Cloning of Serratia marcescens chiA Gene as a Biocontrol Alternative for Plants Targeted by Pathogenic Fungi

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Serratia marcescens is one of the most effective Gram-negative bacteria for degradation of chitin 1, 4-B-glucosamine, which is a major structural component of not only the exoskeletons of insects and crustaceans, but also in the cell wall of several fungi. This links chitinase with large importance in biotechnology due to the enzymes natural function as a biocontrol agent against fungal pathogens. It was therefore hypothesized that if Serratia marcescens' chiA gene can be isolated through Polymerase Chain Reaction, then it’s amplification could be cloned and placed into a vector for expression in plant model system affected by fungal elicitors. The methodology for this project began with extracting the genomic DNA from S. marcescens, which was then used along with whole gene sequencing primers to amplify the chiA gene through PCR. The PCR product was confirmed using gel electrophoresis and later successfully sequenced by a genomic service company. When sequenced, the chiA amplicon showed 99% homology with reported sequences in the NCBI data bases at both the nucleotide and translated amino acid levels. This concluded the successful isolation and sequencing of the ChiA gene. For the gene's ligation into the recipient vector, primers were designed with the purpose of amplifying the sequenced chiA gene along with a simultaneous addition of restriction sites to the ends of the gene in order to facilitate cloning into pSIM24 KanR for evaluation of gene's efficacy as well as chitinolytic activity.