## RHIZOBACTERIA PRODUCING GROWTH PROMOTING SUBSTANCES AS A BIO-CONTROL AGENT AGAINST R. SOLANI AND F. SOLANI CAUSING ROOT ROT DISEASE OF TOMATO SEEDLINGS

[4]

Ebtsam, M. Morsy<sup>1</sup>; Sh.M. Selim<sup>2</sup>; S.A. El-Sayed<sup>1</sup> and M.M. Zaki<sup>2</sup>

#### **ABSTRACT**

A number of 261 bacterial isolates were obtained from different plants rhizosphere. Majority of these isolates showed qualitative abilities to produce indole acetic acid (IAA), siderophores and hydrogen cyanide. Only 31 isolates (11.9%) showed *in-vitro* antagonistic activities against R solani or F. solani the causative agents of root rot disease of tomato seedlings. Efficient isolates against the two pathogens R. solani and F. solani in-vitro were further tested for their abilities as biocontrol agents in a pot experiment. Pre and post emergence damping off, survival rate, shoot and root dry weight of tomato seedlings were determined. Tested isolates varied greatly in their in-vivo antagonistic activities against R. solani or F. solani as indexed by survival rate and also effects on shoot and root dry weights. These isolates were producers of IAA and gibberellins in-vitro and the majority of them produced siderophore and hydrogen cyanide. Most efficient four isolates against R. solani or F. solani and producing reasonable quantities of growth promoting substances as well as sidereophores and cyanide were identified as strains of Bacillus subtilis (3 isolates) and Pseudomonas synxantha (one isolate).

Key words: Plant growth promoting rhizobacteria, Biocontrol agent, Root rot fungi, Auxines, Indole acetic acid, Gibberellins

#### INTRODUCTION

The pollution of environment with pesticides to control plant diseases is a serious problem. To overcome this problem different biocontrol strategies have been applied. These strategies include

organic acid amendments (Ibrahim, 1990), cross protection (Husain et al 1986), crop rotation (Francle et al 1988), plant extracts (Ouf et al 1991).

Soil microorganisms that colonize roots and promote plant growth represent a subset of rhizosphere bacteria called

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<sup>1-</sup> Microbiology Department, Soils Water and Environment Res. Inst., Giza, Egypt.

<sup>2-</sup> Microbiology Department, Faculty of Agriculture, Ain Shams University, Shobra El-Khema, Cairo, Egypt.

plant growth promoting rhizobacteria or PGPR. Plant growth promoting rhizobacteria PGPR can produce direct or indirect effects on host plants. Indirect effects are those related to the production of metabolites, such as antibiotics, siderophores, or HCN. These metabolites increase plant growth by decreasing the activities of pathogens or deleterious microorganisms (biological control of soil-borne pathogens), Kloepper et al (1991) and Dubeikovsky et al (1993). Direct effects on plant growth include production of plant growth regulators (auxin, IAA, GA and cytokinin) that directly promote plant growth, facilitating nutrient uptake by plant promotion of legume nodulation by N2- fixing bacteria, and enhancement of seedling emergence rates (Kloepper et al 1991; Dubeikovsky et al 1993; Amara, 1996; El-Khawas et al 1996; Sedik, 1998 and Garacia de Salamone et al 2001).

Several bacteria of the genera Azoto-bacter, Azospirillum, Bacillus, Entero-bacter, Klebsiella, Sarcina and Pseudo-monas isolated from the rhizosphere of various crops had been reported to produce growth promoting substances (Forlani et al 1995; El-Khawas, 1995 and Amara, 1996).

The present work aims to isolate effective plant growth promoting rhizobacteria as a biocontrol agent against Fuisarium solani and Rhizoctonia solani, infecting tomato seedlings.

#### MATERIAL AND METHODS

#### Source of isolates

Samples from rhizosphere of Tomato, Maize, Clover, Bean, Cowpea, Lupine, Cucumber and Banana plants were collected from different locations, i.e., Giza, Ismallia, Minofia and South of El-Tahrir. A number of two hundred and sixty one bacterial isolates were obtained. These isolates were classified according to their morphological characteristics.

#### Pathogenic fungi

Two strains of fungi; Fusarium solani and Rhizoctonia solai, were used. The fungal strains were kindly obtained from Plant Pathology Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

#### Media used

King's medium (King et al 1954), Hino and Wilson (1958) medium, Abd-El-Malak and Ishac (1968) medium, Dōbereiner and Day (1976) medium were used for isolation of rhizobacteria. King's medium and Tryptic Soy Broth "TSB" (Difco, 1984) medium were used for the preparation of bacterial inocula. Potato glucose agar medium (Waksman and Lechevalier, 1961) was used for invitro testing the antagonism between bacteria and pathogenic fungi.

## In-vitro screening for plant growth promoting rhizobacteria

All bacterial isolates were tested for their qualitative capabilities to produce indole acetic acid according to the modified method described by Bric et al (1991), siderophore according to the method described by Alexander and Zuberer (1991) and hydrogen cyanide according to the method proposed by Bakker and Schippers (1987).

In-vitro antagonistic activities of bacterial isolates against Rhizoctonia solani and Fusarium solani

Isolates were also tested for their antagonistic activity against the two pathogenic fungi i.e., *Rhizoctonia solani* and *Fusarium solani* as described by Silo-Suh *et al* (1994).

## Preparation of pathogenic fungi in-

Glass bottles of 1000 ml capacity containing 95 gm clean moistened sand and 5 g corn meal were autoclaved for 30 minutes at 121 °C, then were inoculated with the tested fungus. Inoculated bottles were incubated at 28-30 °C for 10-days.

## Preparation of plant growth promoting isolates inocula

Conical flasks (250 ml) containing 100 ml of triptic soy broth or King's broth medium sterilized at 121°C for 15 minute. The flasks were inoculated with loop-full of the tested isolates, then incubated at 28-30 °C on rotary shaker (150 rpp) for 2 days.

### Pot experiment

Bacterial isolates producing growth promoting substances and simultaneously antagonizing pathogenic fungi were selected. In a pot experiment under green house conditions, these isolates were evaluated for their efficiencies to promote tomato growth and control root rot disease caused by Fusarium solani and Rhizoctonia solani.

Plastic pots (15 cm. diameter) containing 500 g of sandy-loam soil were used Soil was infested with Fusarium solani or Rhizoctonia solani grown on corn meal sand medium at the rate of 5g/kg soil before sowing. The infested pots were irrigated and left for 5 days before transplanting. Ten tomato seeds (Casel rock variety) free of fungicides were sown in each pot. Each pot was inoculated with 10 ml of bacterial inocula (8 x 10<sup>6</sup> cells/ml). Soluble NPK (1:1:1) mineral fertilizer was added with irrigation water weekly. Pots were maintained under green-house conditions at 28 °C for 30- days and irrigated every 3 days with tap water.

Pre and post emergence damping off were recorded, together with survival ratio after 28 days of planting. Dry weight of tomato shoots and roots were determined.

Disease assessment for incidence of pre and post- emergence damping -off of seedlings after 15 and 30 days of sowing were determined. Survival rate of seedling were also determined after 30 days of sowing as described by Phillips and Hayman (1970) as follows:

Pre emergence damping off (%) =

No. of non emerged
No. of sown seeds

Post emergence damping off (%) =

No. of infected plants

Total plants

Survival plants (%) =

No. of healthy survival plants

Total plants

# Quantitative determinations of plant growth promoting substances

Isolates producing growth promoting substances as well as antagonizing the two root rot fungi were further tested for their quantitative capabilities to produce indoles and gibberellins. Indoles were determined according to Salkowski colorimetric technique, (Glickmann and Dessoux, 1995). Total gibberellins were determined according to the method of Udagwa and Kinoshita (1961).

#### Identification of bacterial isolates

Most active isolates in production of IAA and GA and antagonizing R. solani

and/or F. solani were selected and identified by Bio-log Technique at Plant Pathology Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

#### **RESULTS AND DISCUSSION**

### Isolation of rhizobacteria

A number of 261 bacterial isolates were obtained from rhizosphere of different crops grown in different localities in Egypt. These included 198 bacilli and 63 cocci isolates (Table, 1).

Table 1. Rhizobacteria isolated from different plants

Localities	Source of isolates	No. isolates	Bacilli	Cocci	
Ismallia	Maize	44	34	10	
"	Cucumber	20	18	2	
,,	Lupine	24	9	15	
,,	Cowpea	32	22	10	
Giza	Tomato	33	31	2	
S. El-Tahrier	Banana	21	16	5	
Minofia	Zea maize	20	15	5	
,,	Bean	20	16	4	
,,	Clover	26	18	8	
27	Tomato	21	19	2	
Total		261	198	63	

Rizobacteria producing growth promoting substances and antagonizing root rot fungi

All isolates were *in-vitro* tested for the production of growth promoting substances and abilities to antagonize *R. solani* and *F. solani* pathogens (Tables 2, 3).

#### a- Bacilli isolates

Bacilli isolates were found to include 68 G<sup>+</sup> and 130 G<sup>-</sup> isolates making a total of 198 isolates. Of the G<sup>+</sup> isolates, 48.5% produced indole acetic acid, 51.5% were able to produce siderophoric compounds and finally 72.1% were cyanide producers (Table, 2). As for G<sup>-</sup> bacilli isolates, 55.4%, 66.2% and 71.5% of these isolates showed abilities to produce indole acetic acid, siderophores and cyanide, respectively (Table, 2).

A relatively small percentages of isolates showed in-vitro qualitative inhibiting effects against R. solani being 23.5 and 7.7% of G<sup>+</sup> and G<sup>-</sup> bacilli isolates respectively. Hartman et al (1983); Kapulink et al (1985); Weller (1988) and Young et al (1991) found that Azospirillum succeeded to produce plant growth promoting substances such as auxins, cytokinins and significantly inhibited the Fusarium sp. growth. Smaller percentages of isolates antagonized F. solani, being 2.9% for G<sup>+</sup> bacilli and 0.8% for G<sup>-</sup> bacilli isolates (Table, 2). These findings agree with those of Kaparr and Kar (1989) and Sarhan et al (2001) who reported that the bacilli culture significantly inhibited the growth of Fusarium sp. and inhibited also the conidial germination of the pathogen.

#### b) Cocci isolates

Cocci isolates were found to include 48 G' and 15 G' isolates making a total of 63 isolates. Of the G isolates, 41.7% produced indole acetic acid, 45.8% were able to produce siderophoric compounds and finally 75% were cyanide producers (Table, 3). As for G<sup>+</sup> bacilli isolates, 73.3%, 53.3% and 93.3% of these isolates showed abilities to produce indole acetic acid, siderophores and cyanide respectively (Table, 3). These results are in accordance with the findings of Mahmoud et al (1984) who investigated the ability of different isolates belonging to genera Azotobacter and Bacillus isolated from the rhizosphere of common bean and tomato plants to synthesize plant growth substances. These organisms secreted indoles and gibberellins having different R<sub>F</sub> values. The elutes of different spots of indoles and gibberellins showed stimulatory effect on the length and dry weight of excised barley roots.

As for the antagonistic activities, cocci isolates were generally less active against tested pathogens as compared with bacilli isolates (Tables, 2 and 3).

Only 6.7% of G<sup>+</sup> cocci and 2.1% of G<sup>-</sup> cocci isolates antagonized R. solani. Non of the cocci isolates were able to antagonize F. solani pathogens in-vitro (Table 3). In such direction, Saleh and Ahmed (1988) and Ishac et al (1992) found that inoculation with mixture of Azotobacter and Azospirillum significantly decreased infection of soybean plants with Fusarium solani.

# Selection of most efficient isolates antagonizing R. solani and F. solani

It is clear from the data presented in Tables (2) and (3) that out of the 261

Table 2. Rhizospheric G' and G' bacilli producing Indole acetic acid, siderophors, cyanide and antagonizing R. solani, F. solani

Source	No.		G <sup>+</sup> bacilli				No.		G bacilli			
of	isolates	IAA	Sidero-	Cyanide	Antago	nistic to	isolates	IAA	Sidero-	Cyanide	Antage	nistic to
isolates			phors		R. solani	F. solani			phors		R. solani	F. solani
Maize	17	5	5	14	2	1	32	16	24	24	2	0
Cucumber	3	3	3	2	2	0	15	8	7	11	0	0
Lupine	2	0	2	0	0	0	7	5	2	7	0	0
Cowpea .	. 10	5	3	9	0	0	12	8	7	9	0	0
Bean	4	1	1	3	0	0	12	6	10	10	0	0
Clover	4	2	2	1	0	1	14	9	12	9	1	1
Banana	5	2	2	5	1	0	11	5	6	8	0	0
Tomato	23	15	17	15	11	0	27	15	18	15	7	0
Total	68	33	35	49	16	2	130	72	86	93	10	1
% of total		48.5	51.5	72.1	23.5	2.9		55.4	66.2	71.5	7.7	0.8

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Table 3. Rhizospheric G<sup>+</sup> and G<sup>-</sup> cocci producing IAA, siderophors, cyanide and antagonizing R .solani, F. solani

Source	No.	No. G+ cocci				No.	G. cocci					
of	isolates	IAA	Sidero-	Cyanide	Antago	nistic to	isolates	IAA	Sidero-	Cyanide	Antage	onistic to
isolates	ļ		phors		R. solani	F. solani	1		phors		R. solani	F. solani
Maize	3	2	1	3	0	0	12	4	6	8	0	0
Cucumber	1	0	1	1	0	0	1	1	l	0	0	0
Lupine	] 3	1	2	3	0	0	12	5	10	10	0	0
Cowpea	2	2	1	2	0	0	8	3	3	6	0	0
Bean	0	0	0	0	0	0	4	2	0	2	0	0
Clover	] 1	ĵ	1	0	0	0	7	3	1	6	0	0
Banana	3	3	1	3	0	0	2	0	1	2	0	0
Tomato	2	2	1	2	1	0	2	2	1	2	1	0
Total	15	11	8	14	1	0	48	20	23	36	]	0
% of total		73.3	53.3	93.3	6.7	0.0		41.7	45.8	75.0	2.1	0.0

Rhizobacteria as a biocontrol

rhizosphore isolates only 31 isolates had the abilities to *in-vitro* antagonize *R. solani* and/or *F. solani*. Of these, 26 bacilli and 2 cocci isolates antagonized *R. solani*, 3 bacilli isolates inhibited *F. solani*. In a pot experiment, *in-vivo* efficiency of selected 31 isolates to antagonize *R. solani* and/or *F. solani* which cause root rot disease of tomato seedlings were evaluated.

The survival rate of the seedlings in the untreated control was 95%. Infection with *R. solani* or *F. solani* severely reduced the survival rate to 25% and 30%, respectively (Tables 4, 5). Such inhibitory effect was reflected on deleterious decrease of both shoot and root dry weights being 0.12-0.13 and 0.01-0.02 g/plant, respectively as compared with 0.3 and 0.06 g/plant in the control.

## a) Bacterial isolates antagonizing R. solani

Tested isolates varied greatly in their antagonistic activities against R. solani (Table 4). Only GZT95 and GZT104 isolates showed relatively high in vivo antagonizing activity against R. solani pathogen where survival rate reached 76.7 and 73.3% respectively as compared with 25% noted for R. solani infected control. Shoot and root weight in these two treatments were relatively higher than the untreated control being 0.50-0.53 and 0.08-0.09 g/plant respectively. These results suggest highest activity of these isolates in the producing of growth promoting substances enhancing tomato seedlings growth. This finding was almost near to that obtained by Asaka and Shoda (1996) who pointed that B. subtilis suppressed damping off tomato, seedlings caused by R. solani, in addition to increase plant shoot weight. Meanwhile, *Pseudomonas fluorcences* produced plant growth regulators with highest levels for plant growth promoting substances when this bacteria inhibited *F. oxysporum* and *R. solani* in tomato plants (Amara et al 1996).

On the contrary, the majority of isolates (22 isolates representing 71%) showed moderately inhibitory effect against the pathogen where survival rate ranged between 50 to 70%. In addition, seven isolates showed weak antagonizing effect against *R. solani*, since survival rate did not exceed 50%.

Bacterial isolates showing moderately and weak antagonize activity on survival rates varied in their effects on shoot and root dry weight, which ranged between 0.13-0.47g/plant and 0.02-0.08 g/plant respectively. Such variation might be due to differences in the efficiencies of these isolates in production of growth promoting substances. These findings are in line with those of Gupta et al (1995) and Kamal (1997) who stated that biocontrol agents decreased root-rot severity and increased plant survival.

## b) Bacterial isolates antagonizing F. solani

Out of the 31 selected isolates, six isolates namely ICu47, MZ82, GZT101, GZT97, GZT110 and MT124 showed the higher antagonistic activity against *F. solani* as indexed by survival rate which ranged between 80-90% (Table 5). These isolates also enhanced shoot and root dry weight being obviously higher than in *F. solani* inoculated treatment, ranging between 0.15-0.49 and 0.02-0.10 g/plant, respectively.

Table 4. In-vivo screening for selected bacterial isolates antagonizing R. solani

Treatments	Pre	Past	Survival	Shoot	Root
	emergence da	mping off (%)	%	dry weig	ht g/plant
Contol (1)	5.0	0.0	95.0	0.30	0.06
Control (2)	55.0	20.0	25.0	0.12	0.02
solates+ R. solani	St	arvival rate over	70% *		
GZT95	20.0	3.3	76.7	0.53	0.09
GZT104	26.7	0.0	73.3	0.50	0.08
	Su	irvival rate betw	een 50 to 70 %	*	
17.8	26.7	3.3	70.0	0.47	0.07
GZT105	20.0	13,3	66.7	0.25	0.03
STBa149	30.0	3.3	66.7	0.20	0.02
GZT101	35.0	0.0	65.0	0.41	0.06
MT115	35.0	0.0	65.0	0.44	0.06
ICu47	33.3	3.3	63.4	0.35	0.04
GZT108	30.0	6.7	63.3	0.30	0.07
GZT63	36.7	3.3	60.0	0.37	0.04
MCL84	30.0	10.0	60.0	0.19	0.08
GZT97	35.0	5.0	60.0	0.13	0.02
GZT98	30.0	10.0	60.0	0.41	0.02
MT113	33.3	6.7	60.0	0.34	0.03
MT122	37.3	3.3	59.4	0.32	0.04
MZ82	36.7	6.6	56.7	0.41	0.06
GZT94	36.7	6.6	56.7	0.36	0.02
MT123	30.0	13.3	56.7	0.28	0.05
MZ234	40.0	3.3	56.7	0.31	0.05
GZT106	45.0	0.0	55.0	0.26	0.03
MCL83	40.0	6.7	53.3	0.34	0.07
GZT93	36.7	10.0	53.3	0.22	0.03
MT124	50.0	0.0	50.0	0.26	0.03
MT127	40.0	10.0	50.0	0.32	0.06
	St	urvival rate less	than 50% *		
ICu55	40.0	10.6	49.4	0.41	0.04
<b>IZ</b> 9	50.0	3.3	46.7	0.45	0.04
GZT109	50.0	3.3	46.7	0.41	0.06
GZT103	50.0	6.7	43.3	0.40	0.05
GZT110	56.7	0.0	43.3	0.31	0.04
MT125	40.0	23.3	36.7	0.38	0.05
IZ143	53.3	13.3	33.4	0.38	0.05

<sup>(1)</sup> untreated

<sup>(2)</sup> infested with R. solani

<sup>\*</sup> Survival rate of treatments as compared with that of R. solani infested treatment (25%)

Table 5. In-vivo screening for selected bacterial isolates antagonizing F. solani

Pre	Post	Survival	Shoot	Root
emergence de	amping off (%)	%	dry weig	ht g/plant
5.0	0.0	95.0	0.30	0.06
50.0	20.0	30.0	0.13	0.01
5.0	5.0	90.0	0.46	0.10
ŧ3.3	0.0	86.7	0.49	0.09
		82.9		0.07
		80.0	0.32	0.04
		80.0	0.18	0.02
13.3	6.7	80.0	0.15	0.03
	Survival rate over	r 50% and l	ess than 80%	*
13.3	13.3	73.4	0.23	0.02
26.7	0.0	73.4	0.39	0.04
	10.0	70.0	0.21	0.03
	10.0	70.0	0.18	0.03
	3.3	66.7	0.20	0.02
3 <b>3.3</b>	3.3	63.4	0.26	0.04
30.0	6.7	63.3	0.28	0.03
26.7	10.0	633	0.36	N NK
	1.41.4	VV 14	VWV	V.VO
26.7	10.0	63.3	N 18	0.02
30.0	67			0.02
				0.02
				0.03
26.7				0.06
40.0				0.04
40.0				0.03
43.3				0.03
40.0				0.04
35.0	0.01			0.03
Si	urvival rate 50%			0.00
36.7	13,3	50.0	0.22	0.03
50.0	10.0			0.03
46.7	13.3			0.03
40.0	20.0	40.0		0.06
50.0	13.3			0.04
55.0	10.0	35.0		0.04
63.3	3.3	33.4	0.20	0.04
	5.0 5.0 5.0 13.3 10.4 13.3 13.3 13.3 13.3 26.7 20.0 20.0 30.0 33.3 30.0 26.7 30.0 36.7 30.0 36.7 30.0 40.0 40.0 43.3 40.0 35.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.0 50.	### style="background-color: blue;" style: blue;" style: blue; style:	### Story   St	### Survival rate 80% and over **  5.0

<sup>(1)</sup> untreated

<sup>(2)</sup> infested with F. solani

<sup>\*</sup> Survival rate of treatments as compared with that of  $\overline{F}$ , solani infested treatment (25%)

Other isolates inoculated treatments 25 isolates were less active in inhibiting F. solani growth. This is clear since survival rates in these latter treatments ranged between 33.4-73.4%. However, shoot and root dry weights in these treatments were relatively higher than F. solani inoculated control, probably due to the production of growth promoting substances. Ghonim, (1999) reported that Bacillus subtilis reduced the harmful effect of Fusarium oxysporum, the causative agent of tomato wilt disease. Treatment of tomato seeds with the biocontrol agent Bacillus subtilis and sown in soil infested with F. oxysporum produced less infected plants comparing with those treated with the pathogen only, and improved some growth parameters such as fresh and dry weights of shoots and roots.

# In-vitro production of indole acetic acid (IAA), gibberellins (GA), siderophores and cyanide by bacterial isolates

Activity of the 31 bacterial isolates to produce growth promoting substances, i.e., indole acetic acid (IAA) and gibberellins (GA) and substances inhibiting or suppressing plant pathogens namely siderophores and cyanide were in vitro determined (Table 6). Data reveal that all tested isolates were active producers of IAA and GA in varying amounts ranging between 39.0-108.9 mg/L and 22.6-149.3 mg/L, respectively. These results might indicate the enhancement effect of these isolates in increasing shoot and root weights over R. solani or F. solani inoculated controls. These results are similar to that found by Sadlers (1996) who reported that B. subtilis protected tomato plants against F. solani or R. solani.

In addition, the majority of isolates simultaneously produced siderophores and cvanide. This might reflect the antagonistic activity of the isolates against R. solani or F. solani. On the other direction, the minority of isolates produced only siderophores or cyanide, where only one isolate failed to produce any of these latter two substances. In this concern. Diuff et al (1993): Tuzun and Kloepper (1994) revealed that different mechanisms are involved in the suppression of soil-borne plant pathogens and deleterious microorganisms mediated by plant growth promoting rhizobacteria (PGPR) benefit plants through different mechanisms of action. These mechanisms are including the production of secondary metabolites such as antibiotics, cyanide and hormone-like substances. The production of siderophore mediated competition for iron, competition for carbon and induction of disease resistance. The PGPR Pseudomonas spp. did suppress the pathogenic strain Fusarium oxysporum by different mechanisms.

# Identification of most efficient bacterial isolates antagonizing R. solani or F. solani

As a result of the aforementioned data the most efficient four isolates namely, GZT95, GZT104, ICu47 and MZ82, exhibiting highest survival rates of tomato seedlings sown in soil infested with either R. solani or F. solani, producing reasonable amounts of IAA and GA and simultaneously secreting siderophore and cyanide were selected and identified. According to the Bio-log Technique, ICu 47, MZ82 and GZT 104 were identified as strains of Bacillus subtilis. The fourth isolate, i.e., GZT95 was identified as a strain of Pseudomonase synxantha.

Isolates	G.P.S.	G.P.S. mg/L		Cyanide	Isolates	G.P.S.	mg/L	Sidero-	Cyanide
	IAA	GA	phores			IAA	GA	phores	
IZ8	73.1	40.1	-	+	GZT101	56.0	109.3	+	+
129	75.3	116.9	-	+	GZT103	81.9	43.9	+	+
MZ82	56.3	58.2	+	+	GZT104	77.9	149.3	+	+
IZ143	54.8	50.6	+	+	GZT105	62.4	72.8	+	+
MZ234	70.5	43.9	+	-	GZT106	63.2	54.4	-	-
ICu47	80.2	79.8	<u>;</u> +	+	GZT108	58.2	80.7	÷	+
ICu55	64.4	<i>7</i> 7.9	+	-	GZT109	90.2	37.2	+	+
MCL83	75.4	41.4	+	+	GZT110	64.7	34.7	+	+
MCL84	87.6	127.1	+	-	MT113	63.6	40.1	-	+
STBa149	76.0	76.3	+	+	MT115	39.0	38.8	+	-
GZT63	91.3	<i>7</i> 7.9	+	+	MT122	78.1	37.2	+	+
GZT94	63.2	88.4	-	+	MT123	71.2	26.8	+	+
GZT95	60.1	170.3	-	+	MT124	108.9	22.6	+	+
GZT96	66.3	93.4	+	+	MT125	66.2	23.3	+	+
CZT97	63.8	117.9	•	+	MT127	97.3	69.0	+	+
CZT98	62.8	110.3	+	•					

Table 6. *In-vitro* production of indole acetic acid (IAA), gibberellins (GA), siderophores and cyanide by bacterial isolates

Further work is carried out for the biomass production of these four strains for the use as a biocontrol agent against root rot disease of tomato seedling caused by *R. solani* or *F. solani*.

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بمحلة حوليات العلوم الزراعية ، كلية الزراعة ، حامعة عين شمس ، القاهرة ، م٠٠ ، ع(١)، ٧٧-٦٣ ، ٢٠٠٥

الريز ويكتريا المنتجة للمواد المشجعة للنمو والمقاومة لفطري الريز وكتونيا سولاني والفيوز إريوم سولاني المسببان لعفن جنور شتلات الطماطم

[ 2 ]

إبتسام محمد مرسي ' - شوقي محمود سليم' - سمير على السيد ' -محبود محبد زکی ً

١- قسم المبكروبيولوج معهد بحوث الأراضي والمباه والبينة - مركز البحوث الزراعية - الجيزة - مصر ٣- قسم الميكروبيولجي - كلية الزراعة - جامعة عين شمس- شبرا الخيمة- القاهرة - مصر

ريز وسفير نباتات مختلفة. وقد وجد أن المعملية. وتدم إختبار كفاءة العزلات معظم هذه العز لات لها القدرة على انتاج البكتيرية على تثبيط نمو الفطريات الممرضة إندول حمض الخليك ومركبات السيدروفور تحت ظروف الصوبة. وقدرت نسبة النباتات وسيانيد الهيدروجين. ولقد أظهرت ٣١ عزلة المصابة قبل وبعد الأنبات، ونسبة النباتات فقط (ننسة ١١.٩ % من العدد الكلي) القدرة المتحملة للأصابة كما تم تقدير الوزن الجاف لأشطاء وجذور شتلات الطماطع.

تم عـزل ٢٦١ عزلـة بكتبريـة مـن جذور شتلات الطمياطم تحـت الظـروف على تثبيط نمو فطرى الريز وكتونيا سولاني و الفيوز اريوم سو لاني المسببان لمرض عفن

قدرتها على تثبيط نموفطرى الريزوكتونيا حرفت الاربعة عزلات الأكثر قسدرة على سولاني والفيوزاريوم سولاني بتقدير نسبة تثبيط فطرى الريزوكتونيسا والفيوزاريسوم النباتات المتحملة للأصابة وكذلك تأثيرها وكذلك المنتجة للمواد المشجعة للنمو على الوزن الجاف للأشطاء والجنور. وقــد والسيدروفـــور والسيانيد. وقــد وجــد أن أنتجت هذه العزلات اندول حمض الخليك ثلاثة عزلات منها هي سلالات من والجبرلين كما كان لمعظمها القدرة على Bacillus subtilis والعزلة الرابعة هي سلالة انتاج مركبات السيدروفور وسيانيد من Pseudomonas synxantha

وقد أظهرت النتائج اختلاف العزلات في الهيدروجين تحت الظروف المعملية. ولقـــد

تحكيم: أ.د السيد أحمد صالح أد ابراهيم عيسى غازى