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Biocontrol of the fungus *Botryodiplodia theobromae* Pat. by the yeast *Debaryomyces hansenii* (Zopf) Lodder Kreger – Van Rij.

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The potential use of yeast *Debaryomyces hansenii* was evaluated *in vitro* to control the growth of the fungus *Botryodiplodia theobromae*, causing post harvest rot of sweet potato. The growth of *B. theobromae* was completely inhibited by antagonistic yeast concentration of 1×10^9 CFU. ml^{-1}. However, significant inhibition of *in vitro* mycelial growth was achieved at 1×10^6 CFU. ml^{-1} with co-culturing of the fungus and yeast, irrespective of the carbon source i.e. glucose, sucrose, fructose or maltose.

Key Words : Biocontrol, *Botryodiplodia theobromae*, *Debaryomyces hansenii*, sweet potato, yeasts.

INTRODUCTION

Although there are numerous studies on the biological control of pests and diseases (Droby et al., 1992; Rodgers, 1993), only a few successful studies have been made on microbial control of post-harvest spoilage of fruits and vegetables (Wilson and Wisniewski, 1989), particularly on tuber crops like sweet potato and yams. Our earlier study has shown that *Debaryomyces hansenii* (Zopf) Lodder Kreger-van Rij., a non-antibiotic producing yeast isolated from sweet potato root surface protects wounded sweet potato and yam tubers from infection of putative pathogens like *Botryodiplodia theobromae* Pat. (Ray and Das, 1998). This isolate and another yeast, *Pichia anomola* (Hansen) Kurtzman were the two most effective of 21 isolates screened (Ray and Das, 1998). However, very little information is available on the metabolic aspects of the interactions between *D. hansenii* and *B. theobromae*. This study reports on the effect of antagonistic cell concentrations on the growth of *B. theobromae* in a synthetic culture medium with variable carbon source i.e. glucose, sucrose, fructose and maltose.

MATERIAS AND METHODS

A non-antibiotic producing isolate of *D. hansenii* (Institute of Microbial Technology IMT No.3034) obtained earlier from the root surface of sweet potato (*Ipomoea batatas* L.) (Ray and Das, 1998) has shown to exhibit biocontrol activity against *B. theobromae*, the pathogen causing post harvest rot of sweet potato (Ray and Mishra, 1995). The fungus was routinely maintained on Potato-Dextrose Agar (PDA). The conidiospore suspension of *B. theobromae* were harvested by washing PDA slants with sterile water and adjusted to a concentration of 1×10^6 spores. ml^{-1}. The liquid synthetic medium (SM) used in the experiment contained $NaNO_3$, 3.0g; K_2HPO_4, 1.0g; $MgSO_4$. $7H_2O$, 0.5 g; KCL, 0.5g; $FeSO_4$. $7H_2O$, 0.01g; sodium propionate, 3.0g; distilled water, 1000 ml and a carbon source (sucrose, glucose, fructose or maltose), 10g, in solid medium, agar 20.0g. L^{-1} was added to the above constituents.

One ml of spore suspension (1.0×10^6 spore.ml^{-1}) was inoculated into 50 ml of SM taken in 250 ml Erlenmeyer flasks. The medium contained sucrose, Glucose, fructose or maltose at 10g.L^{-1} as the carbon source. Simultaneously, the flasks were also inoculated (co-cultured) with 1 ml of *D. hansenii* suspension of washed cells (concentration varying from 1×10^9 CFU. ml^{-1}) grown in a liquid yeast maltose medium. Some flasks, which were not inoculated with yeast but with *Botryodiplodia* spore suspension, served as controls. The flasks were incubated at ambient temperature ($30 \pm 2°$ C). Dry weight of the fungus after 8 Days of incubation was determined after collection of the cultures on pre-weighted Whatman No. 1 filter paper and drying the

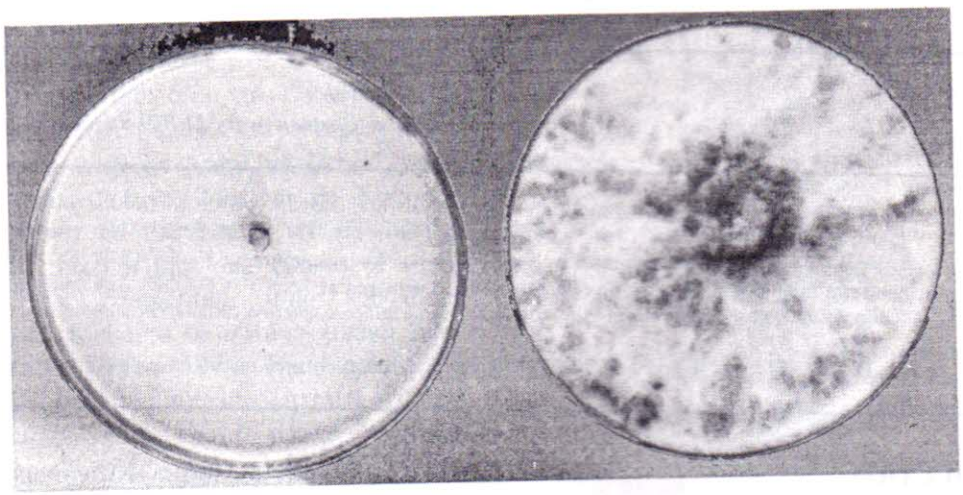

Fig 1. Growth of *B. theobromae* on SMA after 120 hour of incubation at 30 ± 2 ° C. the fungus was inoculated on the center of the plate. The plates contained from left to right, 0 and 10^9 CFU. ml^{-1} of *D. hansenii* cells

mycelial mass overnight at 80° C. Each treatment was replicated five times and the experiment was replicated three times.

In another experiment, a 3 mm diameter disc of PDA containing hyphal tips of *B. theobromae* was taken from a 3-day old culture and placed on the center of Petri-plates (100 mm diameter) containing SM agar (SMA) with sucrose as carbon source. The plates were swabbed with 1 ml of *D.hansenii* suspension served as controls. The growth of *B. theobromae* (hyphal measurement, cm) was made at 24 hour interval up to 120 hour of incubation at 30° C in a B.O.D. incubator. Five replicates were maintained for each treatment and the experiment was replicated thrice.

RESULTS AND DISCUSSION

Complete inhibition of *in vitro* growth of *B. theobromae* was achieved only when 1×10^9 CFU. ml^{-1} of *D. hansenii* suspension was added to the growth medium (Table 1). However, significant inhibition (< 70 per cent) of hyphal growth occurred at next lower concentration (1×10^8 CFU. ml^{-1}). Moreover, growth inhibition of *B. theobromae* was achieved in medium containing different sugars i.e. glucose, fructose, sucrose and maltose that normally promote spore germination and growth (Aneja, 1993). These sugars constitute nearly 100% of the sugars found in sweet potato (Woolfe, 1992) except maltose which is occasionally present (Picha, 1985). The ability of *D. hansenii* to exhibit biocontrol activity in presence of comparatively large amount of sugars ($10g.L^{-1}$) is significant, as there are reports to show biocontrol activity was reduced the presence of sugar (Howell *et al.*, 1988).

Maximum growth (9.00 ± 0.05 cm) of *B. theobromae* was attained on solid SMA after 96 hours of incubation. The growth of the fungus was inhibited 100 percent by *D.hansenii* at a concentration of 1×10^9 CFU. ml^{-1} (Fig.1). There are some reports to show that the yeast *Debaryomyces hansenii* serves as an effective bio control microorganism against post-harvest pathogens. Droby *et al.*, (1989) reported the antagonistic role of *D. hansenii* in the control of *Penicillium digitatum* on grape fruits. Chalutz (1990) reported biocontrol of green and blue moulds and sour rots of citrus fruits by *D. hanseni*. Likewise, *D. hansenii* and certain other osmo-tolerant yeast species such as *Candida sake*, *Pichia guilliermondii* etc were highly effective in controlling wound pathogens such as

Table 1. Per cent inhibition* of *Botryodiplodia theobromae* growth exposed to various concentrations of *Debaryomyces hansenii* in a liquid synthetic medium containing various sugars (10g. L^{-1}) after 8 days of incubation

Sugars	Concentration of *D. hansenii* (CFU. ml^{-1})			
	0	10^3	10^6	10^9
Sucrose	0	30 ± 4	76 ± 8	100
D-Glucose	0	31± 3	76 ± 17	100
D-Fructose	0	28 ± 4	74 ± 15	100
Maltose	0	25 ± 5	70 ± 12	100

*Percent inhibition is the mean (± S.E.) of three experiments each with five replications (n = 15).
Control (no yeast) – mycelial mass of *B.theobromae* was 535 ± 23 mg after 8 days of incubation

Penicillium rots of apples (Wilson and Chalutz, 1989; Wilson *et al.*, 1993; Mehrotra *et al.*, 1996). From our study, it is envisaged that the yeast *D. hansenii* (IMT 3034) has the ability to inhibit growth of *B. theobromea* irrespective of the type of sugar present in the medium. This may act as a boon for biocontrol of *B.theobromae*, a devastating post harvest pathogen of sweet potato (Ray and Mishra, 1995), a crop enriched with starch and sugars.

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