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Antagonistic Effect of Plant Growth Promoting Rhizobacteria (PGPR) as Biocontrol of Plants Damping-Off.

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ABSTRACT

In the rhizosphere can be found that different groups of plant growth promoting rhizobacteria (PGPR). These bacteria including variety genera of nitrogen fixation and potassium silicate minerals solubilization in soils which can promote the growth of plant. In the present work, nine cotton and sugar beet rhizospheric soil samples were collected from two governorates in Egypt.Twenty three bacteria were isolated and screening for antagonism to soil borne fungi i.e.,*Fusarium oxysporum* and growth on potassium- silicate medium, indole acetic acid (IAA) , ammonia productions , cyanid hydrogen (HCN),catalase as biofertilizers and bioagents to soil borne fungi. Four bacterial isolates showed highly antagonistic effects for pathogenic fungus (*F.oxysporum*) and three of them were positive for growth on potassium-silicat medium . IAA production was shown by all the bacterial isolates. Three isolates were positive for ammonia production. Two isolates were positive for HCN production and all isolates were found to be catalase positive. These bacterial isolates as maximum antagonistic for pathogen and growth promotion for plants were identified on the basis colonies morphology on silicate medium, Gram staining , spore formation , capsule forms and biochemical tests as *Bacillus* spp. (*B.subtilis, B.amyloliquefaciens, B.weihensteephanensis* and *B.pseudomycodies*). As PGPR and bioagents are environmental friendly to increase production of crops and health. Therefore, these isolates can be utilized for biofertilizer and biological control under agroclimatic conditions of Egypt.

Keywords: PGPR, Biofertilizers, Bioagents, Biological Control

INTRODUCTION

Of the different soil-borne plant fungi, there are important pathogens that cause many diseases in economically crops such as cotton and sugar beet. *Fusarium oxysporum* repoted from a large number of hosts in the tropical regions of the world (Plaats-Niterink,1981).PGPR have been reported to enhance plant growth by avariety of mechanisms:fixation of nitrogen, solubilization of minerals such as potassium and production of Indole-3-Acetic Acid(IAA) to increase plant growth(Nelson,2004).

Also, In addition mechanisms involves the biological control of plant pathogens through production of lytic enzymes, hydrogen cyanide and catalase to improve plant health and promote growth (Khan,2006). Several soil bacteria belonging to genus Bacillus, possesthe ability to change insoluble forms into soluble form by producing organic acids(Miwa,1980 and Jiang,*et.al*.1999).

The aim of the present study was isolate identificate and screen the various plant growth promoting strains and to find out new potential antagonists against the pathogens in order to make use of these abilities for further biocontrol interventions.

MATERIALS AND METHODS

Soil sampling:

Soil samples were collected from the rhizosphere of cotton and sugar- beet seedlings at different centers at kafr-Elsheikh and Dakahlia Governorates in Egypt. Intact root system was dug out and the rhizospheric soil samples were carefully taken in plastic bags and stored at 4^0C. Nine soil samples were collected for the isolation of rhizosphere bacterial isolates.

Potassium- silicate mineral:

The siliceous mineral used was orthoclase. The orthoclase was kindly supplied by the Egyptian Geological Museum,Cairo, Egypt. Mineral was dried ground finelly in a ball mill to pass a 1mm screen.

Their chemical analysis as regards(Jackson 1973) to potassium and silicon forms in Table(1).

Table 1. Potassium and silicon contents of orthoclase as potassium-silicate mineral(ppm)

Silicate mineral	Potassium(ppm)			Silicon(ppm)	
	Soluble	Exchangeable	Non exchangable	Soluble	Amorphus
Orthoclase	195	317	270	6.0	65

Isolation ,purifaction and maintainance of bacteria:

Ten grams of soil were suspended in 90 ml of sterile tap water and by serially dilution method were isolated and purified twenty three isolates which maintained for further use(Johnson and Curt 1972).

Fungal isolate:

The isolate of *F.oxysporum* Schlech.was obtained from Plant Pathology Research Institute, A.R.C. Giza, Egypt.

Antagonism:

In vitro tests for antagonism of bacterial isolates towords damping-off fungus, *F.oxysporum* were screened using plate assays and observations the inhibition of the fungal growth Sivamani and Gnanamanickan 1988. *In vitro* antagonism,four bacterial isolates showing maximum inhibition of pathogenic fungal growth were chosen.

Selecetion of potassium silicate bacteria from antagonistic bacteria:

The four strongly antagonistic culture were chosen to be tested for spread on (Aleksandrov and Ternovs,ka 1961) agar plate containing orthoclase as potassium silicate mineral. Plates were incubated at 28-300C for 48h. Colonies showed tear shape were considered as k-solubilizer from silicate mineral. The k-solubilizer were purified by repeated streaking and stoked for further use.

Maintainance of isolates:

All the bacterial isolates were maintained at 4^0C in equal volumes of nutrient broth and 3% glycerol.

Identification of antagonistic and potassium-silicate bacteria:

Four antagonistic cultures are usually regarded as a fairly homogenous group of silicate organisms characterized by uniform morphplogical features after 24h. of incubation on nutrient agar.

For comparison both typical repurified strains were used in morphological, biochemical and physiological tests according to the manufacturer,s instructions. Identification was based on the similarity index the biology Microbial System (Biolog,MIRCN, Egypt),and by criteria of Bergy,s Manual of systematic bacteriology(2005).

Determination of plant promotion activities and antagonistic compounds:

Determination of IAA:

Estemation of IAA was done using colorimetric assay according to Loper and Schroth 1986.

Ammonia production:

The bacterial isolates were tested according to Cappuccino and Sherman 1992. The colour was changed when Nessler reagent added.

HCN production:

Testing of bacterial isolates described by Castric1975 for HCN production. Observation of colour brown from light to dark indicated HCN production.

Catalase production:

When added drops of 3% H_2O_2 to old culture of bacteria which used and mixed. If effervescence present indicated that catalase produced.

RESULTS AND DISCUSSION

Twenty three bacterial isolates from the total of nine rhizospheric soil samples cotton and sugar beet crops. All the isolates were designated as shown in Table (2).

Table 2. Description of the bacterial isolates

Soil sample number	Location of sugar beet(S) and cotton(C) Governorate	Center	No. of isolates	Isolate codes
Sample 1	KafrElsheikh	KafrElsheikh	Three	SA1,SA2,SA3
Sample 2	KafrElsheikh	KafrElsheikh	One	CA1
Sample 3	KafrElsheikh	KafrElsheikh	One	CB1
Sample 4	KafrElsheikh	KafrElsheikh	Two	SB1,CC2
Sample 5	Dakahlia	Belqas	Two	SC1,SC2
Sample 6	Dakahlia	Belqas	Two	SD1,CD2
Sample 7	Dakahlia	Mansoura	Three	SE1,SE2,SE3
Sample 8	Dakahlia	Mansoura	Four	CE1,CD2,CD3,CD4
Sample 9	Dakahlia	Mansoura	Five	SF1,SF2,SF3,SF4,SF5

In this study (Table3) showed all the bacterial isolates exhibited varing degree of antagonistic effect againist pathogenic fungus. Only four bacterial isolats showed highest antifungal activity againist fungus cultivated in PDA medium, it may be due to the production and secretion of antifungal compounds that was able to reduce the growth of fungus. This result agrees with Adebayo and Ekpo(2005),because found that *B.subtilis* inhibited fungal growth and also promoted the growth of tomato plant in screen house trial. *Bacillus* spp. are known to reduce fungi-damping off (Ashour and Afify 2016).

Furthermore, our study also showed that a total of four antagonistic bacterial isolates were screened for growth on Alekasandrov agar, of which three isolates showed the highest of growth (Table4& Photo 1).The results in this study are agreement with Glick, 1995 and Lalande,*et.al*.,1989 reported that there are many mechanisms by which PGPR such as activation of mineral nutrient uptake and solubilization.

Four antagonistic bacterial isolates and three of them as k-soluble were characterized for PGPRs activities when all isolates tested for production of plant growth promoting hormone i.e. IAA all isolates positive while,only three isolates ammonia production and two as antagonistic isolates when produce HCN. All isolates showen catalase activity Table (5).

Table 3. Bacterial isolates showing antagonistic effect of *F.oxysporum*.

Isolate codes	Degree of antagonism on fungal vegetative growth *F.oxysporum*
SA1	+++
SA2	+
SA3	+
CA1	-
CB1	-
SB1	-
CC2	+++
SC1	-
SC2	++
SD1	++
SD2	+
SE1	+
SE2	+
SE3	+++
CE1	-
CD2	-
CD3	+
CD4	++
SF1	-
SF2	-
SF3	++
SF4	+
SF5	+++
Control(only fungus)	-

No inhibition: - (Fungal growth was similar to that of control)
Weak inhibition :+(Fungal growth was slightly inhibited by bacteria)
Moderateinhibition :++(Loosely arranged mycelial growth over the bacterial zone)
Strong inhibition :+++(Fungal growth was completely inhibited before the bacterial zone)

Table 4. Photo1.Bacterial isolates showing highly growth on silicate medium (Alekasandrov and Ternovs,ka 1961).

Isolate codes	Degree of growth
SA1	+
CC2	-
SE3	+
SF5	+

(+) = high growth ;(-)=no growth

Table 5. Bacterial isolates showing different plant promotion activities and antagonistic properties.

Isolate codes	K-solubilization	Production of				Catalase activity
		IAA mg/L 1mg	3mg Tryptophan	Ammonia	HCN	
SA1	+	1.60	1.92	+	+	+
CC2	-	0.08	1.05	+	-	+
SE3	+	2.51	3.28	+	+	+
SF5	+	0.24	1.92	-	-	+

Our results are agreement with Brimecombe, *et.al*., (2001) who found that ammonia and hydrogen cyanide are produced by rhizobacteria and play an important role in biocontrol.

These bacterial isolates were identified by a combination of standard tests used to classify and the Biolog Microbial Identification system. The accuracy of each method is limited by the diversity and accurate identification of the bacterial species in each reference database (Table 6&7).

Table 6. Morphological characterization of bacterial isolates.

Isolate codes	Cell shape	Gram stain	Motility	Spore forming	Capsule form	Colony charactaristics on silicate agar medium
SA1	Rod	+	+	+	-	
CC2	Rod	+	+	+	-	Tear shape,mucous,round,lessgrowththan
SE3	Rod	+	+	+	-	on nutrient agar
SF5	Rod	+	-	+	-	

Table 7. Biochemical characterization of bacterial isolates.

Isolate codes	Enzyme production						Sugar fermentation				I	MR	VP	C
	Cat.	Amy.	Cas.	Gela.	Cellu.	Lip.	Glu.	Ma- nn.	Su.	Fra.				
SA1	+	+	+	+	-	-	+	+	+	+	+	+	+	+
CC2	+	+	+	+	-	-	+	-	+	+	-	+	+	+
SE3	+	+	+	+	-	-	+	-	+	+	+	+	+	+
SF5	+	-	+	-	-	-	+	+	+	+	+	+	+	+

Show all isolates were endospore forming cells rods shape, gram positive and fermentated some sugar and colonies like tear shape, mucous and round when growth on potassium silicate medium. The genera and species identified are listed in Table 8.

Table 8. Scientific name of bacterial isolates.

Isolate codes	Scientific name
SA1	*Bacillus subtilis*
CC2	*Bacillus amyloliquefaciens*
SE3	*Bacillus weihensteephanensis*
SF5	*Bacillus pseudomycodies*

The result were agreement with Caroline ,*et.al*.,(2013) and Sharma,*et.al*.,(2013)who found that all the *Bacillus* spp. suppressed the myecelial growth of *F.solani. B.amyloliquefaciens* possessed multiple plant growth promoting trails which included production of IAA, solubilization of zinc, production of HCN. *Bacillus* spp. or and their by- products are applied to plants, the outcome is disease control (Gardener 2004).

Finally,our results shows that *Bacillus* spp. are very important and effective as biocontrol agents. Their effectiveness is also observed in their ability to promote growth in plants. Study is continuing to be able to formulate them into microbial agents that will be health and environmentally friendly (Caroline,*et.al*.,2013).

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تأثير التضاد لبكتريا التربه المشجعه لنمو النبات كمقاومه حيويه لمسببات موت البادرات في النباتات.

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يحتوي الريزوسفير علي مجموعات مختلفه من بكتريا التربه المشجعه لنمو النباتات ،هذه البكتريا تضم أجناس متنوعه من مثبتات النيتروجين وكذلك البكتريا الميسره للبوتاسيوم من معادن الطين السليكاتيه . في هذه الدراسه تم جمع تسعه عينات تربه من محافظتي كفر الشيخ والدقهليه من ريزوسفير نباتات القطن و بنجر السكر و الحصول علي ثلاثه و عشرون عزله بكتيريه و قد اختبرت كفاءتها لتضاد الفطر المسبب لموت البادرات لكثير من النباتات حيث أظهرت أربعه عزلات بكتيريه أعلي قوه تضاد لفطر الفيوزاريوم اكسوسبورام وعند تنميه تلك العزلات البكتيريه الاربعه في البيئه المتخصصه لمعدن السليكات البوتاسيومي حصلنا علي ثلاثه عزلات لها قدره علي النمو في تلك البيئه المتخصصه بالاضافه إلي انتاج بعض منشطات النمو للنباتات مثل انتاج اندول حمض الخليك ومواد تضاد للمسببات المرضية مثل الامونيا و سيانيد الهيدروجين. وبذلك عرفت هذه العزلات علي أنها عوامل مقاومه ومسمدات حيويه وعند تعريف هذه العزلات بالإختبارات القياسيه للتعريف وشكل المستعمرات علي بيئه السليكات وجد انها تنتمي إلي أنواع من جنس الباسلس حيث أنها عصويات موجبه لجرام و متجرثمه.