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Studying the antagonistic activity of some *Gluconacetobacter* isolates and their colonizing ability of rice roots *in vitro*

Eman, O. Hassan[1] and Rasha, M. El-Meihy[2]
Plant Pathology Dep., Fac. of Agric., Benha Univ., Egypt.
Agric. Botany Dep., Microbiology Branch, Fac. of Agric., Benha Univ., Egypt.
Corresponding author: eman.hassan@fagr.bu.edu.eg
Co. author: rashaelmehy@fagr.bu.edu.eg

Abstract

The family Acetobacteriaceae currently includes three known nitrogen-fixing species, *Gluconacetobacter diazotrophicus, G. johannae* and *G. azotocaptans*. In the present study, nitrogen fixing bacteria were isolated from sugarcane and rice roots cultivated in Aswan and Qalubia Governorates, respectively. Seven isolates from sugarcane roots and three isolates from rice roots gave the basis phenotypic characteristics of *Gluconacetobacter* sp. These isolates were examined for plant growth promotion activities such as indole acetic acid (IAA) and gibberellins (GA_3) production in addition to their putative endophytic features such as cellulase and pectinase production. Rice seeds (*Oryza officinalis)* colonizing ability with the ten *Gluconacetobacter* isolates *in vitro* was examined. The ten *Gluconacetobacter* isolates were examined for their antagonistic activity against pathogenic fungi and bacteria in addition to their ability to produce hydrogen cyanide (HCN) and siderophores.Sodium dodecyle sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a technique used for the characterization and analysis of proteins for identification of bacterial isolates and yielding valuable information on the similarity and dissimilarity amongst bacterial cultures.

Key words: Gluconacetobacter, phytohormones, root colonization, antagonistic activity

Introduction

Gluconacetobacter spp. are found to live freely in the intercellular spaces of roots, stems and leaves of sugarcane plants. These endophytic bacteria don't form any specific structures (like the nodules of legume plants) within plant tissues **(Dong *et al*. 1995)**. In early studies *Gluconacetobacter* spp.described as an endophytic nitrogen-fixing bacteria associated with sugarcaneandother sugar-rich plants as sweet sorghum, sweetpotato and pineapple plantsand with sugar-poorplants as coffee **(Tapia-Hernandez *et al*., 2000)**. In the last few years this genus has been found in association with different host plants such as rice (**Muthukumarasamy *et al*., 2005)**. *Gluconacetobacter* spp. are capable of not only supplying its host plant with significant amounts of nitrogen, but also, controlling fungal and bacterial diseases. *G. diazotrophicus* is capable of entering its host plants through the roots, stems, and leaves **(Eskin, 2012).**There are two main types that require associations with host plants are endophytes and rhizobacteria which can be classified as plant growth promoting rhizobacteria (PGPR) because they are beneficial to their host plants. *G. diazotrophicus* has the ability to colonize with large number of cereals crops. **(Saharan and Nehra, 2011).**SDS-PAGE is currently one of the most commonly used techniques for the characterization and analysis of proteins and it has been used as a taxonomic tool for identification of various bacterial species and yielding valuable information on the similarity and dissimilarity amongst bacterial cultures **(Elgaml *et al*., 2014).**

The objectives of this research are to investigate the relationship between the *Gluconacetobacter* spp.isolatedfrom sugarcane and rice roots and examine their abilities to colonize rice seeds *in vitro* in addition, study their antagonistic activity against the pathogenic fungi and bacteria.

Materials and Methods

Isolation of *Gluconacetobacter* spp.

The endophytic bacterial isolates used in this study were isolated from sugar cane and rice roots as the method described by **Mejia *et al.*(2008)** , using semi-solid nitrogen-free LGI medium **(Muthukumarasamy *et al.*, 2005) .** Then, purified by streaking on glucose, yeast extract, calcium carbonate (GYC) agar plates **(Sharafi *et al*., 2010).**

Identification of the obtained isolates

The differentiation and physiological properties of the obtained isolates were employed according to **Bergey's manual of systematicbacteriology (2005).**

Growth of isolates at different glucose concentrations

The nitrogen-free LGI medium supplemented with different concentrations of glucose (10 – 20 and 30%) was used **(Bergey's manual of systematicbacteriology , 2005).**

Putative endophytic features

Apreliminary qualitative analysis for cellulolytic activity was conducted by using Congo red dyeon carboxymethyl cellulose (CMC) agar mediumaccording to **Ariffin *et al*. (2006)**. The tested isolates were inoculated on the pectin agar medium for determining of pectinase activity according to **Hung and Annapurna (2004).**

Plant growth promotion abilities

The ability of isolates for indoles production was determined usingSalkowski's reagent according to the method described by **(Gilickmann and Dessaux, 1995).**Determination of gibberellins is based on the color reaction of gibberellic acid with reagent Folin-ciocalteu**(Lisitskaya and Trosheva, 2013).**

Siderophores and HCN production

The ability of bacterial isolates to produce siderophores was determined using a modification method of **Alexander and Zuberer (1991)**. The selected isolates were screened for the production of hydrogen cyanide (HCN) by adapting the method of **Ghodsalavi *et al*. (2013).**

Root colonization assay

Sterile seeds of rice were sown in glass tubes containing semi-solid mineral medium described by **Mae and Ohira (1981)**. Association of isolated bacteria within the root was confirmed by TTC staining (2, 3, 5-triphenyl-tetrazolium chloride) as mentioned by**Yachana *et al*. (2011)**. Surface sterilized rice roots were inoculated with isolates and incubated overnight in the TTC stain and epidermal layers of section of root were taken and examined under image analyzer microscope (Carl Zeiss) to detect root colonization, the root length and root dry weight were measured.

Antagonistic activities

Inhibition of fungal growth by volatile antifungal compounds was tested according to **Montealegre *et al*. (2003).**All bacterial isolates were tested for their antagonistic activity against some pathogenic fungi namely {*Aspergillus niger*(M1), *Pythium debaryanum*(M2), *Rhizopus nigricans*(M3), *Fusarium oxysporum*(M4), *Helminthosporium sp.* (M5) *and Sclerotium rolfsii*(M6)}whichobtained from Plant Pathology Inst., Agric. Res. Center, Giza, Egyptaccording to the method described by **Hariprasad and Niranjana (2008)**.All bacterial isolates were tested for their ability to inhibit some pathogenic bacterial strains namely (*Bacillus subtilis*(E1), *Ralstonia solanacearum*(E1), *Pseudomonas sp.* (E1), *Ps. syrinage*(E1), *Ps. fluorescens*(E1), *Ps. fluorescens*(E2), *Erwinia caratovora*(E1), *E. caratovora*(E2), *E. caratovora*(E3), *E. atroseptica*(E1), *Xanthomonas sp*(E1), *X. vesicatoria*(E1), *X. vesicatoria*(E2), *X. vesicatoria*(E3) *and X. campestiris*(E1) which obtained from Plant Pathology Dept., Fac. Agric. Benha Univ., Egypt in dual Petri dish culture test as described by **Hariprasad and Niranjana (2008)**.

Protein pattern and electrophoresis analysis for identification of *Gluconacetobacter* isolates

For emphasizing identification of *Gluconacetobacter* isolates,fractionalization of bacterial protein was achieved using sodium dodecyle sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique as described by **Laemmli (1970)** at the Agricultural Genetic Engineering Lab.,Faculty of Agriculture, Benha Univ., Egypt.Protein extracts were prepared according to the method of **Miniatis *et al.* (1989).**Electrophoresis of native protein wasemployed according to **Latorre *et al.* (1995).**

Densitometer scanning

In the electrophoresis studies, Jacard index (I) of any pair densitometer tracing of protein patterns was computed by a computerized program and the resulting matrix of correlation coefficient was used for evaluating the level of similarity between any pair of isolates **(Hadacova, *et al*., 1980)**.

$$\text{Jacard index (I)} = \frac{C}{A + B - C}$$

Where:

C = No. of similar band between the two taxon to be compared.

A = No. of bands present in one taxon.

B = No. of bands present in the compared taxon.

Cluster analysis

Electrophoretic protein patterns of all *Gluconacetobacter* isolates were clustered **(Joseph *et al*., 1992)** by the average linked technique (un-weighed pair-group method). The results were expressed as phonograms. Cluster analysis was performed with a computerized program.

Results and Discussion

Biochemical characteristics and biocontrol activities of *Gluconacetobacter*spp.isolated from sugarcane and rice roots

Ten gram-negative, acid-producing, nitrogen fixers isolates were isolated from sugarcane and rice roots,seven isolates were obtained from sugarcane roots where three isolates from rice roots (Table, 1). These results were in harmony with **Madhaiyan *et al*. (2004)** who reported that *G. diazotrophicus* was an endophyte bacterium firstly isolated from sugarcane roots and with **Loganathan and Nair (2003)** who mentioned that *Gluconacetobacter* sp. as a natural colonizer of the wild rice and of salt tolerant rice varieties. The phenotypic characteristics of the isolates were determined and compared with those of the known nitrogen fixing acetic acid bacteria G. diazotrophicus.

Table 1. Biochemical characteristics of *Gluconacetobacter* spp. isolated from sugarcane and rice roots

Growth characteristics and benefits	*Gluconacetobacter* isolates									
	Sugarcane roots							Rice roots		
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
- Gram stain	G -	G -	G -	G -	G -	G -	G -	G -	G -	G -
- Acid production	+	+	+	+	+	+	+	+	+	+
- Growth on N-free LGI medium	+	+	+	+	+	+	+	+	+	+
- Production of (BWSP) on GYE medium	+	+	+	+	+	+	+	+	+	+
- Growth on glucose:										
• 10 (%)	+	+	+	+	+	+	+	+	+	+
• 20 (%)	+	+	+	+	+	+	+	+	+	+
• 30 (%)	+	+	+	+	+	-	+	+	+	-
- HCN production	-	-	+	-	-	-	+	+	+	+
- Siderophores production	+	+	+	+	+	+	+	+	+	+
- Cellulase activity (cm)	1.5	1.2	1.2	1.8	1.3	1.1	1.2	1.1	1.2	1.1
- Pectinase activity (cm)	1.1	1.1	0.8	0.9	1.0	1.1	1.1	0.8	0.9	1.0
- IAA µg/ml	11.0	10.9	11.5	8.90	10.0	11.1	10.5	10.0	9.04	9.00
- Gibberellins µg/ml	20.2	22.1	22.2	20.0	21.0	18.5	20.0	14.2	18.0	18.0

BWSP: Brown water soluble pigments

Data indicated that all isolates were able to grow on nitrogen free LGI medium. Also, all isolates produce brown water soluble pigments on GYE medium and give dark brown colonies on potato agar medium. On the other hand, data in **Table (1)** indicated the ability of all isolates to grow at different glucose concentrations 10, 20 and 30%. Data showed that all isolates were able to grow at 10 and 20 % glucose whereas, at 30% glucose there are two isolates G6 and G10 were not able to grow.

In nature there are many bacteria those produce multiple bio-control activities against plant pathogenic bacteria and fungi. The obtained bacterial isolates were tested for antagonistic activity against soil-borne plant pathogenic fungi and bacteria.In this respect, data in **Table (1)** illustrated by **Photo (1)** clearly indicated that 50% of the selected bacterial isolates (G3, G7, G8, G9 & G10) were able to produce HCN in culture medium. Whereas, 50% of the selected bacteria (G1, G2, G4, G5 & G6) gave negative results. Also, **Photo(1)** indicates that the isolate number G10 was the highest HCN producer (based on the color intensity) followed by the isolate number G8. This result was in harmony with **Phillips *et al.* (2004)**who mentioned thatHCN is generally considered as a secondary metabolite that has an ecological role and confers a selective advantage to the producer strains. Also, proved that HCN effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at picomolar concentrations, also the production of HCN by beneficial bacteria showed antibiosis against soil-borne pathogenic fungi.

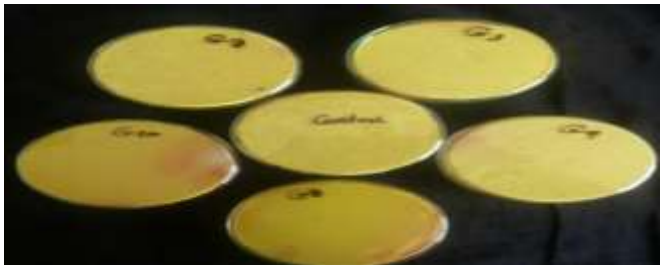

Photo 1. Hydrogen cyanide (HCN) production by *Gluconacetobacter* isolates.

Data presented in **Table (1)** revealed that all isolates were able to produce siderophores in broth culture media. These results were in accordance with the findings of **Sarode *et al.* (2007)** who reported that most of Gram-negative bacteria were able to produce siderophores on Tryptic soy agar (TSA) mediumwhich play an important role in the biocontrol of phytopathogenic microorganisms by sequestering iron, and thereby inhibiting pathogen growth or metabolic activity.

Also, data in **Table (1)**clearly indicated that all isolates have cellulase and pectinase activities but at

different ranges. Isolate G4 has the most cellulase activity with 1.8cm followed by G1 and G5 isolates with 1.5 and 1.3cm, respectively.But, G6, G8 and G10 isolates have the lowest cellulase activity with 1.1cm. On the other hand, data also indicated that pectinase activity of all isolates ranged from 0.8 to 1.1 cm. These results were compatible with **Adriano-Anayal *et al*. (2005)** who reported that the cell-wall degrading enzymes cellulases, hemicellulases and pectinases are implicated in the penetration of roots by beneficial plant microorganisms such as *Gluconacetobacter* spp.

Another important trait beneficial to the plant health is the production of plant growth promotors hormones. Data in **Table (1)** indicated that all isolates were able to produce indole acetic acid and gibberellins at different quantities. G3 isolate produced the highest amounts of IAA and $GA_3$with 11.5 and 22.2µg/ml, respectively. But, the lowest producers of IAA and GA3 were G4 and G8 isolates, respectively. These results were in harmony with **(Pedraza, 2008)** who reported that *G. diazotrophicus* has the ability to produce both auxin and gibberellins which seems to play an importantrole in *Gluconacetobacter*-plant interactions.

Abilityof *Gluconacetobacter* spp. for colonizing rice seeds *in vitro*

The colonization of plant roots by bacteria is a very important in establishment an effective plant-bacterial interaction.Data in **Figs (1&2)** indicated that rice root colonizing with *Gluconacetobacter* isolates led to increase in root length and root dry weigh compared to control. Data graphically illustrated by **Fig (1)** showed thatrice seeds colonized with G1 isolate gave the highest root length 8.0 mm followed by seeds treated with G8 isolate with 7.0 mm. Also, data indicated that rice seeds treated with G4 and G10 isolatesgave the smallest root length and equal with control. On the other hand, Data graphically illustrated by **Fig (2)** showed that rice roots dry weight was the highest when seeds treated with G3 and G8 isolates, but the lowest root dry weight was observed when seed treated with G2, G4 and G10 isolates compared with control.**Photos (2 a & b)** showed that there are differences between the cross section of rice roots treated with G1 isolate compared with control. These results were in agreement with **Rouws *et al*. (2010)** who proved that different strains of *G. diazotrophicus* were able to colonize of rice roots.

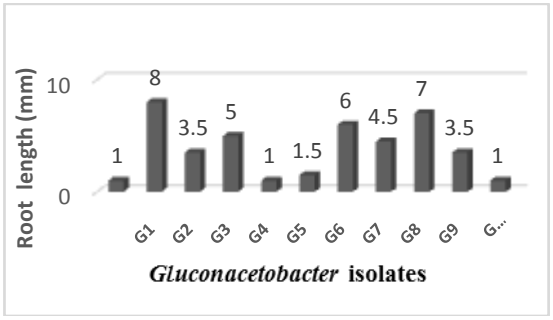

Fig 1. Rice root length (mm)

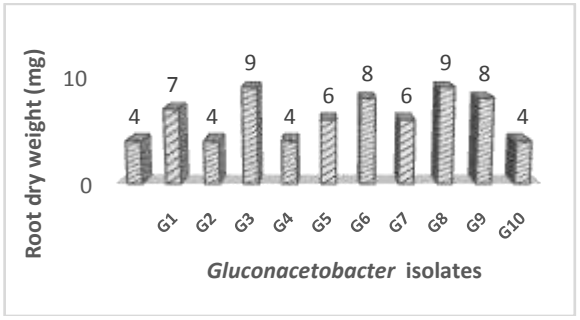

Fig 2. Rice root dry weight (mg)

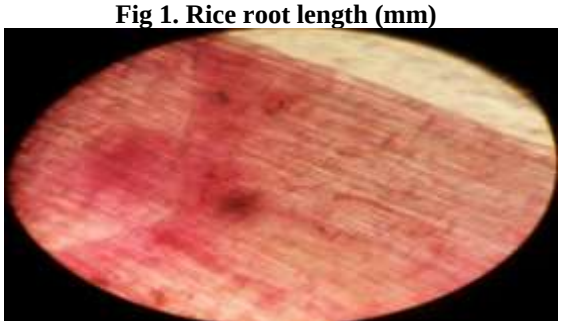

Photo 2a. Cross section of rice root without any treatments (1200x).

Photo 2b. Cross section of rice root treated with G1 isolate (1200x).

Colonization was characterized as the ability of some bacterial cells to develop into a large population attached to the root, plant roots could be observed in a red color with inoculated plants due to reduction of TTC by bacteria associated with the roots while for the un-inoculated plants, the roots of rice (control) were colorless. Moreover, bacteria could be observed as red colored cells under the microscope after TTC staining as shown in **Photos 2 (a & b)**. The presence of bacterial colonies associated with roots could be clearly visualized, as a red spot because it stains living cell (respiring) only, while the dead cell on roots remained colorless. Similar findings were reported by **Yachana *et al*. (2011)** whoconfirmed adhesion and invasion of the isolated strains with the paddy root by 2,3,5-triphenyl-tetrazolium chloride (TTC) staining.

The *Gluconacetobacter*isolates were attracted to roots by chemotactic and air tactic, then colonized the plant roots, so red color was shown.

The area around the point of emergence of lateral roots usually showed deep red color which might due to maximum colonization. Pectinase and exopolysaccharidemay play an important role in the association between thehost plant and bacteria. **Haas and Défago (2005)** clearly indicated that the success of inoculated seeds or seedlings with beneficial bacteria usually depends on the colonization potential of the introduced strains and reported that plant growth-promoting rhizobacteria competitively colonize plant roots and enhance plant growth either by direct or indirect mechanisms.

Volatile antifungal compounds produced by *Gluconacetobacter* isolates

Volatiles compounds are potentially very important to inhibit fungal growth. This experiment was carried out to examine the ability of the selected bacteria to produce antifungal volatiles that could inhibit mycelial growth of six pathogenic fungi.From the obtained data presented in **Table (2)**,all of *Gluconacetobacter* isolates showed inhibition activity by volatile compounds when tested against *A. niger*(M1), *P. debaryanum*(M2), *R. nigricans*(M3), *F. oxysporum*(M4), *Helminthosporium sp.* (M5) *and S. rolfsii*(M6).

Table 2. Fungal biomass dry weight affected with volatile compounds produced by *Gluconacetobacter* isolates**.**

Source of Isolates	Bacterial isolates no.	Fungal strains F. o.	S. r.	H. sp.	P.d.	R. n.	A. n.
		Fungal biomass dry weight (g)					
Sugar cane roots	Control	0.27	0.26	0.30	0.32	0.28	0.30
	G1	0.20	0.19	0.22	0.30	0.22	0.27
	G2	0.20	0.19	0.21	0.30	0.22	0.25
	G3	0.18	0.19	0.22	0.31	0.22	0.27
	G4	0.21	0.18	0.21	0.31	0.24	0.28
	G5	0.20	0.20	0.21	0.29	0.21	0.28
	G6	0.18	0.18	0.20	0.30	0.24	0.29
	G7	0.19	0.18	0.20	0.29	0.22	0.29
Rice roots	G8	0.19	0.20	0.20	0.29	0.21	0.30
	G9	0.19	0.21	0.20	0.29	0.20	0.28
	G10	0.18	0.18	0.20	0.28	0.22	0.29

F. o. ***Fusarium oxysporum*** ***S. r.*** ***Sclerotium rolfsii*** ***H.sp.*** ***Helminthosporium sp***
P.d. ***Pythium debaryanum*** ***R. n.*** ***Rhizopus nigricans*** ***A. n.*** ***Aspergillus niger***

Data also proved that all fungal biomass dry weight were inhibited when cultured face to face with all*Gluconacetobacter* isolates compared with control. From data presented in **Table (2),** it was clearly that when G1 isolate tested against all pathogenic fungi, the lowest inhibition percentage in fungal biomass was observed with *S. rolfsii*(M6)and*P. debaryanum*(M2. Generally, the highest inhibition percentage in fungal biomass of *F. oxysporum*(M4), *S. rolfsii*(M6), *Helminthosporium sp.* (M5) *andP. debaryanum*(M2)were recorded when cultured face to face with G10 isolate. When G9 and G2isolates were cultured face to face with all pathogenic fungi, the lowest fungal biomass of*R.nigricans*(M3)(0.20 g) and *A. niger*(M1) (0.25 g)were recorded,respectively.In this respect, **Weller and Tomashow (1993)** reported that gram-negative bacteria produce several bioactive compounds (antibiotics, siderophores, HCN and volatile compounds) giving one of the broadest spectra of potential biocontrol.

Antagonistic activity of *Gluconacetobacter* isolatesagainst some plant pathogenic fungi

From the obtained data in **Table (3),** it was clearly indicated that all *Gluconacetobacter* isolates showed inhibition activity against the tested pathogenic fungi except *R. nigricans* sinceG1, G2 and G3 isolatesdon't have any inhibition percentage against it.

The isolate G3gave the highest inhibition percentage of *F. oxysporum*, *S. rolfsii* and *P. debaryanum* at a rate of 66.7, 72.2 and 47.1%, respectively. But, G3 isolate gave the lowest inhibition percentage of *A. niger* and *Helminthosporium sp.*being of 5.0 and 27.8%, respectively. On the other hand, the lowest inhibition ratio of *F. oxysporum*, *S. rolfsii* and *P. debaryanum* was observed when treated with G8 isolate being of 22.2, 58.9 and 20.7%, respectively. These results were in agreement with **Logeshwarn *et al.* (2011)** who proved that *G. diazotrophicus* gave antagonistic activity against pathogenic fungi included *F. oxysporum* in sweet potato. **Photos (3 a, b, c & d)** indicated that *Gluconacetobacter* isolates showed high suppression for the pathogenic fungi, since the obtained results emphasized that a clear zones around *Gluconacetobacter* isolates. Such clear zones are likely to be due to the production of antifungal substances by *Gluconacetobacter* isolates.

Table 3. Inhibition ratio of fungal growth by *Gluconacetobacter* isolates**.**

Source of Isolates	Bacterial isolates no.	Fungal strains					
		F. o.	*S. r.*	*H.*	*P.d.*	*R. n.*	*A. n.*
		Inhibition ratio of fungal growth (%)					
Sugar cane roots	G1	44.4	60.0	18.8	36.8	0	46.7
	G2	38.9	66.7	22.5	40.2	0	55.6
	G3	66.7	72.2	5.00	47.1	0	27.8
	G4	50.0	70.0	17.5	47.1	16.7	47.8
	G5	50.0	62.2	5.01	47.1	23.3	51.1
	G6	42.2	62.2	11.3	41.4	22.2	40.0
	G7	38.9	71.1	8.80	39.1	16.7	37.8
Rice roots	G8	22.2	58.9	17.5	20.7	22.2	50.0
	G9	50.0	66.7	20.0	20.7	27.8	33.3
	G10	50.0	67.8	20.0	25.3	5.60	34.4

Abbreviations as described in Table (2)

Photo 3a. Inhibition of *S. rolfsii* growth by *Gluconacetobacter* isolates compared with control.

Photo 3b. Inhibition of *P. debaryanum*growth by *Gluconacetobacter* isolates compared with control.

Photo3c. Inhibition of *R. nigricans* growth by *Gluconacetobacter* isolates compared with control.

Photo 3d. Inhibition of *A. niger* growth by G5.

Siderophores and cyanogenes are the main compounds produced by most plant growth promoting rhizobacteria (PGPR)**(Somers *et al.*, 2005)**. Such substances reduced the mycelium formation and spore germination of *F. oxysporum* **(Al-Kahal *et al.*, 2003).**

Antagonistic activity of *Gluconacetobacter* isolatesagainst some plant pathogenic bacteria

Data in **Table (4)** indicated that G5 and G10 isolates gave antagonistic activity against all pathogenic bacteria except *Ps. fluorescens* (E1) and*X. campestiris* (E1). But G9 isolate gave antagonistic activity against*Ps. fluorescens*(E2)and*E. atroseptica* (E1)only.On the other hand, all *Gluconacetobacter* isolates haven't the ability to antagonize*Ps. fluorescens*(E1) and*X. campestiris*(E1).These results were in harmony with **Arencibia *et al.* (2006)** who reported that*Gluconacetobacter sp.* stimulate plant growth not only by N_2-fixation but also by phytohormones production, biocontrol of phytopathogens, mineral nutrient solubilization and disease resistance induction

Table 4. Antagonistic activity of the selected isolates against pathogenic bacteria.

Pathogenic bacterial strains	*Gluconacetobacter* isolates									
	Sugar cane roots							Rice roots		
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
***Bacillus subtilis* (E1)**	+	-	-	+	+	+	+	+	-	+
***Ralstonia solanacearum* (E1)**	-	-	+	-	+	-	-	+	-	+
***Pseudomonas sp.* (E1)**	+	-	-	+	+	+	+	+	-	+
***Pseudomonas syrinage* (E1)**	-	-	+	-	+	-	-	+	-	+
***Pseudomonas fluorescens*(E1)**	-	-	-	-	-	-	-	-	-	-
***Pseudomonas fluorescens*(E2)**	-	+	+	-	+	-	+	-	+	+
***Erwinia caratovora* (E1)**	+	+	-	-	+	+	+	+	-	+
***Erwinia caratovora* (E2)**	+	-	-	+	+	+	+	+	-	+
***Erwinia caratovora* (E3)**	+	+	-	-	+	+	+	+	-	+
***Erwinia atroseptica* (E1)**	-	+	+	-	+	-	+	-	+	+
***Xanthomonas sp* (E1)**	+	+	-	+	+	+	+	+	-	+
***Xanthomonas vesicatoria* (E1)**	+	-	-	+	+	+	+	+	-	+
***Xanthomonas vesicatoria* (E2)**	+	+	-	-	+	-	+	+	-	+
***Xanthomonas vesicatoria* (E3)**	+	+	-	-	+	-	+	+	-	+
***Xanthomonas campestiris* (E1)**	-	-	-	-	-	-	-	-	-	-

Also, **Raaijmakers *et al.* (1995)** studied the interaction between sugarcane, *Gluconacetobacter* sp. and *Xanthomonas* sp. for the first time, an elicitation of plant defense mechanism against pathogenic bacteria has been demonstrated. The disease suppressive mechanisms of PGPR include siderophores (mediated competition for iron)**.**

Protein pattern and electrophoresis analysis

SDS-PAGE technique was used for the characterization and analysis of proteins and it has been used and yielding valuable information on the similarity and dissimilarity amongst *Gluconacetobacter*isolates. In the present study, protein profiles were very similar and characteristic among the isolates of each group of microorganisms and several isolates exhibited characteristic proteins that may be useful markers for biochemical diversity. Data in **Table (5)** indicated that SDS-PAGE of total cell protein extracts of 10 tested *Gluconacetobacter* isolates produced characteristic patterns containing about 67 discrete bands with molecular weights in the range from 6.89 to 111.75 K_{Da} estimated by polyacrylamide gel electrophoresis. The patterns among all tested isolates were nearly the same; however, there were few differences observed. In the present study, 13 different total cell protein patterns were detected by SDS-PAGE **(Photo 4)**.

The first pattern (111.75 KDa) was represented by 3 isolates (No. G1, G3 and G9), the second pattern (83.60 KDa) was represented by 3 isolates (No. G5, G8 and G9), The fourth pattern (70.50 KDa) was represented by 2 isolates (No. G3 and G5), the fifth pattern(65.60 KDa) was represented by 7 isolates (No. G2, G5, G6, G7, G8, G9 and G20), the sixth fifth pattern (60.55 KDa) was represented by 5 isolates (No. G1, G4, G7, G9 and G10), The seventh pattern (55.15 KDa) was represented by 4 isolates (No. G7, G8, G9 and G10), the ninth pattern(39.15 KDa) was represented by 8 isolates (No.G1, G2, G3, G4, G6, G7, G9 and G10), the tenth pattern (31.98 KDa) was represented by 3 isolates (No. G2, G3 and G4).

Table (5: SDS-PAGE of total cell protein extracts of the tested *Gluconacetobacter* isolates

$MW._{KDa}$	*Gluconacetobacter* isolates									
	Sugar cane roots							Rice roots		
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
111.75	+	-	+	-	-	-	-	-	+	-
83.60	-	-	-	-	+	-	-	+	+	-
75.85	+	+	+	+	+	+	+	+	+	+
70.50	-	-	+	-	+	-	-	-	-	-
65.60	-	+	-	-	+	+	+	+	+	+
60.55	+	-	-	+	-	-	+	-	+	+
55.15	-	-	-	-	-	-	+	+	+	+
46.90	+	+	+	+	+	+	+	+	+	+
39.15	+	+	+	+	-	+	+	-	+	+
31.98	-	+	+	+	-	-	-	-	-	-
23.72	+	+	+	+	+	+	+	+	+	+
17.47	-	-	-	-	-	-	-	-	+	-
6.89	-	-	-	-	-	-	-	-	+	-

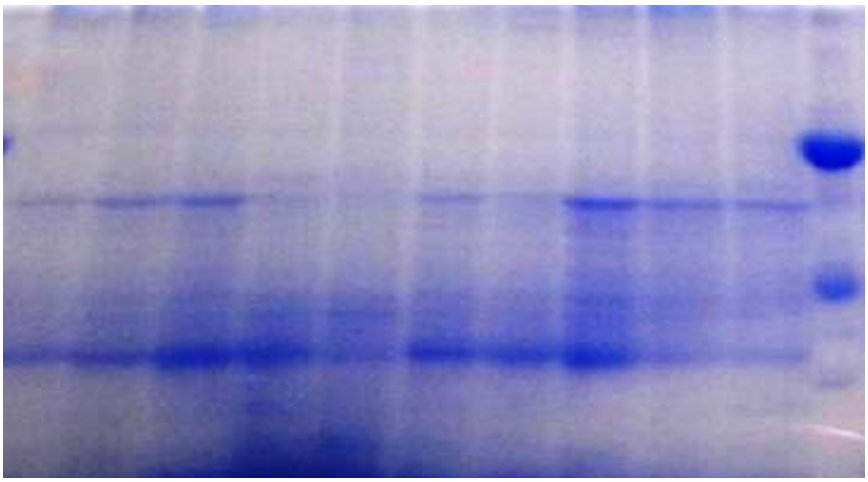

Photo 4. SDS-PAGE of total cell protein extracts of 10 tested *Gluconacetobacter* spp. Isolates

The third, eighth and eleventh patterns (75.85,46.90 and 23.72 KDa) produced one monomorphic band in all isolates; some isolates had some specific bands and could be used to distinguish among then; for instanceisolate(G9) has two positive specific marker at M.W. of 17.47 and 6.89 K_{Da}.With*Gluconacetobacter* isolates, these results figure out the sensitivity of SDS-PAGE as a powerful tool allowing a higher degree of taxonomic discrimination and for typing and subtyping of microorganisms even at the subspecies level.These results were in harmony with **Pedraza (2008)**who proved that some *G. diazotrophicus* strains carry plasmids of sizes varying from 50 to 110 MDa.Also, **Malik *et al.* (2003)** reported that the polyacryl-amide gel electrophoresis (PAGE) of proteins analysis has been used widely in typing of many bacterial strains, and proved that protein patterns offer considerable potential for typing bacterial strains of clinical interest, especially for species with other typing methods are not available.

Nei's similarity coefficient

Similarity indices and two consensus were developed on the basis of the scorable banding patterns of the ten *Gluconacetobacter* spp. isolates shown in **Table (6)** illustrated by **(Figure 3)**. Five most closely related isolates (G1, G2, G4, G7 and G8) with highest genetic distances (0.714)were found. On the other hand, two most closely related isolates(G5 and G10) with low genetic distances (0.200)were found.

Table 6. Similarity index matrix among the tested*Gluconacetobacter* isolates based on SDS-PAGE.

***Gluconace-tobacter* isolates**	***Gluconacetobacter* isolates**									
	Sugar cane roots							**Rice roots**		
	G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**	**G9**	**G10**
G-1	**1.0**	**0.500**	**0.625**	**0.714**	**0.333**	**0.571**	**0.625**	**0.333**	**0.544**	**0.333**
G -2		**1.0**	**0.625**	**0.714**	**0.500**	**0.833**	**0.625**	**0.500**	**0.417**	**0.500**
G -3			**1.0**	**0.625**	**0.444**	**0.500**	**0.400**	**0.300**	**0.385**	**0.300**
G -4				**1.0**	**0.333**	**0.571**	**0.625**	**0.330**	**0.417**	**0.500**
G -5					**1.0**	**0.571**	**0.444**	**0.714**	**0.417**	**0.200**
G -6						**1.0**	**0.714**	**0.571**	**0.455**	**0.375**
G -7							**1.0**	**0.625**	**0.636**	**0.625**
G -8								**1.0**	**0.544**	**0.333**
G -9									**1.0**	**0.417**
G -10										**1.0**

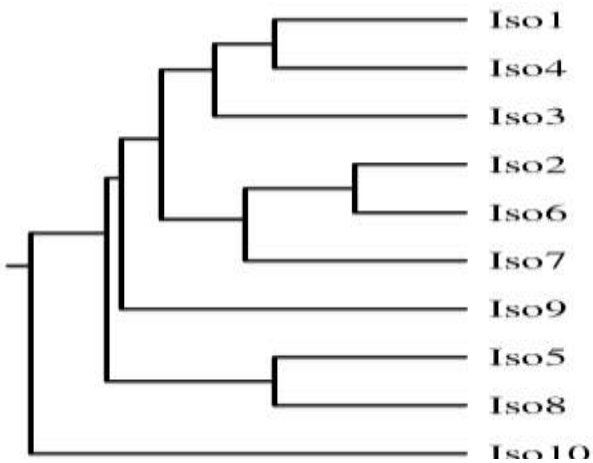

Figure 3. Similarity index matrix among ten *Gluconacetobacter* isolates based on SDS-PAGE

In this respect, **Muthukumarasamy *et al*. (2002)** and**Pedraza (2008)** reported thatthe aforementioned results confirmed that SDS-PAGE profiling is a powerful method for identification and biochemical classification which agreed with results in this paper**.**

Conclusion

In view of the obtained results, this study shows the relationship between *Gluconacetobacter* isolates from sugarcane and rice roots.Obtained *Gluconacetobacter*isolates have abilities to colonize rice roots with antagonistic activity against the phytopathogenic bacteria and fungi. On this basis, it is conceivable that endophytic bacteria capable of producing antagonistic substances and could be used as a biological control agents.

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الملخص العربى

دراسة النشاط التضادى لبعض عزلات *Gluconacetobacter* وقدرتها على إستعمار جذور الأرز فى المعمل

إيمان عثمان حسن[1] – رشا محمد الميهى[2]

1 –قسم أمراض النبات – كلية الزراعة – جامعة بنها – مصر.

2 –قسم النبات الزراعى – فرع الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة بنها – مصر.

eman.hassan@fagr.bu.edu.eg

rashaelmehy@fagr.bu.edu.eg

تشتمل عائلة Acetobacteriaceae على ثلاثة أنواع مثبتة للنيتروجين وتابعة للجنس *Gluconacetobacter* وهى *G. diazotrophicus, G. johannae, G. azotocaptans*. في هذه الدراسة تم عزل بكتريا مثبتة للنيتروجين من جذور نباتات قصب السكر والأرز المزروعة في محافظتى أسوان والقليوبية، على التوالي. أعطت سبع عزلات من جذور قصب السكر وثلاث عزلات من جذور الأرز الخصائص المظهرية لجنس *Gluconacetobacter*. تم إختبار هذه العزلات لقياس قدرتها على إنتاج بعض المواد المنشطة لنمو النبات مثل إندول حمض الخليك (IAA) وحمض الجبريليك (GA_3) بالإضافة إلى إختبار قدرتها على انتاج بعض الانزيمات التى لها علاقة بالقدرة على استعمار جذور النبات مثل السليوليز والبكتينيز. بعد ذلك تم عمل تجربة لقياس قدرة عزلات *Gluconacetobacter* على إستعمار حبوب الارز داخل المختبر. تم إجراء تجربة لقياس النشاط التضادى لعزلات *Gluconacetobacter* ضد بعض الفطريات والبكتيريا الممرضة للنبات، بالإضافة إلى قدرتها على إنتاج سيانيد الهيدروجين ومركبات السيدروفورس. فى نهاية البحث تم استخدام طريقة الصوديوم بولي أكريلاميد الكهربائي (SDS–PAGE) وتحليل البروتينات لتعريف العزلات البكتيرية التى تم الحصول عليها وقياس درجة التشابه والقرابة فيما بينها.