Research Abstract

Evaluation of microbial antagonists for suppression damping-off and root rot diseases of vegetable crops

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A sustainable and eco-friendly crop production system requires a reduction in the use of chemical inputs in agriculture. It has long been recognized that there are many naturally occurring bacteria and fungi that are antagonistic to crop pathogens. These antagonistic microbes have the potential to provide an alternative to chemical fungicides. Hence, the research on eco-friendly adds safer plant protection measures is now focused towards exploring the potential of beneficial microbes in crop disease management. Bio-control agents are eco-friendly, easy to deliver, and safer alternatives to chemicals. These antagonists act through antibiosis, secretion of volatile toxic metabolites, mycolytic enzymes, parasitism, through competition for space and nutrients and by induction of systemic resistance (SAR) in host. Most of the commercially available biocontrol agents are effective not only in controlling plant pest and diseases but also, in plant growth promotion. Genetic engineering of microorganisms has been found to be useful for the commercial production of agricultural products. Biocontrol agents may be improved by genetically engineering them to overexpress one or more anti-phytopathogen traits which can act synergistically without altering the normal functioning of the bacterium. Genetic modification of naturally occurring bacteria strains with genes that are beneficial to plants will lead to accentuate the expression of the genomic products which could alleviate the attack of both pests and diseases. This will facilitate the production of a single bacterium with multiple modes of action to benefit the growers by reducing the inputs invested on plant protection measures. More than 150 bacterial isolates obtained from a commercial potato field were screened for bio-control potential against soilborne pathogens such as *Rhizoctonia solani, Pythium ultimum, Phytophihora capsici* and *Streptomyces scabies,* these pathogens cause diseases of many vegetable crops including radish, cucumber, and potato. Three isolates (9A-14, 8D-45 and 8B-1)

were found to be the most effective in inhibition the growth of above pathogens in laboratory, growth room and under field conditions. The selected bacteria were identified and characterized based on phenotypic characteristics, biochemical tests using AP20 and CHB50 Kits and molecular tests. The 16S-23S rDNA gene of isolates *Pseudomonas fluorescens* strain 9A-14, *Pseudomonas. sp* 8D-45 and *Bacillus subtilis* strain 8B-1 were isolated, cloned, sequenced and submitted to the database of GenBank under the respectively submission numbers (JX905208, JX905209 and JX905210). Mode of action of the isolates to suppress several plant pathogens were studied and their role in promoting plant growth were investigated under growth room and micro- plot trials. Both strains (*P. fluorescens*. 9A-14 and *Pseudomonas* sp. 8D-45) produced HCN and Siderophores in growth media. The culture filtrates of all three strains showed positive activity for Indole Acetic Acid (IAA), Salicylic Acid (SA), and β-1, 3-glucanase. Antibiotic biosynthetic genes of Phenazine and 2, 4-DAPG, Pyrrolnitrin and Pyoluteorin were detected using specific primers and PCR technology. Isolate *P. fluorescens* 9A-14 has 3 types of antibiotics genes (2, 4-DAPG, Pyrrolnitrin and Pyoluteorin) and the isolate *Pseudomonas* sp. 8D-45 has only Phenazine. All isolates showed very good bio-control and plant growth promotion activity in the growth room assays. When applied as pre- or post-planting treatments to the infested peat-based mix, all three isolates of bacteria protected cucumber and radish seedlings from damping-off by improving the percentage of healthy cucumber and radish seedlings and reducing disease severity. The average fresh weights of cucumber and radish seedling produced in pots receiving pre- or post-planting application of the three bacteria were greater than those of plants produced in the pathogen-infested and non-infested control pots. Peat and talc-based formulations of the most effective strains of bacteria were applied as pre-planting or seed treatments and post treatments to the infested substrate also provided effective control of damping-off and root rot of cucumber, damping-off of radish seedling, common scab of potato and promoting plant growth under greenhouse, micro-plot and field conditions. Shelf life studies bacteria isolates in both talc based and peat formulations were studied. All bacteria isolates had shelf life more than 110 days in peat formulation compare with 90 days in talc powder formulation. The combined formulation of *Pseudomonas fluorescens* strain 9A-14 and *Bacillus subtilis* strain 8B-I with specific concentration can be recommended as Bio-control product to control soilborne plant diseases. A patent application may be filed after repeated field trials.