Title Separate and combined disruptions of two exo-β-1,3-glucanase genes decrease the efficiency of *Pichia anomala* (Strain K) biocontrol against *Botrytis cinerea* on apple

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Abstract

The modes of action of the antagonistic yeast *Pichia anomala* (strain K) have been studied; however, thus far, there has been no clear demonstration of the involvement of exo-β-1,3-glucanase in determining the level of protection against *Botrytis cinerea* afforded by this biocontrol agent on apple. In the present study, the exo-β-1,3-glucanase-encoding genes *PAEXG1* and *PAEXG2*, previously sequenced from the strain K genome, were separately and sequentially disrupted. Transfer of the *URA3*-Blaster technique to strain K, allowing multiple use of *URA3* marker gene, first was validated by efficient inactivation of the *PaTRP1* gene and recovery of a double auxotrophic strain (uracil and tryptophan). The *PAEXG1* and *PAEXG2* genes then were inactivated separately and sequentially with the unique *URA3* marker gene. The resulting mutant strains showed a significantly reduced efficiency of biocontrol of *B. cinerea* when applied to wounded apple fruit, the calculated protection level dropping from 71% (parental strain) to 8% (mutated strain) under some experimental conditions. This suggests that exo-β-1,3-glucanases play a role in the biological control of *B. cinerea* on apple. Furthermore, biological control experiments carried out in this study underline the complexity of the host-antagonist-pathogen interaction. Two experimental parameters (yeast inoculum concentration and physiological stage of the fruit) were found to influence dramatically the protection level. Results also suggest that, under some conditions, the contribution of exo-β-1,3-glucanase to biological control may be masked by other modes of action, such as competition.