ORIGINAL ARTICLE

Solubilization of insoluble zinc compounds by *Gluconacetobacter diazotrophicus* and the detrimental action of zinc ion (Zn^{2+}) and zinc chelates on root knot nematode *Meloidogyne incognita*

V.S. Saravanan[1,4], P. Kalaiarasan[2], M. Madhaiyan[3] and M. Thangaraju[4]

1 School of Bio-Engineering and Bio-Sciences, Vellore Institute of Technology (VIT), Vellore, Tamil Nadu, India
2 Department of Plant Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
3 Department of Agricultural Chemistry, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea
4 Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Keywords
Gluconacetobacter diazotrophicus, *Meloidogyne incognita*, zinc oxide, 5-ketogluconic acid.

Correspondence
V.S. Saravanan, School of Bio-Engineering and Bio-Sciences, Vellore Institute of Technology (VIT), Vellore, Tamil Nadu, India.
E-mail: saraphd2003@yahoo.com and vigsaran@gmail.com

2005/0992: received 2 September 2005, revised 1 October 2006 and accepted 19 October 2006

doi:10.1111/j.1472-765X.2006.02079.x

Abstract

Aim: To examine the zinc (Zn) solubilization potential and nematicidal properties of *Gluconacetobacter diazotrophicus*.

Methods and Results: Atomic Absorption Spectrophotometer, Differential Pulse Polarography and Gas Chromatography Coupled Mass Spectrometry were used to estimate the total Zn and Zn^{2+} ions and identify the organic acids present in the culture supernatants. The effect of culture filtrate of Zn-amended *G. diazotrophicus* PAl5 on *Meloidogyne incognita* in tomato was examined under gnotobiotic conditions. *Gluconacetobacter diazotrophicus* PAl5 effectively solubilized the Zn compounds tested and 5-ketogluconic acid was identified as the major organic acid aiding the solubilization of zinc oxide. The presence of Zn compounds in the culture filtrates of *G. diazotrophicus* enhanced the mortality and reduced the root penetration of *M. incognita* under *in vitro* conditions.

Conclusions: 5-ketogluconic acid produced by *G. diazotrophicus* mediated the solubilization process and the available Zn^{2+} ions enhanced the nematicidal activity of *G. diazotrophicus* against *M. incognita*.

Significance and Impact of the Study: Zn solubilization and enhanced nematicidal activity of Zn-amended *G. diazotrophicus* provides the possibility of exploiting it as a plant growth promoting bacteria.

Introduction

Gluconacetobacter diazotrophicus, an acetic acid bacterium, is a gram-negative, nitrogen-fixing endophyte originally isolated from sugarcane (Cavalcante and Döbereiner 1988). Since then numerous reports suggested its occurrence in other crops like *Coffea arabica* (Jiménez-Salgado *et al.* 1997), *Eleusine coracana* (Loganathan *et al.* 1999), pineapple (Tapia-Hernández *et al.* 2000) and from tropical and subtropical plants of India (Madhaiyan *et al.* 2004). *Gluconacetobacter diazotrophicus* has also been reported to possess other plant growth-promoting traits like production of plant growth hormones (Fuentes-Ramírez *et al.* 1993), *in vitro* phosphate solubilization (Mahesh Kumar *et al.* 1999), antagonism against *Colletotrichum falcatum*, and *Xanthomonas albilineans*, the red rot and leaf scald pathogens of sugarcane, respectively (Muthukumarasamy *et al.* 2000; Blanco *et al.* 2005). In this study, we report *G. diazotrophicus* as a potential solubilizer of insoluble zinc (Zn) compounds and the possible mechanism of acid production behind solubilization.

Zinc, an essential micronutrient is a constituent of various metabolic enzyme systems in plants. Deficiency symptoms of Zn that include premature yellowing and drying of leaf tips and leaf margin occur in sugarcane, rice and coffee, the host crops of *G. diazotrophicus*.

Application of Zn alone or in combination with the biocontrol agent *Pseudomonas aeruginosa* significantly decreased the penetration of the root knot nematode *Meloidogyne javanica* in tomato (Siddiqui *et al.* 2002). *Meloidogyne* species considered as one of the most serious nematode pests in sugarcane reduced the cane yield by 9–15 t ha^{-1} (Cadet and Spaull 2003). Infection by *Meloidogyne* sp. has also been reported in coffee and cotton (Spaull and Cadet 1991; Schmitt *et al.* 2001). Although applications of zinc sulfate ($ZnSO_4$) in the form of fertilizers could ameliorate Zn deficiency and improve plant yields, the applied Zn gets transformed into different insoluble forms depending on the soil types and totally become unavailable in the environment within 7 days of application (Rattan and Shukla 1991).

In the present study, we tested the ability of *G. diazotrophicus* to solubilize inorganic Zn compounds *in vitro* and have identified the organic acid aiding in solubilization as 5-ketogluconic acid. Furthermore, the culture filtrate of *G. diazotrophicus* possessed nematicidal activities against *Meloidogyne incognita* juveniles. So, the effect of microbially formed Zn^{2+} and Zn chelates in the culture filtrate was examined for the possible enhancement of nematicidal effects against *M. incognita* in tomato under *in vitro* conditions.

Materials and methods

Gluconacetobacter diazotrophicus strains and culture conditions

Gluconacetobacter diazotrophicus strain PAl5 (ATCC49037) isolated from sugarcane roots was obtained from Johanna Döbereiner (EMBRAPA, Itagui, RJ, Brazil). *Gluconacetobacter diazotrophicus* strains R10, S2 and S7 were obtained from the Centre of Advanced Studies in Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India. Biochemical and molecular characterization of the strains proved their authenticity. *Gluconacetobacter diazotrophicus* strains were maintained in LGI agar slants (Cavalcante and Döbereiner 1988) at 28°C. For solubilization studies, LGI medium with glucose (2%) as carbon source supplemented with insoluble Zn compounds was used. Zinc oxide (ZnO), zinc carbonate ($ZnCO_3$) and zinc phosphate [$Zn_3(PO_4)_2$] (Loba Chemie, Mumbai, India) used as insoluble Zn compounds were added to the medium at 0·12%, 0·21% and 0·21% (w/v) to give a final concentration of 0·1% Zn.

In vitro solubilization of zinc

Gluconacetobacter diazotrophicus strains grown in LGI broth for 48 h (6×10^8 cells ml^{-1}) were spotted in 10-μl volumes to LGI plates amended with insoluble Zn compounds (Fasim *et al.* 2002). Zones of solubilization were observed by incubating the plates at 28°C for 3 days. For liquid cultures, 50 ml of LGI broth supplemented with insoluble Zn compounds were inoculated with log-phase cultures of *G. diazotrophicus* strains and incubated in a gyratory shaker (175 rev min^{-1}) for 10 days. Uninoculated medium served as a control.

Analysis of zinc

The Zn concentration of the culture supernatants and pH were estimated at different periods (Di Simine *et al.* 1998). The total Zn and the free zinc cations (Zn^{2+}) in the samples (diluted 100-fold) were analysed respectively by Atomic Absorption Spectrophotometry (AAS) (Model Varian C) and Differential Pulse Polarography (DPP) (Polarographic Analyser CL 362, Elico Ltd., India). For DPP, the electrolyte used was 1 mol of KCl buffered to pH 6·0 with 10 mmol of piperazine-*N*,*N*′-bis-ethanesulfonic acid and tetramethylammonium hydroxide. Each polarogram was obtained in duplicate, from three consecutive voltage sweeps. Standards were prepared with zinc chloride. The chelated Zn obtained is the difference in concentrations of the total Zn concentration and unbound Zn^{2+}.

Organic acid analysis

Analysis of the sugars and organic acids was carried out using a Gas Chromatography Coupled Mass Spectrometry (GC-MS) system, equipped with Cbp 5 column (25 m, 0·5 mm ID) with helium as carrier gas (Model QP 7000, Shimadzu, Japan). Seven-days-old culture supernatant was lyophilized (Lyophilizer; Christ Alpha 1-4, Germany) and processed to produce trimethylsilyl derivatives. To the methanol extracts (500 μl) of lyophilized samples flushed with nitrogen, 100 μl of *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (Sigma Chemical Co., St. Louis, Missouri, USA) and 100 μl of pyridine were added and again nitrogen was flushed to maintain anaerobic condition. The reactions were carried out in sealed septum vials, heated gently in a water bath at 60°C for 30 min and left overnight for stabilization. The samples were analysed in GC-MS with a source temperature of 200°C and ionizing voltage of 70 eV and operated in a scan mode (50–700 m/z) using a temperature gradient of 70–260°C and compared with known standards.

Nematicidal effects of zinc-amended *Gluconacetobacter diazotrophicus* culture filtrates

Fresh juveniles of *M. incognita* were obtained from the Department of Nematology of Tamil Nadu Agricultural

University, Coimbatore. Egg masses obtained from pure cultures were maintained on susceptible tomato plants (*Lycopersicon esculentum* cv PKM-1) under green house conditions and they were placed in distilled water and incubated for 48 h at room temperature for hatching. Second-stage juveniles thus collected were obtained and used for *in vitro* studies. Seven-days-old *G. diazotrophicus* PAl5 culture filtrate was collected from LGI flasks amended with different Zn sources. It was then filter-sterilized through a bacterial filter of 0·22-μm size, collected and used immediately owing to the possible degradation of the Zn chelates formed. Apart from various Zn compounds amended broth, filter-sterilized 25% gluconic acid solution was also used as a treatment. The commercially available 50% gluconic acid (Sigma-Aldrich Co.) was diluted with water to achieve the desired concentration and used in a volume equal to culture filtrate when mentioned.

To determine the nematode mortality, a 0·5-ml suspension of *M. incognita* containing approximately 100 surface sterilized juveniles was added to the culture filtrate (10 ml) and incubated for 48 h. Each treatment was replicated three times and juveniles kept in uninoculated sterilized LGI broth and sterile distilled water served as controls. After 24 and 48 h, dead juveniles were counted and the percentage of mortality was estimated. The juveniles that showed no movement when probed with a fine needle were considered dead (Cayrol *et al.* 1989).

Tomato seeds (variety Co 3) obtained from the Department of Seed Technology, Tamil Nadu Agricultural University, Coimbatore were surface disinfected (70% ethanol for 3 min; 5% NaOCl with 0·6% Tween 20 for 20 min) and thoroughly rinsed five times in sterile water. The surface-sterilized seeds (0·2 g approximately 100 seeds) were then imbibed in about 2·5 ml of Zn-amended or -unamended culture filtrate of *G. diazotrophicus* in petri dishes (60 × 15 mm) for 2 h. The filtrate was then drained with the aid of micropipette and the seeds were sown in sterilized glass tubes (7·5 × 2·5 cm) filled with sterile quartz sand at four seeds per tube. The tubes were covered and sealed with a rubber septum and incubated in a growth chamber at 27 ± 2°C, 15 000 lux for 9 h, with 70% humidity. At 7 to 8 days after germination, plants were inoculated with 100 *M. incognita* juveniles per tube that were kept in sterile distilled water. Gluconic acid 25% showed the highest mortality of *M. incognita* juveniles among the different concentrations tested (10% and 15%) and hence it was alone included in the treatments to study the effects on nematode penetration. Seeds treated with sterile distilled water served as control. The experiment was arranged in a completely randomized design with three replications per treatment and five tubes per replication. Moisture content was maintained by adding sterile distilled water to the tubes.

The number of nematodes that penetrated the root system was observed 1 day after inoculation (DAI). The roots were washed in tap water and cut into small bits of 1-cm length, immersed in boiled lactophenol-acid fuchsin, destained in clear lactophenol and examined under microscope and the results were expressed as number of nematodes penetrated per gram fresh weight of roots.

Statistical analysis

The data were subjected to statistical analysis and significant difference was calculated at $P \leq 0{\cdot}05$ using SAS version 9·1 (Statistical Analysis Systems version 9·1; SAS Institute Inc., Cary, NC, USA).

Results

Zinc solubilization by *Gluconacetobacter diazotrophicus*

All the four strains of *G. diazotrophicus* used, could effectively solubilize the insoluble Zn compounds used namely, ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$ under the assay conditions. A single exception to this was observed for *G. diazotrophicus* R10, that it could not solubilize $Zn_3(PO_4)_2$ supplemented in the media (Table 1). Solubilization of Zn compounds was higher in *G. diazotrophicus* PAl5 than in other strains and the zone of solubilization was high when ZnO was supplemented.

Gluconacetobacter diazotrophicus PAl5 was able to dissolve Zn compounds in liquid medium, consistent with the observations on solid medium. The total Zn and Zn^{2+} ions concentration in the culture supernatant increased under the assay conditions described, with significant

Table 1 Solubilization of different zinc compounds by *Gluconacetobacter diazotrophicus* strains in the plate assay

	Solubilization zone (mm)		
Strains	ZnO	$ZnCO_3$	$Zn_3(PO_4)_2$
G. diazotrophicus PAl5	48·0 ± 1·89 a	28·0 ± 0·94 b	21·0 ± 1·41 a
G. diazotrophicus R10	25·0 ± 1·89 c	12·0 ± 1·41 d	–
G. diazotrophicus S2	30·0 ± 0·94 b	38·0 ± 1·41 a	20·0 ± 0·94 a
G. diazotrophicus S7	15·0 ± 0·94 d	22·0 ± 1·89 c	14·0 ± 1·41 b
LSD ($P = 0{\cdot}05$)	2·31	1·63	2·83

Each value represents mean ± standard error (SE) of three replicates per treatment. The data are statistically analysed using Duncan's Multiple Range Test (DMRT). In the same column, significant differences according to least significant difference (LSD) at $P = 0{\cdot}05$ levels are indicated by different letters.

Abbreviations: ZnO, zinc oxide; $ZnCO_3$, zinc carbonate; $Zn_3(PO_4)_2$, zinc phosphate.

difference between them suggesting the chelation of Zn^{2+} (Fig. 1c). The total Zn concentration increased from the DAI and at day 10, it was 11 mmol l^{-1} in the ZnO amendment and 6·2, 4·0 mmol l^{-1} in $ZnCO_3$ and $Zn_3(PO_4)_2$ amendments, respectively (Fig. 1a). The free Zn^{2+} analysed by DPP also increased from the DAI and it was higher in the ZnO amendment recording 2·2 mmol l^{-1} at day 2, steadily increasing to 10 mmol l^{-1} at day 10. A similar trend was also observed for $ZnCO_3$- and $Zn_3(PO_4)_2$- amended culture supernatant with the values recorded being lower than the ZnO amendment (Fig. 1b). During the time course of the experiment, a drop in pH was observed in $Zn_3(PO_4)_2$-amended culture and the final pH recorded at day 10 was ~3·5 (Fig. 1d). But the supplementation of ZnO or $ZnCO_3$ caused no significant changes in pH.

GC-MS analysis of organic acids

Analysis of the culture supernatants of *G. diazotrophicus* PAl5 by GC-MS revealed the presence of similar organic acids under both ZnO-amended and -unamended conditions and the organic acid produced in larger amounts was identified as 5-ketogluconic acid by referring to the standards (Fig. 2). But the amount of the acid produced in Zn-amended culture supernatant remained less than that of the unamended control. The other two peaks of the chromatograms obtained could be identified as ribitol pentanoic acid and erythro pentanoic acid.

Nematicidal effects of *Gluconacetobacter diazotrophicus* culture filtrate against *Meloidogyne incognita*

The culture filtrate of *G. diazotrophicus* PAl5 resulted in 24–33% mortality of the second-stage juveniles of *M. incognita* when exposed for 24 and 48 h. The mortality rate was significantly increased when culture filtrate of *G. diazotrophicus* PAl5 grown with Zn amendments was used and the highest mortality accounting to 60% and 74% at 24 and 48 h, respectively was obtained with that of ZnO (Table 2). However, the use of gluconic acid at 25% resulted in the highest mortality rate that could not be observed in any of the treatments imposed with the bacterial filtrate.

Effect of *Gluconacetobacter diazotrophicus* PAl5 on *Meloidogyne incognita* penetration in tomato

The number of nematodes that penetrated the roots of tomato steadily increased in all the treatments over the

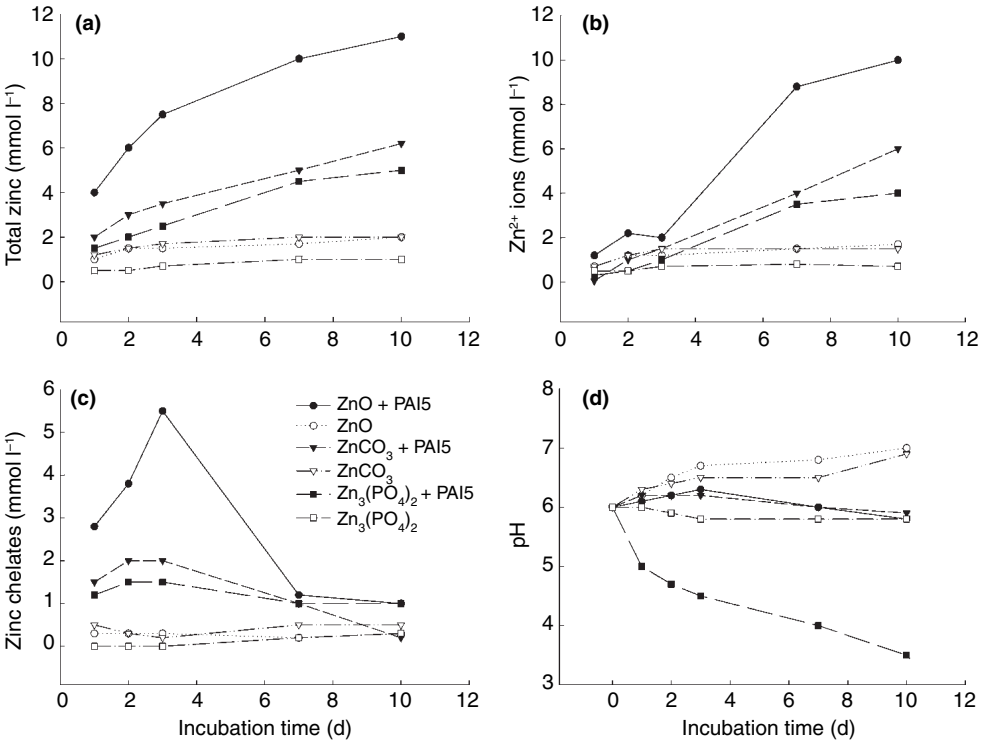

Figure 1 The concentrations of zinc (Zn) and pH variations in the culture supernatant of *Gluconacetobacter diazotrophicus* PAl5 amended with different Zn compounds at different intervals. The total Zn (a) and Zn^{2+} ions (b) in the culture supernatant were analysed by Atomic Absorption Spectrometry and Differential Pulse Polarography, respectively.

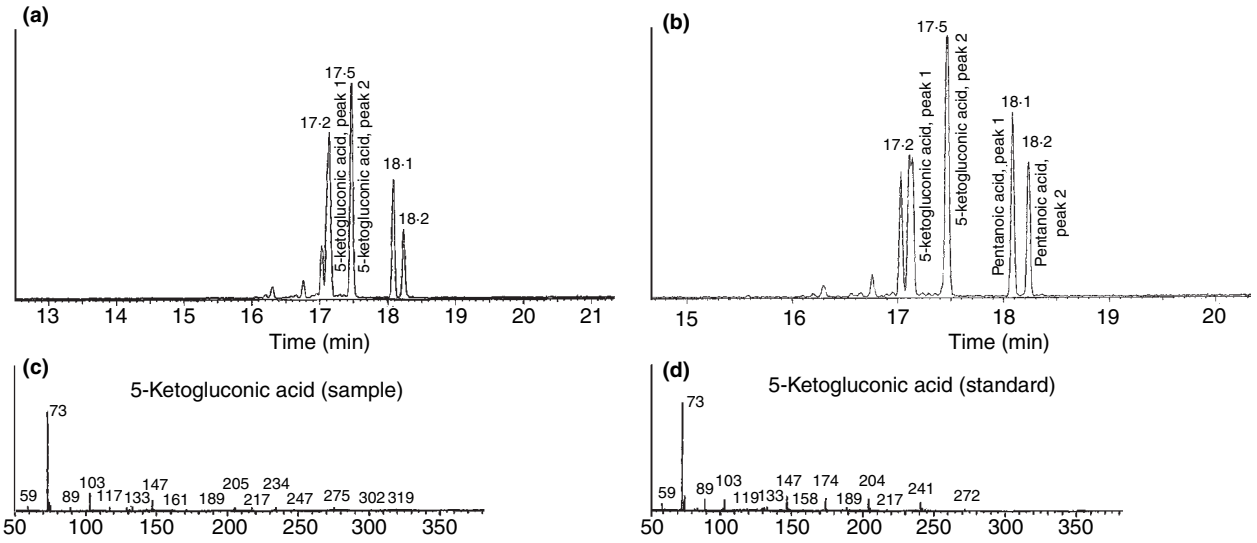

Figure 2 Gas chromatography spectrum of culture supernatant of *Gluconacetobacter diazotrophicus* derivatized with MSTFA and scanned at 50–700 m/z (a) *G. diazotrophicus* unamended (b) *G. diazotrophicus* amended with ZnO. Peaks identified as 5-ketogluconic acid and pentanoic acids (ribitol pentanoic and erythro pentanoic acid) are indicated. 5-ketogluconic acids represented 32% and 34% (a) and 24% and 31% (b) of total peak area of the analysed components; (c and d) comparative ion fragmentation pattern of 17.5-min peak of 5-ketogluconic acid from a sample (c) and standard (d) peak.

Table 2 Effect of culture filtrate of *Gluconacetobacter diazotrophicus* on the mortality and root penetration of *Meloidogyne incognita* under *in vitro* conditions

	Per cent mortality*		Number of nematodes (per gram of fresh root)			
Treatment	24 h	48 h	24 h	48 h	72 h	96 h
Control	1·0 ± 0·0 e	3·0 ± 0·5 e	9·0 ± 0·5 a	40·0 ± 1·4 a	55·0 ± 2·4 a	67·0 ± 2·8 a
G. diazotrophicus PAl5	24·0 ± 1·9 d	33·0 ± 2·8 d	5·0 ± 0·5 a	22·0 ± 0·9 b	34·0 ± 1·4 b	45·0 ± 2·4 b
G. diazotrophicus PAl5 + ZnO	60·0 ± 2·4 b	74·0 ± 3·8 b	3·0 ± 0·0 ba	12·0 ± 0·9 d	23·0 ± 1·4 e	32·0 ± 1·9 d
G. diazotrophicus PAl5 + $ZnCO_3$	39·0 ± 1·4 c	58·0 ± 3·8 c	5·0 ± 0·5 a	21·0 ± 1·4 cb	27·0 ± 1·4 d	36·0 ± 2·4 c
G. diazotrophicus PAl5 + $Zn_3(PO_4)_2$	31·0 ± 1·4 d	55·0 ± 3·3 c	4·0 ± 0·5 ba	19·0 ± 2·4 c	30·0 ± 0·9 c	37·0 ± 0·9 c
25% gluconic acid	82·0 ± 5·7 a	90·0 ± 4·2 a	1·0 ± 0·0 b	3·0 ± 0·5 e	8·0 ± 0·5 f	12·0 ± 1·9 e
LSD ($P = 0·05$)	7·35	8·17	3·56	2·49	2·42	2·49

*The control treatment included treatment with sterilized LGI broth while no mortality was recorded with sterilized distilled water. Each value represents mean ± standard error (SE) of three replicates per treatment. The data are statistically analysed using DMRT. In the same column, significant differences according to LSD at $P = 0·05$ levels are indicated by different letters.
Abbreviations: ZnO, zinc oxide; $ZnCO_3$, zinc carbonate; $Zn_3(PO_4)_2$, zinc phosphate.

period of the experiment. The number of nematodes that penetrated the root system was reduced in plants treated with the culture filtrate of *G. diazotrophicus* PAl5 with further reductions in Zn-amended culture filtrate compared with control (Table 2). Use of 25% gluconic acid was found to be the most detrimental to nematode penetration followed by ZnO-amended *G. diazotrophicus* PAl5 culture filtrate.

Discussion

In our study, preliminary experiments on Zn solubilization by plate assays revealed effective solubilization of Zn compounds by *G. diazotrophicus* strains. Analysis of the culture supernatants of *G. diazotrophicus* PAl5 grown in the presence of insoluble Zn compounds showed high levels of solubilized Zn existing as free Zn^{2+} ions and as Zn chelates, these two forms being potentially bioavailable for plants (Alloway 2004).

Production of protons and organic acids are considered as the significant mechanisms effecting the solubilization of metal compounds though excretion of other metabolites, siderophore and the like also contribute to the solubilization process (Sayer *et al.* 1995). In our study, analyses by GC-MS confirmed that 5-ketogluconic acid was the major organic acid produced in the intermediary

of solubilization while in *Pseudomonas* it was 2-ketogluconic acid that mediated solubilization process (Di Simine *et al.* 1998; Fasim *et al.* 2002). Gluconic acid and its derivatives were predicted to be the mediators of phosphate solubilization in *G. diazotrophicus* (Maheshkumar *et al.* 1999). Gluconic acid and ketogluconates are sugar acids having multiple conformations which chelate the metal cations apart from solubilization (Goldstein 1995). The presence of Zn chelates in our study could be attributed to chelation by gluconic acid and suggest that the solubilization process might be a direct consequence of increased hydrogen ion activity in the solution. The solubilization process was not accompanied by a reduction in the pH of the medium when ZnO or $ZnCO_3$ was supplemented and this might be partly because of their intrinsic buffering potential (Franz *et al.* 1991). Also, ZnO may act as an excellent buffer consuming 2 mol of protons per mole for solubilization.

Culture filtrate of *G. diazotrophicus* PAl5 effectively increased the mortality of second-stage juveniles of *M. incognita* and reduced their penetration in tomato roots under *in vitro* conditions. In our experiments, we have not quantified gluconic acid but were able to detect a derivative of gluconic acid (5-ketogluconic acid). The mechanism behind the nematicidal activity of the culture filtrate could not be explained. With a hypothesis that it might be the excess of gluconic acid and other derivative acids (5-ketogluconic acid detected in the present study) produced by the bacterium assisting in the nematicidal action, we tested a series of concentrations of gluconic acid for their nematicidal activity. Gluconic acid at 25% was found to be the most detrimental to the nematode population, with 5% and 15% gluconic acid, respectively resulting in a mortality rate of 31% and 54% at 48 h. Henceforth, the toxicity inflicted on the nematodes could also be due to some undetermined factors present in the culture filtrate. Recently, it was documented that certain volatile acids and ammonia in *G. diazotrophicus* culture broth were responsible for the nematicidal activities against *M. incognita* (Bansal *et al.* 2005). Additions of Zn enhanced the nematicidal and biocontrol activity of *Pseudomonads* under *in vitro* conditions (Siddiqui and Shaukat 2002; Siddiqui *et al.* 2002). Concurrent results were also obtained in our study, that the use of Zn-amended culture filtrate enhanced the nematicidal activities against *M. incognita* by *G. diazotrophicus*.

The mechanism of Zn solubilization and nematicidal properties by *G. diazotrophicus* could be explained in two steps. When glucose is used, the phosphorylative and direct oxidative pathways of glucose metabolism appeared to be operative in *G. diazotrophicus* and the enzymes pyrrolo-quinoline quinone (PQQ)-dependent glucose and gluconate dehydrogenases seemed to be primarily responsible for the formation of gluconic acid and its derivatives (Attwood *et al.* 1991). The 5-ketogluconic acid so formed could be detected in our study through GC-MS analysis and it aided the solubilization of insoluble Zn compounds making available free Zn^{2+} or Zn chelates in the culture filtrate. The presence of Zn^{2+} or Zn chelates in the culture filtrate enhanced the nematicidal activities against *M. incognita*. If this mechanism would operate under rhizosphere conditions, solubilization of Zn compounds and the nematicidal activities against *M. incognita*, by *G. diazotrophicus* could promote plant growth by converting unavailable Zn to available forms and biocontrol nematode in the rhizosphere. This preliminary study on *G. diazotrophicus*, the first of its kind in reporting Zn solubilization and nematicidal activity of *G. diazotrophicus* was carried under *in vitro* conditions and further studies are necessary to confirm that *G. diazotrophicus* could be better exploited as plant growth-promoting bacteria.

Acknowledgements

We would like to thank Mrs. S. Poonguzhali, Chungbuk National University, Republic of Korea for her valuable suggestions and correction of the manuscript. University Grants Commission, India is gratefully acknowledged for their financial support. The authors wish to thank anonymous referees for their valuable comments on the manuscript.

References

Alloway, B.J. (2004) *Zinc in Soils and Crop Nutrition*. p. 116. Brussels, Belgium: International Zinc Association.

Attwood, M.M., van Dijken, J.P. and Pronk, J.T. (1991) Glucose metabolism and gluconic acid production by *Acetobacter diazotrophicus*. *J Ferment Bioeng* **72**, 101–105.

Bansal, R.K., Dahiya, R.S., Narula, N. and Jain, R.K. (2005) Management of *Meloidogyne incognita* in cotton using strains of the bacterium *Gluconacetobacter diazotrophicus*. *Nematol Medit* **33**, 101–105.

Blanco, Y., Blanch, M., Piñón, D., Legaz, M.E. and Vicente, C. (2005) Antagonism of *Gluconacetobacter diazotrophicus* (a sugarcane endosymbiont) against *Xanthomonas albilineans* (pathogen) studied in alginate-immobilized sugarcane stalk tissues. *J Biosci Bioeng* **99**, 366–371.

Cadet, P. and Spaull, V.W. (2003) Effect of nematodes on the sustained production of sugarcane in South Africa Patrice. *Field Crops Res* **83**, 91–100.

Cavalcante, V.A. and Döbereiner, J. (1988) A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane. *Plant Soil* **108**, 23–31.

Cayrol, J.C., Djian, C. and Pijarowski, L. (1989) Study of the nematocidal properties of the culture filtrate of the nema-

tophagous fungus *Paecilomyces lilacinus*. *Revue Nématol* **12**, 331–336.

Di Simine, C.D., Sayer, J.A. and Gadd, G.M. (1998) Solubilization of zinc phosphate by a strain of *Pseudomonas fluorescens* isolated from a forest soil. *Biol Fertil Soils* **28**, 87–94.

Fasim, F., Ahmed, N., Parsons, R. and Gadd, G.M. (2002) Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. *FEMS Microbiol Lett* **213**, 1–6.

Franz, A., Burgstaller, W. and Schinner, F. (1991) Leaching with *Penicillium simplicissimum*: influence on metals and buffers on proton extrusion and citric acid production. *Appl Environ Microbiol* **57**, 769–774.

Fuentes-Ramírez, L.E., Jiménez-Salgado, T., Abaraca-Ocampo, I.R. and Caballero-Mellado, J. (1993) *Acetobacter diazotrophicus*, an indole acetic acid producing bacterium isolated from sugarcane cultivars of Mexico. *Plant Soil* **154**, 145–150.

Goldstein, A.H. (1995) Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram-negative bacteria. *Biol Agric Hortic* **12**, 185–193.

Jiménez-Salgado, T., Fuentes-Ramírez, L.E., Tapia-Hernández, A., Mascarua, M.A., Martínez-Romero, E. and Caballero-Mellado, J. (1997) *Coffea arabica* L., a new host plant for *Acetobacter diazotrophicus* and isolation of other nitrogen fixing acetobacteria. *Appl Environ Microbiol* **63**, 3676–3683.

Loganathan, P., Sunitha, R., Parida, A.K. and Nair, S. (1999) Isolation and characterization of two genetically distant groups of *Acetobacter diazotrophicus* from a new host *Eleusine coracana* L. *J Appl Microbiol* **87**, 167–172.

Madhaiyan, M., Saravanan, V.S., Bhakiya Silba Sandal Jovi, D., Lee, H.S., Thenmozhi, R. and Hari, K. T.M. Sa. (2004) Natural endophytic occurrence of *Gluconacetobacter diazotrophicus* in tropical and subtropical plants of Western Ghats, India. *Microbiol Res* **159**, 233–243.

Maheshkumar, K.S., Krishnaraj, P.U. and Alagawadi, A.R. (1999) Mineral phosphate solubilizing activity of *Acetobacter diazotrophicus*: a bacterium associated with sugarcane. *Curr Sci* **76**, 874–875.

Muthukumarasamy, R., Revathi, G. and Vadivelu, M. (2000) Antagonistic potential of N_2 fixing *Acetobacter diazotrophicus* against *Colletotrichum falcatum* went, a causal organism of red-rot of sugarcane. *Curr Sci* **78**, 1063–1065.

Rattan, R.K. and Shukla, L.M. (1991) Influence of different carriers on the utilization of micronutrients by rice. *J Indian Soc Soil Sci* **39**, 808–810.

Sayer, J.A., Raggett, S.L. and Gadd, G.M. (1995) Solubilization of insoluble metal compounds by soil fungi: development of a screening method for solubilizing ability and metal tolerance. *Mycol Res* **99**, 987–993.

Schmitt, D.P., Zhang, F. and Meisner, M. (2001) Potential for managing *Meloidogyne konaensis* on coffee in Hawaii with resistance and a nematicide. *Nematropica* **31**, 67–73.

Siddiqui, I.A. and Shaukat, S.S. (2002) Zinc and glycerol enhance the production of nematicidal compounds *in vitro* and improve the biocontrol of *Meloidogyne javanica* in tomato by fluorescent pseudomonads. *Lett Appl Microbiol* **35**, 212–217.

Siddiqui, I.A., Shaukat, S.S. and Hamid, M. (2002) Role of zinc in rhizobacteria-mediated suppression of root-infecting fungi and root-knot nematode. *J Phytopathol* **150**, 569–575.

Spaull, V.W. and Cadet, P. (1991) Studies on the relationship between nematodes and sugarcane in South and West Africa ratoon cane. *Revue Nématol* **14**, 183–186.

Tapia-Hernández, A., Bustillos-Cristales, M.R., Jiménez-Salgado, T., Caballero-Mellado, J. and Fuentes-Ramírez, L.E. (2000) Natural endophytic occurrence of *Acetobacter diazotrophicus* in pineapple plants. *Microb Ecol* **39**, 49–55.