International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 02 Issue 10
October 2015

Growth and vigour improvement of *Lycopersicon esculentum* by a bacterial isolate (*Bacillus sp.*) from *Leonurus sibiricus* rhizosphere: Mini-Review

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Abstract

It is well established that rhizospheric bacteria provide most of the unique and prominent enhancements of the plant health and growth. In our present study, we isolated a Bacillus sp. Bacterium isolated from Leonurus sibiricus rhizospheric zone, which has the ablity to promote Lycopersicon esculentum plant health when used as bio inoculant. Biochemical and morphological characterizations, the isolated stain was identified as belonging to the genus Bacillus. To determine the range of pH (5-9) and temperature (0-60^{0}C) that the bacteria can grow, Nutrient Broth and King's B media were used. SIM medium were used to see that the isolate could degrade tryptophan and H_2S production. Other biochemical characterizations were also done. Inoculation of Lycopersicon esculentum seeds with the Ari^{sis} (3×10^8 cfu/ml) significantly enhanced seed germination (48 hours: 88.40%), plant root & shoot weight, plant root & shoot height and also remained fresh (after 3, 7 and 10 days) in comparison to the control. Plant growth promoting rhizobacteria (PGPR) mediated agriculture is now gaining worldwide importance and acceptance for an increasing number of crops in modified and managed eco-systems all over the world as the safe method of pest control and higher level of crop production.

Keywords: *Leonurus sibiricus, Bacillus sp.,* PGPR, *Lycopersicon esculentum,* Plant health.

Introduction

Plant growth promoting rhizobacterial influence to promote plant growth are not fully understood (Agrawal *et al.,* 2013), but are thought to include the ability to produce or change the concentration of plant growth regulators like indole acetic acid (Patten and Glick, 2002), gibberellic acid, cytokinins and the ability to produce ACC deaminase to reduce the level of ethylene in the roots of the developing plants, thereby increasing the root length and growth (Glick, 1995), asymbiotic N_2 fixation (Kennedy *et al*., 1997), antagonism against phytopathogenic microorganisms by producing siderophores, b-1,3-glucanase, chitinases, antibiotics, fluorescent pigment and cyanide (Sharma *et al.,* 2003), solubilization of mineral phosphates and other nutrients (Johri *et al*., 2003). In last few decades a vast array of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus* and *Serratia* have been reported to enhance plant growth (Kloepper *et al.,* 1989; Glick, 1995). Several attempts were undertaken to manipulate soil microorganisms to improve cereal production. These microorganisms are capable of adding more nitrogen (Çakmakçi *et al.,* 2006), providing plant growth

promoting metabolites to enhance growth (Gutierrez *et al.,* 1996). Some of them produce iron scavenging compounds to improve iron nutrition to the roots of plants, and release secondary antagonistic organic compounds to protect plants from several root disease (Lucy *et al.,* 2004). These microorganisms are generally known as plant growth promoting rhizospheric rhizobacteria (PGPR). Plant growth promoting rhizobacteria (PGPR) accounts for about 2-5% of total the rhizobacteria involved in plant growth promotion (Antoun & Kloepper, 2001). The use of beneficial microbes in agricultural production systems started long time ago, and there is increasing evidence that beneficial microbes can improve plants tolerance to adverse environmental stresses, which include salt stress (Egamberdieva, 2008), drought stress (Zahir *et al.,* 2008), weed infestation (Babalola, 2010), nutrient deficiency, and heavy metal contaminations (Sheng, 2005). The term "induced systemic tolerance" has been used to describe the capacity of PGPR to elicit tolerance to salt and drought (Yang *et al.,* 2009). A range of salt-tolerant rhizobacteria identified so far has shown beneficial interactions with plants in stressed environments.These PGPR utilize osmoregulation; oligotrophic, endogenous metabolism; resistance to starvation; and efficient metabolic processes to adapt under dry and saline environments (Lugtenberg *et al.,* 2001; Egamberdiyeva and Islam, 2008). The bacteria, with their physiological adaptation and genetic potential for increased tolerance to drought, increasing salt concentration, and high temperatures, could improve plant production in degraded sites (Maheshwari *et al.,* 2012; Yang *et al.,* 2009). In the present study we tried to show the PGPR activity of our isolated strain on *Lycopersicon esculentum.*

Materials and Methods

The bacterial strain (Ari^{sis}) was isolated from *Leonurus sibiricus* rhizospheric zone by the serial dilution method. The sample was serially diluted upto dilution 10^{-6} and dilution samples were plated onto nutrient agar medium. After 48 hours, we saw a number of colony. We selected Ari^{sis} strain for our PGPR works. Pure culture was obtained by streaking several times in PYD medium. For future work 20% glycerol suspension of pure culture was prepared and store at -20^0C.

The isolation strain was analyzed for its ability for phosphate solubilization, IAA (indole-3-acetic acid) production, ammonia production and HCN production. Inoculate the tryptophan broth with broth culture or emulsify isolated colony of the test organism in tryptophan broth. Incubate at 37°C for 28 hours in ambient air. Add 0.5 ml of Kovac's reagent to the broth culture (MacFaddin, 1980). Positive: Pink colored rink after addition of appropriate reagent and Negative: No color change even after the addition of appropriate reagent. Colonies were screened for cellulose activity by plotting on CMC (Carboxy Methyl Cellulose) agar and Czapek-mineral salt medium ($NaNO_3$: 2.2g, K_2HPO_4: 1.0g, $MgSO_4.7H_2O$: 0.7g, KCl: 0.5g, CMC: 5.0g, peptone: 2.5g, agar: 18.0g, distilled water: 1000ml, P^H: 6.0) (Cattelan *et al.,* 1999; Aneja, 2003). HCN production was tested according Kremer *et al.* (2001) method described by Kremer and Souissi. Ari^{sis} was tested for salicylic acid, which plays an important role in signaling pathway leading to induced systemic resistance describe by Meyer *et al.,* 1992.

The experiment soil sample was autoclave at 15 lb/inch^2 pressure for 15 mints and bacterized separately in sterilized 100mL culture tubes for 10 days. 250ml of bacteria inoculums, containing 3.2×10^8 cfu/mL, are separately centrifuged at 7500 rpm for 20 mints (REMI, India) supernatant are discarded. Bacterial pallets were washed four times with carboxy-methylcellulose (MERCK, India) solution (1mg CMC in 100ml sterile distilled water) (Ramamoorthy

et al., 2002). Then the bacterial solution is added separately with the sterilized soil sample kept in culture tubes (Abdul *et al.,* 1973). After 3, 7, and 10 days incubation, PGP activities by Ari^{sis} are assessed and results were calculated.

Seeds of *Lycopersicon esculentum* were used to check the effect of isolate. 10 well quality seeds were separately surface sterilized with 95 % alcohol for 10 seconds, followed by 0.1 % sodium hypochlorite for 3–5 min and then washed with sterile distilled water for 6-9 times. Seeds were soaked in inoculum (3 $\times 10^8$ cfu/mL) for 45 min. Seeds with bio inoculant were placed on moist sterile filter paper in Petri plates. For control pot, seeds were soaked in sterile distilled water. Plant growth was measured in centimeters (cm) after each for ten days (Weller & Cook, 1983; Bhagwan, N. 2014).

The VP test shows if the bacterium has butanediol fermentation and can split glucose to acetoin via pyruvate and further to 2, 3-butanediol (Dubey, 2007). If potassium hydroxide is added, acetoin will be converted to diacetyl, which reacts with alpha-naphtol and forms a pink complex. Suspend one colony from the pure culture, which is to be investigated, in VP-MR medium (Mesaros *et al.,* 2007). Incubate at 37ºC for 48 hours Add 0.3 ml of 40% KOH and then 0.6 ml of alpha-naphtol solution. After this time results are appeared.The urease test identifies those organisms that are capable of hydrolyzing urea, Prepare UT medium (Peptone: 1gm, Dextrose: 1gm, Sodium chloride: 5gm, Potassium phosphate: 2gm, Urea: 20gm, Phenol red: 0.012gm, Agar: 15gm, dH_2O: 1lit.) and added 200µl bacterial culture Dubey (2007). After 48 hours results are appeared. The isolates was screened for the production of hydrogen cyanide by performing the method of Lorck (1948). Briefly, nutrient broth was amended with 4.3 g glycine/l and bacteria were streaked on modified agar plate. Whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.4% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated at 28°C for 5 days. Development of orange to red colour indicated HCN production. For catalase test, overnight culture of the Ari^{sis} is smeared on a microscope slide (Aneja, 2003). A drop of 4% hydrogen peroxide is added (Rollins, 2009). If copious bubbles are observed, the microbe is positive for catalase.Temperature range of 0-60°C were mainly used for bacterial growth measurement, a bacterial suspension (100 µl) was placed in a tube containing 10 ml Kings's B broth. The tube was incubated at the predetermined temperatures for 48 hours after which bacterial growth was determined by measuring the optical density (O.D.) at 660 nm. The effect of pH on bacterial growth was studied by using phosphate buffer with various pH values ranged from pH 5-9. Other aspects remained same as with temperature dependence experiment.

In our other work, Ari^{sis} assayed by dual culture plate technique method for Antimicrobial test. For this test, culture Plates were prepared with 15 ml of Muller Hilton Agar medium. About 5 mm diam wells were cut out using cork borer and filled with 100 µl of four differents types of microbes. The plates were incubated at 37±1ºC for 24 hrs except for Ari^{sis} which was incubated at 28±1ºC. The zone of inhibition rated as significant nIz: no inhibition zone, IzL: low inhibition zone and IzH: high inhibition zone.

Results and Discussion

The isolate was gram-positive, rods shape bacteria. By biochemical and morphologically characterization, the isolate stain was identified as belonging to the genus Bacillus. A series of biochemical tests were performed. Results are shown in Table. 1

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Table. 1 Ari^{sis} Biochemical & Morphological characterization

Morphological characteristics	Biochemical test	
Gram-Nature: +ve	*Catalase: +ve*	*Casein: +ve*
Morphology: rods	*Starch: -ve*	*Urease: +ve*
Endospore formation: -ve	*IAA: +ve*	*Ammonia: +ve*
Colour of Colony: white	*Citrate utilization: -ve*	*Phenylalanine utilz.:-ve*
Suggested Bacteria Strain Name: Ari^{sis}	*Oxidase: -ve*	*H_2S: +ve*
Legend: [+ve]: Positive; [-ve]: negative	*MR: +ve*	*CMC: +ve*
	VP: +ve	*HCN: +ve*

The overall performance of Ari^{sis} treatments revealed that seed treatment and soil treatment of *Lycopersicon esculentum* with inoculant, could give significantly performance in respect of control (result are show in table. 2 A. B. C.). We collected and calculated result after 3, 7 and 10 days (fig. 1 to fig. 5) of incubation of PGP in respect of control. Results are shown in tab. 2. A., 2. B. & 2. C.

Table. 2. A. After 3 days incubation of PGP results

Sl. No.	*Seed sample*	*Shoot Height (cm)*
1.	Control	2.00±0.2
2.	Ari-3-I	2.60±0.2
3.	Ari-3-II	2.50±0.1
4.	Ari-3-III	2.60±0.1
5.	Ari-3-IV	2.40±0.2

Control; Ari-3-I, II, III, IV: PGP treated seeds of Lycopersicon esculentum

Table: 2. B. After 7 days growth results of *Lycopersicon esculentum*

Sl. No.	*Seed sample*	*Shoot Height (cm)*
1.	Control	3.00±0.0
2.	Ari-7-I	4.80±0.2
3.	Ari-7-II	4.50±0.0
4.	Ari-7-III	4.80±0.0
5.	Ari-7-IV	4.60±0.2

Control; Ari-7-I, II, III, IV: PGP treated seeds of Lycopersicon esculentum

Table: 2. C. After 10 days final results of *Lycopersicon esculentum* plant

Sl. No.	*Seed sample*	*Root Height (cm)*	*Root Weight (mg)*	*Shoot Height (cm)*	*Shoot Weight (mg)*
1.	Control	2.20±0.2	0.240	3.60±0.1	0.790
2.	Ari-10-I	2.90±0.1	0.350	5.20±0.3	0.930
3.	Ari-10-II	3.00±0.3	0.380	5.00±0.2	0.910
4.	Ari-10-III	2.85±0.0	0.330	4.90±0.3	0.890
5.	Ari-10-IV	2.90±0.1	0.340	5.20±0.2	0.930

Control; Ari-10-I, II, III, IV: PGP treated seeds of Lycopersicon esculentum

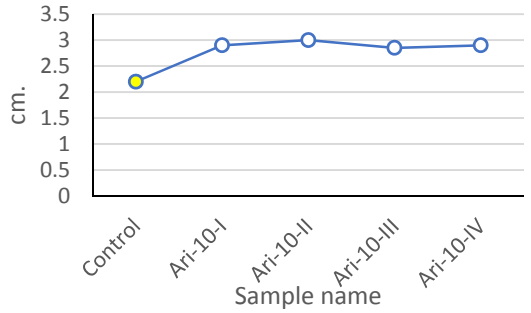

Figure. 1 Root height of *Lycopersicon esculentum* after 10 days

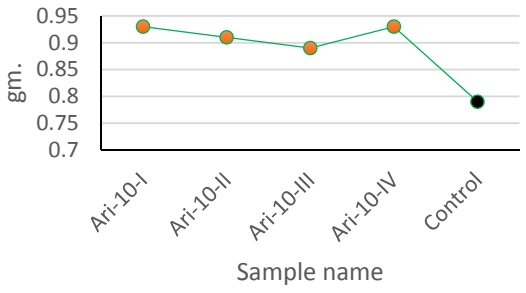

Figure. 2 Shoot weight of *Lycopersicon esculentum*

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e-ISSN: 2348-795X
Volume 02 Issue 10
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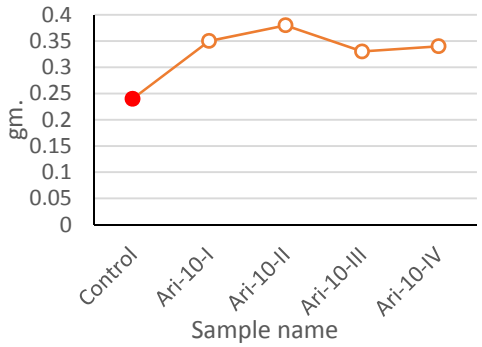


Figure. 3 Root weight after 10 days

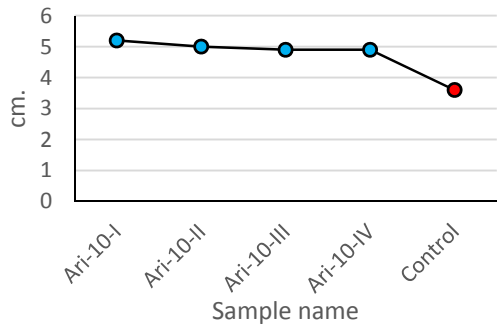


Figure. 4 Shoot height of applied at final time

Figure. 5 well characters leaves and shoots of *Lycopersicon esculentum after 10 days*

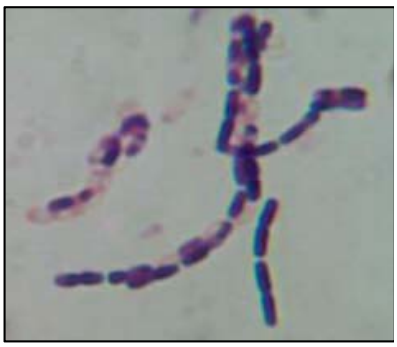


Figure. 6 Ari[sis] Staining and ovserved under light microscope, Olympus, 100X

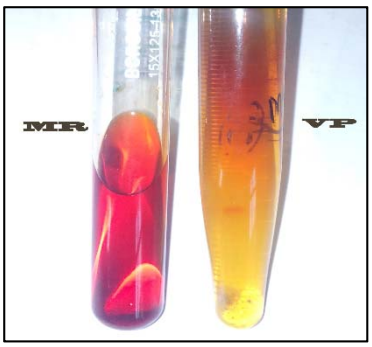
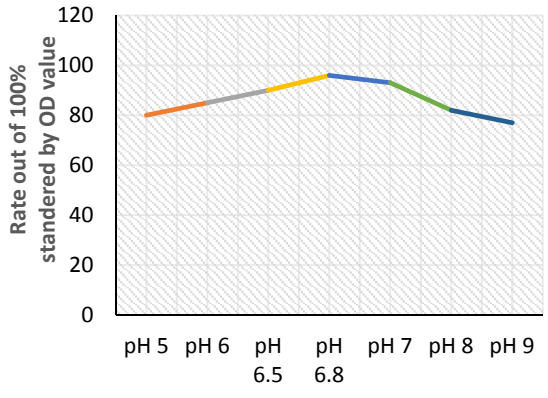


Figure. 7 MR-VP test

To determine the effect of P^H (5-9) and temperature (0-60^{0}C), the extent of growth of the isolate was determined by measuring the $O.D._{660\ nm}$ of spent culture at the aforementioned conditions. All figure are shown in fig.9 and fig. 8. Antimicrobial activity determined by Agar well diffusion metho and apply on four differtents types of microorginsms. Results are shown in table 3.



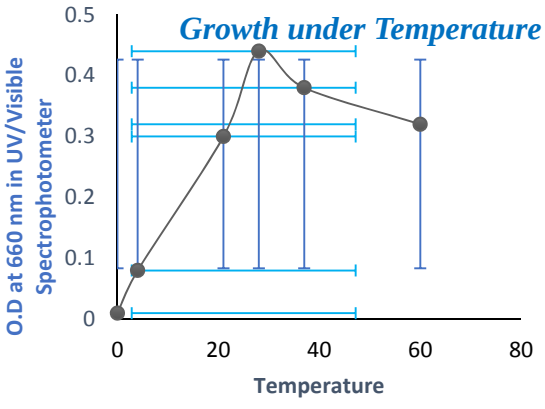


Figure. 8 Ari[sis] growth under pH

Figure. 9 Bacterial growth under stress condition mesured by 660 nm in UV/Visible Spectrophotometer

Table.3 Antibacterial Test

Sl. No.	Bacterial Name	Results
1.	*Pseudomonas sp.*	nIz
2.	*E. coli*	IzL
3.	*Enterobacter sp.*	IzH/IzL

nIz: no inhibition zone; IzL: low inhibition zone; IzH: high inhibition zone

In our other study, experiment of seeds germination of *Lycopersicon esculentum* as describe by Weller and Cook, (1983). In this works are shown in graphically respect of time and calculation of rate (100%). We got significant results which is shown graphically. There is 88.40% germination after 48 hours.

Conclusion

The isolate Ari^{sis} showed significant increased in seed germination after 48hours by tested treatment and also shoot and root length (cm) & weight (mg) improvement as well as enhanced growth of *Lycopersicon esculentum* after 3, 7 and 10 days respectively.

The performance of PGPR has been successful show in results. It survived well under stress condation. Isolate was used as growth promoting agent. Effect of isolate on germination of seeds; their ability of plant growth promotion was also studied. It can be developed as a bio fertilizer and bio-control agent.

Acknowledgment

We are very happy to take this opportunity to express our gratitude to Board of Management & prof. of Oriental Institute of Science and Technology, West Bengal, India. For this publication totally guided & permitted by Dr. Arijit Mukhopadhyay. First author thanks S. Sasmal (BCA, MCA) Dept. of Computer Science & Engineering, Kalyani University India for her helping in graphical tools-technique.

The acknowledgement cannot end without mentioning the constant support rendered by my lovely family members.

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