

# Screening and Efficacy of Biocontrol Agent for Litchi Sour Rot

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## Abstract:

Litchi sour rot is one of the major diseases during the post-harvest storage and transportation of litchi (*Litchi chinensis* Sonn), which seriously affects the quality and economic value of litchi fruits. Yeast strain J-11 with inhibitory effect on *Geotrichum candidum*, the pathogen of litchi sour rot, was screened from papaya soil using the plate confrontation method. Furthermore, the salt tolerance and high-temperature resistance of the strain were determined. The fermentation filtrate of the selected antagonistic yeast was applied to litchi fruits for efficacy tests, and the control effect of the fermentation broth was evaluated. The results showed that yeast J-11 had a significant antagonistic effect on the pathogen of litchi sour rot with a high inhibition rate, and showed good tolerance under different salt concentrations and high-temperature conditions. In the application test on litchi fruits, under normal temperature storage conditions, J-11 filtrate could effectively reduce the disease index of litchi sour rot ( $P < 0.05$ ) with a control effect of 25%. The hardness and sugar concentration of fruits treated with the fermentation filtrate were significantly higher than those of other treatment, indicating that the fermentation broth of this biocontrol agent had a good control effect on litchi sour rot. This result provides new ideas and methods for the biological control of litchi sour rot and has important practical application value.

## Keywords:

Litchi Sour Rot, *Geotrichum Candidum*, Antagonistic Disease Prevention, Fruit Quality

## 1. Introduction

Litchi is a subtropical fruit with high nutritional and commercial value, which is deeply loved by consumers for its unique flavor, juicy flesh and bright-colored peel [1]. However, litchi is extremely prone to sour rot during post-harvest storage and transportation, which seriously affects the edible quality and economic value of litchi and causes great economic losses to fruit farmers [2]. Litchi sour rot usually occurs within a few days after fruit picking. Fruits are prone to mechanical damage or being bitten by pests during harvesting, storage and transportation, resulting in damage to the fruit epidermis, which provides an opportunity for the invasion of litchi sour rot

pathogens. The main pathogen of litchi sour rot is *Geotrichum candidum*. In the early stage, the disease spots are dark brown dots, mainly at the fruit stalk. The disease spots gradually expand and spread to the entire fruit, with white mold on the spots, the peel becomes dark brown and stiff, the diseased flesh softens, oozes sour rot juice, and finally the fruit turns brown and rots [3].

The control of litchi sour rot mainly relied on chemical control. However, this method had disadvantages of pathogens resistance, causing environmental pollution and even threatening human health [4]. Therefore, exploring efficient control methods for litchi sour rot is crucial to the litchi industry. As a green and sustainable prevention and control strategy, biological control has attracted more and more attention from researchers due to its advantages of no environmental pollution and low possibility of pathogens resistance. Many studies had shown that biocontrol microorganisms had effective control effects on pathogens. Ubo Ding et al. evaluated that *Klebsiella aerogenes* DB02 exerted significant inhibitory effects on the mycelial fresh weight and spore production of both *Fusarium oxysporum* and *Rhizoctonia solani* [5]. Wang et al. treated litchi with *Bacillus siamensis* suspension, and Wu et al. soaked litchi fruits in *Bacillus amyloliquefaciens* suspension, showed that the peel browning index of litchi treated with the bacteria was significantly reduced compared with the sterile water control group [6,7]. Li et al. mixed the antagonistic bacteria suspension of *Debaryomyces hansenii* and *Bacillus atrophaeus* isolated from soil in a volume ratio of 1:1 and soaked litchi fruits, which could effectively inhibit the peel browning and fruit disease index [8]. In this study, *Geotrichum candidum* was used as the test sample to isolate and screen yeasts, the salt tolerance and high-temperature resistance of the yeast were further determined, and the efficacy of the fermentation broth was evaluated. The research would provide strain resources for the biological control of *Geotrichum candidum* and provide theoretical reference and new resources for the biological control of post-harvest litchi sour rot.

## 2. Materials and Methods

### 2.1. Materials Samples

Soil was collected from papaya fields in Zhanjiang City, Guangdong Province, China. The pathogen was *Geotrichum candidum* LZ21 preserved in the laboratory. The litchi cultivar “Feizixiao” was purchased from a local market on Zhanjiang. Fruits were selected for uniformity in size, maturity, and absence of physical damage. Potato Dextrose Agar (PDA) was used for pathogen activation and preservation, and LB medium for fermentation broth preparation.

### 2.2. Methods

#### 2.2.1. Screening of antagonistic strain

10 g of papaya soil sample, were suspended into a conical flask containing 90 mL of sterile water, and incubate with shaking. Then, serial dilutions from  $10^{-1}$  to  $10^{-8}$  to obtain soil dilution solutions were performed. Select dilution solutions at appropriate concentrations and spread them onto PDA medium to isolate for yeast. The isolated colonies were inoculated into the corresponding sterile medium and purified by streaking on the plate until single colonies were obtained, transferred to a slant for preservation.

In this experiment, the plate confrontation method was used for screening. The

pathogen of litchi sour rot, *Geotrichum candidum*, was incubated on PDA medium at 28°C for 7 days to complete strain activation. Then, a 5.0 mm diameter *Geotrichum candidum* was taken with a punch and inoculated in the center of the medium. yeast J-11 were inoculated at 4 horizontal points 20 mm away from the center of the medium, with 3 replicates set for each treatment. At the same time, inoculated only with *Geotrichum candidum* as the control group. All plates were incubated at 28°C, and the diameter of *Geotrichum candidum* colonies was measured regularly from the 2nd to the 6th day of incubate to calculate the inhibition rate. Inhibition rate (%) = (control group colony diameter - treatment group colony diameter) / control group colony diameter × 100

### **2.2.2. Identification of strain**

Strain J-11 was inoculated on PDA medium and incubated in a 28°C incubator for 2-3 days for activation. The activated J-11 were picked with an inoculating loop, stained with crystal violet solution, and observed under an optical microscope to record their cell morphology. The purified J-11 strain was sent to Jiade Science and Technology Park, Science City, Huangpu District, Guangzhou for identification to obtain the strain DNA sequence. The sequenced gene sequence was analyzed by homology comparison using the BLAST function of the NCBI database, and MEGA 11.0 software was used to construct a phylogenetic tree by the Neighbor-Joining method.

### **2.2.3. Determination of salt tolerance of strain**

A single colony of yeast J-11 was picked and inoculated into LB medium, and incubated with shaking at 37°C and 180 r/min for 12 h to obtain yeast J-11 suspension. The suspension was inoculated into LB medium with final NaCl concentrations of 0%, 2.5%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5% and 20% at an inoculum size of 1% (volume fraction). Each concentration was set with 3 replicate groups. After shaking incubate at 37°C and 180 r/min for 24 h, the solution was taken to determine the absorbance at a wavelength of 600 nm.

### **2.2.4. Determination of high-temperature resistance of strain**

An appropriate amount of equal volume of J-11 suspension was picked and added to different test tubes. These test tubes were placed in water baths at 85°C, 90°C, 95°C and 100°C for incubation for 10 min, the test tubes were taken out and placed at room temperature to cool. Then, the treated suspension was inoculated into fresh LB medium at an inoculum size of 1%. After shaking at 37°C and 180 r/min for 24 h, samples were taken and the absorbance was measured at a wavelength of 600 nm. Each temperature condition was set with 3 replicate groups, and the suspension without incubation treatment was used as a control for comparative analysis.

### **2.2.5. Determination of the control effect of fermentation filtrate on litchi sour rot**

Yeast J-11 was inoculated into LB medium, incubated at 37°C and 200 r/min for 24 h to obtain seed liquid, which was inoculated into LB medium at a certain inoculum size (100 µL seed liquid into 100 ml medium), incubated at 200 r/min for 48 h, then centrifuged at 9500 r/min for 25 min. The supernatant was collected with a syringe. The supernatant was filtered through a 0.22 µm sterile filter to obtain sterile fermentation broth.

Fresh litchi fruits were soaked and cleaned with water, soaked in 75% alcohol for 10 minutes, then soaked in sterile water for 10 minutes and air-dried. 7 groups were designed. 4 control groups including the uninjured and untreated group, the injured and untreated group, the sterile water treatment group, the LB medium treatment group, and 3 treatment groups including the pathogen treatment group, the sterile fermentation broth treatment group, and the pathogen + fermentation broth treatment group, with 6 replicates set for each treatment group. Except for the uninjured and untreated group, other treatment groups were wounded on the surface with a needle, and then each group was treated as follows. (1) Uninjured and untreated group, no treatment; (2) Injured and untreated group, a 5 mm diameter blank medium was attached to the wound; (3) Sterile water treatment group, 20  $\mu$ L of sterile water was taken at the wound, and a 5 mm diameter blank medium was attached to the wound; (4) LB medium treatment group, 20  $\mu$ L of LB medium was taken at the wound, and a 5 mm diameter blank medium was attached to the wound; (5) Pathogen treatment group, a 5 mm diameter pathogen was inoculated at the wound; (6) Fermentation broth treatment group, 20  $\mu$ L of sterile fermentation broth was taken at the wound, and a 5 mm diameter blank medium was attached to the wound; (7) Pathogen + fermentation broth treatment group, 20  $\mu$ L of sterile fermentation broth was taken at the wound, and after it was dry, a 5 mm diameter litchi pathogen was inoculated at the wound. The treated litchi was incubated in a 28°C constant temperature incubator. From day 2 to day 6, the occurrence of sour rot in litchi was observed every day, and the disease index and efficacy were calculated. According to the ratio of fruit lesion area, it was divided into 4 grades, no lesion was grade 0; lesion area ratio less than 1/10 was grade 1; lesion area ratio 1/10 ~ 1/5 was grade 2; lesion area ratio 1/5 ~ 1/2 was grade 3; lesion area ratio greater than 1/2 was grade 4. The formula for calculating the disease index (D) was as follows. Disease index (D) =  $100 \times \Sigma$  (number of diseased fruits at each grade  $\times$  representative value of each grade) / (total number of investigated fruits  $\times$  representative value of the highest grade) A fruit hardness tester was used to determine the fruit hardness, and the flesh hardness (N) was measured near the equator of each fruit.

### 2.3. Data processing

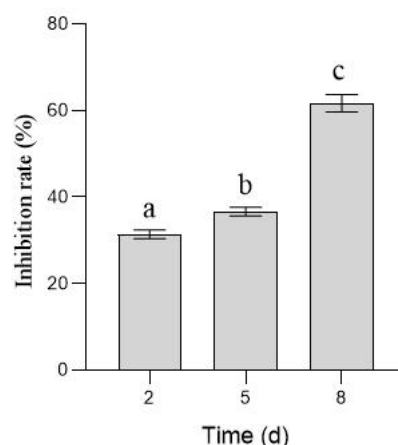
The experimental data were expressed as mean  $\pm$  standard deviation. SPSS 27.0 software was used for statistical analysis of the data, and Duncan's method was used for one-way multiple comparisons. GraphPad Prism 8 and Excel 2019 were used for chart making. Different lowercase letters indicate significant differences between treatments ( $P < 0.05$ ).

## 3. Results and Analysis

### 3.1. Screening of antagonistic strains

Among the 14 strains isolated and screened from papaya soil, strain J-11 had the best antagonistic effect on litchi *Geotrichum candidum*. The experimental results showed that J-11 had a significant inhibitory effect on the growth of *Geotrichum candidum*. On the plate, obvious antigen traces appeared at the edge of pathgen mycelium near J-11, the growth of mycelium was weakened, and the colony was significantly reduced, indicating that J-11 could effectively hinder the expansion of pathgen; the inhibition rate gradually increased with the extension of time, the inhibition rate increased slowly from 2 to 3 days, gradually accelerated from 4 to 7

days, and reached 61.69% after 8 days (Figure 1).

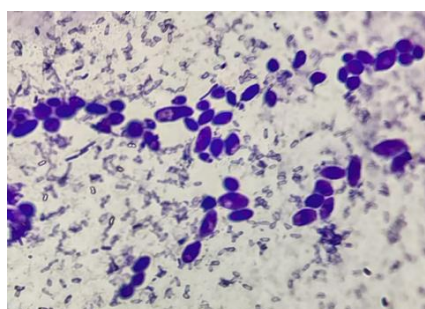


**Figure 1.** Inhibitory effect of yeast J-11 on the growth of *Geotrichum candidum*.

## 3.2. Strain identification results

### 3.2.1. Morphological characteristics

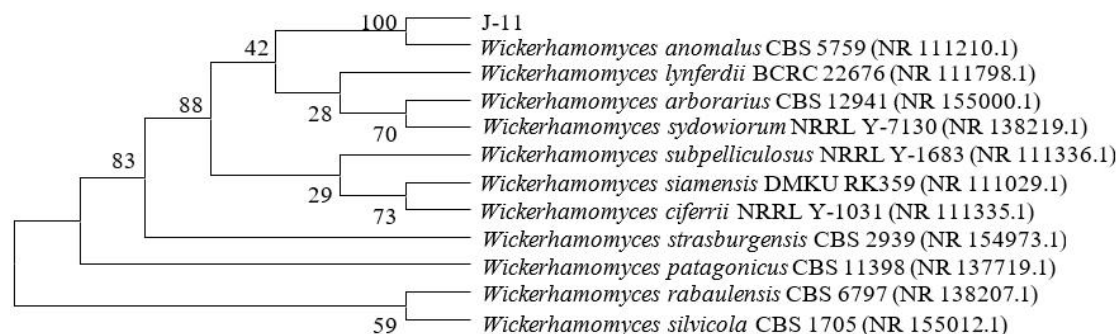
It can be seen from figure 2 that yeast J-11 presented elliptical or round cells with different sizes, thick cell walls, usually transparent cytoplasm, and dark-colored nuclei after staining (Figure 2).



**Figure 2.** Morphology of strain J-11.

### 3.2.2. Molecular biological identification

After PCR amplification and sequencing of the genomic DNA of strain J-11, it was submitted to NCBI for comparative analysis. The yeast sequences with high similarity to the sequence of strain J-11 were selected and downloaded as outgroups to construct a gene phylogenetic tree of yeast (Figure 3).



**Figure 3.** The phylogenetic tree of strain J-11 constructed based on 18S rRNA gene sequence.

The results showed that strain J-11 was clustered with *Wickerhamomyces anomalus*

with a support rate of 100%. Combined with the morphological characteristics of the strain, it was identified as *Wickerhamomyces anomalus*.

### 3.3. Determination of salt tolerance of strain

The salt tolerance results showed that the strain could grow normally in LB medium with final NaCl concentrations of 0, 2.5%, 5%, 7.5% and 10% (Figure 4). when the final NaCl concentration was 7.5%, the strain grew vigorously with an OD value of 0.998; under the conditions of final NaCl concentrations of 5% and 10%, the OD600 values of the strain were 0.866 and 0.875 respectively, with no significant difference. When the final NaCl concentration exceeded 12.5%, the growth of the strain was inhibited, indicating that strain J-11 had a certain salt tolerance.

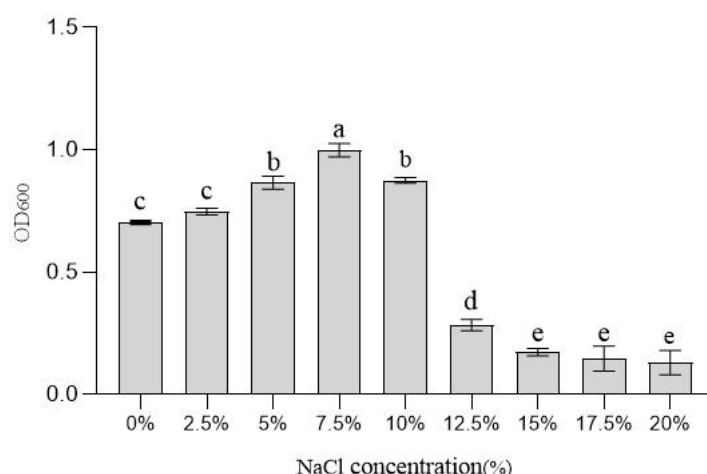


Figure 4. Sensitivity of strain J-11 to different NaCl concentrations.

### 3.4. Determination of high-temperature resistance of strain

The high-temperature resistance results showed that strain J-11 could still maintain a good growth after being treated at 85-95°C; the growth of the strain treated at 100°C was significantly inhibited, with an OD600 value of 0.023, while the OD600 of the treatment group treated at 95°C was 1.415 (Figure 5). This result indicated that J-11 had a certain tolerance to high temperatures, but its growth would be significantly affected under extreme high-temperature conditions (Figure 5).

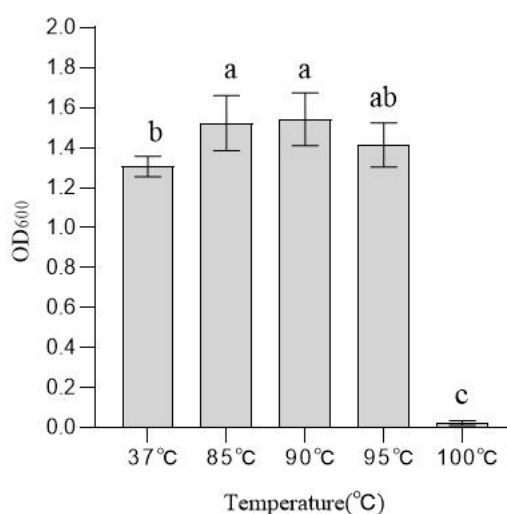


Figure 5. Sensitivity of strain J-11 to different temperatures.

### 3.5. Determination of the control effect of fermentation filtrate on litchi sour rot

#### 3.5.1. Control effect of fermentation filtrate on litchi sour rot

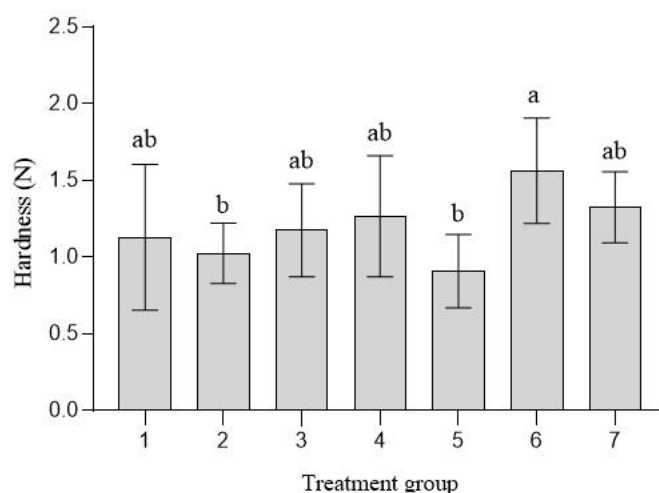
With the extension of storage time, litchi fruits in different treatment groups gradually rotted. The results showed that the fermentation broth had a disease prevention effect on litchi sour rot. brown lesions appeared at the inoculation sites of litchi fruits in the control group and the pathogen treatment group could be seen at the lesions, which spread on the litchi fruits, while the spread of *Geotrichum candidum* in the fermentation filtrate treatment group and the co-treatment group of fermentation broth and pathogen was lighter, and the lesions were smaller; Litchi in the control group and the pathogen treatment group had severe disease, with disease indexes of 41.67, 45.83, 50.00, 58.33 and 58.33 respectively, while the disease degree of litchi in the fermentation broth treatment group and the co-treatment group of fermentation broth and pathogen was alleviated, with disease indexes of 37.50 and 41.67 respectively, and the control effects were 25.00% and 16.66% respectively. The above results indicated that the fermentation filtrate had a certain control effect on litchi sour rot (Table 1).

**Table 1.** Effects of Fermentation Broth from Seven Different Treatments on the Disease Index.

| Group | Storage/d | Disease Index | Group | Storage/d | Disease Index |
|-------|-----------|---------------|-------|-----------|---------------|
| 1     | 0         | 0             | 5     | 0         | 0             |
|       | 2         | 4.17          |       | 2         | 33.33         |
|       | 4         | 29.17         |       | 4         | 37.50         |
|       | 6         | 41.67         |       | 6         | 58.33         |
| 2     | 0         | 0             | 6     | 0         | 0             |
|       | 2         | 16.67         |       | 2         | 0             |
|       | 4         | 41.67         |       | 4         | 12.50         |
|       | 6         | 45.83         |       | 6         | 37.50         |
| 3     | 0         | 0             | 7     | 0         | 0             |
|       | 2         | 12.50         |       | 2         | 33.33         |
|       | 4         | 16.67         |       | 4         | 33.33         |
|       | 6         | 50.00         |       | 6         | 41.67         |
| 4     | 0         | 0             |       |           |               |
|       | 2         | 16.67         |       |           |               |
|       | 4         | 29.17         |       |           |               |
|       | 6         | 58.33         |       |           |               |

#### 3.5.2. Effect of fermentation filtrate treatment on litchi fruit hardness

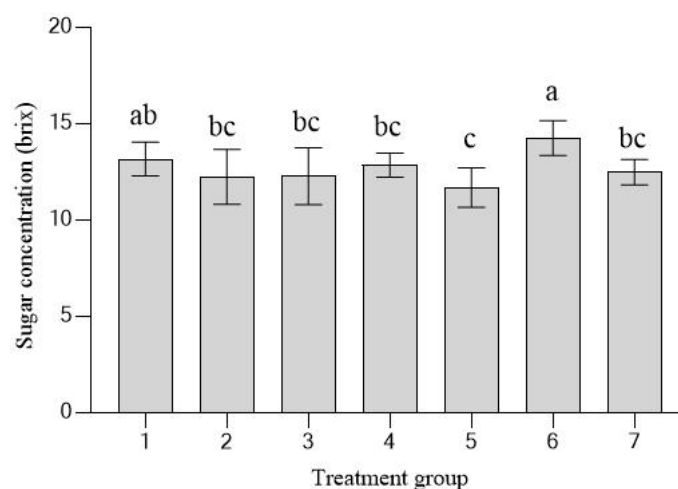
Fruit hardness is a key indicator to measure fruit texture, which can reflect the storage performance and the quality of storage effect. The hardness of litchi fruits in the injured and untreated group and the pathogen treatment group was the lowest, and the hardness of fruits in the fermentation broth treatment group was the highest, which was significantly higher than that in the pathogen treatment group and the injured treatment group; the fruit hardness of other groups was at a medium level, and there was no significant difference between them (Figure 6). It indicated that fermentation filtrate treatment had a certain effect on litchi fruit hardness.



**Figure 6.** Effect of fermentation filtrate treatment on the hardness of litchi fruits.

### 3.5.3. Effect of fermentation filtrate treatment on litchi sugar concentration

The measurement results of fruit sugar concentration showed that the sugar concentration of fruits in the fermentation broth treatment group was the highest, significantly higher than that in other groups; the sugar concentration of fruits in the pathogen treatment group was the lowest, significantly lower than that in other groups; the hardness of other groups was at a medium level, and there was no significant difference between them (Figure 7). It indicated that fermentation filtrate treatment had a certain effect on the litchi sugar concentration.



**Figure 7.** Effect of fermentation filtrate treatment on the sugar content.

## 4. Discussion

Litchi sour rot is one of the main post-harvest diseases, which can cause serious economic losses. At present, post-harvest preservation of litchi mainly relies on chemical pesticides, but Long-term use of chemical pesticides causes many problems. At present, Research on biological control of sour rot is relatively limited. The research on biological control of litchi mainly focuses on bacteria [9,10], and there are no relevant reports on the use of yeast for biological control.

Many yeast strains have been proved to have obvious inhibitory effects on various diseases and have broad application prospects. At present, there are many research

reports on the control of plant fungal diseases by yeasts. For example, yeasts can effectively inhibit and control apple gray mold and blue mold [11], blueberries gray mold and *Alternaria* rot [12], and grape gray mold [13]. It can be seen that yeasts have great potential in the control of fungal diseases. Consistent with previous research results, the yeast J-11 isolated in this experiment has a good inhibitory effect on litchi sour rot, with an inhibition rate of 61.69%. This finding provides new ideas and potential control strains for the biological control of litchi sour rot.

In the determination of salt tolerance and high-temperature resistance, strain J-11 showed strong adaptability. In media with different salt concentrations, J-11 could grow at NaCl concentrations up to 10%, and grew best at 7.5% NaCl concentration. In addition, J-11 could still maintain a good growth at high temperatures of 85°C to 95°C, indicating that it has a certain high-temperature resistance. These characteristics enable J-11 to adapt to different environmental conditions in practical applications, especially in the process of litchi storage and transportation under salt stress and high-temperature environments, with potential application value.

In the litchi fruit efficacy test, J-11 fermentation filtrate had a control effect on litchi sour rot. Compared with the pathogen treatment group, the disease index of the fermentation filtrate treatment group was significantly reduced, with a relative control effect of 25%. In addition, the hardness and sugar concentration of litchi fruits treated with fermentation filtrate were significantly higher than those of other treatment groups, indicating that J-11 fermentation filtrate could not only effectively inhibit the occurrence of litchi sour rot, but also maintain the quality of fruits to a certain extent. These results further confirmed the application potential of J-11 fermentation filtrate in the control of litchi sour rot.

Although this study has achieved certain results, there are still some limitations. First, this study only conducted efficacy tests of fermentation filtrate under laboratory conditions, and has not carried out large-scale application verification in actual post-harvest storage and transportation environments of litchi. Secondly, although strain J-11 showed good salt tolerance and high-temperature resistance, its long-term stability and adaptability under extreme environmental conditions still need further study. In addition, the impact of J-11 fermentation filtrate on other quality indicators of litchi fruits (such as vitamin content, flavor substances, etc.) has not been systematically evaluated, which will be one of the future research directions.

## 5. Conclusions

In this study, a yeast strain J-11 with significant inhibitory effect on *Geotrichum candidum*, the pathogen of litchi sour rot, was screened from papaya soil. The strain showed high inhibition rate and good salt tolerance and high-temperature resistance. In the litchi fruit efficacy test, J-11 fermentation filtrate could effectively reduce the disease index of litchi sour rot and maintain the hardness and sugar concentration of fruits, showing good control effect and fruit quality protection ability. Therefore, J-11 fermentation filtrate is expected to be used as a new biological control agent in the control of litchi sour rot, providing new strategies and methods for the biological control of post-harvest diseases of litchi.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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## References

- [1] Zhang, Z.K.; Huber, D.J.; Qu, H.X. et al. Enzymatic browning and antioxidant activities in harvested litchi fruit as influenced by apple polyphenols. *Food Chemistry*, 2015, 171, 191-199.
- [2] Xu D D.; Deng Y Z.; Xi P G, et al. Biological activity of pterostilbene against *Peronophythora litchii*, the litchi downy blight pathogen. *Postharvest Biological Technology*. 2018, 144, 29-35.
- [3] Vinod, K.; Ajit K D A. *Postharvest Diseases of Litchi and Their Management*. CRC Press, 2021.
- [4] Zhang, N.; Wang, Z.Q.; Shao, J.H. et al. Biocontrol mechanisms of *Bacillus*: improving the efficiency of green agriculture. *Journal Microbiology Biotechnology*, 2023, 16, 2250-2263.
- [5] Ubo, D.; Pan, H.Y.; Wang, Y. et al. Biocontrol mechanism of strain DB02 against watermelon wilt and damping-off diseases caused by *Fusarium oxysporum* f. sp. *niveum* and *Rhizoctonia solani*. *Food Bioscience*, 2025, 71, 107282.
- [6] Wang, X.; Zou, S.C.; Cang, N.P. et al. Enhancement of antioxidant quality by *Bacillus siamensis* N-1 and the role of Lcpod4 in regulating peel browning and senescence of litchi fruit. *Postharvest Biology and Technology*, 2024, 209, 112701.
- [7] Wu, Y.J.; Lin, H.T.; Lin, Y.F. et al. Effects of biocontrol bacteria *Bacillus amyloliquefaciens* LY-1 culture broth on quality attributes and storability of harvested litchi fruit. *Postharvest Biology and Technology*, 2017, 132, 81-87.
- [8] Li, H.; Jia, W.J.; Li, R. et al. The combined application of *Debaryomyces hansenii* and *Bacillus atrophaeus* inhibits disease development and enhances postharvest quality in litchi fruit by activating flavonoid metabolism. *Biological Control*, 2023, 187, 105357.
- [9] Situ, U.J.; Zheng, L.; Xu, D.D.; Gu, C, et al. Screening of effective biocontrol agents against postharvest litchi downy blight caused by *Peronophythora litchii*, *Postharvest Biology and Technology*, 2023, 198, 112249.
- [10] Jiang Y M.; Zhu X R.; LiY B. Postharvest control of litchi fruit rot by *Bacillus subtilis*. *LWT-Food Science.Technology*, 2001, 34, 430-436.
- [11] Li, R.P.; Zhang, H.Y.; Liu, W.M. et al. Biocontrol of postharvest gray and blue mold decay of apples with *Rhodotorula mucilaginosa* and possible mechanisms of action. *International Journal of Food Microbiology*, 2011, 146(2), 151-156.
- [12] Florencia, I.C.; Pedro, E.S.; Santiago, B.G. et al, Unveiling the potential of native Blueberry-derived yeasts as biocontrol agents against *Botrytis cinerea* and *Alternaria alternata*. *Biological Control*, 2025, 209, 105870,
- [13] Nally, M.C.; Pesce, V.M.; Maturano, Y.P. et al. Biocontrol of *Botrytis cinerea* in table grapes by non-pathogenic indigenous *Saccharomyces cerevisiae* yeasts isolated from viticultural environments in Argentina. *Postharvest Biology &*

*Technology*, 2012, 64(1), 40-48.



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