

point in time, and good relationships predictably outgrow their original contracts. For the relationship to grow and mature well over the years requires careful management of expectations' (Ref. 12, and cited in Ref. 10).

References

- 1 UN (1992) *Convention on Biological Diversity*, United Nations
- 2 Posey, D. (1990) *Anthropol. Today* 6, 13–16
- 3 Wolf, R. *Business Monday, San Jose Mercury News* 25 July 1994, pp. 1F & 8F
- 4 Downes, D. (1994) *BioScience* 44, 381–383
- 5 Hobbelink, H. (1991) *Biotechnology and the Future of World Agriculture*, p. 5, Zed Books
- 6 Barton, J. H. and Siebeck, W. E. (1994) in *Materials Transfer Agreements for the International Agricultural Research Centers?*, p. 53, International Plant Genetic Resources Institute
- 7 Reid, W. V. et al. (1993) in *Biodiversity Prospecting: Using Genetic Resources for Sustainable Development* (Reid, W. V. et al., eds), pp. 1–52, World Resources Institute
- 8 King, S. and Tempesta, M. S. (1994) in *Ethnobotany and the Search for New Drugs* (Ciba Found. Symp.) (Vol. 185) (Chadwick, D. and Marsh, J., eds), pp. 197–206, Wiley
- 9 Ryan, J. C. (1991) *World Watch*, July/August, pp. 19–26
- 10 Laird, S. A. (1993) in *Biodiversity Prospecting: Using Genetic Resources for Sustainable Development* (Reid, W. V. et al., eds), pp. 99–130, World Resources Institute
- 11 Escobar, G. *The Washington Post* 30 July 1995 'Rainforest May Hold Prescription for Economic Survival' (Feature Article)
- 12 Sherblom, J. (1991) in *The Business of Biotechnology: From the Bench to the Street* (Ono, D., ed.), p. 223, Butterworth Heinemann

Engineering the rhizosphere: expressing a bias

Kevin P. O'Connell, Robert M. Goodman and Jo Handelsman

The rhizosphere is a largely unexplored frontier for genetic engineering. Processes in the rhizosphere influence plant disease, plant nutrition and root architecture by affecting the dynamics of microbial populations and communities. There is interest in engineering plants to manipulate the rhizosphere for numerous reasons. Such plants might resist soilborne pathogens more effectively, be better hosts to beneficial microorganisms, remediate toxic waste, or attract communities of soil microorganisms that enhance plant health. Central among the strategies to engineer the rhizosphere is the effort to create a 'biased rhizosphere', which involves engineering plants to secrete nutrients that specifically enhance the growth of desirable microorganisms.

The exchange of nutrients and signals between parts of the plant that are above and below ground is the result of a remarkable genetic and developmental integration of roots and shoots. Roots supply inorganic nutrients, including water, to the rest of the plant, and play a crucial role in the hormone gradients that govern shoot ontogeny. Shoots fix carbon through photosynthesis, and transport organic carbon to the roots, which excrete a significant proportion of the plant's carbon into the surrounding environment. This surrounding environment, or the volume of soil that is influenced biologically and biochemically by the living root, is known as the rhizosphere^{1,2}. Root

exudates and secretions create a 'rhizosphere effect' (Ref. 2), which manifests itself in the intense microbial activity that is associated with the immediate vicinity of the root.

Some of the microorganisms in the rhizosphere contribute to plant health by mobilizing nutrients, some are detrimental to plant health because they compete with the plant for nutrients or cause disease, and some stimulate plant growth by producing hormones or suppressing pathogens. Alteration of the biological and chemical composition of the rhizosphere can, therefore, be expected to modify plant health. Historically, various methods have been used to modify the rhizosphere, including the application of organic amendments and microbial inoculants, and the manipulation of plant genotypes^{3–6}.

In this review, we focus on plant genetic engineering to influence the rhizosphere; this involves manipulating

K. P. O'Connell is at the NSF Center for Microbial Ecology, Michigan State University, East Lansing, MI 48824, USA. R. M. Goodman and J. Handelsman (joh@plantpath.wisc.edu) are at the Department of Plant Pathology, 1630 Linden Drive, University of Wisconsin, Madison, WI 53706, USA.

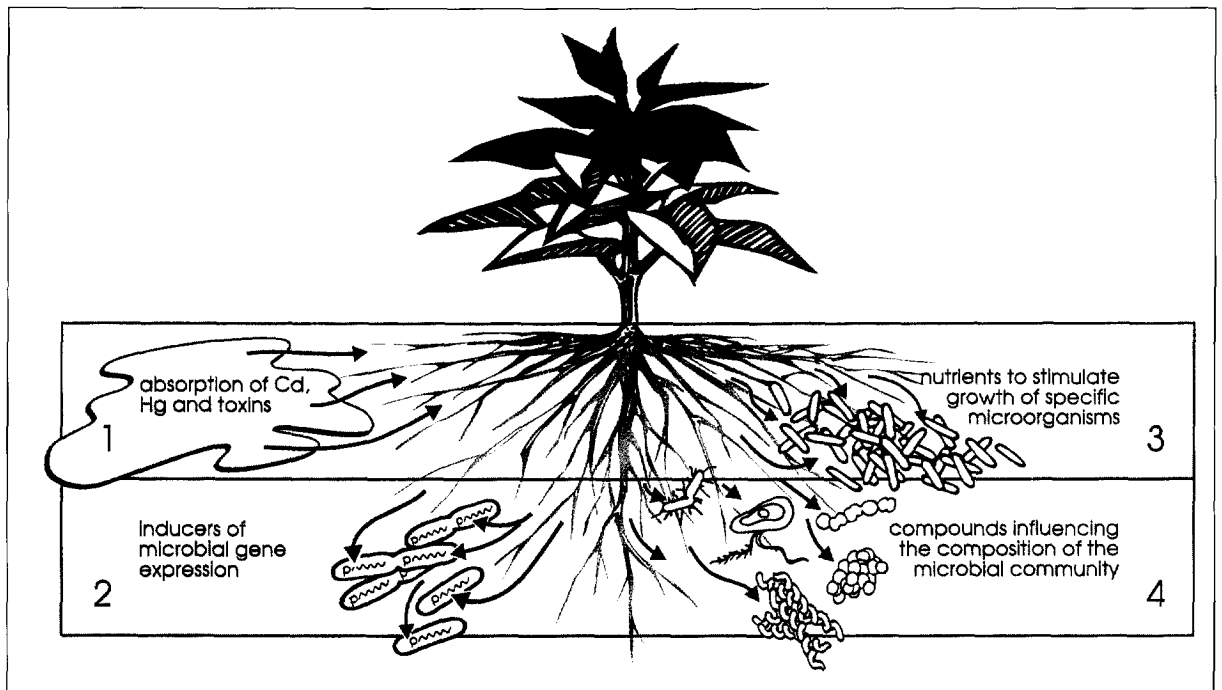


Figure 1

An overview of engineering the rhizosphere. (1) Phytoremediation by engineering plants to take up toxins and metals. (2) Enhancing the effects of beneficial microorganisms by engineering plants to release specific inducers of microbial genes. (3) Enhancing populations of beneficial microorganisms by engineering plants to produce an unusual nutrient and engineering a microorganism to catabolize the nutrient. (4) Engineering plants to produce compounds that affect the overall composition of the community of microorganisms associated with roots.

gene expression in the root for the purpose of modifying the soil or microbial flora surrounding the root to achieve desirable environmental or plant-health effects (Fig. 1). We present an overview of current work in the field, and speculate about other worthwhile approaches that deserve attention.

Engineering the rhizosphere

Engineering of the rhizosphere is an emerging field in which little is known, and in which few of the popular hypotheses that abound have been fully tested. As yet, there have been no field trials that we know of for evaluating plants that have been genetically engineered specifically to alter the function of the rhizosphere. While technical barriers and a lack of knowledge about root gene expression and rhizosphere function may account for the present status of the field, many excellent strategies have been proposed or are being investigated. New techniques, from molecular to optical, enable a more critical study of roots, encouraging an overdue and welcome surge of interest in the biology of roots, the rhizosphere and rhizosphere-microorganism interactions.

Required knowledge: molecular biology

Engineering the rhizosphere requires molecular techniques for introducing and regulating gene expression in roots, as well as an understanding of tissue-specific gene expression. Significant progress toward developing precise methods for engineering roots has been reported in recent years. Promoters whose

regulation is cell- or tissue-type specific in roots have been isolated and studied in some detail⁷⁻⁹. One tissue that offers tremendous potential for the genetic engineering of the rhizosphere is the group of cells known as border cells. These are released from the roots into the surrounding soil where they remain metabolically active for days after release (Fig. 2). These cells effectively increase the dimensions of the rhizosphere, and may play significant roles in mediating rhizosphere processes¹⁰. Border cells are, therefore, particularly attractive targets for engineering because they constitute a distinct tissue type, express a unique complement of genes, and seem to be specifically adapted to mediating rhizosphere processes¹¹.

Required knowledge: community ecology

Knowledge of gene expression in roots is only one facet of successful engineering of the rhizosphere; understanding the ecology of the root and soil microflora is essential for developing sound technical strategies to engineer the rhizosphere. Although we know that rhizosphere communities are dynamic and complex, little is known about the biological rules governing the behavior of these communities. Specific organisms have been the focus of research – an emphasis that has been determined, in part, by current knowledge and available methodology. We know more about the influence of individual microbial species on plant health than we do about the influence of entire communities, and there are better methods available for studying populations of specific

members of the communities than for describing entire communities. However, despite current limitations, there is likely to be more interest in future in manipulating the composition of rhizosphere communities through plant genetic engineering. It is also intuitive that the plant genotype will influence total microbial communities associated with the root, as plant genes control photosynthesis, transport, exudation and root architecture. All of these functions should influence the dynamics and composition of microbial communities associated with roots. Indeed, past work has suggested that the roots of plants of different genotypes harbor communities of different compositions⁶; other work has shown that factors such as soil amendments, cover crops and genetic resistance influence both root disease and the composition of microbial communities associated with roots³⁻⁶. Although a causal relationship between the changes in communities and plant health has not been demonstrated, the correlation that has been shown in many systems is intriguing and worthy of further investigation¹².

Engineering plants to attract and support complex communities of microorganisms that contribute to root health, rather than focusing on single strains or species, might offer advantages. Community-based strategies might be more robust and longer-lasting because, for example, pathogen resistance should be more difficult to select, and the complexity of the community should provide a resilience that resistance genes might not. Exploiting the very complexity that makes studying microbial communities a challenge may provide the antidote to the rapid decline of efficacy observed with many pest-management strategies.

It is likely that there will be an explosion of knowledge about rhizosphere communities as the tools of modern biology are used to dissect the structure and function of communities. Powerful methods in which DNA is isolated directly from bacteria in environmental samples have provided new insights into microbial communities that are difficult to culture^{13,14}. With these methods, the genes for 16S rRNA can be amplified by polymerase chain reaction (PCR), cloned, sequenced and aligned with known sequences to determine the phylogeny of the organisms in the sample. The application of these methods to soil and rhizosphere communities is just beginning¹⁵ but, based on the revolution these methods have caused in our understanding of other environments, they are likely to alter rapidly our understanding of the rhizosphere. Moreover, plant genetic engineering provides the tools to test hypotheses about the role of the plant genotype in shaping rhizosphere communities. Defined changes in the host genotype can be used to study the influence of specific plant functions on communities that develop in the rhizosphere.

In the following sections, we discuss approaches to engineering that focus on manipulating the composition and function of the rhizosphere through changes in root exudation to enhance or inhibit microbial growth, to influence microbial gene expression, or to alter the chemical environment around the root.

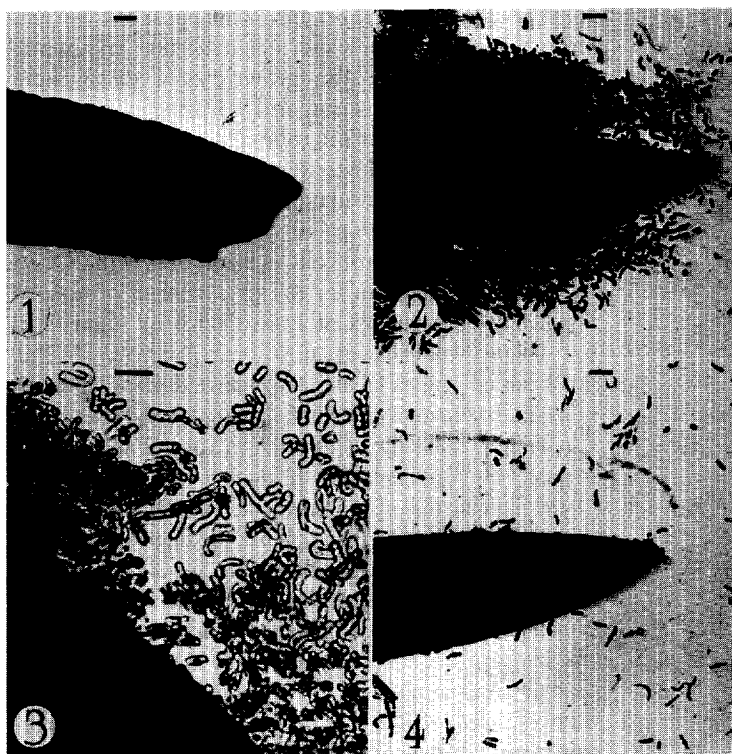


Figure 2

Border cells may be a key to engineering the rhizosphere. Border cells are metabolically active cells that are released from roots; they express a unique complement of genes, thus providing an appropriate target for engineering the rhizosphere. (1) Root of two-day-old cotton seedling germinated on water agar. (2) The same root 60 s after adding a 100- μ l droplet of water. (3) The same root at higher magnification. (4) Release of border cells after agitating the water droplet. In each case, the bar represents 100 μ m. (Reproduced, with permission, from Ref. 10.)

Engineering root exudation

Many types of compounds, such as amino acids, organic acids, sugars, flavonoids, proteins and mucilage, are exuded from roots. Engineering the rhizosphere by altering root exudation could influence microbial populations by inhibiting or enhancing the growth of select microbial members of the rhizosphere community. Current work largely focuses on influencing populations of one species or strain of bacteria on roots and, in particular, on problems for which there is ample evidence of a connection between the abundance of specific bacteria and root health.

Engineering inhibitors

Peptides and proteins that have inhibitory activity against fungi, bacteria and invertebrates such as nematodes and aphids have been described^{16,17}. If they are appropriately engineered for expression in the root, such peptides and proteins should readily diffuse into the rhizosphere; they seem to be logical candidates for genetic engineering, as most are encoded by single genes that could be (and, in a few cases, have been) introduced into plants¹⁷. A more difficult engineering feat would be to introduce into plants the complex biosynthetic machinery for molecules such as antibiotics, the synthesis of which requires many gene products.

Engineering enhancers: origins in the opine concept

Several laboratories are constructing transgenic plants that promote the growth of a rhizosphere inhabitant by synthesizing and releasing compounds that only the desired microorganism can catabolize. This approach has been termed the 'biased rhizosphere' by de Bruijn and colleagues¹⁸; the strategy was suggested by the biology of *Agrobacterium tumefaciens*, which transfers to plant cells a region of plasmid DNA that encodes enzymes for the biosynthesis of opines. Opines are derivatives of amino acids or sugars that few known soil microorganisms can catabolize¹⁹. The synthesis of opines by the transformed plant tumor is believed to create a nutritional niche for the pathogen, specifically favoring its growth in the vicinity of the tumor^{19,20}. This theory is known as the 'opine concept'. To test the opine concept, and to extend it to other plant-microbe interactions, transgenic tobacco²¹ and *Lotus corniculatus*²² plants that synthesize opines and release them into the rhizosphere have been constructed. Guyon *et al.*²² have demonstrated that opines produced by the roots of transgenic plants lead to an increase in the population of opine-catabolizing *Agrobacterium*, compared with a non-catabolizing strain, thus validating the opine concept in the laboratory.

A variation on the current concept of the biased rhizosphere is to engineer plants to overproduce a common nutrient that is limiting for growth of a desirable microorganism in the rhizosphere. Although plants release a rich variety of nutrients in their exudates, the composition may not match the nutritional needs of a desired organism. Therefore, the abundance of a limiting nutrient could be increased through the modification of root gene expression.

Biased rhizospheres: potential problems

A major difficulty in the nutritional biasing of the rhizosphere is that selecting a compound to encourage the growth of a particular microorganism or a desired consortium of microorganisms requires a knowledge of what nutrients are limiting in the rhizosphere. Despite being able to make sole use of accessible nitrogen and carbon, an introduced microorganism might still find itself competing with native inhabitants for other essential nutrients. Furthermore, the diversity of native rhizosphere inhabitants will affect what nutrients are limiting in the rhizosphere. While the opine concept proves valid when two otherwise isogenic *Agrobacterium* strains are the only known rhizosphere inhabitants, there is no published evidence demonstrating that the bias favoring a particular organism in the rhizosphere holds up in the presence of an entire rhizosphere microbial community.

The second problem with the biased rhizosphere is one of uniqueness. The ability to catabolize opines is not exclusive to agrobacteria²³; some pseudomonads can use opines as carbon and nitrogen sources, and have been shown to surpass *Agrobacterium* in competing for the limiting opine in a chemostat²⁴. Extensive

farming with plants that produce novel nutrients, such as opines, will certainly result in increased populations in the rhizosphere of naturally occurring competitors, or will select for the rare organisms that acquire the catabolic genes by horizontal transfer. This problem could be compounded by the transfer of the biosynthetic genes for the novel compound to other plants by pollen movement, thereby increasing the overall environmental exposure to the compound, and providing greater selection for organisms that catabolize it. High populations of native organisms that catabolize the novel nutrient will reduce the selective advantage provided to the desired microorganism by increasing competition for the nutrient in the rhizosphere. Therefore, successful long-term use of the biased-rhizosphere approach will require the development of strategies for minimizing the amount of the compound in the environment. The biosynthetic genes in the plant could be placed under the control of inducible promoters that would be induced by the plant, or by the application of a chemical inducer at the time that growth of the beneficial organisms was desired.

Engineering to induce microbial gene expression

Small molecules other than nutrients also influence the biology of the rhizosphere. Plant and bacterial signal molecules, such as the isoflavonoids and lipooligosaccharides, respectively, of the legume-rhizobial symbiosis, activate gene expression in the other partner at very low concentrations^{25,26}. In addition, some plant signal molecules increase the growth rate of rhizobia at low concentrations²⁷. Plants could be engineered to release a small-molecule inducer of bacterial gene expression into the rhizosphere. In response, an engineered microbial population would synthesize a desired product (such as a siderophore) that discourages the growth of a rhizosphere competitor for iron, an antibiotic effective against root pathogens, or a compound that disrupts the life cycles of insects and nematodes in the vicinity of the root. Although this seems to be a rational approach there are, as yet, no examples of engineering plants to induce microbial gene expression.

Applications of engineering the rhizosphere**Nitrogen fixation**

Rhizosphere phenomena influenced by the size of a particular microbial population are obvious subjects for testing and applying the biased-rhizosphere concept. For example, the proportion of root nodules occupied by a given strain of *Rhizobium* is proportional to that strain's representation in the mixture of rhizobia applied to the plant^{28,29}. Engineering plants to release a nutrient catabolized only by the desired strain might increase that strain's representation in the rhizosphere and, thereby, enhance its nodule occupancy. Implicit in this approach are the assumptions that populations in the rhizosphere (as opposed to in the inoculum) determine nodule occupancy, and that availability of nutrients limits populations of the

rhizobia. Although there is some evidence to support both of these assumptions³⁰⁻³², both remain the subject of controversy. Current work focuses on the construction of transgenic plants that release rhizopine (L-3-O-methyl-scylo-inosamine) into the rhizosphere, and the application of rhizopine-catabolizing bacterial partners to these plants (Fig. 3; Refs 33,34). This strategy could also be used to enhance the growth of free-living nitrogen-fixing bacteria, such as *Azotobacter* and *Azospirillum* to obtain biologically fixed nitrogen for grain crops.

Suppression of plant disease

Genetic engineering can also be used to enhance the association of plants with microorganisms that suppress plant disease or reduce association with pathogenic microorganisms. Work is under way to enhance the nutritive value of root exudates for a bio-control agent. *Bacillus cereus* UW85, which suppresses certain diseases of roots, is a naturally occurring auxotroph for threonine³⁵. As plants have been engineered to express genes that result in increased levels of free threonine^{36,37}, it should be feasible to construct

plants that specifically release excess amounts of threonine into the rhizosphere. This might increase populations of UW85 in the rhizosphere and enhance suppression of root disease. Complementary research is directed toward introducing genes encoding antimicrobial peptides into plants to provide suppression of diseases caused by fungi¹⁷.

Phytoremediation

Plants have potential as agents for remediating soil contamination by toxic heavy metals and organic pollutants. The roots of some plants have an unusually high capacity for heavy-metal uptake, and the net result is a solar-driven bioconcentration of heavy metals into biomass. This biomass can be readily harvested and processed for metal recovery, in effect cleaning the environment while mining the contaminated soil³⁸. The possible roles of rhizosphere processes in phytoremediation are poorly understood. Bacteria can contribute to remediation by catabolizing organic molecules, mobilizing soil-bound metals and excreting metal-chelating organic molecules (siderophores) or protons (decreasing soil pH and solubilizing metal

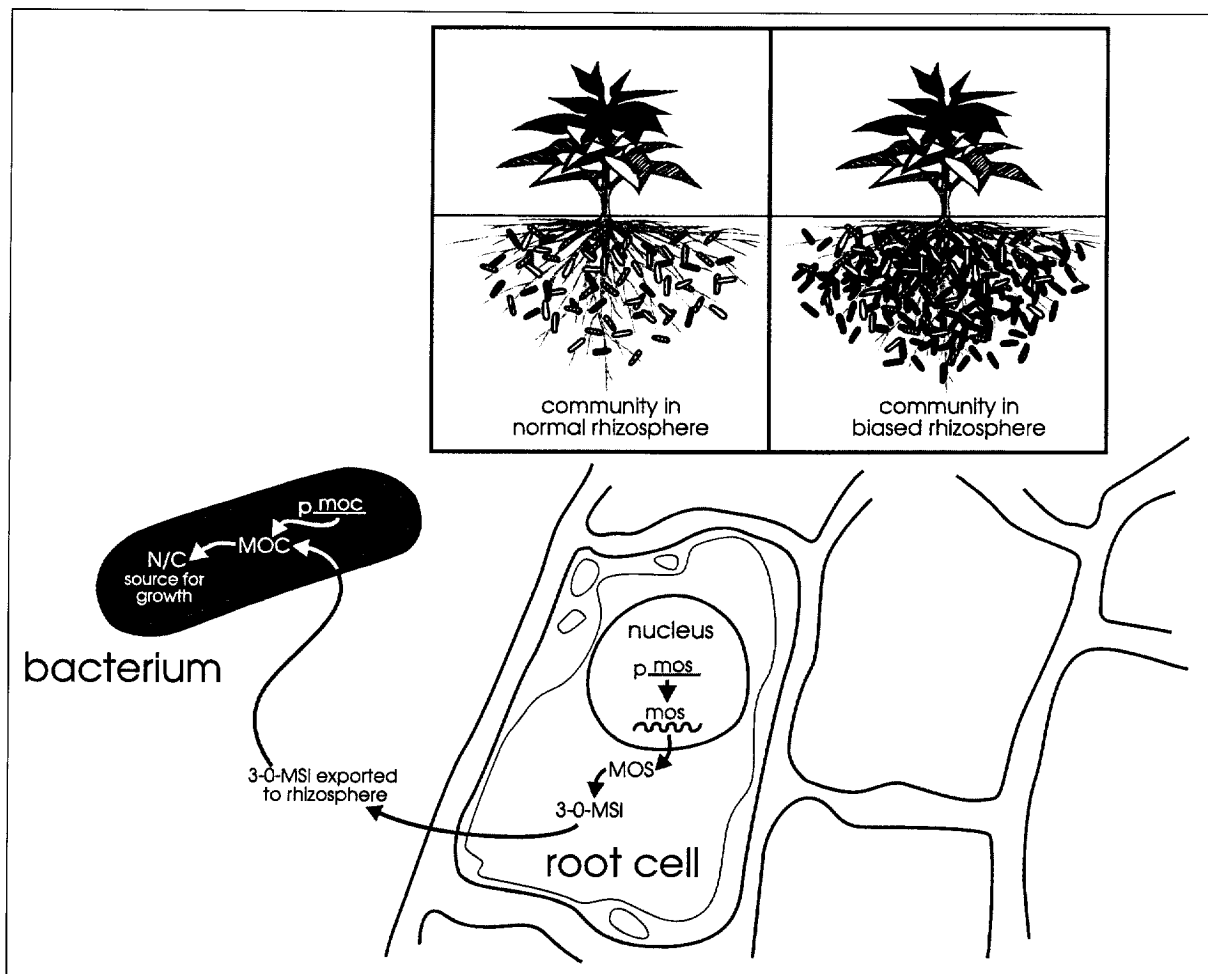


Figure 3

The biased rhizosphere. Plants engineered to contain *mos* genes, which code for the biosynthesis of L-3-O-methyl-scylo-inosamine (3-O-MSI), release the compound into the rhizosphere where only the bacteria containing *moc* genes catabolize 3-O-MSI and use it as a nitrogen and carbon source. This provides a selective growth advantage for bacteria that can catabolize the unusual compound. (Adapted, with permission, from Ref. 18.)

cations)^{39,40}. In fact, remediation activity that is thought to be associated with plants might, in some cases, be due to the activity of plant-associated microorganisms. Thus, the ability of plants to attract and provide nutrients for microbes may play a significant role in phytoremediation in the future.

Conclusions

Plant genetic engineering offers tools to study the rhizosphere and to examine the effect of plant physiology and architecture on individual members of the rhizosphere as well as entire communities. Engineering will also open doors to new strategies for maintaining and enhancing the health of crop plants and the soil they live in by influencing the microorganisms and chemicals in the rhizosphere. Although this field is in its infancy, there is vigorous interest in the genetic component of the interface between the plant root and its environment, and in the next decade we expect to see substantial contributions to fundamental knowledge about the rhizosphere, as well as new strategies for enhancing plant and environmental health.

Acknowledgements

We thank Brian McSpadden for helpful discussions, Liz Stohl and Mark Bittinger for reviewing the manuscript, Andrew Sandvold and Steve Vicen for the illustrations, and Laurie Luther, Laurie Ballentine and Lorraine Kubota Modjeska for assistance in preparing the manuscript.

References

- Hiltner, L. (1904) *Arb. DLG* 98, 59–78
- Bowen, G. D. and Rovira, A. D. (1991) in *Plant Roots: The Hidden Half* (Waisel, Y., Eschel, A. and Kafkafi, U., eds), pp. 349–388, Marcel Dekker
- Chen, W., Hoitink, H. A. J., Schmitthenner, A. F. and Tuovinen, O. H. (1988) *Phytopathology* 78, 314–322
- Hildebrand, A. A. and West, P. M. (1941) *Can. J. Res.* 19, 183–198
- Lochhead, A. G., Timonin, M. I. and West, P. M. (1940) *Sci. Agric.* 20, 414–418
- Neal, J. L., Jr, Larson, R. I. and Atkinson, T. G. (1973) *Plant Soil* 39, 209–212
- John, I., Wang, H., Held, B. M., Wurtele, E. S. and Colbert, J. T. (1992) *Plant Mol. Biol.* 20, 821–831
- de Pater, B. S. and Schilperoort, R. A. (1992) *Plant Mol. Biol.* 18, 161–164
- Yamamoto, Y. T., Taylor, C. G., Acedo, G. N., Cheng, C. and Conkling, M. A. (1991) *Plant Cell* 3, 371–382
- Hawes, M. C. and Pueppke, S. G. (1986) *Am. J. Bot.* 73, 1466–1473
- Brigham, L. A., Woo, H.-H., Nicoll, S. M. and Hawes, M. C. (1995) *Plant Physiol.* 109, 457–463
- Gilbert, S., Handelsman, J. and Parke, J. L. (1994) *Phytopathology* 84, 222–225
- Simon, N., LeBot, N., Marie, D., Partensky, F. and Vaultot, D. (1995) *Appl. Environ. Microbiol.* 61, 2506–2513
- Raskin, L., Stromley, J. M., Rittmann, B. E. and Stahl, D. A. (1994) *Appl. Environ. Microbiol.* 60, 1232–1240
- Liesack, W. and Stackebrandt, E. (1992) *J. Bacteriol.* 174, 5072–5078
- Templeton, M. D., Rikkerink, E. H. A. and Beever, R. E. (1994) *Mol. Plant-Microbe Interact.* 7, 320–325
- Jach, G. et al. (1995) *Plant J.* 8, 97–109
- Rosbach, S., McSpadden, B., Kulpa, D. and de Bruijn, F. J. (1994) *Proc. Biotechnol. Risk Assess. Symp.* (Levin, M., Grim, C. and Angle, J. S., eds), p. 248
- Dessaux, Y., Petit, A. and Tempe, J. (1992) in *Molecular Signals in Plant-Microbe Interactions* (Verma, D. P. S., ed.), pp. 109–136, CRC Press
- Schell, J. et al. (1979) *Proc. R. Soc. London Ser. B* 204, 251–266
- Savka, M. and Farrand, S. (1992) *Plant Physiol.* 98, 784–789
- Guyon, P., Petit, A., Tempe, J. and Dessaux, Y. (1993) *Mol. Plant-Microbe Interact.* 6, 92–98
- Nautiyal, C. S. and Dion, P. (1990) *Appl. Environ. Microbiol.* 56, 2576–2579
- Bell, C. R., Cummings, N. E., Canfield, M. L. and Moore, L. W. (1990) *Appl. Environ. Microbiol.* 56, 2840–2846
- Truchet, G. et al. (1991) *Nature* 351, 670–673
- Downie, J. A. (1994) *Trends Microbiol.* 2, 318–324
- Hartwig U. A., Joseph, C. M. and Phillips, D. (1990) *Plant Physiol.* 95, 797–803
- Amarger, N. and Lobreau, J.-P. (1982) *Appl. Environ. Microbiol.* 44, 583–588
- Beattie, G. A., Clayton, M. K. and Handelsman, J. (1989) *Appl. Environ. Microbiol.* 55, 2755–2761
- Araujo, R. S., Robleto, E. A. and Handelsman, J. (1994) *Appl. Environ. Microbiol.* 60, 1430–1436
- Marques-Pinto, C., Phaik, Y. and Vincent, J. M. (1974) *Aust. J. Agric. Res.* 25, 317–379
- Leung, K., Yap, K., Dashu, N. and Bottomley, P. J. (1994) *Appl. Environ. Microbiol.* 60, 408–415
- Murphy, P. J. et al. (1987) *Proc. Natl Acad. Sci. USA* 84, 493–497
- Rosbach, S., Kulpa, D. A., Rosbach, U. and de Bruijn, F. J. (1994) *Mol. Gen. Genet.* 245, 11–24
- Milner, J. L., Raffel, S. J., Lethbridge, B. J. and Handelsman, J. (1995) *Appl. Microbiol. Biotechnol.* 43, 685–691
- Karchi, H., Shaul, O. and Galili, G. (1993) *Plant J.* 3, 721–727
- Shaul, O. and Galili, G. (1992) *Plant Physiol.* 100, 1157–1163
- Salt, D. E. et al. (1995) *Bio/Technology* 13, 468–474
- Cunningham, S. D., Berti, W. R. and Huang, J. W. (1995) *Trends Biotechnol.* 13, 393–397
- Ehrlich, H. L. (1994) in *Biotechnology for Treatment of Hazardous Waste*, pp. 27–44, Lewis

Overseas Subscriptions

In some countries, the currency needed to pay for a personal subscription (US dollars, or pounds sterling) is not available. If you wish to help a colleague who is not able to benefit from *TIBTECH* for this reason, we will accept your payment for another person's subscription. Simply complete the subscription order card bound into any issue, giving the recipient's name and address labelled 'send to'; after 'signature', give your own name and address and mark this 'bill to'. Renewal notices will be sent to your address, and the recipient will receive their monthly copy of *TIBTECH*. Please inform the recipient of your action.