

## SUSTAINABLE USE OF MICROBIAL GENETIC RESOURCES IN AGRICULTURE: BIOLOGICAL CONTROL AGENTS

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### SUMMARY

Genetic resistance to root diseases of plants is rare, and agriculture controls these diseases by practices such as crop rotation and soil fumigation. However, plants have evolved a strategy of stimulating and supporting specific groups of antagonistic rhizosphere microorganisms as a defense against diseases caused by soilborne pathogens. Antibiotic production plays a significant role in plant defense by many of these bacteria, and detailed information is now available about the genetics, biochemistry, and regulation of synthesis of several commonly-produced antibiotics. Similarly, many genes that contribute to the ability of these strains to colonize roots have been identified, and studies of naturally suppressive soils have provided evidence of preferential interactions between plant hosts and biocontrol bacteria, revealing the existence of functional diversity in protective populations of very closely related strains. Here, we consider how this knowledge can be applied to better manage the indigenous rhizosphere microflora, aid in the selection of more effective microbial amendments, and guide the tailoring of microflora through directed genetic manipulation to enhance crop health and productivity.

**KEY WORDS:** antibiotics, root colonization, rhizosphere, suppressive soils.

### RESUMEN

## USO SUSTENTABLE DE LOS RECURSOS GENÉTICOS MICROBIANOS EN LA AGRICULTURA: AGENTES DE CONTROL BIOLÓGICO

La resistencia genética a enfermedades de raíz es poco frecuente, y en la agricultura estas enfermedades se controlan mediante prácticas tales como la rotación de cultivos y la fumigación del suelo. Sin embargo, las plantas han desarrollado una estrategia para estimular y mantener grupos específicos de microorganismos rizosféricos antagonistas como defensa contra enfermedades causadas por patógenos del suelo. La producción de antibióticos por muchas de estas bacterias juega un papel significativo en la defensa de las plantas, y se dispone actualmente de información detallada sobre la genética, bioquímica y regulación de la síntesis de varios de los antibióticos comúnmente producidos. Además, muchos genes que contribuyen a la habilidad de estas cepas para colonizar raíces han sido identificados y estudios de suelos naturalmente supresivos han aportado evidencias de interacciones preferenciales entre la planta huésped y la bacteria biocontroladora, revelando la existencia de diversidad funcional entre cepas muy relacionadas de las poblaciones protectoras. En el presente trabajo consideramos cómo este conocimiento puede ser aplicado a un mejor manejo de la microflora rizosférica indígena, ayudando en la selección de enmiendas microbianas más efectivas, y guiando en el diseño de la microflora a través de la manipulación genética dirigida para mejorar la salud y productividad de los cultivos.

**PALABRAS CLAVE:** antibióticos, colonización radicular, rizósfera, suelos supresivos.

Whereas genes encoding for resistance to foliar pathogens are abundant in plant species, resistance to common soilborne pathogens, including *Pythium* spp., *Rhizoctonia* spp., and many *Fusarium* spp., is rare or lacking entirely. In agricultural systems, control of these pathogens is achieved mainly through practices including

tillage, crop rotation and the use of chemical pesticides, but plants in undisturbed ecosystems rely on a much older mechanism provided by root-colonizing microorganisms that are supported by rhizodeposition, the release of organic materials from roots as they grow through soil.

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Collectively, these microorganisms provide a basal level of biological buffering and general disease suppression simply because of their metabolic activity. However, many isolates from the rhizosphere of plants can actively antagonize soilborne pathogens, and it is this ability that has been a driving force in biological control research over the past 50 years. Hundreds, if not thousands, of antagonistic strains representing diverse genera and with the potential for use as biological control agents have been described over the past fifty years. Here, we consider some of the lessons learned from these studies, focusing specifically on *Pseudomonas* spp., and show how the knowledge gained can be applied to better serve the needs of agriculture in the 21<sup>st</sup> century.

Much research in recent years has focused on antibiosis, “the inhibition or destruction of one organism by a metabolic product of another,” and especially on antibiotics produced by fluorescent *Pseudomonas* species. Antibiotics include low molecular weight organic compounds that are produced by microorganisms and are deleterious to the growth or other metabolic activities of other microorganisms (Fravel, 1988). Because *Pseudomonas* spp. are amenable to genetic manipulation and the antibiotics they produce are relatively simple in structure, this research has yielded detailed information about the genetics, biochemistry, and regulation of synthesis of phenazines compounds, 2,4-diacetylphloroglucinol, pyrrolnitrin and pyoluteorin (Raaijmakers *et al.*, 1997, 2002). Strains producing these compounds have been isolated from the roots of a wide variety of crops grown on every continent, and have been shown to be antagonistic to a wide variety of pathogens. The biosynthetic genes are chromosomally encoded, mostly clustered into operons, and not surprisingly, their transcription and translation are controlled by global mechanisms responsible for the overall response of microorganisms to their environment as well as by specific, genetically linked regulatory elements (Haas & Keel, 2003). The DNA sequences of the biosynthetic operons for these antibiotics are known, and probes and primers have been developed to facilitate the search for new strains with the capacity to produce these well-characterized compounds (de Souza & Raaijmakers, 2003; McSpadden Gardener *et al.*, 2001; Mavrodi *et al.*, 2001).

Root colonization also has been studied extensively, both as a pre-emptive mechanism to exclude colonization by pathogens and because colonization is a necessary prerequisite for the delivery of antifungal metabolites by biological control agents. It is clear that many strains have the capacity to colonize roots, and genes related to such diverse properties as bacterial cell surface structures

(flagella, fimbriae, and lipopolysaccharides), catabolic activity, and global regulation of gene expression (Lugtenberg *et al.*, 2001) have been implicated in the process. However, it is important to recognize that because investigators have differed in their definitions of “root colonization” and have conducted studies involving diverse crops over varying intervals of time and under conditions ranging from gnotobiotic to field-scale systems, it is not possible to draw general conclusions about the relative importance individual factors contributing to root colonization in relation to biological control. Instead, it may be more useful to consider the importance of colonization in relation to the needs of particular biocontrol systems. Thus, for relatively short-term threats such as those related to seedling pre- and post-emergence damping-off diseases caused by *Pythium* and *Rhizoctonia* spp., inundative applications of highly antagonistic biocontrol agents may eliminate a requirement that the introduced agent be able to aggressively colonize and persist on the roots over an extended period of time. Forage and field crops, in contrast, may require more sustained protection, and in these cases, it is important to understand the factors contributing to both the establishment and the long-term maintenance of protective populations of biocontrol agents. Recent studies of naturally suppressive soils, and particularly, the phenomenon known as take-all decline (Weller *et al.*, 2002), have provided important insight into this area. Of particular note is evidence that soils under long-term cultivation to specific crops and in the presence of pathogens undergo a classical microbial enrichment process resulting in the selection of populations of rhizosphere microorganisms highly adapted to the cultivated crop and protective against certain target pathogens. These and certain other studies (Landa *et al.*, 2002, 2003; Lemanceau *et al.*, 1995) have identified preferential interactions between plant hosts and biocontrol bacteria, revealing the existence of functional diversity in protective populations of very closely related strains and highlighting the need to identify and understand not only the microbial factors, but also those of the host, in these preferential interactions.

How can the knowledge gained from past studies be applied to overcome the constraints that until now have limited more widespread use of biocontrol agents? First, molecular techniques can be utilized to rapidly identify locally-adapted strains capable of producing well-characterized antibiotics effective against known target pathogens. Such strains are more likely to be competitive under the conditions in which they will be used, and in some cases, environmental factors known to limit the

production or activity of these antibiotics can be manipulated to enhance strain performance (Duffy & Defago, 1999; Ownley *et al.*, 1992, 2003). Second, soils that have been subjected to long-term monoculture are a preferred source of host-adapted strains; such strains can be enriched in the laboratory by repeatedly sowing the monoculture crop in a process termed “cycling” (Landa *et al.*, 2002, 2003; Raaijmakers & Weller, 2001) and they can be highly effective biocontrol agents (Raaijmakers & Weller, 2001; Weller *et al.*, 2002). Third, when antibiotic production is limiting to biocontrol, synthesis can be uncoupled from the regulatory mechanisms that normally govern antibiotic gene expression, resulting in increased production and enhanced biocontrol activity (Ligon *et al.*, 2000). Moreover, deregulated biosynthetic operons from strains of *Pseudomonas* can be transferred and expressed in strains with other desirable attributes, extending the range of pathogens controlled without significant loss of ability to colonize roots (Huang *et al.*, 2004; Timms-Wilson *et al.*, 2000). While further research clearly will be required in order to gain the confidence of farmers and the support of the public in the use of genetically modified biocontrol agents, studies to date indicate that introduced wild-type and modified strains produce only small, transient changes in the indigenous microbial community that do not correlate with the genomic background of the introduced strain or the antibiotic genes expressed (Bankhead *et al.*, 2004; Viebahn *et al.*, 2003). Indeed, a single potato crop, crop rotation, seasonal effects, and positional effects in the field had greater effects on ascomycete community structure than did repeated strain introductions (Viebahn *et al.*, 2003). Finally, and perhaps most importantly, naturally suppressive soils (Weller *et al.*, 2002) continue to be an important but underutilized resource for controlling diseases with minimal application of off-farm inputs, and should be considered integral to disease management in sustainable agriculture. By coupling the sophisticated techniques of molecular ecology with classical approaches for studying soil suppressiveness (Weller *et al.*, 2002), there is tremendous potential to more rapidly identify and manage the indigenous rhizosphere microflora to enhance crop health and productivity.

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