Gluconacetobacter azotocaptans: A Plant Growth-Promoting Bacteria

1

Samina Mehnaz and George Lazarovits

Abstract

Gluconacetobacter diazotrophicus is a very well-known and well-studied member of family *Acetobacteraceae*. Strains of this species have been isolated from all over the world. On the other side, only two strains of *Gluconacetobacter azotocaptans* have been isolated, i.e., one from coffee plant in Mexico (type strain) and another from corn plant in Canada; therefore, not much information is available about it. This chapter is an effort to bring this species in the limelight and show its significance as plant growth promoter. Authors have not only isolated the strain DS1, they have also evaluated its plant growth-promoting potential in four varieties of corn, radish, cucumber, tomato, pepper, and potato, under greenhouse, field, and tissue culture conditions, and data is presented in this chapter. Inoculation effect of DS1 was compared with *G. diazotrophicus* PAL5, its *nif* mutant, and strains of other genera including *Pseudomonas*, *Azospirillum*, *Enterobacter*, *Burkholderia*, and *Sphingobacterium*. Work on wheat was done by another group in Canada, and their data has also been added. It is a nitrogen-fixing, indole acetic acid-producing strain with phosphate solubilization ability. It has promoted the plant growth in most cases. Its growth-promoting effect varied in tissue culture medium depending on sucrose concentration as it is linked with IAA and ethylene production. Based on data presented here, authors are recommending its use as a biofertilizer.

S. Mehnaz (✉)
Department of Biological Sciences, Forman Christian College (A Chartered University), Ferozepur Road, Lahore 54600, Pakistan

A & L Research Laboratories, London, ON, Canada
e-mail: saminamehnaz@fccollege.edu.pk

G. Lazarovits
A & L Research Laboratories, London, ON, Canada

S. Mehnaz (ed.), *Rhizotrophs: Plant Growth Promotion to Bioremediation*, Microorganisms for Sustainability 2, DOI 10.1007/978-981-10-4862-3_1

1.1 Introduction

Gluconacetobacter is a gram-negative, acetic acid-producing bacterium. It was initially categorized as sub-genus of *Acetobacter*; however, Yamada et al. (1997) suggested to raise its status to genus level; it was accepted and validated in 1998. This genus has 24 species, and among these *G. diazotrophicus* is the one that is widely isolated from all over the world and extensively studied due to the same reason. *G. diazotrophicus* is an endophytic bacterial species that occurs predominantly in vegetatively propagated plants. It has been isolated from numerous types of plant tissues including the internal tissues of sucrose-accumulating plants such as sugarcane, washed roots and aerial parts of *Pennisetum purpureum*, sweet potato stems and roots, rhizosphere soil of coffee plants, as well as the surface-sterilized stems and roots and inner tissues of *Eleusine coracana*, pineapple plants, and wetland rice varieties (Munoz-Rojaz and Caballero-Mellado 2003; Muthukumarasammy et al. 2005). Recently, a review published by Eskin et al. (2014) listed all reported hosts of *G. diazotrophicus*.

G. diazotrophicus was the only known nitrogen-fixing species of this genus until Jimenez-Salgado et al. (1997) isolated two other acetic acid-producing, diazotrophic bacteria from rhizosphere of coffee plants. These diazotrophs shared features with the genus *Gluconacetobacter* but differ from *G. diazotrophicus* with respect to morphological and biochemical traits as well as genetic and molecular features. Results of intensive taxonomic analysis by Fuentes-Ramirez et al. (2001) led to the recommendation that these new isolates be assigned to novel species within the family *Acetobacteraceae*. These isolates were named as *G. azotocaptans* and *G. johannae*. Like other species of *Gluconacetobacter* (except *G. diazotrophicus*), reports of these two species are also rare.

Mehnaz et al. (2006) reported the isolation of *G. azotocaptans* strain DS1 from corn rhizosphere. Till now there is no other report about the isolation of this species from any other host or other parts of the world. Therefore, work done on this species is also very limited. Focus of this chapter is to throw light on the significance of this species as a plant growth-promoting bacteria.

1.2 *Gluconacetobacter azotocaptans*

It is a gram-negative, nitrogen-fixing bacterium isolated from coffee and corn rhizosphere (Fuentes-Ramirez et al. 2001; Mehnaz et al. 2006). Authors have extensively worked on this bacterium; part of this work has been published (Mehnaz and Lazarovits 2006; Mehnaz et al. 2006). This chapter is presenting the published and unpublished work done on this strain by authors and the University of Saskatchewan. This strain was explored for its plant growth-promoting potential in lab studies, field experiments, and tissue culture conditions. Corn, wheat, potato, and some vegetables were used as host to observe the effect of this bacterium. Detailed information about this work is provided below.

Mehnaz and Lazarovits (2006) screened this strain for plant growth-promoting traits and its potential to be used as biofertilizer. Authors performed qualitative and quantitative assays and reported the nitrogenase activity (40 nmol ethylene/hr/mg protein), indole acetic acid production (106 µg/l), phosphate solubilization, and antifungal activity of this strain, against *Fusarium solani*, *F. solani phaseoli*, *F. moniliforme*, and *F. sambucinum*.

1.3 Growth Promotion of Cereals Inoculated with *G. azotocaptans* DS1

1.3.1 Corn

Authors had worked on the project of bioformulation for corn at Agriculture and Agri-Food Canada, London, Ontario, Canada. Data presented here is part of that project. Corn plants were inoculated with DS1 and other bacterial strains, grown in sterilized sand and unsterilized soil, in pot experiment. DS1 was also used as inoculum for field trials.

For pot experiment, four Pioneer varieties of corn, i.e., 39D82, 39H84, 39M27, and 39T68, were inoculated with *G. azotocaptans* DS1 (corn isolate) and other strains of *Azospirillum* (N7 and N8; corn isolates), *Pseudomonas*, and *G. diazotrophicus* PAL5 and its *nifD* mutant; 10^8 cells per plant at the time of transplantation. Three-days-old seedlings were grown for 4 weeks, in 250 g sterilized sand and NPK fertilizers (20:10:50 kg/ha, respectively). DS1 inoculated plants showed significant and maximum increase (27%) in root weight of 39T68 as compared to all other treatments and other corn varieties used in this study (Table 1.1). For 39T68, *A. brasilense* N8 and *P. putida* CQ179 also increased the root weight, but it was slightly less than DS1. With two varieties, 39H84 and 39M27, inoculation effect for all

Table 1.1 Effect of bacterial isolates on root weight of four corn varieties after 30 days growth, in sterilized sand

	Corn varieties			
Treatments	39D82 (mg/plant)	39H84 (mg/plant)	39M27 (mg/plant)	39T68 (mg/plant)
Control	260 ± 40 bc	210 ± 56 a	270 ± 50 a	220 ± 60 c
A. zeae N7	300 ± 42 a	220 ± 36 a	270 ± 47 a	255 ± 52 abc
A. brasilense N8	290 ± 41 ab	200 ± 59 a	265 ± 63 a	270 ± 64 ab
P. putida CQ179	250 ± 34 c	215 ± 39 a	250 ± 46 a	270 ± 64 ab
***G. azotocaptans* DS1**	**250 ± 32 c**	**220 ± 65 a**	**260 ± 41 a**	**280 ± 90 a**
G. diazotrophicus PAL5 Wt	260 ± 33 bc	210 ± 57 a	290 ± 42 a	230 ± 49 bc
*G. diazotrophicus PAL5 nif*D	250 ± 40 c	200 ± 42 a	250 ± 42 a	240 ± 50 abc

Values are average of 12 replicates. Letters indicate a statistically significant difference between treatments according to Duncan's multiple range test (DMRT) at $P \leq 0.05$. Mean separation within a column followed by the same letters does not differ significantly
Data is taken from Mehnaz and Lazarovits (2006)

bacterial strains on root weight was non-significant as compared to uninoculated plants. For 39D82, *Azospirillum* strains, N7 and N8, showed maximum increase in root weight, 15% and 11%, respectively, as compared to uninoculated and all other inoculated plants. DS1 and all other inoculated strains did not show any difference with uninoculated plants. Interestingly, *G. diazotrophicus* strains did not show significant difference in root weight of any variety.

Shoot weight of all varieties increased when inoculated with DS1 (Table 1.2); however, 39M27 and 39T68 showed significantly high shoot weight, ranging from 23% to 29% (Mehnaz and Lazarovits 2006). *Azospirillum brasilense* N8 and *A. zeae* N7 (Mehnaz et al. 2007a) also significantly increased shoot weight of these two varieties; however, it was less than *G. azotocaptans* DS1, i.e., 15.7%. *G. diazotrophicus* PAL5 strains, i.e., Wt and *nif*D, could not contribute significantly in shoot weight of these varieties. For variety 39H84, increase in shoot weight was non-significant by all bacterial strains. For variety 39D82, shoot weight was significantly increased by both strains of *G. diazotrophicus*, i.e., 12.5–13.7%. *G. azotocaptans* DS1 showed 11.3% increase in shoot weight that was slightly less than *G. diazotrophicus*. *Azospirillum* strains N7 and N8 showed 7.5% and 12.5% non-significant increase, respectively. However, *P. putida* CQ179 showed lowest shoot weight as compared to all other inoculated and control plants.

Same varieties were used for plant experiment in unsterilized soil collected from corn field and inoculated with DS1 and strains of *Azospirillum*, *Pseudomonas*, and *G. diazotrophicus*. DS1 significantly increased the root weight of 39H84, i.e., 19% (Table 1.3), as compared to other varieties and other bacterial strains. None of the bacterial strains could increase the root weight of variety 39T68 as compared to control plants. *P. putida* CQ179 was the only strain that significantly increased the root weight, i.e., 20.8%, of variety 39M27 plants. Performance of DS1 was very poor with this variety as lowest root weight was recorded for plants inoculated with

Table 1.2 Effect of bacterial isolates on shoot weight of four corn varieties, after 30 days growth in sterilized sand

	Corn varieties			
Treatments	39D82 (mg/plant)	39H84 (mg/plant)	39M27 (mg/plant)	39T68 (mg/plant)
Control	400 ± 62 bcd	525 ± 108 ab	310 ± 60 b	510 ± 98 d
A. zeae N7	430 ± 41 abc	510 ± 124 ab	315 ± 54 b	590 ± 66 abc
A. brasilense N8	450 ± 58 ab	490 ± 124 b	330 ± 66 b	590 ± 130 ab
P. putida CQ179	360 ± 54 d	560 ± 100 a	350 ± 60 b	570 ± 74 abcd
***G. azotocaptans* DS1**	**445 ± 71 ab**	**540 ± 86 ab**	**400 ± 71 a**	**630 ± 122 a**
G. diazotrophicus PAL5 Wt	455 ± 65 a	510 ± 98 ab	330 ± 57 b	530 ± 88 cd
*G. diazotrophicus PAL5 nif*D	450 ± 55 a	535 ± 105 ab	320 ± 51 b	560 ± 85 bcd

Values are average of 12 replicates. Letters indicate a statistically significant difference between treatments according to Duncan's multiple range test (DMRT) at $P \leq 0.05$. Mean separation within a column followed by the same letters does not differ significantly
Data is taken from Mehnaz and Lazarovits (2006)

Table 1.3 Effect of bacterial isolates on root weight of four corn varieties, after 30 days growth, in non-sterilized corn field soil

	Corn varieties			
Treatments	39D82 (mg/plant)	39H84 (mg/plant)	39 M27 (mg/plant)	39 T68 (mg/plant)
Control	240 ± 30 c	290 ± 45 bc	240 ± 45 bc	320 ± 61 a
A. zeae N7	290 ± 48 ab	310 ± 53 abc	260 ± 47 abc	340 ± 74 a
A. brasilense N8	250 ± 34 bc	310 ± 70 abc	255 ± 33 abc	330 ± 79 a
P. putida CQ179	300 ± 39 a	330 ± 50 ab	290 ± 52 a	335 ± 63 a
***G. azotocaptans* DS1**	**250 ± 57 bc**	**345 ± 53 a**	**220 ± 69 c**	**320 ± 65 a**
G. diazotrophicus PAL5 Wt	240 ± 37 c	280 ± 66 c	260 ± 64 abc	340 ± 75 a
*G. diazotrophicus PAL5 nif*D	240 ± 25 c	290 ± 75 bc	280 ± 53 ab	340 ± 63 a

Values are average of 12 replicates. Letters indicate a statistically significant difference between treatments according to Duncan's multiple range test (DMRT) at $P \leq 0.05$. Mean separation within a column followed by the same letters does not differ significantly
Data is taken from Mehnaz and Lazarovits (2006)

Table 1.4 Effect of bacterial isolates on shoot weight of four corn varieties, after 30 days growth, in non-sterilized corn field soil

	Corn varieties			
Treatments	39D82 (mg/plant)	39H84 (mg/plant)	39M27 (mg/plant)	39T68 (mg/plant)
Control	500 ± 67 b	570 ± 87 b	450 ± 111 b	690 ± 99 a
A. zeae N7	570 ± 64 a	610 ± 92 b	560 ± 66 a	710 ± 127 a
A. brasilense N8	520 ± 46 ab	590 ± 120 b	450 ± 109 b	670 ± 97 a
P. putida CQ179	560 ± 59 a	675 ± 88 a	560 ± 63 a	720 ± 131 a
***G. azotocaptans* DS1**	**480 ± 42 b**	**570 ± 149 b**	**470 ± 111 b**	**700 ± 144 a**
G. diazotrophicus PAL5 Wt	500 ± 72 b	595 ± 130 b	505 ± 81 ab	710 ± 118 a
*G. diazotrophicus PAL5 nif*D	520 ± 49 ab	580 ± 117 b	590 ± 99 a	710 ± 120 a

Values are average of 12 replicates. Letters indicate a statistically significant difference between treatments according to Duncan's multiple range test (DMRT) at $P \leq 0.05$. Mean separation within a column followed by the same letters does not differ significantly
Data is taken from Mehnaz and Lazarovits (2006)

this strain; however, the difference was non-significant. The rest of the inoculated strains showed non-significant increase in root weight.

G. azotocaptans DS1 could not significantly increase the shoot weight of plants of any variety as compared to uninoculated plants (Table 1.4). *A. zeae* N7 and *P. putida* CQ179 significantly increased the shoot weight of variety 39D82, i.e., 14% and 12%, respectively. The rest of the strains contributed non-significantly in shoot weight. Lowest but non-significant root weight was recorded for DS1 inoculated plants. *P. putida* CQ179 also significantly increased the shoot weight, i.e., 18.4%, of variety 39H84 plants. Shoot weight of the rest of the inoculated plants was far below as compared to CQ179 inoculated plants. For variety 39M27, *G. diazotrophicus nif*D, *P. putida* CQ179, and *A. zeae* N7 significantly increased shoot weight of the

plants as compared to all other treatments. CQ179 and N7 both increased 24.4% shoot weight, and *G. diazotrophicus nif*D showed 31% increase in this parameter.

For field experiment, corn variety 39D82 was used. Bacterial cultures, i.e., *G. azotocaptans* DS1 and *Azospirillum canadense* DS2 (another isolate from corn rhizosphere; Mehnaz et al. 2007b), were individually used as inoculum. Bacterial cell pellets were suspended in 0.85% saline with 1% polyvinyl pyrrolidone K30 (PVP; sticker). Approximately 10^6 cells per seed were applied by soaking them in inoculum. For control, seeds were coated only with sticker. Replicated field plots of corn variety Pioneer 39D82 were established in Southwestern Ontario at the Delhi research farm. All plots received a broadcast application of granular fertilizer containing 55 kg *N* + 20 kg P_2O_5 + 110 K_2O/ha incorporated to a depth of about 10 cm prior to seeding. In the fall, grains were harvested, the moisture content was determined, and the 15% moisture content (MC) yield was calculated. Data was analyzed by using SAS statistical software (ver.9.1). ANOVA was carried out in SAS, and comparison among treatments was done by using Duncan's multiple range test (DMRT).

Grain yield for *G. azotocaptans* DS1 inoculated plants was 9 t/ha as compared to 8.7 and 8.6 t/ha for *A. canadense* DS2 and control plants, respectively. Another field experiment was performed with corn variety, NK N-35 B8, and inoculated with the same organisms (DS1 and DS2) and the same conditions. Grain yield for DS1 was 11.1 t/ha as compared to10.7 and 10.2 t/ha for DS2 and control plants, respectively. For both experiments, DS1 inoculated plants showed highest grain yield; however, the difference was non-significant.

1.3.2 Wheat

The detailed studies with strain DS1 of *G. azotocaptans* on wheat were done at the University of Saskatchewan, Canada, by Morley in 2013. He used DS1 and a strain of *Azospirillum zeae* N7 (Mehnaz et al. 2007a) to inoculate wheat plants cv. Lillian under lab and field conditions and observed their effect on dry matter, %Ndfa, and survival of these strains. Survival of strains was observed on sterilized, non-sterilized, and fungicide-coated seeds. It was reported that DS1 and N7 survived well in the presence of fungicide, Dividend® XLRTA®, on the seed coat, showing their resistance to this product. Survival was better on non-sterilized and fungicide-coated seeds as compared to sterilized seeds as it declined at fast rate on sterilized ones.

DS1 and N7 are nitrogen fixers, and this ability was observed in these experiments. It is known that presence of nitrogen fertilizer reduces the biological nitrogen fixation (BNF). Interestingly, in this study both strains contributed more through BNF, in the presence of nitrogen fertilizer. Nitrogen uptake and %Ndfa increased with the increase of nitrogen fertilizer. Inoculated plants grown in growth chamber with 12.2–24.5 μg N/g had highest %Ndfa, i.e., 25.5%. Inoculated plants fertilized with higher amount showed highest nitrogen uptake, 1.3 g/pot, at maturity, as well. Similar results were obtained in field study as inoculated plants provided with 80 kg N/ha showed highest nitrogen uptake of 47 kg N/ha and significantly

higher ($P < 0.05$) %Ndfa, 10.5%, as compared to other dosage of chemical fertilizer. Accumulation of nitrogen varied in different parts of the plants with bacterial strains.

For pot experiments, *Azospirillum zeae* N7 inoculated plants accumulated significant amount of nitrogen in spikes, and *G. azotocaptans* DS1 inoculated plants had highest amount in stem. This trend was not observed in field experiment. In pot experiments, plants were harvested at 40, 65, and 102 days after sowing. Significant increase in dry matter of *G. azotocaptans* DS1 inoculated plants provided with fertilizer, was observed after 40 days of sowing. At other stages and inoculation with *Azospirillum* N7, it was not as effective as DS1. Among dry matter, the weight of stem for DS1 inoculated plants with different fertilizer doses was higher as compared to uninoculated plants and plants without any fertilizer. However, dry matter for spikes was highest for *Azospirillum* inoculated plants as compared to other treatment.

Field experiments with these two strains were conducted at three sites of Saskatchewan. Field soil was provided with different doses of nitrogen fertilizer. It was observed that presence of nitrogen fertilizers did not inhibit the nitrogen fixation by DS1 and N7, as determined by %Ndfa. Increase in dry matter or grain yield was observed with increase in fertilizer dose. However, increase in yield or dry matter was non-significant in inoculated plants.

1.4 Growth Promotion of Vegetables Inoculated with *G. azotocaptans* DS1

Authors have worked on the *G. azotocaptans* DS1 to observe its growth-promoting effects on different vegetables. For cereals, work was done in greenhouse and field as well. However, for vegetables, experiments were done only in greenhouse under controlled temperature and light conditions. In addition to *G. azotocaptans* DS1, four other bacterial strains, *Azospirillum zeae* N7, *A. brasilense* N8, *A. canadense* DS2, and *Pseudomonas putida* CQ179, isolated from corn rhizosphere were used to inoculate the vegetable crops. Inoculum was applied to cucumber, pepper, radish, and tomato seedlings, at the time of transplantation in promix (mixture of peat and vermiculite) and grown under greenhouse conditions. Plants were harvested after 4 weeks, and root, shoot, and whole plant weights were recorded.

For each crop, 12 replicates were used for each treatment. All experiments were repeated three times. The repeated experiments showed similar trends, and there were non-significant differences between the same treatments in each experiment. The data was analyzed by using SAS statistical software. One-way analysis of variance (ANOVA) was done with the ANOVA procedure in SAS, and comparison among treatments was done by using Duncan's multiple range test (DMRT). All analyses were performed at the $P = 0.05$ level.

Table 1.5 Effect of nitrogen fixers on sweet pepper cultivar "California wonder" and cucumber cultivar "Marketmore 76" plants after 30 days growth in promix, under greenhouse conditions

	Sweet pepper			Cucumber		
Treatments	Total weight (mg/plant)	Shoot weight (mg/plant)	Root weight (mg/plant)	Total weight (mg/plant)	Shoot weight (mg/plant)	Root weight (mg/plant)
Control	113 bc	58 b	53 b	773 bc	603 bc	175 ab
A. zeae N7	103 c	54 b	49 b	756 bc	583 bc	168 b
A. brasilense N8	142 ab	82 a	71 a	829 ab	659 ab	160 b
***G. azotocaptans* DS1**	**161 a**	**89 a**	**72 a**	**931 ab**	**730 a**	**201 a**
A. canadense DS2	177 a	97 a	78 a	635 c	504 c	123 c
P. putida CQ179	163 a	86 a	74 a	844 ab	666 ab	167 b

Values are average of 12 replicates. Letters indicate a statistically significant difference between treatments according to Duncan's multiple range test (DMRT) at $P \leq 0.05$. Mean separation within a column followed by the same letters does not differ significantly

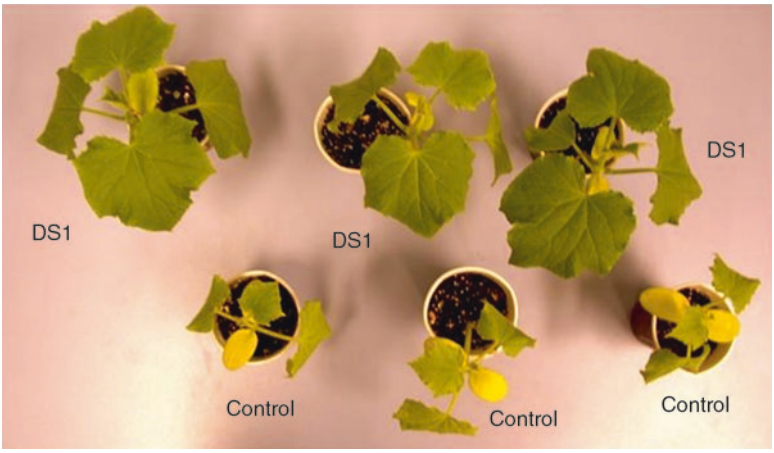

Fig. 1.1 Growth-promoting effect of *G. azotocaptans* DS1 on cucumber plant, under greenhouse conditions

1.4.1 Sweet Pepper

It was observed that *G. azotocaptans* DS1 significantly promoted root, shoot, and total plant weight. Significantly high total weight was recorded for *G. azotocaptans* DS1, *A. canadense* DS2, and *P. putida* CQ179 inoculated plants as compared to uninoculated and two other strains. Although *A. canadense* DS2 inoculated plants showed highest weight as compared to *G. azotocaptans* DS1 and *P. putida* CQ179, the difference was non-significant (Table 1.5; Fig. 1.1). Root and shoot weight for all inoculated plants except *A. zeae* N7 were significantly higher than control plants. N7 inoculated plants had non-significant difference with control. Among other

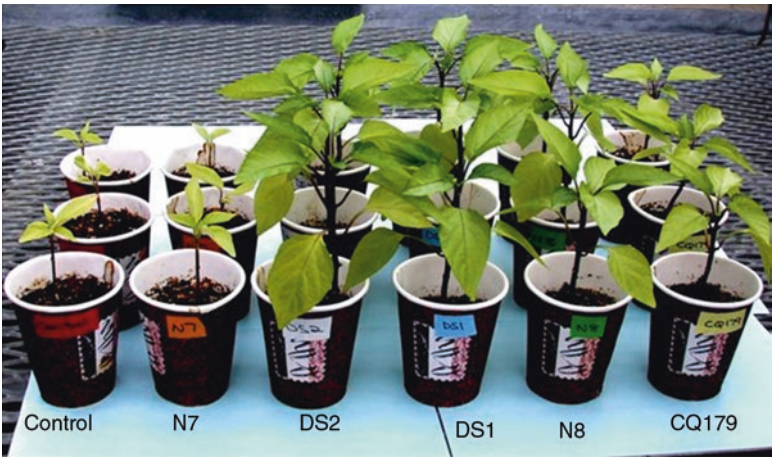

Fig. 1.2 Growth-promoting effect of *G. azotocaptans* DS1 on pepper plant, under greenhouse conditions

strains, highest root and shoot weights were also observed with *A. canadense* DS2 inoculated plants; difference with DS1, N8, and CQ179 was non-significant.

1.4.2 Cucumber

G. azotocaptans DS1 significantly promoted the total plant weight and shoot weight of cucumber plants as compared to uninoculated and plants inoculated with other strains (Table 1.5). Increase in shoot weight was quite visible in DS1 inoculated plants (Fig. 1.2). *A. brasilense* N8 and *P. putida* CQ179 also showed total weight and shoot weight higher than control; however, the difference was non-significant with *G. azotocaptans* DS1 and uninoculated plants. Root weight of DS1 inoculated plants was highest among all inoculated and uninoculated plants, but difference was non-significant with uninoculated plants.

1.4.3 Tomato

G. azotocaptans DS1, *A. canadense* DS2, and *P. putida* CQ179 significantly enhanced the root, shoot, and total weight of tomato plants as compared to uninoculated and rest of the inoculated plants (Table 1.6). Highest shoot weight and total plant weight were recorded for DS2, and root weight was highest for DS1 inoculated plants, however, difference among these three strains was non-significant. Significantly lowest weights among all inoculated and uninoculated plants were recorded for *A. zeae* N7 inoculated plants. *A. brasilense* N8 inoculated plants showed total plant and root weight higher than control plants and N7 but lower than other inoculated plants.

Table 1.6 Effect of nitrogen fixers on radish cultivar "Cherry belle" and tomato cultivar "Bellstar 409" plants after 30 days growth in promix, under greenhouse conditions

	Radish			Tomato		
Treatments	Total weight (mg/plant)	Shoot weight (mg/plant)	Root weight (mg/plant)	Total weight (mg/plant)	Shoot weight (mg/plant)	Root weight (mg/plant)
Control	965 b	308 c	619 bc	320 c	257 b	64 c
A. zeae N7	1,124 a	408 a	711 abc	240 d	190 c	59 c
A. brasilense N8	1,161 a	340 abc	823 a	364 b	282 b	84 b
***G. azotocaptans* DS1**	**1,114 a**	**383 ab**	**680 bc**	**460 a**	**361 a**	**103 a**
A. canadense DS2	925 b	361 abc	585 c	511 a	418 a	102 a
P. putida CQ179	1,041 ab	326 bc	732 ab	472 a	383 a	93 ab

Values are average of 12 replicates. Letters indicate a statistically significant difference between treatments according to Duncan's multiple range test (DMRT) at $P \leq 0.05$. Mean separation within a column followed by the same letters does not differ significantly

1.4.4 Radish

A. brasilense N8, *A. zeae* N7, and *G. azotocaptans* DS1 significantly enhanced total weight of radish plants, as compared to rest of the treatments including control plants (Table 1.6). Among these three, highest weight was recorded for *A. brasilense* N8 inoculated plants; however, difference with *A. zeae* N7 and *G. azotocaptans* DS1 was non-significant. Highest shoot weight was recorded with N7 inoculated plants. DS1 inoculated plants were second highest and had non-significant difference with N7. 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 difference was non-significant.

1.5 Use of *G. azotocaptans* DS1 as Inoculum for Potato Plants in Tissue Culture

Effect of *G. azotocaptans* DS1, on potato cultivar "Kennebec," was observed in tissue culture conditions. Plantlets were grown in MS medium after individual inoculation of *G. azotocaptans* DS1, *E. cloacae* CR1, *S. maltophilia* CR3, *P. putida* CR7, and *S. canadense* CR11. After 8 weeks, it was observed that DS1 significantly reduced the shoot height, shoot weight, and total biomass; root weight was better than control, but difference was not significant (Table 1.7). CR3, CR7, and CR11 significantly enhanced shoot weight and total biomass; however, root weight and shoot height were non-significantly higher than control. CR1 significantly reduced all parameters.

Response of DS1 was quite discouraging in tissue culture. To investigate this effect, three different concentrations of sucrose were used in MS medium, i.e., 7, 15, and 30 g/l. Regular amount of sucrose in MS medium is 30 g/l. Plantlets of same cultivar, "Kennebec," were inoculated with DS1, CR1, and *B. phytofirmans* E24. E24 is

Table 1.7 Effect of bacterial isolates on the "in vitro" growth of potato cultivar "Kennebec" after 8 weeks growth in MS medium

Treatments	Shoot height (cm/plant)	Total biomass (mg/plant)	Root weight (mg/plant)	Shoot weight (mg/plant)
Uninoculated	12.8 ± 1.52 a	57.2 ± 10.3 c	9.05 ± 4.7 b	45.1 ± 12.4 b
E. cloacae CR1	3.0 ± 1.02 c	15.2 ± 6.6 d	0 ± 0 c	15.2 ± 6.6 c
***G. azotocaptans* DS1**	**3.2 ± 0.3 c**	**22.6 ± 5.0 d**	**13.8 ± 2.7 b**	**13.3 ± 3.8 c**
S. maltophilia CR3	13.7 ± 1.99 a	79.5 ± 16.2 b	12.9 ± 5.2 b	66.7 ± 12.0 a
P. putida CR7	14.0 ± 1.32 a	72.1 ± 9.7 b	12.9 ± 4.3 b	59.2 ± 7.3 a
S. canadense CR11	13.5 ± 1.73 a	70.2 ± 15.4 b	9.9 ± 5.5 b	59.7 ± 11.1 a
B. phytofirmans E24	10.9 ± 1.24 b	110 ± 14.5 a	58.4 ± 8.1 a	46.9 ± 12.5 b

Values are average of 10 replicates. Letters indicate a statistically significant difference between treatments according to Duncan's multiple range test (DMRT) at $P \leq 0.05$. Mean separation within a column followed by the same letters does not differ significantly

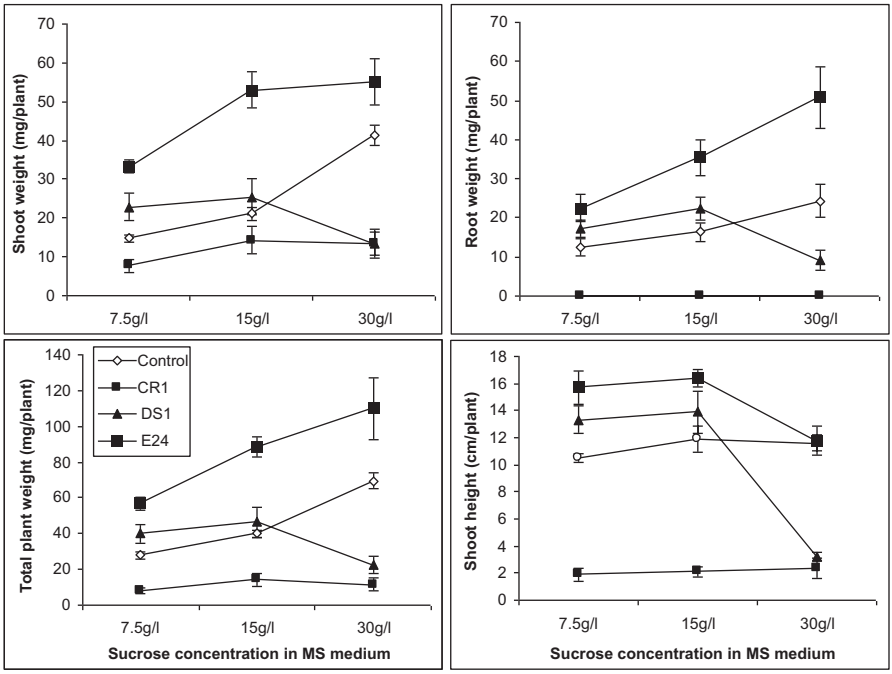

Fig. 1.3 Effect of sucrose concentration in MS medium on different growth parameters of potato cultivar "Kennebec"

known to be a plant growth promoter for corn, potato, tomato, and pepper (Lazarovits and Novak 1997; Mehnaz et al. 2010); it was used as positive control. After 8 weeks, it was observed that at 7 and 15 g/l, DS1 promoted all the parameters, i.e., root weight, shoot weight, total biomass, and shoot height, but it was drastically reduced at 30 g/l (Fig. 1.3). E24 increased all parameters at all concentrations, with maximum positive effect at 30 g/l. CR1 decreased all parameters at all concentrations.

1.6 *Gluconacetobacter azotocaptans* DS1 as Plant Growth Promoter

Role of *G. azotocaptans* DS1 as plant growth promoter is exclusively evaluated by authors. The strain improved the growth of corn, wheat, radish, pepper, tomato, and cucumber, under greenhouse and/or field conditions. Data mentioned above, support that DS1 has potential to be used as biofertilizer. As strain has ability of nitrogen fixation and IAA production, it seems that these two mechanisms are playing major role in plant growth promotion. Ability of this strain was evaluated under greenhouse and field conditions for cereals and vegetables, and in most of the cases results were positive even if non-significant. In soil, sand, and promix, DS1 strain has performed well.

Under tissue culture conditions, results were positive for lower concentration of sucrose. At higher concentration, it almost killed the plantlets. Effect of sucrose on plant growth is reported in literature. Studies have suggested extensive connections between sugar signaling and phytohormone pathways, in which abscisic acid acts positively and ethylene acts negatively (Kozuka et al. 2005). It is known that sucrose increases ethylene production in plant tissues (Meir et al. 1985) and also enhances the sensitivity to auxin (de Klerk et al. 1999). Calamar and Klerk (2002) studied the effect of sucrose concentration on adventitious root regeneration in apple and noticed strong reduction of rooting at higher sucrose concentration. Kozuka et al. (2005) examined the role of photoreceptors and sucrose on differential growth of leaf blade and petiole. They observed the inhibition in leaf blade expansion with increasing sucrose concentration in white light.

Researchers who isolated and named the species, i.e., *G. azotocaptans*, did not analyze the potential of this strain as PGPR; at least authors could not find any report. Therefore, all the information is based on one strain. Work done by Morley (2013) on this strain also validated its plant growth potential.

Unfortunately, only two strains of this species have been reported by now. It seems that more strains might exist but not discovered yet. One strong reason can be that 16S rRNA of *G. azotocaptans* has 98.5% similarity with *G. diazotrophicus* (Fuentes-Ramirez et al. 2001; Mehnaz et al. 2006) that is strong enough to declare it as *G. diazotrophicus*. To confirm it as *G. azotocaptans,* one has to get complete sequence of 1.5 kb, and that is not a regular practice; mostly researchers used a smaller fragment. Another way is to use specific primers designed for this species (Fuentes-Ramirez et al. 2001).

1.7 Conclusions

In this chapter, authors have discussed the role of *G. azotocaptans* in plant growth promotion. Most of the work was carried out by authors on isolate DS1, and data provided here strongly suggests that it should be used as biofertilizer. Now, DS1 strain is with an international company known for bioformulation production, and

authors expect that may be soon we will see the commercially available bioformulation based on this strain alone or as a part of consortium.

In addition, authors recommend that researchers finding new strains of *G. diazotrophicus* should also make sure by doing the sequencing of longer fragment of 16S rRNA that they have the right species.

Acknowledgments Authors are grateful to Brian Weselowski, Tom Kowalik, and Linda Li for lab work. This work was supported by grant from Agriculture and Agri-Food Canada.

References

Calamar A, de Klerk GJ (2002) Effect of sucrose on adventitious root regeneration in apple. Plant Cell Tissue Organ Cult 70:207–212

de Klerk GJ, Paffen A, Jasik J, Haralampieva V (1999) A dual effect of ethylene during rooting of apple microcuttings. In: Altman A, Ziv M, Izhar S (eds) Plant biotechnology and in vitro biology in 21st century. Kluwer Publishers, Dordrecht, pp 41–44

Eskin N, Vessey K, Tian L (2014) Research progress and perspectives of nitrogen fixing bacterium, *Gluconacetobacter diazotrophicus*, in monocot plants.Int J Agron, *Vol 2014, Article ID 208383*

Fuentes-Ramirez LE, Bustillos-Cristales R, Tapia-Hernandez A et al (2001) Novel nitrogen fixing acetic acid bacteria, *Gluconacetobacter johannae* sp. nov. and *Gluconacetobacter azotocaptans* sp. nov., associated with coffee plants. Int J Syst Evol Microbiol 51:1305–1314

Jimenez-Salgado T, Fuentes-Ramirez LE, Tapia-Hernandez A et al (1997) *Coffea arabica* L., a new host plant for *Acetobacter diazotrophicus*, and isolation of other nitrogen fixing acetobacteria. Appl Environ Microbiol 63:3676–3683

Kozuka T, Horiguchi G, Kim GT et al (2005) The different growth responses of the *Arabidopsis thaliana* leaf blade and the petiole during shade avoidance are regulated by photoreceptors and sugar. Plant Cell Physiol 46(1):213–223

Lazarovits G, Nowak J (1997) Rhizobacteria for improvement of plant growth and establishment. Hortic Sci 32:188–192

Mehnaz S, Lazarovits G (2006) Inoculation effects of *Pseudomonas putida, Gluconacetobacter azotocaptans* and *Azospirillum lipoferum* on corn plant growth under green house conditions. Microb Ecol 51:326–335

Mehnaz S, Weselowski B, Lazarovits G (2006) Isolation of *Gluconacetobacter azotocaptans* from corn rhizosphere. Syst Appl Microbiol 29(6):496–501

Mehnaz S, Weselowski B, Lazarovits G (2007a) *Azospirillum zeae* sp. nov., diazotrophic bacteria isolated from rhizosphere soil of *Zea mays*. Int J Syst Evol Microbiol 57(12):2805–2809

Mehnaz S, Weselowski B, Lazarovits G (2007b) *Azospirillum canadense* sp. nov., a nitrogen fixing bacterium isolated from corn rhizosphere. Int J Syst Evol Microbiol 57(3):620–624

Mehnaz S, Kowalik T, Reynold B, Lazarovits G (2010) Growth promoting effects of corn (*Zea mays*) bacterial isolates under greenhouse and field conditions. Soil Biol Biochem 2(10):1848–1856

Meir S, Philosophos-Hidas S, Epstein E, Aharoni N (1985) Carbohydrates stimulate ethylene production in tobacco leaf discs: interaction with auxin and the relation to auxin metabolism. Plant Physiol 78:131–138

Morley RE (2013) Impact of free-living diazotrophs, *Azospirillum lipoferum* and *Gluconacetobacter azotocaptans*, on growth and nitrogen utilization by wheat (*Triticum aestivum* cv. *Lillian*). Dissertation, University of Saskatchewan

Munoz-Rojaz J, Caballero-Mellado J (2003) Population dynamics of *Gluconacetobacter diazotrophicus* in sugarcane cultivars and its effect on plant growth. Microbiol Ecol 46:454–464

Muthukumarasamy R, Cleenwerck I, Revathi G et al (2005) Natural association of *Gluconacetobacter diazotrophicus* and diazotrophic *Acetobacter peroxydans* with wetland rice. Syst Appl Microbiol 28:277–286

Yamada Y, Hoshino K, Ishikawa T (1997) The phylogeny of acetic acid bacteria based on the partial sequences of 16S ribosomal RNA: the elevation of the subgenus Gluconacetobacter to generic level. Biosci Biotechnol Biochem 61:1244–1251 [validation list no. 64. Int J SystBacteriol (1998) 48: 327–328]