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ROLE OF PLANT-MICROBE INTERACTIONS IN ENVIRONMENTAL BIOTECHNOLOGY

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Abstract:

Microbe–plant interactions are complex and are influenced by many factors that contribute to a successful interaction. Also, these microbiota are responsible for many soil physico-chemical, biochemical characterization and activities e.g., decomposing plant residuals and cause diseases with great economic losses, and other can protect crop plants against their phytopathogens or improve the plant nutritional status. Microbial biotechnology addresses the diverse ways in which plant-microbes interactions can be applied to benefit agriculture, industry and the environment. Manipulation of microbial properties and modifications in microbial populations in rhizosphere may lead to new interesting approaches for disease biocontrol and plant growth promotion. The role of plant-microbe interactions can improve soil quality; enhance bioelements sequestration to achieve bioremediation of soil pollutants. Advantages of using plant-microbe interaction for environmental biotechnological application are very important. The use of naturally existing plant-microbe symbiosis for plant growth promotion, biocontrol and organic wastes biodegradation in soil environment can be reduced the application of synthetics of negative impacts on the plant-microbe-soil ecosystem.

Throughout 25 year work with plant-microbes-soil agroecosystem, our results showed plant growth-promoting rhizomicrobiota have been demonstrated positive impacts on plant performance and soil rehabilitations through different mechanisms. For successful application of plant-growth promotion using rhizomicrobial inoculants, many aspects of the plant-soil environment have to be considered in the field. Microbial colonization efficiency is critical for successful plant-soil inoculation. Applications with plant–microbe interactions provide more economical and environmentally sound alternatives to conventional processes.

***Keywords*:**

Plant-microbe interactions, microbial biotechnology, rhizospheric microbes

INTRODUCTION

One major challenge for the twenty-first century will be the production of sufficient food-the United Nations Population Fund estimates that the global human population may well reach 10 billion by 2050 (http://www.unfpa.org). This means increasing agricultural productivity of food crops, as plants form the basis of every food chain.

What has been largely ignored is the important role of microbial communities that interact with plants to influence plant health, productivity and biodiversity. The impact of the microbial world on plants is evident: worldwide each year, microbial diseases cost crop producers billions of Euros. Similarly, the important role of N_2-fixation by rhizobia and other bacteria for plant growth has been known for decades. A greater understanding of how plants and soil microbes live together and benefit each other can therefore provide new strategies to improve plant productivity, while helping to protect the environment and maintain global biodiversity.

Bioremediation is the natural way to cleanup the environment

Regardless of the precise mechanism used by the bacterium to protect plants, the experiments with plant seedlings that certain bacteria may eventually find a use in the development of phytoremediation strategies. In this regard, heavy metals may be removed from polluted soil either by increasing the metal-accumulating ability of plants or by increasing the amount of plant biomass. In heavily contaminated soil where the metal content exceeds the limit of plant tolerance, it may be possible to treat plants with plant growth-promoting bacteria, increasing plant biomass and thereby stabilizing, revegetating, and remediating metal-polluted soils.

Pollution of the biosphere by toxic metals has accelerated dramatically since the beginning of the industrial revolution. The primary sources of this pollution include the burning of fossils fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, and sewage. Toxic-metal contamination of groundwater and soil, which poses a major environmental and human health problem, is currently in need of an effective and affordable technological solution. Moreover, unlike organic pollutants, metals cannot be degraded to harmless products, such as carbon dioxide, but instead persist indefinitely in the environment, complicating their remediation.

"Remediate" means to solve a problem, and "bio-remediate" means to use organisms to solve an environmental problem such as contaminated soil or groundwater.

In a non-polluted environment, bacteria, fungi, and other microorganisms are constantly at work breaking down the contaminant organic matter.

What would occur if an organic pollutant such as oil contaminated this environment?

Some of the microorganisms would die, while others capable of breakdown the organic pollution would survive. Bioremediation works by providing these pollution- breaking down organisms with fertilizer, oxygen, and other conditions that encourage their rapid growth. These organisms would then be able to break down the organic pollutant at a correspondingly faster rate. In fact, bioremediation is often used to help clean up oil spills.

Bioremediation of a contaminated site typically works in one of two ways.

In the case described above, ways are found to enhance the growth of whatever pollution–breaking down microbes might already be living at the contaminated site. In the second, less common case, specialized microbes are added to degrade the contaminants.

Bioremediation provides a good cleanup strategy for some types of pollution, but as you might expect, it will not work for all. For example, bioremediation may not provide a feasible strategy at sites with high concentrations of chemicals that are toxic to most microorganisms. These chemicals include metals such as cadmium or lead, and salts such as sodium chloride.

Bioremediation provides a technique for cleaning up pollution by enhancing the same biodegradation processes that occur in nature. Depending on the site and its contaminants, bioremediation may be safer and less expensive than alternative solutions such as incineration or land-filling of the contaminated materials.

Ground water was leaching such toxic chemicals as benzene from the fuel-saturated soils and carrying them toward a nearby residential area. Contamination had reached the residential area, and the facility was faced with a serious environmental problem. Removing the contaminated soils was technically impractical, and removing contaminated ground water did not address the source of the contaminants. How could contaminated ground water be kept from seeping toward the residential area in the future? One possible solution was a new technology called bioremediation. Studies by the U.S. Geological Survey (USGS) had shown that microorganisms naturally present in the soils were actively consuming fuel-derived toxic compounds and transforming them into harmless CO_2. Furthermore, these studies had shown that the rate of these biotransformations could be greatly increased by the addition of nutrients. By "stimulating" the natural microbial community through nutrient addition, it was theoretically possible to increase rates of biodegradation and thereby shield the residential area from further contamination.

For waste digestion, we can identify several beneficial characteristics that bacteria should have. They must:

Aerobic vs Anaerobic Bioremediation

Pseudomonas These bacteria can be further separated into aerobic types, which require oxygen to live, and anaerobic, which can live without oxygen. (Aerobic bioremediation usually is preferred because it degrades pollutants 10 to 100 times faster than anaerobic bioremediation.) Facultative types can thrive under either aerobic and anaerobic conditions. Certain bacteria belonging to the *Bacillus* and *Pseudomonas* species have these desirable characteristics. They consume organic waste thousands of times faster than the types of bacteria that are naturally present in the waste. They grow and reproduce easily, are non-pathogenic, and do not produce foul odours or gas. These bacteria are cultured on a liquid or dry agar. These cultured bacteria are then freeze dried leaving them in a state of suspension. They remain alive and will function normally as soon as they are dehydrated and put into an acceptable environment. This environment should induce rapid growth and reproduction of these bacteria and must have:

Enzymes

Enzymes are necessary for the proper functioning of the bacteria. An enzyme is a chemical catalyst that breaks up long, complex waste molecules into smaller ones. The smaller particles can be digested directly by the bacteria. Essential nutrients are added to supply the vitamins and minerals required for the growth and activity of the bacteria. These vitamins and minerals might not be present at the contamination site, and a lack of any one of them will inhibit the growth or reproduction of the microbes. They must be added to the site to assure the fastest, most efficient waste digestion.

Bioremediation Processes

Mechanisms of bioremediation include ***bio-augmentation*** in which microbes and nutrients are added to the contaminated site or ***bio-stimulation*** in which nutrients and enzymes are added to supplement the intrinsic microbes. In the injection method, bacteria and nutrients are injected directly into the contaminated aquifer, or nutrients and enzymes, often referred to as “fertilizer”, that stimulate the activity of the bacteria are added. In soil remediation, usually nutrients and enzymes are added to stimulate the natural soil bacteria, though sometimes both nutrients and bacteria are added. When the treatment is stopped, the bacteria die. This technique works best on petroleum contamination.

Bacteria can degrade the following compounds with relative ease

(i) Petroleum or hydrocarbon products: gasoline, diesel, fuel oil
(ii) Hazardous crude oil compounds: benzene, toluene, xylene, naphthalene
(iii) Some polynuclear aromatics
(iv) Some pesticides: malathion
(v) Coal compounds: phenols and cyanide in coal tars and coke waste
(vi) Some industrial solvents: acetone
(vii) Miscellaneous: ethers; simple alcohols such as methanol, and other ground water contaminants including: methylethylketone; ethylene glycol
(viii) Some chemicals are only partially degradable, or sometimes wastes that are so mixed and variable that they degrade at different rates and may leave some toxic chemicals behind. These include:

- TCE (trichloroethylene)
- PCE (perchloroethylene): it degrades to TCE when no oxygen is present
- Pentachlorophenol and other ingredients in coal tar and wood preservatives
- PCBs and dioxin
- Arsenic, chromium and selenium

Currently, experiments are being performed on the bioremediation of certain metals. Heavy metals are not biodegradable, but bacteria can concentrate them into forms that make them more easily disposable. These include: uranium, mercury, cadmium, sulfur, and DDT.

Research on the Degradation of Metals

Cyanobateria

In addition to the research being conducted on the different microbes degrading various metals, research is being performed on algae as well as genetically engineered microbe cultures. Among the algae, blue green algae also known as cyanobacteria (two examples shown to the left), appear to be the most promising. Despite the public outcry against the release of genetically engineered organisms, there are some advantages to these cultures. Many sites have more than one pollutant and genetically engineered microbes are more efficient and do not produce toxic intermediate products. *Pseudomonas* is often used in genetic engineering because certain species have degrative pathways coded for by plasmids. Plasmids are extra chromosomal DNA that are not associated with the nucleus of the cell. By altering the plasmids or adding to them, biodegradation may be accelerated or altered.

In 1992, this theory was put into practice by USGS scientists. Nutrients were delivered to contaminated soils through infiltration galleries, contaminated ground water was removed by a series of extraction wells, and the arduous task of monitoring contamination levels began. By the end of 1993, contamination in the residential area had been reduced by 75%. Nearer to the infiltration galleries, the results were even better. Ground water that once had contained more than 5,000 ppb toluene now contained no detectable contamination.

Why bioremediation works

In the early 1980's, little was known about how toxic wastes interact with the hydrosphere. This lack of knowledge was crippling efforts to remediate environmental contamination under the new super-fund legislation, the Comprehensive Environmental Response, Compensation, and Liability Act. Faced with this problem, USA Congress directed the USGS to conduct a program to provide this critically needed information. By means of this program, known as the Toxic Substances Hydrology Program, the most important categories of wastes were systematically investigated at sites throughout the U.S. One of the principal findings of this program was that microorganisms in shallow aquifers affect the fate and transport of virtually all kinds of toxic substances. For example:

Crude oil: USGS scientists studying the site found that toxic chemicals leaching from the crude oil were rapidly degraded by natural microbial populations. Significantly, it was shown that the plume of contaminated ground water stopped enlarging after a few years as rates of microbial degradation came into balance with rates of contaminant leaching. This was the first and best-documented example of intrinsic bioremediation in which naturally occurring microbial processes remediates contaminated ground water without human intervention.

Sewage effluent: Disposal of sewage effluent in septic drain fields is a common practice throughout the U.S. Systematic studies of a sewage effluent plume led to the first accurate field and laboratory measurements of how rapidly natural microbial populations degrade nitrate contamination (denitrification) in a shallow aquifer.

Chlorinated solvents: Chlorinated solvents are a particularly common contaminant in the heavily industrialized Northeast. Because their metabolic processes are so adaptable, microorganisms can use chlorinated compounds as oxidants when other oxidants are not available. Such transformations, which can naturally remediate solvent contamination of ground water, has been extensively documented by USGS scientists.

Pesticides: Pesticide contamination of rivers and streams is a matter of concern throughout the U.S. Field and laboratory studies have shown the effects of biological and non-biological processes in degrading commonly used pesticides, such as molinate, thiobencarb, carbofuran, and methyl parathion.

Agricultural chemicals: Agricultural chemicals affect the chemical quality of ground water. Studies have traced the fate of nitrogen fertilizers and pesticides in ground and surface waters. These studies have shown that many common contaminants, such as the herbicide atrazine, are degraded by biological (microbial degradation) and non-biological (photolytic degradation) processes.

Gasoline contamination: Gasoline is probably the most common contaminant of ground water in the U.S.. Studies at this site have demonstrated rapid microbial degradation of gasoline contaminants and have shown the importance of processes in the unsaturated zone in degrading contaminants.

Creosote contaminants: Creosote and chlorinated phenols have been used extensively as wood preservatives throughout the U.S. Contaminants leaked to the underlying aquifer through several unlined ponds. Studies have demonstrated that microorganisms can adapt to extremely harsh chemical conditions and that microbial degradation was restricting migration of the contaminant plume.

Technology Transfer: Methods and technology developed in the Toxic Substances Hydrology Program are now being used by private contractors, State environmental managers, and other Federal agencies to address contaminant problems throughout the U.S.

Treating contamination in place: Most of the cost associated with traditional cleanup technologies is associated with physically removing and disposing of contaminated soils. Because engineered bioremediation can be carried out in place by delivering nutrients to contaminated soils, it does not incur removal-disposal costs.

Harnessing natural processes: At some sites, natural microbial processes can remove or contain contaminants without human intervention. In these cases where intrinsic bioremediation (natural attenuation) is appropriate, substantial cost savings can be realized.

Reducing environmental stress: Because bioremediation methods minimize site disturbance compared with conventional cleanup technologies, post-cleanup costs can be substantially reduced.

In the environment, the roots of plants interact with a large number of different microorganisms, and these interactions, together with the soil conditions, are major determinants of the extent to which plants grow and proliferate. We previously reported that many plant growth-promoting bacteria, i.e., free-living soil bacteria that are involved in a beneficial association with plants, contain the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. It was hypothesized that this enzyme, which has no known function in bacteria, might be part of a hitherto undescribed mechanism used by certain bacteria to stimulate plant growth. This could occur by ACC deaminase modulating the level of ethylene in developing plants.

It is well documented that plants respond to a variety of different environmental stresses by synthesizing "stress" ethylene. In fact, a significant portion of the damage to plants from environmental stress as infection with fungal phytopathogens may occur as a direct result of the response of the plant to the increased level of stress ethylene. In the presence of fungal pathogens, not only does exogenous ethylene increase the severity of a fungal infection but also inhibitors of ethylene synthesis can significantly decrease the severity of infection. Since the enzyme ACC deaminase, when present in plant growth-promoting bacteria, can act to modulate the level of ethylene in a plant, we sought in the work reported here to test whether such bacteria might lower the stress placed on plants by the presence of heavy metals and therefore ameliorate some of the apparent toxicity of heavy metals to plants.

Heavy metal contamination of soil causes a variety of environmental problems, and the remediation of heavily contaminated soils often-involves excavation and removal of soil to secured landfills, a technology that is expensive and requires site restoration. Alternatively, heavy-metal contaminated soil may be dealt with by phytoremediation, which is the use of plants to remove, destroy, or sequester hazardous substances from the environment (CUNNINGHAM & BERTI 1993, CUNNINGHAM et al. 1995, CUNNINGHAM & OW 1996, SALT et al. 1995). Metal-tolerant plants have been used to vegetate and control soil erosion on metal mine tailings and waste piles (CUNNINGHAM & OW 1996, REID et al. 1986). Moreover, metal accumulating plants have been used to remove toxic metals from soil (BAKER et al. 1991, CUNNINGHAM & BERTI 1993, CUNNINGHAM et al. 1995, CUNNINGHAM & OW 1996, KUMAR et al. 1995). Certainly, the concept of using plants to remediate heavy metal contaminated soils has been receiving increasing attention (KUMAR et al. 1995). The efficiency of phyto-accumulation is dependent on two main factors:

(i) plants must be able to take up and accumulate high amounts of metal and

(ii) they must be able to produce as much biomass as possible.

Unfortunately, even the growth of metal-resistant metal-accumulating plants can be severely inhibited when the concentration of available metal in the contaminated soil is very high. These results in a decrease in plant biomass and, thereby, in the efficiency of phytoremediation.

One way to relieve the toxicity of heavy metals to plants might involve the use of plant growth-promoting bacteria, free living soil bacteria that exert some beneficial effect on plant development when they are either applied to seeds or incorporated into the soil (GLICK 1995, GLICK et al. 1999). Mechanisms of plant growth-promotion include: N_2-fixation; synthesis of siderophores which can solubilize and sequester iron from the soil; production of phytohormones such as auxins and cytokinins, which can enhance plant growth; and solubilization of minerals such as phosphorus (GLICK 1995, GLICK et al. 1995, GLICK et al. 1999). In addition, some plant growth-promoting bacteria contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (SHAH et al. 1997, GLICK et al. 1998) which can cleave the plant ethylene precursor ACC, and lower the level of ethylene in a developing or stressed plant. Plant growth-promoting bacteria that contain ACC deaminase may act to insure that the ethylene level does not impair root growth (GLICK et al. 1998), and by facilitating the formation of longer roots, these bacteria may enhance seedling survival. A particular bacterium may use any one, or more, of these mechanisms. Moreover, since many plant growth-promoting bacteria possess several of these traits, a bacterium may utilize different traits at various times during the life cycle of the plant, and the impact of the bacterium on plant growth may vary depending upon the soil chemical and physical properties.

Phytoremediation

Living plants have the ability to accumulate heavy metals from soil and water, in particular heavy metals which are essential for their growth and development. Certain plants also have the ability to accumulate heavy metals which have no known biological function. However, excessive accumulation of these metals can be toxic to most plants. Heavy metals ions, when present at an elevated level in the environment, are adsorbed by roots and translocated to different plant parts, leading to impaired metabolism and reduced growth.

Phytoremediation, i.e., the use of green plants to remove, contain, or render harmless environmental contaminants, is considered to be an attractive alternative to the approaches that are currently in use for dealing with heavy metal contamination. Phytoremediation of metals might take one of several forms:

Phytoextraction refers to processes in which plants are used to concentrate metals from the soil into the roots and shoots of the plant; ***rhizofiltration*** is the use of plant roots to remove metals from effluents; and ***phytostabilization*** is the use of plants to reduce the mobility of heavy metals (and thereby reduce the spread of these metals in the environment). Recently, metal-tolerant plants have been used to vegetate and control soil erosion on metal mine tailings and waste piles, i.e., ***phytostabilization***. Moreover, there are a number of reports of using metal accumulating plants to remove toxic metals from soil, i.e., phytoextraction-also called ***phytodecontamination***.

Phytoremediation is the use of plants and trees to clean up contaminated soil and water. This technology is currently in its infancy, and more research needs to be done before it is widely accepted as a remediation technique. However, the future is promising. Currently, the majority of research is concentrated on determining the best plant for the job, quantifying the mechanisms by which the plants convert pollutants, and determining which contaminants are amenable to phytoremediation. Polluted sites are being studied, and phytoremediation looks promising for a variety of contaminants. This technology is useful for soil and water remediation, however, this discussion primarily focuses on groundwater phytoremediation.

Phytoextraction: is the uptake and storage of pollutants in the plants stem or leaves. Some plants, called hyper-accumulators, draw pollutants through the roots. After the pollutants accumulate in the stem and leaves the plants are harvested. Then plants can be either burned or sold. Even if the plants cannot be used, incineration and disposal of the plants is still cheaper than traditional remediation methods. As a comparison, it is estimated a site containing 5000 tons of contaminated soil will produce only 20-30 tons of ash (BLACK 1995). This method is particularly useful when remediating metals.

Phytovolatization is the uptake and vaporization of pollutants by a plant. This mechanism takes a solid or liquid contaminant and transforms it to an airborne vapour. The vapour can either be the pure pollutant, or the pollutant can be metabolized by the plant before it is vaporized, as in the case of mercury, lead and selenium (BOYAJIAN & CARRIERA 1997, BLACK 1995, WANTANBE 1997).

Phytodegradation is plants metabolizing pollutants. After the contaminant has been drawn into the plant, it assimilates into plant tissue, where the plant then degrades the pollutant. This metabolization by plant-derived enzymes such as nitroredictase, laccase, dehalogenase, and nitrilase, has yet to be fully documented, but has been demonstrated in field studies (BOYAJIAN & CARRIERA 1997). The daughter compounds can be either volatized or stored in the plant. If the daughter compounds are relatively benign, the plants can still be used in traditional applications. If the daughter compounds are less harmful than the parent compound, but not benign, then the plants can be burned or used in alternate applications. A proposed degradation mechanism for atrazine (BURKEN & SCHNOOR 1997). The most effective current phytoremediation sites in practice combine these three mechanisms to clean up a site. For example, poplar trees can accumulate, degrade and volatize the pollutants in the remediation of organics.

A number of heavy metals are required by plants as micronutrients to act as cofactors as part of prosthetic groups of enzymes which are involved in a wide variety of metabolic pathways. However, when they are present in high levels, most heavy metals are toxic to plants. Thus, significant phytoremediation of heavy metal contaminated soils can only succeed if this normal phytotoxic effect can be overcome, e.g., by utilizing plant species that are tolerant to high concentrations of various metals (BAKER et al. 1991). Unfortunately, heavy metals can even be toxic for metal accumulating and metal-tolerant plants, if the concentration of metals in the environment is sufficiently high.

Heavy metal contamination of soil is often associated with iron deficiency in a range of different plant species. The low iron content of plants that are grown in the presence of high levels of heavy metals generally results in these plants becoming chlorotic, since iron deficiency inhibits both chloroplast development and chlorophyll biosynthesis.

Once they have bound iron, microbial iron-siderophore complexes can be taken up by plants, and thereby serve as an iron source for plants (BAR- NESS et al. 1991, REID et al. 1986, WANG et al. 1993). It was therefore reasoned that the best way to prevent plants from becoming chlorotic in the presence of high levels of heavy metals was to provide them with an associated siderophore-producing bacterium that could provide a sufficient amount of iron to the plant.

Techniques

Phytoremediation is more than just planting and letting the foliage grow; the site must be engineered to prevent erosion and flooding and maximize pollutant uptake. There are three main planting techniques for phytoremediation:

1. Growing plants on the land, like crops. This technique is most useful when the contaminant is within the plant root zone, typically 3-6 feet, or the tree root zone, typically 10-15 feet.
2. Growing plants in water (aquaculture). Water from deeper aquifers can be pumped out of the ground and circulated through a "reactor" of plants and then used in an application where it is returned to the earth (e.g. irrigation).
3. Growing trees on the land and constructing wells through which tree roots can grow. This method can remediate deeper aquifers *in-situ*. The wells provide an artery for tree roots to grow toward the water and form a root system in the capillary fringe.

Determining which plant to use

The majority of current research in the phytoremediation field revolves around determining which plant works most efficiently in a given application. Not all plant species will metabolize, volatize, and / or accumulate pollutants in the same manner. The goal is to ascertain which plants are most effective at remediating a given pollutant. Phytoremediation has been shown to work on metals and moderately hydrophobic compounds such as BTEX compounds, chlorinated solvents, ammunition wastes, and nitrogen compounds.

Advantages and Disadvantages to Phytoremediation

Advantages:

1. Aesthetically pleasing.
2. Solar driven.
3. Works with metals and slightly hydrophobic compounds, including many organics.
4. Can stimulate bioremediation in the soil closely associated with the plant root. Plants can stimulate microorganisms through the release of nutrients and the transport of oxygen to their roots.
5. Relatively inexpensive-phytoremediation can cost as little as \$10-\$100/cubic yard whereas metal washing can cost \$30-\$300/ cubic yard (WANTANBE 1997).
6. Even if the plants are contaminated and unusable, the resulting ash is approximately 20-30 tons per 5000 tons soil (BLACK 1997).
7. Having ground cover on property reduces exposure risk to the community (i.e. lead).
8. Planting vegetation on a site also reduces erosion by wind and water
9. Can leave usable topsoil intact 10.

Disadvantages:

1. Can take many growing seasons to clean up a site.

2. Plants have short roots. They can clean up soil or groundwater near the surface in-situ, typically 3-6 feet (ECOLOGICAL ENGINEERING 1997), but cannot remediate deep aquifers without further design work..
3. Trees have longer roots and can clean up slightly deeper contamination than plants, typically 10-15 feet, but cannot remediate deep aquifers without further design work.
4. Trees roots grow in the capillary fringe, but do not extend deep in to the aquifer. This makes remediating DNAPL's in situ with plants and trees not recommended.
5. Plants that absorb toxic materials may contaminant the food chain.
6. Volatization of compounds can transform a groundwater pollution problem to an air pollution problem.
7. Returning the water to the earth after aquaculture must be permitted.
8. Less efficient for hydrophobic contaminants, which bind tightly to soil.

Future Challenges

Although bioremediation holds great promise for dealing with intractable environmental problems, it is important to recognize that much of this promise has yet to be realized. Specifically, much needs to be learned about how microorganisms interact with different hydrologic environments. As this understanding increases, the efficiency and applicability of bioremediation will grow rapidly. Bioremediation has proven successful on petroleum and hydrocarbon contamination. Currently research is being performed on the use of microbes to degrade metals. The use of algae and genetically engineered cultures is also being researched.

Because of its unique interdisciplinary expertise in microbiology, hydrogeology, and geochemistry, the agricultural microbiologists and soil biotechologists will continue to be at the forefront of this exciting and rapidly evolving technology.

CONCLUSION

Plant growth promoting rhizobacteria (PGPR) are known to influence plant growth by various direct or indirect mechanisms. In search of efficient PGPR strains with multiple activities, a total of 72 bacterial isolates belonging to Azotobacter, fluorescent Pseudomonas, Mesorhizobium and Bacillus were isolated from different rhizospheric soil and plant root nodules in the vicinity of Aligarh. These test isolates were biochemically characterized. These isolates were screened in vitro for their plant growth promoting traits like production of indoleacetic acid (IAA), ammonia (NH3), hydrogen cyanide (HCN), siderophore, phosphate solubilization and antifungal activity. More than 80% of the isolates of Azotobacter, fluorescent Pseudomonas and Mesorhizobium ciceri produced IAA, whereas only 20% of Bacillus isolates was IAA producer. Solubilization of phosphate was commonly detected in the isolates of Bacillus (80%) followed by Azotobacter (74.47%), Pseudomonas (55.56%) and Mesorhizobium (16.67%). All test isolates could produce ammonia but none of the isolates hydrolyzed chitin. Siderophore production and antifungal activity of these isolates except Mesorhizobium were exhibited by 10–12.77% isolates. HCN production was more common trait of Pseudomonas (88.89%) and Bacillus (50%). On the basis of multiple plant growth promoting activities, 11 bacterial isolates (seven Azotobacter, three Pseudomonas and one Bacillus) were evaluated for their quantitative IAA production, and broad-spectrum (active against X three test fungi) antifungal activity. Almost at all concentration of tryptophan (50-500 mg/ml), IAA production was highest in the Pseudomonas followed by Azotobacter and Bacillus isolates. Azotobacter isolates (AZT3, AZT13, AZT23), Pseudomonas (Ps5) and Bacillus (B1) showed broad-spectrum antifungal activity on Muller-Hinton medium against Aspergillus, one or more species of Fusarium and Rhizoctonia bataticola. Further evaluation of the isolates exhibiting multiple plant growth promoting (PGP) traits on soil-plant system is needed to uncover their efficacy as effective PGPR.

During long-term experiments during the last 25 years, the results revealed that plant-microbe systems may be useful in bioremediation. Rhizomicrobes have evolved to colonize and compete in a rhizosphere environment. Expanding the metabolic functions of such rhizomicrobes to degrade pollutants may prove to be a useful strategy for bioremediation. The mechanisms underlying the colonization of rhizomicrobes are poorly understood. The over-expression of a colonization gene of a rhizobacterium of a plant caused an increase in the extent of colonization. To construct a more efficient bioremediation system, we are now going to analyze the mechanism of interaction between plants and their symbionts. The efficient attachment of microorganisms to host plants as well.

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