

Potential Application of Plant Growth-Promoting Rhizobacteria to Induce Systemic Disease Resistance

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I. INTRODUCTION

Our planet faces new challenges with its ever increasing population, the limited amount of agricultural land available to supply food demand, and the pollution problems associated with the extensive use of agri-chemicals. The need for an increased food supply and the use of intensive farming and mass monoculture have led to the development of new chemicals to control diseases and insects and these have disrupted the balance of agricultural systems. These disruptions may have eliminated some of the biological agents which have helped plants to remain healthy in their environment. Today, we know that

plants have quite sophisticated defense mechanisms that can be activated prior to disease development by environmental factors and microorganisms. Chapter 5 of this book describes such a phenomenon, called induced systemic resistance or plant immunization, that has coevolved with plants and may have contributed to plant survival. Our research has been concerned about the induction of systemic resistance to diseases in plants by manipulation of microbial populations naturally present in the plants' environment. In this chapter, we briefly describe key events in the evolution of the concept of induced systemic resistance, briefly review research on plant growth-promoting rhizobacteria (PGPR) and their role in disease control, describe the research on PGPR-mediated induced systemic resistance, describe current research on the range of diseases controlled by PGPR-mediated induced systemic resistance, and, finally, briefly review the ongoing research to establish the mechanism of PGPR-mediated systemic resistance and to develop their field applications.

II. INDUCTION OF DISEASE RESISTANCE IN PLANTS

Effective mechanisms for resistance to infectious agents have evolved in plants. Such mechanisms, although adequate for the survival of a species, may not satisfy the demands for high crop yield and quality imposed by modern agriculture. As early as 1933, Chester¹ reported development of resistance to diseases in plants following infection. Induced disease resistance can be defined as the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents.² Research in laboratories worldwide has demonstrated induction of disease resistance in susceptible varieties of over 25 crops including cereals, cucurbits, legumes, solanaceous plants, and tree and small fruits³ against a broad spectrum of leaf⁴⁻⁸ and root pathogens.⁹ Today we know that resistance can be induced by prior inoculation with pathogens,^{3,10,11} nonpathogens,^{12,13} seed treatment with specific PGPR strains,¹⁴ and microbial metabolites and other chemicals.¹⁵⁻¹⁹ Plant immunization has also been demonstrated to be induced by previous treatment with plant-derived materials²⁰⁻²⁶ including extracts of compost.²⁷ An important aspect of induced systemic resistance is that the phenomenon is nonspecific; that is, a single inducer can immunize plants against diverse pathogens. In cucumber, treatment of the first true leaf with a necrosis-forming organism protects the plant against subsequent infection by at least 13 pathogens, including fungi, bacteria, and viruses.^{4,6}

III. PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR)

Free-living root and soil bacteria have been studied for the past century as possible inoculants for enhancing crop productively. While early work

indicated that some bacteria, such as *Azotobacter* spp., could periodically increase plant growth, the population density of many inoculated bacteria declined upon introduction to agricultural soils. With the advent of bacterial marking systems, it was demonstrated that select rhizosphere bacteria could colonize plant roots in the presence of an indigenous soil microflora.²⁸ Such bacteria which exhibit root colonization have been termed "rhizobacteria",²⁹ and issues related to the root colonization process have recently been reviewed.³⁰ Effects of rhizobacteria on host plants may be deleterious, neutral, or beneficial, and beneficial rhizobacteria have been termed "plant growth-promoting rhizobacteria (PGPR)".³¹

Throughout the 1980s, many descriptive studies documented that PGPR strains may exert beneficial effects through plant growth promotion³²⁻³⁷ or biological disease control.^{32,34-41} The PGPR reports summarized in these reviews include the effects of many different bacterial groups on many host plants. Therefore, it is difficult to make comprehensive summary statements of common principles. However, some broad conclusions may be drawn from these reports. Most PGPR strains appear to have beneficial effects on plants by more than one mechanism. Growth promotion and biological control are frequently, but not always, expressed by the same PGPR strain. Several bacterial metabolites have been associated with biological control, including siderophores, HCN, volatile and nonvolatile antibiotics, cell-wall degrading enzymes, and antifungal factors. Induced systemic resistance was not included in the list of possible modes of action in most PGPR studies.

IV. PGPR AS INDUCERS OF DISEASE RESISTANCE

Initial suggestions that some beneficial bacteria may act as agents to induce systemic resistance came from two reports in the 1980s. Scheffer⁴² reported that prior inoculation of elm trees with four fluorescent pseudomonad strains led to significant reductions in systemic foliar symptoms of *Ophiostoma ulmi*, the Dutch elm disease pathogen. Some of the bacterial strains exhibited only weak antibiosis *in vitro* to the fungal pathogen, and Scheffer suggested that these strains might cause plant protection by enhancing host resistance. In another system, Voisard et al.⁴³ investigated the mechanisms for biological control of *Thielaviopsis basicola* by PGPR strain CHAO, a strain of *Pseudomonas fluorescens*. Production of HCN was found by molecular analysis to be associated with biological control activity and with promotion of root hair growth. It was suggested that HCN produced by PGPR strain CHAO might induce plant defense mechanisms.

Direct pathological evidence for the induction of systemic resistance by PGPR was published for three systems in 1991. In a carnation system,⁴⁴ applications of *Pseudomonas* sp. strain WCS417 to rockwool cubes resulted in protection from wilt of *Fusarium oxysporum* f. sp. *dianthi*. The pathogen was spatially separated from the PGPR strain by inoculating into stems 1 week after

PGPR application, and separation was confirmed by a failure to isolate WCS417 from stems. In a bean system,⁴⁵ seed treatment with a *P. fluorescens* PGPR strain led to reductions in the numbers of foliar lesions caused by subsequent inoculations of *P. syringae* pv. *phaseolicola*. In another report with cucumber,⁴⁶ 94 known PGPR strains were examined for their ability to control anthracnose caused by *Colletotrichum orbiculare*. Six PGPR strains applied as seed treatments consistently resulted in significant reductions in anthracnose lesion diameter and lesion numbers when the pathogen was applied 21 days after planting. In a subsequent study with the six inducing PGPR,⁴⁷ none of the inducing strains was recovered from leaf petioles, confirming the spatial separation of pathogen and PGPR. Together these reports with carnation, bean, and cucumber demonstrate that saprophytic root-associated bacteria may act as agents of induced systemic resistance, and, hence, they serve to expand the potential mechanisms by which PGPR may exert biological disease control.

V. SPECTRUM OF PGPR-MEDIATED INDUCED SYSTEMIC RESISTANCE

Current work in our laboratories to determine the spectrum of protection was achieved with two of the inducing PGPR strains used by Wei et al.⁴⁶ on cucumber. Both strains, applied as seed treatments, significantly reduce mean diameter of lesions induced by foliar-applied *C. orbiculare*. Protection against cucumber mosaic virus (CMV) is affected by the viral inoculation site. One PGPR strain significantly protected against CMV inoculated onto cotyledons.⁴⁸ Protection was evident as complete blockage of symptom development on PGPR-induced plants, which differs from protection reported against CMV for classical induced systemic resistance.⁴⁹ With classical induced resistance, CMV symptoms were delayed but not completely blocked upon induction. With the PGPR system, both strains delayed, but did not stop, symptom development when CMV was inoculated onto the first, second, or third leaves, which is equivalent to results with classical induced resistance. The same two PGPR strains induce protection of cucumber against *Pseudomonas syringae* pv. *lachrymans* as seen by a significant reduction in mean lesion number and lesion size compared to noninduced controls.⁵⁰ Preliminary studies with *Fusarium* wilt demonstrate that one PGPR strain reduces the rate of symptom development and plant death.⁵⁰ With *Fusarium*, a split root system was used to ensure spatial separation of PGPR and the pathogen. PGPR were applied to one half of the roots after splitting, and *Fusarium* was incorporated into the soil in which the other half of the roots were growing. While this system demonstrates the potential to use PGPR-mediated induced resistance for protection from a soilborne vascular wilt pathogen, it is fundamentally different from the other PGPR systems which use seed treatments. PGPR applied as seed treatments may induce systemic resistance earlier in the plant's life, and, hence, more

work is needed to compare the biochemical responses of plants induced by seed and root treatments of PGPR.

VI. BIOCHEMICAL MECHANISMS

While pathological effects may be considered as direct evidence for induced resistance, some biochemical data are required to conclude that the observed decrease in disease severity resulted from host defense reactions rather than from PGPR-produced antifungal metabolites. Resistance in classically immunized plants is expressed as a reduction both in size and number of lesions and in sporulation.⁶ In cucurbits, appressoria of *C. lagenarium* penetrate much less into immunized cucumber than into control plants.⁵¹ Immunization of tobacco, however, appears to restrict fungal development within leaves after penetration.⁵² In general, immunization sensitizes plants so that they respond rapidly to infectious agents. Treatments with immunizing agents rapidly activate multiple mechanisms of disease resistance which, in susceptible plants, are latent or are expressed too late to control disease. These mechanisms include the accumulation of antimicrobial low molecular weight chemicals (phytoalexins and leaf surface diterpenes)⁵³⁻⁵⁶ and protective biopolymers (lignin, callose, and hydroxyproline-rich glycoproteins).⁵⁷⁻⁵⁹ Increase in activity of enzymes in the pathways leading to production of such products, and increases in the amount of other primary gene products such as chitinases, β -1,3-glucanases, peroxidases, and other pathogenesis related (PR-) proteins, has also been detected.⁶⁰ The induction of systemic resistance to *P. tabacina* in tobacco coincided with the accumulation of β -1,3-glucanases, chitinases, and other PR-proteins,⁶¹ and an anionic isozyme of peroxidase.⁶² Enhanced peroxidase activity,^{57,63} induction of chitinases,^{64,65} and β -1,3-glucanases⁶⁶ have been found in systemically protected cucumber plants. Synthesis of these proteins appears to be regulated at the level of mRNA accumulation.^{67,68} Chitinases and β -1,3-glucanases, which are biologically active against fungi by hydrolyzing cell wall polymers, and peroxidases, which generate H_2O_2 and oxidize phenols, are important in lignin biosynthesis. These enzymes are also likely to be a part of the multicomponent mechanisms effective in the immunization of plants against disease. The multicomponent nature of defense mechanisms associated with immunization result in a very stable resistance.

Four studies have indicated that specific PGPR may stimulate the production of biochemical compounds associated with host defense. Van Peer and his colleagues⁴⁴ observed increased accumulation of phytoalexins in carnation plants treated with PGPR (isolate WCS417) following pathogen inoculation. In a bean system, Hynes and Lazarovits⁶⁹ found that levels of a PR-protein increased in leaves following seed treatment with PGPR strains. Plant root colonization by PGPR was associated with enhanced lignification of stems or leaves in bean⁷⁰ and wheat.⁷¹ Inoculation of bean roots with a *P. putida* PGPR

strain led to an increased abundance of mRNA encoding PR1a protein in leaves.⁷² These reports clearly demonstrate that particular bacteria inoculated onto seeds or roots may elicit systemic physiological changes in plants.

Early investigations with PGPR-mediated induced resistance in cucumbers suggested that the biochemical response of the plant may depend on the inducing PGPR strain. Some inducing PGPR were associated with enhanced peroxidase activity similar to that observed with classically induced controls.¹⁴ Some, but not all, inducing PGPR strains were associated with enhanced mRNA encoding acidic chitinases.⁷³ It will be necessary to conduct further biochemical investigations of how PGPR-mediated and classical induced resistance affect host defense-related compounds.

VII. FIELD APPLICATIONS

Plant immunization can be a natural, safe, effective, persistent, and durable alternative to the use of pesticides in controlling plant diseases. However, the problems associated with utilization of pathogens as inducers and limitation in the application technologies may affect practical application. Chemical inducers, on the other hand, may provide a better means of induction. Some chemicals are rather inexpensive and easily obtainable, such as phosphates used for the induction of resistance in cucumbers,²⁶ while others such as β -ionone derivatives²¹ may not be produced as economically and easily. The method of application, e.g., root treatment with the inducers⁷⁴ or seed treatment, may reduce the cost of application. It is important to note, however, the efficacy of induced resistance in plants, at least in some cases, depends on the environmental conditions.⁷⁵

Most of the research on plant immunization has been conducted in the laboratory and greenhouse; however, there are a number of reports indicating its effectiveness in protecting crop plants under field conditions.^{16,25,76-83} Immunization of tobacco against blue mold was first reported on systemically stem-infected and stunted field-grown tobacco in Australia.⁸⁴ Extensive field tests with a modified technique which employs injection of sporangiospore suspensions of *P. tabacina* into stem tissue external to the xylem were conducted over a 3-year period in Kentucky and Puerto Rico with a metalaxyl-sensitive strain of the fungus.⁸⁰ These tests indicated that immunized plants were protected as well as those treated with metalaxyl. Furthermore, even in the absence of disease, immunized plants grew more vigorously and yields were up to 20% greater than those of the controls.⁸⁰ Further field experiments were conducted in Mexico during 1986 to 1989 to test the effectiveness of immunization against metalaxyl-tolerant strains of *P. tabacina*.^{81,82} Highly significant reductions in the numbers and size of blue mold-induced lesions were observed on plants injected with *P. tabacina* as compared to nonstem-injected controls,

regardless of metalaxyl applications. Some vigorously growing plants with necrotic stem lesions but markedly reduced blue mold were observed in heavily infected commercial fields indicating the natural occurrence of immunization against blue mold in the Gulf Coast of Mexico.⁸² Tolerance to immunization was not evident, although conditions for its development were very favorable. Treatments with chemical inducers such as β -ionone and 3-*n*-butyryl- β -ionone protected tobacco against metalaxyl-sensitive and tolerant strains of the fungus in greenhouse and field trials.^{21,85} In field trials, plants derived by tissue culture from immunized parents were also less affected by blue mold than were plants derived from control parents.⁸⁶ These studies provide evidence that immunization of plants with biotic or abiotic inducers results in effective and stable disease control in the field.

Earlier research indicated that cucumbers can be protected in the field by the application of biological as well as chemical inducers.^{22,26,78} Two field trials were conducted with PGPR on cucumber in 1992 to compare disease protection levels of PGPR-mediated and classical induced resistance. PGPR were applied as seed treatments, and classical induced resistance was achieved by inoculation of the first leaf with *C. orbiculare*. In the first trial, plants were challenge-inoculated with *P. syringae* pv. *lachrymans*. Compared to the noninduced control plants, all those treated with PGPR showed significant reductions in mean lesion diameter, whereas those treated to obtain classical induced resistance showed significant increase in lesion diameter.⁸⁷ Growth promotion, measured as the number of leaves per plant and the weight of fruit per plant, resulted from use of 2 of 3 PGPR strains. In the second field trial, plants were not challenge-inoculated because natural infections of *Erwinia tracheiphila* were observed. All 3 PGPR strains, but not the classical induced resistance treatment, resulted in significant reductions in symptom expression. Hence PGPR-mediated induced resistance can be observed under field conditions and, at least in some cases, may lead to more superior plant protection than classical induced systemic resistance.

Many questions remain to be addressed concerning the use of PGPR as agents of induced systemic resistance. The length of protection period obtained by PGPR-mediated induced resistance is unknown. The bacterial components which trigger induced resistance must be elucidated. In addition, a comparison should be made of the translocatable plant signal between PGPR-mediated and classical induced resistance. Answers to these questions will help assessment of the potential agricultural usefulness of PGPR as agents of induced systemic resistance.

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