A Novel Approach Using H-Mutant Bacteriophages For The Biological Control of Bacterial Blight of Geranium

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Industry Needs and Project Objectives

Geraniums led the nation in wholesale value of all bedding plants in 1994 with a value of 165 million dollars made up of sales from bedding flats ($36 million) and potted geraniums produced from cuttings ($85 million) or seed ($44 million). Perhaps the most difficult problem to overcome in geranium production, and certainly the most serious disease of geraniums, is bacterial blight caused by Xanthomonas campestris pv pelargonii (XCP). Bacterial blight, also referred to as bacterial stem rot, bacterial leaf spot or bacterial wilt, has destroyed entire geranium crops and caused millions of dollars in losses. This deadly disease is feared by geranium growers because it is extremely difficult to control and can be spread through cuttings, soil, water, and insects such as whiteflies.

Current control measures are directed at preventing the disease and include using culture-indexed plants, destroying infected plants, and disinfecting equipment and growing structures. Both seed and cutting geraniums are susceptible, with the outlook for resistant cultivars bleak. Bactericides have not been very effective in controlling this disease; furthermore, bacteria are notorious for developing resistance to new products quickly. In addition, pesticides are not considered environmentally safe by the public and the horticulture industry is constantly under pressure to find alternative control measures.

Our research, which is supported by the American Floral Endowment, is a novel approach utilizing bacteriophages as biological control agents for the prevention and control of bacterial blight of geraniums. Bacteriophages are viruses that kill bacteria. These viruses are very specific for target bacteria and are nontoxic to workers and non-targeted bacteria. Thus, this research is to develop an environmentally safe and effective alternative for prevention and control of bacterial blight of geraniums by utilizing bacterial viruses.

Bacteriophages were once abandoned as biological agents due to the emergence of bacterial mutants resistant to the bacteriophages employed. However, our approach utilizes a mixture of three to eight different bacteriophages including h-mutants. H-mutants are so named because they lyse (kill) not only parent wild-type bacteria, but also bacteriophage-resistant mutants originating from parent bacteria.

Progress to Date

We have successfully isolated two phages from soil samples taken in California and Florida. These phages produced virulent reactions for six of thirty XCP strains, and lysogenic reactions for twenty-two strains. Lysogenic reactions occur when bacteriophages infect the bacteria but do not immediately lyse the cells. This reaction at the microbial level is similar to a parasite living on or within another organism in larger animals. After further purification and selection, the range of virulence for these two bacteriophages was increased. The improved bacteriophage killed three of four previously lysogenic strains.

Different XCP strains can harbor lysogenic-bacteriophages that will kill other XCP strains. So we tested all possible combinations of 25 XCP strains to determine if one XCP strain would inhibit growth of another XCP strain. Three XCP strains inhibited no other strains of XCP. Seven strains inhibited four or fewer strains. Fourteen strains inhibited from 10 to 19 strains. Six strains inhibited 17 to 19 strains. We are currently determining if we can isolate bacteriophages from the six strains which had the greatest effect on growth inhibition of other XCP strains.

Although the focus of our research this year has been to locate, isolate, and improve different strains of bacteriophages, we also initiated studies to test the effectiveness of bacteriophages sprayed on geranium leaves. For example in our latest experiment, geranium plants in 4-inch pots were inoculated with XCP and placed among six healthy, non-inoculated, plants. Plants were sprayed overhead daily to simulate conditions that would spread the disease. Plants were also sprayed daily with a solution containing bacteriophages, bacteriophages with hrp-, hrp-, or water. The hrp- is an additional biological control agent we are testing that is a non-pathogenic strain of bacteria created by chemical mutagenics in the laboratory of Dr. R. E. Stall. These bacteria may improve control by either occupying infection sites on leaf surfaces preventing pathogenic bacteria from infecting the leaf, or by triggering a defense mechanism within the host plant. The bacteriophage + hrp- treatments reduced the number of leaves which had visible spots from bacterial blight by 57% compared to control plants, and the bacteriophage or hrp- alone by about 37%. In order for this control method to be more successful, a mixture of different bacteriophage strains including h-mutant strains will need to be used. However, this level of control with only one bacteriophage is very encouraging, and we believe a good indication that control can be achieved when we develop a mixture of three to eight phages including h-mutants.

Future Plans and Anticipated Benefits to the Industry

We are currently analyzing plant and soil samples from growers around the U.S. that have sent in potted plants with bacterial blight. It is important to find many strains of bacteriophages to insure a broad range of effectiveness against all strains of XCP. We are also developing techniques to evaluate over 70 XCP strains that have been collected from around the world to determine if bacteriophages can be isolated from them. In addition to utilizing laboratory techniques, we have inoculated geraniums in ground beds with 25 XCP strains from around the world in order to study the dynamics of lysogenic strains of XCP under field conditions and the potential for bacteriophage isolation under natural conditions.

As more bacteriophage strains are isolated, we will continue our work to demonstrate the practical feasibility of using appropriate mixtures of h-mutant phages for biological control of bacterial blight of geraniums. Concentration of bacteriophages needed in spray treatments, longevity of bacteriophages on plants, application methods, and effect of environmental factors must all be understood in order to take the concept from the laboratory to the greenhouse. The utilization of h-mutant bacteriophages and the application procedures to be tested in this research on bacterial blight of geraniums have the potential to be developed for control of other bacterial diseases of floricultural crops including cut flowers, flowering potted plants, and bedding plants.