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ORIGINAL ARTICLE

Whole genome analysis of *Gluconacetobacter azotocaptans* DS1 and its beneficial effects on plant growth

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Abstract

Plant-associated bacteria play an important role in the enhancement of plant growth and productivity. *Gluconacetobacter azotocaptans* is an exceptional bacterium considering that till today it has been isolated and reported only from Mexico and Canada. It is a plant growth-promoting bacterium and can be used as biofertilizer for different crops and vegetables. The objective of the current study was to evaluate the inoculation effect of *Gluconacetobacter azotocaptans* DS1, *Pseudomonas putida* CQ179, *Azosprillium zeae* N7, *Azosprillium brasilense* N8, and *Azosprillium canadense* DS2, on the growth of vegetables including cucumber, sweet pepper, radish, and tomato. All strains increased the vegetables' growth; however, *G. azotocaptans* DS1 showed better results as compared to other inoculated and control plants and significantly increased the plant biomass of all vegetables. Therefore, the whole genome sequence of *G. azotocaptans* DS1 was analyzed to predict genes involved in plant growth promotion, secondary metabolism, antibiotics resistance, and bioremediation of heavy metals. Results of genome analysis revealed that *G. azotocaptans* DS1 has a circular chromosome with a size of 4.3 Mbp and total 3898 protein-coding sequences. Based on functional analysis, genes for nitrogen fixation, phosphate solubilization, indole acetic acid, phenazine, siderophore production, antibiotic resistance, and bioremediation of heavy metals including copper, zinc, cobalt, and cadmium were identified. Collectively, our findings indicated that *G. azotocaptans* DS1 can be used as a biofertilizer and biocontrol agent for growth enhancement of different crops and vegetables.

Keywords Plant growth-promoting rhizobacteria · *Gluconacetobacter azotocaptans* · Genome analysis · Nitrogen fixation

Introduction

Plant-associated microbial communities have an important role in the growth and development of host plants. Plant growth-promoting rhizobacteria (PGPR) have the ability to enhance plant growth under normal and various abiotic stress conditions including salinity, drought, heat, cold and heavy metal contamination (Glick 2010; Mehnaz et al. 2006; Mukhtar et al. 2020). A large number of PGP bacterial genera including *Azotobacter, Aeromonas, Azosprillium, Bacillus, Gluconacetobacter, Pseudomonas* and *Serratia* have been isolated and characterized from the rhizosphere and endosphere of various plants (Glick 2010; Mehnaz et al. 2006, 2010; Duan et al. 2013). These PGPR strains can be used as biofertilizers to promote plant growth and productivity of different economically important crops and vegetables such as rice, maize, wheat, sugarcane, cucumber, sugar beet, potato, tomato, and radish (Gonzalez et al. 2015; Mukhtar et al. 2017; Eida et al. 2020). Strains of *P. fluorescens* and *P. putida* have been used as biofertilizers for various crops such as wheat, rice, lettuce, apple, citrus, potato, and biocontrol agents against fungal pathogens (Mehnaz et al. 2010; Shahid et al. 2017; Eida et al. 2020).

Azospirillum is known for its nitrogen fixation and IAA production ability since long and *Pseudomonas* is known for IAA production and most importantly its role as biocontrol agent. *Gluconacetobacter*, although known to have many

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PGP traits, is not used commercially as a biofertilizer as frequently as *Azospirillum* and *Pseudomonas*.

Gluconacetobacter is a Gram-negative bacterium with the ability to produce gluconic acid or vinegar. Twenty-five species have been classified under this genus. They have been isolated from rhizosphere, plant roots, shoots, and flowers of different plants. Most of the species are industrially important due to vinegar production, and some species are well known to be nitrogen fixers such as *G. diazotrophicus* and *G. azotocaptans* (Yamada et al. 2012; Eskin et al. 2014). *G. diazotrophicus* is an extensively studied species of this genus. It has been isolated from vegetatively propagated plants including sugarcane, sweet potato roots and stems, and rhizosphere of coffee plants (Muthukumarasamy et al. 2002, 2005; Eskin et al. 2014).

G. azotocaptans is a rod-shaped, non-spore-forming, non-symbiotic, nitrogen-fixing bacterium. It has been isolated only from two countries, i.e., from the rhizosphere of coffee in Mexico and from the rhizosphere of corn in Canada (Fuentes-Ramirez et al. 2001; Mehnaz et al. 2006). There are no other reports about the isolation of *G. azotocaptans* from other host plants or other countries of the world. Therefore, research done on this bacterium is very limited. It is considered as a good PGPR strain with its abilities to produce indole acetic acid and solubilize phosphate (in addition to fix nitrogen). It has the potential to enhance plant growth of different crops including corn and wheat (Mehnaz and Lazarovits 2017). Although *G. azotocaptans* shows most of the morphological, biochemical, and genetic characteristics, similar to *G. diazotrophicus*, there are some differences in biochemical and genetic traits (Fuentes-Ramirez et al. 2001; Mehnaz et al. 2006).

G. azotocaptans DS1 was previously reported for IAA production (106 µg/L), nitrogenase activity (40 nmol ethylene/h/mg), phosphate solubilization, and antifungal activity against plant pathogens including *Fusarium moniliforme, F. solani* and *F. sambucinum* (Mehnaz et al. 2006). Inoculation effect of *G. azotocaptans* was compared with other PGPR strains such as *Azospirillum*, *Pseudomonas*, *Burkholderia*, and *Enterobacter,* on different crops including corn and wheat (Mehnaz and Lazarovits 2006; Morley 2013). In pot experiment, inoculation of DS1 enhanced the growth of corn, as it showed a significant increase in root and shoot weight (23 and 29%) as compared to other inoculated PGPR strains and non-inoculated control plants (Mehnaz and Lazarovits 2006; Mehnaz et al. 2007, 2010).

In the current study, plant growth-promoting potential of *G. azotocaptans* DS1 was compared with other PGPR strains including *A. zeae* N7, *A. brasilense* N8, *A. canadense* DS2, and *P. putida* CQ179 on cucumber, sweet pepper, radish and tomato, at pot scale, under greenhouse conditions. These bacterial strains have already been identified and characterized for their plant growth-promoting abilities such as nitrogen fixation, IAA production, phosphate solubilization, siderophore production and antifungal activity (Mehnaz and Lazarovits 2006; Mehnaz et al. 2006). This study is the first report on the whole genome analysis of *G. azotocaptans* DS1 to predict the genes and operons involved in plant growth promotion, secondary metabolism, defense mechanisms, bioremediation of heavy metals and abiotic stresses.

Materials and methods

Selection of PGPR strains

Five PGPR strains including *A. zeae* N7, *A. brasilense* N8, *A. canadense* DS2, *G. azotocaptans* DS1 and *P. putida* CQ179, isolated from corn rhizosphere, were selected for plant experiments, on the basis of their previous reports on growth enhancement of corn (Mehnaz and Lazarovits 2006). These strains were previously characterized for their PGP traits and identified on the basis of 16S rRNA analysis. Sequences were deposited at GenBank and Accession numbers were obtained (*A. lipoferum* N7, accession no. AY998242; *A. brasilense* N8, accession no. AY958234; *P. putida* CQ179, accession no. AY958233; *G. azotocaptans* DS1, accession no. DQ073427 and *A. canadense* DS2, accession no. DQ393891; Mehnaz and Lazarovits 2006).

Plant experiments

Pot experiments for four vegetables including cucumber cultivar "Marketmore 76", radish cultivar "Cherry belle", sweet pepper cultivar "California wonder" and tomato cultivar "Bellstar 409", were performed in greenhouse under controlled temperature and light conditions. Surface-sterilized seeds (soaked in 0.1% sodium hypochlorite for 5 min and then washed 3 to 5 times, with autoclaved distilled water) were grown on wet filter papers in Petri plates at 30 °C. Three-day-old seedlings were transferred into coffee cups filled with Pro-Mix 'Bx' (general purpose peat based professional growing medium; Premier Horticulture Ltd., Quebec, Canada; ~ 250 g/cup). Five bacterial strains *A. zeae* N7, *A. brasilense* N8, *A. canadense* DS2, *G. azotocaptans* DS1 and *P. putida* CQ179, isolated from corn rhizosphere, were used to inoculate the vegetable crops. A single colony of each bacterial strain, except DS1, was inoculated individually into 10 mL LB broth and grown overnight at 30 °C, and 125 rpm. DS1 was grown in LGI medium (Cavalcante and Dobereiner 1988). Cells were harvested at 10,000 rpm for 5 min, and pellets were suspended in sterilized saline to get a final concentration of 10^8 cells/ml. One ml of inoculum (approximately 10^8 cells per plant) was applied to individual plants of cucumber, radish, pepper, and tomato seedlings, at the time of transplantation in Pro-mix and grown under

greenhouse conditions. Control plants were provided with 1 mL sterilized saline per seedling. For each vegetable, 12 replicates (12 pots/treatment; 1 plant/pot) were used. All experiments were repeated three times. A photoperiod of 14 h light/10 h dark and thermoperiod of 25/20°C was used. Sterilized water was used to keep the Promix moist throughout the study. Plants were harvested after 30 days of growth, placed in paper bags for drying in an oven at 70°C, for 72 h. Root, shoot, and whole plant weights were recorded. The experimental data were analyzed by using IBM SPSS software (ver. 24). One-way analysis of variance (ANOVA) was done in SPSS, and comparison among treatments was done by using Duncan's multiple range test (DMRT). All analyses were performed at the $P = 0.05$ level.

DNA isolation, genome sequencing and assembly

Genome sequencing was performed with the Illumina HiSeq2000® sequencing platform. 5 µg of genomic DNA was extracted using Genomic DNA isolation kit (Thermo Scientific GeneJET, USA) and prepared for genome sequencing using the Illumina HiSeq2000® library preparation kit (Illumina, Inc.), following the manufacturer's instructions. After sequencing, 8.6 million paired-end reads with 150 nucleotides each were generated. The sequencing data were then assembled into complete contigs with SPAdes assembler Version 3.13.0 (Bankevich et al. 2012). The contigs were arranged against the genome of *G. azotocaptans* (GCF_014174355.1) by using Mauve (Darling et al. 2010). Fast ANI scores of all available species of *Gluconacetobacter* were calculated using Fast ANI (version 0.1.3) (Jain et al. 2018) in order to confirm the species of strain.

Genome annotation of *Gluconacetobacter azotocaptans* DS1

The de novo gene prediction was performed by using GeneMarks and CLC genomics workbench (Besemer et al. 2001). The functional classification was conducted through COG (corresponding cluster of orthologous groups of protein) analysis. The gene function was annotated by BLAST against Kyoto Encyclopedia of Genes and Genomes database KEGG pathway (Kanehisa et al. 2006). KEGG Orthology Based Annotation System (KOBAS 2.0) was used for functional analysis of genes. To predict genes and operons involved in secondary metabolism and antibiotic resistance, antiSMASH 4.0 software was used (Blin et al. 2017). Finally, genomes of *G. azotocaptans* DS1 and *G. azotocaptans* LMG 21311 were compared using FastANI software. The whole genome sequence of strain DS1 was deposited in the GenBank database under the accession number GCF_016916825.1. The metabolic model of *G. azatocaptans* was built by using MedelSEED (Plata et al. 2015).

Antibiotic resistance assay using disc diffusion method

The antibiotic susceptibility of DS1 was studied by using Kirby–Bauer disk diffusion method (Bauer et al. 1966; El-Sayed and Helal 2016). Six antibiotics including ampicillin (10), amikacin (30 µg), ciprofloxacin (5 µg), tetracycline (10 µg), vancomycin (30 µg), and ceftriaxone (10 µg) were used. Antibiotic discs were placed over freshly prepared LGI medium plates seeded with DS1. All antibiotic disks were placed at appropriate distance from one another, and plates were incubated at 30°C for 48 h.

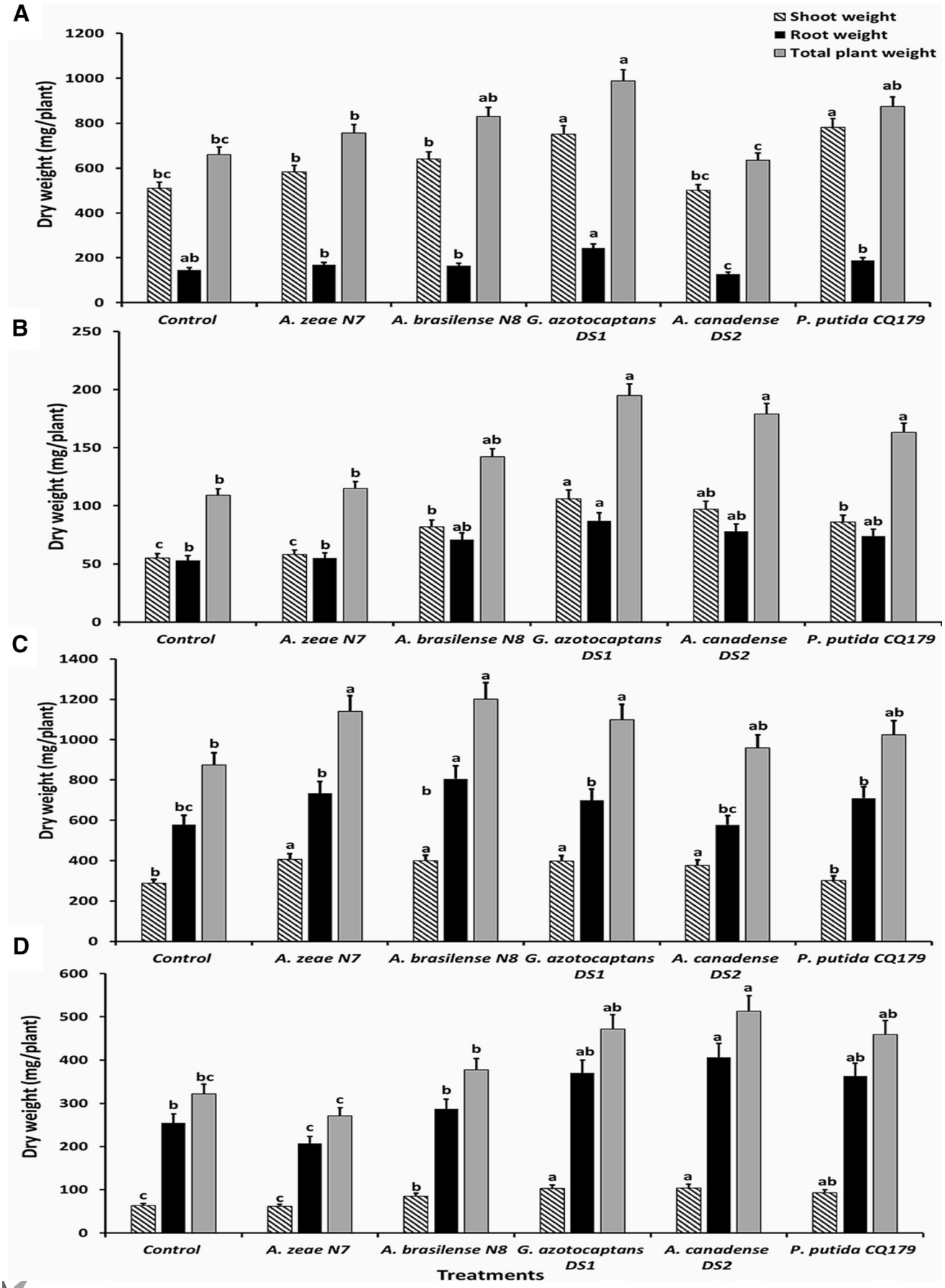
Results

Plant experiments

All strains significantly increased the plant growth of cucumber, sweet pepper, radish and tomato. Cucumber plants inoculated with *G. azotocaptans* DS1 significantly promoted the total plant weight and shoot weight as compared to plants inoculated with other strains and uninoculated control plants (Fig. 1A). These plants showed 47% increase in shoot weight, 65% increase in root weight and 49.6% increase in total plant weight as *G. azotocaptans* DS1 significantly promoted root, shoot, and total plant weight of sweet pepper. Maximum increase in shoot weight (89%), root weight (55%) and total plant weight (72%) was observed with this strain as compared to uninoculated control plants (Fig. 1B). Overall, *G. azotocaptans* DS1, *A. canadense* DS2, and *P. putida* CQ179 promoted growth and increased total plant weight of sweet pepper (78, 64 and 51%, respectively) as compared to uninoculated control plants. All inoculated plants except *A. zeae* N7 showed increase in shoot and root weights as compared to un-inoculated control plants.

G. azotocaptans DS1, *A. brasilense* N8, *A. zeae* N7 and *P. putida* CQ179 exerted a significant influence on the growth of radish as shown by enhanced total plant weight as compared to uninoculated control plants (Fig. 1C). *A. zeae* N7 inoculated plants showed highest shoot weight, DS1 inoculated plants were second highest and had non-significant difference with N7. *A. brasilense* N8 showed highest significant increase in root weight. The rest of the strains, except *A. canadense* DS2, showed higher root weight as compared to control, but the difference was non-significant.

P. putida CQ179, *G. azotocaptans* DS1 and *A. canadense* DS2 promoted the growth of tomato plants as compared to uninoculated control plants. *G. azotocaptans* DS1 and *A. canadense* DS2 inoculated plants showed increase in shoot weight (61 and 65%), root weight (46 and 59%), and total plant weight (47 and 59%) as compared to uninoculated control plants (Fig. 1D). *A. brasilense* N8 inoculated plants


A
Shoot weight
Root weight
Total plant weight
Dry weight (mg/plant)
Control
A. zeae N7
A. brasilense N8
G. azotocaptans DS1
A. canadense DS2
P. putida CQ179
B
C
D
Treatments

◂**Fig. 1** Effect of PGPR strains on **A** cucumber, **B** sweet pepper, **C** radish, and **D** tomato plants, after 30 days growth in Promix under greenhouse conditions. Letters indicate a statistically significant difference between treatments according to Duncan's multiple range test (DMRT) at $P \leq 0.05$ conditions

showed higher root and total plant weight than uninoculated control, but it was lower than other treatments.

General features of *G. azotocaptans* DS1 genome

Maximum fast ANI score of *Gluconacetobacter azotocaptans* DS1 (GCF_016916825.1) was 99.34% against *G. azotocaptans* (GCF_014174355.1) (Table S1; Fig. S1). The draft genome of *G. azotocaptans* DS1 was assembled in 101 contigs and its size was 4,329,144 bp with 66.3% G + C content (Fig. 2). A total of 4042 genes were predicted with 3978 coding DNA sequences (CDs). It was predicted that RNA related genes were 64 (Table 1). The complete ribosomal RNA genes were included, i.e., 1, 2 and 7 for 5S, 16S and 23S, respectively. Fifty genes for transfer RNAs (tRNAs) were identified and four genes were detected as non-coding RNA (ncRNAs). In addition, 80 pseudogenes were also predicted (Table 1). Plasmid sequence was not identified in DS1 strain.

Functional annotation of *G. azotocaptans* DS1 genome

Most of the unique genes were predicted to code hypothetical proteins. Out of 3898 proteins, 2066 (53%) were assigned to COG functional categories (Fig. 3). The functional analysis of these genes using KEGG pathway database showed that they have an important role in various metabolic pathways including plant growth promotion, bioremediation of different toxic compounds, heavy metals, antimicrobial resistance, and other abiotic stresses. The functional analysis of CDSs showed that they could be classified into 25 general COG categories including the metabolism of carbohydrates, amino acids, lipids, transcription, energy, cofactors and vitamins, inorganic ions, signal transduction and cellular processes, glycan biosynthesis and metabolism, cell motility, translation, ribosomal biogenesis, DNA replication and repair, secondary metabolites, defense mechanisms, xenobiotics biodegradation, dormancy, and sporulation (Fig. 3 and S2).

Plant growth-promoting potential of *G. azotocaptans* DS1

The functional annotation of *G. azotocaptans* DS1 identified several genes related to nitrogen fixation such as *nifH, nifD, nifK, knife, nifN, nifX, nfnB, nifB, nifZ* and *nifQ* (Table 2). Nitrogenase protective and regulatory proteins encoded genes (*nifW, glnB, GlnK2* and *NRI*) and putative NAD(P)H nitroreductase gene *ydfN* were also detected. The presence of genes involved in phenylalanine, tyrosine and tryptophan biosynthesis (indole acetic acid production) such as *ATH, ALY* and *CSAT* was confirmed by genome analysis of *G. azotocaptans* DS1. Five genes *THI20, thiE, thiG, thiN* and *THI6* were related to thiamine biosynthesis and metabolism, seven genes *bluB, ribF, folE, cobU, iunH, ACEP, BTER* and *cobT* were involved in nicotinate and riboflavin metabolism and biotin encoded genes were also identified (Table 2 and S3).

Few genes including *fhuA, fecE, ECOH, fitD, echA, FTH1* and *sbnC* related to iron metabolism and siderophore production were predicted in the genome of DS1 (Table 2). PQQ-dependent alcohol dehydrogenase *adh*AB operon, genes related to pentose phosphate pathway *gmhB, rtpR, phoA, phoB, fbaB, gmhA, lpcA, galT,* and phosphate transferases *coaD, kdtB, pgsA, PGS1, yjbB, manC, cpsB, pyrE, bacA,* and *fucA,* were also identified (Table 2). In addition, Na+ symporter phosphatase (*yjbB*) and two genes *araM* and *egsA* related to glycerol-1-phosphate dehydrogenase were also detected (Table 2 and S2).

Production of secondary metabolites

In the genome of *G. azotocaptans* DS1, genes involved in phenylpropanoid biosynthesis (*fabG, oaR1, rfbD, rmlD, ubiX, bsdB, PAD1* and *bglX*), flavone and flavonol metabolism (*uidA, frdA* and *aofH*), siderophore sensor and receptor systems (*entA, EBW* and *ECOK*) and quinone metabolism (*PtR1* and *NQO1*) were identified (Table 3 and S2). Additionally, there were genes related to biosynthesis of streptomycin (*rmlD2, glk, rmlB, rffG, rfbC,* and *rmlC*), phenazine (*phzD, phzE* and *phzF*), staphyloferrin B protein (*sbnC, sbnF, acsA* and *acsC*) and prodigiosin (*fabG* and *OAR1*), which might be involved in plant growth improvement and biocontrol mechanisms (Table 3 and S3).

Prediction of antibiotic resistance genes

Ten genes including *mecR1, mecI, mecA, blaR1, blaI, blaZ, parS, abcA, bmrA* and *penP* that encode beta-lactamase were identified that may play important role in ampicillin resistance of the strain (Fig. S3; Table 4 and S4). Functional annotation of DS1 genome revealed that 11 genes (*rfbB, rmlB, rffG, vanH, vanX, vanB, vanK, vanJ, vanSAc, vanRAc* and *vanW*) related to vancomycin resistance and five genes (*degP, htrA, amiABC, norG* and *mexT*) cationic antimicrobial peptide (CAMP) resistance were also detected. Nine genes *adeA, adeB, adeC, opmE, mexF, mgrA, BLTL, mdfA* and *cmr* involved in multidrug resistance protein and 14 genes (*parR, cusS, copS, silS, cusR, copR, silR, parS, mexT, vanSB, vanRB, vanSAc,*

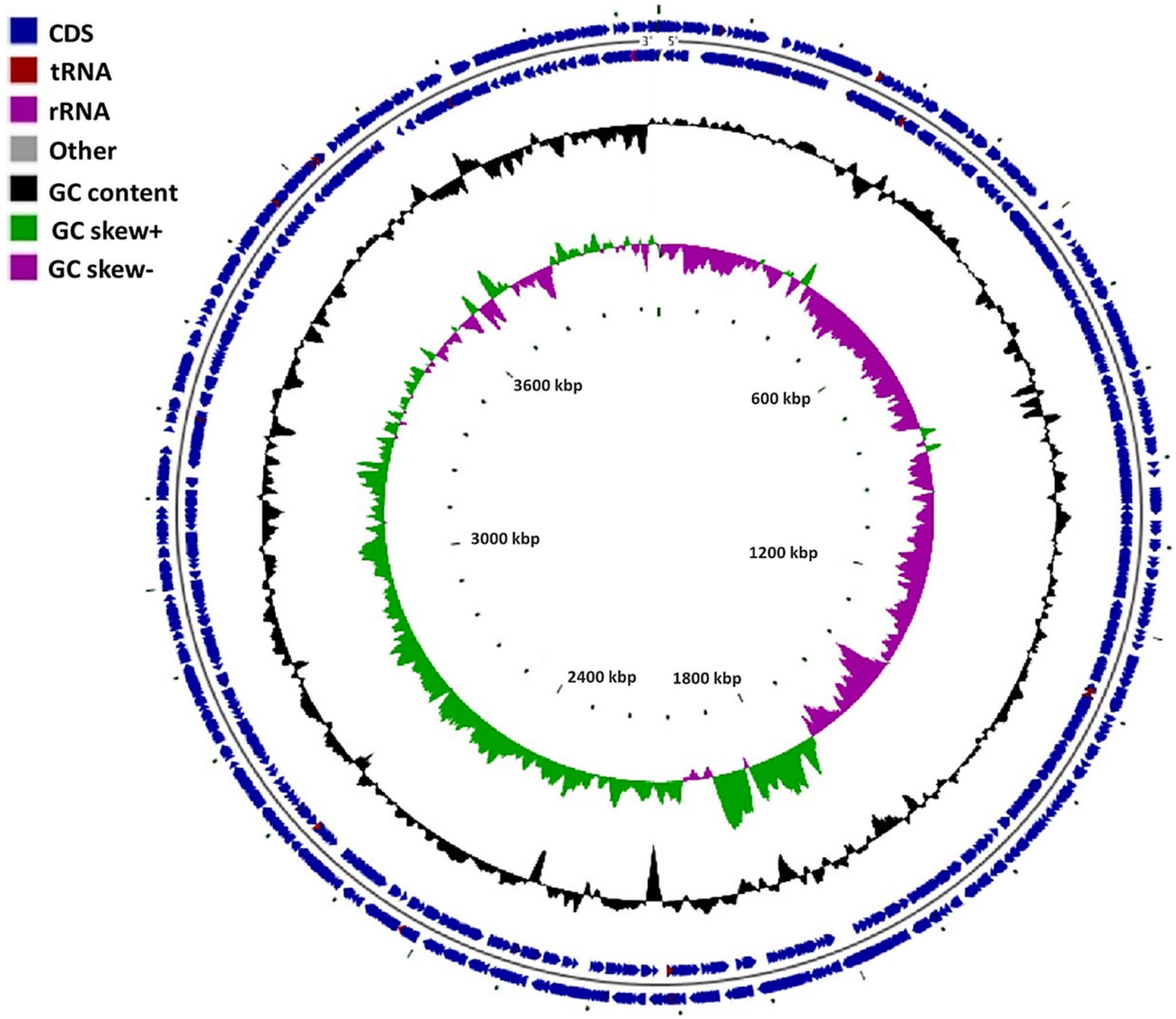


Fig. 2 Graphical circular map of a draft *G. azotocaptans* DS1 genome. The simulated genome is a set of contigs ordered against the complete genome of *G. azotocaptans* LMG 21311. The circular map was generated using CGview. Circles from the outside to the inside show the positions of protein-coding genes (blue), tRNA genes (red), and rRNA genes (purple) on the positive and negative strands (Circle 1 and 2). Circles 3 and 4 show plots of GC content and GC skew plotted as the deviation from the average for the entire sequence

vanRAc and *oprD*) related to two-component system were also identified (Fig. S3; Table 4 and S4).

Antibiotic sensitivity of *G. azotocaptans* DS1 was investigated by using ampicillin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), tetracycline (10 µg), vancomycin (30 µg), and ceftriaxone (10 µg). Inhibition zones from ≥ 15 mm were considered as strong, from 5 to 15 mm as moderate and ≤ 5 mm as weak positive. Overall, DS1 showed sensitivity to all antibiotics at the concentrations used in this study (Fig. S4). But, it showed more sensitivity to ampicillin, amikacin, ciprofloxacin and tetracycline as compared to vancomycin and ceftriaxone.

Bioremediation strategies and resistance to heavy metals

Genes potentially involved in bioremediation of different polluted compounds have been identified. Genes for the degradation of aminobenzoate (*ubiX, bsdB* and *PAD1*), chloroalkane and chloroalkene (*frmA, ADH5, ALDH2* and *adhC*), benzoate (*pcaD, PSYR* and *PSHAa*), aromatic compounds (*gnl, RGN, adhC, hpaB, ubiX, bsdB* and *PAD1*) and xylene (*catE*) were detected (Table 5 and S5). Based on functional analysis of *G. azotocaptans* DS1 genome, various heavy metal resistance genes such as Zn, Cu, Mn and

Table 1 General genome features of *Gluconacetobacter azotocaptans* DS1genome

Genome features	No. of genes
CDS (total)	4042
Genes (coding)	3978
CDS (coding)	3898
Genes (RNA)	64
Complete rRNAs	1, 2, 7 (5S, 16S, 23S)
Partial rRNAs	6 (23S)
tRNAs	50
ncRNAs	4
Pseudo genes (total)	80
Pseudo genes (ambiguous residues)	0 of 80
Pseudo genes (frameshifted)	31 of 80
Pseudo genes (incomplete)	50 of 80
Pseudo genes (internal stop)	10 of 80
Pseudo genes (multiple problems)	10 of 80

Co were identified. Nine genes (*copA, ctpA, ATP7, cobA, copA, ctpA, PPOX, hemY* and *nosF*) related to copper resistance and metabolism, two genes (*mntD* and *ECS*) involved in transportation of manganese, two genes (*SOD1* and *znuB*) with zinc metabolism and three genes (*czcA, czcA,* and *czcC*) involved in cobalt–zinc–cadmium resistance proteins were identified (Tables 5 and S5).

Comparison of *G. azotocaptans* DS1 genome with type strain

Genomes of *G. azotocaptans* DS1 and type strain *G. azotocaptans* LMG 21311 were compared using FastANI software. The comparative analysis showed that 19 genes including glucose-1-phosphate cytidylyltransferase, opine oxidase, adenine-specific methyltransferase, CRISPR-associated helicase Cas3 and other proteins, regulatory protein RecX, HigA protein (antitoxin to HigB), transport ATP-binding protein CydCD and cytochrome C heme lyase subunit CcmH-related genes were only present in the genome of *G. azotocaptans* DS1 (Table S6). Twenty one genes such as creatinine amidohydrolase, DUF1275 domain-containing protein, exoenzymes regulatory protein AepA precursor, isochorismatase family protein, DNA polymerase IV-like protein ImuB, FIG027115:membrane protein, DNA-cytosine methyltransferase, G-T specific endonuclease, putative predicted metal-dependent hydrolase, oligopeptide transport ATP-binding protein OppF, salicylate hydroxylase, phosphate transport system permease protein PstC, acylamino-acid-releasing enzyme, glutathione S-transferase, unnamed subgroup, cation efflux system protein CusA and cobalt–zinc–cadmium resistance protein CzcA related genes were absent in the genome of DS1 while these genes were present in *G. azotocaptans* LMG 21311 genome (Table S7).

Discussion

In the current study, PGPR strains, *G. azotocaptans* DS1, *A. zeae* N7, *A. brasilense* N8, *A. canadense* DS2, and *P. putida* CQ179 showed their potential to promote plant growth of different vegetables including cucumber, sweet pepper, radish and tomato, under greenhouse conditions. Plant experiments showed that *G. azotocaptans* DS1 increased the plant growth of all four vegetables. Response of other strains was variable as *P. putida* CQ179 showed great effect on three

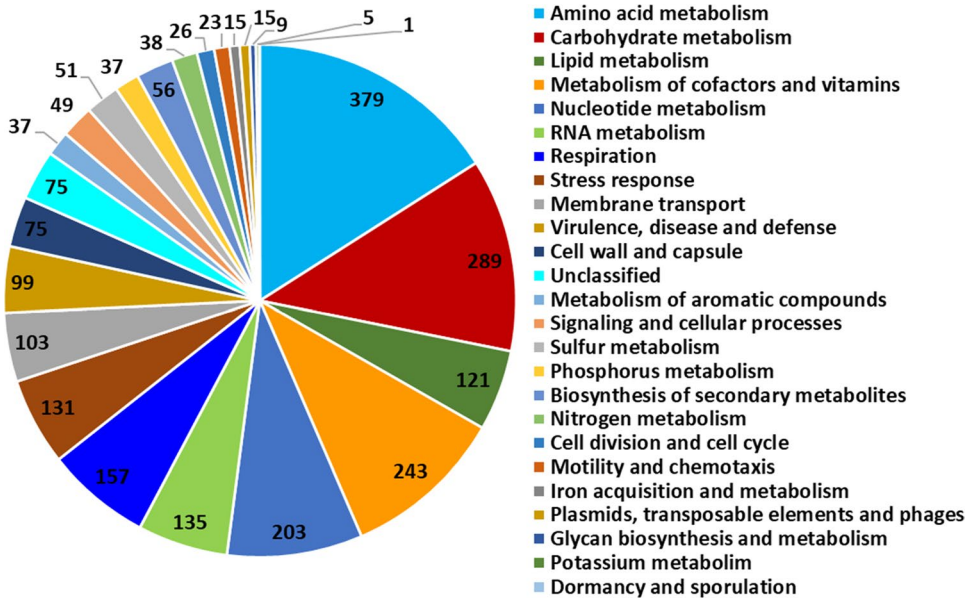


Fig. 3 Functional analysis of *G. azotocaptans* DS1 genome by using KEGG metabolic pathways

Table 2 Prediction of genes linked to plant growth-promoting traits in the genome of *G. azotocaptans* DS1

PGPR traits	KEGG orthology	Metabolic pathways	No. of genes	Name of genes
Nitrogen fixation	00910	Nitrogen fixation proteins and enzymes	10	*nifH, nifD, nifK, knife, nifN, nifX, nfnB, nifB, nifZ, nifQ*
		Nitrogenase-stabilizing/protective protein W	1	*nifW*
		Nitrogen-regulatory proteins	3	*glnB, GlnK2, NRI*
		Putative *NAD(P)H* nitroreductase	1	*ydfN*
Phytohormone production	00400	Phenylalanine, tyrosine, and tryptophan biosynthesis	3	*ATH, ALY* and *CSAT*
Vitamins and cofactors	00730	Thiamine metabolism	5	*THI20, thiE, thiG, thiN* and *THI6*
	00760	Nicotinate and riboflavin metabolism	7	*bluB, ribF, folE, cobU, iunH, ACEP, BTER* and *cobT*
	00780	Biotin metabolism	1	*accC*
Iron metabolism/siderophores production	04978	Iron complex outer membrane proteins	3	*fhuA, fecE, ECOH* and *fitD*
	09183	High-affinity iron transporter proteins	3	*echA, FTH1* and *sbnC*
Phosphorus metabolism	00030	Pentose phosphate pathway	8	*rtpR, phoA, phoB, fbaB, gmhA, lpcA, galT* and gmhB
	02060	Phosphate transferases	10	*coaD, kdtB, pgsA, PGS1, yjbB, manC, cpsB, pyrE, bacA* and *fucA*
	00440	Phosphonate and phosphinate metabolism	2	*fruK* and *phnP*

Table 3 Identification of secondary metabolite genes in the genome of *G. azotocaptans* DS1

KEGG orthology	Metabolic pathways	No. of genes	Name of genes
00940	Phenylpropanoid biosynthesis	8	*fabG, OAR1, rfbD, rmlD ubiX, bsdB, PAD1* and *bglX*
00944	Flavone and flavonol metabolism	3	*uidA, frdA* and *aofH*
001534	Iron siderophore sensor and receptor system	3	*entA, ECO, EBW* and *ECOK*
00524	Quinone metabolism	2	*PtR1* and *NQO1*
00521	Streptomycin biosynthesis	6	*rmlD2, glk, rmlB, rffG, rfbC,* and *rmlC*
00405	Phenazine biosynthesis	3	*phzD, phzE* and *phzF*
00997	Biosynthesis of staphyloferrin B protein	4	*sbnC, sbnF, acsA* and *acsC*
00333	Prodigiosin biosynthesis	2	*fabG* and *OAR1*

Table 4 Identification of antimicrobial genes in the DS1 genome

KEGG orthology	Metabolic pathways	No. of genes	Name of genes
01501	beta-Lactam resistance	10	*mecR1, mecI, mecA, blaR1, blaI, blaZ, parS, abcA, bmrA* and *penP*
01502	Vancomycin resistance	11	*rfbB, rmlB, rffG, vanH, vanX, vanB, vanK, vanJ, vanSAc, vanRAc* and *vanW*
01503	Cationic antimicrobial peptide resistance	5	*degP, htrA, amiABC, norG* and *mexT*
01504	Macrolide resistance genes	3	*ereA_B, mdfA,* and *cmr*
	Multidrug resistance protein	9	*adeA, adeB, adeC, opmE, mexF, mgrA, BLTL, mdfA* and *cmr*
02022	Two-component system	14	*parR, cusS, copS, silS, cusR, copR, silR, parS, mexT, vanSB, vanRB, vanSAc, vanRAc* and *oprD*

Table 5 Prediction of genes potentially involved in bioremediation and heavy metal resistance in the DS1 genome

KEGG orthology	Metabolic pathways	No. of genes	Name of genes
00627	Aminobenzoate degradation	3	*ubiX, bsdB* and *PAD1*
00625	Chloroalkane and chloroalkene degradation	4	*frmA, ADH5, ALDH2* and *adhC*
00622	Xylene degradation	1	*catE*
00362	Benzoate degradation	3	*pcaD, PSYR* and *PSHAa*
01220	Degradation of aromatic compounds	7	*gnl, RGN, adhC, hpaB, ubiX, bsdB* and *PAD1*
09132	Manganese transporter proteins	2	*mntD* and *ECS*
09130	Copper metabolism	9	*copA, ctpA, ATP7, cobA, copA, ctpA, PPOX, hemY* and *nosF*
09131	Zinc metabolism	2	*SOD1* and *znuB*
09183	Cobalt–zinc–cadmium resistance proteins	3	*czcA, czcA,* and *czcC*

out of four vegetables. *A. brasilense* N8 promoted growth of cucumber and radish, and *A. canadense* DS2 increased the growth of sweet pepper and tomato. Previous studies revealed that PGP bacterial genera including *Pseudomonas, Enterobacter, Azospirillum* and *Gluconacetobacter* have potential to enhance plant growth in different crops such as corn, wheat, rice and canola (Xie et al. 1996; Glick 2010; Mehnaz et al. 2006; Mehnaz and Lazarovits 2017; Mukhtar et al. 2020). Only two studies, Morley (2013) and Mehnaz et al. (2006), have previously proved the plant growth-promoting potential of *G. azotocaptans* DS1.

The whole genome analysis of *G. azotocaptans* DS1 revealed that there were 3978 protein-coding sequences and few small proteins identified were annotated as hypothetical proteins. The functional analysis of *G. azotocaptans* DS1 genome using KEGG pathway database showed that it has an important role in signal transduction and cellular processes, cell motility, transcription, translation, ribosomal biogenesis, DNA replication and repair, metabolism of carbohydrates, amino acids, lipids, energy, cofactors and vitamins, inorganic ions, glycan biosynthesis and metabolism, secondary metabolites, defense mechanisms, abiotic stresses and bioremediation of different toxic compounds. Some previous studies on whole genome analysis of *Gluconacetobacter* spp. showed that they have genes for different metabolic pathways identified through KEGG pathway analysis (Mogi et al. 2009; Miura et al. 2013; Matsutani et al. 2014).

Overall *G. azotocaptans* DS1 genome has distinctive plant growth-promoting traits especially nitrogen fixation. Several *nif* genes, nitrogenase protective and regulatory proteins encoded genes and nitroreductase gene were identified in the genome of DS1. Most of the *Gluconacetobacter* strains are free-living bacteria with the ability to fix atmospheric nitrogen and play an important role in the nitrogen and carbon cycles. Genes for nitrogenase and other regulatory proteins were also previously reported for different *Gluconacetobacter, Enterobacter* and *Pseudomonas* strains (Matsutani et al. 2014; Laili et al. 2017; Guo et al. 2020). Genes involved in phytohormone, vitamins and cofactors production such as *thiE, thiG, thiN, bluB, ribF, folE, cobU, iunH* and *THI6* detected in the genome of DS1 were also identified by different studies on the genome annotation of *Gluconacetobacter* strains (Peters et al. 2013; Matsutani et al. 2014).

Genome annotation of DS1 also showed seven genes involved in siderophore production and iron metabolism. Siderophore production is a typical characteristic possessed by some plant growth-promoting bacteria including *Pseudomonas*, *Enterobacter, Gluconacetobacter,* and *Bacillus* (Duan et al. 2013; Singh et al. 2020). Siderophore biosynthesis has also been reported in some other *Gluconacetobacter* strains (Giongo et al. 2010; Peters et al. 2013). PQQ-dependent alcohol dehydrogenase *adh*AB operon identified in the genome of DS1 was also reported in *Gluconacetobacter, Pseudomonas* and *Enterobacter* (Miura et al. 2013; Matsutani et al. 2014). Genes related to different phosphotransferases, such as *pgsA, PGS1, yjbB, manC, cpsB, pyrE, bacA* and *fucA* were identified in the DS1 genome. The phosphotransferase systems were reported in different bacterial genera including *Gluconacetobacter, Pseudomonas*, *Bacillus, Rhizobium* and *Enterobacter* (Xu et al. 2014; Chandra et al. 2020).

Gene clusters for secondary metabolite production such as phenylpropanoid biosynthesis, flavone and flavonol metabolism, phenazine and prodigiosin biosynthesis were also identified in the genome of *G. azotocaptans* DS1. Genes involved in flavone and flavonol metabolism, phenazine and siderophores production have been predicted in *Acetobacter pasteurianus* 386B genome (Illeghems et al. 2013). Previous studies also showed that gene clusters related to secondary metabolites production have been identified in the genome of *Gluconacetobacter, Acetobacter, Rhizobium, Pseudomonas,* and other bacterial genera (Ge et al. 2013; Kang et al. 2020). These gene clusters encoded the secondary metabolites that might be helpful to plants in their growth promotion and improvement in

biocontrol mechanisms (Chandra et al. 2020; Kang et al. 2020; Singh et al. 2020).

The genome annotation of DS1 showed that it has genes for resistance against tetracycline, ampicillin, beta-lactam, cationic antimicrobial peptide (CAMP), vancomycin, aminoglycoside, trimethoprim, rifampin, macrolide resistance genes and multidrugs. Previous studies reported that bacterial genera including *Pseudomonas*, *Gluconacetobacter, Rhizobium*, *Klebsiella* and *Enterobacter* showed resistance to different antibiotics, such as streptomycin, penicillin, tetracycline, kanamycin, vancomycin and chloramphenicol (Wang et al. 2018). Multidrug efflux systems have been identified in the DS1 genome based on sequence analysis, which may play an important role in novobiocin and aminoglycoside resistance (Cardozo et al. 2013; Yssel et al. 2017).

Gluconacetobacter is considered an industrially important bacterial strain due to its ability to accomplish almost complete bioconversions of sugars. It has potential to degrade complex organic pollutants into simpler compounds and play an important role in the bioremediation of polluted environments. Genes related to aromatic compounds, aminobenzoate, chloroalkane and chloroalkene, benzoate and xylene degradation were identified in the genome of DS1. A variety of aromatic compounds have been detected in polluted soils. Some previous studies showed the role of different bacterial genera including *Pseudomonas* and *Gluconacetobacter* to degrade polluted compounds in the soil (Fuchs et al. 2011; Matsutani et al. 2014). Several studies have previously reported the different mechanisms for heavy metal tolerance in *Gluconacetobacter* (Chong et al. 2016; Mukhtar et al. 2019). Gram-negative bacterial strains including *Pseudomonas, Enterobacter* and *Gluconacetobacter* were resistant to cadmium, cobalt, and zinc. These genes are generally working through an efflux mechanism and present in an operon (Burnley 2000; Taghavi et al. 2009; Matsutani et al. 2014). The presence of operon including genes *CzcD*, *CobW*, *CcmF*, and *CutE* that encode cobalt–zinc–cadmium resistance have been identified in *Gluconacetobacter, Rhizobium* and *Pseudomonas* (Fuchs et al. 2011; Chong et al. 2016).

Opine clusters involve in the oxidative cleavage of octopine into L-arginine and pyruvate in the plants through plant–microbe interaction and improve plant growth by reducing the impact of tumor genicity, triggered by different types of opine (Zanker et al. 1994). Additionally, the presence of additional cytochromes encoding genes and high A antitoxin encoding genes enhanced the biofertilizer and plant growth-promoting potential of *G. azotocaptans* DS1 strain.

Conclusion

The current study has elucidated the comparative effects of *G. azotocaptans* DS1, *A. zeae* N7, *A. brasilense* N8, *A. canadense* DS2, and *P. putida* CQ179 on the growth of cucumber, sweet pepper, radish and tomato and provides complete details of the *G. azotocaptans* DS1 genome. PGPR strains used in this work showed the ability to promote plant growth and increase root, shoot and total plant weight of vegetables including cucumber, sweet pepper, radish and tomato. The genomic annotation of *G. azotocaptans* DS1 revealed the identification of genes involved in plant growth promotion, e.g., nitrogen fixation, phosphate solubilization and indole acetic acid production, secondary metabolism, e.g., phenazine, flavonol and siderophore production, antibiotics resistance, e.g., tetracycline, ampicillin, beta-lactam, cationic antimicrobial peptide (CAMP), macrolide resistance genes and multidrugs and bioremediation of heavy metals including copper, zinc, cobalt and cadmium. This versatile PGPR strain may be used as an eco-friendly biofertilizer that will be a better alternative to chemical fertilizers to improve plant growth promotion of important crops and vegetables.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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