Screening of endophytic bacteria antagonistic against *Ustilaginoidea virens* in rice

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Abstract. Rice false smut (RFS) caused by *Ustilaginoidea virens* is a major disease in rice-producing areas in China. Endophytic bacteria have prominent advantages in controlling host plant diseases. To screen and obtain the antagonistic bacteria of RFS and effectively control RFS, the antagonistic strains were screened from 45 rice endophytic bacteria by the method of plate pair culture, the antagonistic activity of the fermentation broth of the antagonistic strains was measured, and the RFS field control test was performed. The results showed that 45 strains of rice endophytic bacteria had antagonistic activity against *U. virens*, of which 23 strains had an antagonistic band of more than 15 mm. The fermented broth of these 23 antagonistic strains had strong antagonistic activity against *U. virens*, of which 17 strains had antagonistic band of more than 20 mm, and 3 of them (REB01, REB32, and REB42) had more than 28 mm. The three strains (REB01, REB32 and REB42) had 81.48%, 72.05% and 76.76% respectively field control effect on RFS, which was very significant (*P* < 0.01) higher than the field control effect of 500-fold dilution of 5% Jinggangmycin (61.72%), and showing great potential for biological control of RFS.

1. Introduction

Rice false smut (RFS), also known as pseudosmut, blackball disease, etc, is a fungal disease caused by a fungal pathogen infecting rice flowers. [1, 2]. The causative agent of RFS is an ascomycete fungal pathogen *Villosiclava virens* (anamorph: *Ustilaginoidea virens* [Cooke] Takahashi) [1], which specifically infects rice flowers and transforms the latter into RFS balls [1, 3-6]. RFS is widely distributed in rice growing areas in Asia, Europe, Africa, and the Americas [7, 8]. In the history of China, the incidence of RFS is relatively low, and it is only a minor disease of rice. [9]. In recent years, under the influence of global climate change, with the replacement of rice varieties, the large-scale popularization of high-quality and high-yielding hybrid rice, and the increase in the application of nitrogen fertilizer, RFS has continued to expand, and the damage has increased year by year, and has increased as the main disease from secondary diseases [10-12], and RFS greatly affected high and stable yield of rice [13]. RFS balls formed by *U. virens* not only affects rice yield and quality, but also contains toxins harmful to humans and animals, which can directly harm people's health [14-17]. Therefore, controlling the occurrence and development of RFS is very important for the safe production of rice and ensuring the health of consumers.
At present, the most common control measures for RFS are chemical control, and mainly use carboxin, propiconazole, organic copper preparations and other fungicides to control RFS [18-21]. However, excessive reliance on chemical pesticides in the prevention and control process not only makes the resistance and resistance of pathogenic bacteria increasingly serious, resulting in little control effectiveness, but also has the disadvantages of environmental pollution [22]. Biological control has the advantages of being environment-friendly, non-residual, and resistant to germs. Therefore, it is of great practical significance to use biological control to explore ways to control RFS. At present, the exploration of the use of biological control to control RFS is mainly conducted from three aspects of antagonistic microorganisms [23-24], genetic engineering technology [25] and biological agents [26-27]. The use of genetic engineering techniques and biological agents to control rice oryzae is currently in the experimental stage. Screening antagonistic microorganisms is the main means of biological control of RFS. Trichoderma can produce secondary metabolites and other biologically active substances to antagonize plant pathogenic fungi or bacteria, as well as promote plant growth and enhance the ability of plants to defend against diseases [28]. In the biological control of RFS, Trichoderma plays a very important role [29-30]. The use of bacteria to control plant diseases is an important part of biological control. The common Bacillus used to control RFS is mainly Bacillus subtilis [23, 31, 32]. Yin et al. [32] isolated bacterial isolates from soil samples collected in Yunnan and Jiangsu, and obtained two faster-growing B. subtilis strains through primary screening and re-screening, and the growth inhibition rates of U. virens reached 97.2% and 85.9%; through field tests, the control effects of these two strains of antagonizing bacteria on RFS were 47.88% and 43.12%, respectively. Chen et al [33] isolated a strain of photosynthetic bacteria cs-1 from Rhodopseudomonas, and found that the fermentation supernatant solution had a 100% inhibition rate on germination of U. virens spores, the inhibitory rate of U. virens reached 57.53%. After the treatment of the original supernatant of the fermentation liquid of cs-1 by U. virens, the distance between the branches of the mycelium of the U. virens was significantly shortened, the fracture was severe, and the mycelia became thin, accompanied by the phenomenon of protoplast coagulation and overflow. In addition, the appropriate concentration of cs-1 fermentation broth can increase the catalase activity in rice and can enhance the resistance of rice to a certain extent. Yian et al. [34] tested the plate inhibitor, field control effect of rice strain U. virens and its colonization effect on U. virens, and found that F26-T strain had stronger effect on RFS The antagonistic effect of F26-T was gradually adapted to the leaf environment and entered the stage of colonization and growth, and the relative control effect of the spore suspension stock solution was as high as 76.93% on the 3rd to 6th days after spray inoculation of rice leaf circumference. However, the types of antagonistic microorganisms currently used for biological control of RFS are insufficient. It is very different from the natural environment in the wild. Therefore, the colonization ability of some biocontrol bacteria in the wild is very poor. At the same time, due to the interference of many factors such as temperature and humidity in the wild environment, the biocontrol effect of the biocontrol bacteria will be serious. decline [35].

Endophytes are non-pathogenic organisms that live inside plant tissues for at least part of their life cycles [36]. Some endophytic bacteria are able to systemically prime the plant’s immune system, Plant endophytic bacteria can have antagonistic effects on plant pathogens due to their ability to produce chitinase, protease and iron carriers [37]. Primed plants do not display major changes in defense-related gene expression in the absence of a pathogen, but mount an accelerated defense response upon pathogen or insect attack, providing broad-spectrum resistance [38-39]. Therefore, a potential to explore the development of endophytic bacteria in agricultural practices should not be ignored. There was 45 strains of endophytic bacteria recently isolated from the stems and seeds of rice grown in Jiuding county and Muchua county, Sichuan province, China. In this experiment, an investigation about the potential of rice endophytic bacteria in suppression of RFS was carried out. The goal of the research outlined in this paper was to explore the potential uses of ric endophytic bacteria in pollution-free prevention and control of RFS.
2. Materials and methods

2.1. The strains and ingredients used in this experiment

45 strains (REB01-REB45) of endophytic bacteria were isolated from the stems and seeds of rice grown in Jiajiang county and Muchuan county, Sichuan province, China recently. *U. virens* used in this experiment was isolated from diseased flowers of rice grown in Muchuan county, Sichuan province, China and was provided and preserved by the Microbiology Laboratory of Leshan Normal University.

2.2. Medium used in this experiment

NA culture medium: beef extract 3.0 g, yeast extract 1.0 g, peptone 5.0 g, glucose 10.0 g, water 1.0 L, agar 20.0 g, pH 7.0.

Potato sucrose (PSA) medium: potato 200.0 g, sucrose 20.0 g, agar 20.0 g, water 1.0 L, pH 7.0.

Wakimoto's medium (XBZ) [40]: potato 300 g, protein 5 g, sucrose 15 g, Ca(NO₃)₂·4H₂O 0.5 g, Na₂HPO₄·12H₂O 2.0 g of, agar 18 g. Add chloramphenicol (50 ug / mL) before sterilize.

2.3. Preliminary screening of endophytic bacteria against *Ustilaginoidea virens*

The purified bacterial isolate was cultured on a NA plate at 28°C for 24 hours. The bacterial isolate was inoculated at the four corners on the plate of Wakimoto's culture medium. The disc of agar (in diameter of 0.70 mm) with the pathogenic *U. virens* incubated at 28°C, 48 h was inoculated in the center of plate and incubated at 28°C in dark. Sterile water was used as a negative control (CK) instead of the endophytic isolate. All treatments and control were set in triplicates. After incubation for five days, the size of pathogen colony was measured. In order to quantitatively evaluate the antagonist activity of the endophytic bacteria, inhibitory bandwidth (IBW) was adopted with the following formula: IBW (mm) = (DCK - DT). Where DT was the diameter of pathogen colony in treatment, DCK was the diameter of pathogen colony in CK [41-42]. The isolates with IBW more than 15.0 mm were considered to be significant of antagonistic activity.

2.4. Antagonistic effect of fermentation broth of antagonistic endophytic bacteria on *Ustilaginoidea virens*

The tested antagonistic endophytic bacteria were inoculated into a test tube containing 5 mL of liquid NA medium, and cultured in a shaker at 28°C and 130 r/min for 36 h. The fermentation broth was filtered through sterilized filter paper, and the filtrate was transferred to a 50 mL centrifuge tube. Centrifuged at 4°C and 8000 r/min for 15 minutes. Take the supernatant and filter it through a 0.22 μm microporous filter for later use. A sterile Oxford cup (inner diameter: 0.7 cm, height: 0.9 cm) was placed in the center of the Wakimoto Zell's medium plate, and 100 μL of the fermentation filtrate of the test antagonistic strain was added to different Oxford cups. Sterile water was used as a negative control (CK) instead of fermentation filtrate of the endophytic isolate. All treatments and control were set in triplicates. After incubation for five days, the size of pathogen colony was measured and IBW was calculated.

2.5. Field control effect of antagonistic endophytic bacteria on rice false smut

The screened antagonistic bacteria were used to prepare bacterial fermentation broth (with a bacterial content of 1 × 10⁸ CFU/mL). Spray inoculated 3 times in a row at the ear differentiation stage of rice. It was advisable to keep the fermented liquid dripping on the leaves of rice. The positive control was 5% Jinggangmycin solution, and the sterile water was negative control. Each treatment was repeated 3 times, each test plot was 8 m² (2 m × 4 m), and the interval of test plot was not less than 2 m, which was randomly arranged. At the yellow ripening stage, a five-point sampling method was used, and 25 clusters of rice were repeatedly surveyed. The survey indicators include the total number of rice ears and diseased rice ears. The number of diseased ears was used as the unit, a 5-level grading method was used to calculate the disease index and control effect [43]. Grading standards for RFS were as follows:
level 0: disease-free; level 1: 1 diseased grain per ear; level 2: 2 to 4 diseased grains per ear; level 3: 5 to 7 diseased grains per ear Level 4: 8-10 diseased grains per ear; Level 5: 10 or more diseased grains per ear.

Diseased index (%) = \( \sum (\text{number of diseased ears at each level} \times \text{relative grade value}) / (\text{total number of ears in the investigation} \times 5) \times 100 \)

Relative control effect (%) = (Control diseased Index - Treatment diseased Index) / Control diseased Index \times 100

DPS 7.05 software was used for statistical analysis [44].

3. Results and analysis

3.1. Preliminary screening of endophytic bacteria against Ustilaginoidea virens

All strains of 45 endophytic bacteria isolated from rice had a antagonistic activity on U. virens (Table 1). The antagonistic activity on U. virens of 45 strains were significantly different. There were 9 strains with IBW below 10 mm, 13 strains with IBW between 10 mm and 15 mm and 23 strains with IBW above 15 mm.

| Strains | Antibacterial bandwidth(mm) | Strains | Antibacterial bandwidth(mm) |
|---------|-----------------------------|---------|-----------------------------|
|         | 1   | 2   | 3   | Average |         | 1   | 2   | 3   | Average |
| REB01   | 15.2| 16.1| 15.4| 15.6  | REB24   | 16.2| 16.8| 17.5| 16.8  |
| REB02   | 12.2| 13.4| 12.6| 12.7  | REB25   | 13.2| 13.5| 13.6| 13.4  |
| REB03   | 11.1| 12.3| 12.2| 11.9  | REB26   | 13.7| 14.1| 14.2| 14.0  |
| REB04   | 10.3| 9.8 | 9.4 | 9.8   | REB27   | 13.5| 14.2| 13.8| 13.8  |
| REB05   | 1.2 | 1.4 | 1.3 | 1.3   | REB28   | 16.1| 16.9| 15.8| 16.3  |
| REB06   | 2.3 | 3.4 | 2.5 | 2.7   | REB29   | 12.2| 12.6| 11.2| 12.0  |
| REB07   | 5.4 | 5.6 | 6.4 | 5.8   | REB30   | 15.2| 15.3| 15.6| 15.4  |
| REB08   | 15.4| 16.6| 16.2| 16.1  | REB31   | 13.1| 12.8| 12.6| 12.8  |
| REB09   | 7.8 | 8.6 | 7.9 | 8.1   | REB32   | 15.2| 15.1| 15.8| 15.4  |
| REB10   | 9.8 | 8.7 | 8.6 | 9.0   | REB33   | 16.3| 16.7| 17.4| 16.8  |
| REB11   | 15.1| 15.7| 15.6| 15.5  | REB34   | 16.5| 17.1| 16.4| 16.7  |
| REB12   | 16.8| 16.5| 17.2| 16.8  | REB35   | 15.3| 15.6| 15.8| 15.6  |
| REB13   | 13.2| 12.6| 13.7| 13.2  | REB36   | 10.2| 11.2| 10.5| 10.6  |
| REB14   | 15.1| 15.2| 15.6| 15.3  | REB37   | 9.8 | 8.6 | 8.2 | 8.9   |
| REB15   | 15.4| 15.6| 16.2| 15.7  | REB38   | 15.6| 16.2| 15.8| 15.9  |
| REB16   | 15.4| 15.7| 15.8| 15.6  | REB39   | 15.6| 16.8| 16.3| 16.2  |
| REB17   | 12.3| 14.5| 14.2| 13.7  | REB40   | 18.2| 17.6| 17.3| 17.7  |
| REB18   | 13.6| 14.2| 13.8| 13.9  | REB41   | 16.7| 17.5| 17.4| 17.2  |
| REB19   | 15.2| 15.4| 15.6| 15.4  | REB42   | 16.8| 16.5| 17.3| 16.9  |
| REB20   | 16.3| 16.5| 15.9| 16.2  | REB43   | 7.4 | 8.3 | 7.6 | 7.8   |
| REB21   | 15.2| 15.2| 15.6| 15.3  | REB44   | 15.6| 15.4| 16.2| 15.7  |
| REB22   | 14.2| 13.7| 14.1| 14.0  | REB45   | 6.2 | 5.4 | 5.1 | 5.6   |
| REB23   | 14.3| 13.7| 13.6| 13.9  |         |       |     |     |       |

3.2. Antagonistic effect of fermentation broth of antagonistic endophytic bacteria on Ustilaginoidea virens

Antagonistic activity of the primary extracellular product of 23 endophytic bacteria strain with with IBW more than 15.0 mm was relatively high (Table 2). There were only 2 strains with IBW less than 15.0 mm, 3 strains with IBW between 15.0 mm and 20.0 mm, 9 strains with IBW between 20.0 mm and 25.0 mm and 8 strains with IBW above 25.0 mm. Among them, 3 strains (REB01, REB32 and
REB42) had significantly higher antagonistic bands ($P < 0.01$) than other strains. These 3 strains (REB01, REB32 and REB42) were used for field experiments in the control of RFS.

### Table 2. Antagonistic effect of rice endophytic fermentation liquid on Ustilaginoidea virens

| Strains   | IBW(mm) | SD  | Strains   | IBW(mm) | SD  |
|-----------|---------|-----|-----------|---------|-----|
|           | 1       | 2   | 3         | Average |     |
| REB01     | 29.3    | 29.1| 28.9      | 29.1    | aA  |
| REB08     | 12.3    | 13.4| 14.1      | 13.3    | iM  |
| REB11     | 13.3    | 12.5| 12.6      | 12.8    | iM  |
| REB12     | 15.6    | 16.4| 17.8      | 16.6    | kL  |
| REB14     | 16.3    | 16.5| 17.4      | 16.7    | kL  |
| REB15     | 18.6    | 19.6| 18.4      | 18.9    | jK  |
| REB16     | 25.8    | 26.2| 27.3      | 26.4    | cdDE|
| REB19     | 20.3    | 22.4| 21.6      | 21.4    | hiU |
| REB20     | 22.5    | 23.1| 22.9      | 22.8    | fgGHI|
| REB21     | 25.7    | 26.5| 27.3      | 26.5    | cdDE|
| REB22     | 24.5    | 24.8| 23.9      | 24.4    | eF  |
| REB24     | 26.9    | 27.8| 27.5      | 27.4    | bcCD|

Notes: IBW means inhibitory bandwidth. SD means significant difference. Different lowercase letters in SD column indicate a significant difference when $P < 0.05$, and different uppercase letters in SD column indicate a significant difference when $P < 0.01$.

3.3. Field control effect of antagonistic endophytic bacteria on rice false smut

The three strains (REB01, REB32 and REB42) had better field control effects on RFS, which was significantly higher ($P < 0.01$) than the positive control, 500-fold dilution of 5% Jinggangmycin (Table 3). The relative control effects of the three strains on RFS were significantly different, and were in order of REB01, REB42, and REB32. The three strains (REB01, REB32 and REB42) has good potential in the control of rice smut disease.

### Table 3 Field control effect of rice antagonistic endophytic bacteria on rice false smut (%)

| Treatment          | Morbidities (%) | Disease index (%) | Relative control effect (%) |
|--------------------|-----------------|-------------------|-----------------------------|
| REB01              | 13.29aA         | 4.33aA            | 81.48aA                     |
| REB32              | 17.54cB         | 6.54cB            | 72.05cB                     |
| REB42              | 15.14bA         | 5.44bAB           | 76.76bAB                    |
| positive control    | 23.35dC         | 8.96dC            | 61.72dC                     |
| negative control    | 57.56eD         | 23.40eD           | -                           |

Note: positive control means 500-fold dilution of 5% Jinggangmycin, negative control means sterile water. Different lowercase letters in the same column indicate a significant difference when $P < 0.05$, and different uppercase letters in the same column indicate a significant difference when $P < 0.01$.

4. Discussion

The use of biocontrol bacteria to control plant diseases was an important part of biological control, and had shown good application prospects. Biocontrol bacteria mainly included Bacillus and Pseudomonas. The common Bacillus used to control RFS was Bacillus subtilis. Endophytic bacteria had a more prominent role in biological control due to their long-term growth and life in the host plant and their interdependence with the host plant. The results of this test showed that 45 strains of rice endophytic bacteria had antagonistic activity against on RFS and the fermentation broth of 23 strains of antagonistic endophytic bacteria also showed good antagonistic activity against on RFS, indicating that the endophytic bacteria were resistant to host plants disease and had a controlling effect.
In recent years, many researches focus on the pathogenicity and prevention of rice diseases [45-47], and RFS had become a major disease in China's rice production, which had caused a significant decrease in the seed setting rate and thousand-grain weight of rice, especially the powdery thick spores of *U. virens*, which had caused major harm to rice quality, human and animal safety, and the natural environment [448]. In addition, researches on the use of antagonistic bacteria to control plant diseases had progressed rapidly, but studies on the use of antagonistic bacteria to control rice blast disease had only stayed in the experimental phase of indoor antagonism [23] or the experimental phase of field control effectiveness was not ideal [24]. At present, related researches on the infection pathway, infection time, and artificial inoculation technology of *U. virens* were in the exploration stage [49-51], and the incidence of artificial inoculation under normal conditions was low, which also given difficult to test the disease control of rice bacterial antagonistic bacteria. In this study, three antagonistic rice endophytic bacteria (REB01, REB32, and REB42) screened in Leshan City were tested in a field trial to control RFS, and the results showed that those antagonistic bacterial strain, which had a strong inhibitory effect on the growth of *U. virens* in a plate test, also had a better control effect in the field, which was significantly higher than the positive control, 500-fold dilution of 5% Jinggangmycin.

The control effect of antagonistic bacteria was greatly affected by various factors [52], such as temperature, humidity, rain, the colonization ability of antagonistic bacteria in the field [53], and the time for antagonistic bacteria to form a dominant group in the field [54, 55], and so on. This also reminds us that we should pay attention to the combination of indoor and outdoor, we should further determine its control effect on plant diseases in the field on the basis of measuring its antagonistic properties, and we must pay attention to studying the mechanism of antagonizing bacteria to control plant diseases [56] and behavioral patterns in a paddy ecological environment. Although three antagonistic endophytic bacteria (REB01, REB32, and REB42) showed good field control effects, those problem including their colonization in rice, whether they can become dominant endophytic bacteria in rice, antagonistic mechanism on Ustilaginoidea virens were further studied on.

5. Conclusion

All strains of 45 endophytic bacteria isolated from rice had antagonistic activity on *U. virens*, and of which IBW of 23 strains was above 15 mm in plate test. The primary extracellular product of 23 endophytic bacteria strain with IBW more than 15.0 mm had antagonistic activity on *U. virens*, and of which IBW of 8 strains was above 25.0 mm. The primary extracellular product of 3 strains (REB01, REB32 and REB42) had significantly higher antagonistic bands (P < 0.01) than other strains. The field control effect of three strains (REB01, REB32 and REB42) was 81.48%, 72.05% and 76.76% respectively on rice false smut, which was very significant (P < 0.01) higher than of 500-fold dilution of 5% Jinggangmycin (61.72%).

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6. References

[1] Tanaka E, Ashizawa T, and Sonoda R 2008 Mycotaxon 106 491
[2] Zou K Q, Hu D W, Wang W M, and Xu X H 2012 Zhejiang Agric. Sci. 5 704
[3] Tang X Y, Jin J and Hu D W 2012 Plant Pathology 62 1
[4] Hu M L, Luo L X and Wang S 2014 European J. Plant Pathology 139 67
[5] Fan J, Guo X Y, Li L, Huang F, Sun W X, Li Y, Huang Y Y, Xu Y J, Shi J, Lei Y, et al. 2015 J. Integrative Plant Biology, 57 577
[6] Fan J, Yang J, Wang Y Q, Li GB, Li Y and Huang F 2016 Molecular Plant Pathology 17 1321
[7] Ou S H 1985 Rice disease (UK: CABI Publishing)
[8] Baruah B P, Senapoty D and Ali M S 1992 Indian J. Mycology and Plant Pathology 22 274
[9] Huang S 2012 Fujian J. Agric. Sci.s 27 452
[10] Li Y S, Zhu Z, and Zhang Y D 2008 Acta Agronomica Sinica 34 1728
[11] Chen Z Y, Nie Y F, and Liu Y F 2009 Jiangsu Agric. Sci. Bulletin 25 737
[12] Xi B G, Wu T, and Zhao Y 2010 Jiangsu Agric. Sci.s 26 170
[13] Dhua U, Dhua S R, and Sahu R K 2015 J. Phytopathol 163 931
[14] Gao J 1978 Plant Protection 13 52
[15] Nakamura K, Izumiyama N, and Ohtsubo K 1993 Mycotoxins 38 25
[16] Koiso Y, Li Y, and Iwasaki S 1994 J. Antibiotics 47 765
[17] Li Y, Koiso Y, and Kobayashi H 1995 Biochemical Pharmacology 49 1367
[18] Zheng M, Xie C F, and Wan W T 2009 Chin. Agric. Tech. Extension 15 40
[19] Li J S, Wang X G, and Dai H X 2008 Modern Pesticides 7 52
[20] Wang A J, Wang G R, Sun L, Li L, Liu L M, and Huang S W 2015 Chin. Rice 21 45
[21] Zheng Q W 2019 Pesticide Market Information 14 40
[22] Jiang S, Tang C S, and Tan Z Q 2010 Tropical Agric. Sci.s 30 62
[23] Lan S L, Chen H R, and Xiao H Y 2004 Plant Protection 30 69
[24] Peng H X, Xi Y D, Liu B W, and Zhu J Y 2008 Southwest Agric. Journal 21 1298
[25] Shao M, Wu Z D, Chen B J, Luo S C R, and Li L 2008 Chinese J. Bio. Control 24 335
[26] Liu Y Z, Chen Z Y, Fu X M, Liu Y F, and Gong Y 2006 Jiangsu Agric. Sci. 22 76
[27] Zheng Q W 2013 Pesticide Market Information 29 38
[28] Weindling R 1932 Phytopathology 22 837
[29] Liu L N, Tu Y L, and Zhang J Z 2010 Chinese Agric. Sci. 43 2031
[30] Liang Z H, Wei L, An Z Y, and Chen Y R 2010 Chinese J. Bio. Control 26 60
[31] Li J S, Zhang M, Peng H X, and Dai H X 2008 Chinese Agric. Sci. Bulletin 24 375
[32] Yin X L, Chen Z Y, Liu Y F, Liu Y Z, Wang X Y, Luo C P, Yu J J, and Nie Y F 2011 Jiang Su Agric. Journal 27 983
[33] Chen S 2012 Determination antagonistic activity photosynthetic bacteria cs-1 against Aspergillus oryzae (Ph.d. thesis. Central South University)
[34] Yan Y X, Liu Q G, Yang X Q, Chen H R, Du Y, Xi L, Wei X W, and Zhang D Y 2015 Hunan Agric. Sci. 7 24
[35] Dou B F 2015 Beijing Agriculture 8 10
[36] Rosenblueth M, and Martinez-Romero E 2006 Mol. Plant Microbe Interact 19 827
[37] Kandel S, Joubert P, and Doty S 2017 Microorganisms 5 77
[38] Pieterse C M, Zamiodis C, Berendsen R L, Weller D M, Vanwees S C, and Bakker P A 2014 Annu. Rev. Phytopathol 52 347
[39] Conrath U, Beckers G J, Langenbach C J, and Jaskiewicz M R 2015 Annu. Rev. Phytopathol 53 97
[40] Xu C Y 2002 Study on the Dynamic Changes Antagonistic Bacteria on Rice Varieties and the Control Rice Blight Disease (Master Degree Thesis, Zhejiang University)
[41] Gong M F, Ma Y H, Li C, Zheng H Y, and Wei G H 2009 Northwest J. Botany 29 0408
[42] Fang Z D 1998 Plant disease research methods (Beijing: Chin. Agriculture Press)
[43] He M Y, Zhang Z H Liu J Y, Zhu C H, Zhang D Y, and Liu Y New 2006 Pesticide 10 26
[44] Tang Q Y 2016 DPS Data Processing System (4th Edition) (Volume 3) Professional Statistics and Others (Beijing: Science Press)
[45] Song, JJ; Sotytong, K; Kanokmedhakul, S; Kanokmedhakul, K; Pooaim, S 2020 Int. J. Agric. Biol. 23 1013.
[46] Cai, CE; Yu, HB; Zhao, HB; Liu, XY; Jiao, BH; Chen, B 2019 Int. J. Agric. Biol. 22 1311.
[47] Nazar, M; Khan, MS; Ijaz, M; Anjum, AA; Sana, S; Setyawan, EMN; Ahmad, I 2018 J. Animal Plant Sci. 28 1034.
[48] Wu Y Y 2004 J. Traditional Chinese Veterinary Medicine 5 20
[49] Zhang J C, Chen Z Y, Zhang B X, Liu Y F, and Lu F 2004 Chin. J. Plant Pathology 34 463
[50] Chen Y J, Xiao Y N, and Zhao Yo J 1994 Hubel Agric. Sci.s, 1 24
[51] Wang S, Li M, and Dong H 2008 J. Phytopathology 7156 55
[52] Mew T W, Collyn B, and Pamplona P 2004 Plant Disease 88 557
[53] Liu Y Z, Chen Z Y, and Liu Y F, 2005 Jiangsu Agric. Sci. 21 48
[54] Liu Y F, Chen Z Y, and Lu F 2004 J. Jinling University Sci. Tech. 20 42
[55] Li X M, Hu B S, and Xu Z G 2003 Chin. Rice Sci. 17 360
[56] Mu C Q, Liu X, and Lu Q G 2007 Chin. J. Plant Protection 34 123