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In vitro antagonistic activity of *Pichia anomala* and *Debaryomyces hansenii* against *Penicillium purpurogenum* strain M-16

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ABSTRACT

This study examines the inhibitory effects of *Pichia anomala* and *Debaryomyces hansenii* on the growth of *Penicillium purpurogenum* strain M-16 under *in vitro* conditions. Results demonstrated that *P.anomala* exhibited significant inhibitory activity, reducing fungal growth by 72.85% in the dual culture test and 39.62% in the volatile compounds assay. In contrast, *D.hansenii* exhibited a lower efficacy, suppressing mycelial growth by 33.49% and 33.85% in the dual culture and volatile compounds tests respectively. These results underscore the potential of this yeasts, particularly *P.anomala*, as promising biological control agents for managing fungal pathogens. Their application could extended to industries such as food preservation and agriculture to mitigate fungi contamination and spoilage.

KEYWORDS: biological control *Penicillium purpurogenum* strain M-16, *Pichia anomala*, *Debaryomyces hansenii*.

INTRODUCTION

Fungi, particularly *Penicillium* species, are regularly found in poultry feeds [6, 8]. The deterioration of food by fungi results in economic losses [12]. Additionally, the production of toxins which can adversely affect animal health and pose harmful effects on humans if transmitted through milk, meat and eggs. To date, more than 300 mycotoxins have been identified [5]. Numerous fungicides have been evaluated for control of pathogenic fungi; however, the development of resistant strains has reduced the efficacy of fungicides [2]. Consequently, biological control strategies could be an alternative to chemical methods.

In recent year, yeasts have emerged as a promising biological solution to fungal contamination control. Different yeast species are being utilized as biocontrol agents; for example *Saccharomycopsis schoenii* against plant pathogenic filamentous fungi *Penicillium expansum*

Link (UFMG 01-2002), *P. italicum* Wehmer (LCP 61.1199), and *P. digitatum* ; *Candida oleophila* Montrocher and *Pichia membranifaciens* Hansen against *Botrytis cinerea* ; *Debaryomyces hansenii* against *P. digitatum* grapefruit; *Pichia guillermondii* Wicherham against *Botrytis*, *Rhizopus*, *Penicillium* and *Alternaria* species [2,14]. Additionally, *Pichia anomala* and *D. hansenii* are reported as safe for use in the food industry [7, 9].

The most common mechanisms of antagonistic yeasts are competition for space and nutrients, production of cell wall-degrading enzymes, antibiotic metabolites, mycoparasitism and production of Killer toxins [14, 3, 11].

This research focusses on the ability of tow yeasts species, *Pichia anomala* and *Debaryomyces hansenii* to inhibit growth of toxigenic fungus *Penicillium purpurogenum* M-16.

MATERIALS AND METHODS

Fungal Pathogen

Penicillium purpurogenum strain M-16 was isolated from 10 samples of poultry feeds collected from factories of Setif region located in east of Algeria .The dilution plating method was used to isolate the fungus . Each sample was diluted and spread on plates containing Potato Dextrose Agar (PDA) medium, then the plates were incubated at 28ºC for 7 days. Taxonomic identification of *P.purpuroenum* strain M-16 was carried out using a standardized media and incubation conditions for macroscopic and microscopic characteristics and then confirmed by Molecular identification. Fungal culture was maintained on slants of PDA at 4ºC.

The Antagonistic Yeasts

The antagonistic yeasts; *Pichia anomala* and *Debaryomyces hansenii* used in this work was isolated and identified in a previous study in the Laboratory of Applied Microbiology at the University of Farhat ABBAS Setif, Algeria. The isolates was maintained on Malt extract agar slants at 4ºC.

Antagonistic Activity of Yeasts by Dual Culture Test

Following Al Shamari (2016) [1] method, fresh cells of antagonistic yeasts were streaked onto PDA medium, in two parallel streaks of 3 cm, and incubated at 30°C for 48 h. Subsequently , a 5 mm diameter plug of *P.purpurogenum* strain M -16 was aseptically obtained from the edge of a growing colony on PDA medium and inoculated into the center of each yeast-containing plate. Plates were incubated at 28°C °C for 7 days. After incubation percentage of fungal growth inhibition was calculated by using the formula as follows:

$$I=(R-T)\times 100/R$$

Where I: inhibition percent of mycelium.

R: radial growth of the fungus without yeast

T: radial growth of the fungus with yeast

Antagonistic Activity of Yeasts by Volatile Compounds (VOCs) Test

Volatile compounds test used which is described by Ghadamgahi et *al*. [4] with few modifications. In brief, yeasts were cultured on a plate with Male extract agar medium, and a 5 mm mycelial disc of *P.purpurogenum* M-16 was separately placed at the center of another plate on PDA culture medium. The Petri dishes containing the yeasts and pathogen were then placed face to face (mouth-to-mouth), sealed with parafilm, and incubated at 28 ◦C for 7 days. A plate containing *P. purpurogenum* only used as a control. The inhibitory activity was calculated as previously described. All the experiments were conducted by triplicates.

STATISTICAL ANALYSES

Statistical analyses were conducted using IBM SPSS Statistics version 25. Data were analyzed through a one-way analysis of variance (ANOVA). A p-values of less than 0. 05 ($P<0.05$) were considered statistically significant.

RESULTS AND DISCUSSION

The antagonistic yeasts showed antifungal activity in a dual culture test against *P.purpurogenum* M-16 (Figure 1 and Figure 2). The highest inhibition rate of 72.85% was observed with *P.anomala* which was statistically significant ($p<0.05$), followed by *D.hansenii* which showed an inhibition rate of 33.49%.

a **b** **c**

Figure 1: Dual culture *in vitro* assays. **a** Control *P.purpurogenum* M-16 monoculture. **b** *P.purpurogenum* challenged with *Pichia anomala*. **c** *P.purpurogenum* challenged with *Debaryomyces hansenii* after incubation at 28ºC for 7days.

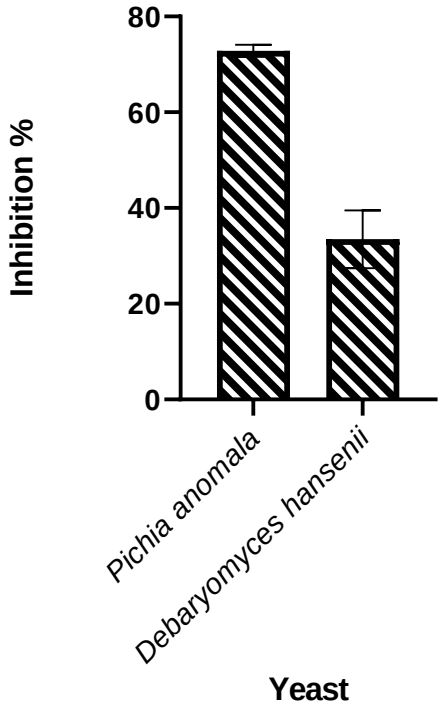

Figure 2: Inhibition effect of *P.anomala* and *D.hansenii* on radial growth of *P.purpurogenum* M16 by dual culture essay.

In the same study, it was reported that the volatile compounds produced by *P. anomala* and *D.hansenii* exhibited antifungal activity against *P.purpurogenum* strain M-16 with inhibition percentages of 39.62% and 33. 85% respectively. Significant difference ($p < 0.05$) was observed between the two strains (Figure 3).

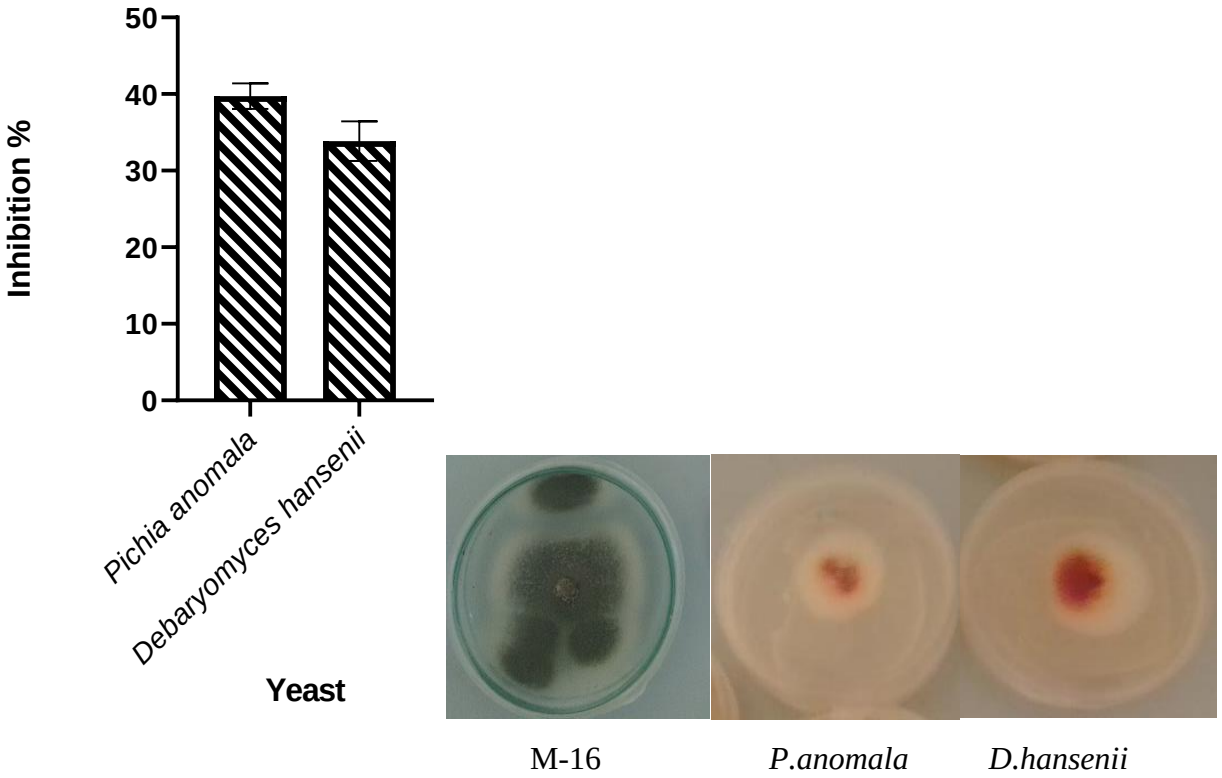

Figure 3: Qualitative and quantitative evaluation of antifungal activity of *P.anomala* and *D.hansenii* by volatile compounds against *P.purpurogenum* strain M-16, after incubation on PDA at 28ºC for 7days.

The antifungal effect of antagonistic yeasts has been previously demonstrated by Droby et *al.* [2] and Druvefors et *al* [3] their study results showed significant inhibition of spore germination and dry weight of mycelium of *Penicillium digitatum* in the presence of *D. hansenii* at concentrations of 10^9cfu/mL and *Penicillium roqueforti* in the presence of *P. anomala* strain J121 *in vitro.*

In addition, the results of Medina-Córdova et *al*. [9] indicates that *D. hansenii* inhibits radial growth of *F. proliferatum* and *F. subglutinans* by 38.9% and 25.9% in a radial inhibition assay and inhibits *F. proliferatum* and *F. subglutinans* by 54.2% and 43.5% respectively in a volatile compounds method. In contrast *M. circinelloides* and *Aspergillus* sp. remained unaffected by the yeast volatile compounds.

The reduction in antifungal activity observed by *D. hansenii* and *P.anomala* was attributed to the chemical nature of the volatile compounds such as ethyl acetate, isoamyl acetate ,2-phenylethyl acetate and geranyl acetate as described by Rojas et *al.* [13] , competition for space and nutriment, secretion of extracellular enzymes such as chitinas and cellulase, and production of killer toxins [3,2, 15,16].

CONCLUSION

In conclusion, poultry feeds is a source of nutrients for several pathogenic fungi like *Penicillium Talaromyces, Fusarium* and *Aspergillus*. Various yeasts such as *P.anomala* and *D.hansenii* have been used as biocontrol agents against different *Penicillium* species .Our results showed that *P.anomal* is effective against *Penicillium purpuogenum* strain M-16, wish use different mechanisms for biological control activity. In contrast, *D.hansenii* showed

moderate efficacy. Further research is recommended to explore its mechanisms of action and evaluate its effectiveness under real-word conditions.

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