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Efficacy of *Wickerhamomyces anomalus* yeast in the biocontrol of blue mold decay in apples and investigation of the mechanisms involved

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Abstract Blue mold decay is the one of most important postharvest disease of apples caused by the fungus, *Penicillium expansum*. This study aimed to investigate the biocontrol efficacy of the yeast, *Wickerhamomyces anomalus*, on postharvest blue mold decay of apples and the relative defense mechanisms. The results indicated that *W. anomalus* could significantly reduce the blue mold decay of apples, and the maximum inhibition was obtained when the concentration of *W. anomalus* was 1×10^8 cells ml^{-1}. Furthermore, *W. anomalus* significantly reduced the fruit decay under ambient conditions, without generating any change in fruit quality. *In vitro* experiments showed that *W. anomalus* greatly inhibited the spore germination and germ tube elongation of *P. expansum*. Besides, its ease of adaptation, stable growth and potential colonization of in apple wounds or surfaces indicated that *W. anomalus* could compete with *P. expansum* for nutrients and space, leading to considerable inhibition blue mold decay. *W. anomalus* significantly induced the activities of polyphenol oxidase (PPO), peroxidase (POD), catalase (CAT), phenylalanine ammonia-lyase (PAL), and ascorbate peroxidase (APX) in apples. Moreover, *W. anomalus* increased the contents of flavonoid and total phenols. All these results suggested that *W. anomalus* has potential biocontrol efficacy to control the postharvest blue mold decay of apples

Keywords Apples · Biocontrol · Blue mold decay · *Penicillium expansum* · *Wickerhamomyces anomalus*

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Introduction

Apples (*Malus domestica*) are one of the most important fruits in traditional diet (Peneau et al. 2006), and are rich in nutrients nutraceuticals and loved by people all over the world. However, they are highly susceptible to pathogenic fungal infections during harvest, packaging, storage and transportation (Wallace et al. 2017). Mechanical damage caused by improper handling leads to different types of diseases in apples, including blue mold, gray mold, anthracnose, etc. (Sholberg et al. 2005). *P. expansum* is one of the most destructive pathogens causes blue mold disease in apples and considerable losses of stored fruit (Vilanova et al. 2014). Along with apple decay, *P. expansum* could produce mycotoxin named patulin which can be harmful to human health (Quaglia et al. 2011).

All along, chemical fungicides were previously used and could effectively control fruit decay caused by fungi (El Ghaouth et al. 2003). However,

the abuse of chemical fungicides causes various negative impacts such as hazardous to human health and environmental pollution (Li et al. 2016). Furthermore, due to the increase of pathogen resistance as well as public awareness in environmental protection, it is necessary to develop alternative strategies in controlling postharvest disease of fruits (Forster et al. 2007).

In recent years, biological control agents are considered as an alternative to chemical fungicides due to their advantages of safety, ecofriendly, and high efficiency. Recently, antagonistic microorganisms such as bacteria, mold and yeast have been screened for their biocontrol activity. Among these antagonistic microorganisms, yeasts have the advantages of genetic stability, simple nutritional requirements, strong survivability, safety, and high inhibitory efficacy against various fungal pathogens. Therefore, yeasts are anticipated as better biocontrol agents for controlling postharvest diseases of fruit. Numerous studies reported that the antagonistic yeasts can multiply and occupy the space of fruits rapidly, and effectively inhibit the growth of pathogens (Spadaro and Droby 2016; Zhang et al. 2019). In addition, antagonistic yeasts can enhance the disease resistance of fruits and induce the activities of defense-related enzymes involved in fruits defense mechanisms (Apaliya et al. 2017).

Over the past few years, several studies demonstrated that antagonistic yeasts could control postharvest diseases of apples. For example, in recent research work, five endophytic yeasts were isolated from fresh apple fruits and showed potential disease control ability to apple postharvest diseases (Madbouly et al. 2020). *Rhodotorula mucilaginosa* is an antagonistic yeast that could inhibit postharvest gray mold, blue mold and natural decay of apples and also degraded patulin (Li et al. 2011; Qian et al. 2020). In the biological control of apple diseases, antagonistic yeasts can be used in combination with other biomolecules to enhance the disease control efficacy of the yeasts. *Meyerozyma guilliermondii* induced by methyl jasmonate (MeJA) showed better disease control ability against blue mold decay of apples (He et al. 2020). As far as we know, the present work is the first attempt to use *W. anomalus* for the biological control of blue mold decay in apples.

Materials and methods

Fruit

Apples (*M. domestica* Borkh, cv. Fuji) were harvested at commercial maturity from an orchard in Yantai of Shandong province, China. Fuji apples are very popular all over the world and have a high market share. The ripening period of Fuji apples generally takes about 170–180 days, and were randomly selected for uniformity of size, bright in appearance, fresh in stems, and without any damage or commercial treatment. Apples were immersed in 0.1% sodium hypochlorite for 2 min to disinfect the surface, and then washed in running tap water, and dried at room temperature for further use.

Yeast

W. anomalus strain was isolated and deposited in the China Center for Type Culture Collection (CCTCC), and received the accession number as CCTCC M 2,018,053. In the laboratory, *W. anomalus* was stored in 30% glycerin at −80 °C. For activation, 1 ml of yeast suspension was inoculated into 50 ml of sterilized nutrient yeast dextrose broth (NYDB, nutrient broth 8 gl^{-1}, yeast extract 5 gl^{-1} and glucose 10 gl^{-1}) and incubated on a constant temperature shaker (180 rpm, 28 °C) for 20 h. The strain was sub-cultured twice before use. Yeast cells were collected by centrifugation and suspended in sterile saline solution. The cells were counted using a hemocytometer and the concentration was adjusted as per our requirement.

Pathogen inoculum

The pathogen, *P. expansum,* was isolated from infected apple fruits and identified by our research team. The spore suspension was stored in 30% glycerol at −80 °C. 1 ml of *P. expansum* spore suspension was inoculated into 50 ml of sterilized potato dextrose broth (PDB, 500 ml of extract from 200 g boiled potatoes, 20 g dextrose, and 500 ml of distilled water), and incubated in a thermostatic shaker (120 rpm, 25 °C) for 20 h. Then, the mold was inoculated on potato dextrose agar medium (PDA, PDB with agar) by streaking a loop full of fungal

suspension and cultured in a constant temperature and humidity incubator (25 °C, RH 95%) for seven days. Spores were scraped from the surface of the plate, suspended in sterile saline and adjusted to an appropriate concentration using a hemocytometer.

Efficacy of *W. anomalus* in inhibiting blue mold decay of apples

Sterile borers were used to create three uniform wounds (4 mm in diameter × 5 mm in depth) equidistantly at the equator of the pretreated apples. Each wound was injected with 30 µl of *W. anomalus* cell suspension at different concentrations (1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 cells ml^{-1}) or equal volume of sterile distilled water as control. After 2 h, 30 µl of *P. expansum* spore suspension (1×10^5 spores ml^{-1}) was injected into each wound. After drying at room temperature, the plastic frame was sealed with plastic wrap and placed in a constant temperature and humidity incubator (20 °C, RH 95%). After five days of incubation, the apples were observed. The rate of decay incidence (number of rotten apple wounds/ total number of wounds × 100%) and lesion diameter were measured. The experiment comprised of three replications (12 apples in a replication) for each treatment and the experiment was repeated twice (for all experiments).

Effect of *W. anomalus* on natural decay and quality of apples

Apples with commercial maturity were selected based on: without any treatment, without mechanical damage, uniform size and similar color. Selected fruits were not washed or disinfected. Then the fruits were sprayed with sterile water (the control) or *W. anomalus* at 1×10^8 cells ml^{-1}. Followed by drying at room temperature, each apple was wrapped in a plastic bag and placed in a clean plastic frame. The plastic frame was sealed with plastic wrap and placed at constant temperature and humidity in an incubator (20 °C, RH 95%) for 35 days. After 35 days of storage, the following indicators were measured.

Natural decay incidence: The natural decay was evaluated by counting the fruits presented with any visible sign of decay, and the percentage of decay was calculated from total apples in each treatment.

Rate of weight loss: The weight loss percentage was calculated by measuring the weight of individual treated apple before and after storage.

Fruit firmness: Firmness was detected using the TA-XT2i Texture Analyzer (Stable Micro Systems, UK). The diameter of the probe was 5 mm, the running speed of the probe was 1.0 mm s^{-1}, and the test depth was 10 mm (Gunness et al. 2009). The maximum resistance when the probe was inserted into the apple was recorded as the firmness (Newton, N).

Total soluble solids (TSS): According to the method of Juan et al. (2016), the TSS content (g per 100 g) was measured using a hand refractometer at room temperature.

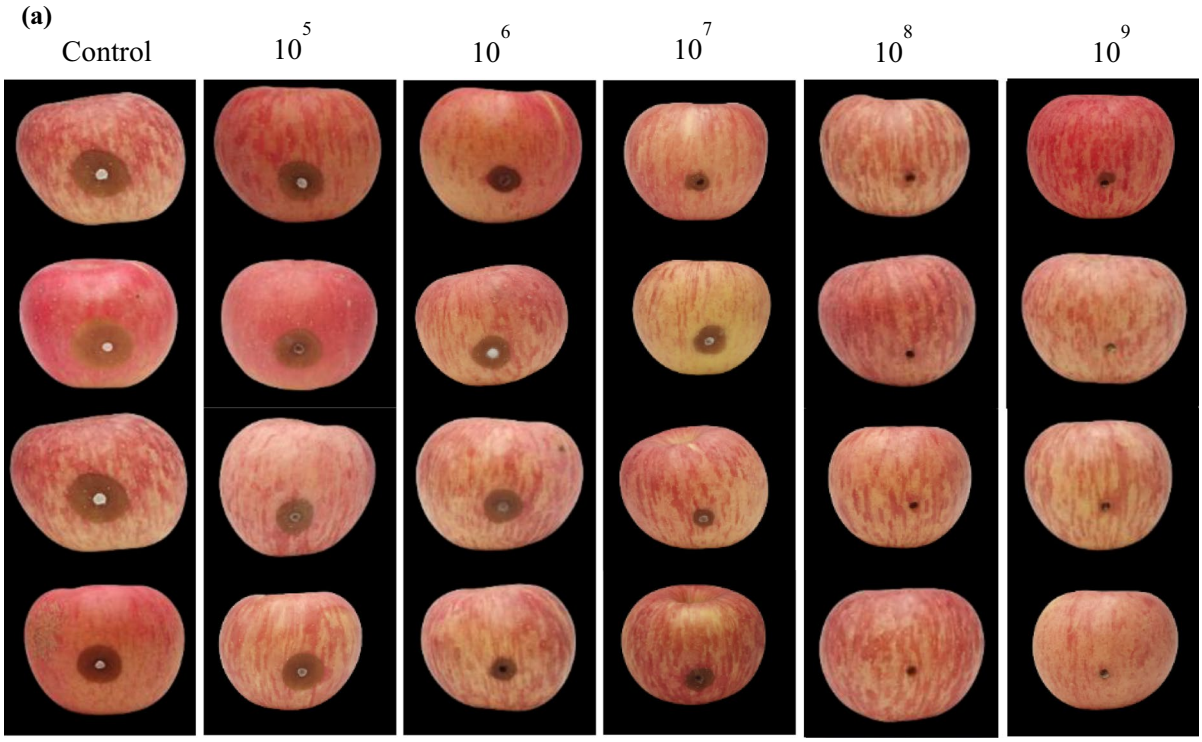
Titratable acids (TA): Acid–base titration method (Wright and Adel 1997) was used for TA determination, with malic acid as the acidity index.

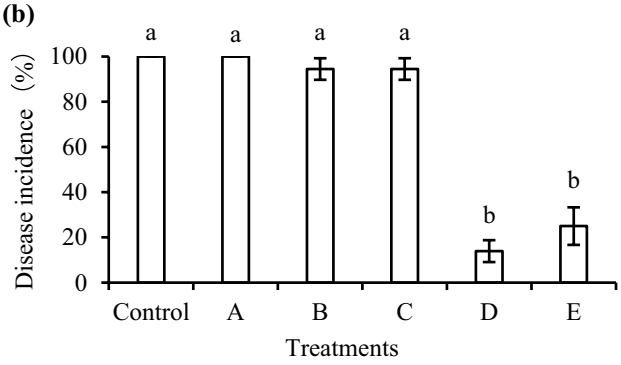
Ascorbic acid (Vitamin C, Vc): Vc content (µg per 100 g) was determined according to the method described by García-Martín et al. (2018).

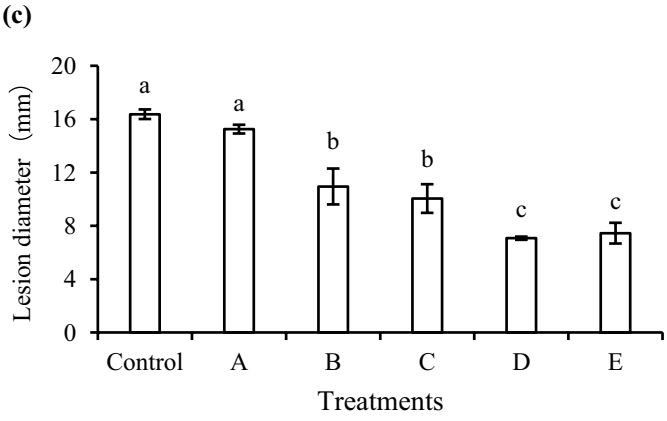
Tissue browning: As described by Lee et al. (1990), the degree of tissue browning was determined and expressed by the absorbance value at 420 nm.

Effect of *W. anomalus* concentration on spore germination and germ tube length of *P. expansum in vitro*

The *P. expansum* spore suspension was inoculated into 50 ml of PDB and the final concentration was adjusted to 1×10^6 spores ml^{-1}. Then the different treatments were as follows: (1) 1 ml of sterilized normal saline (control), (2) 1 ml of different concentrations of *W. anomalus* (1×10^6, 1×10^7, 1×10^8 and 1×10^9 cells ml^{-1}). After incubated in a constant temperature shaker (25 °C, 75 rpm) for 14 h, the growth status of *P. expansum* was observed using a microscope. The spore germination rate and germ tube length were recorded by viewing at least 100 spores at a time. Each treatment consisted of three replications and the experiment was repeated twice.


(a)
Control
10^5
10^6
10^7
10^8
10^9


(b)
Disease incidence (%)
100
80
60
40
20
0
a
a
a
a
b
b
Control
A
B
C
D
E
Treatments


(c)
Lesion diameter (mm)
20
16
12
8
4
0
a
a
b
b
c
c
Control
A
B
C
D
E
Treatments

◂**Fig. 1** Efficacy of *W. anomalus* in inhibiting blue mold decay of apples (**a**). Decay incidence (**b**) and lesion diameter (**c**) of apples infected by *P. expansum* were measured after five days incubation at 20 °C, 95% RH. Control treated with sterilized normal saline, *A* 1×10^5 cells ml^{-1}, *B* 1×10^6 cells ml^{-1}, *C* 1×10^7 cells ml^{-1}, *D* 1×10^8 cells ml^{-1}, and *E* 1×10^9 cells ml^{-1} of *W. anomalus* suspensions. Bars represent SE. Different letters above the columns indicate significant differences among the data according to Duncan's multiple range test at $P<0.05$

Dynamics of *W. anomalus* colonization in apple wounds and on the surface

15 µl of *W. anomalus* suspension (1×10^8 cells ml^{-1}) was inoculated into each wound or coated on each fruit surface (a circle of 15 mm radius) of the pre-treated apples. After dried at room temperature, the plastic frame was sealed with plastic wrap and placed at 20 °C in a constant temperature and humidity incubator (RH 95%). According to the method described by Zhu et al. (2019) for the sample collection, grinding and enumeration of *W. anomalus* cells were performed. The results were expressed as $\log_{10}$ CFU per wound or $\log_{10}$ CFU per circle. Each treatment consisted of three replicates and the experiment was repeated twice.

Effects of *W. anomalus* on enzyme activities, flavonoid content and total phenol content in apples

30 µl of *W. anomalus* suspension (1×10^8 cells ml^{-1}) was inoculated into each wound, and then apples were treated as described above. 2 g of wound tissue from six apples were collected in a mortar. Then 10 ml of 50 mmol l^{-1} buffer with 1.33 mmol l^{-1} EDTA and 1% PVPP (pre-cooled at 4 °C) was added and ground well. The experiments were performed in an ice-cold environment. The samples were centrifuged at $12{,}000\times g$, 4 °C for 15 min. The supernatant was collected and used as crude enzyme solution for further analyses. Each treatment consisted of three replicates and the experiment was repeated twice. The determination of PPO, CAT, APX and PAL activities were carried out as per the method described by Apaliya et al. (2017) with some modifications. Determination of POD activity was performed as per the method designed by Dou et al. (2021) with some modifications. One unit of PPO, POD, PAL and APX was defined as an increase of absorbance (0.01) per minute at 398 nm, 470 nm, 290 nm and 290 nm, respectively. One unit of CAT was defined as a decrease of absorbance (0.01) per minute at 240 nm. All enzyme activities were expressed as U g^{-1} FW (fresh weight).

Flavonoid compounds and total phenols were determined according to the methods designed by Deng et al. (2015) with some modifications. For 2 g of fruit tissue, 30 ml of pre-chilled 1% HCl-methanol solution was added and ground well. The homogenate was centrifuged at 4 °C and $12{,}000\times g$ for 20 min. The supernatant was collected and the OD values was measured at 325 and 280 nm for flavonoid content and total phenol content, respectively.

Statistical analysis

The data were analyzed by ANOVA using the statistical program SPSS/PC version II.x, (SPSS Inc. Chicago, Illinois, USA). Duncan's multiple range test was used to confirm the significance of more than two groups involved and an independent sample t-test was used to confirm the significance of only two groups involved. Data for percentages of decay incidence and spore germination were transformed into the arcsine square root values to normalize distribution before ANOVA, the percentage of germinated spores shown and decay incidence are untransformed data. Significant differences were considered when $P<0.05$.

Results

Efficacy of *W. anomalus* in inhibiting blue mold decay of apples

After five days of incubation, the fruits treated with *W. anomalus,* in the concentration range between 1×10^6 cells ml^{-1} to 1×10^9 cells ml^{-1}, showed a reduction in blue mold decay incidence (Fig. 1a). Especially, *W. anomalus* at the concentration of 1×10^8 cells ml^{-1} showed the lowest decay incidence (13.9%) than control and other treated groups, which was a 86.1% reduction compared to control ($F_{5,12}=72.467$, $P<0.001$) (Fig. 1b). In the case of lesion diameter, *W. anomalus* treated groups

(irrespective of concentration) showed a reduction compared to the control group. Particularly, *W. anomalus* treated group (1×10^8 cells ml^{-1}) showed the least lesion diameter (7.07 mm) than other groups, which was a 56.8% reduction when compared to control ($F_{5,12}=71.321$, $P<0.001$) (Fig. 1c).

Effect of *W. anomalus* on natural decay and quality of apples

After stored at 20 °C for 35 days, the natural decay incidence of *W. anomalus* treated apples was 28.4% which was significantly lower than that of control group (48.5%) ($t=6.854$, $df=4$, $P=0.002$). The fruit quality attributes such as weight loss percentage, total soluble solids, titratable acids, ascorbic acid content and firmness of apples were not shown any adverse effects in *W. anomalus* treated fruits. Moreover, tissue browning in apples treated with *W. anomalus* was significantly lower than the control group ($t=86.000$, $df=4$, $P<0.001$) (Table 1). In general, *W. anomalus* could significantly reduce the natural decay incidence and had no adverse effects on fruit quality.

Effect of *W. anomalus* concentration on spore germination and germ tube length of *P. expansum in vitro*

The spore germination rate and germ tube length of *P. expansum* were both significantly reduced by *W. anomalus* (Fig. 2). Spore germination rate dropped to 0% when treated with *W. anomalus* at 1×10^8 and 1×10^9 cells ml^{-1}, whereas the control group showed 81.4% of spore germination ($F_{4,10}=273.961$, $P<0.001$) (Fig. 2a). The germ tube length of the control group was 35 µm, whereas in *W. anomalus* with 1×10^7 cells ml^{-1} group the length was reduced to 26.4 µm. Besides, in *W. anomalus* (1×10^8 and 1×10^9 cells ml^{-1}) treatment groups, the growth of *P. expansum* was totally inhibited ($F_{4,10}=347.641$, $P<0.001$) (Fig. 2b).

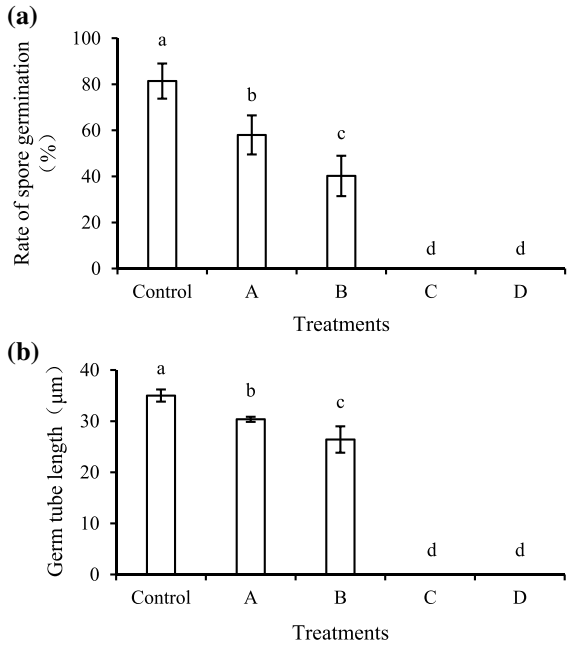


Fig. 2 Effects of *W. anomalus* at different concentrations on spore germination and germ tube length of *P. expansum in vitro*. Rate of spore germination (**a**) and germ tube length (**b**) of *P. expansum* were measured by microscope and micrometer after 18 h of incubation at 28 °C, 75 rpm in PDB. Control treated with sterilized normal saline, *A* 1×10^6 cells ml^{-1}, *B* 1×10^7 cells ml^{-1}, *C* 1×10^8 cells ml^{-1}, and *D* 1×10^9 cells ml^{-1} of *W. anomalus* suspensions. Bars represent SE. Different letters above the columns indicate significant differences among the data according to Duncan's multiple range test at $P<0.05$

Table 1 Effect of *W. anomalus* on natural decay and quality of apples

Treat-ment	Natural decay incidene (%)	Rate of weight loss (%)	Firmness (N)	Total soluble solids (%)	Titratable acids (%)	Ascorbic acid (µg/100 g)	Tissue browning
Control	48.5 ± 2.6^a	1.32 ± 0.10^a	27.15 ± 0.73^a	12.60 ± 0.28^a	0.175 ± 0.007^a	47.0 ± 1.0^a	0.217 ± 0.002^a
Yeast	28.4 ± 4.1^b	1.30 ± 0.13^a	28.42 ± 1.97^a	11.83 ± 0.28^a	0.175 ± 0.007^a	46.7 ± 2.1^a	0.102 ± 0.001^b

The ± values represent the SE. Control: Apples treated with sterilized normal saline; Yeast: Apples treated with *W. anomalus*. Different letters (^{a} and ^{b}) indicate significant differences among the data in column according to an independent sample t-test at $P<0.05$

Dynamics of *W. anomalus* colonization in apple wounds and on the surface

During the first four days of incubation, *W. anomalus* grew rapidly and reached a peak, which was 2.10×10^7 CFU per wound, and then a slight decrease in cell count occurred on day 5. Whereas, on the 6th day, the proliferation was higher and reached the maximum of 2.88×10^7 CFU per wound. The experiment results indicated that *W. anomalus* can grow steadily in apple wounds at 20 °C ($F_{6,14}=15.603$, $P<0.001$) (Fig. 3a).

The population of *W. anomalus* dropped sharply from 1.69×10^6 CFU per circle (0 d) to 6.73×10^5 CFU per circle at 1 d. *W. anomalus* did not adapt to the surface environment of the apples at first. But, in the next two days, *W. anomalus* grown rapidly and reached the pre-inoculation level of 1.77×10^6 CFU per circle. Then, the yeast maintained a stable growth for the remaining days ($F_{6,14}=10.870$, $P<0.001$) (Fig. 3b), which indicated that *W. anomalus* could quickly adapt, colonize and grow on the surface of apples.

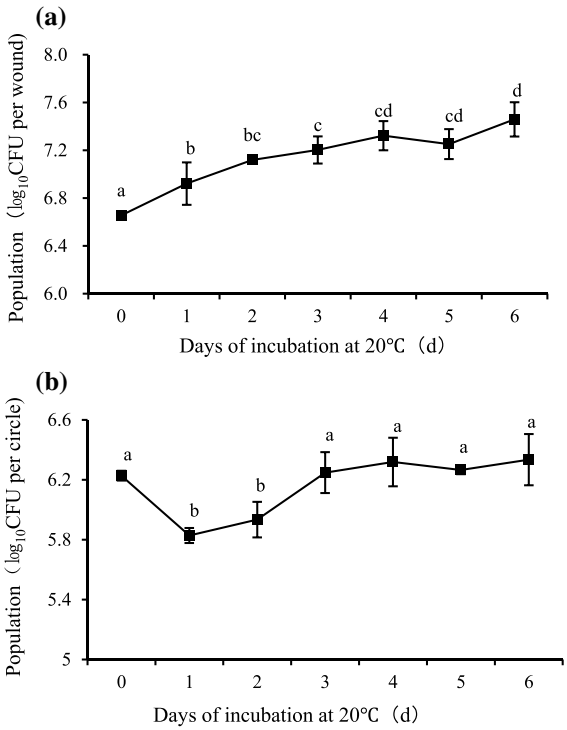
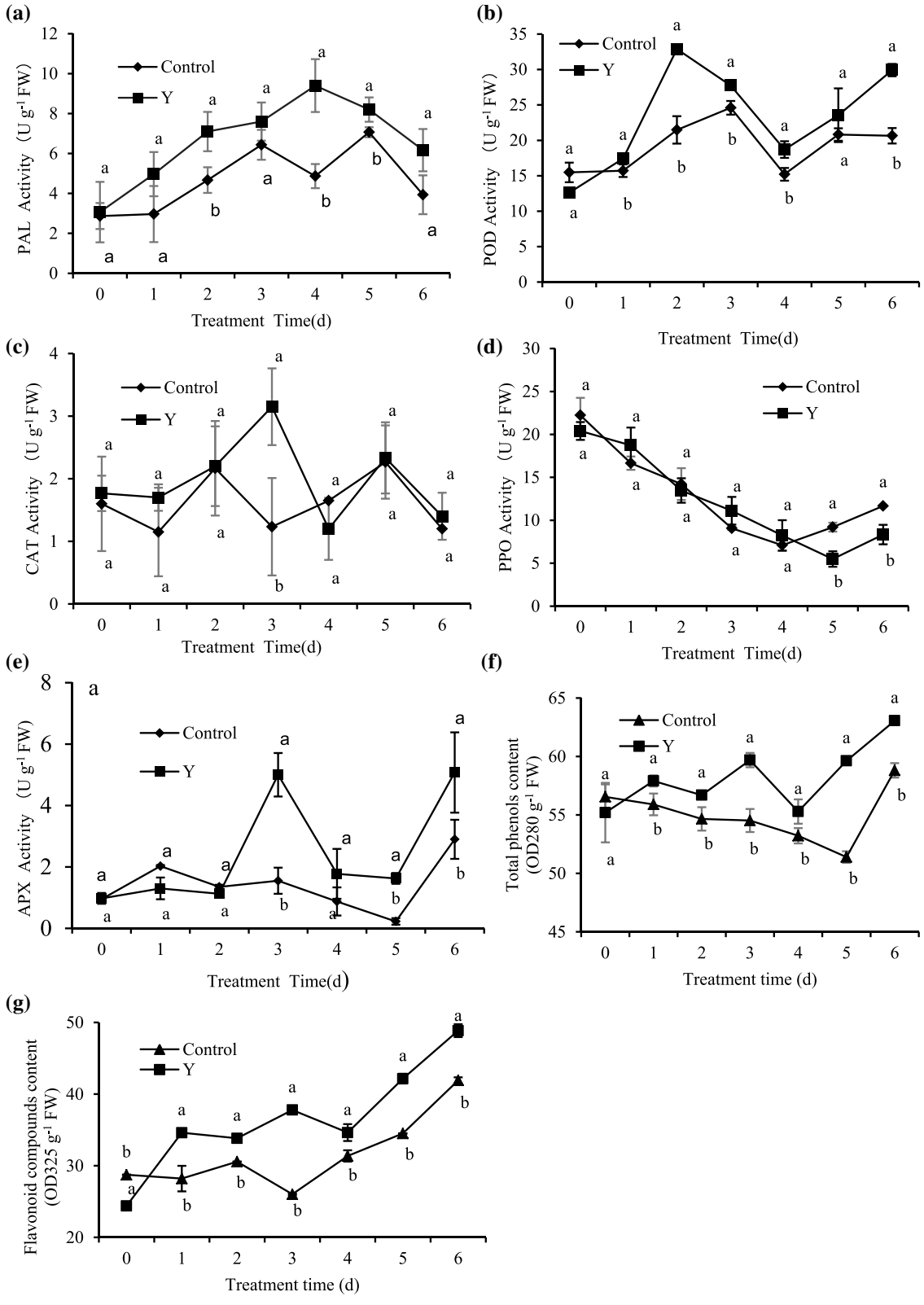


Fig. 3 Dynamics of *W. anomalus* colonization in wounds and on surface of apples at 20 °C from 0 d (2 h after inoculation) to 6 d post-inoculation of the yeast. Population of *W. anomalus* in wounds is expressed by $\log_{10}$ CFU per wound, a wound with a diameter of 4 mm and a depth of 5 mm. Population of *W. anomalus* on surface is expressed by $\log_{10}$ CFU per circle, a circle represents 15 mm radius on the surface. Bars represent SE. Different letters indicate significant differences among the data according to Duncan's multiple range test at $P<0.05$

Effects of *W. anomalus* on enzyme activities, flavonoid compounds content and total phenols content in apples

The PAL activity of *W. anomalus*-treated apples was higher than that of the control group throughout the experimental period (Fig. 4a). The activity reached the maximum value of 9.4 U g^{-1} FW at 4 d, which was 1.93 times higher than the control group ($t=-5.401$, $df=4$, $P=0.006$). The POD activity of *W. anomalus*-treated apples showed a sharp rise initially then fell and then raised again (Fig. 4b). The POD activity reached the maximum value of 32.88 U g^{-1} FW on the second day, which was 1.53 times higher than the control group ($t=-10.177$, $df=4$, $P=0.001$). In the following days, the POD activity of *W. anomalus* treatment group was higher than that of the control group. During the experimental period, the CAT activity of *W. anomalus*-treated apples was higher than the control group except for day 4. Also, on day 3, the CAT activity reached the maximum value of 3.15 U g^{-1} FW, which was 2.56 times higher than the control group ($t=-2.949$, $df=4$, $P=0.042$) (Fig. 4c). The PPO activity of both control and *W. anomalus* treated apples showed a downward trend till day 4 and gradually increased for rest of the days (Fig. 4d). Though it showed a downward trend for the first four days, the PPO activity of the *W. anomalus* treatment group was higher than the control group. Similarly, during the experimental period, the APX activity of *W. anomalus*-treated apples was higher than the control group. On day 3, The APX activity reached a maximum value of 5.0 U g^{-1} FW, which was 3.22 times higher than the control ($t=-0.248$, $df=4$, $P=0.001$) (Fig. 4e).

From day 1, the amount of total phenol content in *W. anomalus*-treated apples was increased and higher than the control group (Fig. 4f). On day 5, the total phenol content of treated apples was 1.16 times higher than that of the control ($t=-28.671$, $df=4$, $P=0.001$). Similarly, the content of flavonoids also increased steadily after treated with *W. anomalus*,


(a)
PAL Activity (U g-1 FW)
Control
Y
Treatment Time(d)
(b)
POD Activity (U g-1 FW)
Control
Y
Treatment Time(d)
(c)
CAT Activity (U g-1 FW)
Control
Y
Treatment Time(d)
(d)
PPO Activity (U g-1 FW)
Control
Y
Treatment Time(d)
(e)
APX Activity (U g-1 FW)
Control
Y
Treatment Time(d)
(f)
Total phenols content (OD280 g-1 FW)
Control
Y
Treatment time (d)
(g)
Flavonoid compounds content (OD325 g-1 FW)
Control
Y
Treatment time (d)

◂**Fig. 4** Effects of *W. anomalus* on enzyme activities of PAL (**a**), POD (**b**), CAT (**c**), PPO (**d**), APX(**e**), total phenols content (**f**) and flavonoid compounds content (**g**) in apples. Enzyme activities were measured from 0 d (2 h after inoculation) to six days post-inoculation of the yeast. Control Apples treated with sterilized normal saline, *Y* Apples treated with *W. anomalus*, *FW* Fresh Weight. Bars represent SE. Different letters indicate differences between the control and *Y* for each time separately, and different letters above the columns indicate significant differences among the data according to an independent sample t-test when $P < 0.05$

from day 1. The flavonoid content of *W. anomalus*-treated apple fruit was always higher than the control. On day 6, the total flavonoid content of *W. anomalus*-treated apple fruit reached a maximum value of 48.85 OD_{325} g^{-1} FW. On day 3, the flavonoid content of *W. anomalus*-treated apples was 1.45 times higher than that of the control ($t = -158.707$, $df = 4$, $P < 0.001$) (Fig. 4g).

Discussion

The biocontrol efficacy of *W. anomalus* was previously reported to significantly reduce the brown rot incidence of sweet cherries at the concentration of 10^7 CFU ml^{-1} (Oro et al. 2014). However, there was no report on the use of *W. anomalus* against postharvest blue mold in apples and on the mechanisms involved. Our study strongly evidenced that *W. anomalus* could significantly inhibit the incidence of blue mold on apples, and it has the best inhibition effect at the concentration of 1×10^8 cells ml^{-1}. (Fig. 1). A similar study was carried out in pears, and proved that *W. anomalus* significantly reduced the disease incidence and lesion diameter of blue mold of pears (Zhang et al. 2019). Furthermore, our results also witnessed that *W. anomalus* remarkably inhibited the natural decay of apples without causing any adverse effects on the quality of apples (Table 1). In general, our experimental results provided a reliable basis for the commercial application of *W. anomalus* in the field of biological control against postharvest diseases of apples.

W. anomalus inhibited the spore germination and shortened the germ tube length of *P. expansum* in a concentration-dependent manner. Our experiments suggested that there was a positive correlation between *W. anomalus* concentration and its inhibitory efficacy (Fig. 2). Similarly, a study by Zhao et al. (2018) showed that *Sporidiobolus pararoseus* Y16 could reduce spore germination and germ tube length of *P. expansum* at higher concentrations. The increased concentrations of antagonistic yeast need more nutrition and space for its growth and eventually inhibit the growth of other pathogenic bacteria/fungi. *W. anomalus* colonization in apples showed a similar result with a study by Zhu et al. (2019), which reported that the number of yeast colonies on the surface of apples simultaneously decreased during storage period (Fig. 3b). Moreover, *W. anomalus* could rapidly proliferate and colonize the wounds of apples, adapt to the surface environment within a short period, and proliferate stably at 20 °C. Competition for nutrition and space with other pathogens is one of the biocontrol mechanisms of *W. anomalus* (Filonow 1998). Antagonistic yeasts can attach and parasitize the mycelium of pathogenic fungi, weaken the nutrition absorption process, and inhibit their growth and metabolism (Bar-Shimon et al. 2004).

Numerous studies have shown that antagonistic yeasts could induce a series of defense-related enzyme activities in host plants. Various resistant enzymes play an important role in protecting fruits and vegetables during post-harvest storage, as well as regulating the content of flavonoids and total phenols (Chen et al. 2019). Our study showed that PPO, POD, CAT, PAL and APX activities as well as the content of flavonoids and total phenols of apples were significantly induced by *W. anomalus* and higher than that of the control group (Fig. 4). PPO and POD are important catalytic enzymes, which can accelerate the oxidation of phenols. In addition, these enzymes also participate to the synthesis of certain hormones, thereby enhance fruit disease resistance (Farkas and Stahmann 1966). PPO participates to the transformation of phenols to quinones, which are extremely toxic to pathogens (Prabhukarthikeyan et al. 2018). POD is a multifunctional enzyme that regulates senescence and physiological changes of fruits, catalyzes the oxidation of H_2O_2 to phenols and formation of quinones (Wu et al. 2017). Furthermore, in apples treated with *M. guilliermondii* and MeJA, the PPO and POD activity was found higher than the control and the disease resistance of apple was enhanced (He et al. 2020), which was in concordance with the results of our studies. CAT contributes to eliminate reactive oxygen species (ROS), block the synthesis of

ROS, and catalyzes the decomposition of H_2O_2 into H_2O and O_2 that are not toxic to fruit cells. O_2 activates the conversion of benzoic acid to salicylic acid and participates in the systemic defense reaction of plants (Jones 1994). PAL is a key and rate-limiting enzyme in the phenylpropane metabolic pathway of fruits and vegetables. It is an important class of resistance-related enzyme that helps to produce resistant substances such as lignin, alkaloids, phenols, and flavonoids, which are essential molecules for fruits to resist to stress and pathogen infection (Toscano et al. 2018). APX can catalyze the reaction of ascorbic acid with H_2O_2, reduce the content of H_2O_2 in the cell, which at the same time increases the reduction potential of the antioxidant system, and cooperate with other antioxidant systems in eliminating free radicals in the cell (Gill and Tuteja 2010). Similar to our results, Ahima et al. (2019) also confirmed that a combination of antagonistic yeast with SA (0.2 mM) led to an upsurge in PAL, CAT and APX activities on oranges, which strengthened the ability of the host to fight against the invasion of pathogens. The total phenol and flavonoid content are related to the metabolism of L-phenylalanine, which can improve the disease resistance of fruits. The interaction between phenols strengthens the cell wall structure of fruits and plays an important role in the resistance of plants to pathogen infection (Deng et al. 2015). The overall results showed that *W. anomalus* could significantly induce the activity of defense-related enzymes, and increase the content of antibacterial substances, thereby enhance the disease resistance of apples.

In conclusion, *W. anomalus* is a potential antagonist which can control the postharvest blue mold of apples effectively, without any side effect on fruit quality. Besides, it could inhibit the spore germination and shortened germ tube length *in vitro*. The biocontrol mechanisms of *W. anomalus* mainly involve competition for nutrients and space with *P. expansum*, induce the defense-related enzyme activities, and increase the flavonoid compounds and total phenols in apples, which ultimately enhanced the disease-resistant ability of apples. We tested the mechanisms of induced resistance only on control and yeast without pathogen inoculation and pathogen + yeast inoculation, so further research is needed to investigate the mechanisms of induced resistance on pathogen inoculation and pathogen + yeast inoculation.

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