



Biocontrol activities of grey mould of grapes with the volatile organic compounds generated by yeast HXMG-1 isolated from grapes

Ning Li¹ · Bujiang Wang¹ · XinYi Cui¹ · Jing Hou¹ · Na Zhang²

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Abstract

The pathogens are reduced by volatile organic compounds (VOCs) generated by yeasts play an important role in controlling postharvest diseases. The yeast HXMG-1, which works effectively against the grey mould pathogen of grapes (*Botrytis cinerea*), was evaluated for its potential to generate volatile organic compounds as one of its modes of action. A double Petri dish assay was used to evaluate the effect of VOCs produced by HXMG-1 on mycelial and spore development of the target pathogens. Compared to the control, the VOCs produced by yeast HXMG-1 significantly reduced the growth of mycelium and spore germination of *Botrytis cinerea*. Specifically, the mycelial growth of *Botrytis cinerea* was completely restricted and the rate of spore germination of *Botrytis cinerea* was only 20.11% at a concentration of 1×10^9 CFU/mL. It was also found that the VOCs could significantly inhibit mycelial growth with an inhibition of 82.46% at a concentration of 1×10^8 CFU/mL. The VOCs caused the mycelium to grow curved, resulting in larger mycelial tips, fewer nuclei, and shorter mycelial septum spacing. In vivo tests, noninjure or injure grapes were artificially inoculated with the pathogen hyphal disc followed by biofumigation with VOCs produced by yeast HXMG-1, and the treatments (Wp2 and Wp3) significantly controlled pathogenic infection, confirming the results of in vitro tests. By molecular biological identification based on comparative sequence analysis of the 18S rDNA gene, the HXMG-1 strain was identified as *Hanseniaspora uvarum*. Through the creation of a phylogenetic tree, HXMG-1 was recognised as a member of the Ascomycota, Hemiascomycota, Yeasts, and *Hanseniaspora* sp. families. In conclusion, the yeast strain HXMG-1 created VOCs that significantly inhibited the development of *Botrytis cinerea* on grapes and is expected to be further developed and utilised. This study lays the foundation for the use of *Hanseniaspora* sp. for biological control of postharvest disease.

Keywords Biocontrol activities · Yeast · Volatile organic compounds · *Botrytis cinerea*

Introduction

Postharvest losses are one of the major problems in grape production, in terms of quality and quantity (Nandhini et al. 2021; Senthil 2011). A 30% cost loss and 39% yield loss in grapes have been recorded as a result of postharvest pathogen infection (Senthil et al. 2011). Grey mould is

one of the most harmful postharvest diseases affecting grapes, caused by *Botrytis cinerea*. The primary strategy for preventing postharvest diseases is currently the use of synthetic fungicides. However, fungicide resistance is a widespread problem with *B.cinerea*. The discovery of novel, non-toxic bio-antagonists to control postharvest losses of grapes.

The use of new environmentally friendly methods to control postharvest pathogens is at the forefront of various strategies, and the use of biological agents to prevent postharvest diseases would be a potential alternative to synthetic chemicals. Yeasts are found in a wide variety of environments and are considered to be effective antagonists of many plant diseases. Due to their antagonistic ability, minimal culture requirements, and limited biosafety concerns, many of these unicellular fungi have been investigated for biocontrol applications (Freimoser et al.

✉ Na Zhang
wuaidehua@163.com

¹ College of Horticulture and Landscape, Tianjin Agricultural University, Tianjin 300392, China

² Institute of Agricultural Products Preservation and Processing Technology (National Engineering Technology Research Centre for Preservation of Agriculture Product), Tianjin Academy of Agricultural Sciences, Tianjin 300392, China

2019). Many studies around the world have shown that yeast spp. can be used to prevent postharvest diseases (Alvarez et al. 2019; Kasfi et al. 2018; Kheireddine et al. 2021; Perez et al. 2017; Zhang et al. 2012), they have been described as biocontrol yeasts in scientific publication and some of them (*C.oleophila*, *A.pullulans*, *M.fructicola*, *C.albidus*, and *S.cerevisiae*) were registered and marketed as plant protection products (Freimoser et al. 2019). Currently, many studies have revealed the mechanism of different yeasts to control plant diseases (Choinska et al. 2020; González-Gutiérrez et al. 2022; Hu et al. 2017; Sangorrín et al. 2007; Zhao et al. 2021): (I) Competition for nutrients or space (González-Gutiérrez et al. 2022; Rosa et al. 2010), this is considered to be the primary mode of action of biocontrol yeasts; (II) Production of extracellular lytic enzymes that can be components in host–pathogen interactions, such as chitinases, glucanases, lipases, or proteases have been regularly reported and highlighted in antagonistic yeasts and implicated in their biocontrol activity (Bar-Shimon et al. 2004; Chan and Tian 2005; Freimoser et al. 2019; Segal et al. 2002); (III) Tolerant reactive oxygen species has also been reported as one of the biocontrol mechanisms of yeasts (Castoria et al. 2003); (IV) Formation of biofilms, which is considered to be an apacific and highly successful strategy to compete for space (Desai et al. 2014; Fiori et al. 2012; Maserti et al. 2015); (V) Toxin production, which provides a competitive advantage activity against bacteria or fungi under in dry, oligotrophic conditions, whereas it has no effect on antagonistic in more humid environments (Droby et al. 2002; Jones and Dangl 2006); (VI) Induced resistance. Biocontrol yeasts can induce systemic resistance in plants against a wide range of pathogens and this activity is thought to contribute to their biocontrol activity. Antagonistic yeast, as a bioactivator, can induce the expression of proteins related to disease progression in fruits and vegetables and improve disease resistance (Freimoser et al. 2019; Di Francesco et al. 2015). In recent years, most research on yeast-mediated control of fruit decay has focussed on the induction of defence enzymes against fungal pathogens (Bar-Shimon et al. 2004; Chan and Tian 2005; Freimoser et al. 2019; Segal et al. 2002); (VII) Emission of volatile organic compounds (VOCs). The VOCs produced by some biocontrol yeasts significantly inhibited the growth of postharvest pathogens (Di Francesco et al. 2015; Khunnamwong et al. 2020).

VOCs are small molecules (usually less than 300 Da) that don't dissolve well in water and have a high gas pressure (Freimoser et al. 2019). Recent experimental evidence suggests that VOCs from yeast play an important role in pathogen interactions (Fialho et al. 2009; Jaibangyang et al. 2020; Khunnamwong et al. 2020). Two *Aureobasidium pullulans* were evaluated for their ability to induce the production of VOCs for the control of five

common postharvest diseases (Di Francesco et al. 2015). Similarly, the citrus black spot pathogen was suppressed in vitro by VOCs produced by *Saccharomyces cerevisiae* (Fialho et al. 2009). The inhibitory effect of *Sporidiobolus pararoseus* against *B.cinerea* spore germination and mycelial development was mainly due to its inhibition of ochratoxin growth and ochratoxin A synthesis in some yeasts during coffee processing. Hua et al. identified 2-phenylethanol as a major component of *P.anomala*, which produces VOCs that inhibit spore germination, mycelial development, and toxin synthesis. To date, More than 20 different VOCs have been found in various biocontrol yeast strains.

Our previous studies have used different yeasts from different materials were used to control postharvest pathogens. amongst these yeasts, a potentially bioactive yeast called HXMG-1 was found on the surface of the most dominant grape varieties grown in the Tianjin region of China, and biofumigation treatments significantly reduced the incidence of decay in various fruits and vegetables. However, little is known about the ability of the HXMG-1 yeast strain to inhibit postharvest grape rot by producing volatile organic compounds (VOCs). Therefore, the objectives of this research were to determine the biological control activity of VOCs produced by HXMG-1 yeast on grape grey mould through laboratory in vitro experiments and in vivo inoculation, and to determine the taxonomic status of this yeast using molecular biology techniques. All this work will provide the theoretical basis for the further development and exploitation of this strain.

Materials and methods

Yeast

At the Laboratory of Plant Pathology (Tianjin Agriculture University, Tianjin, China), the antagonist yeast HXMG-1 was isolated from the surface of grape fruits for in vitro and in vivo testing. The strain was cultured in YPD medium (prepared with 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 20 g/L agar, 1000 mL H₂O). The strain was subcultured on YPD agar at 4-month intervals and kept at 4 °C.

Fruit

Grapes (Jasmine) were harvested in 2020 from an organic farm in Hangu, north China. Samples were selected for homogeneity of shape, size, ripeness, firmness, colour, and absence of mechanical damage or fungal infection. The

fruits were then quickly transported to the laboratory in plastic boxes to prevent mechanical damage, under ambient temperature and humidity conditions (18 °C and 75%RH).

Pathogens

B.cinerea, used in this study was isolated from decaying grapes collected in Tianjin, China. It was grown on Potato Dextrose Agar (200 g of potato, 20 g of dextrose, 18 g agar per 1000 mL of distilled water), incubated at 25 °C for 10 days.

Preparation of conidial suspension of the *Botrytis cinerea*

The surface of the PDA culture was scraped to obtain *B.cinerea* spore suspension, which was then passed through four layers of sterile cheesecloth to remove any mycelium adhering to the surface. This experimental technique was slightly modified from Zhang's method (Zhang et al. 2012).

Preparation of suspension of yeast

Yeast was cultured in liquid yeast glucose medium (yeast extract 5 g/L, glucose 10 g/L) for 24 h, before being collected, cleaned and resuspended in sterile distilled water. The cell concentration was adjusted to 1×10^8 CFU/mL using a hemocytometer.

Effects of volatile organic compounds on the mycelial development of *Botrytis cinerea*

The effect of VOCs on the mycelial development of *B.cinerea* was investigated using a previous approach with slight modifications (Rouissi et al., 2013). One plate was inoculated with 100 µL of yeast suspension ($OD_{660} = 1.0 \times 10^8$ CFU/mL), whilst the other plate, was inoculated with a disc of pathogen mycelium (5×5 mm²). The two plates were placed "mouth to mouth" and covered with parafilm. After incubation of the plates at 25 °C for 5 days, mycelial growth was detected. Plates inoculated with pathogens only were used as controls. Radial mycelial growth in two randomly selected vertical directions was evaluated to assess the efficacy of HXMG-1 in inhibiting *B.cinerea* mycelial growth. The inhibition rate was determined using the following equation:

$$\text{Inhibition of radial growth (\%)} = \frac{C - T}{C - 0.5}$$

where *T* and *C* respectively, stand for the mean colony diameters of the treated and control samples. The experiment was run twice, and three replicates of each treatment were examined.

The influence of volatile organic compounds on the germination of *Botrytis cinerea* spores

A dual culture method was used to determine the effect of VOCs on *B.cinerea* spore germination. 100 µL of *B.cinerea* spore solution was used to inoculate PDA plates (1×10^6 CFU/mL). YPD plates were coated with 100 µL of yeast suspension at concentrations of 10, 1×10^2 , 1×10^4 , 1×10^6 , 1×10^8 and 1×10^9 CFU/mL respectively. Equivalent volumes of sterile distilled water were used for controls. The two plates were placed "mouth to mouth" and covered with parafilm. After 8 h of incubation at 26 ± 1 °C, the percentage of conidial germination was assessed. The experiment was replicated with three duplicates for each treatment.

Influence of volatile organic compounds on *Botrytis cinerea*'s hyphal morphology

The influence of VOCs on the hyphal morphology of *B.cinerea* was studied using a dual culture technique. Mycelial plugs of *B.cinerea* were placed in the centre of a PDA medium plate, and coverslips were placed diagonally and evenly at a distance of 1 cm from the *B.cinerea* mycelial plugs to form a circle. A YPD medium plate with 100 µL of yeast cell suspension (1×10^8 cells/mL) was inoculated, and the two plates were put "mouth to mouth" and covered with parafilm. Yeast-free plates were used and samples were collected after 48 h of incubation at 25 °C. A scanning electron microscope (SEM) was used to examine the hyphal morphology of *B.cinerea*. Each treatment had three replicates, and the experiment was repeated three times to evaluate the effect of VOCs on *B.cinerea* hyphal morphology.

Influence of volatile organic compounds on the nucleus and septum of *Botrytis cinerea*'s mycelia

The influence of VOCs on the nucleus and septum of *B.cinerea* mycelia was investigated using the same approach as previously (Influence of VOCs on *B.cinerea* hyphal morphology). Mycelia were stained with a solution containing 4',6-diamidino-2-phenylindole (DAPI, 11 µg/mL) or Calcofluor White (CFW, 85 g/mL) for nuclei or septum distribution, respectively, and observed with a fluorescence microscope (Olympus BX-51, Japan). The technique can also detect *B.cinerea* hyphal morphology. Each experiment was performed three times, with three replicates of each treatment.

Effects of volatile organic compounds on artificial infection of grapes

The efficacy of the VOCs in the management of *B.cinerea* on grapes was evaluated using the technique proposed by Di Francesco et al. with minor modifications (Di Francesco et al. 2015). Fruits were cleaned with water and disinfected by immersion in sodium hypochlorite solution (0.1 N) for 1 min. The samples were then rinsed twice with distilled water and dried for 30 min at room temperature in a sterilised dry air box. The pre-prepared grapes were divided into 8 groups, 20 grapes in each group, of which 4 groups were noninjured, and the other 4 groups with injury. The noninjured treatments of grapes were inoculated directly with a disc of pathogen mycelium ($5 \times 5 \text{ mm}^2$) in the equatorial region without any treatment. The injured treatment of grapes were inoculated with discs of pathogen mycelium ($5 \times 5 \text{ mm}^2$) at the injured sites ($2 \times 2 \text{ mm}^2$ wound was made with sterile inoculum needle) in the equatorial region. Then, the treated grapes are placed on sterile grids (with a sterile and breathable membrane) in closed glass desiccators (10 cm \times 18 cm, bottom diameter by top diameter \approx 5 L). Aliquots (100 μL) of the yeast cell suspension (about $2\text{--}8 \times 10^8 \text{ CFU/mL}$) were coated onto 9 cm diameter YPD and cultured at 28 °C for 48 h. The lids of the Petri dishes were removed in the noninjured treatments, and zero (one, two, or three) plates containing yeast cultures were placed in each bottom of the glass dryer labelled Control (Noninjured), Wp1 (Noninjured), Wp2 (Noninjured), Wp3 (Noninjured). In the injured treatments, the lids of the Petri dishes were removed and zero (one, two or three) plates with the yeast cultures were placed in each bottle of a glass dryer labelled Control (Injured), Wp1 (Injured), Wp2 (Injured) and Wp3 (Injured) in turn, which were placed in an incubator for 72 h at 25 °C. Measurement of lesion diameter in two randomly selected vertical directions. A completely randomised design was used for the experiment, with each treatment including three replicates. The experiment was performed three times.

Yeast genomic DNA extraction

The yeast strain HXMG-1 on the culture medium was taken up in a mortar and ground under liquid nitrogen next to the flame of an alcohol lamp. The ground yeast strain was placed in a 1.5 mL centrifuge tube bearing the name of the yeast, 0.6 mL Tris-HCl (pH 8.0) buffer containing Ethylene Diamine Tetraacetic Acid (EDTA) (pH 8.0) was added, and the samples were mixed well by sucking and tapping the pipette head. 250 μL of 10% sodium dodecyl sulphate was added to the tube gently inverted and mixed. 3 μL protease K (20 ng/ μL) was added and mixed gently and water bathed at 37 °C for 1 h. 150 μL 5 mol/L NaCl was added and gently mixed. 150 μL 2% hexadecyl trimethylammonium bromide

(CTAB) was added and mixed gently, followed by a water bath at 65 °C, and centrifugation at 12,000 rpm for 20 min at room temperature. The supernatant was carefully drained into a new 1.5 mL centrifuge tube, isopropyl alcohol was added in the same volume, mixed thoroughly, and placed at room temperature for 30 min. Centrifugation was performed at 12,000 rpm for 30 min at 4 °C. The supernatant was aspirated and the liquid was dried on absorbent paper, 750 μL 70% ethanol was added, the tube wall was gently tapped, the precipitate was suspended and inverted several times, centrifuged at 12,000 RPM for 2 min at 4 °C. Add 30 μL of purified water to each tube to dissolve the precipitate (Rnase was added to the water, and the final concentration was 10 ng/ μL). Gently swirl the tube wall and dissolve overnight at 4 °C.

Yeast identification

Genomic ribosomal DNA (rDNA) sequences isolated from yeast cells were identified by polymerase chain reaction (PCR). PCR for amplification of LSU rDNA, including the D1/D2 domain, 5.8S rDNA, and internal transcribed spacer (ITS) segments, using universal primers ITS1 (5'-TCC GTAGGTGAA CCTGCGG-3') and ITS4 (5'-TC CTCCGC TTATTGATATGC-3'). Reaction system: H₂O 17.8 μL , buffer 3 μL , NTP 2 μL , primer 13 μL , Primer 23 μL DNA template 1 μL , enzyme 0.2 μL , total volume 30 μL . PCR is performed as follows: initial denaturation at 95 °C for 5 min, repeated denaturation at 95 °C for 30 s (annealing temperature, 48–55 °C for 30 s) and extension at 72 °C for 1 min for 35 cycles and 72 °C for 10 min, 12 °C forever. DNA sequencing of these samples was performed by BGI Sequencing. Nucleotide sequences were BLAST searched using the National Centre for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov>). A phylogenetic tree based on the 18S rDNA sequence was constructed using the neighbour-joining algorithm in MEGA 2.1,

Statistical analysis

Data are expressed as mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) was used to test the significance of differences between each treatment and control. A value of $p < 0.05$ was considered statistically significant. A randomised design was used for all studies.

Results

Effects of volatile organic compounds on the development of *B.cinerea* mycelium

Since there was no contact between the yeast strain and the pathogen in our study, the antifungal effect of *B.cinerea* on mycelium development could be attributed to the VOCs produced by yeast HXMG-1. Compared to the control, VOCs produced by yeast HXMG-1 suppressed mycelium development of *B.cinerea*, although with varying efficacy (Table 1, Fig. 1). With the application of a treatment that increased the concentration of VOCs, the mycelial development of *B.cinerea* gradually slowed down. At a concentration of 1×10^9 CFU/mL (98.25% inhibition), the mycelial growth *B.cinerea* was almost completely inhibited. The low inhibitory effect of *B.cinerea* was also found ($43.85\% - 1 \times 10^2$ CFU/mL; $59.95\% - 1 \times 10^4$ CFU/mL). There were significant changes between treatments ($p < 0.05$). *B.cinerea* mycelial growth was found to be more sensitive to VOCs produced by yeast HXMG-1.

Table 1 The inhibition rate on mycelial growth of *B.cinerea* of different treatments of VOCs produced by HXMG-1 strain

Treatments (CFU/ mL)	Inhibition rate (%) [*]
1×10^2	43.85 ± 1.54^a
1×10^4	59.65 ± 1.94^b
1×10^6	71.93 ± 2.54^c
1×10^8	82.46 ± 3.17^d
1×10^9	98.25 ± 1.12^e

^{*}Data are presented as mean \pm standard error(SD), of two independent experiments performed in three replicates. The different letters (a, b, c, d, e) indicate significant differences amongst different concentrations of the VOCs according to Duncan' multiple range tests at $p < 0.05$ level

Effects of volatile organic compounds produced by yeast HXMG-1 on the germination of *Botrytis cinerea* spores

Based on the results of mycelial growth, we found that VOCs had the most pronounced suppression effect on mycelial development. To investigate the effect of VOCs on spore germination, Spore germination of *B.cinerea* was estimated after 12 h at 25 °C. It was found that different VOC treatments inhibited *B.cinerea* spore germination (Table 2). *B.cinerea* spore germination decreased steadily with treatment concentrations and showed significant variation between treatments. The treatment of 1×10^9 CFU/mL treatment had the most significant effect on *B.cinerea* spore germination, with only 20.11% of conidia germinating. The 1×10^8 CFU/mL treatment also had a good effect on *B.cinerea* spore germination (44.67% of germinated conidia).

Table 2 The effect on percentage of germinated conidia of *B.cinerea* of different treatments of VOCs produced by HXMG-1 strain

Treatments (CFU/ mL)	Percentage of germinated conidia (%) [*]
0	99.00 ± 1.00^a
1×10	83.33 ± 2.19^b
1×10^2	75.67 ± 2.17^c
1×10^4	65.00 ± 2.93^d
1×10^6	54.00 ± 2.11^e
1×10^8	44.67 ± 1.84^f
1×10^9	20.11 ± 1.65^g

^{*}Data are presented as mean \pm standard error(SD), of two independent experiments performed in three replicates. The different letters (a, b, c, d, e, f, g) indicate significant differences amongst different concentrations of the VOCs according to Duncan' multiple range tests at $p < 0.05$ level

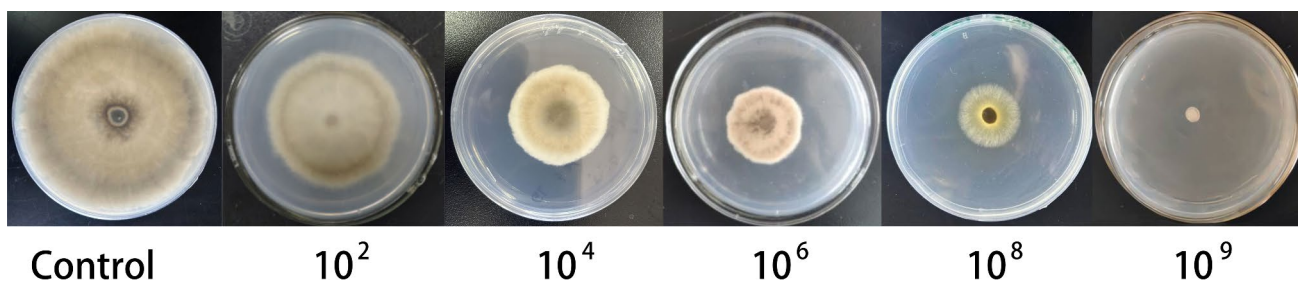


Fig.1 Effect of of VOCs produced by HXMG-1 strain on mycelial growth on *B.cinerea* in the double petri dish assay system

Effect of volatile organic compounds produced by yeast HXMG-1 on hyphae morphology of *B. cinerea*

SEM and fluorescence microscopy examinations were used to examine morphological changes in the hyphae of treated *B. cinerea* to further, investigate the influence of VOCs produced by yeast HXMG-1 on *B. cinerea*. As shown in Fig. 2, untreated control hyphae had normal morphology (Fig. 2a, c and e), whereas treated hyphae had morphological abnormalities with wrinkled and uneven distortions (Fig. 2b, arrows). The average hyphal branching angle reached plateau 82.43° – 100.85° in the VOCs (Fig. 2d, double arrows), whereas the average hyphal branching angle in control was 50.24° – 70.75° (Fig. 2c, double arrows). Microscopic examination showed that the hyphal tissue was twisted and contracted in samples exposed to VOCs produced by yeast HXMG-1 (Fig. 2f, arrow). In conclusion, treatment of yeast strain HXMG-1 can cause abnormal growth and development of the mycelium of *B. cinerea*.

Effects of volatile organic compounds on mycelial nuclei and septum of *B. cinerea*

Figure 3 shows that the mycelial nuclei in *B. cinerea* were densely distributed in control (Fig. 3b), but the number of mycelial nuclei in *B. cinerea* treated with yeast strain HXMG-1 was reduced by about 50% at the same distance compared to the control, and the distribution was not more uniform (Fig. 3a). As shown in Fig. 4, the spacing of the control septa was 33.23 – 69.00 μm , and the spacing of the treated septa was 16.12 – 28.00 μm , indicating that the septum spacing was significantly reduced. It can be concluded that treatment with yeast strain HXMG-1 inhibited nuclear division and mycelial elongation of *B. cinerea*, thereby affecting rapid mycelial reproduction and development of *B. cinerea*.

Effects of yeast strain HXMG-1's volatile organic compounds in vivo on *B. cinerea* on grapes

According to the test (Fig. 5), grey mould on grapes was better controlled by the treatments. The diameter of the lesions decreased significantly in noninjure groups than in injure groups ($p < 0.05$) for the same treatment (Fig. 5a). With increasing VOC concentration, compared to the control, except for the treatment of Wp1 in noninjure groups, the other diameters of lesions decreased significantly ($p < 0.05$) (Fig. 5b). Especially with the treatment of Wp3, the lesion inhibition was inhibited to 55.75% in the noninjure treatment group and 60.48% in the injure treatment group (Fig. 5b). The results of the treatment experiments showed

that the VOCs produced by the yeast strain HXMG-1 were tested and had a good effect on *B. cinerea* on grapes.

Results of the identification of the yeast strain HXMG-1

By molecular biological identification based on comparative sequence analysis of the 18S rDNA gene, the HXMG-1 strain was identified as *Hanseniaspora uvarum*. The 18S rDNA sequence of yeast strain HXMG-1 was submitted to GenBank, and a sequence similarity search was performed by BLAST. Eight strains with high homology were selected, and the phylogenetic tree was constructed by using MEGA 2.1 software. Figure 6 shows that yeast strain HXMG-1 and members of the genus *Hanseniasperu* are on the same branch. The homology was 100%. Therefore, the yeast strain HXMG-1 was identified as Ascomycota, Hemiascomycetes, Saccharomycetales, and the yeast family (Saccharomycetaceae), a member of the genus *Hanseniasperu*.

Discussion

Humans are interested in biological control agents due to concerns about chemical resistance and environmental damage. The use of natural enemies to reduce the number of plant diseases is known as biological control (Chen et al. 2018). Plant protection in the twenty-first century should include biological control as an important component wherever possible. Biological control has been widely used in the process of postharvest disease control of crops (Prendes et al. 2018; Ruiz-Moyano et al. 2016; Tang et al. 2015). The problem of chemical residues can be avoided by using of biological control methods for postharvest fruit diseases, thus eliminating the toxic effect of chemical agents on the human body and improving food safety. The technology of biological control of postharvest fruit and vegetable diseases is being perfected and will eventually replace conventional chemicals. The use of antagonistic yeast in the biological control of postharvest diseases has attracted increasing interest in recent years (Liu et al. 2013; Lutz et al. 2013; Zhang et al. 2019). Currently, research is mainly focussed on the mechanism of biocontrol yeasts, improving the disease control effect of biocontrol yeasts, and using genetic engineering technology to construct new antagonistic yeasts (Cheng et al. 2019; Shao et al. 2019). Yeasts are abundant in nature and widely used in the food industry. Many studies have demonstrated that yeast is safe and non-toxic as a biocontrol bacterium, and no genetic toxicity has been found. These have been used as evidence that it can be used as a biocontrol bacterium

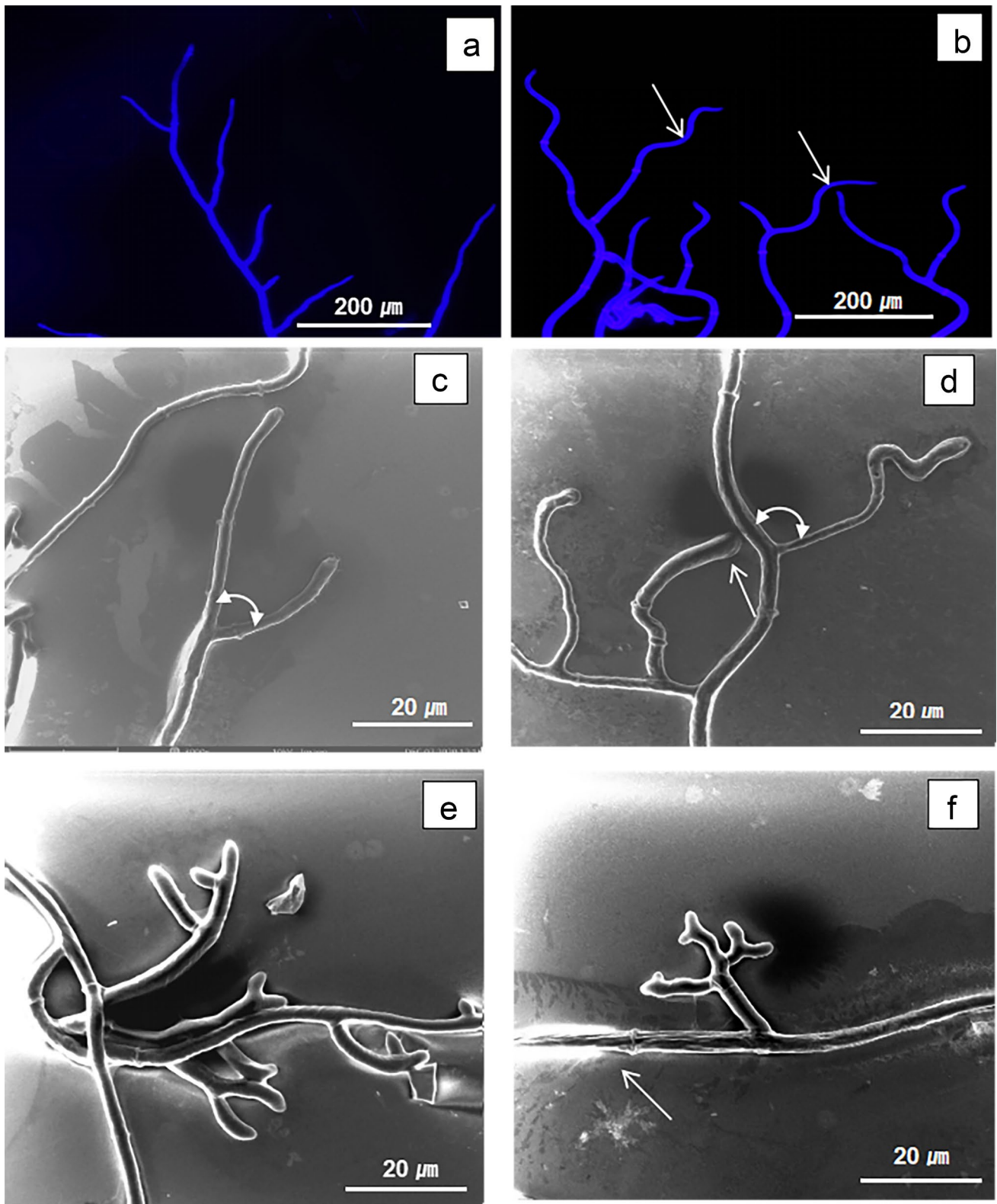


Fig.2 Effect of VOCs produced by HXMG-1 on hyphae morphology of *B.cinerea*

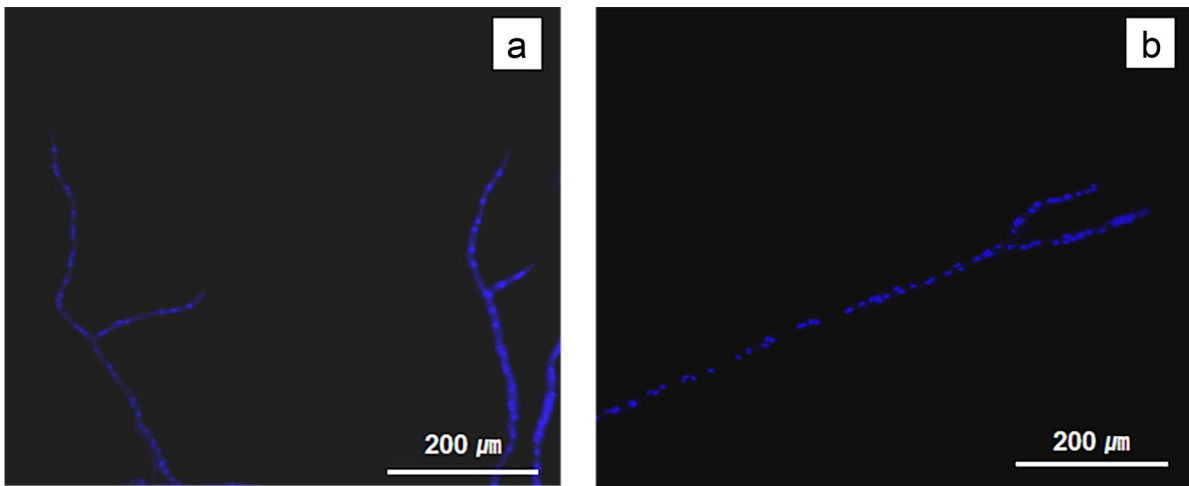


Fig.3 Effects of VOCs produced by HXMG-1 on mycelial nuclei of the *B.cinerea*

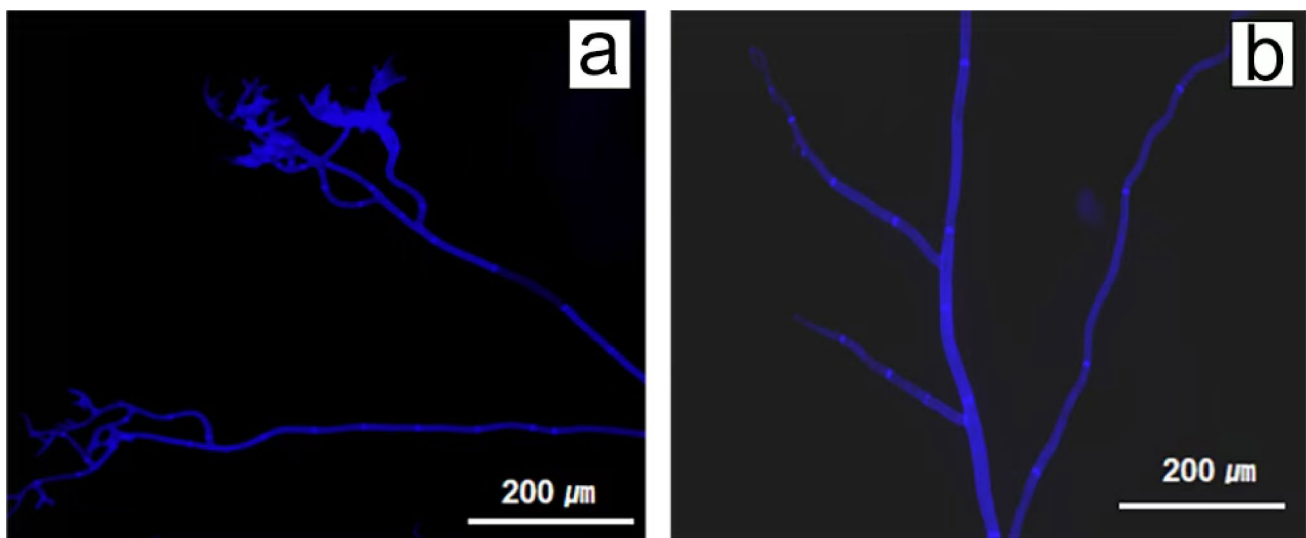


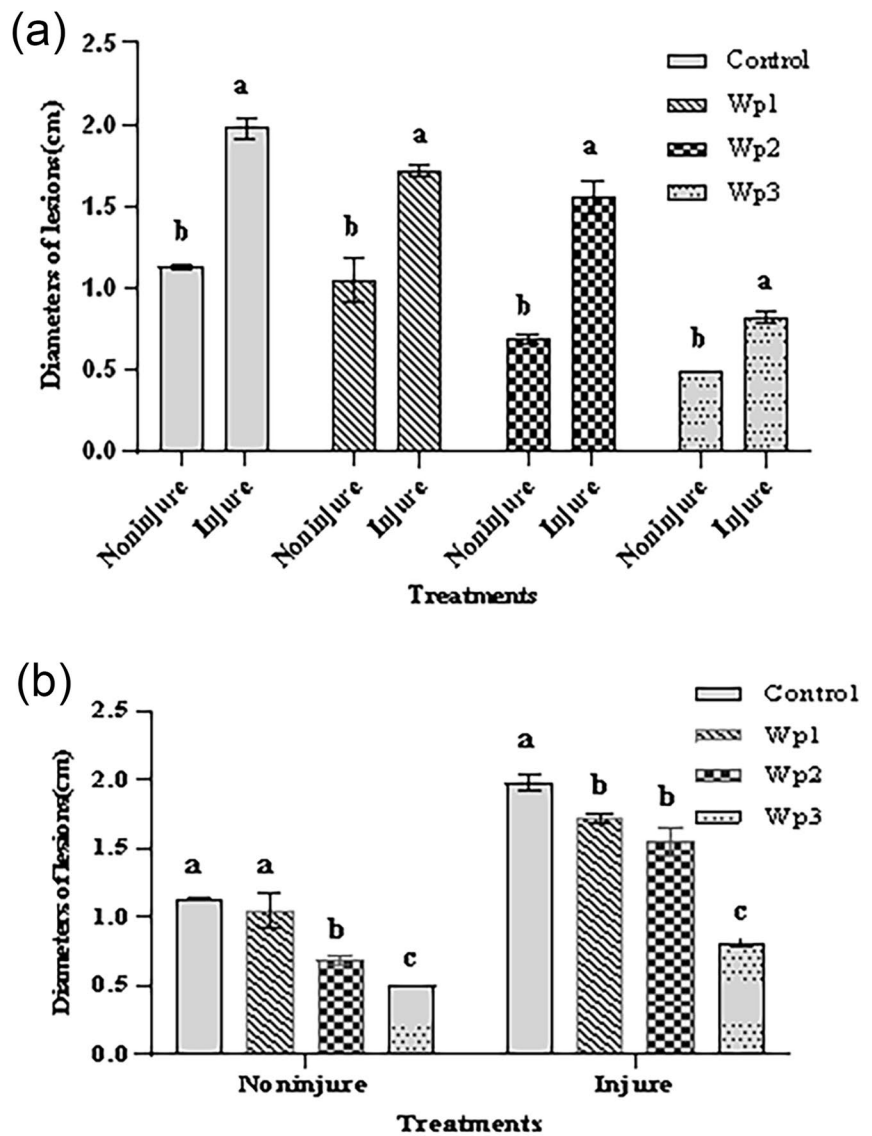
Fig.4 Effects of VOCs produced by HXMG-1 on mycelial septum of the *B.cinerea*

for fruit and vegetable diseases and is safe for the human body. It can also be demonstrated that the yeast is safe to use as a source of biocontrol factor as it was isolated from the surface of the grape fruit for this investigation.

The history of using biocontrol yeasts for biological control of postharvest fruit diseases is relatively short. Currently, more than 10 antagonistic yeasts have been reported, and these yeasts are mainly used to control *penicillium*, green mould, grey mould, etc. during storage. However, there is no published report using *Hanseniasperu* to inhibit *B.cinerea* in grapes. The results of this investigation showed that yeast strain HXMG-1 significantly inhibited *B.cinerea*. The preliminary study on the inhibitory mechanism of VOCs produced by yeast strain HXMG-1 on *B.cinerea*, and other agents needs

further research and confirmation. The effects of VOCs produced by yeast HXMG-1 on *B.cinerea* in grapes were presented in detail. The results of this article indicated that the VOCs were successful in effectively preventing grape grey mould. Numerous recent investigations have shown that yeast-produced VOCs produced by yeast can significantly inhibit the development of several pathogenic microbes (Choinska et al. 2020; Di Francesco et al. 2015; Oro et al. 2018). At the same time, VOCs have potential application value in agriculture, industry and pharmacology due to the relatively small molecular weight of volatiles and their ease of diffusion. However, whether VOCs produced by other yeasts can affect the grey mould of grapes, how yeast-produced VOCs can be effectively applied to the current production practise, and

Fig.5 In vivo antagonistic effect of VOCs produced by HXMG-1on *B.cinerea* in grapes (Diameters of lesions–cm) Fruits were artificially inoculated for 72 h at 25 °C and 80% RH. Control consisted of NYDA without yeast inoculation.each value is the mean \pm standard deviation of three replicates per treatment. Different letters represent significant differences amongst the yeast VOCs concentrations according to one-way analysis of variance(ANOVA).A value of $p < 0.05$



(c)

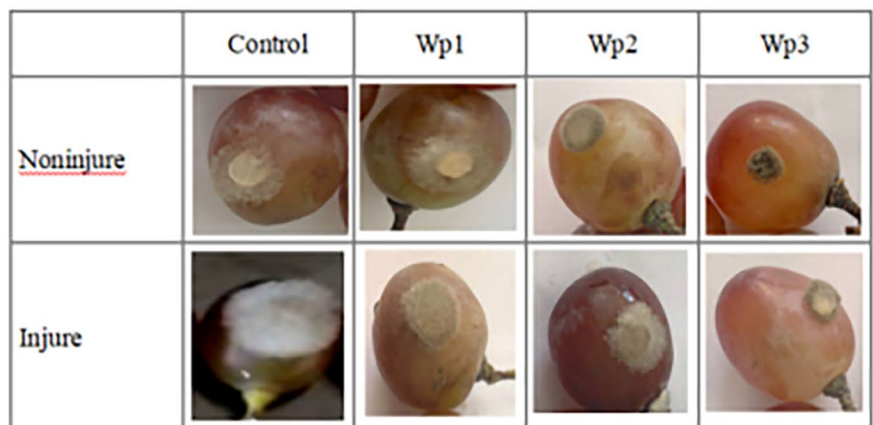
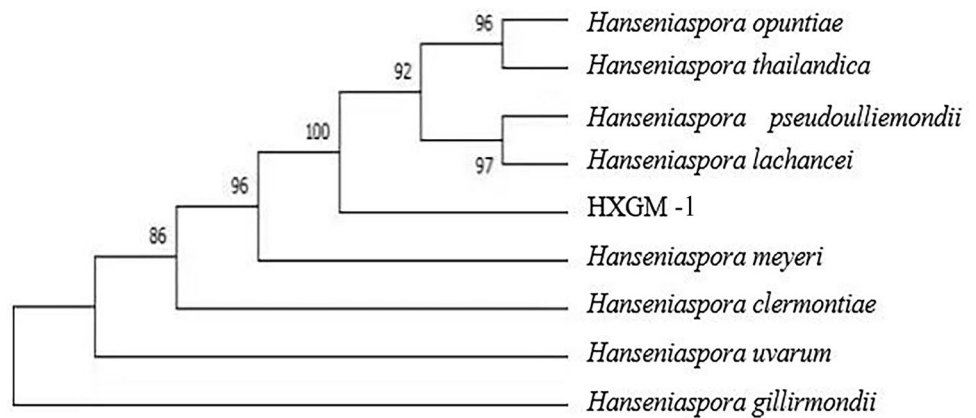


Fig.6 Phylogenetic tree of 18srDNA genes of yeast strain HXMG-1 and its related strains



whether it can also have a good control effect on other fruit and vegetable diseases still need to be further studied and confirmed.

In this study, the effects of VOCs produced by yeast HXMG-1 on *B.cinerea* were investigated in vitro and in vivo experiments without contact between pathogenic bacteria and yeast. The results showed that VOCs by yeast HXMG-1 could significantly inhibit *B.cinerea*. The method rules out antimicrobial activity, nutrient competition, and other modes of action that involve contact. Therefore, this study focuses on the inhibition of volatile compounds produced by yeast on pathogenic bacteria, which may be the primary mode of action of yeast. The study of the active components of volatile compounds of yeast HXMG-1 will be our next important work.

The paper showed that VOCs produced by yeast HXMG-1 could strongly limit *B.cinerea* mycelium development at an inhibition rate of 82.46% with a concentration of 1×10^8 CFU/mL. It caused mycelium bending growth, mycelium tip enlargement, nucleus reduction, and septum spacing increase. According to a study, the volatile chemical produced by *Saccharomyces cerevisiae* 0939–5 prevented the development of grape wilt by 77.67% at a dosage of 1×10^8 CFU/mL, which can also cause the mycelia of *B.cinerea* to appear distorted and deformed, but the distance between the septa increases. These results showed that VOCs generated by yeast HXMG-1 could suppress *B.cinerea*. This research lays the groundwork for future research and applications of VOCs produced by yeast HXMG-1 in control.

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Declarations

Conflict of interest The authors state that they have no conflicts of interest. Each of the study's authors is aware of and consents to the submission of this report.

Ethical approval The authors earnestly guarantee that the study is honest.

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