Biological control of fungal and bacterial plant pathogens
IOBC/wprs Bulletin Vol. 30 (6) 2007
pp. 575-578

Aureobasidium pullulans (1113-5) microbial antagonist for the control of post-harvest decay on apple fruit: development of active biomass formulation at a lab scale

Rabia Mounir[1,2], Alain Durieux[3], Elisabeth Bodo[3], Christophe Allard[3], Jean-Paul Simon[3], El- Hassan Achbani[1], Samir El-Jaafari[4], Allal Douira[2], Mohamed-Haïssam Jijakli[5]

[1] *Laboratory of Phytobacteriology, Research National Institute of Agronomy, BP 579 Meknès VN, Morocco, e-mail: mounir07@hotmail.com;*

[2] *Laboratoire de Botanique et de Protection des cultures, Université Ibn Tofail Faculé des Sciences, BP 133, 14000 Kenitra, Morocco;*

[3] *Biotechnology Unit, Laboratory of Microbiology, Faculté des Sciences, Université Libre de Bruxelles, CP 700, Avenue Emile Gryson 1, B-1070 Bruxelles, Belgium;*

[4] *Laboratoire de Biotechnologie et Amélioration des Plantes (UMI-BAP), University of Moulay Ismail, BP 4010, Meknès;*

[5] *Plant Pathology Unit, Faculté Universitaire des Sciences Agronomiques, Passage des Déportés 2, 5030 Gembloux, Belgium*

Abstract: One strain of *A. pullulans* (de Bary) Arnaud var. *pullulans* 1113-5, was previously selected for its high antagonistic activity against *Penicillium expansum* and *Botrytis cinerea*, two molds responsible of post-harvest decay of apple fruit. The objective of the study to optemize the biomass production of this strain in a lab-scale fermentor. A dried formulation of *A. pullulans* was carried out using a fluidised bed dryer. Stability of the dried product was evaluated during storage and the antagonist activity against *P. expansum* was evaluated at a pilot scale on apple fruit. The high cell density fermentation can be achieved with *A. pullulans* using glucose fed-batch technology resulting in a final biomass dry weight of 107 g/l. A viability of 60% was measured after the drying process allowing the evaluation of this strain for a long period of storage. After 7 months of storage at 4°C, 16% of initial viability corresponding to 1.5×10^{10} CFU/g dry matter was noticed. The best antagonistic activity against *P. expansum* was achieved with the application of a 1×10^{8} CFU/ml suspension of *A. pullulans* on wounded fruit. We can conclude that the biomass formulation did not alter the efficacy of the biological control agent.

Key words: biological control, biomass production, efficacy

Introduction

Apple is one of the most important fruits produced in Morocco. Indeed this country contributes for 30% of the apple production in Africa, which places it in second position after South Africa. Postharvest pathogens cause substantial losses (60%) in this apple production. Fungal diseases are the major factor limiting the storage life of apples. Nowadays, synthetic fungicide treatments are the main means to limit fungal post-harvest diseases but development of resistant strains of the pathogens to many fungicides, and the growing concern for human safety and environmental protection, have resulted in attempts to develop alternative methods as the biological control of postharvest diseases (Jijakli & Lepoivre, 2004).

Aureobasidium pullulans (de Bary) Arnaud var. *pullulans* 1113.5, was previously selected for its high antagonistic activity against *Penicillium expansum* and *Botrytis cinerea*, two wound pathogens causing economically important losses of Golden Delicious apples during storage (Achbani et al., 2005). The yeast-like fungus *Aureobasidium pullulans* is one of the most widespread and well-adapted saprophytes in the phyllosphere and has been frequently considered as an effective biocontrol agent against postharvest diseases (Ippolito et al., 2000; Castoria et al., 2001). In this context, our objectives consisted in the development of active biomass formulation through the optimization of the fermentation and drying steps. Stability and antagonist activity against *P. expansum* of the dried product were evaluated during long term storage.

Material and methods

Biomass production

The biomass production of strain 1113-5 was carried out at 28°C in a 10 litres Biostat ® ED bioreactor (B. Braun Biotech, Germany) using fed-batch technology. Fed batch solution consisted in 50% w/w glucose solution. The medium contained per liter: 5 ml of mineral salts concentrated solution (0.32 g l^{-1} $MnCl_2$ 4 H_2O, 0.49 g l^{-1} $CuSO_4$ 5H_2O, 5.75 g l^{-1} $ZnSO_4$ 7H_2O, 0.48 g l^{-1} $CoCl_2$ 6H_2O, 0.49 g l^{-1} Na_2MoO_4 2H_2O, 15 g l^{-1} EDTA, 2.94 g l^{-1} $CaCl_2$ 2H_2O and 2.78 g l^{-1} $FeSO_4$ 7H_2O), 30 g yeast extract, 30 g soy peptone, 0.37 g Na_2SO_4, 4.5 g K_2SO_4, 6 g KH_2PO_4, 3 g $MgSO_4$ 7H_2O and 700 ml distilled water. 110 g glucose dissolved in 250 ml distilled water and 5 ml vitamins concentrated solution previously sterilised by microfiltration (1 g l^{-1} thiamine HCl, 1 g l^{-1} pyridoxine HCl, 1 g l^{-1} nicotinic acid, 1 g l^{-1} D-biotin, 1 g l^{-1} Ca-D-pantothenat, 0.2 g l^{-1} p-aminobenzoïc acid, 5 g l^{-1} inositol) were added before inoculation. The medium was continuously aerated with 1.5 vvm, the stirring speed was maintained at 600 rpm. The pH of the culture was controlled at 5. The bioreactor was inoculated with 500 ml shake-flasks culture.

Formulation

The biomass produced in the fed batch process has been dried in a fluid bed dryer. The maize starch was used as loading agent (30% w/w). The drying process was accomplished by controlling the air temperature in the bed at 30°C and the air inflow at 150 $m^3 h^{-1}$.

Viability and antagonistic activity

After drying, the samples were stored at 4°C, and after different periods of time (0 to 7 months), the number of viable cells was determined by plating on YEPD medium. After 7 months of storage, the antagonistic activity was conducted on Golden delicious apples by submerging in *A. pullulans* suspension at a concentration of 10^6, 10^7 or 10^8 CFU/ml on wounded fruit (at four equidistant points). Each wound was characterised by a diameter of 2 mm and a depth of 4 mm and a distance of 20 mm inter-wounds). One day after treatment with the antagonist, fruits were pulverised with a *P. expansum* suspension at 1x 10^5 CFU/ml. Each treatment was applied to three replicates of 20 fruits (240 wounds for each treatment). Fruits were placed at 5 or 25°C. The protective level and severity of decay were determined after 5 and 7 days of storage at 25°C and after 20 and 28 days of storage at 5°C. The protective levels (Y %) were calculated with respect to the following formula: $D_T - D_X / D_T \times 100 = Y$ % With D_T = diameter lesion of control and D_X = diameter lesion of treatment. All statistical analysis was performed using (SAS Institute, Cary, NC).

Results and discussion

Biomass production

The high cell density fermentation can be achieved with *A. pullulans* using glucose fed-batch technology resulting in a final biomass dry weight of 107 g/l after 48 hours of fermentation and with a yield coefficient of 0.67. The pH of the culture was controlled at 5. In those fermentation conditions, the yeast-like cell form is predominant.

Formulation

A viability of 60% was measured, after the drying process, corresponding to residual moisture of 10.5% w/w.

Viability and antagonistic activity

After 7 months of storage at 4°C, 16% of initial viability corresponding to 1.5 $\times 10^{10}$ CFU/g dry matter was noticed. This viability reduction occurs in the first 30 days of storage at 4°C. After this period, the viability remains constant. The antagonistic activity against *P. expansum* showed that a protection level of 89% was achieved with the highest biomass preparation after 28 days for apples stored at 5°C and after 7 days for apples stored at 25°C (Fig. 1).

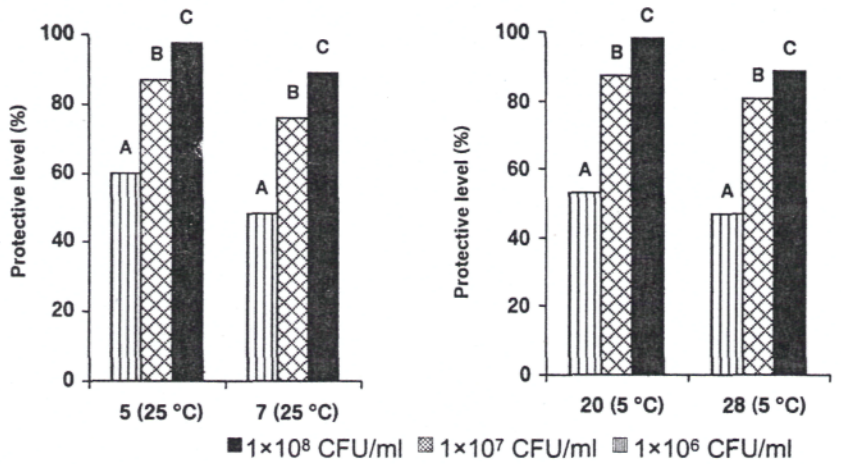

Figure 1. Biocontrol activity of the fluid bed- dried *A. pullulans* cells 1113-5 against *P. expansum* (880) at a pilot scale on wounded 'Golden delicious' apples. *A. pullulans* suspension applied at a concentration of 1×10^6, 1×10^7 and 1×10^8 CFU/ml with untreated control and *P. expansum* (1×10^5 CFU/ml) applied after 24h. The protective level and severity of decay were based on three replicates of 20 fruit each and were determined after 5 and 7 days of storage at 25°C (left) and after 20 and 28 days of storage at 5°C (right). Columns with the same letter within the same time interval are not significantly different according to Duncan's Multiple-Range Test, $P<0.05$ level.

In this study, we can conclude that the biomass formulation of the yeast-like fungus, *A. pullulans* (de Bary) Arnaud strain 1113-5, isolated from the surface of apple fruit, did not affect the efficacy of the biological control agent of apple post-harvest diseases (*Penicillium expansum*) after 7 months of storage at 4°C. The level of efficacy of 1113-5 at 1×10^8 CFU/ml on post harvest apple diseases obtained in our laboratory was high and opens a good opportunity to use this strain as biocontrol agent for apple preservation. Further studies at large scale are needed to confirm these results.

Acknowledgements

Thanks to the C.U.D. in Belgium for funding this study that constitute a part of the "PIC" project "Biological control of apple postharvest diseases". Thanks to A-M Plaisant, A Benbouazza, G Foroni, L Garre and J-P Defroyennes for their excellent technical support.

References

Achbani, E.H., Mounir, R., El Jaafari, S., Douira, A., Benbouazza, A. & Jijakli, H. 2005: Selection of antagonists against *Penicillium expansum* and *Botrytis cinerea*, two post harvest apple parasites. Comm. Appl. Biol. Sci. Ghent Univ. 70 (3): 143-149.

Castoria, R., de Curtis, F., Lima, G., Caputo, L., Pacifico, S. & de Cicco, V. 2001: *Aureobasidium pullulans* (LS-30) an antagonist of postharvest pathogens of fruits: study on its modes of action. Postharvest Biol. Technol. 22: 7-17.

Jijakli, M.H. & Lepoivre, P. 2004: State of the art and challenges of post-harvest diseases management in apples. In: Disease Management of Fruits and Vegetables, Vol.I, ed. Mukerji, Kluwer Academic Publisher, Dordrecht: 59-94.

Ippolito, A., El Ghaouth, A., Wilson C.L. & Wisniewski, M. 2000: Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. Postharvest Biol. Technol. 19: 265-272.