Research Article

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Study on the optimal antagonistic effect of a bacterial complex against Monilinia fructicola in peach

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Abstract: Peach brown rot caused by Monilinia fructicola is one of the most economically destructive diseases of peach (Prunus persica L.) in some orchards of China. Biocontrol is a significant strategy that exhibits strong levels of control and ecologically sound concepts in disease management. The purpose of this study was to investigate the combined suppressive effects of three endophytic bacterial strains (xj-14, xj-15, and xj-16) and two soil rhizosphere bacterial strains (xj-A and xj-C) that were shown to have strong inhibitory activity toward M. fructicola in our previous study. The optimal strains and the optimized combination of strains were determined. The combination of strains xj-15 and xj-C inhibited M. fructicola more intensively for a longer period of time. Following the application of $1 \times 10^9$ CFU/mL bacterial complex to the fruits, leaves, and shoots of peach trees infected with M. fructicola, the rate of inhibition reached 73.80%, 83.33%, and 90.43%, respectively. A pot experiment using lettuce (Lactuca sativa) showed that inoculation with the bacterial complex significantly increased the growth of seedlings. In this study, some compound bacteria were more effective than those in previous study in suppressing disease and promoting growth, which have the potential to be further applied in the field.

Keywords: peach, biocontrol, optimal combination, screening

1 Introduction

Brown rot is a disease caused by the fungus Monilinia fructicola (G. Winter) Honey. It can occur during the whole growth period of peach (Prunus persica L.) and affect blooms, twigs, and fruit [1]. Brown rot affects the leaves, stems, and fruits of peach trees and other stone fruit trees, including apricot, plum, and cherries [2–5]. Monilinia fructicola lives through the winter as mycelia in overwintered fruit mummies or lesions on peach shoots. It produces a large number of conidia in the following spring, and they are disseminated by wind, rain, and insects [1,6]. The fungus infects the plant through wounds caused by insects, mechanical wounds, or natural cavities in the body of the tree, so the plant is susceptible to M. fructicola, particularly under conditions of prolonged rain or high humidity in rainy autumn. Fruits infected with M. fructicola rot at high levels, causing enormous economic losses [7].

Currently, chemical control is still one of the most important measures to control brown rot of peach. The main fungicides used include tebuconazole, propiconazole, fluorosilazole, and nitrile oxazole, among others [8]. However, these agents have been used continuously for many years, and pathogens have become resistant to them, resulting in a reduced ability to control pathogens [9]. Biological control using microbes and their metabolites can inhibit or kill plant pathogens without leaving pesticide residues in fruits and it has the additional functions of improving soil and maintaining its ecological balance; this combination is conducive to the sustainable development of agriculture, so the use of biological control for pests and diseases has attracted much attention [10]. Currently, the primary type of biological control used against brown rot of peach is Bacillus subtilis (Ehrenberg) Cohn, which is frequently used in the field as a biofungicide [11–13].
Despite the sole use of this biofungicide in the biological control of plant diseases, it shows problems of poor adaptability. It has been shown to be ineffective in controlling pathogens in the field, which has resulted in inefficient and inconsistent disease suppression [14]. Therefore, the research and application of complex microbes are urgently needed to increase their utilization in the field. The complementary functions of different combinations of antagonistic bacteria increase their capacity to resist plant diseases. It has been reported that the survival rate of transplanted camphor seedlings treated with complex biofungicides was 30% higher than that of the control group. Moreover, the contents of soluble sugars, chlorophyll, and proline in transplanted camphor seedlings were significantly higher than those of the control group [15]. The effect of complex biofungicides comprising *B. subtilis* and different combinations and proportions of bacterial strains on the control of strawberry anthracnose was much higher than that of a single bacterial agent, and these treatments significantly reduced the incidence of strawberry anthracnose [16]. Additional studies showed that complex biofungicides caused both disease suppression and the promotion of plant growth [17,18]. However, the possibility of competition for space and nutrients among different antifungal agents merits consideration [19]. More research needs to be conducted to explore the optimal combination of different antagonistic bacteria and the effects of the microbial complexes.

Three strains of *B. subtilis* (Ehrenberg) Cohn (xj-14, xj-15, and xj-16), one strain of *B. tequilensis* Gatson (xj-A), and one strain of *B. methylotrophicus* (xj-C) with strong antagonistic activity toward peach brown rot were isolated in our previous research [20,21]. However, each individual strain was effectively antagonistic only for a short time. Therefore, it is necessary to explore the optimal combination of these five antagonistic bacteria to achieve a longer period of disease control. The optimal culture conditions of the bacterial complex were explored. The inhibitory effect of the fermentation broth on tissues of peach trees *in vitro* was studied to obtain a better complex bacterial preparation that is highly effective in controlling brown rot of peach and can lay a foundation for utilization in the field.

## 2 Materials and methods

### 2.1 Source of the antagonistic bacteria and pathogen

Three strains of *B. subtilis* designated xj-14, xj-15, and xj-16 were isolated from the roots of peach trees. One strain of *B. tequilensis* designated xj-A and one strain of *B. methylotrophicus* designated xj-C were isolated from the rhizosphere soil of peach trees. *Monilinia fructicola*, the pathogenic fungus that causes peach brown rot, was isolated from peach orchards in the Pinggu District of Beijing in China and stored at 4–10°C in the Key Laboratory for Northern Urban Agriculture Ministry of Agriculture and Rural Affairs.

### 2.2 Antagonistic activity among bacteria

The Oxford cup method was used to conduct the experiment on the exclusionary effects among bacteria [22]. The fermentation broth of one strain was poured into a culture dish. After the media had cooled and solidified, tweezers were used to place a sterile Oxford cup on the medium. Next, the fermentation broth of other strains was poured into the Oxford cup. The amount of strains in the Oxford cup was 0.5, 1.0, 1.5, and 2.0 times greater than that mixed in the plate. After 2 days of incubation, there was no obvious antagonism between the strains mixed in the plate and the strains poured in the Oxford cup if there was no inhibitory zone around the Oxford cup. In contrast, there was antagonism between the two strains if an obvious inhibitory zone appeared. In this experiment, 30 combinations were examined, and the most promising combination was selected for further study. All the treatments were repeated in triplicate.

### 2.3 Detection of the antagonistic effect of bacteria on *M. fructicola*

The inhibitory effect of the fermentation broth on brown rot of peach was determined using the filter paper method [23]. A block of the pathogenic fungus on agar was placed in the center of a Petri dish that contained Potato Dextrose Agar medium, and six pieces of aseptic filter paper were placed at a distance of 1 cm from the edge of the dish. The same volume of fermentation broth of the single strain or the complex mixture of strains was added to the filter paper at ratios of 1:1, 2:1, and 1:2 to detect the antimicrobial effect. The control group was the uninoculated liquid culture medium. Each treatment was repeated in triplicate. The diameter of the pathogenic colony was measured, and the inhibition rate was calculated. The formula for the calculation of the inhibition rate (%) is as follows: (colony diameter of the
control group – colony diameter of the treatment group)/
(colony diameter of the control group – the diameter of the
original block) \times 100\%.

2.4 Optimization of the fermentation
conditions of complex bacteria

The optimal media for the complex bacteria were studied
using the basic medium as a starting point. An organic
nitrogen source (beef extract, yeast extract, peptone, and
tryptone) and an inorganic nitrogen source (ammonium
sulfate and urea) were used to replace the nitrogen source
in the basic medium [24]. The medium without the
addition of additional nitrogen was used as the control.
Glucose, sucrose, fructose, and soluble starch were used
individually to replace the carbon source in the basic
medium, and the medium without carbon was used as the
control. Each treatment was repeated in triplicate. The bacteria were incubated at 37°C while shaking at 150 rpm
for 24 h. Additional conditions of the fermentation culture
were optimized based on the optimal nitrogen and carbon
source. The temperatures examined were 25, 30, and 35°C.
The optimal volumes for inoculation were 1, 3, 5, 7, and
9% of the culture volume. The volume of liquid in each
250 mL flask was 40, 60, 80, 100, and 120 mL. The initial
pH values were 5, 5.5, 6, 6.5 and 7, respectively. Each
treatment was repeated in triplicate. The antagonistic
activity of the fermentation broth was determined as
described in Section 2.3.

2.5 In vitro direct antagonistic effects of the
bacterial complex on M. fructicola

The antagonistic effect of the bacterial complex on M. fructicola was determined as described in ref. [21]. The
fermentation broth of complex bacteria was studied
using three gradients, i.e., 1 \times 10^7, 1 \times 10^8, and 1 \times
10^9 CFU/mL.

Fresh and disease-free fruits were washed with
sterile water to remove dust from their surface. The
following process was conducted in a laminar flow hood.
The fruits were disinfected in a solution of 1% NaClO for
2 min and rinsed three times with sterile water. The
surface was disinfected with 75% alcohol on a sterilized
bench and rinsed with sterile water an additional three
times before inoculation. A sterile hole punch was used
to make a hole approximately 5 mm deep and wide on
the equator of each fruit. The fruits with wounds were
placed in the fermentation broth for 15 min and then
transferred to a new sterile culture dish. A block of M. fructicola was introduced into the wounds and kept at
28°C for 3 days followed by observation of the extent of
infection.

The surfaces of the leaves and shoots were disinfected by immersion in a solution of 1% NaClO for 2 min
and then rinsed three times with sterile water. Leaves at
similar developmental stages were wounded at six spots
along the main vein at the mesophyll using a sterile scalpel. The wounds were approximately 1 mm in length.
Each healthy shoot was wounded once on the phloem
using a sterile scalpel. The wounded leaves and shoots
were immersed in fermentation broth for 4 h. A block of
M. fructicola was then placed in each wound in the
leaves and shoots before incubation in a 28°C controlled-

2.6 Effect of the bacterial complex on the
growth of lettuce seeds and seedlings

There were the following eight treatments in this
experiment: (I) sterilized water, (II) the culture medium
of the bacterial complex, (III) fermentation broth of the
bacterial complex, (IV) a 50-fold dilution of the
fermentation broth containing the bacterial complex,
(V) a 100-fold dilution of the fermentation broth of the
bacterial complex, (VI) the autoclaved fermentation
broth of the bacterial complex, (VII) a 50-fold dilution
of the autoclaved fermentation broth of the bacterial
complex, and (VIII) a 100-fold dilution of the autoclaved
fermentation broth of the bacterial complex.

Lettuce (Lactuca sativa) seeds of the variety “bei-
sansheng no. 2” were incubated for 3 days at 4°C to
accelerate germination, and the treated seeds were
placed on sterile moistened filter paper in a glass culture dish. After 1 day of culture, the seeds began to germinate. When the embryos appeared, ten seeds of the same size were chosen and placed in glass culture dishes that contained wet filter paper. A total volume of 25 mL of the optimal fermentation broth of the bacterial complex was added to each culture dish. Ten seeds per 25 mL of the optimal fermentation broth of the bacterial strain isolated from the soil or endophytes of peach trees may die when they are mixed with different strains or utilized at different concentrations. An examination of 30 combinations and four different concentration gradients indicated that the antagonistic effect among the five strains, including xj-14, xj-15, xj-16, xj-A, and xj-C, was present only in xj-16 and xj-C when a ratio of 1:2 was applied. No antagonistic effect was found among the other four strains, which indicated that any combination of these strains has the potential to serve as a complex of antimicrobial agents (Figure 1a–d). The combination of xj-15 and xj-C had the strongest antagonistic ability against *M. fructicola* with a rate of antagonism that was as high as 54%, 15%, and 2% greater than that of the single strains xj-15 and xj-C (Table 1). The pathogenic mycelia that were inhibited had a small range of distribution (Figure 1e and f). In the initial stage of culture, the antagonistic effects of the combined bacterial strains xj-15 and xj-C on *M. fructicola* were not observable (Figure 2a and b). However, after 33 days of culture, the combined bacterial mixture still had a strongly antagonistic effect toward *M. fructicola*, while the single xj-15 and xj-C strains completely lost their antagonistic ability (Figure 2c).

### 2.7 Statistical analysis

The collected data were subjected to one-way analysis of variance using SPSS 20.0 software. The comparison of mean effects was based on Duncan’s new multiple range test at a significance level of 0.05.

### 3 Results

#### 3.1 The bacterial complex with the optimal antagonistic effect against *M. fructicola* comprised a combination selected out of five strains

The degree of antagonism of each type of antagonistic bacterial strain isolated from the soil or endophytes of peach trees may differ when they are mixed with different strains or utilized at different concentrations. An examination of 30 combinations and four different concentration gradients indicated that the antagonistic effect among the five strains, including xj-14, xj-15, xj-16, xj-A, and xj-C, was present only in xj-16 and xj-C when a ratio of 1:2 was applied. No antagonistic effect was found among the other four strains, which indicated that any combination of these strains has the potential to serve as a complex of antimicrobial agents (Figure 1a–d). The combination of xj-15 and xj-C had the strongest antagonistic ability against *M. fructicola* with a rate of antagonism that was as high as 54%, 15%, and 2% greater than that of the single strains xj-15 and xj-C (Table 1). The pathogenic mycelia that were inhibited had a small range of distribution (Figure 1e and f). In the initial stage of culture, the antagonistic effects of the combined bacterial strains xj-15 and xj-C on *M. fructicola* were not observable (Figure 2a and b). However, after 33 days of culture, the combined bacterial mixture still had a strongly antagonistic effect toward *M. fructicola*, while the single xj-15 and xj-C strains completely lost their antagonistic ability (Figure 2c).

#### 3.2 Optimization of the culture conditions of the bacterial complex

##### 3.2.1 Optimization of carbon and nitrogen sources for the bacterial complex

Table 2 shows that the highest antagonistic radius of the fermentation broth of 13.2 and 11.1 mm was reached when glucose was used as the carbon source and yeast extract was used as the nitrogen source, respectively. Therefore, glucose and yeast extract were selected as the carbon and nitrogen sources for the bacterial complex.

##### 3.2.2 Optimization of the fermentation conditions of the bacterial complex

To optimize the fermentation conditions of the bacterial complex selected, the optimal conditions were explored through the evaluation of the antibacterial circle diameter and the concentration of bacteria. The optimal concentrations of glucose and yeast extract were 75 and 20 g/L, respectively (Figure 3a and b). The quantity of inoculum was 3% (Figure 3c). The ratio of liquid volume to the flask volume was 100:250 (Figure 3d). The suitable pH was 5.5, and the optimal temperature for the growth of bacteria was 30°C (Figure 3e and f).
Figure 1: Exclusionary effect among strains at different concentrations and antagonistic effect of different ratios of the bacterial complex on *Monilinia fructicola*. (a–d) The exclusionary effect of strain xj-15 in the medium and the other strain in the Oxford cup with different ratios. The numbers 1–4 in the plate represent the ratio of the two strains (0.5:1, 1:1, 1.5:1, and 2:1, respectively). (e–h) The antagonistic effect of the bacterial complex on *M. fructicola*. The numbers 1–6 in the plate represent a single strain or different combinations of the five strains: E1: xj-14; E2: xj-14:xj-15 = 1:1; E3: xj-14:xj-15 = 2:1; E4: xj-14:xj-15 = 1:2; E5: xj-15; E6: the culture medium. F1: xj-15; F2: xj-15:xj-16 = 1:1; F3: xj-15:xj-16 = 2:1; F4: xj-15:xj-16 = 1:2; F5: xj-16; F6: the culture medium. G1: xj-15; G2: xj-15:xj-A = 1:1; G3: xj-15:xj-A = 2:1; G4: xj-15:xj-A = 1:2; G5: xj-A; G6: the culture medium. H1: xj-15; H2: xj-15:xj-C = 1:1; H3: xj-15:xj-C = 2:1; H4: xj-15:xj-C = 1:2; H5: xj-C; H6: the culture medium.

Table 1: Antagonistic effect of five single strains and the bacterial complex on the growth of *Monilinia fructicola*

| Culture mode                  | Plate number | Combination ratio | Strains                | Antagonistic radius (mm) | Inhibition rate (%) |
|-------------------------------|--------------|-------------------|------------------------|--------------------------|---------------------|
| Single strain culture         | E6, F6, G6, H6 | Culture medium    | xj-14                  | 9.05 ± 0.15              | 39.12               |
|                               | E1           |                   | xj-15                  | 9.14 ± 0.15              | 39.23               |
|                               | E5, F1, G1, H1 |                 | xj-16                  | 6.90 ± 0.15              | 30.11               |
|                               | F5           |                   | xj-A                   | 10.47 ± 0.10             | 45.12               |
|                               | G5           |                   | xj-C                   | 12.05 ± 0.22             | 52.21               |
| Different ratios of bacteria in the complex | E2 | 1:1 | xj-14:xj-15 | 10.64 ± 0.11b | 46.12 |
|                               | E3           | 2:1               | xj-14:xj-15            | 9.82 ± 0.09b            | 42.45               |
|                               | E4           | 1:2               | xj-14:xj-15            | 9.79 ± 0.07cd           | 42.14               |
|                               | F2           | 1:1               | xj-15:xj-16            | 9.39 ± 0.171b           | 40.18               |
|                               | F3           | 2:1               | xj-15:xj-16            | 8.48 ± 0.255c           | 36.54               |
|                               | F4           | 1:2               | xj-15:xj-16            | 9.11 ± 0.417c           | 39.23               |
|                               | G2           | 1:1               | xj-15:xj-A             | 8.59 ± 0.245d           | 37.18               |
|                               | G3           | 2:1               | xj-15:xj-A             | 8.34 ± 0.318d           | 36.51               |
|                               | G4           | 1:2               | xj-15:xj-A             | 9.97 ± 0.265b           | 43.62               |
|                               | H2           | 1:1               | xj-15:xj-C             | 12.54 ± 0.167a          | 54.92               |
|                               | H3           | 2:1               | xj-15:xj-C             | 12.37 ± 0.102a          | 53.18               |
|                               | H4           | 1:2               | xj-15:xj-C             | 12.43 ± 0.159a          | 54.12               |

Note: “±” represents the standard deviation, whereas lowercase letters represent a significant difference (Duncan’s test, *P* < 0.05).
3.2.3 The fermentation broth of the bacterial complex cultured with the optimized medium increases the antagonistic ability against *M. fructicola*. The rate of inhibition of the bacterial complex against *M. fructicola* was 46% and 50%, respectively, when the fermentation broth was cultured under the basic conditions. The rate of inhibition reached 71% when the conditions of fermentation were optimized (Table 3). The optimized culture conditions increased the antagonistic activity of the bacterial complex, and they were adopted in the following experiment.

3.3 Antagonistic ability of bacteria against *M. fructicola* on peach plants *in vitro*

Peach fruit, leaves, and branches were treated *in vitro* with the fermentation broth from the bacterial complex at $1 \times 10^7$, $1 \times 10^8$, and $1 \times 10^9$ CFU/mL. The fermentation broth at different concentrations was antagonistic against *M. fructicola* (Figure 4). The antagonistic effect became much stronger as the concentration of the fermentation broth increased. At a fermentation broth concentration of $1 \times 10^9$ CFU/mL, the rate of inhibition reached values as high as 73.80%, 83.33%, and 90.40% for peach fruit, leaves, and branches, respectively; moreover, the incidence was low (Table 4).

### Table 2: Antagonistic effect of the fermentation broth of the bacterial complex with different carbon and nitrogen sources against *Monilinia fructicola*

| Nitrogen source      | Antagonistic radius (mm) | Carbon source     | Antagonistic radius (mm) |
|----------------------|--------------------------|-------------------|--------------------------|
| Tryptone             | 8.26 ± 1.27c             | Glucose           | 13.2 ± 0.97a             |
| Peptone              | 8.01 ± 0.69c             | Sucrose           | 12.2 ± 1.2ab             |
| Yeast extract        | 11.10 ± 0.67a            | Soluble starch    | 9.90 ± 1.64ab            |
| Beef extract         | 10.14 ± 0.72b            | Fructose          | 11.4 ± 1.68b             |
| Ammonium sulfate     | 6.60 ± 0.91d             |                   |                          |
| Urea                 | 10.73 ± 0.83ab           |                   |                          |

Note: “±” represents the standard deviation, and lowercase letters represent a significant difference (Duncan’s test, $P < 0.05$).

3.4 Effect of the bacterial complex on the germination of lettuce seeds and growth of seedlings

Lettuce seeds could not germinate in the following culture media used for the growth of the bacterial complex: the culture medium, fermentation broth, and autoclaved fermentation broth (Figure 5a–c). The lettuce seeds germinated well in the diluted and autoclaved diluted fermentation broths. The increase in radicle length and fresh weight was much higher in seeds treated with the control and autoclaved fermentation broths that had been diluted 100-fold compared to that of the other treatments (Table 5).

The strongest promotion of growth was observed following treatment with the control or autoclaved fermentation broth diluted 100-fold. The root length, plant height, and fresh weight of lettuce plants treated with fermentation broth diluted 100-fold (treatment VI) increased by 1.2, 3.1, and 7.5 times, respectively, compared with the treatment with the culture medium (treatment IV; Table 6). The leaf number and the chlorophyll content of treatment VI were the highest.
among all treatments. No difference in the content of vitamin C was observed between treatments IV and VI.

4 Discussion

Five strains of Bacillus xj-14, xj-15, xj-16, xj-A, and xj-C that were antagonistic toward M. fructicola were utilized in this experiment. A total of 30 experimental combinations were designed at three levels (1:1, 2:1, and 1:2). There was almost no strong exclusionary reaction between any of the two combinations of the five strains. However, when the ratio of strains xj-16 and xj-C was 1 to 2, an obvious inhibitory circle appeared. These results have been reported previously [20]. When multiple strains were cultured together, there was a certain antagonistic effect, which required adjustment. The ratio of strains and order of inoculation were used to reduce the antagonism of each strain in co-culture. Our results showed that the mixed culture of different strains was not suitable for all applications.

The plate confrontation method proved that the combination of strains xj-15 and xj-C was the most active against M. fructicola at all levels. The rate of antagonism reached 54%, which was higher than that of any of the five strains separately (Figure 1). Moreover, this value was determined when the combination of strains xj-15 and xj-C (Figure 2) was cultured for longer duration, which indicated that the combination of the two strains had a strong synergistic effect. Strain xj-C is an isolate of B. methylotrophicus. There are few reports about its application in biological control compared with other B. subtilis, and even fewer reports about its application combined with other strains, particularly for the control of M. fructicola. However, the application of a powdered preparation of B. methylotrophicus WF-3 was more effective than other biofungicides in protecting against cucumber anthracnose [25]; the antagonistic effect was
as high as 77.38% and 72.69% compared with the control, respectively. The effect of strain xj-15 on the biological control of *M. fructicola* was stronger in the previous study [20]. In this experiment, although the single strains xj-14 and xj-16 demonstrated the strongest antagonistic ability, the rate of inhibition of the combination did not exceed that of the single strain xj-16 in any ratio of the two strains. These results showed that the effect produced by the combination of strains was quite different; it was lower or higher than that of a

**Figure 4**: The antagonistic ability of the bacterial complex against *Monilinia fructicola* in the fruits, leaves, and shoots of a peach tree. (a) Peach fruit. (b) Peach leaves. (c) Peach branches. (1) Water treatment as the control; (2) bacterial complex fermentation broth with a concentration of $1 \times 10^7$ CFU/mL; (3) bacterial complex fermentation broth with a concentration of $1 \times 10^8$ CFU/mL; and (4) bacterial complex fermentation broth with a concentration of $1 \times 10^9$ CFU/mL.

**Table 4**: Antagonistic effect of the bacterial complex against *Monilinia fructicola* in fruits, leaves, and shoots treated with different concentrations of fermentation broth

| Different peach tree tissues | Different concentrations of fermentation broth (CFU/mL) | Incidence (%) | Lesion diameter (cm) | Rate of inhibition (%) |
|-----------------------------|--------------------------------------------------------|----------------|----------------------|------------------------|
| Fruits                      | a: control                                             | 100.00a        | 9.24 ± 0.30d         | —                      |
|                             | b: $1 \times 10^7$                                     | 100.00a        | 7.62 ± 0.33d         | 17.53                  |
|                             | c: $1 \times 10^8$                                     | 66.71b         | 5.20 ± 0.28d         | 43.72                  |
|                             | d: $1 \times 10^9$                                     | 33.33c         | 2.42 ± 0.78c         | 73.80                  |
| Leaves                      | a: control                                             | 100.00a        | 0.72 ± 0.31a         | —                      |
|                             | b: $1 \times 10^7$                                     | 81.57b         | 0.38 ± 0.17a         | 47.22                  |
|                             | c: $1 \times 10^8$                                     | 73.78b         | 0.27 ± 0.10a         | 60.01                  |
|                             | d: $1 \times 10^9$                                     | 42.45c         | 0.12 ± 0.05b         | 83.33                  |
| Branches                    | a: control                                             | 100.00a        | 2.50 ± 0.31c         | —                      |
|                             | b: $1 \times 10^7$                                     | 66.67b         | 1.30 ± 0.31b         | 48.13                  |
|                             | c: $1 \times 10^8$                                     | 33.33c         | 0.62 ± 0.08a         | 75.21                  |
|                             | d: $1 \times 10^9$                                     | 33.33c         | 0.24 ± 0.03a         | 90.43                  |

Note: “±” represents the standard deviation, whereas lowercase letters represent a significant difference (Duncan's test, $P < 0.05$).
single strain, or no significant effect was observed [26]. The bacterial complex requires optimization by more experiments.

The conditions of fermentation affected the field application of complex bacteria [27]. In this study, the most suitable carbon and nitrogen sources for the production of the bacterial complex were optimized using a single factor test. The results showed that with different carbon and nitrogen sources, the growth of strains positively correlated with the antimicrobial activity to some extent. Glucose as the carbon source and yeast extract as the nitrogen source aided the growth of the bacterial complex. The other optimal fermentation conditions, including the quantity of inoculum, the liquid volume in flask, the initial pH, and the temperature of the fermentation, were obtained. The antimicrobial activity of the bacterial complex increased under the optimized fermentation conditions.
On the basis of the optimal bacterial complex obtained, the effects of this complex against *M. fructicola* in peach plants *in vitro* and on the growth of lettuce seeds and seedlings were studied. To explore the occurrence of *M. fructicola in vitro*, three healthy types of peach tissues, including fruits, branches, and leaves, were selected for evaluation using three different concentrations of the fermentation broth. The results showed that the fermentation broth of the optimal bacterial complex decreased the incidence of tissue infection and increased the rate of inhibition, which showed that the bacterial complex had a substantial antagonistic effect on *M. fructicola*. However, more research studies need to be developed to verify the antagonistic effect of the bacterial complex in the presence of complex soil microorganisms and under environmental conditions that the bacteria would encounter during application in the field. Additionally, the bacterial complex had a notable effect on the germination of lettuce seeds and seedling growth. The bacterial complex comprising rhizosphere soil isolates of strain xj-C can prevent infections by some plant pathogens [25,28,29]. Moreover, the rhizosphere soil bacteria can promote plant growth [30,31]. The main reason was the production of plant growth-promoting substances synthesized by bacteria or the promotion of the absorption of nutrients in the growing environment [32,33]. Through our study, we obtained a complex biofungicide with strong resistance to *M. fructicola* and growth promoting activity, which can be further applied in the field following additional research.

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**Author contributions:** Y.L. H.C. and F.W. designed the experiments. H.C. and H.Y. performed the experimental procedures. T.H. and H.C. analyzed the data. Y.L. and H.C. wrote the paper.

### Table 5: Effect of different treatments of the fermentation broth of the bacterial complex on the germination and growth of lettuce seeds

| Treatment | Radicle length (mm) | Dry weight (mg) | Fresh weight increase (mg) |
|-----------|---------------------|-----------------|---------------------------|
| I         | 0                   | 0               | 0                         |
| II        | 0                   | 0               | 0                         |
| III       | 0                   | 0               | 0                         |
| IV        | 13.18 ± 0.67b       | 3.76 ± 0.75b    | 35.40 ± 1.49b             |
| V         | 4.53 ± 0.73c        | 6.08 ± 1.22a    | 34.11 ± 2.00b             |
| VI        | 23.94 ± 0.39a       | 6.32 ± 1.26a    | 51.94 ± 1.44a             |
| VII       | 15.96 ± 0.52b       | 4.56 ± 0.42b    | 29.29 ± 1.09b             |
| VIII      | 22.10 ± 0.36a       | 4.66 ± 0.93b    | 51.43 ± 1.18a             |

Note: “s” represents the standard deviation, whereas lowercase letters represent a significant difference (Duncan’s test, *P* < 0.05).

(i) Fermentation broth of the bacterial complex; (II) the autoclaved fermentation broth of the bacterial complex; (III) the culture of the bacterial complex; (IV) sterilized water; (V) 50-fold dilution of the fermentation broth of the bacterial complex; (VI) 100-fold dilution of the fermentation broth of the bacterial complex; (VII) 50-fold dilution of the autoclaved fermentation broth of the bacterial complex; (VIII) 100-fold dilution of the autoclaved fermentation broth of the bacterial complex.

### Table 6: Effect of different fermentation broth treatments of the bacterial complex on the growth of lettuce seedlings

| Treatment | Root length (cm) | Plant height (cm) | Fresh weight (g) | Number of leaves | Chlorophyll content (mg/g) | Vitamin C content (mg/g) |
|-----------|------------------|-------------------|------------------|-----------------|---------------------------|--------------------------|
| I         | 8.93 ± 0.21a     | 9.87 ± 0.19bc     | 2.94 ± 0.19c     | 11 ± 2d         | 11.30 ± 0.67a             | 6.61 ± 1.87b             |
| II        | 9.30 ± 0.51a     | 10.13 ± 0.43bc    | 4.54 ± 0.43d     | 11 ± 2d         | 13.3 ± 2.05ab             | 7.66 ± 0.22bc             |
| III       | 8.86 ± 1.03a     | 8.21 ± 0.54b      | 1.17 ± 0.23a     | 7 ± 1ab         | 12.03 ± 2.95a             | 7.66 ± 0.1bc              |
| IV        | 14.2 ± 0.51bc    | 4.47 ± 3.04a      | 1.22 ± 0.18ab    | 5 ± 1a          | 15.3 ± 3.26ab             | 8.91 ± 0.42c              |
| V         | 13.5 ± 1.75b     | 12.03 ± 0.65cd    | 2.33 ± 0.27c     | 6 ± 1ab         | 13.03 ± 1.17ab             | 5.91 ± 1.85ab             |
| VI        | 16.43 ± 0.95c    | 13.77 ± 1.30d     | 9.14 ± 0.89e     | 14 ± 1e         | 17.93 ± 1.99c             | 9.94 ± 0.58c              |
| VII       | 11.73 ± 0.82ab   | 10.5 ± 0.78bc     | 2.09 ± 0.32bc    | 9 ± 2bc         | 13.27 ± 3.48ab             | 5.67 ± 0.17ab             |
| VIII      | 10.43 ± 2.68a    | 12.77 ± 0.61cd    | 2.42 ± 0.30c     | 8 ± 1bc         | 10.53 ± 2.41a             | 3.68 ± 0.72a              |

Note: “s” represents the standard deviation, whereas lowercase letters represent a significant difference (Duncan’s test, *P* < 0.05).

(i) Fermentation broth of the bacterial complex; (II) the autoclaved fermentation broth of the bacterial complex; (III) the culture of the bacterial complex; (IV) sterilized water; (V) 50-fold dilution of the fermentation broth of the bacterial complex; (VI) 100-fold dilution of the fermentation broth of the bacterial complex; (VII) 50-fold dilution of the autoclaved fermentation broth of the bacterial complex; (VIII) 100-fold dilution of the autoclaved fermentation broth of the bacterial complex.
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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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