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Full Length Article

Burkholderia pyrrocinia JK-SH007 Enhanced Seed Germination, Cucumber Seedling Growth and Tomato Fruit *via* Catecholate-Siderophore-Mediation

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Abstract

Plant growth-promoting bacteria (PGPB), both endophytic and rhizotrophic bacteria, can play a beneficial role for the biocontrol of phytopathogens and promotion of plant growth. *Burkholderia pyrrocinia* JK-SH007, as a poplar endophyte, showed strong antagonistic ability to poplar canker disease. A Siderophore involved in the transport of iron in plants and antifungal activities, inferred that siderophore may be the key factor of JK-SH007 biological control of poplar canker disease and plant-growth-promoting even in non-host plant. The siderophore-producing characteristics and conditions of *B. pyrrocinia* JK-SH007 were investigated, and the impact of JK-SH007-siderophore on plant growth was examined. Chemical analysis indicated that JK-SH007-siderophore was of catecholate-type, as confirmed by HPLC and LC/MS. The antifungal and growth-promoting activities of JK-SH007-siderophore were assessed, using fungal-siderophore desferrioxamine B (DFOB) as a reference. Compared with DFOB, JK-SH007-siderophore showed more effective against phytopathogens and it resulted in high seed germination and seedling growth rates in model plants cucumber (*Cucumis sativus*) and tomato (*Lycopersicon esculentum*), which was similar to JK-SH007 cell re-suspension. Thus, *B. pyrrocinia* JK-SH007 enhanced plant growth *via* siderophore mediation.

Keywords: Siderophore characterization; Antifungal activity; Plant growth promotion

Introduction

The *Burkholderia cepacia* complex (Bcc) is a group of genetically related phenotypically diverse Gram-negative bacteria. It is abundant in soil, water and particularly the rhizosphere of plants (Parke and Gurian-Sherman, 2001). The Bcc strains not only can survive in the external rhizosphere surfaces of roots but also can colonize internal root tissue (Parke and Gurian-Sherman, 2001; Vandamme and Dawyndt, 2011; Chen *et al.*, 2015). And they have attracted attention for their remarkable performance in promoting plant growth. The mechanisms involve in N_2-fixation to improve the host nitrogen availability (Van *et al.*, 2000), producing numerous antibiotics including cepacin, cepaciamide, xylocandins, pseudanes, phenylpyrroles, and phenazine to biocontrol of soilborne plant pathogens.

B. pyrrocinia JK-SH007, a poplar endophyte, was identified as Bcc genomovar IX. Previous reports had highlighted its strong plant growth-promoting and antifungal activities (Ren *et al.*, 2011; 2016). However, the active compounds have not yet been extracted and characterized, therefore their exact nature remains unknown. In this paper, the siderophore-producing characteristics and conditions of JK-SH007 were investigated. Given the efficiency of bacterial siderophores in Fe solubilization and their anti-microbial properties (Saha *et al.*, 2016), it hypothesized that bacterial siderophores may play an important role in the mechanism of JK-SH007 activities. The impact of JK-SH007-siderophore on phytopathogen and plant growth was examined using Desferrioxamine B (DFOB) as a reference. The results from this study will aid the understanding of plant–bacteria interactions, specifically the mechanism involves in bacterial siderophores enhancing plant growth.

Materials and Methods

Siderophore-producing Characteristics of *B. pyrrocinia* JK-SH007

JK-SH007 was originally isolated from *Populus* × *euramericana* cv. NL-895 (poplar NL-895) stems (4-years-

old of the first generation) located at Chenwei Forestry Farm (33°15′ N, 118°18′ E), Sihong County, Jiangsu Province, China and it had been deposited in the Agricultural Culture Collection of China (Accession No. M209028).

To evaluate the ability of JK-SH007 to produce siderophore, solitary colonies were inoculated on the CAS medium (Schwyn and Neilands, 1987) in triplicate and the plates incubated in darkness at 30°C for 60 h. *Escherichia coli* virus T1 was used as the negative control.

After the strain inoculated in sterile nutrient broth (NB) medium 12 h (30°C, 180 rpm), 1 mL of the seed culture (adjusted to OD_{600} = 0.2 with sterile distilled water) was transferred to 100 mL of MSA medium (20 g of sucrose, 2 g of L-asparagine, 1 g of KH_2PO_4 and 0.5 g of $MgSO_4{\cdot}7H_2O$ per one litre distilled water, pH 7.0) in a 150 mL flask and incubated at 24 h (30°C, 180 rpm). When the cultures grew to OD_{600}= 0.8, it was centrifugated (10,000 × g) for 5 min, collected the supernatant and then passed through a 0.22 mm filter. The filtered supernatant contained siderophores (Liu *et al*., 2015).

Qualitative and Quantitative Analysis of Siderophore in JK-SH007 Bacteria-free Fermentation Broth

Qualitative analysis of siderophore types in JK-SH007 filtered fermentation broth were determined by $FeCl_3$ test for hydroxamate (Neilands, 1981), Arnow's test for catecholate (Arnow, 1937) and Shenker's test (Shenker *et al.*, 1992) for carboxylate. Quantitative analysis the catecholate-type siderophore was measured by Arnow reagent (20% (w/v) $NaNO_2$ and $Na_2MoO_4{\cdot}2H_2O$) (Arnow, 1937). Absorbance was measured at λ = 515 nm using a Perkin Elmer Lambda spectrophotometer, and 3, 4-dihydroxybenzoic acid was used as a standard in the range of 2–250 mg mL^{-1} (Thiem*et al.*, 2018). To avoid iron contamination, all glassware was soaked in 10% nitric acid overnight and subsequently washed with deionized water.

Structure Characterization of JK-SH007-siderophore using HPLC Coupled with LC-MS

Prior to analysis, the filtered supernatant (10 mL) was acidified to pH 2 and extracted with equivalent-volume ethyl acetate. The extract was dried by anhydrous Na_2SO_4 and evaporated (Berner *et al.*, 1991). Then, the crude product was further purified using Sephadex LH-20 packed column (3.4 × 39.5 cm) and lyophilized (Liu *et al.*, 2015), and it was referred to as JK-SH007-siderophore.

It was dissolved in methanol for HPLC (Waters Corp., Milford, M.A., U.S.A.) on a C18 reversed-phase column (Nucleosil 5 μm, 4.6 × 250 mm) using gradient elution of methanol / 0.1% phosphoric acid (5–50%, by vol.). The flow rate was 1 mL min^{-1}, and the compounds were detected at a wavelength of 220 nm (Berner *et al.*, 1991).

Liquid Chromatogram (LC): Waters Ultra High Performance Liquid Chromatography (Waters Corp., Milford, M.A., U.S.A.), Agilent ZORBAX-SB C18 (100 mm × 4.6 mm i.d., 1.8 μm) was used in all the chromatographic experiments. The mobile phases were 0.1% formic acid-water (A) and 0.1% formic acid-acetonitrile (B). The linear gradient pro-grams were as follows, 0/5, 2/5, 25/50, 33/95 (min/B%); Sample injection volume, 10 μL; Column oven temperature, 30℃; Flow rate, 0.8 mL min^{-1}; and the UV detector was set at 254 nm.

Mass Spectrometry：AB Triple TOF 5600^{plus} System (AB SCIEX, Framingham, U.S.A.). The optimal MS conditions: scan range *m/z* 100–2000; negative ion mode: source voltage was -4.5 kV, and the source temperature was 550°C; Positive ion mode: source voltage was +5.5 kV, and the source temperature was 600°C. The pressure of Gas 1 (Air) and Gas 2 (Air) were set to 50 psi; the pressure of Curtain Gas (N_2) was set to 35 psi. Maximum allowed error was set to ± 5 ppm. Declustering potential (DP), 100 V; collision energy (CE), 10 V. The IDA-based auto-MS2 was performed on the 8 most intense metabolite ions in a cycle of full scan (1s). The scan range of m/z of precursor ion and product ion were set as 100–2000 Da and 50–2,000 Da, respectively. The CE voltage was set at 20, 40, and 60 V in the positive ESI mode, -20, -40, and -60 V in the negative ESI mode, ion release delay (IRD) at 67, ion release width (IRW) at 25. The exact mass calibration was performed automatically before each analysis employing the Automated Calibration Delivery System (Berner *et al.*, 1991).

Selection of Siderophore-producing Conditions using Single Factor Experiments

The physico-chemical parameters (metal ion, carbon sources, nitrogen sources, carbon to nitrogen ratio, pH, fermentation time, and temperature) were evaluated for their effects on JK-SH007 growth and siderophore production in single factor experiments. The effect of metal ions was tested by supplementing MSA medium with $FeCl_3{\cdot}6H_2O$ and $Zn(NO_3)_2{\cdot}6H_2O$ at respective concentrations of 0, 1, 10, 20, 40, 100, and 200 μmol L^{-1}. Concentrations of 20.0 g L^{-1} fructose, sorbitol, mannitol, xylose, maltose, lactose, and glucose were substituted for sucrose as carbon sources in MSA medium. Concentrations of 2.0 g L^{-1} proline, tyrosine, tryptophan, glycine, alanine, leucine, phenylalanine, lysine, histidine, and glutamic acid were substituted for asparagine as nitrogen sources in MSA medium. The weight ratio of sugar to amino acid was carried out at 3:1, 5:1, 8:1, 10:1, 13:1, and 15:1. The effect of pH within 4.0–8.0 on siderophore production was studied by adjusting pH with 0.1 mol L^{-1} HCl or NaOH before inoculating the strain. The fermentation time was 12–96 h at an interval of 12 h. The fermentation temperature was 20, 25, 30, 35, and 37°C. All culture flasks were incubated at 30°C for 24 h (180 rpm) except those for the temperature and time experiments. The JK-SH007 growth was measured by a spectrophotometer at 600 nm and siderophore production

was determined by Chrome Azurol S Liquid Assay (Schwyn and Neilands, 1987). The absorbance at 630 nm was measured using a PerkinElmer Lambda 365 spectrophotometer. Using the minimal medium as a blank, and the minimal medium plus CAS assay solution plus shuttle as a reference, the siderophore units (SU) are defined as the sample (As) lower than the reference (Ar), which is as follows: SU% = (Ar–As)/Ar ×100%.

All experiments were performed in 50 mL flasks with triplicate replications. All glassware used was cleaned with 1 mol L^{-1} HCl to remove iron traces and then rinsed with double-distilled water.

Bioactivity Analysis of JK-SH007-siderophore

Antifungal activity of JK-SH007-siderophore: *Cytospora chrysosperma*, *Phomopsis macrospora* and *Fusicoccum aesculi* (the poplar cankerpathogens; 0.6 mL) were grown in 20 mL of potato dextrose medium containing 1 mL of 140mg mL^{-1} JK-SH007-siderophore or 140 mg mL^{-1} DFOB in 50 mL flasks and incubated for 4 d. The culture broth was filtered to separate into mycelia and culture filtrates. Then the mycelium was respectively collected and lyophilized. Mycelial weight was calculated by subtracting the microtube weight from the total weight. The inhibition rate was calculated as follows: the inhibition rate (%) = (mycelial weight of control − mycelial weight with treatment)/mycelial weight of control ×100% (Pan *et al.*, 2018). All experiments were performed in triplicate.

Germination and morphological plant responses: Cucumber (*Cucumis sativus* L. cv. Shenqing No.1, obtained from Zhejiang Academy of Agricultural Sciences, China) and tomato (*Lycopersicon esculentum* Mill. cv. Zhefen No. 202, provided by Zhejiang Academy of Agricultural Sciences, China) were used to assess the effect on plant growth of JK-SH007-siderophore. Thirty surface-sterilized seeds were respectively germinated on a double layer of filter paper soaked in 5 mL distilled water, 1:100 diluted JK-SH007-resuspension, 1.40 mg mL^{-1} JK-SH007-siderophore or 1.40 mg mL^{-1} DFOB at 25°C for 3d at dark (Sung *et al.*, 2011). All experiments were performed in triplicate. Germination rate (the percentage of the germinated seeds), radicle length and seedling vigor index were measured. Seedling vigor index (SVI) = (mean shoot length + mean root length) × germination percentage (Pereira *et al.*, 2010).

Effect of JK-SH007-siderophore on cucumber seedling growth: Germinated seeds (cucumber and tomato) were incubated in seedling trays with nursery soil (composition: 75% coco peat, 20% perlite, 4% zeolite and 1% wetting agent). Three-week-old seedlings were transplanted into 30-cm diameter pots containing 3 kg of soil collected locally and placed in a greenhouse condition (15/9 h day/night solar radiation, temperature 25–30°C and relative humidity 60%). No chemical fertilizers were applied due to good soil nutrition; the soil characteristics follows as: pH 6.8, organic matter (39.5 g kg^{-1}), electrical conductivity (0.6 dS m^{-1}) and (all mg kg^{-1}) total N (18.76), P (56.28), K (113.94), Ca (1846.22), As (7.54), Cu (4.4), Pb (26.38) and Zn (7.81). One week after transplanting, 20 mL distilled water, 1:100 diluted JK-SH007-resuspension, 1.40 mg mL^{-1} JK-SH007-siderophore or 1.40 mg mL^{-1} DFOB were, respectively, drenched on the soil every 5 days. The data of total plant height, root length, chlorophyll, fresh and dry weights of cucumber seedling were collected after 4 weeks. Chlorophyll content was calculated using the following formula: total chlorophyll = 0.5 × (20.2 × A_{645} + 8.02 × A_{663}) (Ali *et al.*, 2014). Dry weight was measured after drying the material at 60°C until no weight change was observed (Sung *et al*., 2011).

Effect of JK-SH007-siderophore on harvest of tomato: After transplanting, 200 mL distilled water, 1:100 diluted of JK-SH007-resuspension, 1.40 mg mL^{-1} JK-SH007-siderophore or 1.40 mg mL^{-1} DFOB were added until collecting the harvest. The number of tomato, tomato weight and diameter, and sugar content (degrees Brix, Bx) were measured (Sung *et al*., 2011).

Statistical Analyses

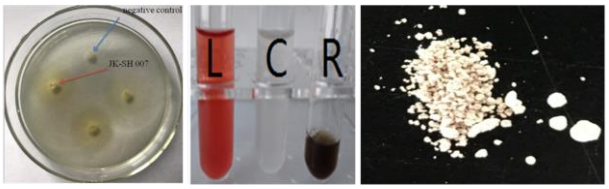
All statistical analyses were performed with S.P.S.S. for Windows, version 17.0 (S.P.S.S. Inc., Chicago, IL, USA). Data were analyzed by one-way analysis of variance, followed by Duncan's test ($P < 0.05$).

Results

Characterization of JK-SH007-siderophore

The CAS medium color change from blue to yellow-orange revealed the presence of siderophores produced by JK-SH007. The size of the orange halo initially increased with culture time and stopped expanding after 17d (Fig. 1a). The chemical testing showed that the JK-SH007-siderophore was of catecholate-type (Fig. 1b). According to Arnow's test, the concentration of catecholate-siderophore in JK-SH007 culture filter was 141.22 mg mL^{-1} based on the standard curve of y= 0.003x (R^2 = 0.997).

The HPLC analysis of ethyl acetate extracts revealed a characteristic two-peak pattern (Fig. 2a). After separating the catecholate fraction on a Sephadex LH-20 column, peak 2 was picked out in a highly purified form and was matched to 3,4-dihydroxybenzoic acid peak (t_R 10.955 min), which was confirmed by LC-MS (Fig. 2b). The LC-MS result, the deprotonated molecules $[M-H]^-$ (m/z 153.0209) was the peak for the monomer of 3,4-dihydroxybenzoic acids which removed $[H]^+$ to become an anion (compound A). Then the negative charge of the carboxylate was transferred to the benzene ring. The oxygen on the phenolic hydroxyl group formed a *p*-π conjugated system with the benzene ring. The electron was transferred to the oxygen atom of the phenolic hydroxyl group *via* the benzene ring, and the hydrogen on the phenolic hydroxyl group lost electron and shifted to the

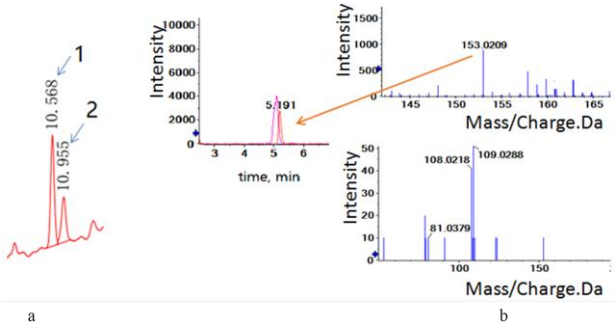


a b c

Fig. 1: a. JK-SH007 grown on CAS-agar plate showing circular halo bands. The change in medium color from blue to orange indicates the presence of siderophores

b. Chemical nature of siderophores produced by JK-SH007 based on different assays. The red (L) solution was positive for catecholate type. The center (C) and right (R) solution were negative for carboxylate and hydroxamate production

c. JK-SH007-siderophore



a b

Fig. 2: a. HPLC separation of catecholate-siderophore from JK-SH007 on a C18 reversed-phase column (Nucleosil 5 μm, 4.6 × 250 mm) using methanol / 0.1% phosphoric acid (5 - 50%) as a gradient eluting solvent. The flow rate was set at 1 ml min^{-1} and the peaks were monitored at a wavelength of 220 nm. Retention times: (1) 10.57 min, (2) 10.96 min

b. Liquid chromatography mass spectrometry of JK-SH007-siderophore showing the deprotonated molecules $[M-H]^-$ m/z 153.0209 and the fragments m/z 109.0288 and 81.0379, which originated from cleavage of carbon dioxide and carbanyl group, respectively. The sample was measured with AB TripleTOF 5600plus System (AB SCIEX, Framingham, U.S.A.) at a flow rate of 0.8 ml min^{-1} using nitrogen as collision gas. The mobile phases were 0.1% formic acid-water (A) and 0.1% formic acid-acetonitrile (B). The linear gradient pro-grams were as follows, 0/5, 2/5, 25/50, 33/95 (min/B%)

benzene ring. And one molecule of CO_2 was removed, that was compound B, the ion peak m/z 109.0288. The other phenolic hydroxyl of B was isomerization to be a carbonyl group (C=O) to form the compound C, the benzene ring stable structure was broken, and then a molecule of carbonyl was removed and the benzene ring was opened to give compound D, which was a free radical, the ion peak m/z 81.0379. D provided one electron, and oxygen atom provided the other to form a bone, and cyclizated to be an oxygen-containing heterocyclic compound (Fig. 3).

Optimization of Fermentation Condition of JK-SH007-siderophore

The cells and siderophore production showed the same trends with time and temperature, increasing in the first 48 h and declining thereafter. The siderophores were detected

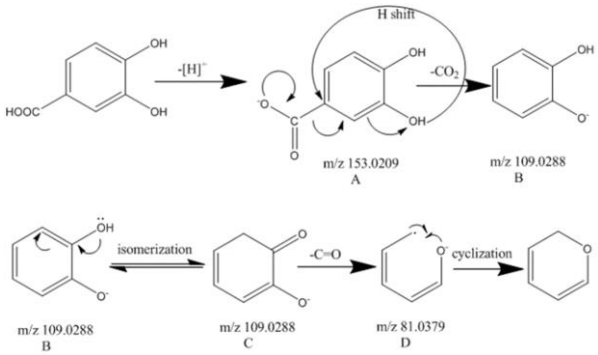


Fig. 3: Analysis of peaks in JK-SH007-siderophore LC-MS spectra

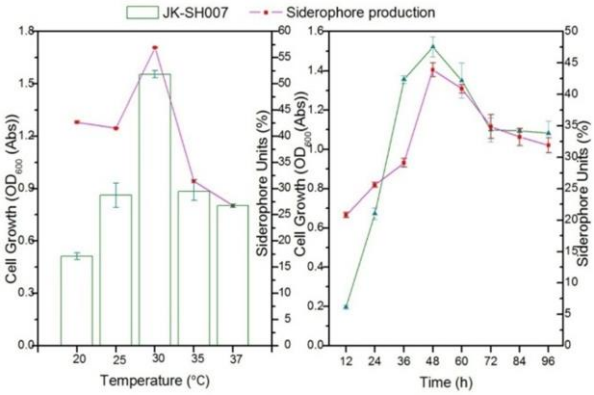


Fig. 4: Effect of fermentation temperature and time on JK-SH007-siderophore production and JK-SH007 growth. Error bars represent standard deviations

after 6 h and maximum siderophore production of 43.9% was achieved after a continuous increase up to 48 h. The highest yield of siderophore (56.9% SU) occurred at 30°C, at which JK-SH007 had the highest cell growth (Fig. 4).

The pH is vital to bacterial growth, and weakly acidic conditions (pH 6) favored JK-SH007 growth but suppressed siderophore production. The optimum siderophore production (33.6% SU) was at pH 8.0, which significantly differed from those obtained at other pH values ($P < 0.05$) (Fig. 5).

As shown in Fig. 6, siderophore production varied with carbon source, the maximum siderophore production (46.3% SU) was obtained with glucose as carbon source, while fructose, sorbitol, mannitol, xylose, maltose, lactose and glucose were 30.3, 34.6, 42.1, 32.9, 14.9, 13.2 and 11.9%. The maximum siderophore production (57.9% SU) was obtained with L-asparagine as the nitrogen source, while tyrosine, tryptophan, phenylalanine, alanine, leucine, glycine, lysine, histidine, and glutamic acid were 28.9, 6.3, 11.2, 29.7, 48, 11.7, 39.2, 32.7 and 41.5%.

Fig. 7 displayed the effect of metal ion (Fe^{3+}, Zn^{2+}) supplementation on siderophore production and JK-SH007 growth. The highest rate of CAS reaction was for JK-SH007 growing in non-iron supplemented medium

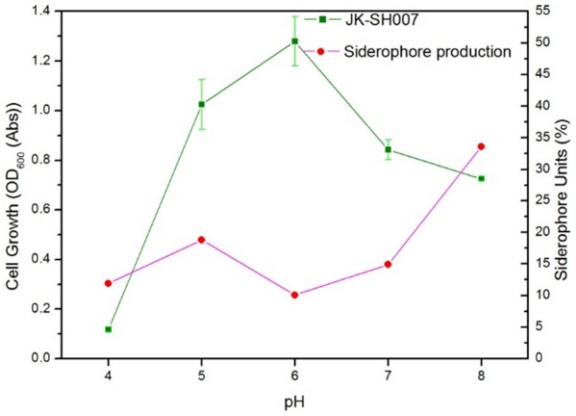


Fig. 5: Effect of culture pH in JK-SH007-siderophore production and JK-SH007 growth. Error bars represent standard deviations

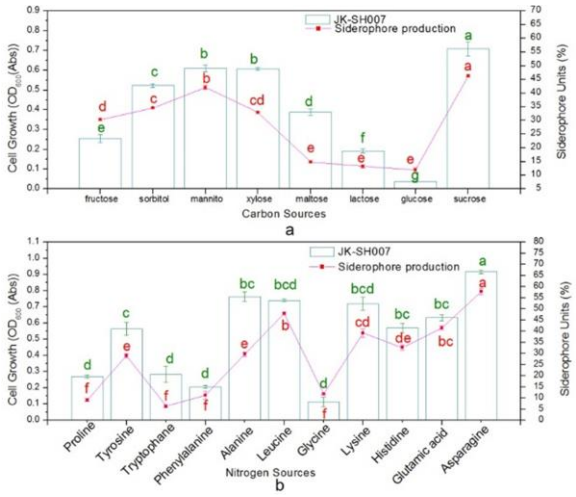


Fig. 6: Effect of carbon (**a**) and nitrogen (**b**) sources on JK-SH007-siderophore production and JK-SH007 growth. Error bars represent standard deviations. Different letters represent significant differences ($P < 0.05$)

(Fig. 7a). When $FeCl_3$ concentration increased from 1 to 200 μmol L^{-1}, the amount of siderophore decreased by 63.7%. It was found that there was an obvious inhibition of siderophore production and cell growth with Zn addition. When Zn concentration increased from 0 to 200 μmol L^{-1}, the siderophore production was reduced by 35.72%. Similarly, JK-SH007 cell growth was reduced by 51.4% at 200 μmol L^{-1} compared with no Zn (Fig. 7b).

Bioactivity of JK-SH007-siderophore

Antifungal activity: The poplar canker pathogens, *C. chrysosperma*, *P. macrospora* and *F. aesculi* were grown for 4 d in PDB plus JK-SH007-siderophore or DFOB. The presence of DFOB and JK-SH007-siderophores inhibited fungal growth, with JK-SH007-siderophore being more effective than DFOB. The DFOB inhibited growth of the

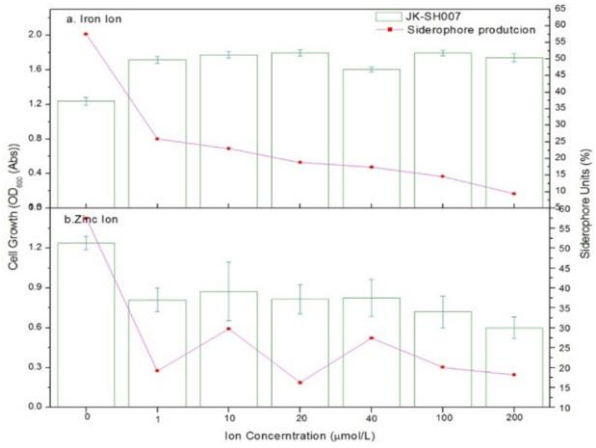


Fig. 7: Effects of (**a**) iron and (**b**) zinc ions on siderophore production and JK-SH007 growth. Error bars represent standard deviations

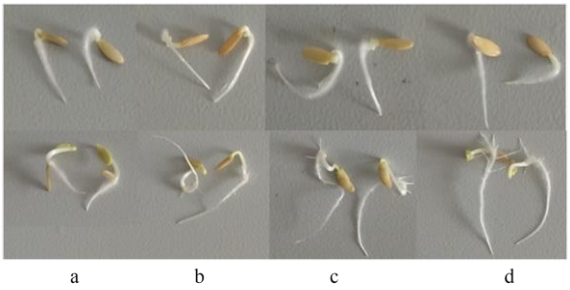


Fig. 8: Comparison of seed germination and seedling vigor under different treatments (**a**. sterile water; **b**. JK-SH007 re-suspension; **c**. JK-SH007-siderophore; **d**. DFOB)

fungi *C. chrysosperma*, *P. macrospora*, and *F. aesculi* by 58 ± 0.6, 46 ± 0.5, and 72 ± 0.5%, respectively. Correspondingly, the JK-SH007-siderophore inhibited their growth by 89 ± 0.9, 76 ± 0.6, and 90 ± 0.7%, respectively.

Promotion plant growth: The pot experiment showed that JK-SH007-siderophores significantly stimulated both cucumber and tomato seed germination (Table 1 and Fig. 8). Compared with sterile water, the other three treatments improved germination rate and seedling vigor index. For cucumber, the effect of promoting germination rate was as follows: JK-SH007-siderophore ≈ DFOB (11%) > JK-SH007-resuspension (7.4%); the effect of promoting seedling vigor index was as follows: JK-SH007-siderophore (72%) > DFOB (56%) > JK-SH007-resuspension (32.4%). As for tomato, the effect of promoting germination rate was slightly: JK-SH007-siderophore (15.3%) > DFOB (4.6%) > JK-SH007-resuspension (0.9%), the effect of promoting seedling vigor index: JK-SH007-siderophore (32%) >JK-SH007-resuspension ≈ DFOB (6.8%).

JK-SH007-siderophore also improved cucumber seedling growth including total plant length, fresh and dry weight of over-ground and underground parts (Table 2). Compared with the control, total plant height was

Table 1: Germination and seedling vigor indices of cucumber and tomato grown in glass Petri dishes for the different treatments

Treatment	Germination index[a] (%)		Radicle length (cm)		SVI[b] (cm)	
	Cucumber	Tomato	Cucumber	Tomato	Cucumber	Tomato
Sterile water (control)	90c	86.7c	4.1c	6.8c	3.6d	5.9c
JK-SH007 re-suspension	96.7b	90.7b	4.3c	6.9c	4.2c	6.3b
JK-SH007- siderophores	100a	100a	6.2a	7.8a	6.2a	7.8a
DFOB	100a	87.5c	5.6b	7.2b	5.6b	6.3b

[a] Germination index, proportion of seeds that germinated by the end of the experiment from total seeds sown. [b] Seedling vigor index, (mean shoot length + mean root length) × germination rate. Different letters represent significant differences ($P < 0.05$)

Table 2: Effect of JK-SH007-siderophore cucumber growth

Treatment	Total plant Height (cm)	Root length (cm)	Total chlorophyll	Wet weight (g)		Dry weigh (g)	
				Shoot	Root	Shoot	Root
Sterile water (control)	43.18c	4.34d	1.74c	83.46d	2.93d	7.62d	0.56d
JK-SH007 re-suspension	56.82b	6.24a	2.74b	86.72c	3.94b	8.84b	0.78b
JK-SH007- siderophores	62.78a	6.16b	2.67b	124.51a	3.82c	9.45a	0.89a
DFOB	55.31b	5.24c	2.95a	93.42b	4.22a	8.42c	0.63c

Different letters represent significant differences ($P < 0.05$)

Table 3: Effect of JK-SH007-siderophore tomato fruit

Treatment	No. of fruits	Fruit weight (g)	Fruit diameter (cm)	Degrees Brix (ºBx)
Sterile water (control)	16c	70.52d	5.19b	3.88c
JK-SH007 re-suspension	18b	84.53c	5.33a	4.24ab
JK-SH007- siderophores	22a	88.78a	5.40a	4.25a
DFOB	17bc	85.37b	5.35a	4.12b

Different letters represent significant differences ($P < 0.05$)

increased by 31.6% (JK-SH007-resuspension), 45.4% (JK-SH007-siderophore) and 28.1% (DFOB). Total chlorophyll in cucumber seedling leaves were also increased by 57.5% (JK-SH007-resuspension), 53.4% (JK-SH007-siderophore) and 68.5% (DFOB). Plant biomass, both wet weight and dry weight, were also increased in the other three treatments compared with the control. Compared with control, both the quantity and quality of tomato were enhanced (Table 3).

Discussion

PGPB have good colonization ability, even in non-host plants (Fan *et al.*, 2012), and can stimulate the growth of plants either directly or indirectly (Fan *et al.*, 2015, 2016). These bacteria can act as biofertilizers, phytostimulators, or stress controllers by producing siderophores, antibiotics, enzymes, and/or fungicidal compounds. Siderophores are small organic molecules produced by microorganisms under iron-limiting conditions which enhance the uptake of iron. Siderophore-producing bacteria have received increasing attention as an alternative to the use of chemical fertilizers and pesticides (Bach *et al.*, 2016).

Appropriate growth conditions are beneficial to bacterial growth and metabolism. Weakly acidic conditions (pH 6) suppressed siderophore production. It is possible that, under acidic conditions, solubilization of iron was improved, and resulted in an adverse impact on siderophore secretion which was previously induced by iron starvation (Muthuselvan and Balagurunathan, 2013). A number of studies have shown that siderophores are iron-specific compounds secreted under low-iron stress and their production in medium is inversely proportional to the iron concentration in the medium. The optimal iron concentration for high siderophore production was without iron added (Rachid and Ahmed, 2005). One report showed that iron concentrations > 10 μmol L^{-1} had a negative effect on siderophore production (Villegas *et al.*, 2002). JK-SH007 showed good growth under both iron-sufficient and -limited conditions. The highest rate of CAS reaction was for JK-SH007 growing in non-iron supplemented medium. When $FeCl_3$ concentration increased from 1 to 200 μmol L^{-1}, the amount of siderophore decreased by 63.7%. However, JK-SH007 still produced a CAS reaction even with a high iron concentration in MSA medium. The results suggested that more than one mechanism was regulating siderophore production. JK-SH007-siderophore production was regulated not only by iron concentration but also by zinc ion which was agreement with the Schalk's observation, and the toxic metals induce production of siderophores suggests that these chelators may play a role in bacterial heavy metal tolerance (Schalk *et al.*, 2011; Rasha, 2017).

Siderophores act as a potential biocontrol agent by inhibiting phytopathogen as well as act as bio-fertilize by enhancing the Fe uptake to plants. As catecholate groups ($K\sim10^{40}$) have greater efficiency than DFOB ($K\sim10^{29}$), and they have a stronger antagonistic action by sequestering iron from the phytopathogen (Folschweiller *et al.*, 2000). JK-SH007-siderophore showed more effective against phytopathogens and it resulted in high seed germination and seedling growth rates, compared with DFOB. JK-SH007-siderophore treatments showed better performances in

improving plant growth which was similar to JK-SH007-resuspension. The result was similar to reports that the chlorophyll content (Imsande, 2010), and general biomass of cucumber plants were increased by microbial siderophores (Weizhen and Lei, 2013). Based on the changes in the physiological, biochemical and photosynthesis of the plant after inoculation with *T. asperellum* Q1, it implied that some protection mechanisms involved in reduction of ROS and improvement of photosynthesis. Mung bean plants inoculated with siderophore-producing *Pseudomonas* strain GRP3 had reduced chlorotic symptoms and enhanced chlorophyll content when grown under iron-limited conditions (Sharma *et al.*, 2003). Moreover, as microbial siderophores can enhance Fe uptake to plant and improving of photosynthesis. Thus, the effect on fruit is another interesting mechanism and should not be neglected. Greenhouse experiments showed that, both application of JK-SH007-siderophore and inoculation with JK-SH007 could increase fruit size (both length and diameter) and weight (Table 3), particularly in JK-SH007-siderophore group(about 4% more than in control), which is similar to the responses reported for other PGPB (Bernabeu *et al.*, 2015; Berger *et al.*, 2017). As no ACC deaminase activity could be detected, the higher production of tomato plants inoculated with *B. tropica* MTo-293 could be attributed to other growth promotion mechanism (Bernabeu *et al.*, 2015). But in the paper, this beneficial effect could be mainly attributed to siderophore production.

Conclusion

Use of siderophore-producing microorganisms is considered a mainstream practice to move toward higher agricultural productivity in a more sustainable and environmentally friendly system. Many siderophore-producing microorganisms have been isolated involved in plant growth-promoting activity, both directly (iron assimilation) and indirectly (biocontrol of plant pathogens). In the present study, the JK-SH007-siderophore was extracted and purified, the main structure was analyzed by LC-MS, the siderophore production process was optimized, and then its effect on phytopathogen growth and plant growth were discussed. JK-SH007-siderophore showed strong inhibition of pathogens and high stimulation of plant growth. To the best of our knowledge, the present work is a new report concerning siderophore, the exact active component of JK-SH007, involves in beneficial effect on non-host plants growth.

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