteria and fungi, and many soil amoebae ingest other microorganisms. Several studies have shown that the introduction of bacteria into sterile soil or similar substrates will permit the growth of the bacteria, while inoculation into similar, but unsterilized, material results in the decline of the introduced populations.

Many recent inoculation experiments with *Azospirillum* have been successful in eliciting favorable plant responses, but it is clear that survival of the inoculant organism is poor in most cases. Our ability to control selectively the growth of root-associated organisms is extremely limited, and the extent to which this ability can be developed is not clear. The most direct approach is development of a modified plant or bacterial genome, which would convey a unique affinity for positive interaction with a beneficial partner. This would be an analog of the symbiotic relationships between plant hosts and microorganisms such as *Rhizobium*, *Frankia* and mycorrhizal fungi.

Some efforts to select such beneficial partnerships have been reported (Neal et al., 1973; Shearman et al., 1979; Bouton and Brooks, 1982; Ela et al., 1982). The combinations identified have not been thoroughly studied. Their beneficial effects are probably not highly significant when compared to those of symbiotic combinations found in nature, but these reports suggest that significant progress has been achieved. In view of the potential benefits, research to identify or develop functional combinations of plant and bacterial genotypes is quite limited. This may reflect a frequently

stated argument that attempts to manipulate soil microorganisms are inevitably doomed to failure. In fact, the general effects of many soil treatments, with respect to their influence on soil microorganisms, are quite well known. Some of the variables which might be manipulated to bring about desired changes in soil microorganism populations will be considered briefly.

Chemical treatment

The alteration or manipulation of populations of soil microbes of potential importance in the rhizosphere is accomplished in various ways. Populations may be selectively or non-selectively reduced by application of biocides to soil, a common approach in control of plant pathogens. The calculated alteration of such environmental factors as soil moisture, pH or organic matter content is frequently used to increase or decrease populations of soil microbes.

The ability of plants to absorb chemicals applied externally to leaves, and to translocate these chemicals to the roots, is well documented. These chemicals are often released into the rhizosphere either in the form in which applied or in altered form. This phenomenon presents the possibility of manipulating populations of rhizosphere microorganisms through foliar application of chemicals. The applied compounds could have, as root exudates, many qualitative and quantitative effects on rhizoplane and rhizosphere microflora. The economic feasibility of this technology on a large scale is an unanswered question.

Plant genetics

Root-microbe associations which are stable to any degree have this characteristic; the microbe is living and functioning in an environment which is both favorable and relatively constant (rhizosphere/rhizoplane). This environment, or microhabitat, is created and maintained largely by the genetically-determined morphology and physiology of the plant root, although environmental effects may also play a critical role. Techniques are developing rapidly by which both plants and microorganisms can be genetically altered, and it is reasonable to speculate that such techniques might be used to produce an improved plant-microbe association. It may be possible to create plants having roots which provide in the soil a natural habitat highly favorable to the survival and functioning of a specific indigenous or inoculant microbe.

Atkinson et al. (1975) have shown that, in the case of wheat, different non-pathogenic bacterial genotypes grow in the rhizospheres of different host-plant genotypes. This has also been shown to be the case with rice (Dommergues, 1978), pearl millet (Bouton and Brooks, 1982) and bahia grass (Döbereiner et al., 1972). Many studies with pathogens have shown

that different concentrations of nutrient elements in the soil affect susceptibility of the potential host (Weste and Thrower, 1971; Grinstead et al., 1982). Competitive interactions among saprophytic organisms, involving a variety of potent, broad-spectrum antibiotic compounds, show clearly that some microorganisms use highly sophisticated means to suppress the growth of their competitors (Bruehl, 1975b).

Thus it is clear that mechanisms based on plant genetic differences exist and function efficiently in nature to bring about and maintain differences in populations among the soil biota. With perseverance, it should be possible to devise suitable analogs to these, which can be used in agriculture to achieve more efficient crop growth.

Microbial genetics

Genetic engineering of microorganisms, in order to optimize biochemical reactions of industrial importance, has been a practical reality for many years. In this instance, there is the distinct advantage that all aspects of the altered system can be closely controlled to favor maintenance of the process. Under natural conditions, genetic changes and the complex selective pressures created by fluctuating conditions of the soil environment generally make the "hybrid" microbe transient in its soil sojourn. The organism must survive, multiply and compete in an environment which is rarely, if ever, optimum for the organism or the process. The optimum conditions for microbial processes of agricultural significance occurring under field conditions are poorly understood and crudely controlled at best. Experience indicates that nature does not usually favor the survival of "engineered" strains of microorganisms. The complexity of the natural system severely increases the difficulty of identifying and manipulating all the characteristics needed to produce an "engineered" bacterial strain for a specific use in agriculture. Thus it remains to be demonstrated whether genetic engineering techniques provide a useful approach in the search for superior strains of soil inoculant bacteria. A persistent program of isolation and screening of potentially superior strains produced through the processes of natural selection under field conditions may be the most effective procedure for the foreseeable future.

Outlook

Efforts to develop associative nitrogen fixation systems have not yet shown clear success, but well-documented instances of occasional success clearly justify continued research effort. It seems likely, with the benefit of hindsight, that some efforts to develop or discover associative nitrogen fixation systems should have been better focused on developing fundamental knowledge. However, many field studies and other applied research projects have yielded much useful new knowledge. Despite its negative aspects,

information developed in the past decade has defined existing limitations and focused attention on areas most critically in need of research. Earlier research in plant pathology, stimulated by similar hopes, served a similar useful purpose. Investigators willing to address the problems should be thoroughly familiar with the successes and failures of studies already performed, in order to avoid repetition of earlier investigations when so many avenues are open for new research.

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