Article

Evaluating the fungal pathogens inhibition efficiency of composite film combined with antagonistic yeasts and sodium alginate on peach

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Abstract: To reduce the indiscriminate use of pesticides and extend the postharvest shelf life of peach fruit (cv. Baihua) from southeast China, microbial antagonism of indigenous yeasts was mainly studied and applied in construction of composite film. After isolation, purification, cultivation and identification, a total of 14 yeast strains from 9 genera were screened out from the surface of peaches. By experimental analysis of *in vitro* inhibition zone and *in vivo* colonizing capacity, *Candida oleophila* sp-ELPY12B and *Cryptococcus laurentii* sp-ELPY15A, which have conservative structure of D1/D2 domain sequences and were considered as new species by phylogenetic analysis, were finally chosen as fungicides against the major pathogens. In combination of Na-alginate film (0.4 % glycerin as plasticizer and 0.1 % Tween-80 as emulsifier), the preservative effects of composite-treated groups (1×10^8 CFU mL^{-1} of *Candida oleophila* sp-ELPY12B and *Cryptococcus laurentii* sp-ELPY15A) showed best antifungal effects, which significantly delayed the postharvest preservation period about 6 - 7 d under ambient temperature of 25 ± 3°C and relative humidity of 50 - 70%.

Keywords: peach (*Prunus persica*); postharvest preservation; antagonistic yeasts; Na-alginate film; Antifungal activity

1. Introduction

For a long time, the main methods to control peach (*Prunus persica*) postharvest diseases are the use of chemical fungicides, such as Imazalil, Tecto, Carbendazim, thiophanate-methyl, and Nurelle-D505. As environmental pollution becomes more and more severe, the indiscriminate use of these chemical fungicides does not meet the requirements of safety and sanitary conditions, which is increasingly limited by government. Otherwise, it also further lead to drug resistance problems in pathogens and greatly reduce the efficiency. Therefore, many countries are committed to the development of biological prevention and control, including antagonistic microorganism, enzyme, resistance gene and natural product [1], trying to gradually replace or reduce the use of chemical fungicides. Among them, the research on antagonistic yeast in control of postharvest diseases of fruits and vegetables has more generalizable practice and experience. It started in the 1980s, and has started to be commercialized from the laboratory. At present, some antagonistic yeasts have been successfully developed and patented, such as "Aspire™" (*Candida oleophila*, U.S.A), "Biosave" series (*Pseudomonas syringae*, U.S.A), "Shemer" (*Metschnikowia fructicola*, Antigua-Barbuda), "Serenade™" (*Bacillus subtilis*, U.S.A), "Pantovital™" (*Pantoea agglomerans*, Spain), "Boniprotect™" (*Aureobasidium Pullulans*, Germany), and so on. A promising antagonistic yeast needs following desirable characteristics [2], mainly includes: 1) genetic stability; 2) low demand for nutrition; 3) good adaptability under specific environment; 4) antifungal broad-spectrum and always has growth advantage compared with pathogens; 5) easy to dilute for application and can prolong the shelf life obviously; 6) harmless to

 doi:10.20944/preprints202212.0021.v1

human body; 7) no pathogenicity to the host; 8) can coexist with other common treatments. So, identification, development and commercialization of a biological control product is a long and costly process requiring continuous input and market demands.

In addition to the individual applications of antagonistic yeast, the combination of edible film based on biopolymers could further enhance the total preservation efficiency mainly by changing the surface morphology of fruit, reducing water evaporation and oxygen permeability in the pericarp [3]. As a green packaging material, sodium alginate (Na-alginate) were applied in this study. It is an anionic natural polymer polysaccharide, soluble in water, with properties of biodegradability, biocompatibility, non-toxicity, adhesion and good toughness [4]. Its film form can be easily cleaned by water, which is very suitable for the development of edible films [5].

Currently, the fresh peach fruit (*Prunus persica,* cv. Baihua) from southeast China has the characteristics of high yield in large scale and concentrated ripening from July to August. As the aroma and taste are all excellent and rich in nutrition, it is greatly appreciated by the market. However, its melting-flesh pericarp is liable to decay during harvest which resulted in huge economic losses, and the shelf life is relatively short, only approximately 2 - 3 d [6]. To solve the above-mentioned problem, this study firstly carried out detailed research on isolation, purification, cultivation and identification of indigenous yeasts on peach. Subsequently, the candidate antagonistic yeasts against major fungal pathogens were screened out by the experiments of inhibition zone and colonizing capacity. On this basis, Na-alginate was added in yeast suspension to prepare the film solution for further improvement in practical application, and the comprehensive preservation effects were evaluated by physiological and organoleptic indicators of weight loss, total soluble solids content, respiration rate and decay index under ambient temperature of 25 ± 3 °C and RH of 50 - 70 %.

2. Materials and Methods

2.1 Peach Sampling and Cultivation of Microorganisms

Honey peaches (*Prunus persica,* cv. Baihua) at approximately 80% maturity in similar size and weight (about 7 cm diameter and 200 g) with no mechanical injury or pests were selected as experimental samples from Fenghuang town, Zhangjiagang, Jiangsu, China (31°45′ N, 120°36′ E). To isolate the antagonistic yeasts on surface, peaches were suspended in 300 mL sterile deionized water and fully oscillated on rotary shaker at 200 rpm (revolutions per minute) for 40 min. Afterwards, 60 μL of above suspension was extracted and evenly coated on YPDA plates [Yeast Extract Peptone Dextrose Agar Medium. 1 % yeast extract, 2 % peptone, 2 % dextrose (glucose) and 2 % agar, respectively sterilized at 115 °C for 20 min and then mixed together] contains 40 μg mL^{-1} ampicillin and 37.5 μg mL^{-1} methicillin in order to prevent contamination of bacteria. The plates were incubated at 28 °C for 60 h, and the recognized colonies were subsequently purified twice by streaking plate method under the same conditions.

Six pathogenic fungal strains of *Aspergillus (A. tubingensis), Penicillium (P. expansum), Botrytis (B. elliptica), Rhizopus (R. stolonifer), Alternaria (A. alternate)* and *Monilinia (M. fructicola)*, identified and isolated from decay peaches in our past studies [6], were selected as antagonistic objectives in inhibition experiments for antagonistic yeasts. They were priory stored on PDA slants (Potato Dextrose Agar Medium. 20 % potato, 2 % dextrose, and 2 % agar, respectively sterilized at 115 °C for 20 min) contains 50 μg mL^{-1} streptomycin at 4 °C. The fungal spores on surface of agar medium were then gently scraped off for PD liquid medium cultivation (400 mL). After cultured at appropriate temperatures (26 ± 2 °C for *Aspergillus*, *Penicillium*, *Rhizopus*, *Alternaria* and *Monilinia*, 20-22 °C for *Botrytis*) for 72 h, the spore suspensions of each strain were collected by filtering through sterilized 7 layers of cheese cloth for 3 times with mixing and thorough oscillation, and the appropriate dilutions (gradient dilution method, 10^{-1}-10^{4} times) were pipetted into hemocytometer for counting and diluted to obtain the final required concentrations.

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2.2 Molecular Identification of Antagonistic Yeasts

The yeast strains were identified by sequencing the genes of internal transcribed spacer (ITS) of 5.8S ribosomal RNA (forward primer: 5′-TCCTCCGCTTATTGATATGC-3′; reverse primer: 5′-GGAAGTAAAAGTCGTAACAAGG-3′) and D1/D2 domain (forward primer: 5′-GCATATCAATAAGCGGAGGAAAAG-3′; reverse primer: 5′-GGTCCGTGTTTCAAGACGG-3′) at the 5′ end of the 26S rRNA. The polymerase chain reaction (PCR) was conducted according to White [7] by using universal primers. The products were then sent to Sangon Biological Engineering Technology Co., Ltd (Shanghai, China) for sequencing, and the sequences were identified by BLASTn (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi). After being aligned by MEGA 7, related phylogenetic trees were constructed by neighbor-joining method [8] from evolutionary distance, and Kimura′ 2-parameter model [9] was applied. The test of phylogeny was measured by bootstrap method (1,000 replications) [10]. The referential standard strains were represented as genus and species by binomial nomenclature and accession numbers from GenBank (genetic sequence database, https://www.ncbi.nlm.nih.gov/genbank/).

2.3 In Vitro Antifungal Test of Antagonistic Yeasts

To preliminarily evaluate the antifungal effect of screened yeasts, the *In-vitro* inhibition zone experiments were conducted by beating holes method that referred to the follows: 100 μL of different pathogenic fungal suspensions at 1×10^6 CFU mL^{-1} were respectively prepared and evenly smeared on Mueller-Hinton agar mediums (0.6 % beef extract, 2 % peptone, 0.5 % sodium chloride, pH = 7.4, sterilized at 121 °C for 20 min). The agar of mediums were then perforated by sterilized punch or steel pipe to make holes (6 mm) for containing the yeast suspensions (60 μL, 1×10^8 CFU mL^{-1}) to be tested. Referring to the Standard of Clinical and Laboratory Standards Institute (CLSI) 2022, the wounds filled with sterile water was defined as negative control, and amphotericin B was defined as positive control. After cultured at ambient temperature of 25 ± 3 °C and RH of 50 - 70 % for 48 h, the diameters of the inhibition zones were determined by crossing measurement with vernier caliper [11]. The total relative inhibitory rate (TRI) were calculated as Equation (1).

$$TRI = (\overline{d}_{ex} - \overline{d}_{nc})/(\overline{d}_{pc} - \overline{d}_{nc}) \times 100\% \quad (1)$$

Where *TRI* is total relative inhibitory rate, $\overline{d}_{pc}$, $\overline{d}_{nc}$ and $\overline{d}_{ex}$ respectively represents the averaged inhibition zone diameters under six pathogens stress from positive control (amphotericin B), negative control (sterile water) and experimental groups. Each yeast treatment was represented by 2 plates, and each plate was measured 3 times for average.

2.4 Colonizing Capacity of Candidate Antagonistic Yeasts

After *in-vitro* antifungal Test, the yeasts that have remarkable antagonistic effect were selected for subsequently colonizing capacity test and preservative effect analysis on peach. The experiments were mainly conducted according to Zhang *et al* [12] with appropriate modifications. Peaches were punctured six equally distributed wounds (6 mm diameter and 3 mm deep) by sterilized steel pipe on surface for containing 40 μL each pathogenic fungal suspension applied in this study at 1×10^6 CFU mL^{-1}. After 2h absorbing and drying, yeast suspensions (1×10^8 CFU mL^{-1}) was applied into each wound (15 μL) and sprayed to cover the whole surface, including applying the sterile water as Control Check (CK).

To estimate the colonization ability and growth dynamic of the antagonistic yeasts, 2 g pulp tissues (0 - 5 mm from junction area) of CK group in next every 10d were cut off in sequence and grounded into appropriate dilutions with 30 mL 0.7% saline loading. The yeasts from tissues were respectively recovered by spreading 50 μL of each dilutions on YPDA and culturing them at ambient temperature of 25 ± 3 °C and RH of 50 - 70 % for 36h. The colonies on plates were then counted and calculated by gradient dilution method with hemocytometer, and the population densities of the antagonistic yeasts were described as $\log_{10}$ CFU per wound.

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2.5 Antagonistic Yeasts Preservative Effect Analysis on Fungi Inoculated Peach

The *in-vivo* preservative effect of antagonistic yeasts were also evaluated in above experiments. After inoculation of fungi and yeasts, related quality attributes of weight loss, respiration rate, soluble solids content and decay index were measured every 2d during 14d storage under the same circumstance referring to past research methods [6] with minor modifications. In decay index measurement, the statistics of disease speckle coverage should ignore the area of initial punched wounds.

2.6 Evaluating the fungal pathogens inhibition efficiency of composite films

For further application in production, Na-alginate were also applied with antagonistic yeast solution to form edible films to enhance the preservative effect. The edible film solutions were mainly prepared according to the procedure used by Foteini *et al* [13] with modifications. Firstly, 1 g Na-alginate was added and sufficiently dissolved in 100 mL pre-warmed (50 - 70 °C) sterile water by dispersion homogenizer (3000 rpm). Following gradual addition of 0.4 % glycerin (w/v, hereinafter inclusive) as plasticizer and 0.1 % Tween-80 as emulsifier, the coating solution was then fully vibrated by ultrasonic cleaner for 20 min. after degassing with vacuum pump and cooling down to 25 ± 3 °C, the candidate yeast isolate was added with agitation, to yield final concentration of 1×10^8 CFU mL^{-1}, and the pH of the mixed solution was adjusted to 6 - 7 using 0.1 mol L^{-1} acetic acid. To verify the antifungal effects of yeasts with edible films, the relative solutions were applied on fungi inoculated peach. All experiments were conducted at ambient temperature of 25 ± 3 °C and RH of 50 - 70 %, and details of all groups were demonstrated as **Table 1**. Seven fruits were regarded as a replicate, and 3 replicates were measured per treatment for average.

Table 1. Treatments of different groups.

Group Name	Treatments	Instructions
Control Check (CK)	Sterile Water	● Fungi-inoculated peach without yeast treatment
Group - 1	Na-alginate film	● The composite Na-alginate film was combined with 0.4 % glycerin as plasticizer and 0.1 % Tween-80 as emulsifier
Group - 2	*Cryptococcus laurentii*	● 1×10^8 CFU mL^{-1} yeast suspension was applied
Group - 3	*Candida oleophila*	
Group - 4	Na-alginate film composite by *Cryptococcus laurentii*	● composite Na-alginate coating solutions were mixed with 1×10^8 CFU mL^{-1} yeast
Group - 5	Na-alginate film composite by *Candida oleophila*	

2.7 Statistical Data Processing

The experimental results were analyzed by SPSS 22.0 for Windows. Analysis of variance (ANOVA) and Duncan's multiple ranges at confidence interval of $p < 0.05$ were determined to be significant different among different treatments. The related analysis graphics were created by OriginPro 2020.

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3. Results

3.1 Isolation and Identification of Yeasts on Peach

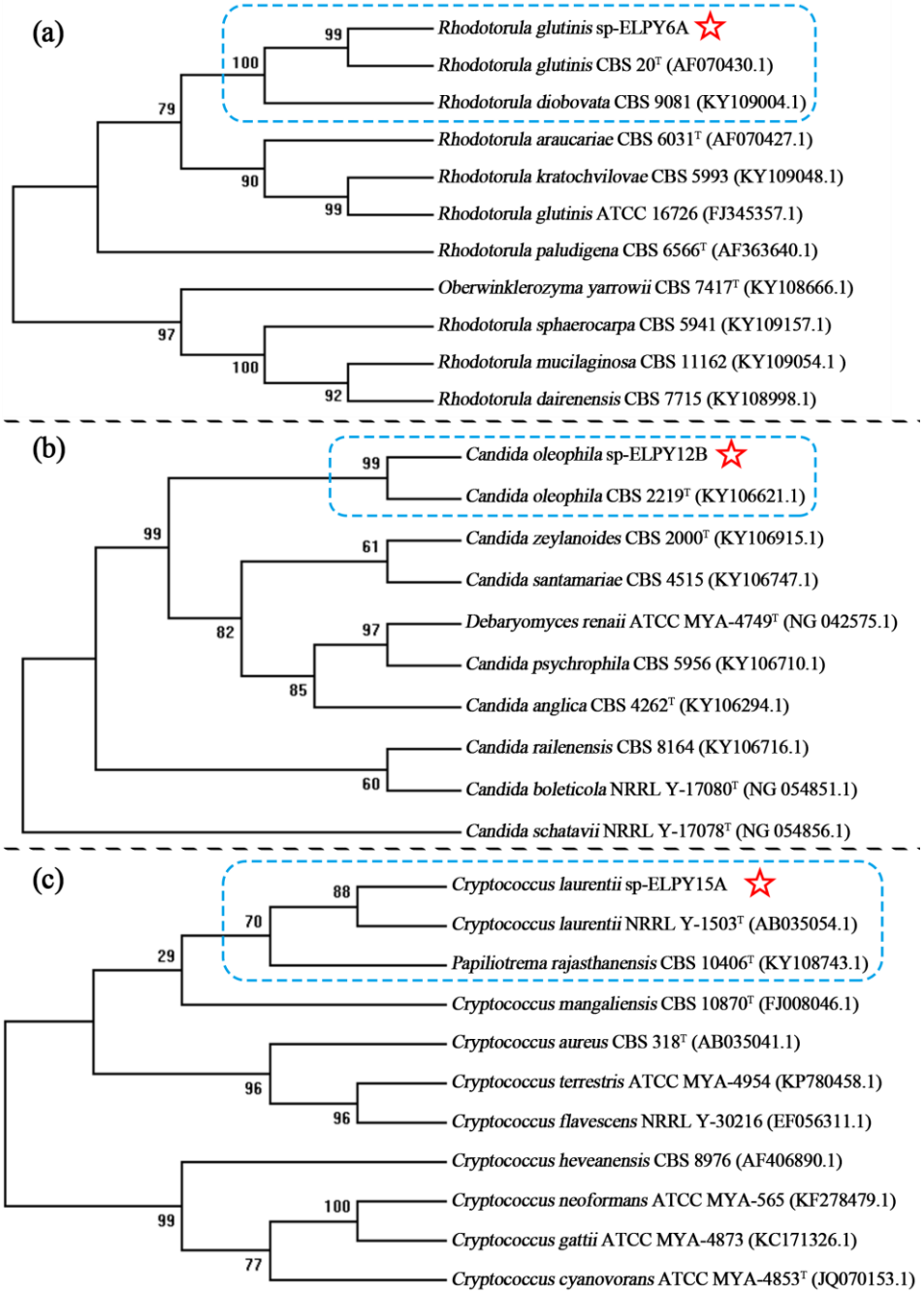

Figure 1. The phylogenetic trees of 26S rRNA D1/D2 domain sequences from the three stains, constructed by neighbour-joining method (Kimura' 2-parameter model), represent the evolution distance among close typical species. The bootstrap values (%, 1,000 replications) were given at each branch. All the reference strains were shown as accession numbers from GenBank, and the type strains from American Type Culture Collection (ATCC), Agricultural Research Service Culture Collection (NRRL) and Centraalbureau voor Schimmelcultures (CBS) were marked with a superscript T letter.

In this study, a total of 14 yeast strains from 9 genera were isolated respectively, and most of them were identified and confirmed as currently recognized yeast species by ITS and D1/D2 domain sequence alignment with GenBank database. They are *Metschnikowia* (*M. citriensis*, *M. zizyphicola*), *Pichia* (*P. fermentans*, *P. anomala*), *Meyerozyma guilliermondii*, *Candida* (*C. glabrata*, *C. inconspicua*, *C. oleophila*), *Torulaspora delbrueckii*, *Clavispora lusitaniae*, *Rhodotorula glutinis*, *Cryptococcus* (*C. laurentii*, *C. flavescens*) and *Sporobolomyces roseus*, in

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which three strains were found to have significant different gene sequences from corresponding known species. To further identify the three strains, relative phylogenetic trees were constructed by D1/D2 domain sequences comparison and illustrated as **Figure 1**.

As shown in **Figure 1-a**, the new isolated strain belongs to the clade of *Rhodotorula* from overall comparison. Within closely related taxa, it is most similar with *R. glutinis* CBS 20T (AF070430.1) and *R. diobovata* CBS 9081 (KY109004.1). As the strain has lower sequence coverage with *R. diobovata*, it was eventually identified as *Rhodotorula glutinis* sp-ELPY6A (assigned by specific laboratory number). From **Figure 1-b**, the unidentified strain has significant sequence similarities and high bootstrap value with *Candida oleophila* CBS 2219T (KY106621.1), so it was named *Candida oleophila* sp-ELPY12B. In **Figure 1-c**, the unidentified strain was proved to have more similarity to *Cryptococcus laurentii* NRRL Y-1503T (AB035054.1) than *Papiliotrema rajasthanensis* CBS 10406T (KY108743.1), and the bootstrap value is acceptable. The strain was identified as *Cryptococcus laurentii* sp-ELPY15A. Past research has established the standard for identification that more than 1 % sequence difference from 26S rDNA D1/D2 domain in yeast strains were defined as different species [14]. Therefore, the strain of *Rhodotorula glutinis* sp-ELPY6A (2.49 %, 14 out of 561 nucleotides), *Candida oleophila* sp-ELPY12B (2.03 %, 12 out of 589 nucleotides) and *Cryptococcus laurentii* sp-ELPY15A (1.76 %, 10 out of 568 nucleotides) are confirmed as new species of corresponding genus.

3.2 In Vitro Antifungal Test for Screening the Isolated Yeasts

After isolation, cultivation and sequence identification, *in vitro* antifungal test of all the 14 yeasts were tested with by inhibition zone method, the measuring data were collected, and the TRI were calculated in **Table 2**. According to CLSI 2021 standard, the test yeasts which have over 15 mm diameter inhibition zone are considered to be extremely susceptive to the target pathogens. As a common efficient polyene fungicide, amphotericin B showed high degree of fungistatic activity and the inhibition zone is obviously lager than other groups, so it is well suited for apply as evaluating reference in this experiment. Overall, the top 8 yeasts (from number 3 to number 10) exhibited good antagonistic effects as the inhibition zone results for most fungi are acceptable (10-15 mm are considered to be moderate susceptive) and the TRI values are relatively higher (over 40 %) then the remaining 6 stains. They have almost half the antifungal properties as amphotericin B. However, *Sporobolomyces roseus, Metschnikowia citriensis* and *Metschnikowia zizyphicola* are not so effective at inhibiting *M. fructicola* (6-10 mm are considered to be resistant). So *Cryptococcus laurentii, Candida oleophila, Meyerozyma guilliermondii, Rhodotorula glutinis,* and *Cryptococcus flavescens* were chosen as primary candidates for the following experiments. Otherwise, it is obvious to find that yeasts from the same genus does not necessarily show similar results in antagonistic efficiency [15], such as the three strains of *Candida* in this research.

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Table 2. The results of inhibition zone with standard deviations.

No.	The tested items	The diameter of inhibition zone (mm)						Total relative inhibitory rate (%)
		A. tubingensis	*P. expansum*	*B. elliptica*	*R. stolonifer*	*A. alternate*	*M. fructicola*	
1	**Sterile Water (d_{nc})**	6.00 ± 0.00g	6.00 ± 0.00f	6.00 ± 0.00f	6.00 ± 0.00g	6.00 ± 0.00e	6.00 ± 0.00g	N/A+
2	**Amphotericin B (d_{pc})**	20.53 ± 2.12a	18.76 ± 1.56a	19.88 ± 1.44a	18.94 ± 1.76a	19.07 ± 2.11a	19.85 ± 2.69a	N/A
3	***Cryptococcus laurentii***	12.73 ± 1.25bcd	16.38 ± 1.37ab	15.04 ± 1.66b	11.15 ± 1.16def	14.42 ± 1.58b	14.93 ± 1.36b	60.04
4	***Candida oleophila***	13.88 ± 1.43b	14.62 ± 2.59c	12.97 ± 1.79bcde	12.64 ± 1.52bc	12.58 ± 2.39bc	13.35 ± 1.48b	54.35
5	***Meyerozyma guillier-mondii***	12.48 ± 0.85bcd	13.39 ± 1.93c	13.25 ± 1.64bcd	14.23 ± 0.68b	12.36 ± 2.85bc	10.70 ± 1.14bcd	49.87
6	***Sporobolomyces roseus***	11.52 ± 1.29cd	13.83 ± 2.04bc	13.75 ± 2.15bc	14.22 ± 1.77b	13.11 ± 2.53bc	9.54 ± 1.02def	49.32
7	***Metschnikowia citriensis***	13.65 ± 2.01b	13.96 ± 1.83bc	10.63 ± 1.00de	12.94 ± 2.21bc	13.75 ± 2.22bc	8.58 ± 1.31defg	46.29
8	***Rhodotorula glutinis***	10.63 ± 1.13de	11.96 ± 1.38cd	13.67 ± 1.68bc	13.56 ± 1.67bc	12.18 ± 1.37bc	10.97 ± 2.01bcd	45.62
9	***Cryptococcus flavescens***	10.59 ± 0.96cd	12.77 ± 1.76c	11.86 ± 1.47cde	12.02 ± 1.73bcd	11.35 ± 1.33bc	12.54 ± 2.37bc	43.35
10	***Metschnikowia zizyph-icola***	12.74 ± 1.48bc	14.11 ± 1.65bc	11.42 ± 1.59cde	13.07 ± 0.91bc	12.24 ± 2.55bc	7.35 ± 0.82fg	43.11
11	***Pichia anomala***	8.24 ± 0.76f	12.42 ± 1.79c	10.27 ± 1.06e	12.51 ± 1.16bc	7.63 ± 1.19de	13.46 ± 1.39b	35.21
12	***Candida glabrata***	9.11 ± 0.47ef	9.20 ± 1.06e	11.89 ± 1.79cde	11.34 ± 0.88cde	10.61 ± 0.95cd	8.34 ± 0.73efg	30.22
13	***Candida inconspicua***	7.86 ± 0.43fg	8.78 ± 0.95e	10.56 ± 2.35de	8.29 ± 1.26f	11.57 ± 1.35bc	7.43 ± 0.66fg	22.82
14	***Pichia fermentans***	6.00 ± 0.00g	9.47 ± 0.84de	10.56 ± 1.75de	9.68 ± 1.14ef	7.93 ± 0.82de	10.34 ± 1.39cde	22.19
15	***Torulaspora delbrueckii***	6.00 ± 0.00g	6.00 ± 0.00f	7.49 ± 0.59f	6.00 ± 0.00g	12.43 ± 2.77bc	8.82 ± 0.98def	13.25
16	***Clavispora lusitaniae***	6.00 ± 0.00g	7.36 ± 0.99ef	6.00 ± 0.00f	9.81 ± 0.77def	6.00 ± 0.00e	7.59 ± 1.17fg	8.34

Note: N/A+: not applicable. Detailed information about the species is provided in the text above. The data in the table are shown as the mean ± SD, and the different normal letters in the same column indicate significant differences among treatments at the p = 0.05 level.

3.3 Colonizing Capacity of Candidate Antagonistic Yeasts on Peach

Stored at ambient temperature of 25 ± 3 °C and RH of 50 - 70 % for 10d, the growth conditions of candidate yeasts on peach were evaluated every day and illustrated in **Figure 2**. After short adaptation of lag phase, it is worth noting that all the yeasts had rapid proliferations in the first few days of logarithmic phase [16]. The populations of *Cryptococcus laurentii*, *Candida oleophila*, *Meyerozyma guilliermondii*, *Rhodotorula glutinis*, and *Cryptococcus flavescens* reached their corresponding maximum values at 5d, 8d, 3d, 9d and 6d, they were 3.98×10^8 CFU per wound, 1.35×10^8 CFU per wound, 7.59×10^7 CFU per wound, 4.37×10^7 CFU per wound and 1.82×10^8 CFU per wound, respectively. The yeast densities then basically remained at the same level or declined slightly for the strains growth gradually stepped into stationary phase and decline phase. Although all the strains had problems of growth stagnation after 5 - 8 d referring to the trend lines, *Cryptococcus laurentii*, *Candida oleophila* and *Cryptococcus flavescens* still maintained rather high density levels that are over 1×10^8 CFU per wound, and were confirmed to have better colonizing capacities than *Rhodotorula glutinis* and *Meyerozyma guilliermondii*, apparently. Therefore, the three strains were considered as proper antagonistic strains which can be applied in preparation of preservative productions. In addition, the colonization capacity of yeast on fruit were

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also affected by growth or postharvest conditions, such as temperature, humidity, illumination, air composition and host species [17]. So this screening result is mainly determined by peach (*Prunus persica*, cv 'Baihua') used in this experiment.

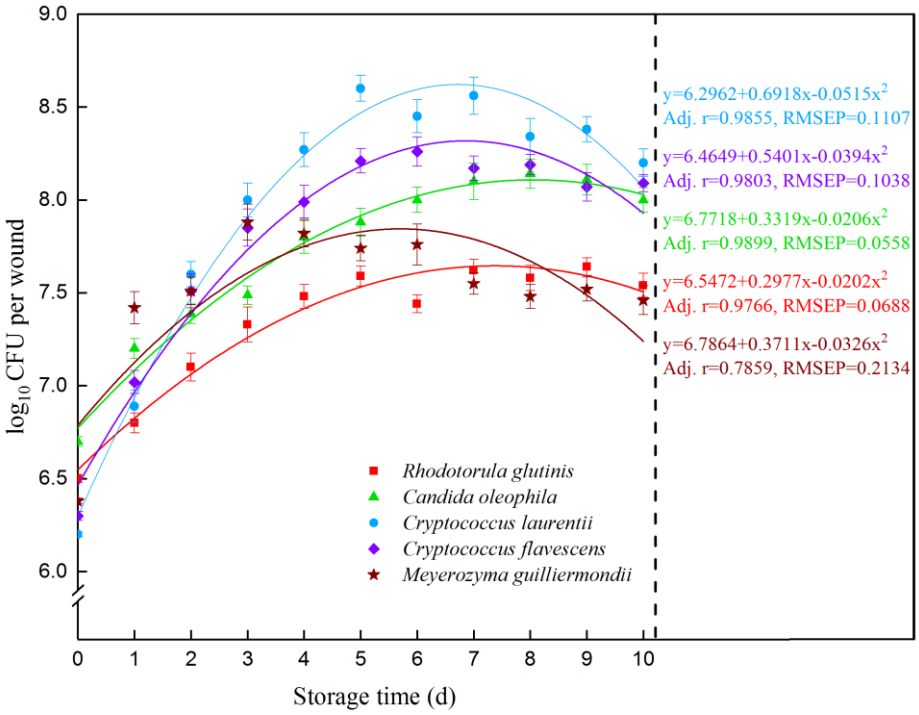

Figure 2. Population dynamics of five candidate yeasts in wounds of peach that stored at ambient temperature of 25 ± 3 °C and RH of 50 - 70 %. The yeast concentration data are shown as the mean ± SD, and the trend lines were constructed by polynomial regression.

3.4 In vivo Preservative Effects of Candidate antagonistic yeasts on Fungi-inoculated Peaches

Taking into consideration of the *in vitro* antifungal effect and colonizing capacity in film, *Candida oleophila* sp-ELPY12B and *Cryptococcus laurentii* sp-ELPY15A were finally chosen as microbiological additive in combination of Na-alginate film solution (0.4 % glycerin as plasticizer and 0.1 % Tween-80 as emulsifier) for *in vivo* preservative effect analysis in practice. After evaluating the quality attributes of weight loss (WL), respiration rate (RR), soluble solids content (SSC) and decay index (DI) every 2d during storage, the relative statistical graph were illustrated as **Figure 3**.

Past studies have shown that most of the postharvest WL is mainly caused by water loss in transpiration, while the rest is caused by respiration in nutrition consumption [18]. In this study, peaches from different groups suffered total WL ranging from 16 % to 25 % after 14 d storage. From the data shown in **Figure 3-a**, all the curves have initial upward trends and then decrease and remain relatively flat near 2 % in general. During physiological post ripeness, especially the untreated peaches from CK group lose weight most quickly by 5 % - 7 % with high respiration activity (**Figure 3-b**), and then maintained lowest weight-loss rate after 6d. Otherwise, the yeast suspension treated groups (group-2 and group-3) still cannot restrain the initial rapid WL compared with those film-treated groups (group-1, group-4 and group-5). All these above phenomena suggested that the edible Na-alginate film on surface of peaches can isolate themselves from the external environment to a certain extent to effectively reduce the water transpiration, which could greatly reduce the WL in the early stages of harvesting. Although the subsequent WL variability remains low, many peaches have lost weight so much in the first few days, leading to poor quality. As a kind of juicy fruit, when the WL is greater than 8 %, the peel of the peach pulp began to shrink, thus affecting the flavor and appearance in practice. By summing each measurement, the total WL of peaches from group-CK, group-1, group-2 and

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group-3 reached the critical value on 3d, 6d, 4d and 5d, respectively. In contrast, the acceptable period of peaches from group-4 and group-5 can be deferred to 10d in storage.

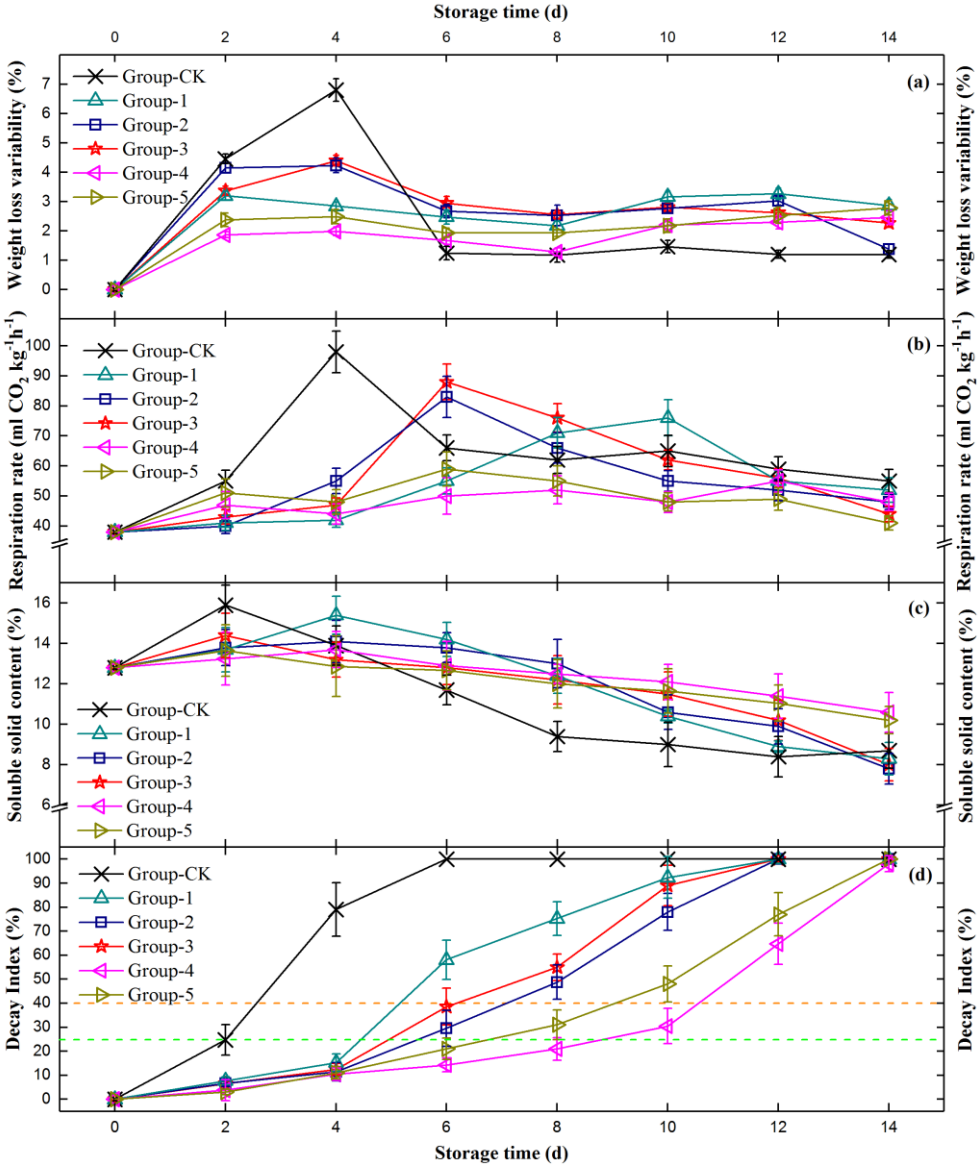

Figure 3. Physiological and organoleptic variations with standard deviation of peaches from different treatment groups within 15d postharvest storage. **(a)** Weight loss variability (WL, %); **(b)** Respiration rate (RR, ml CO_2 kg^{-1} h^{-1}). **(c)** Soluble solid content (SSC, %); **(d)** Decay index (DI, %). Detailed treatment information is provided in Table 1.

Shown as **Figure 3-b**, the respiratory peaks of group-CK, group-1, group-2 and group-3 were found, and they reached the maximum values of 98 mL CO_2 kg^{-1} h^{-1}, 76 mL CO_2 kg^{-1} h^{-1}, 83 mL CO_2 kg^{-1} h^{-1} and 88 mL CO_2 kg^{-1} h^{-1} on 4d, 10d, 6d and 6d, respectively. The approach of the respiration climacteric of coated peaches from group-1 were delayed about by 6d compared to those of the bare peaches form group-CK. It is worth noting that the simple application of antagonistic yeasts seems to have little effect on respiratory inhibition by comparing group-2 and group-3 with group-CK. Furthermore, by combining the antagonistic yeasts and the Na-alginate film, the RR of the peaches in group-4 and group-5 was greatly inhibited and remained below 60 CO_2 kg^{-1} h^{-1} all over the time, which is mainly due to air separation provided by film and the inhibition of fungi. Associated with data in **Figure 3-a**, changes in respiratory intensity have a relatively small effect on the WL variability, probably because most WL is caused by water transpiration.

As the results shown in **Figure 3-c**, the overall trend of SSC decreases steadily with the exception of a small initial increase. This is mainly because the peach samples used in the experiment were not fully ripen at the beginning (0d), and the SSC gradually increased

 doi:10.20944/preprints202212.0021.v1

to the maximum value along with the hydrolysis of polysaccharides [19]. The untreated peaches from group-CK showed a rapid decline in SSC, while the declines in other treatment groups were more gradual, especially in groups-4 and groups-5. The SSC of the untreated peaches from group-CK decreased rapidly, while that of the other treatment groups decreased more slowly, especially the group-4 and group-5. In the balance of polysaccharide hydrolysis and SSC consumption, the curve of groups-4 and groups-5 did not even show an initial increase, but remained stable around 12 %.

From the results in **Figure 3-d**, it is obvious that the DI of group-CK increased rapidly and reached 100 % by only 5d comparing the other groups. The application of antagonistic yeasts efficiently reduces the DI by comparing the results from group-CK, group-2 and group-3. In addition, the isolation effect of Na-alginate film (group-1) on the surface of the peach also inhibits the contact of the external fungi to some extent, thus reducing the DI. By combining the antagonistic yeasts and the Na-alginate film, the peaches from groups-4 and group-5 remained as fresh as possible in first 7d and 9d, and were still acceptable (DI < 40 %) before 9d and 10d, respectively. Compared with the control group, the effective preservation period was extended by about 6 - 7d.

4. Discussion

Overall, compared with naked peaches, the application of edible Na-alginate film reduces the occurrence of rot through isolating the outside air, at the same time, avoiding oxidation and respiration to a certain extent. Peach is a fruit that has typical respiration climacteric and short after-ripening. Therefore, the RR stands for the metabolism intensity for the most part, and it is usually associated with other indicators such like firmness, soluble sugar content and electrical conductivity [20]. The peach applied in this study is melting-flesh species which is more perishable, and it consumes less time to reach respiratory peak than common species (about 5 - 6 d) after completely ripening [21], so suppression of respiration is especially necessary. The SSC mainly defines the total soluble saccharides largely consumed as respiratory substrate in postharvest period, such as sucrose, glucose and fructose. As the tested peach species 'Baihua' possesses strong fragrant flavor and high sweetness, the maximum value of SSC could reach to about 16 %, relatively higher than those general varieties, which is usually 11 - 14 % [22]. In practice, we found that the SSC of well-preserved peaches from these two groups would recover to the high level again in short period after washed off the edible wrapping film, speculating that the physiological activity has changed certain degree, which needs further investigation. DI is a direct and useful organoleptic parameter for assessing the comprehensive disease severities of peaches in visual. Referring to past research experiences [6], a peach with a DI below 25 % is considered having good preservative status, having a DI being between 25% and 40% is acceptable, while being inedible as the DI is over 40 %. Combined with the previous analysis, the change of physiological parameters above (WL, RR and SSC) will become relatively flat after the peaches are completely rotten, which is mainly because the fruit itself lose its physiological activity, giving rise to cell membrane rupture and cell disassembly, and then the fungi takes over the following metabolism dynamics [23]. The composite biological preservatives prepared by combining antagonistic yeasts with Na-alginate film are more environmental friendly and easily obtained. Related middle-scale experimental applications of upon treatments have been conducted in producing areas of Jiangsu province and obtained good results.

Author Contributions: Conceptualization, Xiaolong Du and Jianlong Li; methodology, Xiaolong Du and Shaobin Li; software, An Luo; validation, Kai Fan and Linyun Mou; formal analysis, Xiaolong Du; investigation, Xiaolong Du; resources, Xiaolong Du and Jianlong Li; data curation, Xiaoli Yin; writing—original draft preparation, Xiaolong Du; writing—review and editing, Xiaolong Du. All authors have read and agreed to the published version of the manuscript."

Funding: This research was funded by "the Suzhou Science and Technology Project of China (grant number: SNG201447)", and "the nutrition physiology, decay mechanism and comprehensive preservation research of Fenghuang honey peach in Zhangjiagang City (grant number: ZKN1002)".

 doi:10.20944/preprints202212.0021.v1

Data Availability Statement: Not applicable.

Acknowledgments: I would like to acknowledge the assistance of the Fenghuang honey peach processing factory in Zhangjiagang City for providing experimental sites and materials.

Conflicts of Interest: The authors declare no conflict of interest.

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