

Biological control of *Penicillium expansum* causing blue mould of apple by application of *Candida* spp

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Abstract : Two *Candida* spp. viz. *C. sake* and *C. oleophila* were screened in vitro against *Penicillium expansum* Link. ex F.S. Gray, the causal agent of blue mould disease of apple (*Pyrus malus* L.). These antagonists significantly inhibited the mycelial growth of the pathogen when compared with the control. The inhibition in the growth of pathogen by *C. sake* and *C. oleophila* was about 76.5% and 56.7% respectively. The treatment of inoculated fruits with mycelial extract of *Candida* spp. showed significantly low disease severity. The treatment reduced lesion diameter by 65.5% and 46.5% respectively. (**Key words :** *Biological control, Candida* spp, *P. expansum*, *Blue mould, Apple*).

India, N. America, Australia, New Zealand, Europe etc. are the chief apple growing countries. During storage, often fruits are severely affected by *Penicillium expansum* Link. ex F.S. Gray causing blue mould disease. At Udgir (Maharashtra) this disease was found to cause about 18-20% loss during storage. The disease often appears in the form of brown soft rot on the fruits. In advanced stage it forms brown sudden patches of blue green coremia. The present investigation was carried out to test the efficacy of *Candida* spp. against *P. expansum*.

Materials and methods

Experiments were conducted in Botany department of M.U. College, Udgir during Oct.-Dec. 1998. *Penicillium expansum* was isolated from the spoiled apple fruits. On potato dextrose agar medium colonies grew rapidly which were normally yellow green in colour. The colonies showed well marked zonation with concentric rings of small coremia. The conidiophores are normally smooth but occasionally rough, 3-3.5 μ m in diameter. The conidia are elliptical at first, remaining so or becoming sub-globose, smooth, 3.0-3.5 μ m in long axis.

Antagonists, *Candida sake* and *C. oleophila* were isolated from the surface of apple fruits. Stock cultures were stored at 8°C and were subcultured on Maltose Peptone Agar medium. The apple fruits (cv. Golden Delicious) obtained from Udgir fruit market were used in all experiments.

Dual culture technique was used to test the efficacy of *Candida* spp. against the pathogen on PDA plates. To obtain large and distinct inhibition zones, 15 ml of PDA was poured in 6cm Petriplates. The agar medium was seeded

with the potential antagonists and test pathogen (5 mm culture discs of three days old culture) opposite each other near the periphery of petriplates. The medium inoculated with pathogen alone served as control. The plates were incubated at $28 \pm 1^\circ\text{C}$ temperature. After six days of inoculation, the diameter of mycelial growth of both antagonist and test pathogen was measured.

In another set of experiments, antagonists were grown in Czapek Dox broth and after 10 days of growth, mycelium extracts were obtained. Surface sterilized healthy apple fruits were wounded covering 2 x 2 mm areas and 2mm deep. The wounded fruits were inoculated with the pathogen. After 24 hours of inoculation these fruits were dipped into mycelial extract of antagonists for five minutes. The inoculated fruits without dipping in mycelial extract of antagonists served as control. The fruits thus treated were stored at $28 \pm 1^\circ\text{C}$. After six days the disease severity in lesion diameter caused by the pathogen was determined.

Results and Discussion

Antagonism between the potential antagonists (*Candida sake* and *C. oleophila*) and test pathogen (*Penicillium expansum*) in dual culture experiment was very clear. It indicated that the test pathogen stopped its growth in petriplates upon contact with the antagonists and the hyphae began to lie back while the antagonist continued its growth over the test fungus colony. *Candida sake* followed by *C. oleophila* exerted maximum growth inhibition of pathogen. *C. sake* caused 76.5% growth inhibition while *C. oleophila* showed 56.7% inhibition over control (Table 1). The plates inoculated with test pathogen only showed maximum growth (8.1cm.) covering entire medium.

Table 1. *In vitro* assay of fungal antagonists against *Penicillium expansum*

Fungal antagonist	Mycelial growth of pathogen (in cm)	Growth inhibition over control (%)
<i>Candida sake</i>	1.9	76.5
<i>Candida oleophila</i>	3.5	56.7
Control	8.1	—

The disease severity (lesion diameter) was significantly lower in apple fruits treated with mycelial extract of antagonists. The fruit treatment with the extract of *C. sake* and *C. oleophila* reduced disease severity (lesion diameter) by 65.5% and 46.5% respectively (Table 2).

Table 2. Inhibition percentage of disease severity by percentage mycelial extract of antagonists

Fungal antagonist	Disease severity (lesion diameter in cm)	% inhibition disease severity
<i>Candida sake</i>	2.0	65.5
<i>Candida oleophila</i>	3.1	46.5
Control	5.8	—

The development of resistance in fungal pathogens to fungicides and the growing public concern over health and environment hazards associated with high level of pesticides have resulted in a significant interest in the development of alternative, non-chemical methods of disease control. Biological control using microbial antagonists has emerged as one of the most promising alternatives either alone or as part of an integrated control strategy to reduce pesticide inputs. Many antagonists of fungal pathogens of fruits have been reported earlier. The biocontrol of post-harvest pathogens has been very successful as reported by a Vinas *et al.* (1996). The studies

in Spain have demonstrated that *Candida sake* strain CPA-1 is an effective antagonist of major post-harvest pathogens of pome fruits including *P. expansum* and *Botrytis cinerea* Pers Fr. as reported by Usall (1995). The effect of *Candida* spp. has been tested against apple pathogens by Mercier and Wilson (1994), McLaughlin *et al.* (1992) and Teixido *et al.* (1998). The present results are more or less in agreement with these workers. The investigations indicated the advantage of application of biological agent for the control of blue mould disease of apple. This application would also avoid additional contamination by pathogenic fungi from drenching solution usually used during chemical treatments.

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