Host Specificity in Microbe–Microbe Interactions

Biological control agents vary in specificity for hosts, pathogen control, ecological habitat, and environmental conditions

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Agriculture in the United States and other developed countries is in transition from conventional monocultural crop systems often supported by high inputs of pesticides and fertilizers to sustainable agriculture. Although precise definitions of sustainable agriculture vary, one key aspect is a reduction in agrichemical inputs with a corresponding shift to alternative crop protection strategies, such as cultural practices, organic amendments, resistant or tolerant crop varieties, and biological control. Biological control of plant diseases may be accomplished by several different means (Cook and Baker 1983), including the use of introduced microbial biological control agents. Because most plant diseases are caused by pathogenic microorganisms, using a microbial biological control agent to control a plant pathogen involves microbe–microbe interactions.

Examples of biological control agents in experimental systems include viruses, fungi, and bacteria. With such a diversity of agents, it is difficult to address the subject of host specificity across all microbe–microbe interactions. Therefore, this article focuses on one group of biological control agents, plant-associated bacteria; however, the issues discussed are broadly applicable to other biological control agents. In contrast to some other biological control systems discussed in other articles of this special issue of *BioScience*, a simple definition of host specificity is not possible for microbe–microbe interactions. Biological control agents of plant pathogens are seldom actual parasites or predators, so one could ask: What is meant by the *host* in host specificity? Various answers are possible and correct. The plant is the host of the pathogen and is also often the host of the biological control agent. Because action of biological control agents is aimed at one or more pathogens, host specificity may alternatively be discussed from the viewpoint of what specific pathogens are affected by a biological control agent. Hence, the underlying premise of this article is that host specificity in microbe–microbe interactions is a complex and multifaceted issue.

The various specificities are not impediments to the development of biological control products but rather advantages for product development

To illustrate the multiple aspects of this subject, I have selected a group of plant-associated bacteria, the root-colonizing bacteria, termed *plant growth–promoting rhizobacteria* (PGPR). PGPR strains may exhibit biological disease control or plant growth promotion (Kapulnik 1991). This group of bacteria is currently available as several crop protection products and is currently being developed into commercial products for use in sustainable agricultural approaches.

Although a large variety of fungi and bacteria have been identified as biological control agents in various crop–pathogen systems (Cook and Baker 1983), I focus on PGPR to discuss issues related to microbial host specificity. It is often difficult to separate definitively growth promotion from biological control in agricultural ecosystems, and from a product development viewpoint, it may be beneficial to have both traits. Therefore, I refer to both plant growth promotion and biological control, although I emphasize biological control.

Specific strains of PGPR have demonstrated biological control against many soilborne plant pathogenic fungi, including *Aphanomyces* spp., *Fusarium oxysporum, Fusarium solani, Gaeumannomyces graminis, Phytophthora* spp., *Pythium* spp., *Rhizoctonia solani, Sclerotium rolfsii*, and *Thielaviopsis basicola* (reviewed in Kloepper 1991). Mechanisms for biological control by many PGPR strains are believed to involve production of bacterial metabolites that adversely affect the pathogen

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(Weller 1988). These metabolites include antibiotics, iron chelators, cell wall degrading enzymes, and hydrogen cyanide. An alternative mechanism, which does not involve antagonism of the PGPR against the pathogen, is induced systemic resistance (Kloepper et al. 1993). In this case, PGPR stimulate the host plant's defenses, thereby reducing the level of disease resulting from infection by pathogens throughout the plant. Induced systemic resistance offers a considerable expansion of the potential uses of PGPR in agriculture in that a seed treatment could be used to control multiple pathogens in both the rhizosphere and phyllosphere.

PGPR are typically applied to crops at planting as seed treatments. PGPR may also be applied as root dips during transplanting. Several PGPR-based products are now marketed for biological control and growth promotion. In China, eight fermentation facilities are devoted to producing PGPR products, which are used annually on more than 3 million hectares (Chen et al. 1996). At least two PGPR strains have been registered by the US Environmental Protection Agency and are now available commercially in the United States (Mahaffee and Kloepper 1994). Many more PGPR strains have demonstrated efficacy in reducing crop diseases but are sitting in culture collections unused in agriculture, partly because of regulatory and development costs.

Bacterial strain concept

When discussing specificity, it is important to consider a key concept of bacteriology—the concept of strain. According to this concept, each isolate of a particular bacterial taxon has the potential to be unique because of minor genetic variations that can confer unique phenotypes. At least 12 genera include strains that have been reported to function as PGPR (Mahaffee and Kloepper 1994), and unlike the other biological control agents discussed in this special issue, taxonomic or phylogenetic relationships are of little value for predicting which bacteria in a given microbial habitat may function as PGPR. As our knowledge of bacterial genetics continues to grow, it is clear that individual bacteria and bacterial communities in the environment exhibit genotypic plasticity (Metting 1993). Because of their smaller genome size, the phenotypes of bacteria are more likely to be affected by small changes in DNA regions than are insects or plants. Genomic changes resulting from random mutation, viral infection, conjugation, or transformation may convert a PGPR strain to an ineffective strain. Hence, although PGPR are a broad functional group that may be found within many genera and species of bacteria, only specific strains of a given taxon cause plant growth promotion or biological disease control.

Specificity in host colonization. For PGPR strains to be effective biological control agents, they must colonize roots. The introduced bacteria must establish and grow in an ecological habitat that includes indigenous microorganisms (Schroth and Becker 1990). Hence, root colonization is a competitive process that is affected by characteristics of both the PGPR and the host. Specificity of colonization can be observed at various levels. For instance, several different PGPR strains may colonize one host, such as tomato, at various population densities. The strain that colonizes the tomato hybrid at the highest level may not be the best colonist of a different tomato hybrid. One strain that effectively colonizes many different tomato hybrids may be a poor colonist of cotton. Hence, individual PGPR strains may be crop specific, cultivar specific, or nonspecific for root colonization (Chanway et al. 1991, Schroth and Becker 1990, Weller 1988).

Specificity in efficacy on different hosts. After colonization, PGPR produce metabolites that inhibit the pathogen or interact with the host to induce defenses. Therefore, PGPR may also show crop specificity independent of root colonization. Some PGPR exhibit narrow specificity (e.g., Chanway et al. [1988] found that six of seven *Bacillus subtilis* strains enhanced growth of wheat cultivar Katepwa, but none promoted growth of cultivar Neepawa). Other PGPR may exhibit much broader specificity. With one commercial PGPR-based product, Kodiak, the active agent (*B. subtilis* strain GB03) has demonstrated biological control against *R. solani* on cotton, peanut, and soybean (Backman et al. 1994). Therefore, as with root colonization, individual PGPR strains exhibit specificity for efficacy of growth promotion and biological control ranging from narrow to broad.

Specificity of pathogen control. Few systematic studies have used whole plant disease assays to determine the variety of plant pathogens that the PGPR strain is able to control. This likely relates to the fact that specific PGPR are usually selected for control of a particular disease. In contrast, antibiosis (production of inhibitory compounds) in vitro against several pathogens is often used as a part of selection strategies or studies on mechanisms and is known to be specific against multiple pathogens. These studies show that many PGPR strains have the potential to inhibit multiple pathogens in vitro; however, it is important to consider that pathogen inhibition in vitro does not predict disease control in vivo. PGPR that induce systemic resistance in the host can also lead to control of multiple pathogens.

An example of a PGPR strain with a wide spectrum of pathogen control is *Pseudomonas fluorescens* strain CHAO (Défago et al. 1990). Strain CHAO was originally isolated from a Swiss tobacco field suppressive to black root rot disease, which is caused by *T. basicola*. This strain has demonstrated biological control activity against *T. basicola* on tobacco, cotton, and cherry; against *Pythium ultimum* on wheat and sugar beet; against *G. graminis* var. *tritici* on wheat; and against *F. oxysporum* on tomato. In contrast, *B. subtilis* strain GB03 has biological control activity against damping-off disease caused by *R. solani* but not by *P. ultimum* (Backman et al. 1994). The underlying reason for the different spectrum of pathogen control with strains CHAO and GB03 probably relates to the mechanisms of biological control employed by each strain. Although strain CHAO produces numerous antimicrobial compounds, including at least two antibiotics as

well as HCN and siderophores, and also induces systemic plant resistance to disease (Défago et al. 1990), strain GB03 is thought to produce only one antibiotic.

Recent work with induced systemic resistance mediated by PGPR (Kloepper et al. 1993) demonstrates clearly that some PGPR strains that do not act through production of antibiosis may act as biological control agents against a broad spectrum of plant pathogens. Two PGPR strains, when applied to cucumber seeds, reduced damage to a foliar fungal pathogen (*Colletotrichum orbiculare*), a foliar bacterial pathogen (*Pseudomonas syringae* pv. *lachrymans*), a soilborne fungal wilt pathogen (*F. oxysporum* f. sp. *cucumerinum*), an insect-vectored bacterial wilt pathogen (*Erwinia tracheiphila*), and a systemic viral pathogen (cucumber mosaic virus).

Specificity of ecological habitat. Another aspect of specificity with biological control agents is that of survival and growth in different microbial habitats. In the case of PGPR, most studies indicate that these bacteria preferentially colonize root zones (Chanway et al. 1988, Schroth and Becker 1990). PGPR populations on roots generally decline during the season and are undetectable in soil after harvest. Some PGPR strains, especially spore-forming bacteria such as *Bacillus* spp., have the potential to survive in the absence of plant roots but may show strain-specific ability to survive in crop-free soil. In contrast, asporogenous PGPR depend on the continual association with plant roots for survival. A subgroup of root-colonizing bacteria may enter plant roots and live as endophytes inside the plant (McInroy and Kloepper 1994). Data on indigenous endophytic bacteria indicate that more bacterial taxa live in roots than in stems, suggesting specificity for niche colonization within plants.

Few reports have examined the fate of root-colonizing bacteria outside the root and soil zone. Because soil- and root-zone bacteria generally lack mechanisms to protect against ultraviolet radiation, they are not expected to survive on plant stems or leaves. However, in China, some of the commercialized PGPR strains are applied both as seed treatments and as midseason foliar sprays (Chen et al. 1996). Studies on the population dynamics of the bacteria indicate that these PGPR survive, and sometimes multiply, on the foliage.

Specificity in sensitivity to environmental parameters. Limited studies (Schroth and Becker 1990) have been conducted to determine the effects of various environmental parameters, such as soil moisture, soil texture, seed pH, and organic matter content, on root colonization or efficacy of PGPR. Although it is much too early to draw overall definitive conclusions, and much of this work is published only in abstract form, it appears that PGPR strains vary widely in their responses to specific environmental parameters. Some strains are well adapted to diverse conditions. For example, PGPR strain GB03 colonizes cotton roots and provides biological control against *R. solani* throughout the Cotton Belt, from Texas to the southeastern United States. Similarly, the same strain acts as a biological control agent against *R. solani* on peanut from the Southeast to Texas and Oklahoma. The same group of PGPR strains is reported to control soilborne pathogens throughout the country of China. Hence, although a commonly held view of biological control agents is that they are likely to be specific to various regions because of differential responses to the environment, there are exceptions.

Implications for commercial development

The various specificities may seem to be impediments to development of biological control products; however, these specificities can also be viewed as advantages for product development. Antagonists with narrow ranges of pathogen control can be combined with other antagonists or with existing fungicides active against other pathogens to develop a customized product. Broad pathogen control activity of some bacteria is generally useful for product development, allowing individual biocontrol agents to be developed for relatively broad markets. Similarly, the finding that specific PGPR strains may be efficacious in biological control across diverse regions greatly expands the potential market size of an individual product.

Conclusions

Is there host specificity in the use of microorganisms to control plant diseases? Yes and no. Although confusing, this answer has some practical significance. It would be wrong from a regulatory view to consider that all microbial disease biocontrol agents share common features of host specificity. Regulations should allow for "either/or" scenarios, in which biological control agents with some host specificity are treated separately from those without host specificity. Despite the demonstrated potential of PGPR to act as plant growth promoters and biological control agents, there are still few successful commercial products containing PGPR. Knowing that some PGPR strains lack tight host specificity, future selection of new PGPR should be designed to test for broad host ranges for pathogens controlled and crops colonized, thereby increasing the chances of routine use of PGPR in future crop protection strategies.

References cited

Backman PA, Brannen PM, Mahaffee WF. 1994. Plant reponse and disease control following seed inoculation with *Bacillus subtilis*. Pages 3–8 in Ryder MH, Stephens PM, Bowen GD, eds. Improving plant productivity with rhizosphere bacteria. Adelaide (South Australia): CSIRO Division of Soils.

Chanway CP, Nelson LM, Holl FB. 1988. Cultivar-specific growth promotion of spring wheat (*Tricticum aestivum* L.) by coexistent*Bacillus species*. Canadian Journal of Microbiology 34: 925–929.

Chanway CP, Turkington R, Holl FB. 1991. Ecological implications of specificity between plants and rhizosphere microorganisms. Advances in Ecological Research 21: 121–169.

Chen Y, Mei R, Lu S, Liu L, Kloepper JW. 1996. The use of yield-increasing bacteria (YIB) as plant growth-promoting rhizobacteria in Chinese agriculture. Pages 165–184 in Gupta CK, Utkehde R, eds. Management of soilborne disease. New Delhi (India): Kalyani Publishers.

Cook RJ, Baker K. 1983. In the nature and practice of biological control of plant pathogens. St. Paul (MN): APS Press.

Défago G, Berling CH, Burger U, Haas D, Kahr G, Voisard C, Wirthner P, Wüthrich B. 1990. Suppression of black root rot of

tobacco by a *Pseudomonas* strain: potential applications and mechanisms. Pages 93–108 in Hornby D, ed. Biological control of soil-borne plant pathogens. Oxon (UK): CAB International.
Kapulnik Y. 1991. Plant growth-promoting rhizobacteria. Pages 717–729 in Waisel Y, Eshel A, Kafkafi U, eds. Plant roots, the hidden half. New York: Marcel Dekker.
Kloepper JW. 1991. Plant growth-promoting rhizobacteria as biological control agents of soilborne diseases. Pages 142–152 in Bay-Petersen J, ed. The biological control of plant diseases. Taipei (Taiwan): Food and Fertilizer Technology Center.
Kloepper JW, Tuzun S, Liu L, Wei G. 1993. Plant growth-promoting rhizobacteria as inducers of systemic disease resistance. Pages 156–165 in Lumsden RD, Vaughn JL, eds. Pest management: biologically based technologies. Washington (DC): American Chemical Society Books.
Mahaffee WF, Kloepper JW. 1994. Applications of plant growth-promoting rhizobacteria in sustainable agriculture. Pages 23–31 in Pankhurst CE, Doube BM, Gupta VVSR, Grace PR, eds. Soil biota: management in sustainable farming systems. Melbourne (Australia): CSIRO.
McInroy JA, Kloepper JW. 1994. Studies on indigenous endophytic bacteria of sweet corn and cotton. Pages 19–28 in O'Gara F, Dowling DN, Boesten B, eds. Molecular ecology of rhizosphere microorganisms. New York: VCH Publishers.
Metting FB Jr. 1993. Structure and physiological ecology of soil microbial communities. Pages 3–25 in Metting FB Jr., ed. Soil microbial ecology. New York: Marcel Dekker.
Schroth MN, Becker JO. 1990. Concepts of ecological and physiological activities of rhizobacteria related to biological control and plant growth promotion. Pages 380–414 in Hornby D, ed. Biological control of soil-borne plant pathogens. Oxon (UK): CAB International.
Weller DM. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology 26: 379–407.