

Title Cell death and regrowth of biocontrol yeast *Cryptococcus laurentii* regulated by sodium citrate
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Abstract

The yeast *Cryptococcus laurentii* has been selected to be an effective biocontrol agent against postharvest diseases, and the model of action of this antagonist is to compete for nutrition particularly important to conserve cell viability for the mass production of shelf-stable formulated product. Here we show that yeast cells incubated in water remain viable for weeks, whereas sodium citrate (100mM, pH 5.8) induces rapid loss of viability within 72 h. The induction of cell death is dependent on citrate and sodium ions as well as the concentration and pH value of the medium, is accompanied by membrane damage, nucleus fragmentation and rapid production of reactive oxygen species (ROS), and is delayed by addition of ascorbic acid and GSH to sodium citrate-incubated cells or by overexpression of antioxidant enzymes such as superoxide dismutases or catalase. Small clones appear along with the rapid loss of cell viability, showing altered carbon metabolism pathway as compared with wild strains. In addition, adaptive regrowth is observed at 10d of post-incubation, but the regrowth mutants show biocontrol activity similar to that of the wild clones. Extracellular metabolite profiles significantly change between cell death and adaptive regrowth. The present study raises a convenient and successful method for commercial production of biocontrol yeast with right quality and shelf-life.