Effect of $CaCl_2$ and Various Wild Yeasts From Plant Origin on Controlling *Penicillium expansum* Postharvest Decays in Golden Delicious Apples

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VH Tournas[1] and EJ Katsoudas[2]

[1]Division of Microbiology, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD, USA. [2]Northeast Food and Feed Laboratory, Office of Regulatory Affairs, U.S. Food and Drug Administration, Jamaica, NY, USA.

ABSTRACT: The biocontrol potential of four wild yeast strains (*Meyerozyma guilliermondii* – strain YS-1, *Meyerozyma caribbica* – strain YS-3, *Cryptococcus albidus* – strain YS-4, and *Cryptococcus* sp. – strain YS-5) against *Penicillium expansum* was studied *in vivo* (on Golden Delicious apples). The test yeasts were applied to the fruits alone as well as in combination with 2% $CaCl_2$. Treated apples were stored at room temperature (~21°C) for up to 2 weeks or under refrigeration (3°C) for up to 2 months. *Candida oleophila* was used as positive biocontrol agent. Biocontrol activities were expressed as percentages of lesion size reduction caused by the test yeasts or by test yeasts + $CaCl_2$ as compared with decays on apples treated with *P. expansum* alone. All strains tested during this study showed some degree of biocontrol activity against *P. expansum*. When the test yeasts were applied alone, they effected moderate pathogen inhibition reducing the decay size by 28% to 52% at day 7 and 11% to 27% at day 14 of incubation at room temperature. When the treated apples were stored at 3°C, lesion size reduction was between 48% and 63% after 1 month and 24% to 41% after 2 months of incubation. Addition of $CaCl_2$ to yeast suspensions facilitated much higher pathogen inhibition. At room temperature, lesion size reduction ranged between 74% and 77% during the first week. After 2 weeks of incubation, decays on yeast + $CaCl_2$-treated apples were still substantially smaller (49%-73% lower) than those on apples treated with *P. expansum* alone. At refrigeration, lesion size reduction ranged between 76% and 92% in the first month of storage and between 75% and 87% after 2 months of incubation. Decay incidence was 75% to 100% in apples stored at room temperature and 30% to 85% in those kept under refrigeration. The inhibitory activities of the wild yeast strains were similar to those exhibited by *C. oleophila* for the most part. These strains, when combined with $CaCl_2$, showed high potential as biocontrol agents against *P. expansum* on stored apples.

KEYWORDS: apples, postharvest disease, ***Penicillium expansum***, biocontrol yeasts, $CaCl_2$

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CORRESPONDING AUTHOR: VH Tournas, Division of Microbiology, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 5001 Campus Drive, College Park, MA, 20740, USA. Email: vasiliki.tournas@fda.hhs.gov

Introduction

Apples are consumed in substantial amounts in the western societies due to their favourable sensory and nutritional attributes. Therefore, they constitute a big part of the fresh produce-related economy. Postharvest invasion and spoilage by *Penicillium expansum* is the cause of great economic losses.[1,2] More importantly, this mould is capable of producing the highly toxic and mutagenic mycotoxin, patulin (PAT) in apples and other fruits rendering them hazardous to human health. Past studies have reported the presence of PAT in apple products such as apple juice and apple purees at levels exceeding the limits established by the United States Food and Drug Administration (USFDA) and other regulatory agencies.[3-6] More troublesome about the occurrence of PAT in apple products is that high amounts of these products are consumed by the most sensitive populations, infants, and young children.

Mainly chemical pesticides have been used for the prevention of postharvest spoilage by *P. expansum*. Use of these compounds, however, has some drawbacks such as possibly harmful effects on human and animal health and the contamination of the environment.[7,8] Moreover, some *P. expansum* strains acquire resistance to synthetic fungicides and they can no longer be controlled by them.[9,10] To solve these problems, researchers turned their efforts towards finding alternative means and measures for controlling *P. expansum* and other postharvest invaders. A seemingly feasible approach is the use of non-pathogenic, non-toxigenic organisms that have the ability to inhibit or hinder the growth of the pathogen(s). These organisms are collectively known as biocontrol agents (BCAs). Several such BCAs have been tested and a few are commercially used.[1,11-14] Several possible mechanisms of inhibitory action of BCAs against the pathogens have been reported; those include competition for nutrients and space, antibiosis, production of cell wall-degrading enzymes by the BCAs, induction of host resistance to the pathogen and oxidative response.[15-18] BCAs, however, have shown some deficiencies in controlling *P. expansum* such as inability to control already established decays and inconsistency in antagonistic activities.[19,20]

BCAs with improved and better-sustained antagonistic activities are needed. Past studies have showed that combination of treatments such simultaneous use of BCA yeasts and certain compounds (eg, calcium chloride, sodium bicarbonate, silicon, chitosan, ascorbic acid) achieved a synergistic inhibitory effect against postharvest pathogens *in vivo*.[21-31] Therefore, this study was undertaken in search of a more efficient

biocontrol of *P. expansum* using a combination of treatments. In a previous study, we had isolated a few yeast strains that exhibited inhibitory potential against the pathogen. The current research was an effort to increase the antagonistic activity of those strains by the incorporation of $CaCl_2$ in the BCA yeast suspensions.

Materials and Methods

Materials

Four yeast strains (*Meyerozyma guilliermondii* – strain YS-1 from Red Delicious apples, *Meyerozyma caribbica* – strain YS-3 from pineapple fruit salad, *Cryptococcus albidus* – strain YS-4 from red seedless grapes, and *Cryptococcus* sp. – strain YS-5 from *Pyrus calleryana* fruit) were previously isolated in our laboratory and stored in 15% glycerol at −80°C until used. Organic, ripe, sound quality Golden Delicious apples were purchased from local supermarkets; *P. expansum* (ATCC 7861) and *Candida oleophila* (ATCC MYA 1208) were obtained from the American Type Culture Collection (Manassas, VA, USA). Culture media (PDA – DIFCO, Detroit, MI, USA) were prepared in house according to manufacturer's instructions.

Preparation of yeast cell suspensions

Yeast cultures were freshly prepared by growing the isolates on PDA agar for 48 h at 25°C. Subsequently, yeast cell suspensions were prepared in sterile distilled water and cell concentration was determined using a hemocytometer; final yeast concentrations were adjusted to 1.0×10^8 cells/ml. For determining the combined effects of yeast antagonists and $CaCl_2$, the yeast suspensions were prepared in sterile 2% $CaCl_2$ and adjusted to concentrations of 1.0×10^8 cells/ml.

Penicillium expansum *spore suspension*

P. expansum (ATCC 7861) was cultured on PDA for 7 days at 25°C. Subsequently, its spores were collected by flooding the sporulating cultures with sterile distilled water containing 0.05% Tween 80. The spore concentration was determined by a hemocytometer and culturally and adjusted to 1.0×10^4 spores/ml. Spore suspension was stored at 5°C until used. Fresh suspensions were made weekly.

In vivo *testing for biocontrol activity of yeast isolates alone and yeast + $CaCl_2$*

In vivo testing was conducted according to the method described by He et al[32] with minor modifications as follows: Golden Delicious apples were surface disinfected with 10% commercial bleach in sterile distilled water for 2 min and subsequently rinsed twice with sterile distilled water. The apples were allowed to dry at room temperature and then they were wounded in the equator area (3 wounds per apple) with a sterile 6-mm cork borer. Next, each wound was inoculated with 30 µL yeast suspension in distilled water (1.0×10^8 cells/ml) (treatment 1) or 30 µL yeast suspension in 2% $CaCl_2$ (treatment 2). Two hours after the yeast inoculation, 20 µL of *P. expansum* spore suspension (1.0×10^4 spores/ml) was added to each wound and the apples were stored in covered containers at room temperature for up to 2 weeks or at 3°C for up to 2 months. Control apples inoculated with 2% $CaCl_2$ + *P. expansum* or sterile distilled H_2O + *P. expansum* were also included in the experiment. *P. expansum*-caused decays were measured at weekly intervals for fruits stored at room temperature and at monthly intervals for the ones incubated at 3°C. Lesion sizes were compared with those on apples infected with *P. expansum* alone. *C. oleophila* was used as positive BCA control. Comparisons were also made between *P. expansum* decays of the two treatments (comparing the activities of each yeast strain alone and respective strain combined with $CaCl_2$). Four fruits were inoculated per treatment and the experiment was repeated three times. Results are the averages of *P. expansum* decay diameters of 36 fruit wounds per treatment.

Statistical analysis

T-tests (equal variance) were conducted to compare the two treatments (application of yeast alone vs yeast + $CaCl_2$).[33]

Results

This research focused on the examination of the effect of four wild yeast strains (*M. guilliermondii* – strain YS-1, *M. caribbica* – strain YS-3, *C. albidus* – strain YS-4, and *Cryptococcus* sp. – strain YS-5) and $CaCl_2$ on the growth of *P. expansum* on Golden Delicious apples. The results of the study are summarized in Tables 1 to 5. Lesion sizes on yeast-treated apples were compared with those on apples infected with *P. expansum* alone and biocontrol activity was measured as percent lesion size reduction. Application of these yeasts on fruit wounds prior to pathogen inoculation resulted in reduced growth of the pathogen, therefore smaller decays. Decrease in lesion size depended on yeast strain and incubation temperature and time (Tables 1 and 2). At room temperature, lesion size (decay severity) on yeast-treated apples was reduced by 28% to 52% after 1 week and by 11% to 27% after 2 weeks of incubation, while decay incidence (percent infected wounds) was 100% during both incubation periods (Table 1). At 3°C, yeast application facilitated lesion size reduction of 48% to 63% after 1 month of incubation and 24% to 41% after 2 months of incubation. Decay incidence ranged between 69% and 88% during the first month and between 94% and 100% after 2 months of incubation (Table 2).

Addition of 2% $CaCl_2$ (final concentration) facilitated an increase in biocontrol activity of the tested yeasts. When incubation took place for 1 week at room temperature, lesion reduction caused by the wild yeasts combined with $CaCl_2$ ranged between 74% and 77% and was for the most part similar to that exhibited by the control BCA yeast, *C. oleophila*.

Table 1. Biocontrol activity of wild BCA yeasts against *Penicillium expansum* on Golden Delicious apples at 21°C.

TREATMENT	INCUBATION AT 21°C					
	7 DAYS			14 DAYS		
	DECAY DIAMETER (mm)[a]	LESION REDUCTION (%)[b]	DECAY INCIDENCE (%)	DECAY DIAMETER (mm)	LESION REDUCTION (%)	DECAY INCIDENCE (%)
Meyerozyma guilliermondii (YS-1)	18.25	46	100	43.58	24	100
Meyerozyma caribbica (YS-3)	16.25	52	100	41.67	27	100
Cryptococcus albidus (YS-4)	24.17	28	100	51.08	11	100
Cryptococcus sp. (YS-5)	19.58	42	100	47.58	17	100
Candida oleophila[c]	17.83	47	100	46.58	19	100
P. expansum (alone)	33.50	N/A	100	57.17	N/A	100

Abbreviation: BCA, biocontrol agent; N/A, not applicable.
[a]Decay diameter values are the means of 36 measurements.
[b]Percent lesion reduction was calculated in relation to decays caused by *P. expansum* alone.
[c]*C. oleophila* served as the positive BCA control.

Table 2. Biocontrol activity of wild BCA yeasts against *P. expansum* on Golden Delicious apples at 3°C.

TREATMENT	INCUBATION AT 3°C					
	1 MONTH			2 MONTHS		
	DECAY DIAMETER (mm)[a]	LESION REDUCTION (%)[b]	DECAY INCIDENCE (%)	DECAY DIAMETER (mm)	LESION REDUCTION (%)	DECAY INCIDENCE (%)
M. guilliermondii (YS-1)	14.69	56	75	52.50	41	94
M. caribbica (YS-3)	17.25	48	81	66.13	26	100
C. albidus (YS-4)	17.38	48	88	67.50	24	100
Cryptococcus sp. (YS-5)	15.94	52	69	56.25	37	100
C. oleophila[c]	12.38	63	81	54.25	39	100
P. expansum (alone)	33.31	N/A	100	88.94	N/A	100

Abbreviation: BCA, biocontrol agent; N/A, not applicable.
[a]Decay diameter values are the means of 36 measurements.
[b]Percent lesion reduction was calculated in relation to decays caused by *P. expansum* alone.
[c]*C. oleophila* served as the positive BCA control.

When the incubation was extended for a second week, the inhibitory activities of all yeast strains were reduced resulting in lesion reduction between 49% and 73% as compared with lesions on apples inoculated with *P. expansum* only. When the treated apples were stored at 3°C, the test yeasts combined with $CaCl_2$ caused high inhibition of *P. expansum* with lesion size reduction ranging between 76% and 92% after a 1-month storage and between 75% and 87% after a 2-month refrigeration. Decay incidence was 30% to 60% during the first month and 70% to 85% after 2 months at refrigeration. T-test comparisons between the two treatments (yeast alone vs yeast + $CaCl_2$) showed that significant increase in biocontrol activity was achieved with the addition of $CaCl_2$ for all tested strains (Table 5).

Discussion

The aim of this research was to determine the combined effects of wild BCA yeasts (*M. guilliermondii*, *M. caribbica*, *C. albidus* and *Cryptococcus* sp.) and $CaCl_2$ on the growth of *P. expansum* on Golden Delicious apples. These strains, when applied to apple fruit wounds alone, exhibited substantial antagonistic activity against *P. expansum*; when they were inoculated onto the fruits in combination with $CaCl_2$, however, their inhibitory activities were increased markedly. Some of the wild yeast strains facilitated higher inhibition of the pathogen than the control BCA yeast, *C. oleophila*, during refrigerated storage; this indicates that those strains maintained higher viability and/or grew more abundantly under reduced temperatures. Inhibition of postharvest pathogens by BCA yeasts has been

Table 3. Inhibitory activity of wild BCA yeasts + $CaCl_2$ against *P. expansum* on Golden Delicious apples at 21°C.

TREATMENT	INCUBATION AT 21°C					
	7 DAYS			14 DAYS		
	DECAY DIAMETER (mm)[a]	LESION REDUCTION (%)[b]	DECAY INCIDENCE (%)	DECAY DIAMETER (mm)	LESION REDUCTION (%)	DECAY INCIDENCE (%)
M. guilliermondii (YS-1) + $CaCl_2$	6.85	76	75	19.25	70	85
M. caribbica (YS-3) + $CaCl_2$	6.80	76	75	21.85	66	90
C. albidus (YS-4) + $CaCl_2$	7.20	74	80	28.15	56	90
Cryptococcus sp. (YS-5) + $CaCl_2$	7.15	75	75	32.25	49	100
C. oleophila[c] + $CaCl_2$	6.50	77	75	17.35	73	80
P. expansum + $CaCl_2$	22.90	19	100	56.15	12	100
P. expansum (alone)	28.25	N/A	100	63.60	N/A	100

Abbreviation: BCA, biocontrol agent; N/A, not applicable.
[a]Decay diameter values are the means of 36 measurements.
[b]Percent lesion reduction was calculated in relation to decays caused by *P. expansum* alone.
[c]*C. oleophila* served as the positive BCA control.

Table 4. Inhibitory activity of wild BCA yeasts + $CaCl_2$ against *P. expansum* on Golden Delicious apples at 3°C.

TREATMENT	INCUBATION AT 3°C					
	1 MONTH			2 MONTHS		
	DECAY DIAMETER (mm)[a]	LESION REDUCTION (%)[b]	DECAY INCIDENCE (%)	DECAY DIAMETER (mm)	LESION REDUCTION (%)	DECAY INCIDENCE (%)
M. guilliermondii (YS-1) + $CaCl_2$	2.25	92	30	10.10	87	70
M. caribbica (YS-3) + $CaCl_2$	3.05	90	35	11.75	85	75
C. albidus (YS-4) + $CaCl_2$	7.10	76	60	18.05	77	85
Cryptococcus sp. (YS-5) + $CaCl_2$	4.25	86	50	15.95	80	75
C. oleophila[c] + $CaCl_2$	4.50	85	45	19.60	75	70
P. expansum + $CaCl_2$	20.80	29	100	65.75	18	100
P. expansum (alone)	29.45	N/A	100	79.70	N/A	100

Abbreviation: BCA, biocontrol agent; N/A, not applicable.
[a]Decay diameter values are the means of 36 measurements.
[b]Percent lesion reduction was calculated in relation to decays caused by *P. expansum* alone.
[c]*C. oleophila* served as the positive BCA control.

reported in past studies.[34-37] Restriction of *P. expansum* growth by *M. guilliermondii* was reported by Yan et al.[34] These investigators artificially inoculated pear fruit with the BCA yeast and the pathogen and incubated the treated fruits at 4°C and 20°C. The results of this experiment showed that the yeast colonized the wounds of the fruits quickly, thus inhibiting pathogen growth. In addition, activities of the antioxidant enzymes of the pear, catalase and peroxidase, and phenylalanine ammonia lyase were increased after addition of the yeast; these enzymes are associated with disease resistance in pear. Cao et al[35] studied the ability of *Pichia caribbica* (synonym of *M. caribbica*) to control *P. expansum* decays and PAT degradation in Fuji apples stored at 4°C and 20°C. These researchers concluded that *P. caribbica* colonized fruit wounds rapidly, significantly reduced the incidence of blue mould decays, and was able to degrade PAT at both temperatures. *In vitro* experiments showed that the yeast inhibited pathogen spore germination. Mbili,[36] on the other hand, postulated that YieldPlus, a biofungicide

Table 5. Statistical comparisons (t-test) of *P. expansum* decay severity (lesion diameters) on Golden Delicious apples between the two treatments (yeast alone vs yeast + $CaCl_2$).

TREATMENT	DECAY DIAMETER (mm)[a] AT 21°C				DECAY DIAMETER (mm)[a] AT 3°C			
	7 DAYS		14 DAYS		1 MONTH		2 MONTHS	
	– $CaCl_2$	+ $CaCl_2$	– $CaCl_2$	+ $CaCl_2$	– $CaCl_2$	+ $CaCl_2$	– $CaCl_2$	+ $CaCl_2$
M. guilliermondii (YS-1)	18.25a	6.85b	43.58c	19.25d	14.69e	2.25f	52.50g	10.10h
M. caribbica (YS-3)	16.25a	6.80b	41.67c	21.85d	17.25e	3.05f	66.13g	11.75h
C. albidus (YS-4)	24.17a	7.20b	51.08c	28.15d	17.38e	7.10f	67.50g	18.05h
Cryptococcus sp. (YS-5)	19.58a	7.15b	47.58c	32.25d	15.94e	4.25f	56.25g	15.95h
C. oleophila[b]	17.83a	6.50b	46.58c	17.35d	12.38e	4.50f	54.25g	19.60h

Abbreviation: BCA, biocontrol agent.
Means in same row and incubation temperature and time period followed by different letters are significantly different ($P < .01$). The data from each incubation temperature and incubation period were analysed separately.
[a]Decay diameter values are the means of 36 measurements.
[b]*C. oleophila* served as the positive BCA control.

containing *C. albidus*, was very effective against *P. expansum* and *Botrytis cinerea* artificially inoculated on Golden Delicious apples. Guerrero et al[37] tested three *C. oleophila* strains and noted that all strains exhibited substantial inhibition against *P. expansum* artificially inoculated on Golden Delicious apples.

Enhancement of the biocontrol activities of *C. oleophila*, *Candida guilliermondii* (teleomorph *M. guilliermondii*) *Pichia* spp., and *Cryptococcus laurentii* with the addition of $CaCl_2$ was reported in previous studies.[25-29,38,39] Wisniewski et al[26] examined the effect of Ca++ on the BCA activity of two *C. oleophila* isolates (strains 182 and 247) against *P. expansum* and reported a substantial increase in inhibitory activity of isolate 182. The biocontrol enhancement of this yeast was attributed to inhibition of spore germination and reduction of the pectinolytic activity of *P. expansum caused* by calcium ions, while these ions had no adverse effect on the yeast survival and growth. McLaughlin et al[25] tested the inhibitory activity of two *Candida* strains combined with $CaCl_2$ and found that addition of this salt improved the biocontrol activity of the yeasts. Yu et al[27,28] reported increased antagonistic activity of a *C. laurentii* strain against *P. expansum* and *B. cinerea* when it was combined with $CaCl_2$. These investigators found that $CaCl_2$ increased the biocontrol of mould rots by educing fruit resistance to pathogens when it was applied on the fruit wounds 24 h prior to mould inoculation. Scherm et al,[38] on the other hand, showed that inclusion of $CaCl_2$ to *C. guilliermondii* (strains 3C-1b and F1) suspensions enhanced *P. expansum* decay control on Fuji and Golden Delicious apples. Gramisci et al[39] studied the effects of various nutrients and additives (including $CaCl_2$) on the biocontrol activities of *Pichia membranifaciens* and *Vishniacozyma victoriae* against *P. expansum* and found that $CaCl_2$ significantly improved the antagonistic activity of the yeasts. Our research demonstrated that addition of $CaCl_2$ substantially increased the antagonistic activities of *M. guilliermondii*, *M. caribbica* and the two *Cryptococcus* species studied here against *P. expansum*. Integration of these yeast strains with $CaCl_2$ could provide a significant improvement in long-term storage of fruits like apples.

Conclusions

The wild yeast strains isolated in our laboratory and studied here showed substantial inhibitory activity against *P. expansum in vivo* (on Golden Delicious apples) during the first week of incubation at 21°C. The highest activity was observed in strains isolated from apples and a fruit salad. These strains had activities similar to that of *C. oleophila*. Biocontrol activity was higher and better preserved during refrigerated storage (at 3°C) of the treated apples. Incorporation of $CaCl_2$ in the yeast suspensions increased the inhibitory activities markedly. Testing of these strains for pathogenicity is necessary before considering them for use in food commodities. Therefore, future research efforts will include rigorous toxicological testing to determine whether these BCA yeasts are safe for use in products destined for human consumption, and studies to ascertain whether they possess antagonistic activity against other postharvest apple pathogens.

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Author Contributions

VHT conceived and designed the experiments, conducted the experimental work, analysed the data, and wrote the first draft. EJK made significant contributions to manuscript writing. Both authors agreed with the manuscript results and conclusions, and reviewed and approved the final version.

REFERENCES

1. Usall J, Torres R, Teixido N. Biological control of postharvest diseases on fruit: a suitable alternative? *Curr Op Food Sci*. 2016;11:51–55.
2. Nunes CA. Biological control of postharvest diseases of fruit. *Eur J Plant Pathol*. 2012;133:181–196. doi:10.1007/s10658-011-9919-7.
3. United States Food and Drug Administration (USFDA). Patulin in apple juice, apple juice concentrates and apple juice products. Compliance policy guidance for FDA staff: apple juice, apple juice concentrate, and apple juice products: adulteration with patulin. https://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074427.htm.
4. Baert K, De Meulenaer B, Kamala A, Kasase C, Devlieghere F. Occurrence of patulin in organic, conventional, and handcrafted apple juices marketed in Belgium. *J Food Prot*. 2006;69:1371–1378.
5. Food and Agriculture Organization of the United Nations (FAO). FAOSTAT – agricultural statistics database. http://faostat.fao.org. Published 2006.
6. Ritieni A. Patulin in Italian commercial apple products. *J Agric Food Chem*. 2003;51:6086–6090.
7. United States National Research Council, Board Agric. *Regulating Pesticides in Food. The Delaney Paradox*. Washington, DC: National Academy Press; 1987. http://www.worldcat.org/title/regulating-pesticides-in-food-the-delaney-paradox/oclc/893646871/editions?editionsView=true&referer=br.
8. United States National Research Council, Board Agric. *Pesticides in the Diets of Infants and Children*. Washington, DC: National Academy Press; 1993. http://psep.cce.cornell.edu/issues/nas-exec.aspx.
9. Eckert JW, Sievert JR, Ratnayake M. Reduction of imazalil effectiveness against citrus green mold in California packinghouse by resistant biotypes of *Penicillium digitatum*. *Plant Dis*. 1994;78:791–794.
10. Holmes GJ, Eckert JW. Sensitivity of *Penicillium digitatum* and *P. italicum* to postharvest citrus fungicides in California. *Phytopathology*. 1999;89:716–721.
11. Droby S, Wisniewski M, Teixido N, Spadaro D, Jijakli MH. The science, development and commercialization of postharvest biocontrol products. *Postharvest Biol Technol*. 2016;122:22–29.
12. Sharma RR, Singh D, Singh R. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: a review. *Biol Control*. 2009;50:205–221.
13. Tournas VH, Katsoudas EJ. Bacteria and fungi as biocontrol agents against pome fruit postharvest pathogens. *Int J Adv Biol Res*. 2014;4:93–112.
14. Wisniewski M, Droby S, Norelli J, Liu J, Schena L. Alternative management technologies for postharvest disease control: the journey from simplicity to complexity. *Postharvest Biol Technol*. 2016;122:3–10.
15. Spadaro D, Droby S. Development of biocontrol products for postharvest diseases of fruit: the importance of elucidating the mechanisms of action of yeast antagonists. *Trends Food Sci Technol*. 2016;47:39–49.
16. Sun S, Fu D, Lu H, Zhang J, Zheng X, Yu T. Autoclaved yeast enhances the resistance against *Penicillium expansum* in postharvest pear fruit and its possible mechanisms of action. *Biol Control*. 2018;119:51–58.
17. Amin SS, Ahmed AI, Sidiq SA, Mahmood HA. Biological control of post-harvest disease of blue mould (*Penicillium expansum*) of pear fruit by using antagonistic microorganisms under laboratory and cold storage conditions. *Int J Curr Res Biosci Plant Biol*. 2017;4:20–31. doi:10.20546/ijcrbp.2017.401.004.
18. Nadai C, Fernandes Lemos WJ Jr, Favaron F, Giacomini A, Corich V. Biocontrol activity of *Starmerella bacillaris* yeast against blue mould disease on apple fruit and its effect on cider fermentation. *PLoS ONE*. 2018;13:e0204350. doi:10.1371/ journal.pone.0204350.
19. Blachinsky D, Antonov J, Bercovitz A, et al. Commercial applications of 'Shemer' for the control of pre- and postharvest diseases. *IOBC/WOPRS Bull*. 2007;30:75–78.
20. Droby S, Wisniewski M, Macarisin D, Wilson C. Twentyyears of postharvest biocontrol research: is it time for new paradigm? *Postharvest Biol Technol*. 2009;52:137–145.
21. Conway WS, Gross KC, Boyer CD, Sams CF. Inhibition of *Penicillium expansum* polygalacturonase activity by increased apple cell wall calcium. *Phytopathology*. 1988;78:1052–1055.
22. Conway WS, Leverentz B, Janisiewicz WJ, Saftner RA, Camp MJ. Improving biocontrol using antagonist mixtures with heat and/or sodium bicarbonate to control postharvest decay of apple fruit. *Postharvest Biol Technol*. 2005;36:235–244.
23. Janisiewicz WJ, Conway WS. Combining biological control with physical and chemical treatments to control fruit decay after harvest. *Stewart Postharvest Rev*. 2010;6:1–16.
24. Lima G, Spina AM, Castoria R, De Curtis F, De Cicco V. Integration of biocontrol agents and food grade additives in enhancing protection of stored apples from *Penicillium expansum*. *J Food Prot*. 2005;68:2100–2106.
25. McLaughlin RJ, Wisniewski ME, Wilson CL, Chalutz E. Effect of inoculum concentration and salt solutions on biological control of postharvest diseases of apple with *Candida* sp. *Phytopathology*. 1990;80:456–461.
26. Wisniewski M, Droby S, Chalutz E, Eilam Y. Effects of Ca^{2+} and Mg^{2+} on *Botrytis cinerea* and *Penicillium expansum* in vitro and on the biocontrol activity of *Candida oleophila*. *Plant Pathol*. 1995;44:1016–1024.
27. Yu T, Yu C, Chen F, et al. Integrated control of blue mold in pear by combined application of chitosan, a biocontrol yeast and calcium chloride. *Postharvest Biol Technol*. 2012;69:49–53.
28. Yu T, Yu C, Lu H, et al. Effect of *Cryptococcus laurentii* and calcium chloride on control of *Penicillium expansum* and *Botrytis cinerea* infections in pear fruit. *Biol Control*. 2012;61:169–175.
29. Bastiaanse H, de Bellaire L, Lassois L, Misson C, Jijakli M. Integrated control of crown rot of banana with *Candida oleophila* strain O, calcium chloride and modified atmosphere packaging. *Biol Control*. 2010;53:100–107.
30. Farahani L, Etebarian HR, Sahebani N, Aminian H. Effects of two strains of antagonistic yeasts in combination with silicon against two isolates of *Penicillium expansum* on apple fruit. *Int Res J Appl Basic Sci*. 2012;3:18–23.
31. Li C, Zhang H, Yang Q., Komla MG, Zhang X, Zhu S. Ascorbic acid enhances oxidative stress tolerance and biological control efficacy of *Pichia caribbica* against postharvest blue mold decay of apples. *J Agric Food Chem*. 2014;62:7612–7621.
32. He D, Zheng X-D, Yin Y-M, Sun P, Zhang H-Y. Yeast application for controlling apple postharvest diseases associated with *Penicillium expansum*. *Bot Bull Acad Sin*. 2003;44:211–216.
33. Motulsky H, ed. *Intuitive Biostatistics*. New York: Oxford University Press; 1995.
34. Yan Y, Zhang X, Zheng X, et al. Control of postharvest blue mold decay in pears by *Meyerozyma guilliermondii* and its effects on the protein expression profile of pears. *Postharvest Biol Technol*. 2018;136:124–131.
35. Cao J, Zhang H, Yang Q., Ren R. Efficacy of *Pichia caribbica* in controlling blue mold rot and patulin degradation in apples. *Int J Food Microbiol*. 2013;162:167–173. doi:10.1016/j.ijfoodmicro.2013.01.007.
36. Mbili NC. *Evaluation of Integrated Control of Postharvest Grey Mold and Blue Mold of Pome Fruit Using Yeast, Potassium Silicate and Hot Water Treatments* [master's thesis]. Pietermaritzburg, Republic of South Africa: University of Kwazulu-Natal; 2012.
37. Guerrero V, Guigon C, Berlanga D, Ojeda D. Complete control of *Penicillium expansum* on apple fruit using a combination of antagonistic yeast *Candida oleophila*. *Chil J Agric Res*. 2014;74:427–431.
38. Scherm B, Ortu G, Muzzu A, Budroni M, Arras G, Migheli Q. Biocontrol activity of antagonistic yeasts against *Penicillium expansum* on apple. *J Plant Pathol*. 2003;85:205–213.
39. Gramisci BR, Lutz MC, Lopes CA, Sangorrín MP. Enhancing the efficacy of yeast biocontrol agents against postharvest pathogens through nutrient profiling and the use of other additives. *Biol Control*. 2018;121:151–158.