EFFECT OF CHITINASE ENCODING GENES IN BIOCONTROL PSEUDOMONAS SPP.

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INTRODUCTION

Chitin is an unbranched polysaccharide composed primarily of beta 1,4 linked N-acetylglucoseamine residues. It can be regarded as a cellulose analog, in which the hydroxyl groups have been replaced by N-acetylglucoseamine groups. Chitin is a major component of the cell walls of most fungi, except for the class Oomycetes. Insects, nematodes and other invertebrates have chitin as a structural component of their exoskeleton. Vascular plants and mammals lack chitin. The enzymatic digestion of the chitin components of plant pathogens and plant pests could present an effective method for their control. At the specific sites of infection in the rhizoplane or phylloplane, chitin degradation can be stimulated by addition of chitin substrates or bacteria with high chitinolytic activity.

The degradation of chitin is catalyzed by chitinases, which hydrolyze chitin to chitodextrins. Bacteria, fungi, animals and higher plants commonly have chitinases. The production and secretion of chitinases by microorganisms may be important in the biological control of plant pathogenic fungi. Addition of chitin-containing amendments to soil has been reported to affect fungi and nematodes (Mitchell and Alexander, 1962). Chitinase positive Serratia spp. have been used for biocontrol of plant pathogens (Sneh et al., 1985; Ordentlich et al., 1988). Also, chitinase-positive strains of Escherichia coli have shown biocontrol potential (Shapira et al., 1989).

Serratia marcescens, a Gram negative, enteric soil bacterium produces and secretes large amounts of chitinases. It has been the object of investigations into the genetic basis of chitinase production. Five different chitinolytic proteins have been isolated from S. marcescens with molecular sizes of 21, 36, 48, 52, and 57 KD respectively. The data indicate that chitinases of S. marcescens are endolytic enzymes that solubilize chitin more rapidly than the exolytic chitinases (Montreal and Reese, 1969).

Chitinase production in S. marcescens is inducible by the substrate. However, there is probably a small background of insolubility of chitin, it is unlikely to serve as an inducer for chitinase activity. Both D-glucoseamine and N-acetylglucoseamine act as inducers of chitinase production (Smith and Grula, 1983).
RESULTS

A genomic library of *S. marcescens* was constructed in the broad host range cosmid pLAFR3. Following partial digestion with EcoRI and separation of the fragments on a sucrose gradient, fractions containing 15 to 30 kb were pooled and ligated into the cosmid. The ligated DNA was packaged using Packagene (Promega, WI, USA) and transduced into HB101. Chitinase-positive clones were selected on a chitin medium (Sundheim, 1987).

More than 90% of the random clones contained inserts. Of 5686 transductants, 21 expressed chitinase activity as indicated by clearing of a chitin medium. Four of the chitinase-encoding clones had an 18-kb EcoRI fragment and seventeen had a 9.4-kb EcoRI fragment. Southern hybridization experiments showed no homology between the two groups, and restriction enzyme maps indicated no similarity.

Cosmids of the two groups were mobilized from *E. coli* into root-colonizing, fluorescent *Pseudomonas* strains with demonstrated biocontrol potential. A triparental mating protocol was employed using pRK2013 as helper plasmid. The mating yielded *Pseudomonas* transconjugants that expressed chitinase activity on the chitin medium (Sundheim et al., 1988).

Effect of Chitinase-positive Bacteria on Fungi

Hyphal growth of the two plant pathogenic fungi, *Rhizoctonia solani* and *Magnaporthe grisea*, was determined. In the presence of cosmid containing strains, growth of the two fungi was inhibited. In an other experiment, culture filtrate of a cosmid containing strain inhibited germ tube growth of *Fusarium oxysporum* f. sp. *redolens* and *F. oxysporum* f. sp. *conglutinans*. Cosmid-containing strains showed greater inhibition of germ tube growth than did the parental strains.

Effect of Chitinase-positive Bacteria on Disease

Radish seed was coated with bacteria and planted in sand. When the plants were one week old, they were inoculated with a conidial suspension of *F. oxysporum* f. sp. *redolens*. One chitinase-positive *Pseudomonas* strain reduced disease more than did the parental strains.

In a tube assay, soil was inoculated with the take-all fungus *Gaeumannomyces graminis* var. *tritici* and wheat seed treated with bacteria were planted. The plants were maintained in a growth chamber with 12 h light per day. Four weeks after inoculation the plants were harvested and rated for take all severity. The chitinase positive strains reduced disease, but the effect was low and not significantly different from the parental strains. When 100 random colonies were analyzed at the end of the experiment, all were sensitive to tetracycline. This indicated that the cosmids were lost from the bacteria during the course of the experiment (Sundheim et al., 1988).

Research is in progress to stabilize the chitinase encoding genes in *Pseudomonas*. Two strategies are possible. One is to clone the chitinase genes into a more stable plasmid. Another strategy is to integrate the chitinase encoding genes into chromosomal DNA by homologous integration.

LITERATURE CITED