International Journal of Food Microbiology 141 (2010) 173–176

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Combination of *Pichia membranifaciens* and ammonium molybdate for controlling blue mould caused by *Penicillium expansum* in peach fruit

Shifeng Cao [a,*], Yongjun Yuan [b], Zhichao Hu [a,*], Yonghua Zheng [c]

[a] Nanjing Research Institute for Agricultural Mechanization, Ministry of Agriculture, Liuying 100, Nanjing 210014, PR China
[b] College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo 315100, PR China
[c] College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, Jiangsu, PR China

ARTICLE INFO

Article history:
Received 4 January 2010
Received in revised form 28 March 2010
Accepted 12 May 2010

Keywords:
Biological control
Pichia membranifaciens
Peach fruit
Ammonium molybdate
Blue mould

ABSTRACT

The potential enhancement of *Pichia membranifaciens* by ammonium molybdate (NH_4Mo) to control blue mould caused by *Penicillium expansum* on peach fruit was investigated. Combining *P. membranifaciens* at 1×10^8 cell/ml with 1 mM NH_4Mo provided a more effective control of blue mould rot than applying the yeast or NH_4Mo alone. Addition of 1 mM NH_4Mo significantly increased the growth of *P. membranifaciens* in peach wounds, but did not affect the population in nutrient yeast dextrose broth medium. The *in vitro* experiment showed that the combined treatment inhibited spore germination and germ tube elongation of *P. expansum* in comparison with the treatment of *P. membranifaciens* or NH_4Mo alone. Moreover, *P. membranifaciens*, NH_4Mo, and the combination of them did not impair the quality parameters including fruit firmness and content of total soluble solids, titratable acidity and vitamin C of peach fruit after 6 days of storage at 20 °C. These results suggested that the use of NH_4Mo is a useful approach to improve the efficacy of *P. membranifaciens* for postharvest disease control in peach fruit.

1. Introduction

Blue mould caused by *Penicillium expansum*, is the major postharvest disease of peaches in China (Fan and Tian, 2000; Zhang et al., 2007). Currently, chemical fungicides are commonly used to prevent peach fruit from fungal infection and extend shelf life (Fernandez-Trujillo et al., 1998; Karabulut et al., 2002). However, problems related to development of pathogen resistance to many site specific fungicides and potentially harmful effects on the human safety and environment protection have stimulated research to look for alternative methods for disease control (Ragsdale and Sisler, 1994).

Biological control with microbial antagonists has emerged as a promising alternative, either alone or as part of integrated pest management to reduce synthetic fungicide usage (Wisniewski and Wilson, 1992). At present, a new yeast antagonist, *Pichia membranifaciens* Hansen, has been evaluated as a potential biological control agent for suppressing *Rhizopus* rot of peach and nectarine fruit (Fan and Tian, 2000; Tian et al., 2002), and mould decay in sweet cherry fruit (Qin et al., 2004; Chan and Tian, 2006), as well as anthracnose rot in loquat fruit (Cao et al., 2008a). However, when used alone, the biocontrol efficacy of *P. membranifaciens* is not as great as that of fungicides (Tian et al., 2002). Therefore, from a practical view, the effectiveness of the yeast antagonist must be enhanced.

Great interest has recently been focused on combining antagonists with some food additives. Among them, ammonium molybdate (NH_4Mo) shows broad-spectrum antifungal activity (Nunes et al., 2002a,b). The potential of NH_4Mo for the enhancement of biocontrol ability of antagonists has been investigated in apple, pear and jujube fruit (Nunes et al., 2002a,b; Wan et al., 2003; Wan and Tian, 2005). However, there is no information concerning the effect of NH_4Mo with the yeast *P. membranifaciens* on control of the postharvest diseases in fruit.

The objectives of this study were to evaluate (a) the effect of NH_4Mo at various concentrations and the antagonistic yeast *P. membranifaciens* used separately or in combination, on controlling postharvest blue mould decay of peach fruit caused by *P. expansum*; (b) the effect of NH_4Mo, used alone or in combination with *P. membranifaciens* on spore germination of *P. expansum in vitro*; (c) the effect of NH_4Mo on population dynamics of *P. membranifaciens in vivo* and *in vitro*; and (d) the efficacy of NH_4Mo and *P. membranifaciens*, separately or in combination, on quality of peach after storage, including firmness and total soluble solids (TSS) content, titratable acidity (TA) and vitamin C.

2. Materials and methods

2.1. Microorganisms, fruit and treatments

P. expansum was isolated from infected peach fruit. The culture was maintained on potato-dextrose agar medium (PDA: extract of

* Corresponding authors. Cao is to be contacted at Tel.: +86 25 84346251; fax: +86 25 84346256. Hu, Tel.: +86 25 84346256; fax: +86 25 84346256.
E-mail addresses: njcaoshifeng@yahoo.com.cn (S. Cao), nfzhongzi@163.com (Z. Hu).

0168-1605/$ – see front matter. Crown Copyright © 2010 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2010.05.009

boiled potatoes, 200 ml; dextrose, 20 g; agar, 20 g and distilled water, 800 ml). Spores of *P. expansum* were obtained from 2-week-old cultures incubated at 25 °C by flooding the cultures with sterile-distilled water containing 0.05% (v/v) Tween 80, and filtered through four layers of sterilised cheesecloth. The concentration of spores was adjusted to 1×10^5 spores/ml with a haemocytometer.

A strain of the yeast *P. membranifaciens* was obtained from the Institute of Microbiology, Chinese Academy of Science (Beijing, P.R. China). The yeast cultures were maintained at 4 °C on Nutrient Yeast Dextrose Agar (NYDA) medium containing 8 g nutrient broth, 5 g yeast extract, 10 g glucose and 20 g agar, in 1 l of distilled water. The yeast was transferred from NYDA with a sterile bacteriological loop to NYD Broth (NYDB: NYDA without agar), and then cultured in 250 ml conical flasks containing 50 ml of NYDB in a rotary shaker at 28 °C for 48 h. Following incubation, cells were centrifuged at $6000\times g$ for 10 min and washed twice with sterile-distilled water in order to remove the growth medium. The yeast was resuspended in sterile-distilled water and adjusted to a concentration of 1×10^8 cell/ml with a haemocytometer.

Peach fruit (*Prunus persica* Batsch cv Baifeng) were hand-harvested at firm-mature stage from a commercial orchard in Nanjing, China, and selected for uniform size, colour and absence of defects. Fruits were disinfected with 2% (v/v) sodium hypochlorite for 2 min, washed with tap water, and air dried prior to wounding. Disinfected fruits were wounded at two sites with a dissecting needle (2 mm diameter × 4 mm deep). An aliquot of 20 µl of each treatment was applied to the wounds, followed by inoculation with 15 µl of 1×10^5 spores/ml suspension of *P. expansum*. Treatments consisted of (i) sterile-distilled water; (ii) *P. membranifaciens* at 1×10^8 cell/ml; (iii) aqueous solutions of NH_4Mo at 1, 5 or 15 mM alone or in combination with *P. membranifaciens* at 1×10^8 cell/ml. The fruits were sealed in polyethylene bags to retain about 95% relative humidity and incubated at 20 °C for 6 days. The percentage of infected wound and lesion diameter were measured 3 and 6 days after inoculation. There were three replicates of 15 fruit each per treatment, and the experiment was conducted three times.

2.2. In vitro effect of NH_4Mo on growth of *P. membranifaciens*

Following the method of Wan and Tian (2005), aliquots of 50 ml of NYDB, with or without NH_4Mo at 1, 5 or 15 mM, in 250 ml conical flasks were autoclaved (121 °C, 15 min) and 100 µl of the suspension of *P. membranifaciens* (1×10^8 cell/ml) was added in the above solutions. The number of colony-forming unit (CFU) of the yeast was determined by dilution-plating at 0, 1, 2, and 3 days after incubation on a rotary shaker at 160 rpm at 28 °C and expressed as Log_{10} CFU/ml. Each treatment was replicated three times and the experiment was repeated twice.

2.3. Effect of NH_4Mo on growth of *P. membranifaciens* in peach wounds

Two wounds were made on each fruit, then 20 µl of *P. membranifaciens* (1×10^8 cell/ml), alone or in combination with NH_4Mo at 1, 5 or 15 mM was injected into each wound. Fruits were incubated at 20 °C (90% relative humidity). *P. membranifaciens* was recovered from the wounds 1 h after inoculation at 20 °C (time 0) and after 2, 4 and 6 days. Wounded tissue was removed with an ethanol-flamed, 5 mm (internal diameter) cork borer and ground in an autoclaved mortar with 5 ml of sterile 0.05 M phosphate buffer (pH 7.0), then plated 0.1 ml of a 10-fold dilution on NYDA. The plates were incubated at 28 °C for 2 days and the colonies were counted. Population densities of *P. membranifaciens* were expressed as Log_{10} CFU/wound. There were three single fruit replicates per treatment, and the experiments were repeated three times.

2.4. In vitro effect of NH_4Mo and *P. membranifaciens* on spore germination of *P. expansum*

The effect of NH_4Mo and *P. membranifaciens* on spore germination of pathogen was tested in potato-dextrose broth (PDB). Aliquots of 100 µl of spore suspensions of *P. expansum* (1×10^5 spores/ml) were added into 10 ml glass tube containing 5 ml of PDB. The PDB contained different concentrations of NH_4Mo (0, 1, 5 and 15 mM) with or without 100 µl of *P. membranifaciens* (1×10^8 cell/ml). All treated tubes were placed on a rotary shaker (100 rpm) at 26 °C. After 12 h incubation, at least 100 spores per replicate were observed microscopically to determine germination rate and germ tube length. Spores were considered germinated when germ tube length was equal to or greater than spore length. Each treatment was replicated three times and the experiment was repeated twice.

2.5. Effect of NH_4Mo and *P. membranifaciens* on quality parameters of peach fruit

Quality parameters of peach fruit treated with NH_4Mo, *P. membranifaciens*, or in combination were measured after storage. The testing methods used were described below.

The firmness of five fruits from each replicate was measured at three points of the equatorial region by using a FT327 firmness tester (Facchini FG, Alfonsine, Italy) fitted with a 5 mm diameter probe. The same five fruits from each replicate were then wrapped in cheesecloth and squeezed using a hand press. The resulting juice was analysed for its TSS and TA. TSS was determined at 20 °C using a portable refractometer (WYT-4; Quanzhou Zhongyou Optical Instrument Co., Ltd., Fujian, China). TA was determined by titrating 20 ml juice to pH 8.2 using 0.1 M NaOH. The vitamin C content of each sample of juice was measured using 2,6-dichloro-indophenol titration, as described by Jones and Hughes (1983). The results were expressed in mg/100 g fresh weight (FW).

2.6. Statistical analysis

Experiments were performed using a completely randomised design. Experimental data were the mean ± SE. All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA) for this experiment. The data were analysed by one-way analysis of variance (ANOVA) to test the difference of the treatments. Mean separations were performed by Duncan's multiple range tests. Differences at $P<0.05$ were considered as significant.

3. Results

3.1. Effect of NH_4Mo and *P. membranifaciens* on the control of blue mould rot

As shown in Fig. 1, treatment with *P. membranifaciens* at 1×10^8 cell/ml or 1 mM NH_4Mo alone both resulted in significantly ($P<0.05$) smaller lesion diameter and lower disease incidence of blue mould rot on peach fruit wounds inoculated with *P. expansum* compared with the controls during 6 days of incubation at 20 °C. No significant difference of lesion diameter and disease incidence was observed between the two treatments. Treatments with NH_4Mo at 5 or 15 mM alone were not effective in reducing blue mould rot incidence and severity in fruit wounds. However, the combined treatment of *P. membranifaciens* with 1 mM NH_4Mo markedly ($P<0.05$) reduced the lesion diameter and disease incidence in comparison with the treatment of *P. membranifaciens* or NH_4Mo alone. In this combined treatment, the percentage of infected wounds and lesion diameter were reduced significantly ($P<0.05$) from 38.5 to 10.6% and 8.3 to 2.2 mm, respectively, compared with *P. membranifaciens* alone. With increase of the concentration of

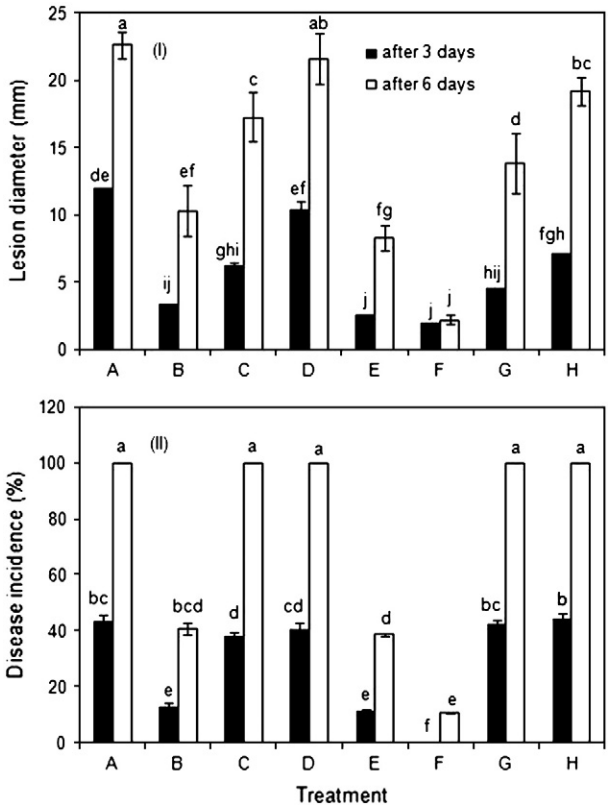

Fig. 1. Effects of NH_4Mo and *P. membranifaciens* on lesion diameter (I) and disease incidence (II) of peach fruit during incubation at 20 °C for 6 days. (A) Control; (B) 1 mM NH_4Mo; (C) 5 mM NH_4Mo; (D) 15 mM NH_4Mo; (E) *P. membranifaciens* (1×10^8 cell/ml); (F) *P. membranifaciens* (1×10^8 cell/ml) combined with 1 mM NH_4Mo; (G) *P. membranifaciens* (1×10^8 cell/ml) combined with 5 mM NH_4Mo; (H) *P. membranifaciens* (1×10^8 cell/ml) combined with 15 mM NH_4Mo. Each column represents the mean of three replicates samples. Vertical bars represent the standard errors of the means. Values with different letters at the same time within the same figure are significantly different according to Duncan's multiple range test at $P=0.05$ level.

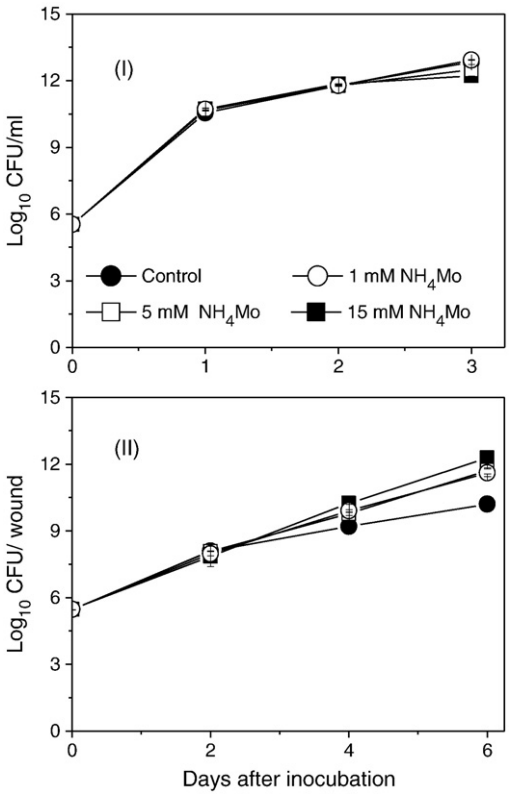

Fig. 2. Effects of NH_4Mo on population dynamics of *P. membranifaciens* in NYDB medium (I) or wounds of peach fruit (II) during incubation. Data are expressed as the mean of triplicate assays. Vertical bars represent the standard errors of the means.

NH_4Mo to 5 or 15 mM, no benefit from the combined treatment effect was observed.

3.2. Effect of NH_4Mo on population dynamics of P. membranifaciens in NYDB medium or fruit wounds

The populations of *P. membranifaciens* increased rapidly in NYDB or in wounds in peach fruit. Adding NH_4Mo did not influence the population of *P. membranifaciens in vitro* (Fig. 2I). However, in peach fruit treatment with NH_4Mo at selected concentrations enhanced the growth dynamics of *P. membranifaciens* after 2 days of inoculation at 28 °C compared with the control. Within 6 days in the absence of NH_4Mo, the population of *P. membranifaciens* reached approximately 1.6×10^{10} CFU/wound. In the presence of NH_4Mo at 1, 5 and 15 mM, the numbers of CFU of the yeast were 4.8×10^{11}, 5.7×10^{11}, and 1.8×10^{12} per wound, respectively (Fig. 2II).

3.3. Effect of NH_4Mo and P. membranifaciens on spore germination and germ tube elongation of P. expansum

Treatment with NH_4Mo at selected concentrations alone markedly ($P<0.05$) inhibited spore germination and germ tube elongation of *P. expansum* in a concentration-dependent manner: the higher the concentration of NH_4Mo used, the greater the degree of inhibition. Meanwhile, the spore germination rate and germ tube length were significantly ($P<0.05$) lower in the samples treated with *P. membranifaciens* alone than in the control after 12 h incubation at 26 °C. The inhibitory effect of *P. membranifaciens* on spore germination and germ tube growth of *P. expansum* was enhanced by the addition of NH_4Mo, the combined treatment of *P. membranifaciens* with NH_4Mo (1, 5 or 15 mM) completely inhibited the growth of *P. expansum* (Table 1).

3.4. Effect of NH_4Mo and P. membranifaciens on quality parameters in peach fruit after 6 days of storage

NH_4Mo, yeast antagonist, and the combination had no significant effect on fruit firmness, TSS and TA after 6 days at 20 °C. However, NH_4Mo at 1 mM with or without *P. membranifaciens* maintained a higher level of vitamin C content in peach fruit, when compared to the control fruit (Table 2).

Table 1
Effects of NH_4Mo and *P. membranifaciens* on spore germination and germ tube length of *P. expansum in vitro* after 12 h incubation at 26 °C in potato-dextrose broth.

Treatment	Spore germination (%)	Germ tube length (μm)
A	100.00 ± 0.00a	61.42 ± 19.67a
B	8.50 ± 2.20c	19.25 ± 2.78bc
C	4.00 ± 1.00d	12.25 ± 2.50c
D	0.25 ± 0.07de	8.16 ± 1.33c
E	16.73 ± 3.00b	33.25 ± 1.28b
F	0.00 ± 0.00e	0.00 ± 0.00c
G	0.00 ± 0.00e	0.00 ± 0.00c
H	0.00 ± 0.00e	0.00 ± 0.00c

Means in a column followed by a different letter differ significantly at $P=0.05$ by Duncan's multiple range tests. Data are accompanied by standard deviations of the means. (A) Control; (B) 1 mM NH_4Mo; (C) 5 mM NH_4Mo; (D) 15 mM NH_4Mo; (E) *P. membranifaciens* (1×10^8 cell/ml); (F) *P. membranifaciens* (1×10^8 cell/ml) combined with 1 mM NH_4Mo; (G) *P. membranifaciens* (1×10^8 cell/ml) combined with 5 mM NH_4Mo; (H) *P. membranifaciens* (1×10^8 cell/ml) combined with 15 mM NH_4Mo.

Table 2
Effects of NH_4Mo and *P. membranifaciens* on quality parameters of peach fruit after 6 days of incubation at 20 °C.

Treatment	Firmness (kg/cm^2)	TSS (%)	Vitamin C (mg/100 g FW)	TA (%)
A	1.50 ± 0.23a	10.67 ± 0.44a	40.40 ± 0.10c	0.15 ± 0.03a
B	1.63 ± 0.20a	11.00 ± 0.00a	46.17 ± 1.25a	0.15 ± 0.02a
C	1.52 ± 0.13a	10.67 ± 0.34a	44.37 ± 2.41ab	0.15 ± 0.03a
D	1.52 ± 0.24a	10.33 ± 0.57a	37.70 ± 0.62c	0.13 ± 0.02a
E	1.50 ± 0.32a	10.87 ± 0.34a	37.57 ± 0.64c	0.15 ± 0.01a
F	1.50 ± 0.21a	11.00 ± 0.00a	45.57 ± 0.20a	0.15 ± 0.01a
G	1.38 ± 0.17a	11.00 ± 0.25a	41.42 ± 1.89bc	0.14 ± 0.02a
H	1.61 ± 0.21a	10.00 ± 0.67a	40.38 ± 1.88c	0.10 ± 0.00a

Means in a column followed by a different letter differ significantly at $P = 0.05$ by Duncan's multiple range tests. Data are accompanied by standard deviations of the means. (A) Control; (B) 1 mM NH_4Mo; (C) 5 mM NH_4Mo; (D) 15 mM NH_4Mo; (E) *P. membranifaciens* (1×10^8 cell/ml); (F) *P. membranifaciens* (1×10^8 cell/ml) combined with 1 mM NH_4Mo; (G) *P. membranifaciens* (1×10^8 cell/ml) combined with 5 mM NH_4Mo; (H) *P. membranifaciens* (1×10^8 cell/ml) combined with 15 mM NH_4Mo.

4. Discussion

The yeast, *P. membranifaciens* is a useful biocontrol agent for suppressing postharvest diseases in various fruits (Fan and Tian, 2000; Qin et al., 2004; Cao et al., 2008a). However, the antagonistic yeast, when used alone, is not as effective as fungicides (Tian et al., 2002). Thus, great interest has recently been focused on the methods of enhancing biocontrol ability of *P. membranifaciens*. It has been shown that the performance of *P. membranifaciens* can be improved by calcium chloride and methyl jasmonate (Tian et al., 2002; Cao et al., 2008b, 2009). The results from the present study demonstrated that *P. membranifaciens* at 1×10^8 cell/ml combined with 1 mM NH_4Mo was more effective in reducing *P. expansum* infection in peach fruit wounds than treatment with *P. membranifaciens* alone. Similar results were reported by combining NH_4Mo with antagonists such as *Candida sake* and *Cryptococcus laurentii* (Nunes et al., 2002a,b; Wan et al., 2003; Wan and Tian, 2005).

In general, the biological activity of the antagonist is based on its capacity for rapid colonisation and competition for space and nutrients in fruit wounds (Janisiewicz and Korsten, 2002). There is a direct relationship between the population of the antagonist and the biocontrol efficacy (Janisiewicz and Korsten, 2002). *P. membranifaciens* has been shown to be able to rapidly colonise and grow in the surface wounds, and subsequently to compete with the pathogen for nutrients and space, which plays a major role in the mode of action of *P. membranifaciens* in control of postharvest diseases (Fan and Tian, 2000; Cao et al., 2008a). In the present study, the results showed that 1 mM NH_4Mo could enhance the population of *P. membranifaciens* in the wounds of the peach fruit, which indicated that enhancement of the efficacy of *P. membranifaciens* was positively correlated with the population of the yeast. However, this result was contrary to previous reports of Nunes et al. (2002a,b) and Wan and Tian (2005), who found that although NH_4Mo could enhance the biocontrol efficacy of antagonistic yeasts, the addition of NH_4Mo did not lead to the increase of population of the yeast in fruit wounds. The reason for the above results might be due to differing sensitivity of antagonistic yeasts to NH_4Mo.

Our experiments revealed the direct antagonistic activity of *P. membranifaciens* or NH_4Mo against *P. expansum*. The application of *P. membranifaciens* or NH_4Mo alone could directly inhibit spore germination and germ tube length. The presence of NH_4Mo in yeast suspensions enhanced the inhibition on spore germination and germ tube length of *P. expansum*, which resulted in a more effective control of peach decay using mixture of antagonist and 1 mM NH_4Mo. However, it must be pointed that in this study we observed that high concentrations of NH_4Mo, i.e. 5 or 15 mM, decreased the biocontrol ability of *P. membranifaciens*, even if the population of the yeast in fruit wounds and direct inhibition of *P. expansum* growth were increased by the addition of the chemical. It has been reported that the basis of NH_4Mo's biological activity is its ability to inhibit acid phosphatase activity (PTPase), which interferes with phosphorylation and dephosphorylation, one of the most important processes of cell regulation (Hunter, 1995). Nunes et al. (2002a) concluded that PTPase could be involved in enhancing the biological effectiveness. On the other hand, one of the possible mechanisms related to the antagonistic effect of *P. membranifaciens* is the secretion of lytic enzymes such as β-1,3-glucanase and chitinase (Chan and Tian, 2005). Therefore, the decrease in biocontrol ability of the yeast by high concentrations of NH_4Mo might be associated with its effect on these microbial enzymes. However, further research is needed to elucidate the precise mechanisms by which higher concentrations of NH_4Mo decreased the biocontrol activity of yeasts.

Toxicological experiments showed that NH_4Mo did not lead to mortality or adverse effects in test animals, and was also environmentally safe (Nunes et al., 2002a). Thus, the integration of NH_4Mo and *P. membranifaciens* was a safe approach to enhance the efficacy of the yeast. In addition, the combination of antagonistic yeast and NH_4Mo did not impair fruit quality parameters including firmness, TSS, TA and vitamin C content of peaches. Therefore, a combination between our antagonist and NH_4Mo could be a reliable solution to control postharvest diseases on peach fruit.

References

Cao, S.F., Zheng, Y.H., Tang, S.S., Jin, P., Wang, K.T., 2008a. Biological control of postharvest anthracnose rot of loquat fruit by *Pichia membranifaciens*. Journal of Horticultural Science and Biotechnology 83, 816–820.

Cao, S.F., Zheng, Y.H., Tang, S.S., Wang, K.T., 2008b. Improved control of anthracnose rot in loquat fruit by a combination treatment of *Pichia membranifaciens* with $CaCl_2$. International Journal of Food Microbiology 126, 216–220.

Cao, S.F., Zheng, Y.H., Tang, S.S., Wang, K.T., Rui, H.J., 2009. Effect of yeast antagonist in combination with MeJA treatment on postharvest anthracnose rot of loquat fruit. Biological Control 50, 73–77.

Chan, Z.L., Tian, S.P., 2005. Interaction of antagonistic yeasts against postharvest pathogens of apple fruit and possible mode of action. Postharvest Biology and Technology 36, 215–223.

Chan, Z.L., Tian, S.P., 2006. Induction of H_2O_2-metabolizing enzymes and total protein synthesis by antagonistic yeast and salicylic acid in harvested sweet cherry fruit. Postharvest Biology and Technology 39, 314–320.

Fan, Q., Tian, S.P., 2000. Postharvest biological control of *Rhizopus* rot of nectarine fruits by *Pichia membranifaciens*. Plant Disease 84, 1212–1216.

Fernandez-Trujillo, J.P., Martinez, J.A., Artes, F., 1998. Modified atmosphere pack affect the incidence of cold storage disorder and keep 'flat' peach quality. Food Research International 31, 571–579.

Hunter, T., 1995. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. Cell 80, 225–236.

Janisiewicz, W.J., Korsten, L., 2002. Biological control of postharvest diseases of fruits. Annual Review of Phytopathology 40, 411–441.

Jones, E., Hughes, R.E., 1983. Foliar ascorbic acid in some angiosperms. Phytochemistry 22, 2493–2499.

Karabulut, O.A., Cohen, L., Wiess, B., Daus, A., Lurie, S., Droby, S., 2002. Control of brown rot and blue mold of peach and nectarine by short hot water brushing and yeast antagonists. Postharvest Biology and Technology 24, 103–111.

Nunes, C., Usall, J., Teixido, N., Vinas, I., 2002a. Improvement of *Candida sake* biocontrol activity against post-harvest decay by the addition of ammonium molybdate. Journal of Applied Microbiology 92, 927–935.

Nunes, C., Usall, J., Teixido, N., Abadias, M., Vinas, I., 2002b. Improved control of postharvest decay of pears by the combination of *Candida sake* (CPA-1) and ammonium molybdate. Phytopathology 92, 281–287.

Qin, G.Z., Tian, S.P., Xu, Y., 2004. Biocontrol of postharvest diseases on sweet cherries by four antagonistic yeasts in different storage conditions. Postharvest Biology and Technology 31, 51–58.

Ragsdale, N.N., Sisler, H.D., 1994. Social and political implications of managing plant diseases with decreased availability of fungicides in the United States. Annual Review of Phytopathology 32, 545–557.

Tian, S.P., Fan, Q., Xu, Y., Jiang, A.L., 2002. Effects of calcium on biocontrol activity of yeast antagonists against the postharvest fungal pathogen *Rhizopus stolonifer*. Plant Pathology 51, 352–358.

Wan, Y.K., Tian, S.P., 2005. Integrated control of postharvest diseases of pear fruits using antagonistic yeasts in combination with ammonium molybdate. Journal of the Science of Food and Agriculture 85, 2605–2610.

Wan, Y.K., Tian, S.P., Qin, G.Z., 2003. Enhancement of biocontrol activity of yeasts by adding sodium bicarbonate or ammonium molybdate to control postharvest disease of pear fruits. Letter in Applied Microbiology 37, 249–253.

Wisniewski, M.E., Wilson, C.L., 1992. Biological control of postharvest diseases of fruits and vegetables: recent advances. Hortscience 27, 94–98.

Zhang, H.Y., Zheng, X.D., Yu, T., 2007. Biology control of postharvest diseases of peach with *Cryptococcus laurentii*. Food Control 18, 287–291.