The persistence and performance of phosphate-solubilizing *Gluconacetobacter liquefaciens* qzr14 in a cucumber soil

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Abstract The persistence and performance of plant growth-promoting microorganisms (PGPMs) in soil are considered critical features for effectiveness, yet they are poorly understood. Here, we investigated the colonization and activity of a new PGPM, phosphate-solubilizing *Gluconacetobacter liquefaciens* qzr14, in a pot culture experiment using cucumber as test crop for 20 days. The number of *G. liquefaciens* and bacterial diversity in the rhizosphere and bulk soil were monitored by real-time PCR and DGGE, respectively. Soil phosphorus and cucumber biomass were also examined. *G. liquefaciens* qzr14 effectively colonized the rhizosphere soil (bacterial density ranging from 2.70×10^8 to 1.18×10^9 copies per gram dry soil). *G. liquefaciens* qzr14 inoculation had significantly positive effects on bacterial diversity (BD) of the rhizosphere and bulk soil and the ratio of soluble phosphorus to total phosphorus (SP/TP). The number of *G. liquefaciens* in the rhizosphere soil was significantly related to SP/TP and the BD of the rhizosphere and bulk soil. BD in rhizosphere soil was significantly related to SP/TP and BD in bulk soil. Based on the results of correlation analysis, we inferred that the introduced *G. liquefaciens* qzr14 effectively

colonized the rhizosphere of cucumber, and then expanded its bacterial community by solubilizing soil phosphorus. The expanded bacterial communities might promote cucumber growth by some new functions.

Keywords Bacterial community · Colonization · *Gluconacetobacter liquefaciens* · Phosphate-solubilizing activity · Plant growth-promoting microorganisms (PGPMs)

Introduction

The application of plant growth-promoting microorganisms (PGPMs) has been considered as a highly attractive agricultural method, because it substantially reduces the use of chemical fertilizers and pesticides (Baris et al. 2014; Kumar et al. 2009; Sathya et al. 2016). PGPMs promote plant growth by (i) supplying nutrients (such as nitrogen-fixing bacteria and phosphate-solubilizing bacteria); (ii) producing plant hormones (such as *Bradyrhizobium* spp. and *Rhizobium* spp.); (iii) controlling or inhibiting the activity of plant pathogens (such as *Bacillus* spp. and *Pseudomonas* spp.); (iv) improving soil structure; and (v) inducing systemic resistance (Bhattacharyya and Jha 2012; Bloemberg and Lugtenberg 2001). Apart from these features, PGPMs also promote plant growth by modifying the structure and function of microbial communities (Trabelsi and Mhamdi 2013). Recently, an increasing number of PGPMs being commercialized for various crops worldwide (Owen et al. 2015). Several applications of PGPMs into soils had been successful; i.e., these have resulted in the colonization of soil and plant roots to a level sufficiently high for the intended purpose (Bhattacharjee et al. 2012; Chabot et al. 1996; Luna et al. 2012). However,

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various failures or inconsistencies in achieving the objective have also been reported (Holmberg et al. 2012; Vanelsas et al. 1986; Von Felten et al. 2010). For example, *Burkholderia tropica* increased tomato fruit yields by 17% in the first year, but only 6% in the second year (Bernabeu et al. 2015). Some ACC-utilizing or N₂-fixing bacteria effectively promoted the growth of plants in a sterilized environment, whereas its plant growth-promoting effects were weaker in a non-sterilized environment (Liu et al. 2014). These inconsistencies and failures have raised concerns about the perspective of the great practical potential offered by microbial releases into soils. A key factor involved in the lack of success was the rapid decline of the size of the populations of inoculants to levels ineffective to achieve the objective following its introduction into soil. Population declines have been observed in a wide variety of newly introduced bacteria in rhizosphere soil, including *Pseudomonads fluorescent* (Von Felten et al. 2010), *Bradyrhizobium* sp. (Dudeja and Khurana 1989), *Azospirillum* sp. (Bashan et al. 1995), and *Rhizobium* spp. (Postma et al. 1990). It has been suggested that the scarcity of available nutrient sources to soil microbes and the hostility of the soil environment to incoming microbes due to a myriad of adverse abiotic and biotic factors might be reasons for the decline in PGPM populations in rhizosphere soil (vanVeen et al. 1997). Another factor involved in the lack of success may be related to the activity of PGPMs in soil. Some reports showed that the plant growth-promoting activities can be affected by fungicides, substrate, temperature, pH, microorganism, and so on (Liu et al. 2014).

Previously, our group isolated new PGPM bacteria, phosphate-solubilizing bacterial qzr14, from the rhizosphere soil of eggplant (Yin et al. 2015). Pot experiments showed that qzr14 inoculation can significantly increase the height (27.56%), dry (98.46%), and fresh (57.01%) weight of cucumber seedlings. Here, we investigated the colonization and activity of the qzr14 in soil for 20 days. This study may facilitate in further understanding of the colonization and activity of PGPMs in soil.

Materials and methods

Microorganisms

The bacterial strain qzr14 was isolated from the rhizosphere soil of eggplant in Anshan, Liaoning, China (N 40°59'59.41", E 123°0'29.22") in a previous study (Yin et al. 2015). The pot experiment showed that strain qzr14 can significantly increase the height (27.56%) as well as dry (98.46%) and fresh (57.01%) weight of cucumber seedlings (Yin et al. 2015). 16S rDNA sequence analysis

using primers 27F/1492R was performed to identify strain qzr14 (Tanner et al. 1999). Specific primers of *Gluconacetobacter diazotrophicus*, *G. sacchari*, and *G. liquefaciens* were used to verify the species of strain qzr14 (Franke-Whittle et al. 2005). Growth in the presence of 0.01% malachite green was tested (Franke et al. 1999). Nitrogen fixation, phosphate solubilization and potassium solubilization capacity of strain qzr14 were assessed using selective mediums (Jiménez et al. 2011; Nautiyal 1999). P-solubilization was further determined using the Mo-blue method (Chen et al. 2006). Indoleacetic acid (IAA) and siderophore production were measured using Salkowski reagents and CAS medium, respectively (Rana et al. 2011).

Pot experiments

Soil was collected from the 0 to 20 cm depth of a cropped field in Anshan, Liaoning, China (N 40°59'59.41", E 123°0'29.22"). The soil had a pH of 6.8, organic matter content of 23.5 g kg⁻¹, total nitrogen content of 14.2 g kg⁻¹, total phosphate content of 107 mg kg⁻¹, total potassium content of 31 g kg⁻¹, available nitrogen content of 147 mg kg⁻¹, available phosphate content of 6 mg kg⁻¹, and available potassium content of 376 mg kg⁻¹. The soil was ground and passed through a 1-mm sieve prior to use in the pot experiment. The plant species used in the present study was *Cucumis sativus* L. (Chinese Academy of Agricultural Science, Beijing, China). The experiment was conducted in a plant growth chamber (RXZ, Ningbo Jiangnan Instrument Plant, Ningbo, China) at the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Science. Plastic cylinder pots with a diameter of 8 cm and a height of 9 cm were used. Each pot contained a uniform mix of 118.9 g dry soil and 1.1 g Ca₃(PO₄)₂. Cucumber seeds (No. 8 Zhongnong, China) were soaked in sterilized distillation–distillation H₂O for 10 min. The seeds were then placed on nutritive soil for 7 days to allow germination. Seven-day-old seedlings were removed from the nutritive soil and transplanted into pots (1 seedling per pot). The bacterial strain qzr14 was cultivated overnight in beef extract peptone broth containing 5% glucose at 30 °C. The cultures were centrifuged at 3000 rpm for 5 min. The resulting pellets were resuspended in a 0.9% NaCl solution and diluted to a density of about 1 × 10⁸ cells mL⁻¹. The experimental setup included two treatments: inoculated bacterial strain qzr14 (Q) and a control check (CK). Approximately 20 mL of bacterial suspension (Q) or 0.9% NaCl solution (CK) was applied to the seedlings after transplantation. Fifty plants were grown for each treatment. The plants were grown for 20 days in the plant growth chamber under controlled environmental conditions of 14-h days at 28 °C and 10-h nights at 12 °C. Irrigation was

manually performed every second day. Every 4 days, three plants of each treatment were harvested. Rhizosphere and bulk soil were collected and stored at 4 °C (for soil characterization) or −80 °C (for soil DNA isolation) until analysis.

DNA extraction

DNA was extracted from 0.25 g of frozen rhizosphere or bulk soil using a PowerSoil® DNA isolation kit (Mo Bio Laboratories, CA, USA). The quantity and purity of DNA were determined using a NanoDrop (NanoDrop-1000, Thermo Scientific, USA). The DNA was then stored at −20 °C before use.

Real-time PCR assay

Quantification of copy number of strain qzr14 was performed using a real-time PCR assay. Real-time PCR experiments were conducted in a 7500 Fast Real-Time PCR System (Applied Biosystem, Foster City, CA, USA). Strain qzr14-specific primers (GLF 5'-GGCTGCATTTGATACGTCCA-3'; GLR 5'-GCGTTAACTACGACA CTGAATGA-3') were used (Franke-Whittle et al. 2005). Each PCR was performed in a total reaction volume of 20 µL, which consisted of using 10 µL of SYBR Select Master Mix (2×) (Applied Biosystem, Foster City, CA, USA), 250 nM of each primer, 1 µL of the template DNA ($\sim 3 \text{ ng } \mu\text{L}^{-1}$, $A_{260/280} \sim 1.9$, $A_{260/230} \sim 2.0$), and 7 µL of ddH₂O. The final two-step cycling program included a 2-min initial pre-incubation at 95 °C followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

Standards for real-time PCR assays were prepared as described elsewhere (Wang et al. 2014). Briefly, the specific *16S rRNA* gene of strain qzr14 was PCR-amplified from extracted DNA with the primers GLF/GLR, and the PCR products were cloned into a pMD19-T Simple Vector (TaKaRa, Dalian, China). Plasmids used as standards for quantitative analyses were extracted from the correct insert clones of each target gene using a Plasmid Miniprep Kit (BIOMIGA, Shanghai, China). The concentration of plasmid DNA was determined on a NanoDrop (NanoDrop-1000, Thermo Scientific, USA), and the copy numbers of the target genes were calculated directly from the concentration of the extracted plasmid DNA. Ten-fold serial dilutions of each known copy number of the plasmid DNA were subjected to a real-time PCR assay in triplicate to generate an external standard curve.

Denaturing gradient gel electrophoresis (DGGE)

Denaturing gradient gel electrophoresis analysis of bacterial community of the rhizosphere and bulk soil was

performed with the DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, USA) and primers 341F/GC-518R. Similar amounts of the PCR products were loaded onto polyacrylamide gradient gels with a denaturing gradient of 40–70% (100% denaturant containing 7 M urea and 40% formamide). Electrophoreses were run at 180 V for 6 h. The gels were analyzed using the software Quantity One (Bio-Rad Laboratories) (Wang et al. 2014). UPGMA algorithms were used to cluster the DGGE patterns. The bands in the DGGE gel were excised and amplified with the primers 341F/518R. The purified PCR products were ligated into the pMD19-T Simple Vector (TaKaRa, Dalian, China). The resulting ligation mix was transformed into *Escherichia coli* DH5α competent cells following the instructions of the manufacturer. The positive clones were amplified using the above primers with GC clamp, and then verified by DGGE. The correct clone was selected for sequencing. The sequences were aligned with BLAST, and a phylogenetic tree was constructed using the neighbor-joining method provided in MEGA version 5.0 with a bootstrap value of 1000 replicates. The Shannon diversity index *H* was used to calculate the diversity of bacterial communities.

Soil phosphorus

The bulk soil in each pot was mixed and sampled. All soil samples were air-dried and sieved through a 150-µm mesh sieve before storing for analysis. Soil total phosphate was determined using a Soil Total Phosphate Kit (COMIN, Suzhou Comin Biotechnology Co., Ltd, China). Solubilized phosphorus was determined using the molybdenum blue method (Xie et al. 2013).

Fresh weight of cucumber seedlings

Every 4 days, cucumber seedlings of three pots were harvested. Soils adhering to the rhizosphere were washed with ddH₂O. After air-drying, the fresh weight of the cucumber seedlings was measured on an analytical balance.

Statistical analysis

The copy numbers of strain qzr14 quantified by real-time PCR assay were log-transformed and normalized to per gram dry soil prior to statistical analysis. All statistical analyses were performed with SPSS version 17.0. Mean comparisons were performed between inoculation treatment (Q) and control check (CK) using an independent *t* test at individual time points. Pearson correlation analysis was adopted to analyze the correlations among *G. liquefaciens* populations, bacterial diversity, soil phosphorous,

and fresh weight of cucumber seedlings. $P < 0.05$ was considered statistically significant.

Results and discussion

Properties of bacterial strain qzr14

16S rDNA sequence analysis of bacterial strain qzr14 (Accession no. KP715459) showed high similarity with *G. liquefaciens* (99%), *G. sacchari* (99%), and *G. diazotrophicus* (98%). Among genus *Gluconacetobacter*, only *G. liquefaciens* and *G. sacchari* showed growth on malachite green medium (Franke et al. 1999). Strain qzr14 can grow on 0.01% malachite green medium (Fig. S1). PCR analysis showed that strain qzr14 only can be amplified by the primer of *G. liquefaciens* (Fig. S2). These assays validated that strain qzr14 was *G. liquefaciens*. *G. diazotrophicus* has been widely applied in sugarcane fields based on its nitrogen-fixing ability (Munoz-Rojas and Caballero-Mellado 2003; Saravanan et al. 2008). However, the plant growth-promoting activity of *G. liquefaciens* has never been reported except our previous work. Chemical assays showed that strain qzr14 could grow on Ashby medium, P-solubilization medium, and K-solubilization medium, and that strain qzr14 had IAA secreting ability, and could not form orange holes on CAS medium. The P-solubilization capacity of strain qzr14 was 270 mg L^{-1} (Table S1).

Persistence of *G. liquefaciens* qzr14 in the bulk and rhizosphere soil of cucumber

The colonization and persistence of introduced PGPM in soil are the bases to exert their plant growth-promoting abilities (Compant et al. 2010). The colonization and persistence of introduced PGPM in rhizosphere soil had been widely investigated (Bashan et al. 1995; Couillerot et al. 2010; Pellegrino et al. 2012; Troxler et al. 2012). Population declines have been observed in a wide variety of newly introduced bacteria in rhizosphere soil (Vanelsas et al. 1986; Von Felten et al. 2010). However, our results showed that in the rhizosphere soil, the number of *G. liquefaciens* was significantly higher in the inoculation treatment [Q(R)] (bacterial density ranging from 2.70×10^8 to 1.18×10^9 copies per gram dry soil) than that in the control check [CK(R)] (bacterial density ranging from 8.89×10^7 to 1.92×10^8 copies per gram dry soil) at 4–20 days after inoculation ($P < 0.001$) (Fig. 1). The number of *G. liquefaciens* significantly increased over time in both Q(R) and CK(R). The number of *G. liquefaciens* showed a four-fold increase in Q(R), but only a two-fold enhancement in CK(R) (Fig. 1). Population increases of introduced bacteria

Fig. 1 Persistence of *Gluconacetobacter liquefaciens* qzr14 in the bulk (B) and rhizosphere (R) soil of cucumbers monitored during 20 days by real-time PCR (Asterisks indicate differences between *Gluconacetobacter liquefaciens* qzr14 inoculation (Q) and control check (CK), *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; Means in the line followed by the same letter are not significantly different at a 5% level of significance)

in rhizosphere soil were only observed in some rhizobia (Robert and Schmidt 1983; Steinberg et al. 1989). There might be three reasons for the successful colonization and persistence of *G. liquefaciens* qzr14 in the rhizosphere soil. First, the number of *G. liquefaciens* increased by fourfold in Q(R) but only twofold in CK(R) after inoculation, indicating that the introduced strain qzr14 can reproduce in rhizosphere soil. Second, strain qzr14 is an indigenous microorganism, since it can be detected in CK. It might have the advantage of adapting to the abiotic and biotic factors in the rhizosphere soil environment (vanVeen et al. 1997). Third, the increase in the number of strain qzr14 whether in Q or in CK may be related to the enhancement

Fig. 2 Cluster analysis of denaturing gradient gel electrophoresis (DGGE) results of 16S rDNA amplicons from the rhizosphere soil of cucumber using UPMGA. (Bands marked with letters were excised from the polyacrylamide gel and sequenced. Q, *Gluconacetobacter liquefaciens* qzr14 inoculation; CK, control checks; Q4, Q8, Q12, Q16, and Q20 represent 4, 8, 12, 16, and 20 days after *G. liquefaciens* qzr14 inoculation; C4, C8, C12, C16, and C20, represent corresponding five control checks)

Table 1 Effect of *Gluconacetobacter liquefaciens* qzr14 inoculation on bacterial diversity

Shannon–Weaver index	Treatment	Days after inoculation					Treatment		Time	
		4	8	12	16	20	<i>t</i>	<i>P</i>	<i>F</i>	<i>P</i>
Rhizosphere soil	CK	2.14	2.15	2.14	2.13	2.12	−8.521	0.000	0.056	0.992
	Q	2.24	2.24	2.32	2.27	2.27				
Bulk soil	CK	2.08	2.06	2.03	2.23	2.27	−3.664	0.021	0.277	0.881
	Q	2.31	2.31	2.32	2.30	2.32				

Bold values indicate statistically significant

Q *Gluconacetobacter liquefaciens* qzr14 inoculation, CK control checks

Fig. 3 Cluster analysis of denaturing gradient gel electrophoresis (DGGE) results of 16S rDNA amplicons from the bulk soil of cucumber using UPMGA. (Bands marked with letters were excised from the polyacrylamide gel and sequenced. Q, *Gluconacetobacter liquefaciens* qzr14 inoculation; CK, control checks; Q4, Q8, Q12, Q16, and Q20 represent 4, 8, 12, 16, and 20 days after *G. liquefaciens* qzr14 inoculation; C4, C8, C12, C16, and C20, represent corresponding five control checks)

in plant secretions with cucumber growth as these can supply enough nutrients to soil microorganisms (Vacheron et al. 2013).

Studies on the colonization and persistence of introduced PGPM in bulk soil were limited. Some have shown that the inoculants' number is significantly higher in the rhizosphere than that in unplanted soil (Bashan et al. 1995; Steinberg et al. 1989). Our results showed that the number of *G. liquefaciens* in bulk soil was significantly higher than that in rhizosphere soil of cucumber except for Q(R) and Q(B) at the 20th day after inoculation ($P < 0.01$) (Fig. 1). The lower number of *G. liquefaciens* in the rhizosphere soil might due to the reassembly of microbial communities in the rhizosphere as the original microbial community had been disrupted during transplant.

In bulk soil, the number of *G. liquefaciens* was significantly higher in inoculation treatment [Q(B)] than that in control check [CK(B)] ($P < 0.01$) only at the 4th day after inoculation. Time had minimal effects on the number of *G. liquefaciens* in Q(B) and CK(B) (Fig. 1). This indicated

that the number of *G. liquefaciens* did not differ between Q and CK in the bulk soil, and the number of *G. liquefaciens* in the bulk soil did not change with cucumber growth, which might due to soil capacity. It had been suggested that each soil ecosystem had its own distinctive biological space with respect to the maximum level of microbial biomass (King and Parke 1996).

The effects of *G. liquefaciens* qzr14 inoculation on soil bacterial community

The clustering of DGGE banding patterns of the rhizosphere soil showed a clear separation, with two main groups, one formed by Q and another by CK (Fig. 2). Q had higher bacterial diversity ($P < 0.001$) than CK in rhizosphere soil (Table 1). This indicated that *G. liquefaciens* qzr14 inoculation had a significant effect on bacterial community of rhizosphere soil. Twelve bacterial bands were chosen for further analysis. Bands 1–2, 1–3, 1–4, 1–5, 1–6, 1–7, 1–8, 1–9, 1–10, and 1–12 were present in all treatments; However, these bands showed a four-fold higher intensity in Q (70–250) than that in CK (0–220). The clustering of DGGE banding patterns of bulk soil resulted in three main groups (Fig. 3). CK4, CK8, and CK12 constituted one main cluster (CK), Q8, Q12, Q16, and Q20 comprised the second main cluster (Q), and Q4, CK16, and CK20 made up the third cluster (Q-CK). Q had higher bacterial diversity ($P < 0.001$) than CK in bulk soil (Table 1). This indicated that *G. liquefaciens* qzr14 inoculation had some effects on bacterial community of bulk soil. A total of 11 bands were detected. Bands 2–2 (*Acidithiobacillus*) and 2–10 (*Alicyclobacillus*) were only detected in the CK cluster. Bands 2–7 (*Mesorhizobium*), 2–8 (*Gluconacetobacter*), and 2–11 (Uncultured Firmicutes) were only present in the Q cluster (Fig. 3). Effects on plant growth are not necessarily resulting from a direct effect of the inoculants and may be related to induction or repression of resident microbial populations. Some works showed that inoculants had no effect or a transient effect on microbial community; however, others evidenced a long-term effect. For example, *Sinorhizobium* spp. inoculation

Fig. 4 Neighbor-joining phylogenetic tree of bacteria from bulk (blue rectangle) and rhizosphere (red triangle) soil of cucumber, based on the 16S rRNA gene sequences of DGGE fragments. Values shown in each node of the phylogenetic tree are bootstrap values; 1000 bootstrap replicates were performed

significantly affected bacterial communities in the rhizosphere of alfalfa (Babic et al. 2008; Schwieger and Tebbe 2000). Some rhizosphere microbiome inoculations could modify bacterial communities and change plant flowering time (Panke-Buisse et al. 2015). *R. gallicum* 8a3 and *E. meliloti* 4H41 inoculation showed significant effects on

bacterial structure and diversity in the bulk soil of common bean (Trabelsi and Mhamdi 2013). In this study, we investigated the effect of *G. liquefaciens* qzr14 inoculation on bacterial community in rhizosphere and bulk soil simultaneously. This will further understood the promote mechanisms of inoculants on plant.

Fig. 5 Effects of *Gluconacetobacter liquefaciens* qzr14 inoculation on soil total phosphate (TP), soluble phosphate (SP), and the ratio of soluble phosphate to total phosphate (SP/TP) during 20 days after inoculation. (Asterisks indicate differences between *G. liquefaciens* qzr14 inoculation (Q) and control check (CK), *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; Means in the line followed by the same letter are not significantly different at a 5% level of significance)

Phylogenetic analysis of bacterial genes grouped all sequences into five clusters, namely, alpha, beta, gamma, epsilon Proteobacteria, and Firmicutes (Fig. 4). Epsilon proteobacteria were only present in rhizosphere soil. Gamma proteobacteria appeared in both rhizosphere and bulk soil. However, the only band in the rhizosphere belonged to genus *Halomonas* and all four bands in the bulk soil belonged to genus *Acidithiobacillus*. Beta proteobacteria also appeared in both rhizosphere and bulk soil. However, all two bands in the rhizosphere belonged to uncultured beta proteobacteria. The only band in the bulk soil was identified as genus *Propionivibrivo*. Alpha proteobacteria, especially *G. liquefaciens*, were shared by both rhizosphere and bulk soil except for two bands (identified with uncultured alpha proteobacteria) in the rhizosphere. Genus *Sulfobacillus* and one uncultured bacteria of Firmicutes were only observed in the rhizosphere soil. Another uncultured bacteria of Firmicutes were only identified in bulk soil. The difference in bacterial community composition of the rhizosphere and bulk soil might be related to variations in functions.

The effects of *G. liquefaciens* qzr14 inoculation on soil phosphorus

TP was higher in CK (958.93–1132.53%) than that in Q (764.67–1004.40 mg kg⁻¹) during a period of 20 days after inoculation. TP was significantly lower at the 12th and 16th days after inoculation than that at the 4th and 20th days after inoculation in CK. TP was significantly higher at the 4th day after inoculation than the others in Q (Fig. 5a). SP was higher in Q (172.56–196.84 mg kg⁻¹) than in CK (145.63–170.73%) during a period of 20 days after inoculation. Time had no significant effect on SP in both Q and CK (Fig. 5b). SP/TP was significantly higher in Q (19.62–22.57%) than that in CK (12.53–17.35%) during a period of 20 days after inoculation. The SP/TP was lowest at the 4th day after inoculation in Q and then significantly

Table 2 Effects of *Gluconacetobacter liquefaciens* qzr14 inoculation on the fresh weight of cucumber seedlings during 20 days

Days after inoculation	Q (g)	CK (g)	Rate of increase (%)
4	0.22 ± 0.04d	0.13 ± 0.02d	69.23
8	0.47 ± 0.09c	0.41 ± 0.06c	14.63
12	0.71 ± 0.08b*	0.51 ± 0.02c	39.22
16	0.87 ± 0.16b	0.76 ± 0.04b	14.47
20	1.15 ± 0.04a	1.05 ± 0.18a	9.52

Values with different letters represent significant differences

Asterisks indicate differences between *Gluconacetobacter liquefaciens* qzr14 inoculation (Q) and control check (CK), * $P < 0.05$

Table 3 Correlations of fresh weight of cucumber seedlings, *Gluconacetobacter liquefaciens* population, bacterial diversity, and soil phosphorus

	FW	NR	NB	DR	DB	TP	SP	SP/TP
FW	1							
NR	0.563	1						
NB	−0.041	−0.144	1					
DR	0.204	0.813**	0.022	1				
DB	0.494	0.905**	0.150	0.701*	1			
TP	−0.286	−0.644*	−0.121	−0.849**	−0.541	1		
SP	−0.303	0.476	0.065	0.625	0.384	−0.488	1	
SP/TP	0.130	0.712*	0.081	0.912**	0.598	−0.950**	0.724*	1

Bold values indicate statistically significant

FW fresh weight of cucumbers seedlings, NR log copy numbers of *Gluconacetobacter liquefaciens* qzr14 per g cucumber rhizosphere soil, NB log copies numbers of *G. liquefaciens* qzr14 per g cucumber bulk soil, DR the bacterial diversity of cucumber rhizosphere soil, DB bacterial diversity of cucumber bulk soil, TP total phosphate, SP solubilized phosphate

** $P < 0.01$; * $P < 0.05$

Fig. 6 Performance of *Gluconacetobacter liquefaciens* qzr14 in the rhizosphere and bulk soil of cucumber

increased at the 8th day after inoculation. The SP/TP in CK dramatically decreased at the 20th day after inoculation (Fig. 5c). After a successful colonization, PGPMs would exert its ability in soil. Our results showed that the introduced *G. liquefaciens* qzr14 can exert its P-solubilizing ability in soil, as soil TP significantly decreased and SP/TP significantly increased after *G. liquefaciens* qzr14 inoculation. SP/TP was an effective index for the evaluation of phosphorus solubilizing ability of microorganisms. TP and SP are usually influenced by plants. The absorption of available phosphorus by inoculated plant in soil was more than that by non-inoculated plant as inoculated plant growth was faster than that of non-inoculated plants.

The effects of *G. liquefaciens* qzr14 inoculation on cucumber seedlings

The fresh weight of cucumber seedlings with *G. liquefaciens* qzr14 inoculation (Q) was higher than that of CK

during a period of 20 days after inoculation. *G. liquefaciens* qzr14 inoculation (Q) increased cucumber seedling fresh weight from 9.52 to 69.23% (Table 2). After 12 days inoculation, the fresh weight of cucumber seedlings in Q was significantly higher than that in CK ($P < 0.05$) (Table 2). The fresh weights of cucumber seedling significantly increased over time in both qzr14 ($P < 0.01$) and CK ($P < 0.01$) (Table 2).

Correlations on the number of *G. liquefaciens*, bacterial diversity, soil phosphorus, and the fresh weight of cucumber seedlings

The number of *G. liquefaciens* in the rhizosphere soil was significantly positively related to bacterial diversity of rhizosphere ($r = 0.813$, $P < 0.01$) and bulk ($r = 0.905$, $P < 0.01$) soil and SP/TP ($r = 0.712$, $P < 0.05$) and was significantly negatively related to TP ($r = -0.644$, $P < 0.05$) (Table 3). The bacterial diversity in the

rhizosphere soil was significantly positively related to bacterial diversity in the bulk soil ($r = 0.701$, $P < 0.05$) and SP/TP ($r = 0.912$, $P < 0.01$) and was significantly negatively related to TP ($r = 0.849$, $P < 0.01$). TP was negatively related to SP/TP ($P < 0.01$). SP was positively related to SP/TP ($P < 0.05$). The fresh weight of cucumber seedlings and the number of qzr14 in cucumber bulk soil had no significant correlation with other parameters (Table 3). The use of phosphate-solubilizing bacteria as inoculants always increases P uptake by the plant and crop yield (Rodríguez and Fraga 1999). However, *G. liquefaciens* qzr14 did not promote cucumber growth by solubilizing phosphate, because the fresh weight of cucumber seedlings had a lower correlative with phosphorus and higher correlative with *G. liquefaciens* number in the rhizosphere soil. *G. liquefaciens* qzr14 inoculation had significant positive effects on bacterial diversity and biomass. Several reports indicated that the application of microbial inoculants can influence, at least temporarily, the resident microbial community's composition and function by directly inducing trophic competitions and antagonistic/synergic interactions with resident microbial populations or indirectly mediating via enhanced root growth and exudation (Trabelsi and Mhamdi 2013). It was inferred that the introduced *G. liquefaciens* qzr14 effectively colonized the rhizosphere soil of cucumber and then expanded the rhizosphere bacterial communities by solubilizing soil phosphorus and supplying soluble phosphorus (Fig. 6). The expanded bacterial communities might promote cucumber growth by some new functions (Saikaly and Oerther 2011).

Conclusion

In summary, we found that the introduced *Gluconacetobacter liquefaciens* qzr14 effectively colonized in rhizosphere other than bulk soil of cucumber. The effectively colonized *G. liquefaciens* qzr14 might expand rhizosphere bacterial community by solubilizing soil phosphorus. The expanded bacterial communities might promote cucumber growth by some new functions.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no financial/commercial conflicts of interest.

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