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BIOCONTROL OF *Colletotrichum gloeosporioides* IN OLIVE FRUITS BY ANTAGONISTIC YEASTS

V.M. Pesce[1 3], G.P. Carrizo[1 3], M.C. Nally[1 3], M. Brizuela [1 4], L.A. Rodríguez Assaf[1 4], M.E. Toro[1 3], L.I. Castellanos de Figueroa[2], F. Vazquez[1 4].

[1] *Instituto de Biotecnología. FI. UNSJ. San Juan.* [2] *Planta Piloto de Procesos Microbiológicos (PROIMI) - CONICET. Tucumán.* [3] *Dpto de Agronomía. FI: UNSJ. San Juan.* [4] *Dpto de Biología. FCEFyN. UNSJ. San Juan.*

virgi_pesce@yahoo.com.ar

Anthracnose, caused by *Colletotrichum* sp., is the major fungal disease in all olive producing areas of the world. In Argentina, this disease was reported in Catamarca, La Rioja, Córdoba, Mendoza and San Juan. Biological control using antagonistic microorganisms has been developed as an alternative to synthetic fungicide treatment. Yeasts have characteristics that give them advantages over other microorganisms to exert biocontrol of pathogenic fungi. However, yeasts-like fungi (*Aureobasidium*) have been reported against *Colletotrichum* species in olive fruits. The aim of this work was to evaluate the ability of autochthonous yeasts to control *C. gloeosporioides* in mature olive fruits, at different concentrations. Methodology: 92 antagonistic indigenous yeasts were previously selected in *in vitro* tests: 45 yeasts from viticultural environments and 47 from olivicultural environments. An isolate of *C. gloeosporioides* from the collection of INTA IMyZA, Castelar was used as pathogen. The pathogenicity of *C. gloeosporioides* was evaluated in olive fruits. The fungus was inoculated at different concentrations (10^2 to 10^6 conidia/mL) in mature fruits, and incubated during 5 days at 25°C. Percentage incidence [(number of decayed wounds/number of total wounds) x 100] and the mean of lesion diameter were determined. A suspension of yeasts (10^8 cells/mL) and 10^5 conidia/mL of fungi were inoculated in wounded fruits for biocontrol at *in vivo* tests (5 days, 25°C). Wounded fruits inoculated with fungal spore suspension were included as negative controls. The incidence of disease and %severity [(mean of diameter lesion in treated fruits/mean of diameter lesion in negative control) x 100] was calculated. A minimum of 50% reduction in the incidence of the disease was considered as antagonistic activity. The influence of yeast concentration at 10^7 and 10^6 cells/mL on biocontrol activity was evaluated. All experiments were made by triplicate. Nine indigenous yeasts (*Pichia membranifaciens* BPm6; *Saccharomyces chevalieri* BSch25; *Torulaspora delbrueckii* BTd126; *Candida tropicalis* Bo13b; *Cryptococcus albidus* Bo86; *Pichia kudriavzevii* Bo91, Bo108; *Wickerhamomyces anomalus* Bo107, Bo156) reduced the incidence of *C. gloeosporioides.* Biocontrol efficacies of these yeasts were: 50%, 50%, 63.34%, 90%, 76.67%, 53.34%, 70%, 60% and 90%, respectively. All antagonistic yeasts (except *W. anomalus* Bo107) were significantly effective in reducing the lesion diameter at 10^8 cells/mL. Only *C. tropicalis* Bo13b was antagonistic at lower concentration (10^7 cells/mL) with 70% incidence reduction. It can be concluded from this study that indigenous yeasts were effective as *in vivo* biocontrol agents against *C. gloeosporioides*. Concentrations of antagonists significantly affected their biocontrol activity. This is the first report that informed autochthonous biocontrol yeasts against *C. gloeosporioides* in olive.

BIOCONTROL DE *Colletotrichum gloeosporioides* EN FRUTOS DE OLIVO POR LEVADURAS ANTAGONISTAS

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Biotecnología
San Juan

Pesce V.M.[1,3], Carrizo G.P.[1,3], Nally M.C.[1,3], Brizuela M.[1,4], Rodríguez Assaf L.A.[1,4], Toro M.E.[1,3], Castellanos de Figueroa L.I.[2] y Vazquez F.[1,4]

virgi_pesce@yahoo.com.ar
[1] Instituto de Biotecnología. FI.UNSJ. San Juan.
[2] Planta Piloto de Procesos Microbiológicos-CONICET. Tucumán.
[3] Dpto de Agronomía. FI. UNSJ. San Juan.
[4] Dpto de Biología. FCEFyN. UNSJ. San Juan.

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INTRODUCCIÓN:

La antracnosis, causada por *Colletotrichum* sp., se presenta en todos los países donde se cultiva el olivo, tanto en la cuenca mediterránea como en América y Asia. En Argentina, se ha detectado, esporádicamente, en las provincias de Catamarca, La Rioja, Córdoba, Mendoza y San Juan, cuando las condiciones climáticas son propicias para el desarrollo de la enfermedad. Existen numerosas investigaciones sobre el empleo de microorganismos como agentes de control biológico de enfermedades fúngicas de productos agrícolas. En este contexto algunas levaduras poseen cualidades adicionales que las sitúan como agentes microbianos con gran potencial para su uso como biofungicidas. Sin embargo, hasta el momento, no existen antecedentes sobre el uso de levaduras como antagonistas de *C. gloeosporioides* en frutos de olivo. Sólo hongos levaduriformes del género *Aureobasidium* se han reportado como biosupresores de la antracnosis en olivo.

OBJETIVO

Evaluar la habilidad de levaduras autóctonas para biocontrolar a *C. gloeosporioides* en frutos de olivo, con diferentes concentraciones de antagonista

Metodología: Se utilizaron 92 aislamientos de levaduras autóctonas antagonistas (45 vitivinícolas y 47 olivícolas), que en estudios previos mostraron actividad inhibitoria, *in vitro*. El fitopatógeno utilizado fue *C. gloeosporioides*, perteneciente al cepario de INTA-IMyZA, Castelar. Se determinó la fitopatogenicidad de este aislamiento inoculando en heridas artificiales de frutos de olivo diferentes concentraciones del hongo (10^2 a 10^6 conidios/mL). Los frutos se incubaron 5 días a 25°C. Se determinó el %Incidencia [(n° de heridas con síntomas de pudrición/n° de heridas totales)*100] y la severidad calculada como el promedio del diámetro de la lesión. Para determinar la acción biosupresora de levaduras frente a *C. gloeosporioides*, *in vivo*, heridas realizadas en frutos de olivo se inocularon con una suspensión de 10^8 cel/mL de levadura y posteriormente 10^5 conidios/mL del patógeno. El control consistió en frutos inoculados sólo cono conidios fúngicos. Luego de 5 días a 25°C, se calculó el % Incidencia y el %Severidad [(promedio diámetro de la lesión de frutos inoculados con patógeno-antagonista/ promedio del diámetro de la lesión en frutos inoculados sólo con el patógeno)*100]. Las levaduras que redujeron 50% más la incidencia se consideraron antagonistas. Finalmente se evaluó la capacidad antagonista de las levaduras seleccionadas en concentraciones menores (10^7 y 10^6 cel/mL). Todos los experimentos se realizaron por triplicado.

Resultados

Nueve levaduras autóctonas: 3 vitivinícolas (*Pichia membranifaciens* BPm6; *Saccharomyces chevalieri* BSch25; *Torulaspora delbrueckii* BTd126) y 6 olivícolas (*Candida tropicalis* Bo13b; *Cryptococcus albidus* Bo86; *Pichia kudriavzevii* Bo91, Bo108; *Wickerhamomyces anomalus* Bo107, Bo156) redujeron el 50% o más la incidencia de la pudrición causada por *C. gloeosporioides*. La reducción de la incidencia por las levaduras fue: 50%, 50%, 63.34%, 90%, 76.67%, 53.34%, 70%, 60% y 90%, respectivamente (Gráfico 1). Todas las levaduras seleccionadas como antagonistas (excepto *W. anomalus* Bo107) redujeron significativamente el %Severidad con una concentración de 10^8 cel/mL, en comparación con el control negativo. Sólo una levadura biosupresora, *C. tropicalis* Bo13b, presentó actividad inhibitoria en concentraciones menores. Este aislamiento redujo el 70% la Incidencia de la enfermedad con una concentración de 10^7 cel/mL (Gráfico 1). Ninguna levadura fue biosupresora con una concentración de 10^6 cel/mL (Gráfico 1).

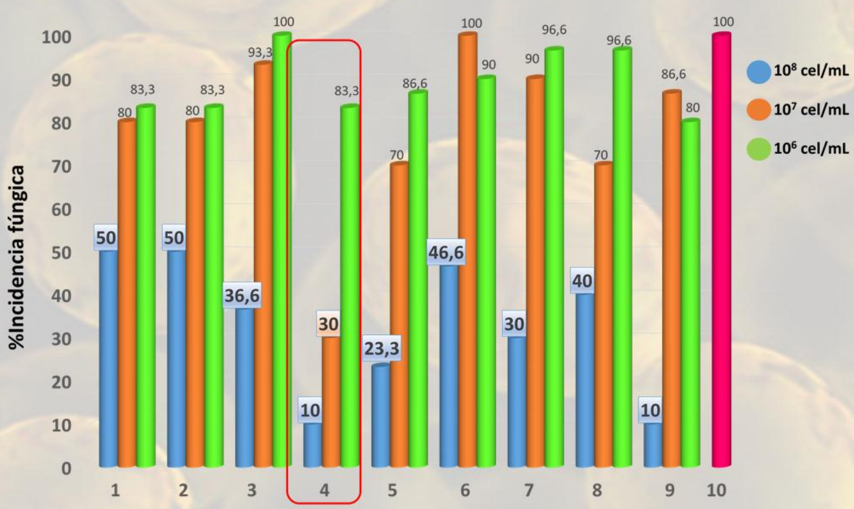

Gráfico 1: Efecto de diferentes concentraciones de levaduras antagonistas (10^8, 10^7 y 10^6 cel/mL) sobre el % Incidencia de *C. gloeosporioides* en frutos de olivo. 1-*P. membranifaciens* BPm6; 2-*S. chevalieri* BSch25; 3-*T. delbrueckii* BTd126; 4-*C. tropicalis* Bo13b; 5-*Cr. albidus* Bo86; 6-*P. kudriavzevii* Bo91; 7-*P. kudriavzevii* Bo108; 8-*W. anomalus* Bo107; 9-*W. anomalus* Bo156; 10-Control *C. gloeosporioides*. Se destaca con rojo la levadura *C. tropicalis* Bo13b que inhibió a *C. gloeosporioides* más del 50% con diferentes concentraciones (10^8 y 10^7 cel/mL).

CONCLUSIONES

- **Levaduras autóctonas son efectivas como agentes de control biológico de *C. gloeosporioides* en olivo.**
- **La concentración de los antagonistas afecta significativamente la acción biosupresora.**
- **Este estudio es el primer reporte de levaduras indígenas como antagonistas de *C. gloeosporioides* en frutos de olivo.**