

Isolation and Identification of Low-Temperature Tolerant-Yeast Strains from Apple with Biocontrol Activity

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Abstract : Various microbes, such as fungi and bacteria species, are naturally found in the fruit microbiota, and some of them act as a pathogen and result in fruit rot. Among non-pathogenic microbes, yeasts (single-celled microorganisms belonging to the fungi kingdom) can colonize fruit tissues and interact with them without causing any damage to them. Although yeasts are part of the plant microbiota, there is little information about their interactions with plants in comparison with bacteria and filamentous fungi. According to several existing studies, some yeasts can colonize different plant species and have the biological control ability to suppress some of the plant pathogens. It means those specific yeast-colonized plants are more resistant to some plant pathogens. The major objective of the present investigation is to isolate yeast strains from apple fruit and screen their ability to control *Penicillium expansum*, the causal agent of blue mold of fruits. In the present study, psychrotrophic and epiphytic yeasts were isolated from apple fruits that were stored at low temperatures (0-1°C). Totally, 42 yeast isolates were obtained and identified by molecular analysis based on genomic sequences of the D1/D2 and ITS1/ITS4 regions of their rDNA. All isolated yeasts were primarily screened by 'in vitro dual culture assay against *P. expansum* by measuring the fungus' relative growth inhibition after 10 days of incubation. The results showed that the mycelial growth of *P. expansum* was reduced between 41-53% when challenged by promising yeast strains. The isolates with the strongest antagonistic activity belonged to *Metschnikowia pulcherrima* A13, *Rhodotorula mucilaginosa* A41, *Leucosporidium Scottii* A26, *Aureobasidium pullulans* A19, *Pichia guilliermondii* A32, *Cryptococcus flavescens* A25, and *Pichia kluyveri* A40. The results of seven superior isolates to inhibit blue mold decay on fruit showed that isolates *A. pullulans* A19, *L. scottii* A26, and *Pi. guilliermondii* A32 could significantly reduce the fruit rot and decay with 26 mm, 22 mm and 20 mm zone diameter, respectively, compared to the control sample with 43 mm. Our results show *Pi. guilliermondii* strain A13 was the most effective yeast isolates in inhibiting *P. expansum* on apple fruits. In addition, various biological control mechanisms of promising biological isolates against blue mold have been evaluated to date, including competition for nutrients and space, production of volatile metabolites, reduction of spore germination, production of siderophores and production of extracellular lytic enzymes such as chitinase and β -1,3-glucanase. However, the competition for nutrients and the ability to inhibit *P. expansum* spore growth have been introduced as the prevailing mechanisms among them. Accordingly, in our study, isolates A13, A41, A40, A25, A32, A19 and A26 inhibited the germination of *P. expansum*, whereas isolates A13 and A19 were the strongest inhibitors of *P. expansum* mycelia growth, causing 89.13% and 81.75 % reduction in the mycelial surface, respectively. All the promising isolates produced chitinase and β -1,3-glucanase after 3, 5 and 7 days of cultivation. Finally, based on our findings, we are proposing that, *Pi. guilliermondii* as an effective biocontrol agent and alternative to chemical fungicides to control the blue mold of apple fruit.

Keywords : yeast, yeast enzymes, biocontrol, post harvest diseases

Conference Title : ICYB 2023 : International Conference on Yeast Biotechnology

Conference Location : San Francisco, United States

Conference Dates : June 05-06, 2023