Title Biocontrol of *Botrytis cinerea* in table grapes by non-pathogenic indigenous *Saccharomyces cerevisiae* yeasts isolated from viticultural environments in Argentina

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Citation Postharvest Biology and Technology, Volume 64, Issue 1, February 2012, Pages 40-48

Keywords Biocontrol; Table grape; *Saccharomyces cerevisiae*; *Schizosaccharomyces pombe*; *Botrytis cinerea*; Pathogenic yeasts

Abstract

Botrytis cinerea, the causal agent of gray mold, is an important disease of grapes. Yeasts are members of the epiphytic microbial community on surfaces of fruits and vegetables and because some yeasts inhibit fungi they are used as biocontrol agents. The major objective of the present work was to isolate yeasts from grapes, vineyard soil, and grape must and select them for their ability to prevent gray mold onset after harvest. Yeasts that were found effective against the fungus were also assayed for their possible pathogenicity in humans. Two antagonism experiments were performed to study the effect of yeasts on *B. cinerea*, an *in vitro* study with Czapeck Yeast Extract Agar and an *in vivo* study with grape berries at 2 °C and 25 °C; both experiments were conducted at different yeast concentrations (10^5, 10^6 and 10^7 cfu/mL). Antagonists were subsequently assayed for their ability to colonize and grow in fruit wounds. The biocontrol yeasts were also examined for their possible pathogenicity in humans: phospholipase and proteolytic activity, growth at 37 °C and 42 °C, pseudohyphal formation and invasive growth. A total of 225 yeasts belonging to 41 species were isolated from must and grape berries and 65 of them, representing 15 species, exhibited *in vitro* inhibition of *B. cinerea* at 25 °C. These 65 biocontrol yeasts were subsequently assayed *in vivo* and 16 of them (15 *Saccharomyces cerevisiae* and 1 *Schizosaccharomyces pombe*) showed antagonistic properties against *B. cinerea* at 25 °C. Only one isolate (*S. cerevisiae* BSc68) was able to inhibit mycelial growth of *B. cinerea* on grape berries at both 2 °C and 25 °C. The biomass of this strain in grape wounds increased 221.5-fold at 25 °C after 3 d and 325.5-fold at 2 °C after 10 d of incubation. An increase in the concentration of certain yeasts significantly enhanced their antagonistic activity. All yeast isolates determined as biocontrol agents under *in vivo* conditions were isolated from fermenting musts. Twelve biocontrol agents (*S. cerevisiae*) revealed one or more phenotypical characteristics associated with pathogenicity in humans but none of them showed all characteristics together. The fact that there exist few reports on *S. cerevisiae* and none on *Sch. pombe* as biocontrol agents against *B. cinerea* makes our results even more relevant.