A Study of Pseudomonas Bio Control Strains for Environmental Bio Control Inoculants Applications

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Abstract

The use of chemicals like fertilisers and pesticides on an indiscriminate basis has wreaked havoc on the environment and ecology, including animals and humans. Nature provides a biological remedy in the form of microbes that can boost plant development while causing minimal environmental harm. Biocontrol plant growth-promoting rhizobacteria (PGPR), which is capable of reducing or preventing phytopathogen damage, is one of the biological techniques for the control of diverse phytopathogenic agents. Pseudomonas is the genus of bacteria that has the best biocontrol PGPR. Because of their large population in natural soils and plant root systems, and their capacity to use numerous plant exudates as fertiliser, fluorescent pseudomonads are suited for use as biological control agents. Fluorescent pseudomonads feature crucial bacterial characteristics include the capacity to cling to soil particles and the rhizoplane, motility and prototrophy, antibiotic synthesis, and hydrolytic enzyme production. Despite the fact that a number of Pseudomonas biocontrol products have been approved for use in agriculture, several limitations remain. Many strains perform well in trials, but this does not always translate into consistent, effective biocontrol in a variety of field conditions.

Keywords: *Biocontrol; Rhizobacteria; Fluorescent; Pseudomonas.*

I. INTRODUCTION

To sustain consistently high yields, conventional agriculture relies significantly on the use of chemical inputs such as fertilisers and pesticides. Alternatives to this system, on the other hand, are becoming increasingly popular. This stems from two points of view: environmental conservation and human health concerns. In terms of the environment, concerns include chemical residue pollution of soil and water, habitat destruction and loss, the harmful impact of pesticides on non-target species, and biodiversity consequences. Chemical residues in food products and in the environment are also causing worry among consumers and health authorities. Of course, there is precedence for harmful consequences, since several formerly approved fungicides and insecticides have now been outlawed. The EU monitors chemicals allowed under Directive 91/414/EC because of these environmental and health concerns. This is a never-ending procedure that has included the need to defend certain previously approved substances (COM1112/2002). Some member states have exerted significant pressure to limit the use of certain fungicides (e.g., Sweden, thiabenzadole), and it is probable that more prohibitions on fungicide usage in agriculture will be enacted at the EU level. If fungicide reduction is to be achieved, viable alternatives to pesticides must be supplied.

Many naturally occurring bacteria and fungi have long been known as antagonistic to crop diseases, and hence have the potential to provide an alternative to artificial fungicides. The control of Lepidoptera by the bacterium Bacillus thuringiensis (Bt toxin) is perhaps the most well-known example, but there are numerous more well-documented examples of bacteria and fungi that can prevent the spread of soil-borne bacterial and fungal diseases of crops. These diseases, including as root rot, damping off, and take-all, are presently managed, at least in part, by seed coatings or liquid formulations containing chemical fungicides. It's highly enticing to replace these fungicides with bacteria that have inherent intrinsic action against fungal infections.

II. MICROBIAL BIOCONTROL INOCULANTS

Phytostimulation, bio fertilization, bioremediation, and biocontrol are just a few of the potential uses of microbial inoculants for a sustainable agri sector that have been highlighted in recent studies. Biocontrol is a multifaceted phenomenon whose success is determined by a variety of circumstances. These include the capacity of the microbial inoculant to persist in the rhizosphere and compete with native microbial populations, as well as the ability to protect the plant host from pathogens at both the time and location of infection. Many naturally occurring bacteria and fungi have long been known as antagonistic to crop diseases, and hence have the potential to provide an alternative to artificial fungicides. Potential biological control agents have been identified as bacteria from the Bacillus and Trichoderma genera, as well as fungus from the Trichoderma genus. Bacillus has been used in the development of a variety of biocontrol products (mainly in the US). Pseudomonas bacteria have lately received attention, and a variety of commercially available Pseudomonas-based biocontrol inoculants have been created. The soil-borne fluorescent pseudomonads have been identified as promising candidates for use in biocontrol applications based on their genotypic and phenotypic traits.

The innate capacity of specific strains of Pseudomonas bacteria to colonise the rhizosphere at a high density, compete well with microorganisms, and create secondary metabolites with potent antifungal action such as 2, 4-diacetylphloroglucinol is of special interest (Phl). Phl is a broad-spectrum antibiotic generated by Pseudomonas fluorescents spp., with biological action against a variety of fungal and bacterial plant diseases. Inoculants of Pseudomonas that generate Phl have also been linked to the development of systemic disease resistance in plants.

III. PSEUDOMONAS' APPLICATION AS A BIOCONTROL AGENT

In laboratory and greenhouse research, the idea of regulating crop rhizosphere microbial populations by inoculating beneficial bacteria, such as P. fluorescens, to boost plant development has shown tremendous promise. This approach's potential environmental advantages, such as a reduction in the usage of agricultural pesticides, align with sustainable management techniques. As bio fungicides, we should anticipate to see new P. fluorescent products available to farmers. The capacity to control the rhizosphere to improve the survival and competitiveness of these beneficial microbes will be critical to the success of these goods. The genome's sequencing revealed more about the organism's environmental connections and metabolic capacities, which may be utilised to combat plant illnesses. Despite the fact that P. fl fluorescent is the most extensively used biocontrol agent, it has a number of drawbacks, including a short shelf life and uneven field effectiveness.

IV. RISK ASSESSMENT OF PSEUDOMONAS BIOCONTROL INOCULANTS

In Europe the deliberate release into the environment of genetically modified organisms is legislated by Council Directive 2001/18/EC, which repeals Council Directive 90/ 220/EEC. Before market approval, consideration must be given to

(1) case-by-case environmental risk assessment prior to any release,

(2) Deliberate release at the research stage as a necessary part of new product development,

(3) Progressive authorized release ‘step by step’, and

(4) Satisfactory field testing at the research stage in ecosystems that could be affected.

In North America release into the environment of genetically modified organisms is under the authority of the USDA, FDA and EPA. The successful utilization of microbial inoculants with improved biocontrol traits such as the ability to overproduce secondary metabolites relies on their introduction to the rhizosphere posing no risk to the environment. Prior to assessing the impact of these genetically modified strains on nontarget microorganisms, the influence of wild-type strains must be investigated to establish appropriate comparative baselines. In independent field-based trials, the influence of P. fluorescens F113, a Phlproducing wild-type strain, on sugarbeet yield parameters and on the ecologically significant resident fluorescent Pseudomonas population in the rhizosphere of sugarbeet was assessed (Moe¨nne-Loccoz et al., 2001). Plant parameters such as germination, root yield/ quality and sugar yield were not reduced by the addition of P. fluorescens F113. Inoculation with this strain also had no influence on size, genetic diversity or distribution of phylogenetic groups (based on amplified ribosomal DNA restriction analysis, ARDRA) of the resident fluorescent population in the rhizosphere of sugarbeet. Shifts (based on carbon utilization) in this resident population occurred following inoculation, but these were spatially limited to the rhizoplane and did not result in a deleterious alteration of ecosystem function. The residual impact of this P. fluorescens F113 Phl-producing strain was measured in field-based studies by investigating the effect of a previous inoculation of sugarbeet on the resident rhizobia population nodulating a subsequent red clover crop rotation. Addition of the P. fluorescens F113 Phl-producing strain did not reduce genetic diversity, but resulted in a shift in the population where an enrichment of Rhizobium leguminosarum bv. trifoli strain isolates that belonged to the dominant rapid amplified polymorphic DNA profile occurred. However, this perturbation did not result in the ability of the Rhizobium–clover symbiosis to function or reduce clover yield. As expected, in some cases inoculation with P. fluorescens F113 resulted in perturbations in the soil microbial community structure; however, this did not translate into deleterious consequences for plant yield.

A rigorous assessment of the impact of P. fluorescens F113 genetically modified to overproduce Phl, on both nontarget microbial communities in the rhizosphere and plant yield parameters, was carried out within the framework of two multidisciplinary EU collaborative networks, IMPACT 1/II and ECO-SAFE. Notification for field release of P. fluorescens F113 inoculants genetically modified to overproduce Phl occurred in November 1997 and May 1998 for field sites cropped to maize and sugarbeet, respectively. In the field trials carried out in Granada, Spain, the impact of these strains on mycorrhizal fungal communities in the rhizosphere of maize was investigated. Inoculation had a beneficial influence on mycorrhizal symbiosis where it stimulated mycelial development from germinated Glomus mosseae spores and the degree of overall mycorrhizal colonization of maize roots. In addition, inoculation resulted in an increase in both maize shoot and root yield compared with wild-type-inoculated and uninoculated control plants (Barea et al., 1998). In the parallel study carried out in Ravenna, Italy, inoculation with the Phloverproducing P. fluorescens F113 strain had no influence on the population sizes of resident microbial populations, namely microfungi, Streptomycetes and fluorescent pseudomonads, in the rhizosphere of sugarbeet with respect to the wild-type control. In addition, inoculation by this strain did not have a harmful effect on crop yield or quality, with germination, root length and biomass comparable to sugarbeet plants inoculated with the wild type strain and the uninoculated control plants. Other key studies have been conducted to investigate the impact of other Pseudomonas inoculants with a range of genetically modified biocontrol relevant traits on nontarget microrganisms in the rhizosphere. In some instances, the scientific literature suggests that the addition of a microbial inoculant genetically modified for improved biocontrol traits such as secondary metabolite production can cause perturbations in the microbial community structure in the rhizosphere; however, this did not translate into deleterious consequences for plant yield.

V. FUTURE DIRECTIONS FOR THE EXPLOITATION OF PSEUDOMONAS BIOCONTROL INOCULANTS

A considerable body of research has been done on the exact methods by which Pseudomonas controls phytopathogenic fungi on a global scale, and a variety of biocontrol products based on these microorganisms have been sold (mainly in the US). Despite this, there are still several significant barriers to the general use of this technology in agriculture, one of which being the product's effectiveness. Many strains perform well in specialised studies, but this does not always transfer into consistent, effective biocontrol in a variety of field conditions. Some of this is due to external variations like soil or climatic circumstances, but a significant portion is due to microbe intrinsic features like fluctuating synthesis of essential metabolites or poor colonisation under specific conditions.

The rhizosphere is a zone where bacteria and plants interact intensely. Although there is a wealth of scientific understanding regarding the biology and biochemistry of this ecologically important niche, little is known about the molecular interactions that occur between plants and bacteria in the rhizosphere. The result of the many relationships between these biological systems in nature is influenced by the flow of signals between bacteria and plant hosts. Signal molecules released by the plant impact both the main initiation and subsequent behaviour of bacteria in complex plant–bacteria interactions. These signalling pathways are important for the start of positive interactions like biocontrol. For the formation of successful Pseudomonas biocontrol strains, aggressive colonisation and the capacity to compete with existing microbes are required. Molecules emitted by plant roots may function as signals, influencing microbial strains' capacity to colonise and survive in the rhizosphere. By altering root cell leakage, cell metabolism, and plant nutrition status, microbes can impact the composition of these root exudates.

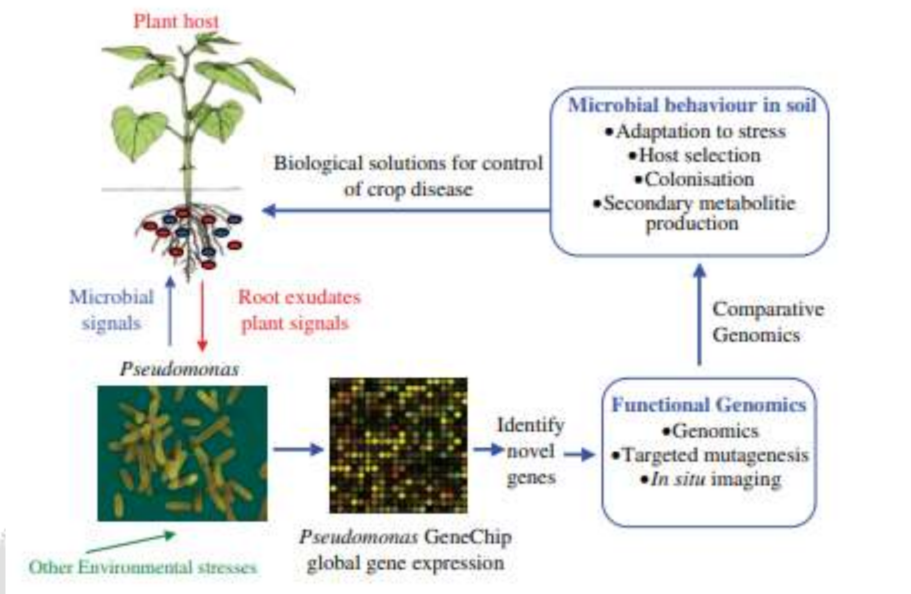

Figure 1: A systems-based approach to investigate molecular-based signalling mechanisms in Pseudomonas–plant interactions.

Several plant diseases have well-documented plant host-specific responses. Mycorrhizal fungi and Rhizobium, for example, may create symbiotic connections with a high degree of host specificity. Many additional beneficial interactions between microorganisms and plants now appear to have some degree of host specificity. In the rhizosphere, different plant species can actively select for different microbial communities. In field-based studies, researchers used a culture-independent technique to discover the presence of plant-host-specific endophytic bacterial populations. This plant host selection can also happen within the same plant species' variations or cultivars. Different sugarbeet types can select for genetically and functionally distinct populations of resident culturable fluorescent pseudomonads in the rhizosphere, according to field experiments (G. L. Mark, unpublished data). The stimulation of bacterial gene expression in response to molecular signals sent by the host roots plays a role in plant-mediated selection. Despite widespread agreement that plant-derived extracellular signals can influence bacterial behaviour in the rhizosphere, little is known about their impact on bacterial gene expression patterns and the involvement of genes with changed expression in the plant–microbe interaction.

VI. CONCLUSION

Biocontrol agents, unlike chemical pesticides, require ongoing assistance to establish themselves in a specific niche. As a result, for biological control to be successful, one must assure not only the quality of the biocontrol agent used, but also its establishment in the natural environment so that it may survive and compete effectively with diseases. Another area of research is the development of improved formulations to ensure field activity survivability and compatibility with chemical and biological seed treatments. P. fluorescens as a bioagent has a promising future because of its high cost-benefit ratio. In light of this, the first hypothesis is to isolate P. fl uorescens bacteria with enhanced antagonistic activity against soil-borne fungal pathogens from the rhizo sphere of various field crops under native environmental conditions, and then test the ability of selected bacterial isolates to suppress soil-borne fungal pathogens in vitro.

VII. REFERENCES: -

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