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Study of the Diverse Bio controlling Potentials of *Bacillus* Species for Plant Growth Promotion

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KEYWORDS

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A B S T R A C T

A total of 27 bacteria belonging to the *Bacillus spp*. were isolated from the rhizosphere and associated regions of the sunflower plant. This study was undertaken to isolate the beneficial bacteria called the plant growth promoting rhizobacteria (PGPR) from the rhizosphere of an important oleaginous plant *Helianthus annuus. L.* Among these isolates nine belonged to bulk soil, ten rhizosphere and eight were endophytic. Production of various hydrolytic enzymes protease, lipase, cellulose and amylase are some of the factors that help the plant against various pathogens. Other bio control factors such as HCN, ammonia were also studied. These isolates were then studied for their antagonistic activities against plant pathogenic fungi. The results showed that protease production was observed in 37%, lipase 52%, cellulose 48%, amylase 41% of the isolates. HCN production was detected in 11% of isolates and ammonia was observed in 93% of the isolates. These bacteria were also possessing antagonistic activity against various tested plant pathogenic fungi *Fusarium, Aspergillus, Curvularia* and *Helminthosporium* species. From among these, two *Bacillus* species were promising and were able to promote plant growth in the plate bacterization assay and even during the early stages of plant growth. Both the isolates were identified and were submitted to Genebank under the accession numbers(KF 562000 and KF981437) and showed similarity with *Bacillus* spp. These bacterialspecies could be used along with other Biofertilizer preparations as biocontrolling agents in agriculture.

Introduction

The sunflower crop has gained importance due to its short duration of maturity, containing excellent quality of oil, photo-insensitivity and wide adaptability into

different kinds of cropping pattern, high-energy hull and drought tolerance. It is a short duration crop and can be grown as inter cropping with other crops such as groundnut, pigeon pea, castor, soya bean and urd bean (*Vignamungo*). The crop has the ability to adjust to grow successfully in different agro climatic conditions. Since it is a photo-insensitive crop, it can be grown throughout the year. India is one of the largest producers of oilseed crop in the World. Oilseeds occupy an important position in the Indian agricultural economy because of being a source of high quality edible oil. Sunflower oil is one of the healthiest and popular oil in the world. It is often considered to be premium oil due to its light colour, mild flavour, low level of saturated fat and ability to withstand cooking temperature.

Its importance increases as sunflower oil is considered as heart friendly oil (Akbari et al., 2011). Besides oil, almost every part of sunflower has commercial value. It is used in manufacturing paints, resins, plastics, soap, cosmetics and many other industrial products. Consequently the sunflower area has crossed 20 million hectares producing around 30 million tons of seeds annually. Sunflower is the third important major edible oil seed crop in the world after soybean and ground nut.

Moreover sunflower has the ability to store heavy metals in the roots as it is a hyper accumulator plant. The inoculation with plant growth-promoting rhizobacteria (PGPR) which reside in the rhizosphere vicinity may facilitate plant growth and thus increase its phytoremediation efficiency.

Among the microbes that inhabit the rhizosphere, the population that exerts stimulatory effects on plant growth and health are the plant growth-promoting rhizobacteria – PGPR (Glick, 1995). The PGPR affect plant growth either directly or indirectly, indirect promotion occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic microorganisms. While direct promotion of plant growth involves either providing plants with the compound synthesized by the bacterium or facilitating the uptake of certain nutrients from the environment. General mechanisms include associative nitrogen fixation, lowering of ethylene levels, production of siderophores, production of phytohormones, induction of pathogen resistance in the plant, solubilization of nutrients, promotion of mycrrohizal functioning, decreasing pollutant toxicity etc. (Glick et al., 1999).

The table shows the plant growth promoting substances produced by various Bacilli. The production of hydrolytic enzymes is an important mechanism of the ability of the bacteria to produce hydrolytic enzymes for the entry of the bacteria into plant roots. Endoglucanase (EG) classified as cellulase enzyme can hydrolyse the internal bonds of the cellulose and pectin degrading enzymes,these enzymes could assist the beneficial microbes to enter plant roots (Asilah, Radziah and Radziah, 2009). Production of fungal cell wall degrading enzymes like proteases, lipases, chitinases also helps in fungal inhibition (Muleta, Assefa and Granhall, 2007).

PGPR also possess antagonistic activity against phytopathogenic fungi. These rhizobacteria have the ability to inhibit the soil borne pathogens. Thus it becomes possible that antibiotics produced by microorganisms in the rhizosphere can be used to control plant pathogens with variable degree (Weindling, Katznelson and Beale, 1950). Root colonization, with the idea that fast growing rhizobacteria might outcompete

fungal pathogens by competition for Carbon and Energy sources and provide a basis for biological control. Biological control may be an alternative to chemicals in the control of some pathogenic fungi or to reduce environmental pollution. Cyanide (Hydrocyanic acid)is a low molecular weight, volatile metabolite which can act as a biocide with antifungal activity. Cyanide is a chemical produced by the rhizobacteria has toxic properties. It is synthesized and excreted by hundreds of organisms including bacteria, algae, fungi, plant and insects as a means to avoid predation and competition. Cyanide producing bacterial strains and host specific rhizobacteria act as biological weed control agent.

In the present study the main aim was to study the indirect mechanisms of the plant growth promoting bacteria mainly Bacillus spp. isolated from plant's own rhizosphere to increase the crop yield and improve crop quality which will be helpful to increase seed production and indirectly the oil production.

Materials and Methods

Sample Collection

An intact plant *Helianthus annuus L.* was uprooted with a surface sterilized garden trowel and placed in a sterile plastic bag along with the adhering soil.Three samples were collected. Non rhizosphere (bulk) soil was collected from about 6-8 cm depth under the root surface. Suspension was prepared in sterile 0.1 M MgSO4 to disperse the microorganisms (Wocomo, 2008). Root systems from the experimental plants with adhering soil were used to isolate rhizosphere microorganisms. Root segments of the plant were shaken vigorously for 3-5 min. in a test tube of sterile 0.1 M MgSO4 to dislodge the adhering soil and attendant microorganisms. These suspensions were heated to isolate the spore forming bacteria. The suspensions were plated onto specialized media for Bacillus species.Plates were then incubated at ambient temperature after which growth of bacteria allowed differentiation by morphological characteristics of colonies. All three types of samples were processed and analyzed as follows.

Isolation and Characterization of Isolates

Colony morphology was observed on various media. Cell morphology, Gram reaction and motility were determined by standard microbiological procedures.

Assaying for Antagonistic Substances

Qualitative Detection of Siderophores by Plate Assay

The chrome azurolsulfonate (CAS) assay – [universal assay – Schwyn&Neilands]was used. For the qualitative assay cultures were spot inoculated onto the blue agar and incubated at 28°C for 3-5 days The results were interpreted based on the colour change from intense blue to orange. The sizes of yellow-orange haloes around the growth indicated total Siderophore activity. The result was scored either negative or positive.

Quantitative Assay

For the quantitative estimation of Siderophores, the cultures were inoculated onto Trypticase soy broth (TSB). The tubes with the cultures were then incubated at 28°C for 24-72 h with constant shaking at 120 rpm. After incubation, the culture broths were centrifuged in a centrifuge at 10,000 rpm for 10 min. The cell free supernatants were subjected to quantitative estimation of Siderophores by CAS-shuttle assay (Payne

1994). In this assay, 0.5 ml of CAS reagent and 0.5 ml of supernatant were taken against uninoculated broth as reference. Spectrometric readings were taken and percent decolorisation was calculated by the following formula (Rane et al. 2005).

% decolorization = Ar-As/Ar x 100 where, Ar = absorbance of reference at 264 nm (CAS reagent) and As = absorbance of sample at 264 nm.

Determination of Protease

Protease production was determined using skimmed milk agar. Bacterial cells were spot inoculated and incubated for 2 days at 28°C. Proteolyticactivity was identified by clear zone around the colonies (Smibert and Kreig, 1994).

Determination of Cellulase

Production of cellulase was determined using carboxymethyl cellulose (CMC) agar method (Cattelan et al., 1999; Jha et al., 2009). Bacteria were spot inoculated onto M9 medium agar amended with 10 g of carboxymethyl cellulose, 1.2 g of yeast extract, congo red (0.02%) per liter of distilled water. After 8 days of incubation at 28°C, colonies surrounded by clear halos were considered positive for cellulase production

Determination of Lipase

Itwas determined on modified Sierra agar. Lipolytic activity was recorded as white precipitation around the colonies (Egamberdiyeva, 2007).

Determination of Amylase was determined as described by Smibert and Kreig (1994). The cultures were spot inoculated onto starch agar plates and hydrolysis of starch was observed by addition of iodine solution.

Assessment of Antifungal Activity

To assess the ability of the isolates to inhabit fungi, each isolate was tested against four different fungi with the circle method (Da Luz, 1990). The bacterial isolates were seeded in a 5 cm diameter circle on a PDA plate. After 24 h at room temperature, a 5 mm plug of each fungus was placed on the plates. And the plates were incubated at 28°C for 3-6 days. Fungal growth inhibition was assessed by measuring the mycelial radial growth (Cattelan, 1999).

Assay for Hydrogen Cyanide Production

Hydrogen cyanide production was assayed by the method suggested by Lorck (1948) and Castric (1977). For the production of HCN, bacteria were streaked into King's B agar plates supplemented with glycine. After this, petriplates were inverted and a piece of filter paper impregnated with 0.5% picric acid and 2% of sodium carbonate was placed on the lid.

Petri plates were sealed with parafilm and incubated at 28°C for 96 h. Discoloration of the filter paper from orange to brown after incubation was considered as microbial production of cyanide (Suresh et al. 2010).

Ammonia Production

All the bacterial isolates were tested for the production of ammonia as described by Cappuccino and Sherman (Kumar et al., 2012). Overnight grown bacterial cultures were inoculated in 10 ml peptone broth and incubated at 30±0.1°C for 48 h in Incubator Shaker. After incubation 0.5 ml of Nessler's reagent was added. The development of

faint yellow to dark brown color indicated the production of ammonia.

Seed Bacterization Experiment

Seed Germination Plate Assay

In the experiments, seeds of sunflower were treated with 20 M ethanol for 5 seconds and surface sterilized in 0.21 M NaOCl for 5 min. The seeds were washed once in sterile distilled water and soaked for 10 minutes in 0.01 M HCl (to remove traces of NaOCl) and washed 5 times in sterile distilled water to remove traces of HCl. All the bacterial isolates were grown on 0.1 x TSB for 24 to 48 hr, agitated on a rotary shaker (120 rpm, 24°C) and before harvest, centrifuged. The pellets were collected and suspensions prepared in 0.1 M $MgSO_4$ to give an absorbance of 0.1 at 620 nm. These suspensions were used for the bacterization. The sunflower seeds were bacterized with the selected test strains bysoaking 10 seeds in the above prepared bacterial suspensions for 30 minutes. Control seeds were treated with 0.1 M $MgSO_4$ only. After treatment the seeds were placed, 10 seeds per plate in 9 cm diameter glass petriplates lined with 2 filter papers, moistened with sterile de-ionized water (SDW) to test for germination. Petri plates were covered and incubated in the dark at ambient temperature. Sterile Distilled water was added to the plates to provide moisture for germination when necessary. Germination was recorded at every 24 hr and measurement of growth parameters was recorded after 7d (Wocoma, 2008; Egamberdiyeva, 2007).

Seed Bacterization Pot Assay

The seeds were surface-sterilized with 0.1% $HgCl_2$ [Mercury (II) chloride] for 3-5 min and then washed and rinsed in sterilized distilled water for 3-4 times and dried overnight under a sterile air stream. Cells were grown in TSB for 24 hr at 28-30°C under shaker conditions and were finally centrifuged at 7000 x g for 15 min. The supernatant was discarded and pellet was washed with sterilized distilled water and re-suspended to obtain a population density of 10^7-10^8 CFU/ml. This suspension was mixed with 1% carboxymethylcellulose (CMC). Seeds were allowed to air dry overnight under aseptic conditions after coating with CMC slurry of bacterial culture. Seeds coated with slurry of CMC (without bacteria) served as control (Bhatia et al., 2008).The bacterized seeds and control seeds were grown in pots containing sterilized and non-sterilized soils. After planting the seeds in pots the pots were randomly placed under fluorescent illumination of 12-14 hr a day length at ambient temperature. Each pot received the same amount of water. Emergence was recorded daily. After germination, at an interval of 10 d inoculation with the selected strains was carried out. The plants were harvested after 10 dand data regarding the growth parameters (root length, shoot length, fresh root weight and fresh shoot weight) were recorded.

Results and Discussion

A totalof 27 bacterial strains were isolated among these isolates nine belonged to bulk soil, ten rhizosphere and eight were endophytic. Their colonial characteristics morphology and Gram reaction were studied (Table:1, 2 and 3) Production of various hydrolytic enzymes protease, lipase, cellulose and amylase are some of the factors that help the plant against various pathogens. The results showed that protease production was observed in 37%, lipase 52%, cellulose 48%, amylase 41% of the isolates. HCN production was detected in 11% of isolates and ammonia was observed

in 93% of the isolates (Table 4, 5 and 6). Siderophores were also observed in many of the isolates. These bacteria were also possessing antagonistic activity against various tested plant pathogenic fungi *Fusarium, Aspergillus, Curvularia* and *Helminthosporium* species (Table: 7, 8 and 9). From among these, two *Bacillus* species were promising able to produce siderophores and hydrolytic enzymes and were able to promote plant growth in the plate bacterization assay Figure:1). and even during the early stages of plant growth (Table:10). Both the isolates were identified and were submitted to Genebank under the accession (KF 562000 and KF981437) and showed similarity with *Bacillus* spp. These bacterialspecies could be used along with other Biofertilizer preparations or as as biocontrolling agents for plant growth promotion along with other agents in agriculture.

These results also showed that Bacillus species harboring in the close vicinity of plant roots were able to produce several antagonistic substances that helped to promote plant growth during the early stages of the sunflower plant. Our results are in accordance with several other scientists.*Bacillus* is the most abundant genus in the rhizosphere, and the PGPR activity of some of these strains has been known for many years, resulting in a broad knowledge of the mechanisms involved (Probanza et al., 2002; Gutierrez Manero et al., 2003). There are a number of metabolites that are released by these strains (Charest, Beauchamp and Antoun, 2005), which strongly affect the environment by increasing nutrient availability of the plants (Barriuso et al., 2008).

The Table Shows the Plant Growth Promoting Substances Produced by Various Bacilli

PGPR	PGRs	Crop	Response	Reference
Bacillus cereus RC18	Indole-3-acetic acid	Wheat & Spinach	Strains efficient in IAA production increased growth	Cakmakci et al., 2007
Bacillus&Paenibacillus spp.	Indole-3-acetic acid	Rice	Significant increase in root and shoot parts of rice plants	Beneduzi et al., 2008
Bacillus subtilis GB03, *Bacillus pumilus* SE-34, *Paenibacillu spolymyxa* E681, *P. fluorescens* 89B-61	Biocontrol	Arabidopsis		Ryu et al., 2005
Alcaligenes faecalis, *Bacillus cereus*, *P. aeruginosa*	Biocontrol, ACC deaminase	Wheat	Improved plant growth and nutrition under salt stress,	Egamberdiyeva, 2008
Burkholderiagladii strain BA7, *P. putida* strain BA8, *B. subtilis* strain BA142, *B. megaterium* strain M3	Fix N_2, Biocontrol, Release of enzymes	Radish	Improved percentage of seed germination under saline conditions	Kaymak et al., 2009

Table.1 Observation of Morphological Characteristics of Bulk Soil Bacterial Species

Isolate No.	colony Size and shape	edge	surface	opacity	gram reaction and motility	
M9S1	Intermediate, irregular	Erose	Glistening	Opaque	Gram positive, Short rods	Motile
M9S2	Intermediate, Irregular	Undulate	Dull	Opaque	Gram positive, Short rods	Motile
M10S1	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive Slender rods	Motile
M10S2	Intermediate, Circular	Erose	Glistening	Opaque	Gram positive, Thick rods	Motile
M10S3	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, rods	Motile
M11S1	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, Short rods	Motile
M11S2	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, Short rods	Motile
M12S1	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, Short, Thick rods	Motile
M12S2	Large, Irregular	Filamentous	Dry	Opaque	Gram positive, Thick rods	Motile

Table.2 Observation of Morphological Characteristics of Rhizosphere Bacterial Species

Isolate No.	Size and shape	edge	surface	opacity	gram reaction and motility	
M9R1	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, Thick, Short rods	Sluggish
M9R2	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, Long, Thick rods	Motile
M9R3	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, rods	Motile
M10R1	Large, Circular	Filamentus	Dry	Opaque	Gram positive, Long, Thick rods	Motile
M10R2	Small, Irregular	Undulate	Wrinkled	Opaque	Gram positive, Short, Thick rods	Motile
M11R1	Intermediate, Rhizoidal	Undulate	Dry	Opaque	Gram positive, Rods occuring Singly	Motile
M11R2	Large, Circular	Entire	Dry	Opaque	Gram positive, Long rods	Motile
M12R1	Medium, Circular	Undulate	Butyrous	Opaque	Gram positive, Slender rods	Motile
M12R2	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, Thick rods	Motile
M12R3	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, Short, Thick rods	Motile

Table.3 Observation of Morphological Characteristics of Endophytic Bacterial Species

Isolate No.	Size and shape	edge	surface	opacity	gram reaction and motility	
M9ER1	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, Rods	Motile
M9ER2	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, Rods	Motile
M10ER1	Intermediate, Irregular	Undulate	Smooth	Opaque	Gram positive, Short, thick rods	Motile
M10ER2	Intermediate, Irregular	Erose	Dry	Opaque	Gram positive, Short, thick rods	Motile
M10ER3	Intermediate, Irregular	Undulate	Smooth	Opaque	Gram positive, ling rods	Motile
M12ER1	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, Short rods in chain	Motile
M12ER2	Intermediate, Irregular	Entire	Dry	Opaque	Gram positive, bacillary rods	Motile
M12ER3	Intermediate, Irregular	Entire	Dull	Opaque	Gram positive, Short, thick rods	Motile

Table.4 Results of Siderophore and Enzymatic Analysis of Various Bulk Soil Bacterial Species

Isolate No.	Bacterial Species	Siderophore		Enzyme Production			
		Qualitative	Quantitative	Protease	Lipase	Cellulase	Amylase
M9S1	*Bacillus pasteurii*	+	27.12	++	++	-	Trace
M9S2	*Bacillus cereus*	-	ND	+	++	-	Trace
M10S1	*Bacillus subtilis*	+	36	+	+++	+	+
M10S2	*Bacillus pasteurii*	+	27.12	-	+	+	++
M10S3	*Bacillus subtilis*	+	36	-	+	+	+++
M11S1	*Bacillus subtilis*	+	36	+	++	-	-
M11S2	*Bacillus subtilis*	+	36	+	+	-	+
M12S1	*Bacillus subtilis*	+	36	+	-	-	-
M12S2	*Bacillus badius*	-	ND	-	+	-	-

Table.5 Results of Siderophore and Enzymatic Analysis of Various Rhizosphere Soil Bacterial Species

Isolate No.	Bacterial Species	Siderophore		Enzyme Production			
		Qualitative	Quantitative	Protease	Lipase	Cellulase	Amylase
M9R1	*Bacillus subtilis*	++	31.1	++	-	-	++
M9R2	*Bacillus subtilis*	++	31.1	++	+++	+	+++
M9R3	*Bacillus subtilis*	++	31.1	-	+	-	++
M10R1	*Bacillus badius*	-	ND	-	++	+	-
M10R2	*Bacillus pasteurii*	-	ND	-	+	+	+
M11R1	*Bacillus badius*	-	ND	-	++	+	+
M11R2	*Bacillus sp. RHS-st.*	++	31.1	++	++	+	+
M12R1	*Bacillus badius*	-	ND	-	+++	+	-
M12R2	*Bacillus subtilis*	++	31.1	-	-	-	+
M12R3	*Bacillus subtilis*	++	31.1	-	-	+	-

Table.6 Results of Siderophore and Enzymatic Analysis of Various Endorhizosphere Soil Bacterial Species

Isolate No.	Bacterial Species	Siderophore		Enzyme Production			
		Qualitative	Quantitative	Protease	Lipase	Cellulase	Amylase
M9ER1	*Bacillus subtilis*	-	32.5	++	++	-	++
M9ER2	*Bacillus subtilis*	-	32.5	++	+++	-	++
M10ER1	*Bacillus coagulans*	++	32.35	-	+	+	++
M10ER2	*Bacillus polymyxa*	++	31.7	-	+	+	++
M10ER3	*Bacillus coagulans*	-	ND	-	+	-	+
M12ER1	*Bacillus subtilis*	ND	32.5	+	++	-	++
M12ER2	*Bacillus sphaericus*	-	ND	+	++	-	-
M12ER3	*Bacillus subtilis*	-	32.5	++	++	+	++

Table.7 Results of Antagonistic Activities of Bulk Soil Bacterial Species against Plant Pathogenic Fungi

Isolate no.		Antifungal Activity (Radial diam. In mm)							HCN Prod.	NH_3 Prod.
	Control	*Fus.*	Control	*Asp.*	Control	*Curv.*	Control	*Hel.*		
M9S1	12	5	40	5	25	5	25	5	-	+
M9S2		6		10		8		6	-	+
M10S1		6		6		5		5	-	+++
M10S2		6		7		5		5	-	+
M10S3		6		7		5		5	-	+++
M11S1		6		12		8		8	-	+
M11S2		5		5		5		5	-	+
M12S1		8		14		8		5	-	+
M12S2		12		16		11		14	-	+

Table.8 Results of Antagonistic Activities of Rhizosphere Soil Bacterial Species against Plant Pathogenic Fungi

Isolate no.		Antifungal Activity (Radial diam. In mm)							HCN Prod.	NH_3 Prod.
	Control	*Fus.*	Control	*Asp.*	Control	*Curv.*	Control	*Hel.*		
M9R1	12	6	40	25	25	18	25	16	-	+
M9R2		5		9		5		5	-	+++
M9R3		5		6		5		5	-	+
M10R1		5		5		5		5	-	+
M10R2		5		5		5		5	-	+
M11R1		5		10		5		5	-	+
M11R2		5		10		7		5	-	++
M12R1		6		12		6		6	+	+
M12R2		7		25		16		14	ND	+
M12R3		6		15		8		16	+	+

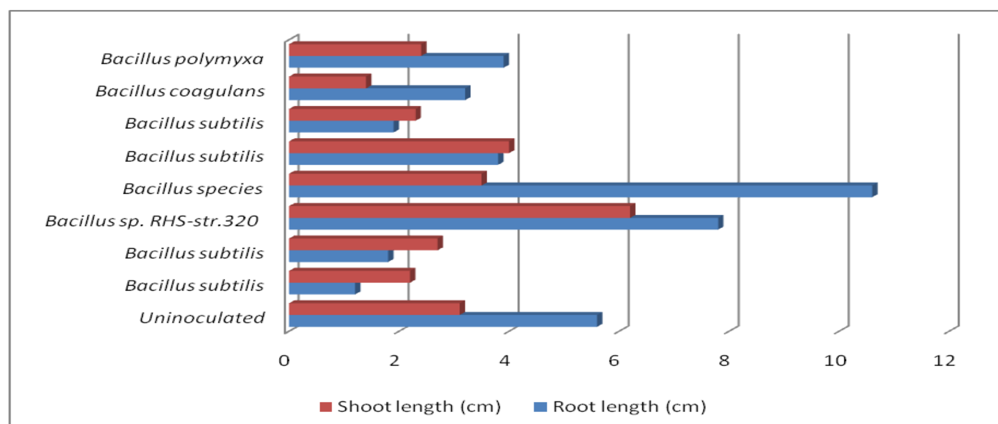
Table.9 Results of Antagonistic Activities of Endophytic Soil Bacterial Species against Plant Pathogenic Fungi

Isolate no.		Antifungal Activity (Radial diam. In mm)							HCN Prod.	NH_3 Prod.
	Control	*Fus.*	Control	*Asp.*	Control	*Curv.*	Control	*Hel.*		
M9ER1	12	5	40	8	25	5	25	5	ND	+
M9ER2		5		5		5		5	-	++
M10ER1		5		6		5		5	-	++
M10ER2		5		6		5		5	-	++
M10ER3		5		6		7		10	ND	ND
M12ER1		8		25		15		16	ND	ND
M12ER2		8		25		8		10	ND	+
M12ER3		6		8		8		6	-	++

Table.10 Results of Growth Parameters in Bacterization Pot Assay Experiments of Selected Isolates

Isolate No.	Bacterial species	Root length (cm)	Shoot length (cm)	Weight (g)
control	Uninoculated	4	17	0.592
M9 ER2	*Bacillus subtilis*	3.8	15.4	0.317
M10 S1	*Bacillus subtilis*	6.2	11.6	0.329
M10 S3	*Bacillus subtilis*	6.8	11	0.375
M10 ER1	*Bacillus coagulans*	6.8	16.5	0.686
M10 ER2	*Bacillus polymyxa*	7	14.5	0.563
M11 R2	*Bacillus sp. RHS-str.320*	7	21.3	0.787
M12 R1	*Bacillus species*	11.5	18.1	0.665
M12 R3	*Bacillus subtilis*	4.5	18.2	0.679
M12 ER3	*Bacillus subtilis*	4.1	17	0.731

Figure.1 Graphical Presentation of Growth Parameters Plate Experiments



Bacillus is also found to have potential to increase the yield, growth and nutrition of raspberry plant under organic growing conditions (Orhan et al., 2006). *Bacillus megaterium*is very consistent in improving different root parameters (rooting performance, root length and dry matter content of root) in mint (Kaymak et al., 2008). The PSB *Bacillus megaterium* var. *Phosphaticum* and Potassium Solubilising Bacteria (KSB) *Bacillus mucilaginosus* when inoculated in nutrient limited soil showed that rock materials (P and K rocks) and both bacterial strains consistently increased mineral availability, uptake and plant growth of pepper and cucumber, suggesting its potential use as fertilizer (Han, Supanjani and Lee, 2006; Supanjani et al., 2006). The *Bacillus pumilus* 8N-4 can be used as a bio-inoculant for biofertilizer production to increase the crop yield of wheat variety *Orkhon*in Mongolia (Hafeez et al., 2006). Naturally present in the immediate vicinity of plant roots, *Bacillus subtilis* is able to maintain stable contact with higher plants and promote their growth. In a micropropagated plant system, bacterial inoculation at the beginning of the acclimatisation phase can be observed from the perspective of the establishment of the soil microbiota rhizosphere. *Bacillus licheniformis* when inoculated on tomato and pepper shows considerable colonisation and can be used as a bio fertiliser without altering normal management in greenhouses (Garcia, 2004).

Jaizme-Vega, Rodriguz-Romero and Guerra (2004) evaluated the effect of a rhizobacteria consortium of *Bacillus* species on the first developmental stages of two micropropagated bananas and concluded that this bacterial consortium can be described as a prospective way to increase plant health and survival rates in commercial nurseries.

About 95% of Gram-positive soil bacilli belong to the genus *Bacillus* (Garbeva, van Veen and van Elsas, 2003). Members of *Bacillus* species are able to form endospores and hence survive under adverse conditions; some species are diazotrophs such as *Bacillus subtilis* (Timmusk et al., 1999), whereas others have different PGPR capacities (Garcia et al., 2004; Kokalis-Burelle et al., 2002; Probanza et al., 2002; Barriusoet al., 2008). While working with two *Bacillus* strains, Orhanet al.(2006)found that *Bacillus* M3 alone or in combination with *Bacillus* OSU-142 have the potential to increase the yield, growth and nutrition of raspberry plant under organic growing conditions.

Conclusion

Hence we can conclude that the *Bacillus* species abundant in the rhizosphere and possessing the capability of producing many antagonistic and plant growth promoting abilities can be used as Biofertilizers or used as adjuncts with agrichemicals for improving plant growth of such an important oleaginous crop.

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