Growth promotion effects of the endophyte *Acinetobacter johnsonii* strain 3-1 on sugar beet

Yingwu Shi · Kai Lou · Chun Li

Abstract An endophytic bacteriumn identified as *Acinetobacter johnsonii* strain 3–1 was isolated from surface-sterilized roots of *Beta vulgaris*. Its effect on sugar beet seedling growth was studied using pot assays and field experiments. This strain promoted beet seedling growth following seed inoculation by seed dipping. Plant height and dry weight of beet increased by 19% and 69%, respectively, compared with controls. Strain 3–1 exhibited the ability to increase absorption of N, P, K, and Mg elements from soil and increase the content of vitamins B and C, and protein within beet. In addition, the strain also produced a phytohormone-auxin, produced nearly twice as much IAA as that produced by strain 2–2, and was able to *solubilize phosphates*. The concentration of dissolved P in the medium was 180.5 mg L^{-1} after 4 days of incubation. In field experiments, strain 3–1 significantly increased the content of sucrose, fructose, and the yield of the beet. The growth-promoting properties of *Acinetobacter johnsonii* strain 3–1 indicates that this promising isolate merits further investigation into its symbiosis with beet plants and its potential application in agriculture.

Y. Shi · K. Lou (✉)
Institute of Microbiology,
Xinjiang Academy of Agriculture Science,
Urumqi, Xinjiang, China
e-mail: loukai02@mail.tsinghua.edu.cn

Y. Shi
Key Laboratory of Green Chemical Processes of Xinjiang
Production and Construction Corps, College of Agronomy,
Shihezi University,
Shihezi, Xinjiang, China

C. Li
Department of Biotechnology, School of Life Science
and Technology, Beijing Institute of Technology,
Beijing, China

1 Introduction

Sugar beet is the most important sugar crop in the developing world. Fertilizers and pesticides are the most important inputs for beet production but using growth-promoting bacteria can contribute to making beet cultivation sustainable and less dependent on fertilizers. Among such bacteria are rhizobacteria, which colonize plant roots and consume root exudates and lysates (Pieterse et al., 2002; Antoun and Prevost, 2006). Some strains, the growth-promoting rhizobacteria (PGPR), can promote plant growth. They improve the cycling of nutrients and minerals such as nitrogen, phosphate and can be used as biofertilizers (Kennedy et al., 2004). The growth-promoting effects of PGPR are affected by environmental conditions and therefore are not stable. Endophytes including a variety of bacteria, fungi, and actinomycetes and can be isolated from cultivated crops (Liu and Tang 1996) as well as from wild plants (Brooks et al. 1994) both monocotyledons (Fisher et al. 1992) and dicotyledons (El-Shanshoury et al. 1996).

Healthy roots of sugar beet have been shown to harbour endophytic bacteria and healthy leaves to harbour endophytic fungi (Jacobs et al. 1985; Larran et al. 2004), little is known about the behaviour of these microorganisms during the growing season. Endophytes occupy microniches within plant tissues and some have been found to be growth-promoting endophytes (PGPE). These endophytes are unaffected by competition from other microorganisms or conditions in the rhizosphere. As a result, a new approach has been developed in recent years to reduce fertilizer use which consists of treating seeds and seedlings with PGPE.

Thus, inoculation of pepper with endophytic *Pseudomonas fluorescens* significantly increased fresh weight, height, and stem diameter (Lucas-Garca et al. 2003). In an previous study, we isolated 221 endophytic bacterial isolates from tissues sterilized sugar beets from a widely planted beet cultivar (*Beta vulgaris* L. var. *saccharifera*) variety KWS2409 in a semi-arid region in east Urumuqi in Xinjiang,China. In the present study, we assess the effect of *Acinetobacter johnsonii* strain 3–1 on the growth of beet seedlings and on the ability of their root system to absorb mineral nutrients.

2 Materials and methods

2.1 Sugar beet variety, bacterial strain, and soil

The soil for the pot experiments was collected from a fertile field in northern Xinjiang. Chemical and physical properties of soil were analysed (Table 1). Total carbon, C, was determined by muffle furnace heating the soil to 500°C; total nitrogen, N, by the Kjeldahl method; and total phosphorus, P, by molybdenum blue colorimetry (Riehm 1985). Calcium, extractable magnesium, potassium, and sodium were measured using an atomic absorption spectrophotometer (Schachtschnabel and Heinemann 1974); pH was determined with a pH meter; and total salt content was assessed by measuring the electrical conductivity of soil.

Sugar beet (*Beta vulgaris* L. var. *saccharifera*) variety KWS2409 and *Acinetobacter johnsonii* strain 3–1 (GenBank accession number EU594557) were used, with *Chryseobacterium indologenes* strain 2–2 (GenBank accession number EU594563) as control for 3–1, in the study assessing the effect of bacteria on plant growth. Sugar beet seeds (*Beta vulgaris* L. var. *saccharifera*) variety KWS2409 were obtained from Toutunhe farm in a semi-arid region in east Urumuqi in Xinjiang,China.

2.2 Plant growth and inoculation in pots

Both strains 3–1 and 2–2 were grown in a liquid medium (3 g beef extract, 5 g peptone, 5 g NaCl, and 1 g glucose, in 1 L water, pH 7.0–7.2) shaken at 200 rpm at 30°C for 48 h, after which the bacterial cells were precipitated by centrifugation at 5,000×g for 5 min. The pellets were suspended in sterile distilled water and the concentration of the suspension

adjusted to 10^8 cfu ml^{-1} with a spectrophotometer (Mayak et al. 2004). Sugar beet seeds were surface-sterilized with 70% alcohol for 8 min followed by 5% hydrogen peroxide solution for 2 h and then rinsed in sterile water (Caitriona et al. 2004). The disinfected seeds were immersed in the bacterial suspension for 6 h and then sown in pots, 10 seeds per pot. After germination, the stand was thinned to keep six plants in each pot. Ten replicates were maintained, with beet seeds immersed in distilled water instead of the bacterial suspension serving as control (CK). The pots were kept in a growth chamber with the day/night temperatures set at 30°C/25°C and 200 μmol photons m^{-2} s^{-1} of light supplied throughout the 12 hday. Soil moisture was maintained at 60% of the soil's moisture-holding capacity. Germination percentage was recorded 7 days later and data on plant height, fresh weight and dry weight, and the contents of N, P, K, and Mg and of Vitamin B,Vitamin C and protein recorded 4 weeks after germination.

2.3 Re-isolation of endophytic bacteria

Endophytic bacteria from the seedling plant tissues were re-isolated 4 weeks after germination. To do this, fresh samples of sugar beet plants were washed thoroughly with tap water to remove adhering soil and debris, rinsed in 95% ethanol, and then flamed. This minimised carry-over of external bacteria when cross-sectioning at various locations using a sterile knife. Samples were aseptically removed from the core, periphery, and secondary root emergence zone (crease) areas. Each sample of root tissue was aseptically weighed, then added to 100 mL of sterile saline (0.85%), and blended for 2 min in a Waring blender. The blended samples were initially diluted to standardise all preparations. This was followed by additional serial decimal dilution in sterile saline. Endophytic bacteria were isolated using an aerobic spread-plate method. Dilution volumes of 0.1 mL were plated in triplicate on nutrient agar (NA) supplemented with 2.0 g/L sucrose. The spread plates were incubated at room temperature for 7 d and colony counts recorded by standard methods (Kodaka et al. 2005). Pure cultures were subsequently isolated and bacterial isolates were stored on agar slants.

2.4 Identification of endophytic bacteria

Pure cultures of the bacterial isolates were prepared for identification purposes. The bacterial re-isolates 3–1 and

Table 1 Chemical and physical properties of soil^a in the top (0–25 cm) layer

Parameter	C_t g/kg	N_t	P	K	Mg	Na	Ca	Total salts	pH
Value	24.1	1.28	1.1	0.15	0.04	0.14	0.12	1.32	7.85

^{a} The soil was yellow brown

Fig. 1 Effect of Strain 3–1 on growth of sugar beet. From left CK-W, seeds soaked in water; 3-1-N, seeds soaked in a suspension of Strain 3–1 in nutrient solution; CK-N, seeds soaked in nutrient solution

2–2 were identified using the Vitek AutoMicrobic System (Vitek AMS; Vitek Systems, Inc., Hazelwood, MO.). The Vitek test was repeated twice. Isolates were characterized by Vitek AMS, colony morphology, catalase production, oxidase test, and gram stain.

2.5 Phylogenetic analysis of the isolated bacteral strains

The 16S rRNA gene of genomic DNA isolated was extracted from pure bacterial colonies following standard protocols. The primers used for PCR were pF (5 -AGA GTT TGATCC TGG CTC AG-3) and pR (5 -AAG GAG GTG ATC CAG CCG CA-3).The PCR products with the expected size (about 1,500 bp) were purified using a DNA Gel Extraction Kit and cloned into pMD18-T vector followed by sequencing. Sequence analysis was performed using the BLAST algorithm (http://www.ncbi.nlm.nih.gov). Bacterial identifications were based on 16S rRNA gene sequence similarity. For further characterization of

isolates 3–1 and 2–2, a neighbor-joining phylogenetic tree was constructed with the MEGA 4.0 program.

2.6 Measuring the ability of strain 3–1 to solubilize phosphates

Strains 3–1 and 2–2 were grown in PKO ($Ca_3(PO_4)_2$) medium Pikovaskaia's medium, Yao 2004, per liter: $Ca_3(PO4)_2$ 3.0~5.0 g, sucrose 10.0 g, $(NH_4)_2SO_4$0.5 g, NaCl 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, KCl 0.2 g, yeast extract 0.5 g, $MnSO_4$1 mL (0.004 g/L), $FeSO_4$ (Fe-EDTA) 0.1 mL (0.002 g/L), agar 15 g, pH value 7.0±0.2 () and incubated at 30°C for 12 h with vigorous shaking. The concentration of the bacterial suspension was adjusted to 10^8 cfu ml^{-1} and the suspension used as the primary inoculum. Two millilitres of the primary inoculum were added to 200 ml PKO medium whereas 2 ml distilled water added to 200 ml PKO medium served as control. Each treatment was repeated three times. The phosphates-solubilizing capability of Strains 3–1 and 2–2 were measured every 24 h for 7 days. Each time, a sample of the culture medium was centrifuged at 10,000 rpm for 20 min, and pH of the supernatant determined with a pH meter. Dissolved P ($H_2PO_4^-$) was determined using molybdenum blue colorimetry (Zhao et al. 2003; Zhang 2008). The pellet was suspended in 0.1 mol·L^{-1} HCl, rinsed three times in sterilized water, and optical density (OD660nm) of the suspension was measured.

2.7 Measuring the ability of strain 3–1 to secrete IAA

The IAA assay was performed using the method described by Patten and Glick (2002) with some modifications. Both the strains, namely strains 3–1 and 2–2, were multiplied overnight in 5 ml of DF salts minimal medium (Dworkin and Foster 1958) which had the following composition (for 1 L): 4.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$,
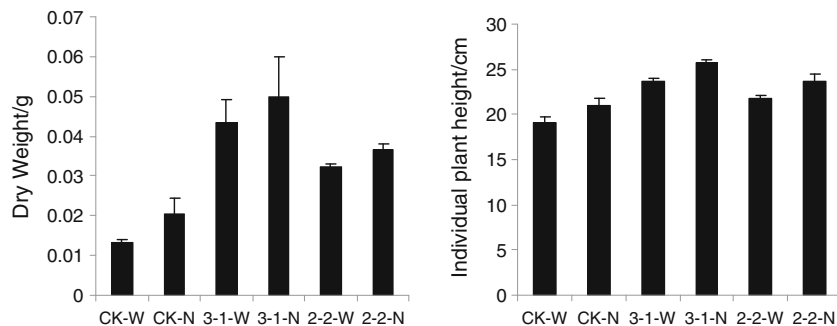

Fig. 2 Effect of seed inoculation on growth of beet seedlings. CK-W, seeds soaked in water; CK-N, seeds soaked in Hoagland solution; 3-1-W, seeds soaked in a suspension of Strain 3–1 cells in water; 3-1-N, seeds soaked in a suspension of Strain 3–1 cells in

Hoagland solution; 2-2-W, seeds soaked in a suspension of Strain 2–2 cells in water; 2-2-N, seeds soaked in a suspension of Strain 2–2 cells in Hoagland solution. The results are representative of 48 plants. Error bars show the standard deviation

Table 2 Effect of inoculation with Strain 3–1 on the content (mg/kg fresh weight) of N, P, K, and Mg and of Vb$_2$,Vc, and protein in beet

Treatment	N$_t$	P	K	Mg	VB$_2$(10^{-3})	VC	Protein
CK-W	1789.23±13.56	14.44±0.59	3629.99±61.41	659.61±17.59	1.62±0.05	0.82±0.01	964.01±0.88
CK-N	1828.06±35.09	15.39±0.51	3657.90±9.06	669.64±8.80	1.79±0.07	0.86±0.01	1002.43±1.90
3-1-W	2005.11±5.72*	19.63±0.44*	3823.65±88.53*	857.05±3.30*	1.94±0.07*	0.93±0.02*	1497.25±1.46*
3-1-N	2118.56±14.35*	21.78±0.63*	3949.72±50.98*	877.41±8.65*	2.06±0.05*	0.96±0.01*	1546.67±3.49*
2-2-W	1824.20±28.58	16.39±0.61	3653.14±10.16	664.85±7.75	1.86±0.07	0.85±0.02	1354.39±3.70
2-2-N	1852.63±18.27	17.63±0.62	3701.36±29.83	687.13±10.03	1.95±0.05	0.9±0.02	1405.92±2.19

Average ± standard error from three separate experiments. Values with the symbol * indicate significant differences at $P<0.05$ from corresponding control

2.0 g glucose, 2.0 g gluconic acid, and 2.0 g citric acid; trace elements: 1 mg FeSO$_4$·7H$_2$O, 10 mg H$_3$BO$_3$, 11.1 mg MnSO$_4$·H2O, 124.6 mg ZnSO$_4$·7H$_2$O, 78.22 mg CuSO$_4$·5H$_2$O, and 10 mg MoO$_3$; pH 7.2; and 2.0 g (NH$_4$)$_2$SO$_4$ as the nitrogen source. The next day, 20 μL of the primary inoculum was transferred into 5 ml of the DF medium supplemented with 100 μg ml^{-1} L-tryptophan (Sigma Chemicals) taken from a filter-sterilized 2 mg/ml stock prepared in warm water. After incubation for 48, 72, 96, and 120 h, the density of each culture was measured spectrophotometrically at 600 nm. The bacterial cells were then removed from the culture medium by centrifuging at 6,000×g for 15 min; a 1 ml aliquot of the supernatant was mixed vigorously with 4 ml of Salkowski's reagent (Gordon and Weber 1951) consisting of 150 ml of concentrated H$_2$SO$_4$, 250 ml of distilled H$_2$O, and 7.5 ml of 0.5 M FeCl$_3$·6H$_2$O, and allowed to stand at room temperature for 20 min before the absorbance at 535 nm was measured. The concentration of IAA in each culture medium was determined by comparing with a standard curve.

### 2.8 Estimation of internal root colonization

Pot trials were designed to assess the internal colonization of sugar beet roots after seedling inoculation through the seed soaking method described above. Rifampicin-resistant mutants of isolates 3–1 and 2–2 were prepared as described by He et al. (2004). The obtained mutants were compared with their wild types in relation to their ability to produce auxins. None of these mutants differed morphologically from their parental strains, and all mutants had identical growth rates and auxin-production capability with their parental strains. Sugar beet seedlings were prepared as described above and inoculated with isolates 3–1 and 2–2. Every week after planting (1–8), roots were sampled from the soil, washed thoroughly in tap water, surface disinfested as described above and the population densities of isolates 3–1 and 2–2 (log10 CFU g−1 fresh root weight) were determined using NA amended with rifampicin (300 μgml^{-1}, Sigma). Each treatment was replicated five times with two plants in each replicate for each sampling.

2.9 Field experiments to evaluate performance of strain 3–1

Field experiments were conducted at the Toutunhe Farm in Urumuqi, Xinjiang province, China, from 2009 to 2010. Strain 3–1 inoculation was made the same way as pot experiment, inoculated by using the root drench method. Beets were harvested shortly after the 7th week of testing, at which time the plants were about 15 cm at the crown.

Fig. 3 Phylogenetic analysis of partial 16S rDNA sequence of isolates *Acinetobacter johnsonii* strain 3–1 obtained from sugar beet. Numbers in parentheses represent the sequencesaccession number in GenBank. The number at each branch points is the percentage supported by bootstrap
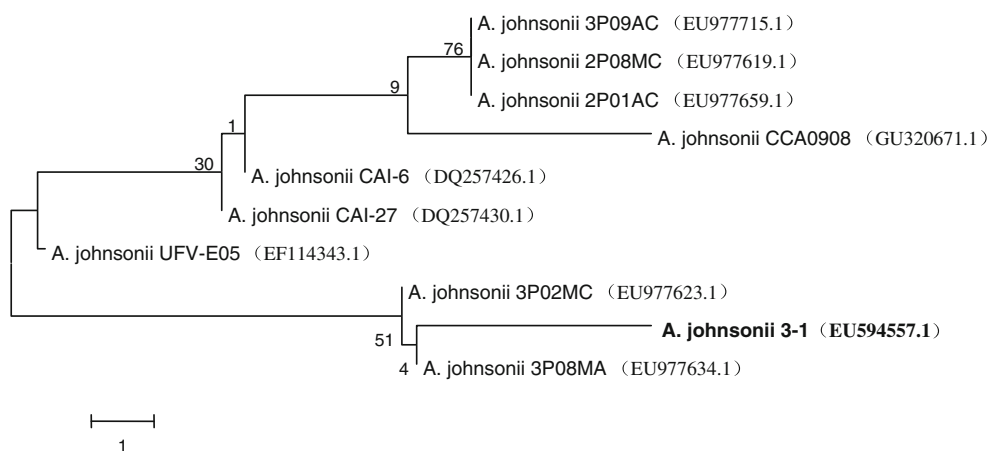
Beets were fertilized, irrigated, and weeds controlled following typical production practices in northwest, China. The sucrose and fructose assay was performed using a spectrophotometric method (Taylor, 1995). Yield comparisons among treatments were made at harvest date, and sugar content was determined by refractive method.

2.10 Statistical analysis

All experiments were arranged in completely randomised block designs. Population data were transformed into log10 CFU g^{-1} fresh weight. Data were subjected to analysis of variance, and treatment means were compared using Fisher's Protected LSD Test at $P<0.05$.

3 Results

3.1 Effect of strain 3–1 on plant growth

Results of seed inoculation are shown in Figs. 1 and 2. Germination percentage was significantly higher in seeds inoculated with *Acinetobacter johnsonii* strain 3–1 (3–1-W) and so were the two other growth parameters, namely dry weight and plant height. Germination rate of inoculated seeds was 92%, 28.6% (in the text just give percentages to the nearest whole percentage i.e. 92% and 29%) greater than that of seeds that had been soaked in water (CK-W). Dry weight of seedlings from the inoculated seeds was about 3-fold greater, and the seedlings were 18.3% taller, than those from seeds that had been soaked only in water before sowing. Plants from seeds inoculated with strain 3–1 grew significantly better than those growing from seeds that had been soaked in water (W) or in Hoagland solution (N). Plants from seeds inoculated with strain 3–1 were 19.4% taller and 69.8% heavier than those from uninoculated seeds. In comparison, the 2–2 strain did
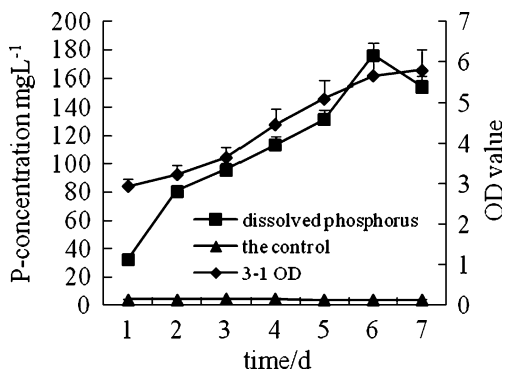

Fig. 4 Relationship between growth of Strain 3–1 and level of dissolved phosphorus. The results are representative of three experiments, with distilled water added to PKO medium as control. Error bars indicate standard deviation
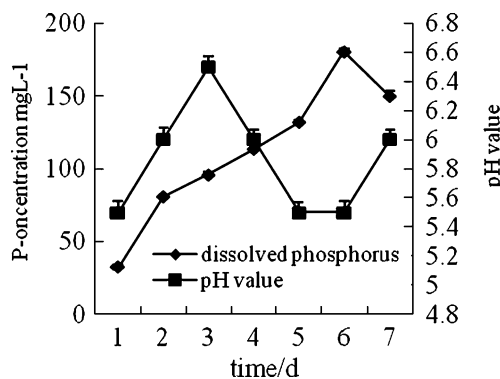

Fig. 5 Relationship between dissolved phosphorus (in the culture medium inoculated with Strain 3–1) and pH of the culture medium. The results are representative of three experiments. Error bars indicate standard deviation

increase the dry weight of beets, and it had little effect on plant height, however.

3.2 Effect of strain 3–1 on plant nutrient uptake

The content of N, P, K, and Mg and of vitamin B, vitamin C, and protein was determined 4 weeks after germination (Table 2). Inoculation with Strain 3–1 significantly increased the uptake of all the elements that were analysed: total N and P following inoculation with strain 3–1 were greater by 10.8% and 26.4% respectively than their levels in CK-W, and the levels of K and Mg were also higher. It was also found that salt, that is soaking the seeds in a nutrient solution, affected the uptake of nutrients from soil: levels of N and P in CK-W were lower by 2.1% and 6.2% (In the test just give the % to the nearest whole percentage, i.e. 2% and 6%) respectively than the levels in CK-N. Inoculation with strain 3–1, however, counteracted this effect, levels of K^+ and Mg^{2+} in strain 3–1-N being 7.4% and 23.6% higher respectively than those in CK-N and levels of total Vitamin B,Vitamin C, and protein being 13.1%, 10.4%, and 35.2%

Table 3 Production of IAA by Strain 3–1 in a medium supplemented with tryptophan

Time (h)	IAA production ($\mu g/ml/OD_{600}$ unit)	
	Strain 3–1	Strain 2–2
24	21.53+2.17	10.12+1.01
48	42.19+2.87	15.97+1.60
72	95.84+3.00	20.49+2.05
96	126.97+3.23	25.64+2.56
120	119.45+3.40	33.23+3.32
144	51.07+3.86	33.20+3.32
168	49.56+3.36	29.10+2.91

A average + standard error from three separate experiments

Table 4 Average quantities of bacterial isolate 3–1 at different times of sampling of beet roots grown under glasshouse conditions

Time after inoculation (weeks)	Average quantity ($\log_{10}$ CFU g^{-1} fresh weight)
1	4.32b A
2	5.86a A
3	6.04a A
4	4.36b A
5	3.79c AB
6	3.75c AB
7	3.72c AB
8	3.58c B

Values are means of five replicates for each sampling, and the values with the same lower or same upper case letter are not significantly different according to LSD

higher respectively in strain 3–1-N than those in CK-N. Strain 2–2 also influenced the ability of sugar beet to accumulate ions, although not to the same extent as strain 3–1: levels of N, P, and K^+ in 2–2-W were 1.9%, 11.9%, and 0.6% higher respectively than those in CK-W.

3.3 Re-isolation and identification of endophytic bacteria

Endophytic bacteria were re-isolated from in vitro inoculated beet tissues. The strain 3-1-1 strain had the phenotypic properties of *Acinetobacter johnsonii* strain 3–1. Thus the cells were Gram-negative rods measuring $0.5–1.0 \times 0.9$–5.8 μm. They were motile at a growth temperature of 4–41°C. The cytochrome oxidase test was positive. Nitrate reduction was negative. Phenotypic data of morphological, physiological, and biochemical characteristics combined with 16S rRNA gene (GenBank accession number EU594563) analysis (Fig. 3) allowed the identification of the strain 3-1-1 as *Acinetobacter johnsonii*. Strain 3-1-1 presented high similarity values with *Acinetobacter johnsonii* (100%) in the BLASTn search, and were recovered in a tight cluster with *Acinetobacter johnsonii* in the phylogenetic tree (Fig. 3).

3.4 Effect of strain 3–1 on the level of solubilized phosphates

The relationship between plant growth, measured by the number of days after germination, and (a) the levels of

dissolved P in the culture medium and (b) the pH of the culture medium is shown in Figs. 4 and 5 respectively. Without strain 3–1, the mean P content of the culture medium was very low and the pH remained at 8.0. However, with strain 3–1, the levels of dissolved P increased (reaching 180.5 mg L^{-1} on the sixth day) and the pH decreased to 5.5. However, in the first 144 h, as the strain multiplied, the level of dissolved P decreased significantly from 180.5 mg L^{-1} to 150.0 mg L^{-1}, a decrease that could be attributed to the use of P by the bacteria for their growth.

3.5 Secretion of IAA by strain 3–1

When cultured in a medium supplemented with 100 μg ml^{-1} tryptophan for approximately 48–168 h, strain 3–1 produced roughly up to twice as much IAA as that produced by strain 2–2 (Table 3). The rate of IAA production increased with time up to Day 6 (up to 144 h) but slowed down on Day 7 (at the end of 168 h).

3.6 Estimation of internal root colonisation

The rifampicin-resistant mutants of isolates 3–1 were isolated from the *in vitro* inoculated beet tissues, indicating that the strain was an endophytic bacteria. Isolate 3–1 maintained their endophytic colonising abilities and were isolated from healthy beet roots in all samplings until week 8 (Table 4). An initial increase in colonies after 1, 2 and 3 weeks was followed by a decrease at the 4th week. However, at and after week 5 and up to week 8, the populations were relatively stable (Table 4).

3.7 Impact of strain 3–1 on yield

In field experiments, the content of sucrose, fructose, and the biomass were determined at harvest. Sugar beets were harvested by hand in October 2006. Inoculation with *Acinetobacter johnsonii* strain 3–1 significantly increased the content of sugar that were analysed: the content of sucrose and fructose following inoculation with strain 3–1 were greater by 9.0% and 41.2% respectively than their levels in CK. Inoculation with strain 3–1, levels of biomass

Table 5 Effect of indophytic bateria inoculation on sugar increasing of sugar beet

Strain number	Sucrose (%) Mean ± SE	Fructose (%) Mean ± SE	Fresh leaf mass per plant (g·plant^{-1})	Fresh root mass per plant (g·plant^{-1})	Sugar content (%)	Sugar yield (g·plant^{-1})
3–1	1.21+0.022 a	0.62+0.008 a	589a	1709a	19.50a	333.26a
CK	0.43+0.008b	0.41+0.009 b	420b	1550b	15.75b	244.13b

Data are an average of 10 plants for each treatment + SE. Comparisons followed by different letters are significant at the 0.05 probability level (Tukey's test). Different letters meant significant difference at 0.05 level

and sugar content being 16.65% and 23.81% higher respectively than those in CK (Table 5).

4 Discussion

Endophytes are known to promote the growth of plants (Compant et al. 2005a, b), primarily by increasing the supply of nutrients to the host plant. The results of the present study support this as inoculation of sugar beet seeds with *Acinetobacter johnsonii* strain 3–1 increased the levels of N, P, K^+, Mg, VB_2,Vc and the protein content of the plants (Table 2). The growth may also be enhanced by the ability of strain 3–1 to solubilize phosphate. Phosphorus is very important for normal plant growth and development. PGPR, which have the ability to solubilize P, increased the phosphate content and plant yield of maize and lettuce (Chabot et al. 1996; Yao 2004). Our study confirms this and revealed that strain 3–1 was alabe to solubilize $Ca_3(PO_4)_2$ a major type of phosphorus. Further studies are needed using other forms of phosphorus that are difficult to solubilize.

Bacteria that can produce IAA and siderophores that can stimulate plant growth and help plants to acquire sufficient iron and phosphate for optimal growth (Glick et al. 1995; Chabot et al. 1996; Rajkumar et al. 2006). For example, *Trianthema aestivum* secretes IAA, which enhances root development and improves the uptake of mineral nutrients (Ali et al. 2009). Bacteria of the genus *Methylobacterium* can benefit plants by promoting nitrogen metabolism, producing vitamin B12 (Basile et al. 1985; Holland and Polacco 1992, 1994; Holland 1997). Indeed, many beneficial effects on plant growth have been attributed to endophytes including osmotic adjustment, stomatal regulation, modification of root morphology, enhanced mineral uptake of minerals, and altered nitrogen accumulation and metabolism (Compant et al. 2005a, b). However a great deal remains to be done to understand the symbiosis between endophytes and their host plants. Only when this research has been completed will we be able to use endophytes optimally in agriculture, forest regeneration or phytoremediation of contaminated soils.

References

Ali B, Sabri AN, Ljung K, Hasnain S (2009) Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of Triticum aestivum L. Lett Appl Microbiol 48:542–547

Antoun H, Prevost D (2006) Ecology of plant growth promoting rhizobacteria. In: Siddiqui ZA (ed) PGPR: Biocontrol and Biofertilization. Springer, Dordrecht, pp 1–38

Basile DV, Basile MR, Li QY, Corpe WA (1985) Vitamin B12-stimulated growth and development of Jungermannia leiantha Grolle and Gymnocolea inflata (Huds.) Dum. (Hepaticae). Bryologist 88:77–81

Brooks DS, Gonzalez CF, Appel DN, Filer TH (1994) Evaluation of endophytic bacteria as potential biological control agents for oak wilt. Biol Control 4:373–381

Caitriona D, Wilson LW, McFadden H (2004) Gene expression profile changes in cotton root and hypocotyl tissues in response to infection with Fusarium oxysporum f. sp. Vasinfectum Mol Plant Microbe In 17:654–667

Chabot R, Antoun H, Cescas MP (1996) Growth promotion of maize and lettuce by phosphate solubilizing Rhizobium leguminosarium biovar phaseoli. Plant Soil 184:311–321

Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005a) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Compant S, Reiter B, Sessitsch A, Nowak J, Clement C, Barka EA (2005b) Endophytic colonization of Vitis vinifera L. by a plant growth-promoting bacterium, Burkholderia sp. Strain PsJN. Appl Environ Microbiol 71:1685–1693

Dworkin M, Foster J (1958) Experiments with some microorganisms which utilize ethane and hydrogen. J Bacteriol 75:592–601

El-Shanshoury AR, El-Sououd SMA, Awadalla OA, El-Bandy NB (1996) Effects of Streptomyces corchorusii, Streptomyces mutabilis, pendimethalin and metribuzin on the control of bacterial and Fusarium wilt of tomato. Can J Bot 74:1016–1022

Fisher PJ, Petrini O, Lazpin SHM (1992) The distribution of some fungal and bacterial endophytes in maize (Zea mays L.). New Phytol 122:299–305

Glick BR, Karaturovic DM, Newell PC (1995) A novel procedure for rapid isolation of plant growth promoting pseudomonas. Can J Microbiol 41:533–536

Gordon SA, Weber RP (1951) Colorimetric estimation of indoleacetic acid. J Plant Physiol 26:192–195

He H, Qiu SX, Cai XQ, Guan X, Hu FP (2004) Colonization in plants and identification of endophytic bacteria BS-1 and BS-2 from Capsicum annuum. Acta Microbiol Sinica 44:13–18

Holland MA (1997) Occams razor applied to hormonology (Are cytokinins produced by plants?). J Plant Physiol 115:865–868

Holland MA, Polacco JC (1992) Urease-null and hydrogenase-null phenotypes of a phylloplane bacterium reveal altered nickel metabolism in two soybean mutants. J Plant Physiol 98:942–948

Holland MA, Polacco JC (1994) PPFMs and other covert contamination: is there more to plant physiology than just plant? Ann Rev Plant Physiol Plant Mol Biol 45:197–209

Jacobs MJ, William MB, David AG (1985) Enumeration, location, characterization of endophytic bacteria within sugar beet roots. Can J Bot 63:1262–1265

Kennedy IR, Choudhury ATMA, Kecskes ML (2004) Nonsymbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? Soil Biol Biochem 36:1229–1244

Kodaka H, Mizuochi S, Teramura H, Nirazuka T (2005) Comparison of the compact dry TC method with the standard pour plate method (AOAC official method 966.23) for determining aerobic colony counts in food samples: Performance-tested method. J AOAC Int 88:1702–2713

Larran S, Monaco C, Alippi HE (2004) Endophytic fungi in beet (Beta vulgaris var. esculenta L.) leaves. Adv Hortic Sci 4:193–196

Yao T (2004) Associative nitrogen-fixing bacteria in the rhizosphere of Avena sativa in an alpine region II Phosphate-solubilizing power and auxin production. Acta Pratacultural Science 3:85–90

Liu SF, Tang WH (1996) The study on endophytic streptomyces of cotton. In: Tang WH, Cook RJ, Rovira A (eds) Advances in biological control of plant diseases. China Agricultural University Press, China, pp 212–213

Lucas-Garca JA, Schloter M, Durkaya T, Hartmann A, Gutirrez-Maero FJ (2003) Colonization of pepper roots by a plant growth promoting *Pseudomonas fluorescens* strain. Biol Fertil Soils 37:381–385

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572

Patten CL, Glick BR (2002) Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–3801

Pieterse CMJ, Van Wees SCM, Ton J, Van Pelt JA, Van Loon LC (2002) Signalling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. Plant Biol 5:535–544

Rajkumar M, Nagendran R, Lee KJ, Lee WH, Kim SZ (2006) Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard. Chemosphere 62:741–748

Riehm H (1985) Arbeitsvorschrift zur Bestimmung der Phosphorsaure und des Kaliums nach der Laktatmethode. Zeitschrift fur Pflanzen Dungung und Bodenkunde 40:152–156

Schachtschnabel P, Heinemann CG (1974) Beziehungen zwischen den Kaliumgehalten in Boden und in jungen Haferpflanzen. Zeitschrift fur Pflanzen Dungung und Bodenkunde 137:123–134

Taylor KACC (1995) A colorimetric method for the quantitation of fructose. Appl Biochem Biotechnol 53:215–227

Zhang XS (2008) Analysis of the factors affecting the available P content in the fermentation liquid of P bacteria determined by Mo-Sb colorimetry. Journal of Anhui Agri Sci 36:4822–4823

Zhao XR, Lin QM, Li BG (2003) The relationship between rock phosphate solubilization and pH and organic acid production of microorganisms. J Microbiol 23:5–7