Title Characterization of extracellular lytic enzymes produced by the yeast biocontrol agent *Candida oleophila*

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Abstract

The yeast *Candida oleophila*, the base of the commercial product Aspire, is recommended for the control of postharvest decay of citrus and pome fruit. Competition for nutrients and space is believed to be the major mode of action. Involvement of fungal cell wall-degrading enzymes is also suggested to play a role in the mechanism of action of yeast antagonists. The present study showed that the yeast *C. oleophila* is capable of producing and secreting various cell wall-degrading enzymes, including exo-β-1,3-glucanase, chitinase and protease. Exo-β-1,3-glucanase and chitinase were produced and maximized in the early stages of growth, whereas protease reached a maximum level only after 6–8 days. Production of exo-β-1,3-glucanase, chitinase and protease was stimulated by the presence of cell wall fragments of *Penicillium digitatum* in the growth medium, in addition to glucose. This study also provided evidence that *C. oleophila* is capable of secreting exo-β-1,3-glucanase into the wounded surface of grapefruit. The role of exo-β-1,3-glucanase (*CoEXG1*) in the biocontrol activity of *C. oleophila* was tested using *CoEXG1*-knockouts and double-*CoEXG1* over-producing transformants. In vitro bioassays showed that wild-type *C. oleophila* and exo-β-1,3-glucanase over-expressing transformants had similar inhibitory effects on spore germination and germ-tube elongation; and both were more inhibitory to the fungus than the knockout transformant. In experiments conducted on fruit to test the biocontrol activity against infection by *P. digitatum*, no significant difference in inhibition was observed between transformants and untransformed *C. oleophila* cells at the high concentrations of cells used, whereas at a lower concentration of yeast cells the knockout transformants appeared to be less effective.