Front. Agr. Sci. Eng. 2024, 11(3): 485–498 https://doi.org/10.15302/J-FASE-2024537

RESEARCH ARTICLE

Paenarthrobacter nitroguajacolicus can promote cucumber growth and control cucumber corynespora leaf spot

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KEYWORDS

Adaptability, antagonistic bacterium, disease resistance, growth promotion, secondary metabolite

HIGHLIGHTS

- First report of bacteria *Paenarthrobacter nitroguajacolicus* for effective control of cucumber corynespora leaf spot and promotion of cucumber growth.
- *P. nitroguajacolicus* strain BJ-5 is well-adapted for biocontrol being tolerant of saline environments.
- *P. nitroguajacolicus* produces a variety of secondary metabolites that promote plant growth.

Received March 30, 2023;
Accepted December 7, 2023.

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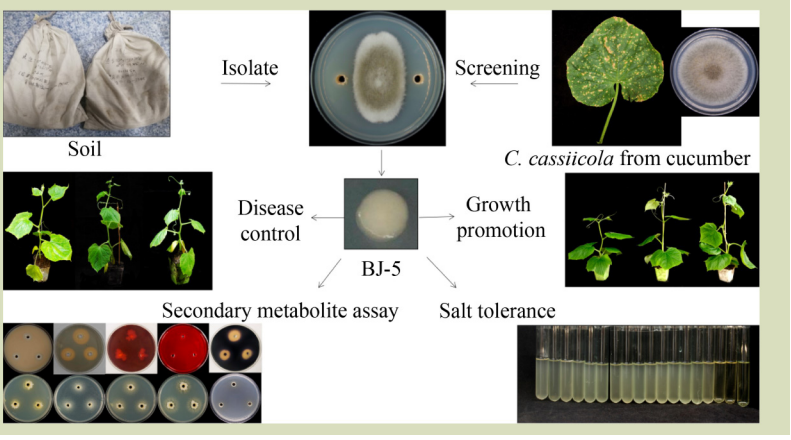
GRAPHICAL ABSTRACT



ABSTRACT

Currently, the disease control in cucumber mainly depends on agrochemicals, which is not an environmentally benign strategy. Biocontrol bacteria not only resist plant pathogens but also promote plant growth, which is ecofriendly and sustainable option. A biocontrol bacterial strain BJ-5 was screened using *Corynespora cassiicola* as the target pathogen, and BJ-5 was determined to be *Paenarthrobacter nitroguajacolicus* by morphological and molecular methods. The effect of BJ-5 on *C. cassiicola* was studied, including the spore germination, cell membrane permeability and infected cucumbers. BJ-5 inhibited the germination of *C. cassiicola* spores *in vitro* and led to atrophy and deformation of the *C. cassiicola* budding tubes. BJ-5 caused the relative extracellular conductivity of *C. cassiicola* mycelia to increase compared with the control. Additionally, BJ-5 reduced the severity of cucumber corynespora leaf spot of cucumber infected with *C. cassiicola*. The inhibition efficacy of BJ-5 suspension as a foliar spray against cucumber corynespora leaf spot reached 63% inhibition, which is higher than a 5000-fold dilution of Luna-Son SC fungicide. In addition, BJ-5 was tested on the emergence of cucumber seedlings, recording the biomass and photosynthesis of cucumber during the growth period. BJ-5 at 1.5×10^5 CFU·mL^{-1} promoted the germination of

cucumber seeds and increased biomass and photosynthesis at the adult plant stage. Also, the secondary metabolites of BJ-5 were determined. BJ-5 could produce chitinases, siderophore, cellulase, amylase and protease in the respective medium. Finally, adaptation assay of BJ-5 showed good salt tolerance and good adaptability in alkaline conditions, and that BJ-5 retains inhibition of fungi activity at higher temperatures. This is the first report of the biocontrol by *P. nitroguajacolicus* with antagonism to *C. cassiicola* and promote cucumber growth. This study indicates that *P. nitroguajacolicus* may serve as potential biocontrol agents against cucumber corynespora leaf spot fungus.

1 Introduction

Cucumbers are grown for their crisp and refreshing characteristics. To meet demand, greenhouse production of cucumbers has increased, however, repeated production with such facilities can easily lead to the accumulation of pathogens. Cucumber corynespora leaf spot is a major foliar disease which causing yield loss in cucumbers. The disease was first recognized in North Carolina, USA in the 1950s[1]. Subsequently, the disease has been reported around the world[2]. Cucumber corynespora leaf spot is caused by the fungus *Corynespora cassiicola*. Under natural conditions, *C. cassiicola* has a wide host range and worldwide distribution, covering important cash crops[3] and infects ornamentals[4].

The fungal pathogen from diseased leaves collected in the cucumber greenhouse was isolated and purified in the laboratory (Fig. 1(a)). The pathogen mostly caused irregular spots on the leaves (Fig. 1(b)). The isolated pathogen was identified as the causal agent of cucumber corynespora leaf spot using Koch's rule (Fig. 1(e)). The pathogen was identified by morphology (Fig. 1(c,d)) and molecular biology (Fig. 1(f,g)) as *C. cassiicola*[5]. In high temperature and high humidity environments, the conidia can directly penetrate the leaf epidermis of the host by germination and production of shoot tubes, or invade through the stomata, natural wounds and cause host disease[6].

As the scale of cucumber cultivation continues to expand, the occurrence of the disease significantly increased, with varying degrees of dependence on fungicide susceptibility, cucumber corynespora leaf spot risen to one of the main diseases in cucumber production[7]. Fungicides currently used against *C. cassiicola* include Trifloxystrobin, Carbendazim, Fluopyram, Boscalid, Pydiflumetofen, Isopyrazam and Fluxapyroxad[8]. It is likely that with the use of these fungicides, fungicide resistance of pathogen will increase.

Currently, the control of fungal plant diseases mainly relies on agrochemicals and cultural practices, but due to the emergence of resistance, the efficacy of fungicides has declined. Meanwhile, consumers have growing concerns about the environmental impact and food residues associated with fungicides. The advantages of environmentally-friendly biological control methods are gradually being recognized as more important[9]. Consequently, there is increased interest in biological control as an alternative approach to managing plant diseases. Biological control offers a viable solution for managing plant diseases, serving as an alternative to current methods. It is an environmental-friendly approach that promotes disease management while maintaining a balanced ecosystem. By employing biological control, it is possible to effectively control plant diseases without disrupting the natural balance of flora and fauna. Also, this approach enhances soil fertility, making it a valuable tool in sustainable agriculture.

Antagonistic bacteria have the ability to inhibit the germination of ascospores, either by producing antimicrobial substances or directly colonizing the ascospores. Jisha et al.[10] demonstrated the effectiveness of the bacterial biocontrol agent *Pseudomonas aeruginosa* against the pathogenic fungus *Colletotrichum capsici*, which causes anthracnose in chili plants. *Pseudomonas* spp. produce various antimicrobial compounds such as pyoluteorin, pyrrolnitrin, phenazines, siderophores, and cyanide, 2,4-diacetylphloroglucinol[11], as well as enzymes that can breakdown fungal cells, including cellulose, chitinase, proteases and β-glucanase[12]. Using these metabolites as biocontrol agents offers the advantage of controlling other pathogenic microorganisms in an environmentally friendly manner. Also, they enhance soil fertility, promote the growth of beneficial microorganisms, and contribute to plant growth in a significant way.

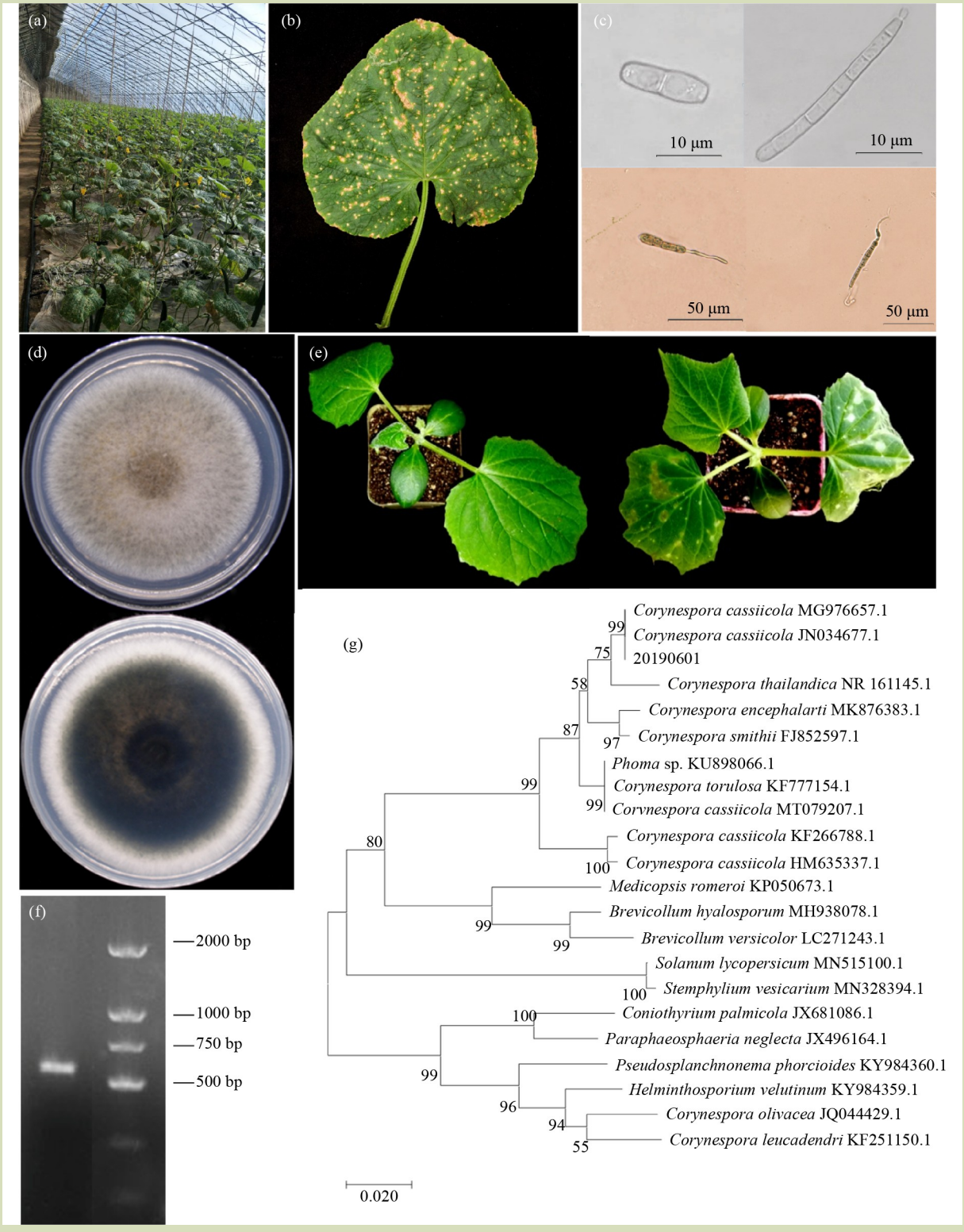


Fig. 1 Introduction to cucumber corynespora leaf spot: (a) infection of cucumber with corynespora leaf spot in greenhouses; (b) diseased cucumber leaves; (c) morphological characteristics of condia of *C. cassiicola*; (d) colony morphology of *C. cassiicola*; (e) symptoms 7 days after inoculation of cucumber leaves; (f) polymerase chain reaction products of rDNA-ITS amplified 564 bp; and (g) phylogenetic tree constructed with Neighbor-Joining method based on the rDNA-ITS of *C. cassiicola* and other 21 related strains.

Microorganisms that aid in nutrient acquisition by plants, known as biofertilizers, act through various mechanisms such as increasing the surface area accessible by plant roots, nitrogen fixation, phosphorus solubilization, siderophore production and hydrogen cyanide production[13]. Additionally, the availability of essential nutrients like Fe and Zn can limit crop yields. Bacterial strains can enhance the availability of iron by producing organic acids or siderophores[14]. Chakraborty et al.[15] discovered that rhizobacterial strains *Bacillus amyloliquefaciens*, *B. pumilus* and *Serratia marcescens*, which exhibited multiple attributes of plant growth-promoting (PGP) rhizobacteria, such as siderophore production, phosphate solubilization, indole-3-acetic acid (IAA) production, and antagonism against fungal pathogens, can increase growth in tea plants in terms of leaf number, biomass, and shoot number in both nursery and field conditions. Many PGP strains can produce auxins, particularly IAA, which is crucial in plant-microbe interactions and has significant effects on root growth[16] and architecture.

With control of cucumber corynespora leaf spot relying on agrochemicals, this could lead to increased planting costs, environmental pollution, pesticide residues, resistance and other problems, so it is particularly important to find safe and effective means of control. Biological control has the advantages of safety, no residues, no resistance and good environmental compatibility, and has a broad application prospect. In this study, we screened a biocontrol bacterium that was potentially antagonistic to cucumber corynespora leaf spot fungus by biological control methods to achieve the goal of pollution-free and environment-friendly.

2 Materials and methods

2.1 Materials

Cucumber (*Cucumis sativus*) cv. Zhongnong 6 (Chinese Academy of Agricultural Sciences, Beijing, China)[17] was disinfected with 75% alcohol for 30 s and rinsed 3–4 times with sterile distilled water. *C. cassiicola* was isolated from cucumber leaves with corynespora leaf spot collected from greenhouse cucumbers at 28 °C on potato dextrose agar (PDA, Laboratory M Limited, Lancashire, UK). The test fungicide used as a comparator (positive control) was Luna-Son SC (21.5% Trifloxystrobin and 21.5% Fluopyram; Bayer Crop Science Co., Leverkusen, Germany)[18]. A cucumber corynespora leaf spot isolate was cultured on PDA medium, incubated at 28 °C in the dark until sporulation. Spores were suspended in sterile distilled water and adjusted to 1×10^6 per milliliter by hemocytometer plate.

2.2 Methods

2.2.1 Screening and identification biocontrol bacteria

Soils for screening biocontrol bacteria strains were collected from nine provinces in China, and three soil samples were collected from each location. One g of soil per sample was added to 10 mL of sterile distilled water, and 100 μL of soil dilutions 10^5 and 10^6 fold were applied to Luria-Bertani medium for bacterial culture. Bacterial strains were isolated and purified by streaking, and the single colonies with large differences were picked according to colony morphology and color, numbered and initially stored at 4 °C. Using conduct standoff experiments[19] between the biocontrol bacteria isolated from soil samples and *C. cassiicola*, and the inhibition effect of all strains was determined, and the strains with the greater inhibition effect was selected as the target biocontrol agent in subsequent experiments.

The strains to be tested were grown on LB medium plates and incubated at a constant temperature of 28 °C for 24 h. The size, morphology, color, glossiness, colony shape, transparency and edge characteristics of the colonies were observed under the light microscope. Gram staining was performed and observed by microscopy. The physiological and biochemical characteristics of biocontrol bacteria test reference[20], the second part of the content of the method described physiological and biochemical performance. Growth media and physiological tests: PDA medium, LB (Luria-Bertani) medium, gelatin liquefaction medium, Tresner medium, tyrosine medium, sugar alcohol fermentation test medium, milk plate, tyrosine medium, methyl red test, peroxidase assay, oxidase assay and vp reaction basic culture medium.

DNA was extracted using bacterial genomic DNA rapid extraction kit (Sangon Biotech, Shanghai, China) with the polymerase chain reaction 16S rDNA amplification primer: 27F, 5′-ATGCCATTCGTGCGGAGGTTG-3′ ; and 1492R, 5′-CGTCTCTGCTGTCGATCATCACTTCGTAT-3′. PCR reaction system: A total volume of 25 μL, with 12.5 μL of 2× Reaction Mix, 8.5 μL of ddH_2O, 1 μL each of forward and reverse primers, and 2 μL of template DNA. The amplification conditions: 94 °C predenaturation for 5 min, 94 °C denaturation for 30 s, 56 °C annealing for 30 s, 72 °C extension for 90 s over 30 cycles, and 72 °C for 10 min[21]. The sequences obtained were analyzed for sequence similarity in EzBioCloud and a phylogenetic tree was constructed by Neighbor-Joining method using MEGA6.0 software.

2.2.2 Determination of the ability of BJ-5 to inhibit *C. cassiicola*

C. cassiicola spore suspension (1×10^6 CFU·mL^{-1}) was mixed

with 1×10^8 CFU·mL^{-1} of BJ-5 suspension in a 1:1 ratio, and 10 μL drops were pipetted onto water agar slides using a micropipette, with sterile distilled water as a control (1 CFU·mL^{-1} represents a quantity of 1 bacterium per milliliter of liquid). The culture was humidified at 25 °C in the dark and the germination of *C. cassiicola* spores counted at 2, 4, 6, 8, 10, 12, 24 and 48 h. Germination criterion was that the length of the germ tube was greater than half of the spore length. The number of germinated spores and the total number of spores in each treatment were recorded in more than 10 arbitrary fields of view, recording at least 200 spores per observation time. Finally, the inhibition of spore germination was calculated[22].

Conductivity can be used as an indicator of electrolyte leakage and loss of membrane permeability, reflecting cell membrane integrity. *C. cassiicola* grown in PDA medium using a 5-mm agar plug added to 500-mL conical flasks with 200-mL of PDB, with pure bacterial suspension was used as a control. The conical flasks were placed on a rotary shaker at 180 r·min^{-1}, incubated at 28 °C for 36 h and then co-cultivated with 1×10^8 CFU·mL^{-1} of BJ-5 suspension. 10 mL culture samples were taken at 0, 2, 4, 6, 8, 10 and 12 h, with the conductivity of the medium determined using a conductivity meter FC20 (Ohaus, Guangdong, China). All tests were repeated three times[23].

To determine the effect of the BJ-5 on the control of cucumber corynespora leaf spot. Cucumber seeds with consistent germination were selected and sown in pots of the same size, and used when four true leaves grew. Twenty cucumbers were sprayed with 1×10^6 CFU·mL^{-1} of *C. cassiicola* spores. After 24 h, 1×10^8 CFU·mL^{-1} of BJ-5 suspension was applied as a spray, 5000-fold dilution of Luna-Son (21.5% Trifloxystrobin and 21.5% Fluopyram) SC was used as a fungicide comparator, and clear water was used as a control. The cucumber was placed in a humidor at 95% relative humidity and 28 °C for 24 h and then transferred to normal seedling greenhouse culture[24]. The treatment test was set up in three treatments. The disease severity percentage and relative control effect for each replication was calculated using the equation described by Madden[25].

2.2.3 Growth promotion of cucumber by BJ-5

The BJ-5 was cultured in Luria-Bertani medium to a concentration of 1.5×10^8 CFU·mL^{-1} and diluted 7.5×10^7, 3×10^7, 1.5×10^7, 3×10^6, 1.5×10^6, 1.5×10^5, and 1.5×10^4 CFU·mL^{-1} from the original concentration as the treatment, with sterile distilled water as a control. 20 mL of each concentration of BJ-5 suspension was added to Petri dishes lined with filter paper, 20 seeds of each group were supplemented with 10 mL of treatment solution each day and the germination was recorded after 5 days. The seeds treated with 1.5×10^6 CFU·mL^{-1} of BJ-5 suspension and sterile distilled water were transferred to germination bags and supplemented with 10 mL of treatment solution each day, and the growth of cucumber in each group was observed after 10 days. All the experiment was repeated three times.

The BJ-5 suspension applied to seed was diluted to 1.5×10^6 and 1.5×10^5 CFU·mL^{-1} as the treatments, and the sterile substrate without inoculation was used as a control. Cucumber seeds were germinated and transplanted into three replicate pots per treatment. After 45, the net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate of the leaves were measured using a LI-6400XT (LICOR, Dalla, USA) photosynthesizer from 9 am to 12 pm in full sunlight by selecting the same growing true leaves of the cucumber plants[26]. The shoot height and root length, and fresh and dry weights of the cucumber plants were measured 45 days after sowing[27].

2.2.4 Detection of secondary metabolites of BJ-5

The methods of Awla et al.[28] were used to determine the secondary metabolites of BJ-5. Test included chitinase, carboxymethylcellulase, protease, β-1,3-glucanase, starch hydrolase, lipase, HCN (hydrocyanic acid), ACC deaminase, iron phagocytosis, growth hormone IAA and solubilized organophosphorus capacity.

2.2.5 Adaptation assay of BJ-5

BJ-5 was inoculated onto LB medium and incubated at 10–60 °C for 7 days observing the growth. Also, BJ-5 was cultured at different NaCl concentrations and pH environments, with growth measured after 7 days at 28 °C. The three treatments repeat three times in this research.

2.2.6 Statistical analysis

Data were analyzed using the analysis of variance (ANOVA) and means were separated using least significant difference (LSD) test was employed to compare. Statistical analysis was performed using IBM SPSS Statistics 20.0 (IBM Corporation, State of New York, USA) with significance at $P < 0.05$.

3 Results

3.1 Screening and identification of biocontrol bacteria

In total 286 bacterial strains were tested for inhibition of

C. cassiicola, and Table S1 shows some of the confrontation results (Fig. 2(a)). The screening showed that 39 strains had different degrees of inhibition against *C. cassiicola*. In this screening, strain BJ-5 was selected as the study subject based on

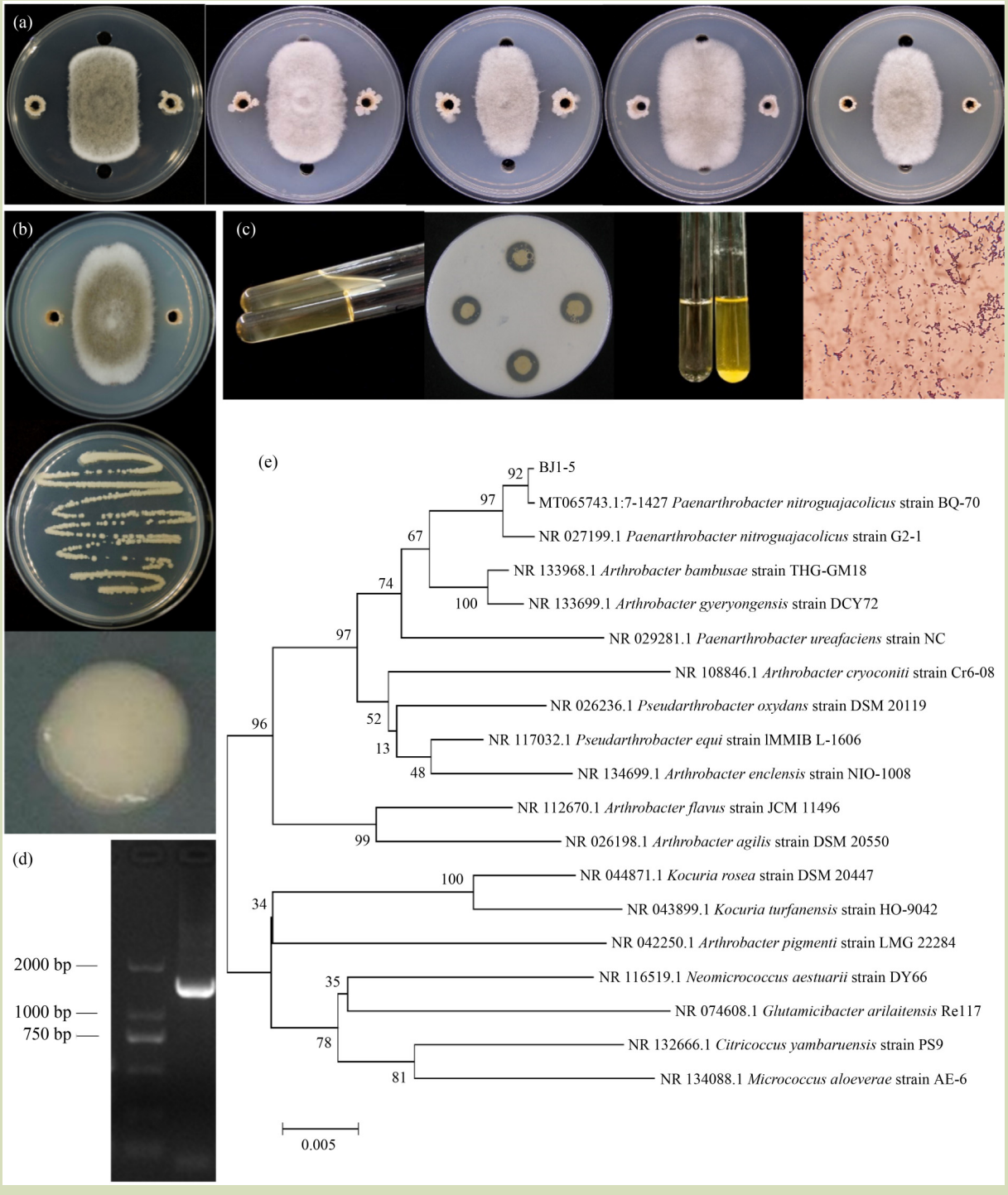


Fig. 2 Screening and identification biocontrol bacteria: (a) confrontation results of different biocontrol bacteria against *C. cassiicola* in a dishes; (b) physiological and biochemical characteristics of BJ-5 (part)-gelatin liquefaction, tyrosine hydrolysis, ammonium production and Gram test in that order; (c) BJ-5 colony morphology; (d) polymerase chain reaction products of 16S rDNA amplified 1424 bp; and (e) phylogenetic tree constructed with Neighbor-Joining method based on the 16S rDNA of BJ-5 and 17 related strains.

relative inhibition activity (Fig. 2(b)) with its inhibition rate of 62% (Table S1).

Figure 2(c) shows that BJ-5 is a Gram positive and rod-shaped. In LB medium, single colonies BJ-5 are white-yellowish, smooth with a regular edge, and in the liquid medium static culture, it forms a film on the surface of the medium (Fig. 2(b)). Characteristics of physiological and biochemical indicators of biocontrol bacteria are given in Table 1. Constructing a phylogenetic tree based on the 16S rDNA gene sequencing (Fig. 2(d), BJ-5 was closest in evolutionary distance to the *Paenarthrobacter nitroguajacolicus* (Fig. 2(e)).

3.2 Results of the control of cucumber corynespora leaf spot by strain BJ-5

BJ-5 was found to initially inhibited (with 12 h) the germination of *C. cassiicola* spores but the spores gradually continued to germinate. This indicated that the BJ-5 suspension could inhibit and delay spore germination (Table 2). Microscopic observations revealed that the spores treated with BJ-5 suspension had obvious deformation, and the budding tubes were atrophied and deformed (Fig. 3(a)).

Conductivity can be used as an indicator of electrolyte leakage and loss of membrane permeability, reflecting the integrity of the cell membrane. BJ-5 increased *C. cassiicola* membrane permeability and damaged the cell membrane. The conductivity of the *C. cassiicola* growth medium treated with BJ-5 was always higher than the control (Fig. 3(b)). These results indicated that BJ-5 had a direct effect on the integrity of the cell membrane of the mycelium of *C. cassiicola.*

Table 3 shows that both BJ-5 suspension treatment and fungicide application can effectively reduce the incidence and disease index of cucumber corynespora leaf spot. The effect of BJ-5 suspension (Fig. 3(f)) on cucumber corynespora leaf spot in pot cultures was 63%, which significantly reduced the disease index compared with the water control (Fig. 3(d)) and was better than the 5000-fold dilution of Luna-Son SC fungicide treatment (Fig. 3(e)).

3.3 Growth promotion of cucumber by BJ-5

According to the screening results in the previous section, different concentration of the BJ-5 suspension was used to soak cucumber seeds, and the seedling emergence rate of each

Table 1 Physiological and biochemical characteristics (positive (+) and negative (–) reactions) of *Paenarthrobacter nitroguajacolicus* strain BJ-5

Test	Result	Test	Result
Methyl red test	–	Tyrosine hydrolysis	+
Gelatin liquefaction	+	Oxidase	+
Ammonium production	+	VP reaction	+
Contact enzyme	+	Sugar alcohol fermentation	+
Catalase	+	Melanin production	–
Hydrogen cyanide	–	Gram stain	+

Table 2 Effects of *Paenarthrobacter nitroguajacolicus* strain BJ-5 on conidial germination of *Corynespora cassiicola*

Treatment time (h)	Germination rate (%)		Inhibition rate (%)
	Control	BJ-5	
2	5.6 ± 0.4	0 ± 0	100
4	13.7 ± 0.5	0 ± 0	100
6	32.0 ± 0.2	0 ± 0	100
8	42.6 ± 0.4	16.9 ± 0.3	83.1
10	57.6 ± 0.8	26.8 ± 0.4	73.2
12	77.2 ± 0.6	43.2 ± 0.8	56.8
24	100 ± 0	49.5 ± 0.5	50.5
48	100 ± 0	54.5 ± 0.5	45.5

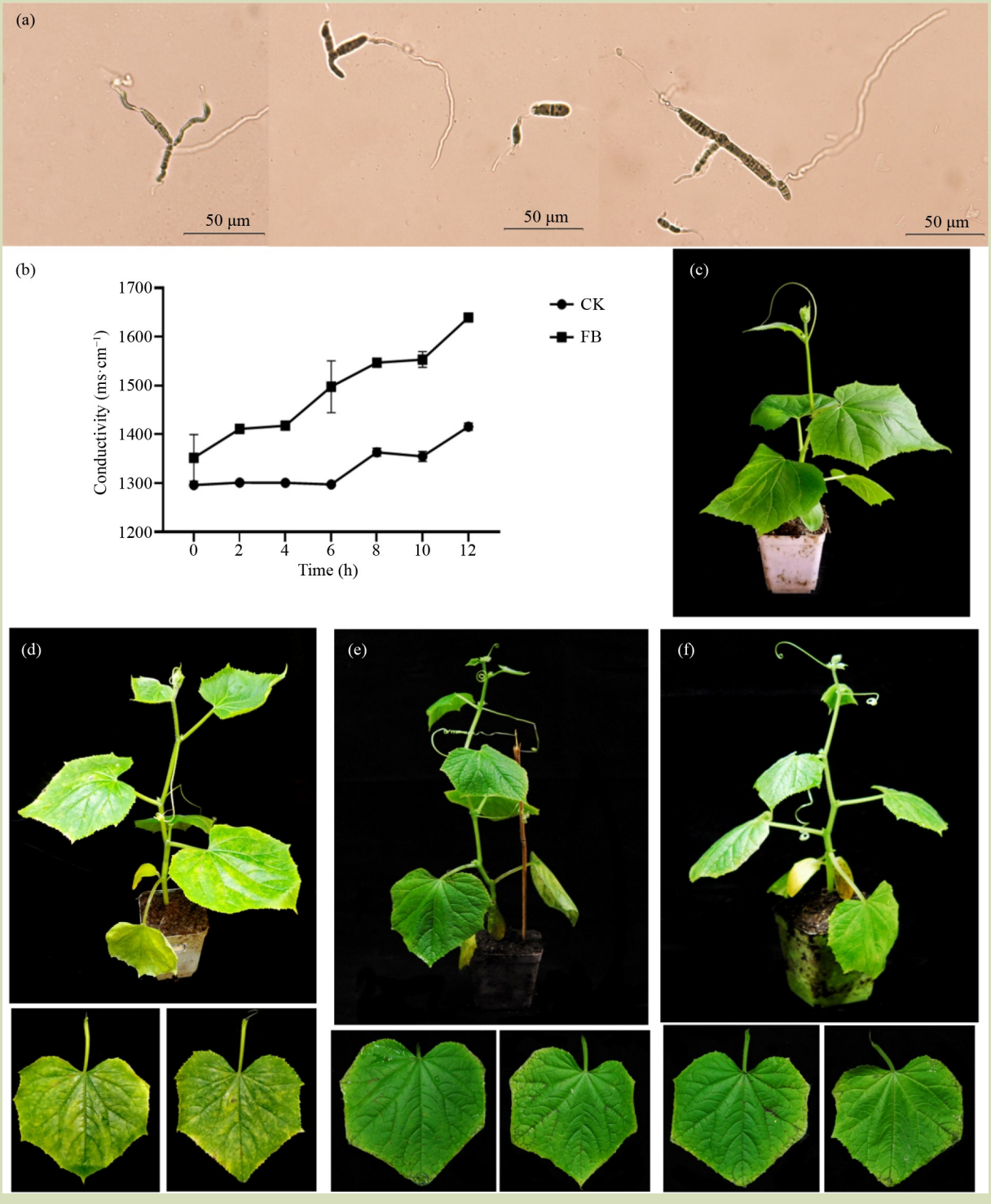


Fig. 3 Inhibit *C. cassiicola* by *P. nitroguajacolicus* strain BJ-5: (a) effect of BJ-5 on *C. cassiicola* spore germination; (b) determination of conductivity; (c) normal cucumber; (d) inoculated cucumbers with *C. cassiicola*; (e) 5000-fold dilution of Luna-Son SC (21.5% Trifloxystrobin and 21.5% Fluopyram) was used as the fungicide experimental group; and (f) 1×10^8 CFU·mL^{-1} of BJ-5 suspension apllied as a spray.

treatment was counted. Compared with the control, the BJ-5 suspensions of 1.5×10^5 and 1.5×10^4 CFU·mL^{-1} slightly promoted the emergence of cucumber seeds, root length and fibrous roots of germinated seeds, whereas the germination of cucumber seeds was inhibited in the original solution and at higher dilutions (Fig. 4(a,b)).

Table 3 Control effect of *Paenarthrobacter nitroguajacolicus* strain BJ-5 on cucumber corynespora leaf disease

Treatment	Disease index	Relative control effect (%)
Control	75 ± 4.1 a	none
Luna-Son	34 ± 3.7 b	54.3
BJ-5 (1.5×10^8 CFU·mL^{-1})	28 ± 3.8 c	62.9

Note: Data were reported as the mean value ± standard deviation of three replicates. Different lower case letters in the same row indicated significant differences at $P < 0.05$ level.

Seeds germinated with 1.5×10^5 CFU·mL^{-1} BJ-5 suspension and sterile distilled water were transferred to medium germination bags, and plant height, root length and fresh weight were measured in both after 10 days. Figure 4(c) shows that the root length and plant height of cucumber seeds germinated with sterile distilled water were higher than those for the BJ-5 treatment after 10 days, while the fresh weight was the opposite, and the main roots of cucumber seedlings treated with the BJ-5 suspension were thicker and more dense with fibrous roots (Fig. 4(d)).

After 45 days, the photosynthetic indexes of cucumber treated with BJ-5 were enhanced. The net photosynthetic rate, stomatal conductance and transpiration rate were significantly higher than those of the control at 1.5×10^6 CFU·mL^{-1} (Fig. 4(h)), and the photosynthetic promotion effect on cucumber was more significant than that with 1.5×10^5 CFU·mL^{-1}. This indicates that BJ-5 has the potential to promote the growth of cucumber. At day 45 after sowing the fresh weight, dry weight, plant height and root length of cucumber treated with BJ-5 were significantly greater than for the control group (Table 4).

3.4 BJ-5 secondary metabolite assay

We found by testing secondary metabolite cultures that BJ-5 can produce metabolites that promote plant growth and inhibit pathogenic fungus (Fig. 5(a)). BJ-5 produced cellulase, lipase and protease, which lyses cell walls, as well as siderophore, solubilized organophosphorus and starch hydrolase, which promote plant growth (Table S2).

3.5 Adaptation assay of BJ-5

BJ-5 was found to have good salt tolerance (Fig. 5(c)), can grow normally at 14% salt concentration (Table S3). BJ-5 exhibits heat resistance (Table S3), as its suspension's filtrate retains antifungal activity even after being heated at 80 and 100 °C (Fig. 5(b)). This suggests that it may produce heat-resistant antifungal substances. BJ-5 has good adaptability in acid and alkaline conditions (Fig. 4(d,e)), which indicates the potential of this strain in saline context and provides the foundation for further salt tolerance related research (Table S3).

4 Discussion

With control of cucumber corynespora leaf spot relying on agrochemicals, this could lead to increased planting costs, environmental pollution, pesticide residues, resistance and other problems, so it is particularly important to find safe and effective means of control. Biological control has the advantages of safety, no residues, no resistance and good environmental compatibility, and has a broad application prospect. In this study, we screened a biocontrol bacterium that was potentially antagonistic to cucumber corynespora leaf spot fungus by biological control methods to achieve the goal of pollution-free and environment-friendly.

With the continuous pursuit of environment-friendly agriculture, biological control with less pollution and less residue is expected to become a growing concern for pest and disease control. For plant disease control, the use of antagonistic bacteria, which compete with and resist plant pathogens, has emerged as a safe and effective research focus with significant potential for development. As a result, biological control through beneficial microorganisms has gained considerable importance.

For all year round supply of produce, the use of solar greenhouse allows cucumber to be planted every year, which gradually leads to the worsening of various diseases. In recent years, cucumber corynespora leaf spot has increased in greenhouse cucumbers; a leaf spot disease of cucumber affecting the plant photosynthesis, gradually leading to the death of the whole cucumber plant, and with serious disease in the seedling stage, the seedlings cannot grow. Over the recent decades, many scholars have screened biocontrol agents for fungus control.

P. nitroguajacolicus strains (like BJ-5 isolated in this study), have not been widely studied or reported to be pathogenic to

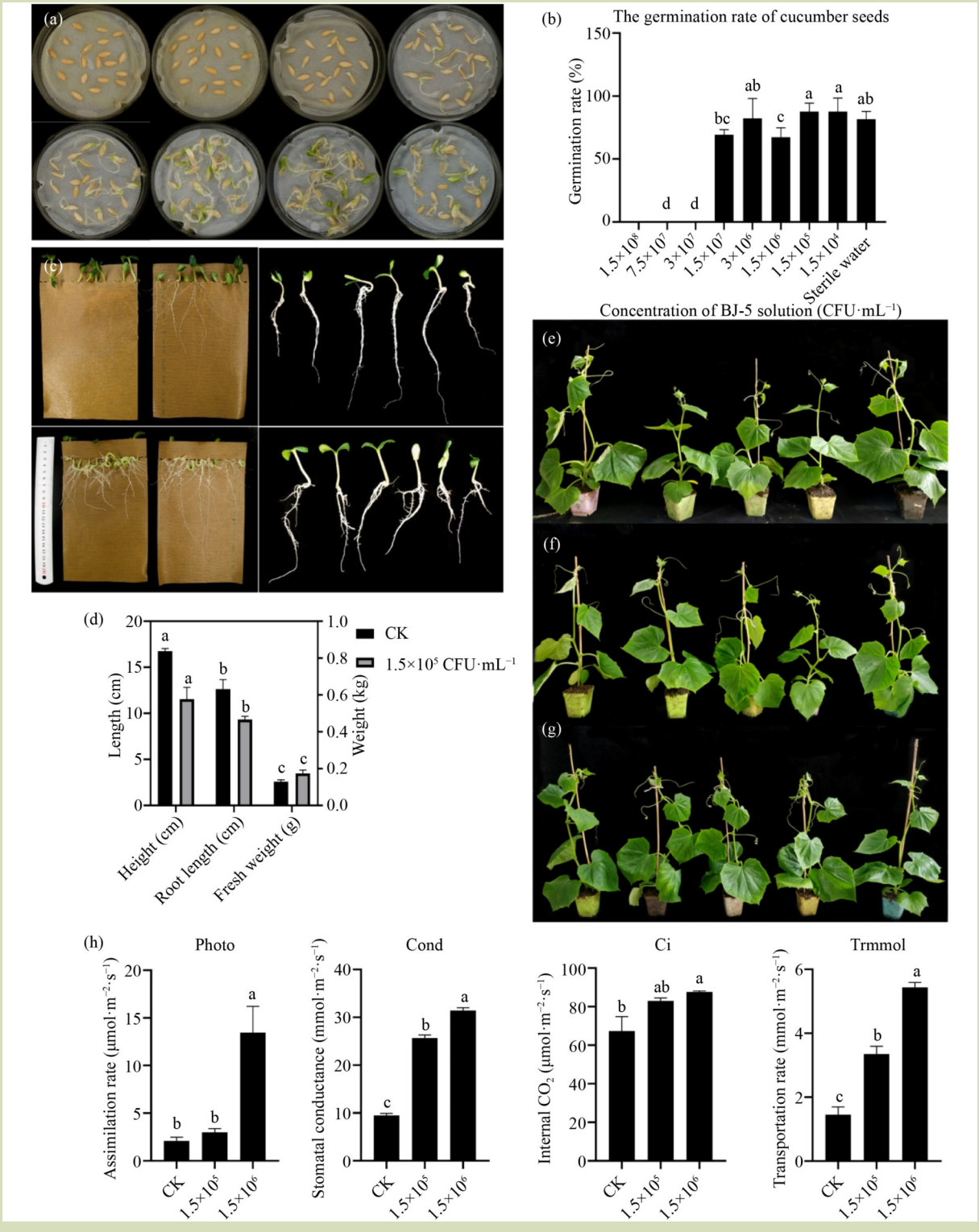


Fig. 4 The growth promotion of cucumber by *P. nitroguajacolicus* strain BJ-5: (a) cucumber seeds treated with different concentrations of BJ-5 suspension; (b) germination rate of cucumber seeds soaked in BJ-5 suspension at different concentrations; (c) germination bag growth of cucumber seed biomass of BJ-5 suspension and sterile water for 10 days; (d) effects of BJ-5 on cucumber biomass; (e) control; (f) seeds treated with 1.5 × 10^5 CFU·mL^{-1} dilution of BJ-5 suspension; (g) seeds treated with 1.5 × 10^6 CFU·mL^{-1} dilution of BJ-5 suspension; and (h) effects of BJ-5 on photosynthesis in cucumber leaves.

Table 4 Effects of *Paenarthrobacter nitroguajacolicus* strain BJ-5 on cucumber biomass

Treatment	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
Control	30.2 ± 1.41 c	19.4 ± 0.28 c	9.04 ± 0.81 c	0.501 ± 0.02 b
1.5×10^5 CFU·mL^{-1}	38.7 ± 0.84 a	26.4 ± 0.49 a	13.3 ± 0.61 a	0.554 ± 0.02 a
1.5×10^6 CFU·mL^{-1}	36.4 ± 0.98 b	22.6 ± 0.85 b	10.1 ± 0.48 b	0.524 ± 0.03 ab

Note: Data were reported as the mean value ± standard deviation of three replicates. Different lower case letters in the same row indicated significant differences at $P < 0.05$ level.

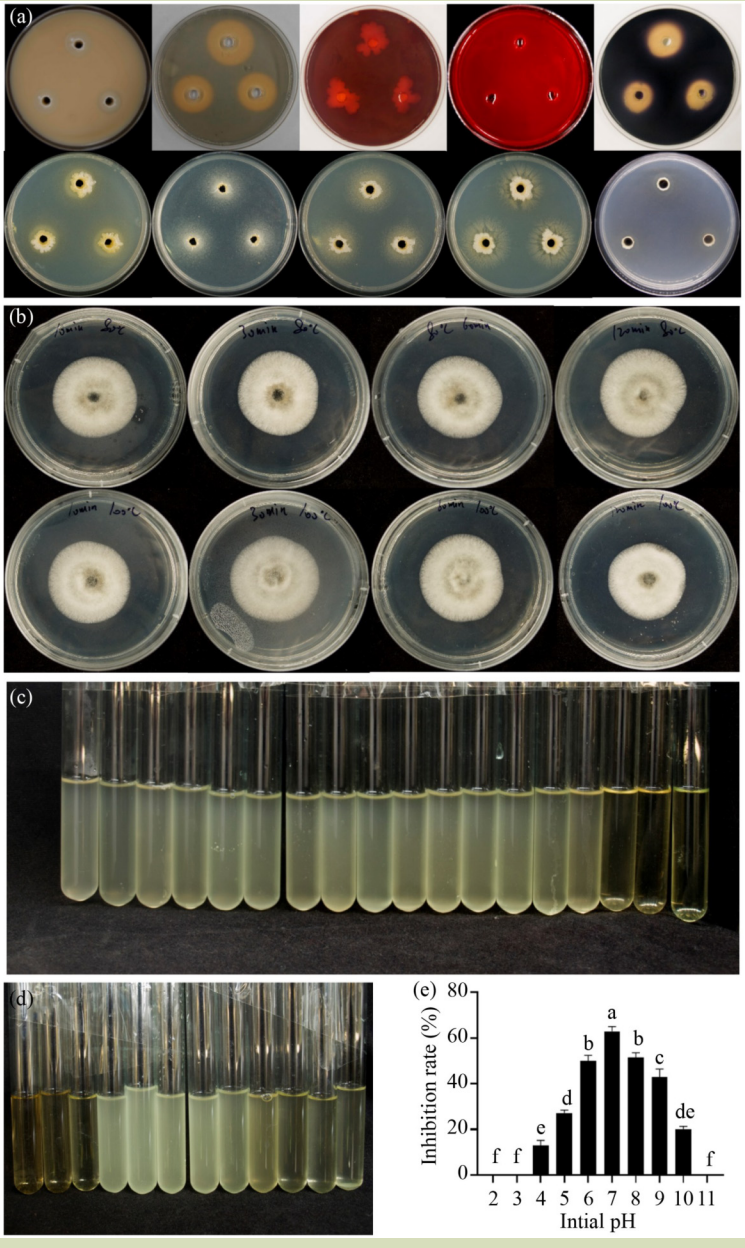


Fig. 5 Detection of secondary metabolites and adaptation assay of *P. nitroguajacolicus* strain BJ-5: (a) secondary metabolites detection (enzyme secretion detection); (b) high temperature stability of the BJ-5 suspension; and (c) NaCl tolerance range of BJ-5; (d, e) pH tolerance range of BJ-5.

crops. According to the research, *P. nitroguajacolicus* demonstrates both environmental friendliness and efficacy in remediating Cd-contaminated soil[29]. Also, it has shown potential in enhancing water use efficiency and alleviating plant water stress[30]. Additionally, it exhibits resistance to UV radiation[31] and displays higher PGP activity compared to other soil bacteria[32]. In our study, we observed that BJ-5 suspension caused deformation of the spores of the target pathogen and shriveling its germination tubes. Similarly, conductivity indicated that the BJ-5 suspension directly affected the cell membrane integrity of *C. cassiicola*. BJ-5 exhibited a 63% inhibition rate, which was superior to the 5000-fold dilution of the comparator fungicide, Luna-Son SC. The control effect of BJ-5 was better than that of other biocontrol bacteria[33].

To fully utilize the raw control effect in actual production, it is crucial to determine the optimal treatment of raw control bacteria on plants. Consistent with previous studies, BJ-5 demonstrated significant inhibition of cucumber seed germination both in the cultured stock and 2- to 5-fold dilutions. However, when applied at a 1000-fold dilution, it exhibited a remarkable growth promotion effect on cucumber, resulting in a significant increase in biomass compared to the control. This effect was further supported by the photosynthesis assay for BJ-5. Currently, researchers have reported that many biotrophic bacteria produce secondary metabolites, some of which directly inhibit the growth of pathogenic microorganisms while also containing substances that promote plant growth[34]. BJ-5 was able to produce cellulase, which dissolves cell walls, and iron chelators and organic phosphorus, which promote plant growth. This further supported the results of the disease suppression and growth promotion tests. Meanwhile, to understand the environmental adaptability of BJ-5, the salt, temperature and pH tolerance of BJ-5 were measured. It was found that BJ-5 has strong salinity tolerance and can grow normally in high salt and alkaline environment, the strain has useful salinity tolerance and has the value of further research and use.

5 Conclusions

In the control harmful microorganisms, attention needs to be given to impact of agrochemicals on the environment. Finding natural ways to control harmful microbes has great potential and is also environmentally friendly. It achieved reductions in pesticide application and to increase efficiency, the use of biocontrol bacteria to for plant diseases has become an important are of research and development. In this context, BJ-5 is a promising biocontrol bacterium, which is highly adaptable to saline environments, has ability to promote the growth of cucumber and to control the *C. cassiicola*. Most importantly BJ-5 can be use in production to help cucumber resist the damage of cucumber corynespora leaf spot.

Supplementary materials

The online version of this article at https://doi.org/10.15302/J-FASE-2024537 contains supplementary materials (Tables S1–S3).

Acknowledgements

This work was funded by the Shaanxi Province Key Research and Development Plan Project (2019ZDLNY03-07).

Compliance with ethics guidelines

Min Li, Yahui Huang, Xiaomin Dong, Xu Zhang, and Qing Ma declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any study with human or animal subjects performed by any of the authors.

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