Nitrogen fixation by *Paenibacillus polymyxa* WLY78 is responsible for cucumber growth promotion

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**Abstract**

*Aims* To study nitrogen contribution to cucumber derived from nitrogen fixation of *Paenibacillus polymyxa* WLY78.

*Methods* The *nif* gene cluster deletion mutant (Δ*nifB-V*) of *Paenibacillus polymyxa* WLY78 was constructed by a homologous recombination method. The effects of plant-growth promotion were investigated by greenhouse experiments. The nitrogen fixation contribution was estimated by 15N isotope dilution method (also being called the 15N natural abundance technique).

*Results* Deletion of *nif* gene cluster of *P. polymyxa* WLY78 resulted in complete loss of nitrogenase activity. Greenhouse experiments showed that inoculation with *P. polymyxa* WLY78 could significantly enhance the lengths and dry weights of cucumber roots and shoots, but inoculation with Δ*nifB-V* mutant could not. 15N isotope dilution experiments showed that cucumber plants derive 25.93% nitrogen from nitrogen fixation performed by *P. polymyxa* WLY78, but the Δ*nifB-V* mutant nearly could not provide nitrogen for plant growth.

*Conclusions* This present study demonstrated that nitrogen fixation performed by *P. polymyxa* WLY78 contributes to plant growth.

**Keywords** Plant growth-promoting bacteria · *Paenibacillus polymyxa* WLY78 · cucumber · nitrogen fixation · 15N isotope dilution

**Introduction**

Nitrogen is one of the most important nutrients in plant growth, but plants can not directly use nitrogen in the atmosphere. Biological nitrogen fixation (BNF) is a process in which nitrogen-fixing microorganisms reduce nitrogen in the air to ammonia through nitrogenases. Biological nitrogen fixation is an important part of the natural nitrogen cycle (Dart 1986) and plays an important role in the sustainable development of agriculture (Raymond et al. 2004). Nitrogen-fixing microorganisms include symbiotic nitrogen-fixing bacteria, free-living nitrogen-fixing bacteria and associative nitrogen-fixing bacteria (Xu et al. 2017). Associative nitrogen-fixing bacteria can colonize root surface cells, invade plant roots, and form close contact with plants, thereby promote plant growth (Baldani et al. 1997). Associative nitrogen-fixing bacteria promote the absorption of nitrogen by non-legume plants (Geddes et al. 2015). Biological
nitrogen fixation not only reduce the use of nitrogen fertilizer, but also improve soil fertility and the absorption of nutrients by crops (Farrar et al. 2014).

It has been reported that the associative nitrogen-fixing bacteria play an important role in promoting growth of non-legumes by fixing nitrogen and producing phytohormone (Chalk 1991). In earlier research, $^{15}\text{N}$ isotope and N balance studies had shown that several sugarcane varieties obtain over 60% of their nitrogen (>150 kg N ha$^{-1}$ year$^{-1}$) from biological nitrogen fixation performed by diazotrophs (Boddey et al. 1995). Diazotrophic bacteria present in the mucilage of aerial roots contributed 29–82% of the N nutrition of Sierra Mixe maize (Van Deynze et al. 2018). Inoculation with nitrogen-fixing Klebsiella pneumoniae 342 (Kp342) increased total N and N concentration in the wheat plant (Iniguez et al. 2004). Inoculation of the rhizobacteria including Azospirillum brasilense and Azospirillum lipoferum contributed up to 20-50% of the total nitrogen requirement of the oil palm seedlings through nitrogen fixation (Amir et al. 2003). Diazotrophic Paenibacillus beijingensis BJ-18 provided nitrogen for wheat, maize and cucumber plants and promoted plant growth, nitrogen uptake and metabolism (Li et al. 2019). A recombinant nitrogen-fixing Pseudomonas protegens Pf-5 X940 was constructed by introducing the $nif$ genes of Pseudomonas stutzeri A1501 via the X940 cosmid to the beneficial rhizobacterium Pseudomonas protegens Pf-5, and inoculation of Arabidopsis, alfalfa, tall fescue and maize with Pf-5 X940 increased the ammonium concentration in soil and plant productivity under nitrogen-deficient conditions (Fox et al. 2016; Setten et al. 2013). Inoculation with Azospirillum brasilense Ab-V5 cells enriched with exopolysaccharides and polyhydroxybutyrate enhances the productivity of maize under low N fertilizer input (Oliveira et al. 2017).

Paenibacillus polymyxa WLY78 is a nitrogen-fixing bacterium containing a compact $nif$ gene cluster consisting of 9 genes ($nifBHDKENXhesAnifV$) (Wang et al. 2013; Xie et al. 2014). In addition to nitrogen fixation, this bacterium has the ability of phosphate solubilization and indole-3-acetic acid (IAA) production (Xie et al. 2016). Also, this bacterium can produce fusaricidins that are a class of cyclic lipopeptide antibiotics to inhibit plant pathogenic fungi (Li et al. 2019). These specific traits suggest that P. polymyxa WLY78 is a member of plant growth-promoting bacteria (PGPB) and has great potential as an inoculant in agriculture. However, the nitrogen contribution to plants derived from nitrogen fixation of P. polymyxa WLY78 is unclear. In this study, a $nif$ gene cluster deletion mutant ($\Delta nifB-V$) of P. polymyxa WLY78 was constructed. Comparisons of P. polymyxa wild-type and $\Delta nifB-V$ mutant in plant-growth promotion and nitrogen fixation contribution rate were investigated. Our study was to analyze the role of $nif$ gene in $\text{N}_2$ fixation to stimulate plant growth.

### Materials and methods

Bacterial strains, plasmids, media and growth conditions

Bacterial strains and plasmids used in this study are summarized in Table 1. Paenibacillus polymyxa WLY78 was isolated from the rhizosphere of bamboo in Beijing (Wang et al. 2013). P. polymyxa

| Table 1 | Bacterial strains and plasmids used in this study. |
|---------|--------------------------------------------------|
| Strain or plasmid | Genotype and/or relevant characteristics | Source or reference |
| **strains** | | |
| *Paenibacillus polymyxa* | | |
| WLY78 | Wild-type strain | (Wang et al. 2013) |
| $\Delta nifB-V$ | $nif$ gene cluster deletion mutant | This study |
| E. coli JM109 | General cloning host; *recA1, endA1, gyrA96, thi-1, hsdR17, supE44, relA1, Δ(lac-proAB)/F′[traD36, proAB+, lacIq, lacZΔM15] | Sangon Biotech Co. |
| **Plasmid** | | |
| pRN5101 | Temperature-sensitive *E. coli-Bacillus* shuttle vector, Em$^R$ | (Zhang et al. 2013) |
WLY78 and ΔnifB-V mutant were routinely grown in LB medium (per liter contains: 10 g NaCl, 5 g yeast and 10 g tryptone) at 30°C with shaking. Nitrogen-limited medium (per liter contains: 10.4 g Na2HPO4, 3.4 g KH2PO4, 26 mg CaCl2·2H2O, 30 mg MgSO4·7H2O, 0.3 mg MnSO4, 36 mg Ferric citrate, 7.6 mg Na2MoO4·2H2O, 10 mg p-aminobenzoic acid, 10 mg biotin, 4 g glucose as carbon source and 0.3 g glutamic acid as nitrogen source) (Wang et al. 2013) was used for assay of nitrogenase activity (Wang et al. 2013).

Escherichia coli JM109 was used as routine cloning. Thermo-sensitive vector pRN5101 (Zhang et al. 2013) was used for gene disruption in P. polymyxa WLY78. When appropriate, antibiotics were added in the following concentrations: 100 μg/ml ampicillin and 5 μg/ml erythromycin for maintenance of plasmids.

**Construction of ΔnifB-V mutant of P. polymyxa WLY78**

The nitrogen-fixing gene cluster deletion mutant ΔnifB-V of P. polymyxa WLY78 was constructed by a homologous recombination method. For doing this, the upstream fragment and downstream fragment flanking nif gene cluster were amplified by PCR using Phanta®Max Super-Fidelity DNA Polymerase (Vazyme Biotech Co., Ltd., Nanjing, China), respectively. Primer 1 (5' CGGCCACGATGCGTCCGG CGTAGAGGATCCGCCTGTTG)

GATTGGA CG 3') and Primer 2 (5' AACGCT TTTTCGTTATCTATCCTTCACATCTATTT TC TTCGTC 3') were used to amplify a 950 bp-long sequence located upstream of nifB. Primer3 (5' GAAGGAAATGATA ACCGAAAA)

AGCGTTCGGCTC 3') and Primer 4 (5' GACTGC GCAAAAGACATAATCGAAAGCTTCTGTGAT AAGGCAG

ACAGGCCTTC 3') were used to amplify a 1107 bp-long sequence located downstream of nifV. The two fragments were then fused with BamHI/Hind III digested pRN5101 vector using Gibson assembly master mix (New England Biolabs), generating the recombinant plasmid pRDnifB-V. Then, the recombinant plasmid pRDnifB-V was transformed into P. polymyxa WLY78 as described by Wang et al. (2018), and the marker-free deletion mutant (the double-crossover transformant) ΔnifB-V mutant was selected from the initial erythromycin resistant (EmR) transformants after several rounds of nonselective growth at 39°C and confirmed by PCR using the primer 5 (5' GCATAAAATTGACACGTGTA 3') and primer 6 (5' AGGCTCATAAACCCGTATC 3').

**Growth and nitrogenase activity of the wild type and mutant strains**

To measure growth, P. polymyxa WLY78 and ΔnifB-V mutant were grown in 20 mL of LB media in 50 mL flasks shaken at 200 rpm at 30°C to logarithmic growth phase. The cultures were collected by centrifugation, washed three times with sterilized water and then resuspended in sufficient nitrogen medium (nitrogen-limited medium supplemented with 100 mM NH4Cl) to a start OD600 of 0.02. Every 2 h, the growth of P. polymyxa WLY78 and ΔnifB-V mutant strains were determined by absorbance at 600 nm.

Acetylene reduction assays were performed as described previously to measure nitrogenase activity (Wang et al. 2013). P. polymyxa WLY78 and ΔnifB-V mutant strains were grown in 50 mL of LB medium overnight. The cultures were collected by centrifugation, washed three times with sterilized water, and then resuspended in a nitrogen-limited medium to a final OD600 of 0.4. Then, 4 mL of the culture was transferred to a 26-mL test tube and the test tube was sealed with a rubber stopper. The headspace in the tube was then evacuated and replaced with argon gas. Then, approximately 2.2 mL of C2H2 (10% of the headspace volume) was injected into the test tubes. After incubating the cultures at 30°C for 8 h, a 100 μL gas sample was taken out and injected into gas chromatography to quantify ethylene (C2H4) production. The nitrogenase activity was expressed in nmol C2H4/mg protein/h.

**Preparation of soil, seeds and bacterial suspension**

The soil (0-20 cm deep topsoil) was taken from the Shangzhuang Experimental Station of China Agricultural University. The soil was low nitrogen (7.8 mg kg⁻¹) sandy soil. After the soil was air-dried and crushed, the debris were removed with a 2 mm sieve to reduce heterogeneity, and then packed into plastic pots with a diameter of 20 cm and a height of 14 cm.
Each pot was filled with 2 kg of soil to grow cucumbers. No trace elements were applied during plant growth.

Cucumber seeds (“Zhongnong 8” of Beijing Shengfeng Garden Agricultural Technology Co., Ltd.) were first disinfected with 10% sodium hypochlorite for 10 minutes, then were rinsed with sterile water three times, and finally were distributed in a sterile petri dish with damp filter paper at room temperature (25°C) for 3-5 days in the dark.

The bacterial suspension of *P. polymyxa* WLY78 and Δ*nifB-V* used in inoculation was prepared as follows. *P. polymyxa* WLY78 and Δ*nifB-V* were inoculated into LB liquid medium, cultured at 30°C and 180 rpm to logarithmic growth phase, and then harvested by centrifugation and finally suspended with physiological saline (0.89% w/v NaCl in deionized water). The cell concentration was set to 10^8 cells mL⁻¹.

**Greenhouse pot experiment**

The research was conducted in the greenhouse of China Agricultural University using greenhouse potting. The experimental design was arranged by random factors, with three inoculation treatments (two bacterial inoculation and one mock inoculation) and two nitrogen level treatments. Each treatment was repeated three times, for a total of 18 pots of cucumber plants. Nitrogen level treatment included high nitrogen and low nitrogen levels. Nitrogen fertilizer was applied in the form of 15N labeled (NH₄)₂SO₄ (10.16% 15N atom, Shanghai Research Institute of Chemical Industry, China). The high nitrogen level was 250 mg N kg⁻¹ soil, and the low nitrogen level was 83 mg N kg⁻¹ soil. Nitrogen fertilizer was applied in three times, one-third each time, and the first time was applied as a base fertilizer, followed by 7 and 14 days after transplantation.

The inoculation included three treatments: inoculation of *P. polymyxa* WLY78 (WT), Δ*nifB-V*, and equal amount of physiological saline (as a control). The germinated cucumber seeds with robust and consistent growth were picked and immersed in the bacterial suspension for 20 minutes. The seeds were immersed in physiological saline for 20 minutes as a control group, and then transplanted into plastic pots. Four seeds were planted in each pot, and three repetitions were set for each treatment. In the first and second weeks after planting, the 80 mL of the bacterial suspensions of *P. polymyxa* WLY78 and Δ*nifB-V* were applied to pot containing inoculated cucumbers, respectively, and 80 mL of physiological saline was applied to pot containing non-inoculated cucumbers. Pots were placed in the greenhouse under optimum conditions (15 h light/9 h dark cycle, 25–30/15–20°C day/night temperature and 40% day/60% night humidity). The seedlings were regularly watered every 3 days until the relative humidity of the soil reached 40%.

**Plant sample collection**

On 30th day of cucumber planting, the plants were collected by destructive sampling. The whole seedling was first uprooted, and rinsed with deionized water to remove the soil attached to the root system, then the root and shoot samples were separated, and the length of the root and shoot were measured. The root and shoot samples were killed in an oven at 105°C for 30 minutes, and then dried at 65°C until constant weight for dry weight analysis. Then the dried samples were immediately frozen in liquid nitrogen for subsequent analysis.

**Quantification of biological nitrogen fixation (BNF) contribution**

The plants un-inoculated, inoculated with *P. polymyxa* WLY78 (WT) and Δ*nifB-V* mutant were collected on 30th day of cucumber planting as described above. The dried plant samples were ground, sieved with a 1 mm sieve and placed in a bag. The δ^{15}N values were assayed by using DELTA V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., United States) in Institute of Botany, the Chinese Academy of Science, China.

The 15N natural abundance technique (also being called the 15N isotope dilution method) was used to quantitatively determine the contribution of BNF (Boddey et al. 2001; Bremer and van Kessel 1990). This method is based on difference of the relative abundances of the stable isotope 15N in the atmosphere and soil, with 15N abundance in the soil being higher than in the air. Atmospheric N₂ shows a natural abundance of 0.3663 atom% 15N. Natural 15N abundance was calculated as follows: δ^{15}N (‰) = 1000 × (atom% 15N sample − 0.3663)/0.3663. The
percentage of nitrogen derived from BNF (%Ndfa) was calculated using the following formula.

\[
\% \text{Ndfa} = \frac{\delta^{15}N_{\text{reference}} - \delta^{15}N_{\text{fixing plant}}}{\delta^{15}N_{\text{reference}} - B} \times 100
\]

In the formula, “\(\delta^{15}N\)” is stable nitrogen isotopes, “ref” is the value from non-N-fixing reference plants, “fixing plant” is plant inoculated with nitrogen-fixing bacteria, and “B” is the N abundance in the air, assumed to be 0.0‰.

Statistical analysis

Graphs were prepared using GraphPad Prism software v. 8.0 (GraphPad Software Inc., San Diego, CA, USA). Statistical analysis was performed using SPSS software version 20 (SPSS Inc., Chicago, IL, United States). Two-way analysis of variance (ANOVA) was employed to check the significant differences between treatments. Means of different treatments were compared using the least significant difference (LSD) at the 0.05 or 0.01 level of probability.

**Results**

The nif gene cluster deletion mutant (\(\Delta\text{nifB-V}\)) of *P. polymyxa* WLY78 resulted in complete loss of nitrogenase activity.

*P. polymyxa* WLY78 contains a compact nif gene cluster consisting of 9 genes (nifB nifH nifD nifK nifE nifN nifX hesA nifV) located within a 10.5 kb region. The nif gene cluster deletion mutant (\(\Delta\text{nifB-V}\)) was constructed by recombination as described in Fig. 1a. The disruption of nif gene cluster was confirmed by PCR (Fig. 1b). The \(\Delta\text{nifB-V}\) and the wild-type strains exhibited similar growth phenotypes on nitrogen-limited medium with ammonium as nitrogen sources (Fig. 1c). However, the \(\Delta\text{nifB-V}\) mutant did not have nitrogenase activity (Fig. 1d), indicating that the nif gene cluster is essentially required for nitrogen fixation under nitrogen limitation.

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**Fig. 1** Construction and characterization of nif gene cluster deletion mutant (\(\Delta\text{nifB-V}\)) of *P. polymyxa* WLY78. a. General scheme of construction of nif gene cluster deletion mutant. b. The nif cluster disruption was confirmed by PCR using the primer 5 and primer 6. c. Comparison of growth of the WT (the wild-type) and \(\Delta\text{nifB-V}\) (deletion mutant) strains. Growth of the WT and \(\Delta\text{nifB-V}\) strains in nitrogen-limited medium supplemented with 100 mM NH\(_4\)Cl as the nitrogen source. d. Nitrogenase activities of WT and \(\Delta\text{nifB-V}\) grown anaerobically in nitrogen-limited medium.
Effects of *P. polymyxa* WLY78 and Δ*nifB-V* mutant on the growth of cucumber

Cucumber samples were collected on the 30th day after plantation, and the length and dry weight of cucumber shoots and roots were measured to evaluate the effects of inoculation with *P. polymyxa* WLY78 and Δ*nifB-V* mutant on plant growth under low and high nitrogen conditions. The non-inoculated cucumber served as a control. Compared to the non-inoculated control group, cucumber inoculated with *P. polymyxa* WLY78 under low nitrogen conditions showed increase of 68.18% in shoots dry weight, of 59.15% in root dry weight, of 37.61% in shoot length and of 38.52% in root length, but they showed a little increase in lengths and weights under high nitrogen conditions (Fig. 2a-d). Compared with the non-inoculated control group, the cucumber inoculated with the Δ*nifB-V* mutant showed a little increase in the dry weight and length of the shoots and roots under both low and high nitrogen conditions (Fig. 2a-d). Fig. 3a, c is an experimental diagram of the greenhouse cultivation. The data indicated that the diazotrophic *P. polymyxa* WLY78 can effectively promote plant growth under low nitrogen conditions and disruption of *nif* genes encoding nitrogenase results in almost loss of the ability of promoting plant growth (Fig. 3b, d).

Nitrogen fixation in cucumber provided by *P. polymyxa*

To estimate the contribution of BNF, the natural abundance $^{15}$N measurement technique was used to analyze the inoculated and un-inoculated cucumber plants grown in soil containing $^{15}$N-labeled (NH$_4$)$_2$SO$_4$ as N fertilizer in greenhouse conditions. As shown in Table 2, the plants without inoculation (reference control) have the highest δ$^{15}$N value (7699.37). The δ$^{15}$N value (7485) of the plants inoculated with Δ*nifB-V* mutant is similar with that of reference control. Whereas, the plants inoculated with *P. polymyxa* WLY78 have the lowest δ$^{15}$N value (5705.87). Here, the δ$^{15}$N value is stable nitrogen isotopes in plants

![Fig. 2](image-url)

Fig. 2 Biomass of cucumber inoculated with *P. polymyxa* WLY78 and Δ*nifB-V* mutant, with non-inoculated cucumber as control. Length of shoots (a) and roots (b). Dry weights of shoots (c) and roots (d). The error bars in the figure indicate SD values. Statistical analysis is performed using Two-way ANOVA. * Indicates P <0.05, ** indicates P <0.01
containing both $^{14}$N and $^{15}$N which were absorbed from soil containing $^{15}$N-labeled (NH$_4$)$_2$SO$_4$. If biological nitrogen fixation occurs, atmospheric $^{14}$N will be converted to NH$_4^+$ and thus $\delta^{15}$N value will become smaller. Therefore, the $\delta^{15}$N value of plants without nitrogen-fixing bacteria is higher than that of plants with nitrogen-fixing bacteria.

The percentage of nitrogen derived from BNF ($\%$Nd$_{dfa}$) was estimated from the $\delta^{15}$N value as described in Materials and Methods. The percentage of nitrogen derived from BNF ($\%$Nd$_{dfa}$) in the $P$. polymyxa WLY78 inoculated cucumber under low N conditions was calculated as follows: $(7699.37 - 5705.87) / 7699.37 \times 100 = 25.9\%$. According to this calculation, the plants inoculated with $P$. polymyxa WLY78 under high nitrogen condition obtained N from BNF is 2.8%. The nitrogen derived from BNF in the plants inoculated the $\Delta$nifB-V strain under low N and high N conditions is 1.5% and 0.2%, respectively. These results indicate that the cucumber plant has incorporated the nitrogen provided by BNF of $P$. polymyxa WLY78 under low N conditions and BNF was inhibited by high concentration of available nitrogen in the environment. The $\Delta$nifB-V strain-inoculated cucumbers only derived a very low amount of nitrogen from BNF, consistent with the $\Delta$nifB-V strain had no nitrogenase. The data also indicate that $P$. polymyxa WLY78 can be used to provide nitrogen nutrition to plants and reduce the use of nitrogen fertilizers.

The results came from three biological replicates, the error represents SD, lowercase letters a and b indicate that there is a significant difference between the groups ($P < 0.05$), while the same letter indicates that there is no significant difference.
Discussion

To estimate the contribution of BNF, the cucumbers inoculated with wild-type *P. polymyxa* WLY78 and with a Δ*nif*B-V mutant and the cucumbers without inoculation (reference control) were grown in soil containing 15N-labeled (NH₄)₂SO₄ as N fertilizer in greenhouse conditions. The contribution of BNF was determined by using the natural abundance 15N measurement technique (Boddey et al. 2001; Bremer and van Kessel 1990). The δ¹⁵N value is stable nitrogen isotope. Plants shall have both 14N and 15N which was absorbed from soil containing 15N-labeled (NH₄)₂SO₄. If biological nitrogen fixation occurs, atmospheric 14N will be changed into NH₃ and thus δ¹⁵N value will become smaller. Therefore, the δ¹⁵N value of plants without nitrogen fixing bacteria is higher than that of plants with nitrogen fixing bacteria. The percentage of nitrogen derived from BNF (%Ndfa) was estimated from the δ¹⁵N value described in Material and Methods. *P. polymyxa* WLY78 provided 25.9% N for cucumber plants by nitrogen fixation under low nitrogen condition, but the Δ*nif*B-V mutant hardly fixed nitrogen. Similar reports are found that nitrogen fixation of *P. beijingensis* BJ-18 provided 27.8% nitrogen for cucumber under low nitrogen conditions (Li et al. 2019) and *P. polymyxa* P2b-2R provided 15% nitrogen for maize (Padda et al. 2017).

Nitrogen fixation is a highly energy-consuming process in which reduction of 1 mol of N₂ requires 16 mol of ATP in vitro (Seefeldt et al. 2009; Hu and Ribbe 2016). Thus, nitrogen fixation is strictly regulated according to ammonium concentration. Most of the N-fixing bacteria fix nitrogen only in absence of ammonium or in presence of low concentration (1-5 mM) of ammonium. Thus, *P. polymyxa* WLY78 and the Δ*nif*B-V mutant showed the similar growth rates in the presence of 100 mM NH₄⁺ (Fig 1c), since nitrogen-fixing ability of *P. polymyxa* WLY78 is inhibited by high concentration of ammonium.

The contribution of nitrogen-fixing bacteria on plant growth via BNF has been reported in *Klebsiella pneumoniae* 342, *Pseudomonas stutzeri* A1501 and *Acetobacter diazotrophicus* PA15 (Iniguez et al. 2004; Ke et al. 2019; Sevilla et al. 2001). A difference between our study with other’s is that the Δ*nif*B-V mutant of *P. polymyxa* WLY78 has a deletion of a compact *nif* gene cluster comprising 9 genes (*nifBHDKENXhesAnifV*) and the other’s mutant (such as the *nifH* mutant or *nifK* mutant or *nifD* mutant) has a deletion of a single *nif* gene.

The effects of *P. polymyxa* WLY78 and Δ*nif*B-V mutant on cucumber growth under different nitrogen concentrations were studied through greenhouse cultivation experiments. Compared to the uninoculated control cucumber plants and the inoculated cucumber plants with the Δ*nif*B-V mutant, inoculated cucumbers with *P. polymyxa* WLY78 were significantly increased in the dry weights and lengths of cucumber shoots and roots under low nitrogen conditions. These results have revealed that the nitrogen fixation of *P. polymyxa* WLY78 plays an important role in promoting plant growth. Phosphate solubilization and IAA production of *P. polymyxa* WLY78 may exhibit a minor role in promoting plant growth. Similarly, inoculation with *Pseudomonas stutzeri* A1501 strain can increase the root and shoot dry weight of maize, but this effect is not found in the maize inoculated with *nifH* mutant (Ke et al. 2019). In N₂-deficient conditions, Kallar grass inoculated with *Azoarcus* sp. BH72 grew better and accumulated more nitrogen than plants inoculated with the *nifK* mutant strain (Hurek et al. 2002.). Inoculation with *K. pneumoniae* 342 resulted in increased dry weight, chlorophyll content, total N, and N concentration of wheat in comparison with the uninoculated and *nifH* mutant-inoculated controls (Iniguez et al. 2004).

Authors’ contributions

Conceptualization: Chen SF; Experiments: Liu S and Li Q; Methodology: Liu S, Li Q, Li YB and Hao TY; Writing: Liu S, Li Q and Chen SF; Funding acquisition: Chen SF. All authors read and approved the final manuscript.

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Data Availability

All data generated or analyzed in this study are included in this published article.

Declarations

Conflict of interest

The authors have no conflicts of interest to declare.

Ethics approval

Not applicable
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