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RESEARCH ARTICLE
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Biocontrol Activity of *Metschnikowia pulcherrima* Strains Isolated from Local Varieties of Apples in Kazakhstan

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ABSTRACT

Wild yeasts, as part of the natural microbiome of fruits and vegetables, are promising candidates for applications as biocontrol agents due to their biological activities, low demand for nutrient sources, and broad spectrum of antifungal activity. In the present research, 27 yeast strains were isolated from the carposphere of apples and pears stored in a private gardening farm in Southeastern Kazakhstan at the end of the cold storage period. Various in-vitro plate tests showed high inhibitory activity against *Penicillium expansum, Alternaria alternata* and *Acremonium alternatum* in eight strains, defined by ITS region sequence as *Metschnikowia pulcherrima*. Experiments with inoculation of two apple varieties identified the strain MP-03 as the most effective. Spraying the local apple tree varieties "Aport", "Voskhod" and "Talgarskoe" with a lyophilisate solution of the MP-03 strain during flowering and fruiting periods reduced the incidence and severity of scab (*Venturia inaequalis*) in fruit compared to the control. Postharvest treatment of apples of three cultivars resulted in an increase in the yield of healthy fruits at the end of the 120-day storage period in comparison to the control groups. In addition, the firmness and weight retention indices also showed better results in treated fruits.

Keywords: Post-harvest spoilage; Fungicidal activity; Microbiome; Storage

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INTRODUCTION

Abundant harvest serves as the basis for the security and proper function of food chains of different levels. But prominent problem arises in the amount of general post-harvest loss that can reach up to 30% of the crop yield (Lemanceau et al., 2017). There are many reasons for this, one of the main being pathogenic fungi that causes post-harvest spoilage (Arras & Arru, 1997; Dukare et al., 2018). The use of chemical fungicides can address this issue, but the potential harm from their use can lead to unpredictable consequences for both the environment and consumer health (Spadaro & Droby, 2016; Zhang et al., 2017; Carmona-Hernandez et al., 2019). Current research on the fruit microbiome has expanded our knowledge of the post-harvest pathology and provided valuable information necessary for the development of safe and effective biocontrol systems based on antagonistic bacteria (Berg et al., 2014; Tang et al., 2015; Lugtenberg et al., 2017). Yeasts perform as strong antagonists with flexible metabolism that do not require special nutrient sources for their growth and reproduction and are resistant to chemical treatment agents (Freimoser et al., 2019; Wisniewski & Droby, 2019). Additionally, yeasts themselves do not form spores but are capable of forming colonies and biofilms under low humidity conditions (Pawlikowska et al., 2019). With those factors in consideration, native yeast strains are regarded as well-suited for creating biocontrol agents for specific plant species (Pawlikowska et al., 2019; Turkel et al., 2014).

European Food Safety Authority (EFSA) has officially recognized some yeast strains of *Metschnikowia* as plant protection agents against fungal diseases (Palou et al., 2016). One of them is *Metschnikowia pulcherrima,* which belongs to the Saccharomycetales order and can be isolated from different phyllosphere samples worldwide (Liu et al., 2018). Pre-harvest treatment using these yeasts is becoming

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increasingly popular as they successfully colonize the surface of fruits, preventing pathogen proliferation (Marian & Shimizu, 2019). In several studies by (Janisiewicz et al. 2008, Janisiewicz & Conway 2010), the antimicrobial activity of *Metchnikowia pulcherrima* T5-A2 and *Cryptococcus laurentii* ST-4-E14 strains (isolated from apples) was shown to inhibit the growth of mold and rot pathogens *Colletotrichum acutatum* and *Penicillium expansum* during apple storage. Significant results were achieved when the crop was treated with a biopreparation combined with the influence of physical factors (exposure to warm air) and sodium bicarbonate solution. Tian et al. (2018) demonstrated that *Metschnikowia pulcherrima* maintains the high quality of mango fruits during storage. This yeast prevents skin discoloration, preserves fruit firmness and the overall content of soluble solids, acids, and vitamin C, and inhibits the growth of the pathogen *Colletotrichum gloeosporioides*. In the works of (Droby & El-Gerberia, 2006; Droby et al., 2016; Biasi et al., 2021), pre-harvest treatment with a *Metschnikowia fructicola*-based preparation demonstrated changes in the apple microbiome composition which lead to the formation of several potentially beneficial bacterial taxa associated with *M. fructicola*. The nature of the apparent antifungal activity of *Metschnikowia pulcherrima* and *M. fructicola* strains is currently under intensive study. Until recently, it was believed that the primary antagonistic property of these yeasts was provided by pulcherrimin, a red iron chelate formed during the interaction of pulcherriminic acid and iron ions (Sipiczki, 2020). This process leads to iron deficiency in the environment of the crop, which negatively affects the growth of pathogenic fungi *B. cinerea*, *A. alternata*, and *P. expansum* (Pawlikowska et al., 2020). Thus, pulcherrimin plays an important part in establishing the role of microorganisms at the ecosystem level, controlling the pathogen growth and biofilm formation. However, recent studies have shown that there are also other non-iron-related mechanisms responsible for yeast antagonism. Millan et al. (2022) identified and examined new extracellular antifungal compounds of two *M. pulcherrima* strains. The first compound is 3-amino-5-methylhexanoic acid, which demonstrated high levels of protection against *B. cinerea* and *A. alternata* in vivo infection models of tomato, apple, and grape. Biphenyl-2,3-diol is the other metabolite that showed very high antifungal activity. This metabolite was the strongest inhibitor of in vitro mycelial growth. Sinapaldehyde is the third compound that showed significant in vitro and in vivo protection against *B. cinerea*, although it was only found in culture filtrates from the MP-03 strain. Moreover, researchers Büyüksırıt and Kuleaşan (2022) managed to isolate and characterize a killer toxin from the *Metschnikowia pulcherrima* strain, which inhibits the growth of the pathogenic microorganism *M. luteus*. These findings suggest that yeasts of the *Metschnikowia* genus are excellent candidates for use as biocontrol agents, especially in crops like apple (Abdullabekova et al., 2024).

Kazakhstan, specifically the foothills of the Alatau Mountains, is the homeland of the wild apple (*Malus sieversii*), which became the foundational material for the development and development of modern apple varieties worldwide. The foothills of the Zailiysky, Zhetysu (Dzungarian), and Talas Alatau continue to serve as the primary zones for industrial horticulture in Kazakhstan (Panyushkina et al., 2017). From 1940 to 1990, Kazakh breeders developed a number of local apple varieties characterized by excellent taste quality, bright aroma, winter hardiness, and high yield, extremely important in the climatic conditions of Southern Kazakhstan. Among those varieties are: Zarya Alatau, Voskhod, Talgarskoe, Maksat, Nazym, Zailiyskoe, Kazakh Jubilee, Ainur, Aport, etc. (Yefremova et al., 2023). After a decade of stagnation following the collapse of the Soviet Union, government measures began to support scientific research in horticulture sphere and attemps to restore Kazakhstan's former volumes of crop production. Among those efforts special attention is given to the revival and preservation of native apple varieties (Ministry of National Economy of the Republic of Kazakhstan, 2020).

However, the owners of horticultural farms in Kazakhstan now face another problem: the uncontrollable distribution of new diseases and pests that cause significant damage to perennial plants, reducing their yield by up to 35% in some cases. The main reason for this situation is suspected to be the copious import of infected planting material from abroad. Currently, the pathogens of such diseases as apple and pear scab (*Venturia inaequalis Wint*, *V. pirina*), powdery mildew (*Podosphaera leucotricha*), fruit rot (*Monilia fructigena*), phyllosticta leaf spot (*Phyllosticta mali*), rust (*Gymnosporangium tremelloides*), cytospora canker (*Cytospora spp*.), European canker (*Nectria galligena*), phytophthora root rot (*Phytophthora cactorum*), fusarium wilt (*Fusarium spp*.), fire blight (*Erwinia amylovora*), bacterial bark necrosis (*Pseudomonas syringae*), etc., are widespread in Kazakh gardens. Farms engaged in "intensive horticulture" are particularly affected (Isina et al., 2024). Because of this widespread problem, Kazakhstan now needs to develop and implement safe and effective methods to reduce crop losses and protect plants. In connection to that, this study is aimed at the development of biocontrol agents based on local Kazakh yeast strains that will be viable for the protection of the fruits of local apple varieties against disease pathogens.

MATERIALS & METHODS

The study was conducted over the course of two years, from 2022 to 2024, when the treated fruits were last examined. Facilities used in research were located in Southeast Kazakhstan, Almaty region. To determine the antifungal properties of yeasts isolated from local apple varieties, researchers performed in vitro and in vivo testing, employing a multitude of standard practices.

Fruits

Several cultivars of apple ("Aport", "Grushovka Vernenskaya", "Zarya Alatau") and pear ("Beauty of Talgar", "Aromatic") grown in commercial fruit farming in Essyk of Almaty region (latitude: 43°21′18″ N, longitude: 77°27′08″ E, altitude: 1029 m) in Southeast Kazakhstan were taken for microorganism isolation at the end of the storage period in February of 2022. Five fruits were taken for each of the five varieties, 25 fruits in total. In vivo assay was

performed on apples of the Golden Delicious and Red Delicious varieties, produced in Poland and purchased from grocery stores in Astana. Field trials were conducted during the spring-autumn period of 2023 in the pomological garden and storage facilities of LLP "KazF&VRI" in Talgar, Almaty region, southeastern Kazakhstan, on apple trees of the "Aport", "Voskhod", "Talgarskoe" varieties, and their fruits.

Local Cultivars of Apples

"Aport": was introduced to Southeast Kazakhstan in the mid-19th century, first coming from Russia (Voronezh region). It is well adapted to the winter conditions of Southeast Kazakhstan but is not resistant to diseases. The best results in fruit production are achieved when the plant of this variety is grafted onto the wild apple *Malus sieversii* rootstock. The flesh of the produced fruits is tender, juicy, with an excellent sweet-sour taste and strong aroma. The fruits ripen in early September and can be stored until February-March.

"Grushovka Vernenskaya": a local folk selection variety found in the gardens of Almaty region. The variety is winter-hardy and moderately resistant to common diseases. The flesh is dense, juicy, with a sweet-sour taste and aroma. The fruits ripen in mid-September and can be stored until March-April.

"Zarya Alatau" ("Alatau dawn"): a variety selected by "Kazakh Fruit and Vegetable Research Institute". It is winter-hardy, with fruits ripening in late September and storing until May. It is moderately resistant to diseases. The fruit flesh is dense, creamy, with a sweet-sour taste and a distinctive aroma.

"Voskhod" ("Sunrise"): a variety selected by "Kazakh Fruit and Vegetable Research Institute". It is well adapted to the winter conditions of Almaty region and moderately resistant to diseases. This variety is also commonly grafted onto the wild apple *Malus sieversii* rootstock. The yield is high. The fruits are large, light-yellow with juicy flesh, having an excellent sweet-sour taste and pleasant aroma.

"Talgarskoe": a variety selected by "Kazakh Fruit and Vegetable Research Institute". It is winter-hardy and resistant to common diseases. The fruits are large, greenish with a burgundy blush. The flesh is creamy, juicy, dense, with a sweet-sour taste and moderate aroma. The yield is high, with fruits ripening in late September and storing until May (Yefremova et al., 2023).

Pear

"Beauty of Talgar": A variety selected by "Kazakh Fruit and Vegetable Research Institute" obtained from seeds of the "Forest Beauty" variety through open pollination. It is well adapted to the winter conditions of Southeast Kazakhstan, resistant to powdery mildew and scab. The flesh is creamy, crunchy, juicy, and sweet. The fruits ripen in mid-September and store until the end of February.

"Aromatic": A variety selected by "Kazakh Fruit and Vegetable Research Institute". It is well adapted to the winter foothill conditions of Southeast Kazakhstan. The yield is high, resistant to major diseases, ripening in late September and storing until the end of February (Yefremova et al., 2023).

Isolation of Yeasts

Yeasts were isolated from the peel surface of apple and pear. 5mM in diameter peel samples were taken using sterile pipette tips. Ten peel samples from each fruit were then placed into tubes with 10 mL of sterile distilled water. After shaking for 5-7mins, the mixtures were serially diluted to reduce the cell concentration. Then 0.1 mL of each dilution was plated on nutrient Yeast Dextrose Agar (Sigma Aldrich) and Sabouraud Dextrose Agar (HiMedia, India) and incubated at 28±2°C for 48 hours. Re-cultivation was used until pure cultures of yeast were obtained.

Screening of Antagonist Yeast

Pathogenic molds *P. expansum*, *Alternaria alternata*, and *Acremonium alternata* were isolated from decayed fruits from the same storage of commercial fruit farming in Essik. To detect surface contamination, apples were stored in closed boxes at 24±1°C and 95% relative humidity for 16 days. These unfavorable storage conditions were specifically created to increase the intensity of mold growth on the apple surface. Molds were isolated from infected apple and pear fruits and subsequently cultivated on Czapek medium at 27°C for 14 days. PCR (Polymerase Chain Reaction) was performed with primers ITS 5 5′–ggaagtaaaagtcgtaacaagg–3′ and ITS 4 5′–tcctccgcttattgatatgc–3′ in a total volume of 25μL. The PCR mixture contained 15ng of DNA, 1 unit of Taq DNA Polymerase (Fermentas), 0.2mM of each dNTP, 10x KCl buffer (Fermentas), 2.5mM MgCl2, and 10pmol of each primer. The PCR amplification program included an initial denaturation at 95°C for 6min; 35 cycles of 94°C for 30s, 52°C for 30s, and 72°C for 1min; and a final elongation at 72°C for 9min. The PCR program was performed using a Simpli Amp Thermal Cycler (Applied Biosystems). The sequencing reaction was carried out using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions, followed by fragment separation on an automated genetic analyzer 3730xl DNA Analyzer (Applied Biosystems) (Berdimuratova et al., 2020). For screening fungicide activity both quality and quantity methods were used.

Study of Fungicidal Activity of Yeast

Preliminary in vitro screening was conducted for all yeast isolates to evaluate their antagonistic capabilities against three pathogenic molds *P. expansum*, *A. alternata*, and *Acremonium alternata* according to the agar method described by Hassan et al. (2021), with minor modifications. Phytopathogenic fungi were streaked onto plates with appropriate agar medium, and then agar blocks cut from the lawn of the tested yeast strain were placed equidistantly on the agar surface. The prepared Petri dishes were incubated at 28°C, optimal for the test culture. The growth inhibition of phytopathogenic microorganisms by the tested yeast strains was monitored after 7 days.

Dual Culture Assay

For assessment of antagonistic activity, the yeast isolates were cultured with the pathogen mold on potato dextrose agar (PDA) medium. Two-day-old yeast culture

was streaked on the same media as mold. A 6mM mycelial plug was taken from a 10-day-old *P. expansum*, *A. alternata*, and *Acremonium alternata* cultures and placed at the center of the plate. Mold placed on agar without the yeast served as the control. The plates were then incubated at room temperature (28±2°C) for seven days.

Percent inhibition of radial growth (PIRG) was recorded based on the following formula (Sariah, 1994):

$$PIRG = (R1 - R2)/ R1 \times 100,$$

In which R1 = Radial growth of *P. expansum* or *A. alternata* in the control plate; and R2 = Radial growth of *P. expansum* or *A. alternata* cultivated with potential antagonistic yeast.

Only isolates with PIRG >60% were selected to the next experiment. This study was done in triplicates for each treatment.

Identification of Yeasts

Genomic DNA extraction, PCR (Polymerase Chain Reaction), and sequencing methods were applied for the determination of taxonomic positions of 8 selected yeast strains. Sequences of the ITS1-5.8 s rDNA-ITS2 regions were amplified by ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′- TCC TCC GCT TAT TGA TAT GC-3′) primers. The PCR fragments were purified and sequenced using the same primers. The taxonomic positions of the strains were accepted when ≥99.6% identity was found in the BLAST analysis to sequences of the type strains (Turkel & Ener, 2009; Berdimuratova et al., 2020). MEGA11 software package was used for phylogenetic analysis of the obtained results.

Fruit Wounding and Inoculation

Healthy apple fruits of Golden Delicious and Red Delicious varieties were washed before being soaked in sodium hypochlorite 0.5% for 5 min, followed by soaking in sterile distilled water for 1 min. After being air dried, the fruits were surface sterilized with 70% (v/v) ethanol. After that, apples were wounded using a sterile cylindrical wounding tool (4mM deep x 4mM dia.) and the tissue plugs were removed. All fruit were wounded once per fruit at the mid-point between calyx and stem end. Then 0.02mL of antagonistic yeast suspension (5×108 cells/mL) was applied to the wounds. After 2 hours, 25µL of the 104 or 105 conidia/mL suspension harvested from 9-10-day-old cultures of *P. expansum* was placed on the wounds. For the control treatment, only *P. expansum* or yeast culture was inoculated onto the wounds. After inoculation, boxes were covered with lids and stored at room temperature (24±2°C) for 10 days. The severity (lesion expansion) of blue mold decay was determined daily beginning 4 days post-inoculation until the end of the experiment. There were three replications of 5 fruit for each treatment and the experiment was repeated. Disease development was recorded after ten days by measuring the diameter of the lesions formed. Calculations of Disease Reduction Over Control Percentage (DRCP) were performed after 10 days of storage using the following formula:

Disease reduction over control percentage (%) = (Lesion diameter of control fruit (cm) − Lesion diameter of treated fruit (cm)/ Lesion diameter of control) × 100 (Hassan et al., 2021).

Field Trials

Dry Formulation of *M. pulcherrima* MP-03

The dry *M. pulcherrima* formulation was produced by LLP "National Center for Biotechnology" (Astana, Kazakhstan). Cultures were grown in Erlenmeyer flasks on a shaker-incubator KS 4000 control (IKA, Germany). Precultures were grown in 100 mL Erlenmeyer flasks (26–27°C, 200 rpm). Fermentation was performed in a BioTech 500 (Shanghai, China). The culture medium consisted of 10% sucrose, magnesium, iron, copper, manganese, molybdenum, zinc, and 0.4% yeast extract. Rapeseed oil was used as an anti-foaming agent. After a run time of 48 hours, *M. pulcherrima* cells were harvested by centrifugation (20 min, 8°C, 4000 rpm) using a continuous flow centrifuge Fuji Separators WGQ-75 (Nanjing, China). The resulting yeast cell suspension was mixed with 10% skim milk powder and 10% sucrose. The formulation was then frozen at −20°C and freeze-dried. Lyophilization of microbial mass was performed in BETA 2-8 LDplus (Christ, Germany). The dried cells were powdered and quality checked. The number of viable cells was determined at all stages of production. The dry product was stored at 4°C (Bühlmann et al., 2021).

Treatment

Field trials were held in the pomological garden of the "Kazakh Scientific Research Institute of Fruit and Vegetable Growing" in the Talgar district of Almaty region (Southeast Kazakhstan, 43°18′0″N 77°14′0″E). The soil of the experimental plot is dark chestnut, with a loamy texture, humus content of 1.4-2.0%, and pH equaling to 7.64-7.55. The altitude of the orchard is 1000 m above sea level, and about 490mM of precipitation falls annually. 8-year-old apple trees of the cultivar *Malus pumila* local var. Talgarskoe, Voskhod, Aport were used for all experiments within this study. Each treatment was carried out in four replicates comprised of 5 trees of each variety. The replicates were randomly distributed in the orchard. Treatments were compared to an untreated control (C). *M. pulcherrima* was applied in concentrations of approximately 10^9-10 CFU/L. Biopreparation treatment was conducted by spraying a working solution of the biopreparation Bioconservant MP-03 during flowering in April. The treatment was carried out using a MIURA backpack sprayer with a capacity of 12 liters. The working fluid consumption was 400 L/ha. After treatment, the prevalence and development of scab was monitored three times: 1 – at the time of excess ovary shedding (mid-May), 2 – one month after the first (mid-June), and 3 – at the harvest of ripe fruits. Monitoring of the spread of fruit tree pathogens and their identification was conducted by specialists from the "Laboratory of Plant Quarantine and Protection" of LLP "KazF&VRI".

Incidence and Severity of Apple Scab Quantification

For assessment of parameters such as "incidence" and "severity," each tree was investigated for disease by examining 200 leaves and fruits, with 50 taken from each side (N, E, S, W).

Incidence was expressed as the proportion of infected parts (leaves and fruits) with scab lesions to the total number of investigated parts and was calculated as follows:

$$P=n/Nx\ 100.$$

"Severity" was determined as the degree to which the symptoms of pathogenic infestation are expressed. Disease severity of apple scab on each tree was calculated by the visual method of Croxall et al. (1952), which employs a scale of 0 to 7 for leaves, where 0-1% of scabbed surface equals to 1; ≤ 5% – 2; ≤ 10% – 3; ≤ 25% – 4; ≤ 50% – 5; ≤ 75% – 6; >75% – 7. And on a scale of 0 to 4 for fruits, 0 equals to no visible lesion, 1 – less than 10% of fruit surface infected; 2 – 10-25% of fruit surface infected; 3 – 25-50% of fruit surface infected and 4 – more than 50% of fruit surface infected (apples are skewed and deformed) (Lahlali et al., 2019). Then the scaled results were defined in a formula:

$$S = \sum(n \times v)/ (N \times Z) \times 100.$$

Treatment efficacy (%) was calculated by Abbot's formula (1925) (Lahlali et al., 2019):

$$E = (X-Y)/X \times 100,$$

In which X is the apple scab severity of the control, and Y is the disease severity after treatment.

Harvest and Storage

Apples without visible damage and no signs of disease were harvested at optimal ripeness in mid-September 2023 and stored in regular cold storage chambers of LLP "KazF&VRI". The storage temperature in the cold chamber was maintained at +1…+2°C with a relative humidity of 90-95%. To assess the effect of the biopreparation on apple yield, the fruits were treated by soaking them in the biopreparation for 30s before storage. Apples in the control groups were not treated. Apples of the Talgarskoe, Voskhod, and Aport varieties were sorted by weight and grouped into 150 apples per group, with each group in three replicates. The control groups of apples were not treated. After 30, 60, 80, 100, and 120 days of shelf life, the percentage of infected fruits was scored visually. The percentage of diseased apples was calculated.

Fruit Firmness and Weight Determination

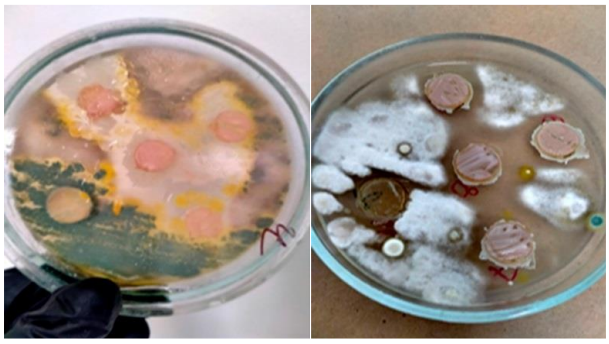
Flesh firmness was measured with a firmness tester – penetrometer. The peel from apples was removed in a strip 2 cm wide on both sides. The plunger of the penetrometer was forced into the apple to a depth of 7.9mM (scribed line on the plunger). The results were given in kilopond, which were converted to N/cm^2. The harder the fruit flesh is, the more force is necessary to penetrate it. Measurements were taken from both sides, and the value of one fruit was calculated as the average of two readings. Fruit weight was measured by individually weighing each fruit on electronic scales. Ten fruits were measured in three replicates for each variant. The average fruit weight was calculated (Lachapelle et al., 2013; Orosz-Tóth & Kincses, 2019).

Statistical Analysis

All experiments were independently repeated three times. Data were represented as the mean±SD and calculated using Statgraphics software. Student's t-test to evaluate the differences of the mean between treated and control groups were performed for all experiments with alpha value at 0,05.

RESULTS

In total, 27 yeast isolates from the surface of apples and pears of different cultivars at the end of the winter storage period were obtained. Initial identification was carried out considering the growth and morphology of the colonies and microscopy. An important factor was the viability assessment of the isolates. Pathogens causing fruit spoilage were also isolated from the fruits stored on this farm. Four isolates of molds differing in morphological characteristics were selected for genetic identification. The ITS gene sequence of 2G and 1L isolates showed 100% similarity with *Penicillium expansum* (GenBank acc #MT582774.1, MT294667.1), the isolate 3T showed 100% similarity with *Acremonium alternatum* (GenBank acc #MT529342.1), and isolate 1G showed ≥99.9% sequence similarity with *Alternaria alternata* (GenBank acc #KY075667.1). Out of 27 yeast isolates, only 8 isolates showed positive inhibitory activity against all three phytopathogenic fungi (Fig. 1). As a result of the genetic identification of the selected 8 yeast isolates, all 8 yeast isolates were identified as *Metschnikowia pulcherrima*. Gene sequence similarity with *Metschnikowia pulcherrima* strains in the NCBI database is shown in Fig. 2.



(1) (2)

Fig. 1: Presence and absence of lysis zones around agar blocks with different yeast strains on lawn mold of *Penicillium expansum* (1) and *Alternaria alternata* (2).

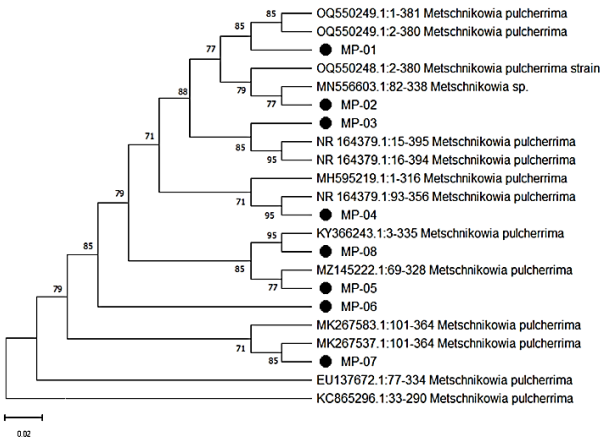


Fig. 2: Phylogenetic tree of the isolated strains MP-01, MP-02, MP-03, MP-04, MP-05, MP-06, MP-07, MP-08, based on the analysis of nucleotide sequences of the ITS1-5.8 s rDNA-ITS2 gene. Tree generated using N-J analysis with 1000 bootstrap simulations. Numbers show bootstrap values.

The obtained values from further assessment of fungicidal activity with consideration of the Percentage Inhibition of Radial Growth (PIRG) against all three pathogens are shown in Table 1. All 8 yeast strains showed different degrees of antagonistic activity (PIRG values) after seven days of incubation. However, only 2 of them, MP-03 and MP-07, had more than 60% inhibitory effects against all pathogenic fungi compared to the control (0%) (Table 1 and Fig. 3). These 2 strains were selected for further studies. As a result of DRCP calculations, the maximum delay in fruit spoilage of both apple varieties (Golden Delicious and Red Delicious) was observed in the group where the wound was pre-treated with strain MP-03. For the Red Delicious variety, this indicator was 53%, and for the Golden Delicious variety, it was almost 65% (Fig. 4 and 5).

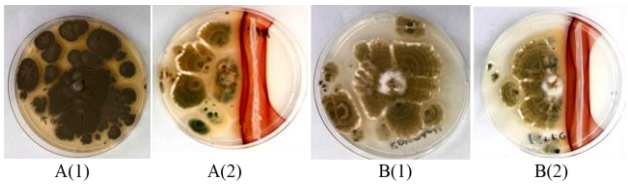


Fig. 3: Reduction in radial growth of pathogenic fungi *Penicillium expansum* A(2) and *Alternaria alternata* B(2) compared to the control A(1) and B(1) when co-cultivated with the yeast strain *M. pulcherrima* MP-03 (the medium is colored red due to yeast's pulcherimin).

Fig. 4: Radial growth of *P. expansum* in apple (var. Red Delicious) wounds treated with antagonist yeast after 10 days of inoculation; (a) apple wound treated only with MP-03 yeast, (b) apple wound with yeast MP-03 treatment and pathogen, (c) apple wound treated only with pathogen.

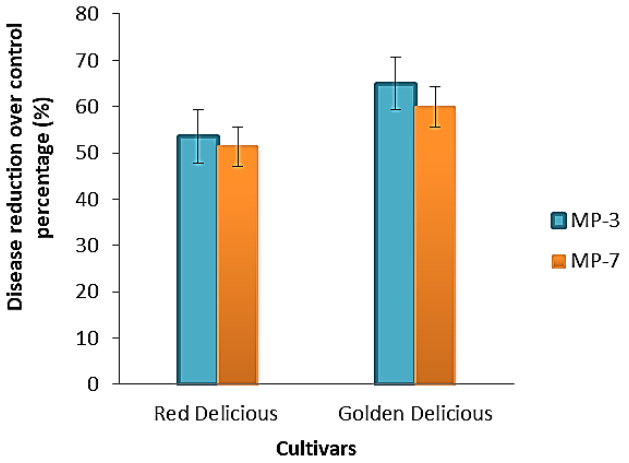


Fig. 5: Comparison of disease reduction over control percentage (%) by treatment with MP-03 and MP-07 of apple wounds var. Red Delicious and Golden Delicious.

Since the DRCP values were higher for strain MP-03 on both apple varieties, it was chosen as the basis for producing a dry formulation. Incidence and severity of apple scab on fruits and leaves of different cultivars of *Malus domestica* in untreated trees in the control groups are shown in Table 2. Among the three cultivars in control groups, the "Talgarskoe" cultivar was the most susceptible to apple scab, with an incidence of scab in leaves of 4.65% recorded in the first examination and 8.9% in the third examination. On fruits, this index was lower: 2.9% in the first examination and 6.05% in the third examination. The other cultivars had lower rates, but they also increased by the time of the ripe fruit harvest. Severity values of scab on leaves and fruits were higher in groups of the "Talgarskoe" cultivar: on leaves, it was 2.32% in the first examination and 4.45% in the third examination. On fruits, severity values were 1.45 and 3.02%, respectively. In the experimental groups of trees treated with MP-03, the incidence and severity values in leaves and fruits of all three cultivars were lower and continued to decrease by the third examination (Table 3).

Table 1: Effects of antagonist yeasts *Metchnikowia pulcherrima* on radial growth of *Penicillium expansum*, *Alternaria alternata* and *Acremonium alternatum* in dual culture assay after 7 days incubation at 28±2◦C (P<0.05)

Pathogenic mold	Percentage Inhibition of Radial Growth (PIRG) (Mean±SD%)							
	MP-01	MP-02	MP-03	MP-04	MP-05	MP-06	MP-07	MP-08
Penicillium expansum	43.33±3.82	45.83± 2.89	66.67±3.81	39.2±2.89	47.5±5	49.16±1.44	71.67±1.44	65.83±2.89
Alternaria alternata	65.86± 3.36	54.76±3.36	74.43±0.98	51.8±3.4	52.53±3.4	52.53±3.41	69.56±3.42	54.03 ±2.54
Acremonium alternatum	45.3±3.92	41.83±5.39	64.06±4.45	50.36±1.44	62.36±6.44	42.7±3.96	64.1±2.6	39.23±3.91

Table 2: Incidence (I) and severity (S) of apple scab of three cultivars of *Malus domestica* in south region of Kazakhstan (Talgar region) in control groups (2023)

Cultivated variety	Leaves (Mean±SD %)						Fruits (Mean±SD %)					
	1st survey		2nd survey		3rd survey		1st survey		2nd survey		3rd survey	
	I	S	I	S	I	S	I	S	I	S	I	S
Voskhod	1.82±0.12	0.9±0.063	2.4±0.216	1.2±0.1	3.2±0.16	1.6±0.08	1.55±0.13	0.7±0.065	2.2±0.13	1.1±0.06	2.8±0.16	1.4±0.08
Talgarskoe	4.65 ±0.38	2.32±0.25	5.97±0.54	2.98±0.27	8.9±1.17	4.45±0.58	2.9±0.39	1.45±0.19	4.17±0.47	2.08±0.23	6.05±0.8	3.02±0.4
Aport	2.73±0.28	1.36±0.14	3.8±0.14	1.9±0.07	4.6±0.16	2.3±0.08	1.95±0.13	0.97±0.06	3.27±0.09	1.64±0.05	4.17±0.17	2.08±0.08

Table 3: Incidence (I) and severity (S) of apple scab of three cultivars in south region of Kazakhstan (Talgar region) after treatment *M. pulcherrima* MP-03

Cultivated variety	Leaves (Mean±SD %)						Biological efficacy	Fruits (Mean±SD %)						Biological efficacy
	1st survey		2nd survey		3rd survey			1st survey		2nd survey		3rd survey		
	I	S	I	S	I	S		I	S	I	S	I	S	
Voskhod	1.87±0.1	0.9±0.048	1.05±0.13	0.56±0.048	0.4±0.08	0.2±0.041	87.5±1.9	1.22±0.17	0.61±0.085	0.87±0.22	0.4±0.11	0.32±0.05	0.16±0.025	88.2±2.46
Talgarskoe	1.9±0.17	0.98±0.11	1.44±0.2	0.71±0.1	1.26±0.18	0.63±0.09	85.8±3.87	1.55±0.2	0.77±0.1	1.17±0.17	0.59±0.08	0.95±0.13	0.47±0.06	83.8±4.1
Aport	2.7±0.18	1.35±0.91	1.53±0.1	0.76±0.04	0.67±0.96	0.33±0.048	85.3±1.9	2.07±0.09	1.03±0.05	1.15±0.13	0.57±0.065	0.72±0.1	0.36±0.05	82.63±1.9

The highest incidence values of scab on leaves after treatment were recorded in the "Aport" cultivar, being 2.7% in the first examination, decreasing to 0.67% by the third examination. Incidence values of scab on fruits were 2.0 and 0.72%, respectively. The severity values of scab on leaves in Aport were 1.35% in the first examination and 0.33% in the last examination. On fruits, the severity values for "Aport" were 1.03 and 0.36%, respectively. According to the obtained data, it can be concluded that treatment with a solution of the dry formulation of *M. pulcherrima* MP-03 allowed to reduce scab disease to varying degrees in apple trees of the three cultivars. The efficacy in all cases exceeded 80%. The maximum efficacy of MP-03 was recorded in the "Voskhod" cultivar, inhibiting scab growth by 88.2% (Table 3).

Inspection of apples on the 120th day after harvest showed that the spoilage of fruits in all groups was associated with the development of *Penicillium expansum*. In the control groups, the yield of healthy fruits in the "Voskhod" variety averaged 45.3%, in the "Talgarskoe" variety – 56.6%, and in the "Aport" variety – 52.3%. The indicators in the *M. pulcherrima* MP-03 treated groups were on average 20% higher: in the "Voskhod" variety, it was 67%, in the "Talgarskoe" variety – 74.3%, and in the "Aport" variety – 73% (Fig. 6). Fig. 7 shows the decrease in flesh firmness values of the three apple varieties during the storage period. The most significant changes were observed in the "Voskhod" variety in the control groups. If the average values were at 7.57 N/cm² immediately after harvesting,

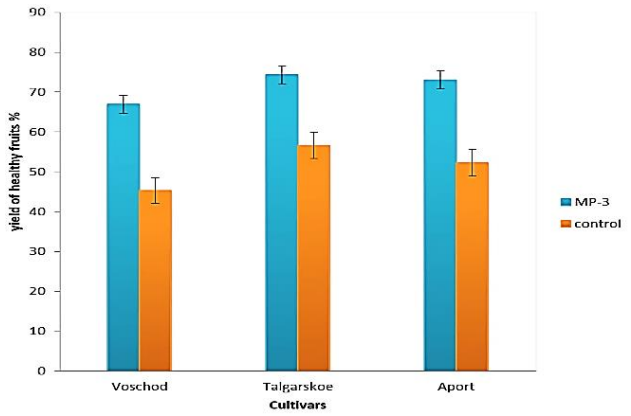


Fig. 6: Ratio of healthy fruits in control and experimental groups (MP-03) of apple varieties "Voskhod", "Talgarskoe", and "Aport" after 120 days of storage (T +1-+2°C) .

they decreased to 3.83N/cm² by the 120th day. In the "Aport" variety, the indicator decreased from 6.77 to 3.77N/cm² during storage in the control groups. For the "Talgarskoe" variety, it decreased from an average of 7.93 to 4.77N/cm². In the groups treated with the *M. pulcherrima* MP-03 solution, the average flesh firmness indicator for all varieties was higher than in the control groups at the end of the storage period. In the "Voskhod" variety, it was 4.27N/cm², in the "Talgarskoe" variety – 5.27N/cm², and in the "Aport" variety – 4.77 N/cm². Thus, treatment with the MP-03 strain solution allows better preservation of flesh firmness compared to untreated fruits. Natural weight loss of fruits also significantly affects fruit quality. Fig. 8 shows the comparison of weight changes in percentage terms in the fruits of the three varieties in control groups and groups treated with the MP-03 solution before storage. The maximum weight loss by the 120th day of storage was observed in the control groups of the "Voskhod" variety fruits, amounting to 40%. In comparison, the weight loss in the "Voskhod" variety fruits treated with the biocontrol agent solution before storage equaled to about 20%. The smallest difference in weight loss compared to the control groups was found in the "Aport" variety: in the control groups, the weight loss averaged 30%, while in the MP-03 treated fruits, the weight loss was 20%. In the "Talgarskoe" variety, the weight loss in the control groups averaged 38%, while in the experimental groups, it averaged 18%.

DISCUSSION

Apples, as part of the daily diet for most people, rank among the top five fresh fruits and vegetables exported globally (Kistechok et al., 2024). The primary issues related to their quality transportation and storage are fungal diseases (pathogenic fungi) that lead to spoilage (Zhang et al., 2020). Over the last 30 years, the number of studies focused on finding and studying new biocontrol agents (BCA) for protecting fruits during storage has consistently increased (Massart et al., 2015). A good biocontrol agent must meet several key requirements: their properties must be stable, they must maintain viability under various, even unfavorable conditions (temperature, humidity), be able to adhere to and colonize the surface of the host plant, suppress a wide range of pathogens, be environmentally friendly, be easy to apply, have a long shelf life, be produced using inexpensive nutrient media, and be unable to produce toxins as secondary metabolites. According to many studies, native yeasts isolated directly from fruit crops fully meet these requirements (Bühlmann et al., 2021; Hassan et al., 2021).

In the study of the carposphere microbiome of several apple varieties during fruit development and storage, Zhimo et al. (2022) revealed significant changes in the microbiome composition, particularly in core taxa members, characteristic of the fruitlet, maturation, and harvest periods. But during storage, the main microbiome groups and their quantitative ratios remained more or less stable. The researchers linked this to the effect of external factors; in orchards, environmental factors constantly change, while in storage, temperature and humidity are maintained at stable levels. They also observed a strict correlation of the microbiome with the host genotype (Zhimo et al., 2022). In present research, the isolation of antagonist yeast strains was carried out at the end of the storage period. It seemed to us that during this period, the carposphere microbiome has a stable structure, with dominant species clearly expressed, providing an opportunity to obtain antagonist strains with high fungicidal activity.

Correlated to that, yeasts with potential biocontrol properties were isolated from the carposphere of whole and healthy-looking local apple cultivars at the end of winter. Pathogenic fungi, *Penicillium expansum*, *Alternaria alternata*, and *Acremonium alternatum*, were isolated from the same farm. These phytopathogenic fungi are the main

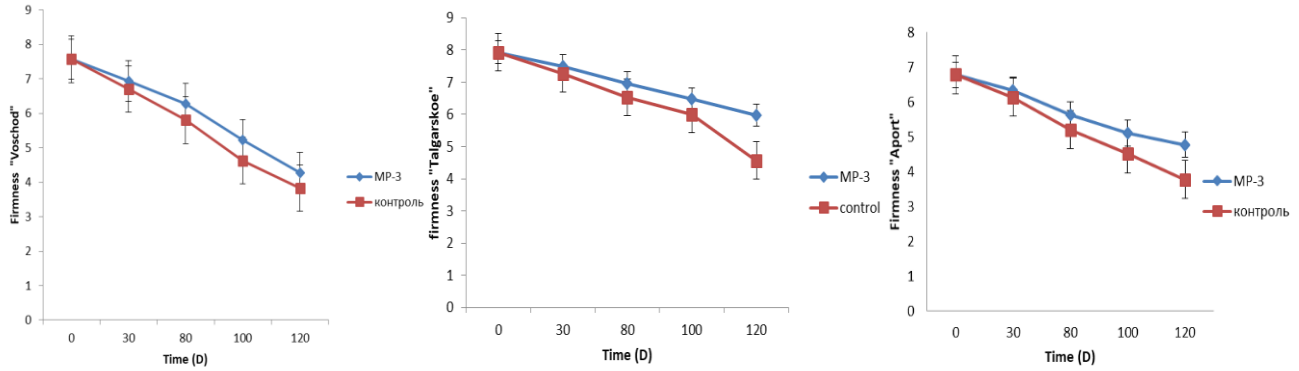


Fig. 7: Decrease in flesh firmness values of three apple varieties "Voskhod", "Talgarskoe", "Aport" during storage period (120 days) in control and MP-03 treated groups.

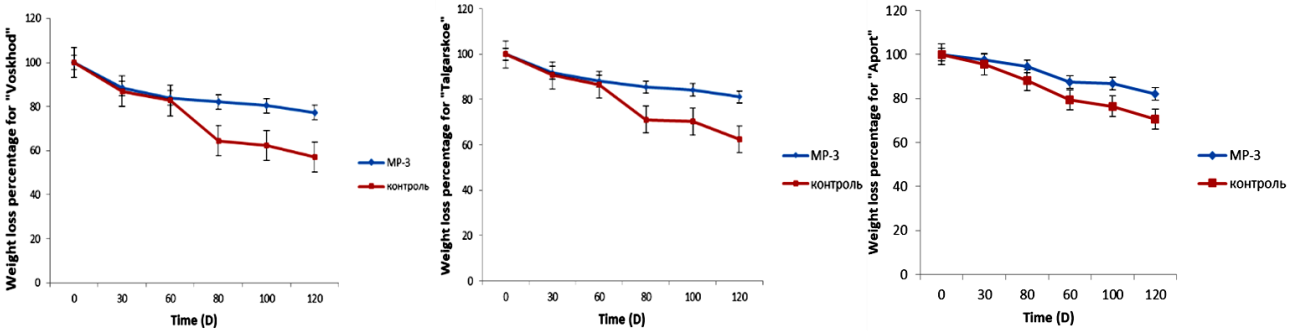


Fig. 8: Comparison of weight loss percentage in the fruits of three apple cultivars "Voskhod", "Talgarskoe", "Aport" in control and MP-03 treated groups during storage period (120 days).

cause of fruit crop spoilage during storage worldwide and pose a potential health risk by producing toxins (Dean et al., 2012; Sanzani et al., 2016). The study of fungicidal activity against the main spoilage pathogens of apples in the storage facilities of Kazakhstan's orchards showed that most yeast strains had either low fungicidal activity or antagonism directed against one or two types of pathogens. In the work of Hassan et al. (2021), only five out of 110 yeast strains isolated from papaya showed high antimicrobial activity against the phytopathogen *Colletotrichum gloeosporioides*. Although they did not study the nature of antagonism, they associated it with different yeasts having different antagonist modes of action. In our study, only 8 out of 27 yeast isolates showed visible zones of inhibition against all three pathogens in the agar block experiment. Genetic identification of the selected 8 isolates showed that they all belong to the species *Metschnikowia pulcherrima*. The experiment calculating the Percentage Inhibition of Radial Growth (PIRG) allowed us to select 2 out of the 8 strains, with PIRG values against all three pathogens being above 50%. These were *M. pulcherrima* MP-03 and *M. pulcherrima* MP-07. However, in artificial infection experiments on two apple varieties, Red and Golden Delicious, the Disease Reduction Over Control Percentage (DRCP) values of strain MP-03 exceeded those of strain MP-07 by a few percentage points in both cases. Therefore, only strain *M. pulcherrima* MP-03 was selected for field trials and the production of a dry formulation.

In most studies, different strains of *M. pulcherrima* and *M. fructicola* showed biological efficacy against postharvest diseases caused by *Botrytis cinerea*, *Penicillium expansum*, *Alternaria sp.*, *Monilia sp.*, *Colletotrichum gloeosporioides* during the storage of apples, mangoes, grapes, berry crops, and some stone fruits (Tian et al., 2018; Freimoser et al., 2019; Zhang et al., 2020; Hassan et al., 2021; Zajc et al., 2022). Bühlmann et al. (2021) studied and proved the biocontrol efficacy of the application of *M. pulcherrima*'s two formulations against the important apple storage pathogen *Neofabraea vagabunda* over several years. However, some yeasts can also be successfully used for preharvest protection of wheat, vegetables, and fruit crops. For example, the effectiveness of using *Aureobasidium pullulans* against *Fusarium*, *Acremonium*, and *Penicillium*; *Cryptococcus sp.* against *Monilia fructicola*; *Saccharomyces sp.* against *Botrytis cinerea*; yeasts of the genus *Rhodotorula* reduced symptoms of potato virus Y, etc. (Amprayn et al., 2012; Wachowska & Głowacka, 2014; Ponsone et al., 2016; Kowalska et al., 2022; He et al., 2024). The dominant pathogen for the cultivars "Aport", "Voskhod" and "Talgarskoe" was *Penicillium expansum* in our research. The high antagonistic activity of *M. pulcherrima* against this pathogen is explained by its ability to destroy the mycotoxin patulin. Some strains of *M. pulcherrima* are able to reduce the patulin content in the environment completely within 120 hours (Settier-Ramírez et al., 2021; Guler, 2024).

In our study, early spraying of trees at the flowering stage with an aqueous solution of the dry formulation based on *M. pulcherrima* MP-03 reduced the severity of apple scab (*Venturia inaequalis*) on three local apple varieties compared to the control. This form of the *M. pulcherrima* MP-03 was chosen because, dry formulations, powders or granules, are often used for yeast biocontrol. However, liquid formulations are simpler to produce, handle and apply, and less likely to generate dust (Marian & Shimizu,

2019). Typically, liquid formulations typically contain a buffer that helps maintain cell viability or even form robust cell types, substances that reduce sedimentation, components that reduce water activity, and additives that improve adhesion or establishment in the field (Chaudhary et al., 2020). More complex formulations include microencapsulation, Pickering emulsions stabilized by insoluble nanoparticles, or microemulsion, a popular technology with beneficial characteristics (such as water solubility, stability, low surface tension, and ultramicroscopic droplet size) for pesticide formulation. Bühlmann et al. (2021) found that the best conditions for maintaining viability in aqueous solution were 5g/L xanthan gum, 20% glycerol and a temperature of 22°C. Five years of field trials using yeast have shown mixed results, but some years have shown a reduction in Neofabreae infestations in stored apples. Application of *M. pulcherrima* after fungicide pretreatment has repeatedly shown an additive effect compared to fungicide treatment alone.

In this study, we investigated the efficacy of *M. pulcherrima* against scab at first time. Scab is associated with significant economic losses for commercial orchards as it negatively affects the quality and appearance of the fruits. Therefore, controlling this disease often involves multiple applications of synthetic fungicides during the growing season, with up to twenty treatments per year in some high-humidity regions, where the climate greatly affects future yield (Lahlali et al., 2019). The abundance of precipitation during the spring-summer period, leading to high humidity, in some years can result in 100% infection of fruit trees with scab. In the spring of 2023, the weather in the region of study was unstable with heavy rainfall during the first half of the growing season. The average air temperature ranged from 19.2 to 21.8°C. In the second half of the growing season, the air temperature was high. The summer was hot and humid, with the average daily temperature in the summer months ranging from 22.8 to 25.5°C. Because of those weather fluctuations, the scab pathogen, *Venturia inaequalis*, formed characteristic lesions and spots on fruits and leaves. Even with low disease development rates, the marketable appearance of the fruits suffered, reducing their price. To control the spread of this disease, synthetic fungicides are usually used (Orosz-Tóth & Kincses, 2019; Bühlmann et al., 2021). With that, several studies have shown a reduction in scab severity when using the following non-synthetic BCAs: *Bacillus spp.*, *Trichoderma spp.*, *Trichoderma viride*, *Pseudomonas syringae* (Doolotkeldieva & Bobusheva, 2017; Jimenez et al., 2018). In our study, the efficacy of *M. pulcherrima* MP-03 against scab compared to the control ranged from 82% in Aport fruits to 88% in Voskhod fruits. It should be noted that scab levels were generally low in the region in 2023. An ideal biocontrol agent is considered to be highly effective (>95%) (Zhang et al., 2020). However, alternating the use of BCAs with synthetic fungicides shows high effectiveness in disease control while significantly reducing the negative impact on the environment.

The proposed BCA treatment reduced weight loss and maintained flesh firmness, which is an important characteristic of fruits in all three varieties at the end of the storage period. The fruit flesh softens due to the activity of pectinesterase and polygalacturonase enzymes, which change the structure of the cell wall (Lachapelle et al., 2013). But because of the effects of *M. pulcherrima* treatment, fruits preserved in good quality. Similar BCA was also previously shown to be effective during the storage of ripe mangoes (Tian et al., 2018).

The decrease in efficiency should be explained by the existing limitations in the use of yeast to improve fruit quality. *Drosophila suzukii* causes significant economic damage to fruit crops worldwide. Laboratory studies showed that four of 12 yeast isolates were attractive to *D. suzukii*, with *Metschnikowia pulcherrima* and *Hanseniaspora uvarum* also showing attractiveness in field trials. Of ten yeast combinations including *Candida zemplinina, Pichia pijperi, M. pulcherrima* and *H. uvarum*, four were attractive in the laboratory. Although the *M. pulcherrima* + *H. uvarum* combination attracted the highest numbers of *D. suzukii* in the field, its efficiency was only slightly higher than that of traps containing *H. uvarum* alone. Although volatile compounds from *M. pulcherrima* and *H. uvarum* isolates may act as baits for *D. suzukii*, further research is needed to understand the mechanisms by which flies are attracted to specific baits and to optimize control methods (Jones et al., 2021). A possible solution to the attractiveness of yeast to insects may be to isolate antibacterial substances from *M. pulcherrima*. These yeast produce toxins that were tested against *Escherichia coli* type I, *Micrococcus luteus*, and *Candida albicans*. The toxin showed inhibitory activity only against *M. luteus*. Optimum conditions for growth and toxin production were 20°C, pH 7 and glycerol as a carbon source. The toxin was partially purified with ethanol and showed inhibitory activity against *M. luteus* (36mM). The molecular weight of the toxin was about 7.4kDa by SDS-PAGE and 10.3kDa by MALDI-TOF mass spectrometry (Büyüksırıt-Bedir & Kuleasan, 2022). Thus, the dry formulation of *M. pulcherrima* increased the resistance of local varieties of Kazakhstani apples to diseases both during growth and in the postharvest period. MP-03 strain of *M. pulcherrima* may potentially become a new bioactive agent in this area. However, to improve the effectiveness of the agent, further research is needed using multi-omics technologies that will shed light on the functioning of the antibacterial defense system at various molecular levels. For instance, advanced techniques have revealed that in the commercially used yeast-like fungus *Aureobasidium pullulans*, biocontrol genes encode secreted hydrolases or are part of secondary metabolite clusters (e.g., NRPS-like, NRPS, T1PKS, terpene and β-lactone clusters) (Rueda-Mejia et al., 2021). A similar approach to studying *M. pulcherrima* MP-03 would allow the strain's shortcomings to be corrected.

Conclusion

During the course of this study, the dry formulation based on a MP-03 strain of *M. pulcherrima*, isolated from the surface of local apple and pear varieties grown in Kazakhstan, showed effectiveness for preserving valuable qualities in pathogen-infected fruits. All experiments, including isolation, laboratory, and field trials, were conducted on different apple varieties, most of which are

local cultivars, but export varieties were also used. On all varieties, *M. pulcherrima* MP-03 maintained its biocontrol activity against major post-harvest spoilage pathogens regardless of environmental conditions. Postharvest application of an aqueous solution of *M. pulcherrima* MP-03 on ripe fruits increased the yield of healthy fruits at the end of the storage period by an average of 20% compared to the control groups. Thus, the fungicidal activity of *M. pulcherrima* MP-03 against *Penicillium expansum* during long-term storage was demonstrated, as this pathogen was the main cause of fruit spoilage. Fruit varieties continue to face new diseases' growth and spread within this decade, as the use of only synthetic fungicides does not provide sustainable protection and does not improve the situation. With the proven effectiveness of proposed BCA, it is recommended to investigate its simultanious use along with existing fungicides. Research on the biocontrol activity of this and new antagonist strains will continue, with plans to study their effectiveness on new varieties and other fruit crops, expanding the range of pathogens targeted by the BCA.

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REFERENCES

Abdullabekova, D.A., Magomedova, E.S., Magomedov, G.G., & Kachalkin, A.V. (2024). Yeasts of the Georgian honeysuckle (Lonicera iberica) and grapes (Vitis vinifera) in Dagestan. *Mycology and Phytopathology*, *58*(2), 108-116.

Acar, E.G., Dikmetas, D.N., Devecioglu, D., Ozer, E.M., Sarikece, H., & Karbancioglu-Guler, F. (2024). Antagonistic Activities of *Metschnikowia pulcherrima* Isolates Against Penicillium expansum on Amasya Apples. *Current Microbiology*, *81*(7), 180. https://doi.org/10.1007/s00284-024-03700-1

Amprayn, K.O., Rose, M.T., Kecskés, M., Pereg, L., Nguyen, H.T., & Kennedy, I.R. (2012). Plant growth promoting characteristics of soil yeast (*Candida tropicalis HY*) and its effectiveness for promoting rice growth. *Applied Soil Ecology, 61*, 295-299. https://doi.org/10.1016/j.apsoil.2011.11.009

Arras, G., & Arru, S. (1997). Mechanisms of action of some microbial antagonists against fungal pathogens. *Annali di Microbiologia Ed Enzimologia, 47*(1), 97- 120.

Berdimuratova, K., Amirgazin, A., Kuibagarov, M., Lutsay, V., Mukanov, K., & Shevtsov, A. (2020). Optimization of PCR purification using silica-coated magnetic beads. *Eurasian Journal of Applied Biotechnology, 1*, 81-89. https://doi.org/10.11134/btp.1.2020.8

Berg, G., Grube, M., Schloter, M., & Smalla, K. (2014). The plant microbiome and its importance for plant and human health. *Frontiers in Microbiology, 5*, 491-497. https://doi.org/10.3389/fmicb.2014.00491

Biasi, A., Zhimo, V.Y., Kumar, A., Abdelfattah, A., Salim, S., Feygenberg, O., Wisniewski, M., & Droby, S. (2021). Changes in the fungal community assembly of apple fruit following postharvest application of the yeast biocontrol agent *Metschnikowia fructicola*. *Horticulturae, 7*, 360-367. https://doi.org/10.3390/horticulturae7100360

Bühlmann, A., Kammerecker, S., Müller, L., Hilber-Bodmer, M., Perren, S., & Freimoser, F.M. (2021). Stability of Dry and Liquid *Metschnikowia pulcherrima* Formulations for Biocontrol Applications against Apple Postharvest Diseases. *Horticulturae*, 7(11), 459. https://doi.org/10.3390/horticulturae7110459

Büyüksırıt-Bedir, T., & Kuleaşan, H. (2022). Purification and characterization of a *Metschnikowia pulcherrima* killer toxin with antagonistic activity against pathogenic microorganisms. *Archives of Microbiology*, *204*(6), 337. https://doi.org/10.1007/s00203-022-02940-8

Carmona-Hernandez, S., Reyes-Pérez, J.J., Chiquito-Contreras, R.G., Rincon-Enriquez, G., Cerdan-Cabrera, C.R., & Hernandez-Montiel, L.G. (2019). Biocontrol of postharvest fruit fungal diseases by bacterial antagonists: A review. *Agronomy, 9*(3), 121. https://doi.org/10.3390/agronomy9030121

Chaudhary, T., Dixit, M., Gera, R., Shukla, A. K., Prakash, A., Gupta, G., & Shukla, P. (2020). Techniques for improving formulations of bioinoculants. *3 Biotech*, *10*, 1-9. https://doi.org/10.1007/s13205-020-02182-9

Croxall, H., Gwynne, D., & Jenkins, J. (1952). The rapid assessment of apple scab on leaves. *Plant Pathology, 1*(2), 39-41. https://doi.org/10.1111/j.1365-3059.1952.tb00022.x

Dean, R., Van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G.D. (2012). The top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology, 13*(4), 414-430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Doolotkeldieva, T., & Bobusheva, S. (2017). Scab disease caused by venturia inaequalis on apple trees in Kyrgyzstan and biological agents to control this disease. *Advances in Microbiology, 7*(6), 450-466. https://doi.org/10.4236/aim.2017.76035

Droby, S., & El-Gerberia, B. (2006). Yeast Metschnikowia Fructicola NRRL Y-30752 for inhibiting deleterious microorganisms on plants. U.S. Patent No. 6994849B2, February 7, 2006.

Droby, S., Wisniewski, M., Teixidó, N., Spadaro, D., & Jijakli, M.H. (2016). The science, development, and commercialization of postharvest biocontrol products. *Postharvest Biology and Technology*, *122*, 22-29. https://doi.org/10.1016/j.postharvbio.2016.04.006

Dukare, A.S., Sangeeta, P., Eyarki, N.V., Ram, K.G., Rajbir, S., Kalyani, S., & Rajesh, K.V. (2018). Exploitation of microbial antagonists for the control of postharvest diseases of fruits: A review. *Critical Reviews in Food Science and Nutrition*, *59*(9), 1498-1513. https://doi.org/10.1080/10408398.2017.1417235

Freimoser, F.M., Rueda-Mejia, M.P., Tilocca, B., & Migheli, Q. (2019). Biocontrol yeasts: Mechanisms and applications. *World Journal of Microbiology and Biotechnology, 35*, 154. https://doi.org/10.1007/s11274-019-2728-4

Hassan, H., Mohamed, M.T.M., Yusoff, S.F., Hata, E.M., & Tajidin, N.E. (2021). Selecting antagonistic yeast for postharvest biocontrol of *Colletotrichum gloeosporioides* in papaya fruit and possible mechanisms involved. *Agronomy, 11*(4), 760. https://doi.org/10.3390/agronomy11040760

He, Y., Degraeve, P., & Oulahal, N. (2024). Bioprotective yeasts: Potential to limit postharvest spoilage and to extend shelf life or improve microbial safety of processed foods. *Heliyon, 10*(3), e24929. https://doi.org/10.1016/j.heliyon.2024.e24929

Isina, Z.M., Koigeldina, A.K., Tursunova, A., Kopzhassarov, B., Sardar, A., & Boltaeva, L.A. (2024). Impact of environmental degradation on the development of moniliosis: A case study of apple orchards in the Almaty region, Kazakhstan. *Caspian Journal of Environmental Sciences*, *22*(1), 211-220.

Janisiewicz, W.J., & Conway, W.S. (2010). Combining biological control with physical and chemical treatments to control fruit decays after harvest. *Stewart Postharvest Review, 6*(1), 1-16. https://doi.org/10.2212/spr.2010.1.3

Janisiewicz, W.J., Saftner, R.A., Conway, W.S., & Forsline, P.F. (2008). Preliminary evaluation of apple germplasm from Kazakstan for resistance to postharvest blue mold in fruit caused by *Penicillium expansum*. *HortScience, 43*(2), 420-426. https://doi.org/10.21273/HORTSCI.43.2.420

Jimenez, M.C.M., Hernandez, F.D., Alcala, E.I.L., Morales, G.G., Valdes, R.A., & Reyes, F.C. (2018). Biological effectiveness of *Bacillus spp.* and *Trichoderma spp*. on apple scab (*Venturia inaequalis*) in vitro and under field conditions. *European Journal of Physical and Agricultural Sciences, 6*(2), 7-17.

Jones, R., Fountain, M.T., Günther, C.S., Eady, P.E., & Goddard, M.R. (2021). Separate and combined Hanseniaspora uvarum and *Metschnikowia pulcherrima* metabolic volatiles are attractive to *Drosophila suzukii* in

the laboratory and field. *Scientific Reports*, 11(1). https://doi.org/10.1038/s41598-020-79691-3

Kistechok, A., Wrona, D., & Krupa, T. (2024). Effect of storage conditions on the storability and nutritional value of new Polish apples grown in Central Poland. *Agriculture, 14*(1), 59. https://doi.org/10.3390/agriculture14010059

Kowalska, J., Krzymińska, J., & Tyburski, J. (2022). Yeasts as a potential biological agent in plant disease protection and yield improvement - A short review. *Agriculture, 12*(9), 1404. https://doi.org/10.3390/agriculture12091404

Lachapelle, M., Bourgeois, G., & DeEll, J. (2013). Effects of postharvest weather conditions on firmness of McIntosh apples at harvest time. *HortScience, 48*(4), 474-480. https://doi.org/10.21273/HORTSCI.48.4.474

Lahlali, R., Moinina, A., Ezrari, S., Maclean, D., & Boulif, M. (2019). Apple scab disease severity in the Sais region of Morocco and its sensitivity to three commercial fungicides. *Notulae Scientia Biologicae, 11*(2), 249-257. https://doi.org/10.15835/nsb11210434

Lemanceau, P., Blouin, M., Muller, D., & Moënne-Loccoz, Y. (2017). Let the core microbiota be functional. *Trends in Plant Science, 22*(7), 583-595. https://doi.org/10.1016/j.tplants.2017.04.008

Liu, Y., Yao, S., Deng, L., Ming, J., & Zeng, K. (2018). *Metschnikowia citriensis sp*. nov., a novel yeast species isolated from leaves with potential for biocontrol of postharvest fruit rot. *Biological Control, 125*, 15-19. https://doi.org/10.1016/j.biocontrol.2018.05.018

Lugtenberg, B., Rozen, D. E., & Kamilova, F. (2017). Wars between microbes on roots and fruits. *F1000Research, 6*, 343. https://doi.org/10.12688/f1000research.10696.1

Marian, M., & Shimizu, M. (2019). Improving performance of microbial biocontrol agents against plant diseases. *Journal of General Plant Pathology, 85*, 329-336. https://doi.org/10.1007/s10327-019-00866-6

Massart, S., Martinez-Medina, M., & Haissam, J.M. (2015). Biological control in the microbiome era: Challenges and opportunities. *Biological Control, 89*, 98-108. https://doi.org/10.1016/j.biocontrol.2015.06.003

Millan, A.F.S., Gamir, J., Farran, I., Larraya, L., & Veramendi, J. (2022). Identification of new antifungal metabolites produced by the yeast *Metschnikowia pulcherrima* involved in the biocontrol of postharvest plant pathogenic fungi. *Postharvest Biology and Technology, 192*, 111995. https://doi.org/10.1016/j.postharvbio.2022.111995

Ministry of National Economy of the Republic of Kazakhstan, (2020). Forecasts of socio-economic development of the Republic of Kazakhstan for 2021-2025. URL: https://www.gov.kz/memleket/entities/economy/documents/details/62731?lang=ru&ysclid=m3ybdax1yj603457665 (accessed on 4 May 2020).

Orosz-Tóth, M., & Kincses, S. (2019). The examination of flesh firmness in different apple varieties. *Acta Agraria Debreceniensis, 2*, 103-107. https://doi.org/10.34101/actaagrar/2/3686

Palou, L., Ali, A., Fallik, E., & Romanazzi, G. (2016). GRAS, plant and animal-derived compounds as alternatives to conventional fungicides for the control of postharvest diseases of fresh horticultural produce. *Postharvest Biology and Technology, 122*, 41-52. https://doi.org/10.1016/j.postharvbio.2016.04.017

Panyushkina, I.P., Mukhamadiev, N.S., Lynch, A.M., Ashikbaev, N.A., Arizpe, A.H., O'connor, C.D., & Sagitov, A.O. (2017). Wild apple growth and climate change in southeast Kazakhstan. *Forests*, *8*(11), 406.

Pawlikowska, E., James, S.A., Breierova, E., Antolak, H., & Kregiel, D. (2019). Biocontrol capability of local *Metschnikowia sp*. isolates. *Antonie Van Leeuwenhoek, 112*, 1425-1445. https://doi.org/10.1007/s10482-019-01272-w

Pawlikowska, E., Kolesinska, B., Nowacka, M., & Kregiel, D. (2020). A new approach to producing high yields of pulcherrimin from *Metschnikowia* yeasts. *Fermentation, 6*(4), 114. https://doi.org/10.3390/fermentation6040114

Ponsone, M.L., Nally, M.C., Chiotta, M.L., Combina, M., Kohl, J., & Chulze, S.N. (2016). Evaluation of the effectiveness of potential biocontrol yeasts against black sur rot and ochratoxin A occurring under greenhouse and field grape production conditions. *Biological Control, 103*, 78-85. https://doi.org/10.1016/j.biocontrol.2016.07.012

Rueda-Mejia, M.P., Nägeli, L., Lutz, S., Hayes, R.D., Varadarajan, A.R., Grigoriev, I.V., Ahrens, C.H., & Freimoser, F.M. (2021). Genome, transcriptome and secretome analyses of the antagonistic, yeast-like fungus Aureobasidium pullulans to identify potential biocontrol genes. Microbial Cell, 8(8), 184–202. https://doi.org/10.15698/mic2021.08.757

Sanzani, S.M., Reverberi, M., & Geisen, R. (2016). Mycotoxins in harvested fruits and vegetables: insights in producing fungi, biological role, conducive conditions, and tools to manage postharvest contamination. *Postharvest Biology and Technology, 122*, 95-105. https://doi.org/10.1016/j.postharvbio.2016.07.003

Sariah, M. (1994). Potential of *Bacillus* spp. as a biocontrol agent for anthracnose fruit rot of chilli. *Malaysian Applied Biology, 23*, 53-60.

Settier-Ramírez, L., López-Carballo, G., Hernández-Muñoz, P., Fontana, A., Strub, C., & Schorr-Galindo, S. (2021). New Isolated *Metschnikowia pulcherrima* Strains from Apples for Postharvest Biocontrol of Penicillium expansum and Patulin Accumulation. *Toxins*, 13(6), 397. https://doi.org/10.3390/toxins13060397

Sipiczki, M. (2020). *Metschnikowia pulcherrima* and related pulcherrimin-producing yeasts: Fuzzy species boundaries and complex antimicrobial antagonism. *Microorganisms, 8*(7), 1029. https://doi.org/10.3390/microorganisms8071029

Spadaro, D., & Droby, S. (2016). Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists. *Trends in Food Science & Technology, 47*, 39-49. https://doi.org/10.1016/j.tifs.2015.11.003

Tang, J., Liu, Y., Li, H., Wang, L., Huang, K., & Chen, Z. (2015). Combining an antagonistic yeast with harpin treatment to control postharvest decay of kiwifruit. *Biological Control, 89*, 61-67. https://doi.org/10.1016/j.biocontrol.2015.04.025

Tian, Y., Li, W., Jiang, Z., Jing, M., & Shao, Y. (2018). The preservation effect of *Metschnikowia pulcherrima* yeast on anthracnose of postharvest mango fruits and the possible mechanism. *Food Science and Biotechnology, 27*, 95-105. https://doi.org/10.1007/s10068-017-0213-0

Turkel, S., & Ener, B. (2009). Isolation and characterization of new *Metschnikowia pulcherrima* strains as producers of the antimicrobial pigment pulcherrimin. *Zeitschrift für Naturforschung, 64*, 405-410. https://doi.org/10.1515/znc-2009-5-618

Turkel, S., Korukluoglu, M., & Yavuz, M. (2014). Biocontrol activity of the local strain of *Metschnikowia pulcherrima* on different postharvest pathogens. *Biotechnology Research. International, 2014*(2), 397167. https://doi.org/10.1155/2014/397167

Wachowska, U., & Głowacka, K. (2014). Antagonistic interactions between *Aureobasidium pullulans* and *Fusarium culmorum*, a fungal pathogen of winter wheat. *BioControl, 59*(5), 635-645. https://doi.org/10.1007/s10526-014-9596-5

Wisniewski, M., & Droby, S. (2019). The postharvest microbiome: The other half of sustainability. *Biological Control, 137*, 104025. https://doi.org/10.1016/j.biocontrol.2019.104025

Yefremova, M.Y., Urazayeva, V.M., & Kazybaeva, S.S. (2023). Rootstocks and Varieties of Fruits, Berry Crops, and Grapes, Used for Intensive Gardening in Kazakhstan. IntechOpen. London. https://doi.org/10.5772/intechopen.108360

Zajc, J., Cernoša, A., Di Francesco, A., Castoria, R., De Curtis, F., Lima, G., Badri, H., Jijakli, H., Ippolito, A., GostinCar, C., Zalar, P., Gunde-Cimerman, N., & Janisiewicz, W.J. (2022). Characterization of *Aureobasidium pullulans* isolates selected as biocontrol agents against fruit decay pathogens. *Fungal Genomics & Biology, 10*, 163.

Zhang, H., Mahunu, G.K., Castoria, R., Apaliya, M.T., & Yang, Q. (2017). Argumentation of biocontrol agents with physical methods against postharvest diseases of fruits and vegetables. *Trends in Food Science & Technology, 69*(Part A), 36-45. https://doi.org/10.1016/j.tifs.2017.08.020

Zhang, X., Li, B., Zhang, Z., Chen, Y., & Tian, S. (2020). Antagonistic yeasts: A promising alternative to chemical fungicides for controlling postharvest decay of fruit. *Journal of Fungi, 6*(3), 158. https://doi.org/10.3390/jof6030158

Zhimo, V.Y., Kumar, A., Biasi, A., Abdelfattah, A., Sharma, V.K., Salim, S., Feygenberg, O., Bartuv, R., Freilich, S., Whitehead, S.R., Wisniewski, M., & Droby, S. (2022). Assembly and dynamics of the apple carposphere microbiome during fruit development and storage. *Frontiers in Microbiology, 13*, 928888. https://doi.org/10.3389/fmicb.2022.928888