Biocompatibility testing of yeast mixtures to select potential biocontrol agents

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Introduction

The Mexican states where citrus crops are important are: Nuevo León, Tamaulipas, San Luis Potosí, Colima, Michoacán, Puebla, and Oaxaca. Among the districts of Tamaulipas state, Victoria contributes with 71% and 90% of limes and oranges of the state production[1], respectively. Citrus production in Mexico is affected by many fungal diseases. The most important pre-harvest fungal diseases of *Citrus* sp. in Mexico are Fusarium dry rot (*Fusarium* sp.), and Postharvest diseases are Clear rot or Green/Blue mold (*Penicillium*), and Anthracnose (*Colletotrichum gloeosporioides*).

In this work, the compatibility of the combination of yeasts was assessed *in vitro* to be used as biocontrol agents[2] to control main citrus fungal diseases, and to select those with biological features suitable to be used as active ingredients of a biocontrol product in the future.

Experimental part

Compatibility amongst yeasts: The yeast used are the following gives the good results LCBG-03 (P*ichia guilliermondii*), LCBG-27 (*Macalpinomyces bursus*), LCBG-30 (*Pseudozyma sp.*), L10B2 ***(****Rhodotorula mucilaginosa)* The combinations to be tested were the following way 03+03, 03+27, 03+30, 03+L10B2, 03+49, 27+27, 27+30, 27+L10B2, 27+49, 30+30, 30+L10B2, 30+49, L10B2+L10B2, L10B2+49, 49+49. Aliquots of 100 µL of each yeast combinations tested at initial total inoculum concentration of $1x10^8$ cells/mL, and were evenly distributed on plates of 50% potato-dextrose agar (PDA) using a glass rod, incubated at 29°C. Each treatment was replicated two times. combined growth were observed CFU/ml calculated.

Biocontrol effect of mixed yeasts: Biocontrol effect of mixed yeast were done by testing the different combinations of yeasts mentioned above. Aliquots of 100 µL of each yeast combinations tested at initial total inoculum concentrations of 10^6, 10^7 and 10^8 cells/ L were evenly distributed on plates of 50% potato-dextrose agar (PDA) using a glass rod, and then an agar plug of 0.5 cm of diameter of the most infectious citrus fungus, obtained from the edge of a 7 d colony of each of the fungi tested growth on PDA were placed facing the center of the plates and incubated at 29°C. Fungi were labelled AL 13, AL 21, AL 38, and positive control H3A corresponding to *C. gloeosporioides*, *Fusarium* sp., *Penicillium digitatum* and *Phoma* sp., respectively. The colonies radii were recorded periodically for 5 d and the radial growth rate calculated accordingly. Each treatment was replicated three times.

Results and Discussion

The best performing yeast combination were L10B2+L10B2, 27+L10B2, 03+30, 03+27, 03+L10B2. Based on the percentage of inhibition of radial growth the best combinations were selected.

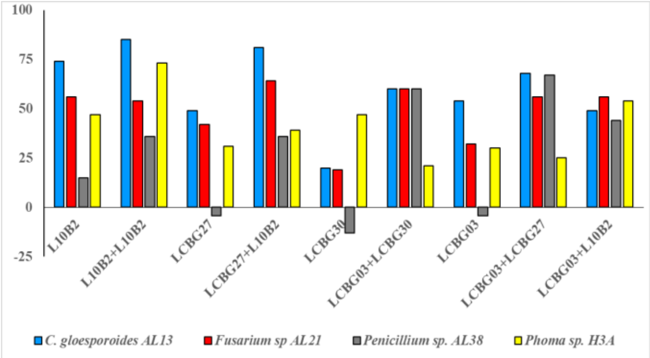

Figure 1. Percentage of radial growth rate inhibition by selected yeast combinations as compared with the individual results

The antagonistic mixture of *Candida sake* CPA-1 ($2×10^7$ CFU/ml) and bacterium *Pantoea agglomerans* ($2×10^7$ CFU/ml) control rot on Blanquilla pear and blue mold rot on Golden Delicious Apples[3]. The yeast *Pichia guillermondii*, with combination of five yeast isolates control black rot (*Ceratomyces paradoxa*) of pineapple at a low temperature (8-10°C)[4]. Our results demonstrate that not all combination of yeasts are compatible, and also the biocontrol efficacy depends on the fungus being controlled.

Conclusions

There are few reports on the literature assessing the performance of yeast combinations as biocontrol agents in horticulture crops but almost none in citrus sp. This work contributes in the search of those compatible yeast combinations aimed to diminish the fungal losses of citrus fruits.

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