

Title Improved biocontrol of fruit decay fungi with *Pichia pastoris* recombinant strains expressing Psd1 antifungal peptide

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Abstract

Future expansion of biological control of postharvest diseases will depend largely on improving its effectiveness under a broader range of conditions and expanding its activity to new commodities and new diseases. Plasmid pGAPZ α C/Psd1, a binary vector encoding the constitutive expression of the gene for the pea defensin Psd1, was used to transform the yeast *Pichia pastoris*, and transformed strains were evaluated for enhancing biocontrol potential by Psd1. Two *P. pastoris* strains, X-33 and GS115, were successfully transformed by electroporation and produced the active rPsd1 peptide. Nontransformed strain X-33 grew faster than strain GS115 in Golden Delicious apple wounds and was chosen as the host for plasmid pGAPZ α C/Psd1 in biocontrol tests. The severity and incidence of blue mold decay caused by *Penicillium expansum* were significantly reduced on apples treated with X-33(pGAPZ α C/Psd1/X-33) when compared to apples inoculated with this fungus alone or in combination with the nontransformed parental strain X-33, or the X-33(pGAPZ α C/X-33) recombinant containing the empty binary vector. Four selected transformants reduced decay in repeated studies, but were effective only when applied at a lower (6.3×10^5 CFU mL⁻¹) cell concentration. This study demonstrates the potential of Psd1 for enhancing suppression of postharvest diseases. However, the full potential of the Psd1 defensin may be achieved after optimizing its expression and activity on the fruit.