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Field Level Studies On The Association Of Plant Growth Promoting Rhizobacteria (PGPR) In *Gloriosa Superba L.* Rhizosphere

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Abstract:-The rhizobacteria, saprophytic bacteria that live in the plant rhizosphere and colonize the root system, have been studied as plant growth promoters for increasing agricultural production and as biocontrol agents against plant diseases. The variety of microbes present in rhizosphere soil. The plant growth promoting rhizobacteria presence is being observed from rhizosphere soil in Gloriosa superba. The field level studies revealed predominance of four PGPR microbes viz., *Azospirillum, Azotobacter, Bacillus* and *Pseudomonas* in twenty four locations of Tamil Nadu. These studies bring out the significant association of PGPR in the rhizosphere of *Gloriosa superba. L.*

Keywords:*Gloriosa superba* L, rhizosphere soil, PGPR.

INTRODUCTION

Gloriosa popularly known as “Glory lily”. Glory lily a perennial tuberous climbing herb is widely distributed in tropical and sub-tropical parts of India including footh hills of Himalayas. The plant thrives very well from arid Bundelkhand to the humid Assam valley, India. It is one of the most important medicinal plants of Asia and Africa (Sivakumar and Krishnamurthy 2000; Jana and Shekhawat, 2011).

Herbal medicine recommends G. superba for the treatment of urinary and reproductive systems, respiratory, skin diseases, cardiovascular troubles, and many other disorders. The seeds of G. superba are highly priced in the world market as sources of colchicine, chemical that has been used in the past as a remedy against gout, a disease caused by deposits of uric acid in the joints (Sivakumar and Krishnamurthy, 2002).

Plant growth promoting rhizobacteria enhance plant growth by direct and indirect means, but the specific mechanism involved have not all been well-characterized (Glick, 1995). Many studies have demonstrated that soil-borne microbe interact with plant roots and soil constituents at the root-soil surface (Bowen and Rovira, 1999).

The great array of root-microbe interaction results in the development of a dynamic environment known as the rhizosphere where microbial communities also interact. The different physical, chemical, and biological properties of the root – associated soil compared with those of the root-free bulk soil are responsible for changes in microbial diversity and for increased numbers and activity of microorganism in the rhizosphere microenvironment (Toal and Tennedy, 2000).

Plant growth promoting rhizobacteria have been reported to directly enhance plant growth by a variety of mechanism fixation of atmospheric nitrogen that is transferred to the plant root, solubilization of minerals such as phosphorous and synthesis of phytohormones such as auxins (Lucas Garcia et al., 2004).

Plant growth promoting rhizobacterial (PGPR) associations range in degree of bacterial proximity to the root and intimacy of association. In general, these can be separated into extracellular PGPR (EPGPR) existing in the rhizosphere and rhizoplane or in the spaces between cells of the root cortex and intracellular PGPR (IPGPR), which exist inside root cells, generally in specialized nodular structures (Gray and Smith, 2005).

MATERIALS AND METHODS

Survey for the collection of rhizosphere soil sample

Survey was conducted at different locations of Tamil Nadu viz., Ariyalur, Salem, Namakkal, Villupuram and Erode Districts. Nearly twenty four rhizosphere soil samples were carefully collected from well grown plants of Gloriosa superba. Then the rhizosphere soils were stored in refrigerator at 4ºC and used for the further microbiological study.

Enumeration of plant growth promoting rhizobacteria from rhizosphere soil

The rhizosphere soil samples were used for isolation and enumeration by following the standard procedure using NFB medium for Azospirillum (Day and Dobereiner, 1976) Waksman's No.77 medium for Azotobacter (Allen, 1953), Pikovskaya's medium for Phosphate solubilizing bacteria (Bacillus) (Pikovskaya, 1948) and King's medium for Pseudomonas (King's et al., 1954).

Enumeration of Azospirillum sp

Azospirillum population was enumerated from rhizosphere soil. Tenfold serial dilutions of each soil sample, ranging from 10-1 to 10-4 were made in mineral salts solution of Day and Dobereiner (1976). One ml of each dilution was inoculated in a set of five tubes containing 9 ml of nitrogen free semisolid malate medium (Day and Dobereiner, 1976). Atleast three consecutive dilutions were inoculated and tubes were incubated for three days at 30 ± 2ºC. Tubes showing sub-surface, thin pellicle growth were identified as positive tubes for Azospirillum. The MPN counts of Azospirillum were calculated on the basis of positive tubes by referring the MPN table (Cochran, 1950).

Enumeration of Azotobacter sp.

Azotobacter population was enumerated from rhizosphere soil. Sample collected from G. superba plant was performed serial dilution plate technique. Serial dilutions of the rhizosphere soil by adding 10 g of soil to 100 ml sterile water. Serially dilute upto 10-5 using water blanks. Then pipetted out 1 ml of sample from 10- 4 and 10-5 to sterile petridish and poured Waksman's base medium 77. The plates were incubated at 30ºC for a week. Azotobacter colony is initially observed colourless, transparent and later became brown pigment. The obtained population was expressed as cfu g-1 of oven dry soil (Allen, 1953).

Enumeration of phosphate solubilizing bacteria

Phosphate solubilizing bacteria were enumerated from the rhizosphere soil. The sample collected from G. superba plant was performed serial dilution plate technique according to Pikovskaya (1948). The soil samples were serially diluted upto 10- 6. Then pipetted out 1 ml of aliquots in 10-5 and 10-6 dilution poured to sterile petridishes were plated in Pikovskaya's medium. The plates were incubated upto two weeks at 28 ± 2ºC. The bacterial colonies showing clear zone were enumerated and expressed as cfu g- 1 of oven dry soil.

Enumeration of Pseudomonas sp.

Pseudomonas was enumerated from the rhizosp here soil. Sample collected from G. superba plant was performed serial dilution plate technique using King's B medium (King's et al., 1954). Taken a 10 g of soil added to 100 ml of sterile water. Then serial dilutions up to 10-6 under aseptic conditions. One ml of 10-5 and 10-6 dilutions is transferred to a King's B medium. The plates were incubated under room temperature for 48 hrs and observed for the development of Pseudomonas colonies. The population was expressed as cfu g-1 of oven dry soil.

Purification of Azospirillum sp

The inoculated test tubes were then incubated at 35°C for 3-5days. After incubation, the tubes were observed for the growth of Azospirillum. Azospirillum growth was observed by change in colour of the medium from greenish yellow to blue and the presence of white dense subsurface pellicles. The pellicles were streaked on N -free malic acid medium containing 0.3% NH_4Cl and after growth they were stored at 4°C until further use.

Purification of Azotobacter sp.

Azotobacter colonies were obtained from the soil sample after 72 hrs of incubation at room temperature and further purified by streaking a single colony in Waksman's base medium 77. The typical colonies were examined microscopically and transferred to Waksman's base medium 77 agar slants and stored in a refrigerator at 4ºC and used for further studies.

Purification of Bacillus sp.

The isolated culture was purified by streaking a single colony in Sperber's hydroxy apatite medium and colonies were examined microscopically and maintained in Pikovskaya's slant and stored in a refrigerator at 4ºC for further studies.

Purification of Pseudomonas sp.

The isolated culture was purified by streaking a single colony in King's B medium and colonies were examined microscopically and maintained in King's B medium agar slant and stored in a refrigerator at 4ºC for further studies.

RESULT AND DISCUSSION

The plant growth promoting rhizobacteria such as Azospirillum, Azotobacter, Bacillus and Pseudomonas were determined from the rhizosphere soils of G.superba collected from 24 different locations of Tamil Nadu. The plant growth promoting rhizobacterial population was found to be higher in Moolanoor of Erode District. The populations were (8.00 $x10^5$cfu g^{-1}), for Azospirillum, (7.66 x10 cfu g^{-1}), for Azotobacter, (7.33 $x10^5$cfu g^{-1}), for Bacillus and (8.33 $x10^5$ cfu g^{-1}), for Pseudomonas followed by samples collected from Kolli hills of Namakkal District, Udayarpalayam of Ariyalur District, Kappiyampuliur of Villupuram District and Moolanoor of Erode District. The minimum population in (2.33 $x10^5$cfu g^{-1}) for Azotobacter, (2.00 $x10^5$cfu g^{-1}) for Bacillus and (2.66 x10 cfu g^{-1}) for Pseudomonas were recorded in Kolathur of Salem District (Table - 1).

The designation of plant growth promoting rhizobacterial isolates obtained from Gloriosa superba soils collected from twenty four different locations was done and the assigned isolate code were given in (Table 2). The twenty four Azospirillum isolates were designated as GAz-1 to GAz-24, the twenty four Azotobacter isolates were designated as GAt-1 to GAt-24, the twenty four isolates Bacillus were designated as GBm-1 to GBm-24 and the twenty four Pseudomonas isolates were designated as GPf-1 to GPf-24.

Characterization of Azospirillum isolates

All the twenty isolates produced sub surface pellicles in Nitrogen free malate medium, (Nfb) showed Gram negative reaction, produced PHB and developed pink coloured wrinkled colonies on Potato infusion agar medium which helped to identify as Azospirillum (Plate- 1).

The isolates of Azospirillum were further subjected to various physiological and biochemical tests viz., acid production from glucose, utilization of different carbon sources, biotin requirement, nitrite reductase (NiR) and

nitrate reductase (NR) activity. The results obtained were used for further characterization (Table 3).

The isolates viz., GAz-4, GAz-6, GAz-7, GAz-8, GAz-11, GAz-12, GAz-15, GAz-16, GAz-18, GAz-22, were identified as Azospirillum brasilense. The isolates viz., GAz-1 to GAz-5, GAz-9, GAz-10, GAz-13, GAz-14, GAz-17, GAz-21, GAz-23 were found to be as Azospirillum lipoferum and GAz-19, GAz-20 and GAz-24 were identified as Azospirillum species.Characterization of Azotobacter isolates

The isolates viz., GAt-1, GAt-2, GAt-3, GAt-5, GAt-8, GAt-10, GAt-12, GAt-13, GAt-15, GAt-17, GAt-20 and GAt-23 were observed negative for Gram reaction, positive for motility, catalase activity, starch and raffinose utilization and brown to black colour *pigmentation*. Based on the result these twelve isolates were characterized based on bergey's manual of systematic bacteriology as *Azotobacter chroococcum* (Plate-2).The isolates GAt-4, GAt-7, GAt-16 and GAt-24 show negative for Gram reaction, motility, catalase activity, starch and raffinose utilization and pale colour.

These four isolates were characterized based on bergey's manual of systematic bacteriology as *Azotobacter vinelandii*. The isolates GAt-6, GAt-9, GAt-14, GAt-18 and GAt-22 were showed negative for Gram reaction, motility, catalase activity, starch and raffinose utilization and positive for yellowish pigmentation. Which helped to these five isolates was characterized as *Azotobacter beijerinckii.* The isolate GAt-11, Gat-19 and GAt-21 showed negative for Gram reaction, motility and catalase no growth, starch utilization negative and raffinose utilization and no growth for yellowish pigmentation. Based on the result of the experiment these three isolates were tentatatively characterized as *Azotobacter species* (Table- 4).

Characterization of Bacillus isolates

The isolates viz., GBm-1, GBm-5 and GBm-22, showed positive for Gram reaction, acid production, gas formation from glucose broth, hydrolysis of starch, hydrolysis of gelatin and Voges-Proskauer test and positive for utilization of citrate and these characters makes the isolate belonged to Bacillus subtilis.

The isolates viz., GBm-2, GBm-4, GBm-6, GBm-10, GBm-14, GBm-15, GBm-18, GBm-20 and GBm-23 showed positive for Gram reaction, acid production, *hydrolysis* of starch, hydrolysis of gelatin and utilization of citrate and negative for gas production from glucose broth, and Voges-Proskauer test which helped to characterize these isolates as Bacillus megaterium (Plate-3).

The isolate viz., GBm-8, GBm-12, GBm-16 and GBm-21 showed positive for Gram reaction, acid production, gas formation from glucose broth, hydrolysis of starch, hydrolysis of gelatin and Voges-Proskauer test and negative for utilization of citrate which helped to characterize these isolates as Bacillus cereus.

The isolates viz., GBm-9, GBm-13, GBm-17 and GBm-19 showed positive for Gram reaction, acid production, gas formation from glucose broth, hydrolysis of starch, hydrolysis of gelatin and Voges-Proskauer test and negative for utilization of citrate, based on the results these isolates tentatively characterized as Bacillus polymyxa.

The isolates viz., GBm-3 and GBm-7 showed positive for Gram reaction, acid production, hydrolysis of starch, hydrolysis of gelatin, casein hydrolysis, catalase, oxidase and negative for Indole, methyl red, utilization of citrate which helped to characterize these isolates as Bacillus circulans (Table - 4).

The isolates viz., GBm-11 and GBm-24 showed positive for Gram reaction, acid production, hydrolysis of starch, hydrolysis of gelatin, casein hydrolysis, urease test, Voges proskauer and negative for methyl red, utilization of citrate which helped to characterize these isolates as Bacillus alvei.

Characterization of Pseudomonas isolates

The isolates viz., GPf-1, GPf-7, GPf-11, and GPf-15 observed negative for Gram reaction, starch hydrolysis and hydrolysis of gelatin and positive result for egg yolk reaction and fluorescent pigment production which helped them identify as Pseudomonas putida.

The isolates viz., GPf-2, GPf-8, GPf-18, and APf-23 showed negative for Gram reaction, starch hydrolysis and hydrolysis of gelatin and negative result for egg yolk reaction and fluorescent pigment production are the characters make these isolates to identify as Pseudomonas striata.

The isolates GPf-3, GPf-6 and GPf-24 showed negative for Gram reaction, starch hydrolysis and hydrolysis of gelatin and positive for egg yolk reaction and fluorescent pigment production which helped to identify as Pseudomonas aeruginosa. The isolates viz., GPf-4, GPf-5, GPf-9, GPf-10, GPf-12 to 14, GPf-17, GPf-19, GPf-21 and GPf-22 observed negative for Gram reaction, starch hydrolysis and egg yolk reaction and positive for hydrolysis of gelatin and fluorescentpigment production which helped to identify as Pseudomonas fluorescens.(Table - 5) (Plate - 4).

The isolate GPf-16 and GPf-20 showed Gram reaction, hydrolysis of gelatin, pigment production, indole, methyl red, H2S production and positive for egg yolk reaction, casein hydrolysis, catalase, oxidase, citrate utilization based on the results these isolates tentatively characterized as Pseudomonas fragi.

CONCLUSION

The plant growth promoting rhizobacteria belonging to the genus of Azospirillum, Azotobacter, Bacillus and Pseudomonas is known to increase the crop yields. Further, these studies provide a clue for the improvement of alkaloid contents of Gloriosa superba. L., by selectively enriching the PGPR in their rhizosphere region.

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Table-1 Enumeration of Plant growth promoting rhizobacteria in the rhizosphere soil of Gloriosa superba

Sl. No.	Location	Population (1x 10^5 cfu g^{-1} on oven dry weight)			
		Azospirillum	*Azotobacter*	*Bacillus*	*Pseudomonas*
	AriyalurDistrict				
1.	Udayarpalayam	7.00	7.66	6.33	7.33
2.	Thathanur	4.00	4.33	3.33	3.66
3.	Kachiperumal	3.33	3.66	5.00	5.33
4.	Moorthiyan	4.00	6.00	7.00	3.33
5.	Thularankuruchi	3.33	3.66	3.33	3.00
6.	Kallankulam	5.00	4.33	4.66	4.33
	Salem District				
7.	Attur	4.00	3.66	4.33	6.33
8.	Muluvi, Yercaud	7.33	6.66	6.00	7.66
9	HRS, Yercaud	6.33	6.00	6.66	6.66
10.	Thalaivasal	4.66	5.00	4.33	5.33
11.	Kolathur	3.00	3.33	2.00	5.66
12.	Ayodhiya pattinam	3.66	2.66	5.33	6.66
	Namakkal District				
13.	Kolli hills	8.00	6.33	7.33	7.66
14.	Varagur	4.00	2.33	5.00	5.66
15.	Rasipuram	3.66	5.00	4.66	6.66
16.	Tiruchengodu	5.00	5.66	5.33	5.33
	Villupuram District				
17.	Vedur	3.66	5.33	2.66	6.00
18.	Kappiyampuliur	5.00	4.33	7.33	2.66
19.	Salaiagaram	6.00	5.66	4.00	7.66
20.	Kanai	4.33	3.33	4.66	5.66
	Erode District				
21.	Moolanoor	3.33	5.33	6.66	8.33
22.	Perundurai	2.00	2.66	2.66	6.00
23.	Vallipuruthanpalayam	5.33	4.66	5.66	6.66
24.	Verapan sathiram	4.33	5.00	3.66	4.66

TABLE -2 Plant growth promoting rhizobacteria (PGPR) isolates obtained from the rhizosphere soil of Gloriosa superba collected from different locations of Tamil Nadu

Sl. No.	Location	*Azospirillum*	*Azotobacter*	*Bacillus*	*Pseudomonas*
	AriyalurDistrict				
1.	Udayarpalayam	GAz -1	GAt -1	GBm -1	GPf -1
2.	Thathanur	GAz -2	GAt -2	GBm -2	GPf -2
3.	Kachiperumal	GAz -3	GAt -3	GBm-3	GPf -3
4.	Moorthiyan	GAz -4	GAt -4	GBm-4	GPf -4
5.	Thularankuruchi	GAz -5	GAt -5	GBm -5	GPf -5
6.	Kallankulam	GAz -6	GAt -6	GBm-6	GPf -6
	Salem District				
7.	Attur	GAz -7	GAt -7	GBm -7	GPf -7
8.	Muluvi, Yercaud	GAz -8	GAt -8	GBm-8	GPf -8
9	HRS, Yercaud	GAz -9	GAt -9	GBm -9	GPf -9
10.	Thalaivasal	GAz -10	GAt -10	GBm -10	GPf -10
11.	Kolathur	GAz -11	GAt -11	GBm-11	GPf -11
12.	Ayodhiya pattinam	GAz -12	GAt -12	GBm -12	GPf -12
	Namakkal District				
13.	Kolli hills	GAz -13	GAt -13	GBm-13	GPf -13
14.	Varagur	GAz -14	GAt -14	GBm -14	GPf -14
15.	Rasipuram	GAz -15	GAt -15	GBm-15	GPf -15
16.	Tiruchengodu	GAz -16	GAt -16	GBm-16	GPf -16
	Villupuram District				
17.	Vedur	GAz -17	GAt -17	GBm -17	GPf -17
18.	Kappiyampuliur	GAz -18	GAt -18	GBm-18	GPf -18
19.	Salaiagaram	GAz -19	GAt -19	GBm-19	GPf -19
20.	Kanai	GAz -20	GAt -20	GBm-20	GPf -20
	Erode District				
21.	Moolanoor	GAz -21	GAt -21	GBm -21	GPf -21
22.	Perundurai	GAz -22	GAt -22	GBm -22	GPf -22
23.	Vallipuruthan pallayam	GAz -23	GAt -23	GBm-23	GPf -23
24.	Verapan sathiram	GAz -24	GAt -24	GBm-24	GPf -24

TABLE – 3 General characteristics of Azospirillum isolates obtained from the rhizosphere soil of Gloriosa superba

	Sl. No	Name of the Isolate	Subsurface pellicle formation in Nfb Semi-solid medium	Cell shape	Motility	Gram reaction	Acid production from glucose	Utilization of different carbon sources: Malate	Succinate	Lactose	Mannitol	Pectin/ glutamate	Biotin requirement	Nitrite reductase activity	Nitrate reductase activity	Tentative identification
1.	1	GAz-1	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
	2	GAz-2	+	Rod	+	- ve	+	+	+	+	+	+	+	+		*A. lipoferum*
2.	3	GAz-3	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
3.	4	GAz-4	+	Curved rod	+	- ve	+	+	+	+	+	-	+		-	*A. brasilense*
4.	5	GAz-5	+	Rod	+	- ve	+	+	+	+	+	+	+	+		*A. lipoferum*
5.	6	GAz-6	+	Rod	+	- ve	+	+	+	+	+	-	+	-		*A. brasilense*
6.	7	GAz-7	+	Rod	+	- ve	+	+	+	+	+	-	+		-	*A. brasilense*
7.	8	GAz-8	+	Rod	+	- ve	+	+	+	+	+	-	+	-		*A. brasilense*
8.	9	GAz-9	+	Curved rod	+	- ve	+	+	+	+	+	+	+	+		*A. lipoferum*
9.	10	GAz-10	+	Curved rod	+	- ve	+	+	+	+	+	+	+	+		*A. lipoferum*
10.	11	GAz-11	+	Curved rod	+	- ve	+	+	+	+	+	+	+	-		*A. brasilense*
11.	12	GAz-12	+	Curved rod	+	- ve	+	+	+	+	+	+	+	-		*A. brasilense*
12.	13	GAz-13	+	Rod	+	- ve	+	+	+	+	+	+	+		+	*A. lipoferum*
13.	14	GAz-14	+	Rod	+	- ve	+	+	+	+	+	+	+	+		*A. lipoferum*
14.	15	GAz-15	+	Curved rod	+	- ve	+	+	+	+	+	-	+	-		*A. brasilense*
15.	16	GAz-16	+	Curved rod	+	- ve	+	+	+	+	+	-	+		-	*A. brasilense*
16.	17	GAz-17	+	Rod	+	- ve	+	+	+	+	+	+	+	+		*A. lipoferum*
17.	18	GAz-18	+	Curved rod	+	- ve	+	+	+	+	+	-	+		-	*A. brasilense*
18.	19	GAz-19	+	Curved rod	+	- ve	+	+	+	+	+	-	-	-		*A.* species
19.	20	GAz-20	+	Curved rod	+	- ve	+	+	+	+	+	-	-	-		*A. species*
20.	21	GAz-21	+	Rod	+	- ve	+	+	+	+	+	+	+	+		*A. lipoferum*
21.	22	GAz-22	+	Curved rod	+	- ve	+	+	+	+	+	-	+	-		*A. brasilense*
22.	23	GAz-23	+	Rod	+	- ve	+	+	+	+	+	+	+	+		*A. lipoferum*
23.	24	GAz-24	+	Curved rod	+	- ve	+	+	+	+	+	-	-	-		*A.* species

(+) Showed positive growth; (-) Showed No growth

TABLE – 4
Characterization of Azotobacter isolates obtained from the rhizosphere soil of *Gloriosa superba*

Motility	Pigment: Water soluble	Pigment: Water insoluble	Catalase	Oxidase test	Indole test	Methyl test	Citrate utilization test	Utilization of different Carbon source: Starch	Raffinose	Tentative Identification
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
+	+	Pale color	+	+	+	+	+	+	+	*A.vinelandii*
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
-	+	Yellowish	-	+	+	+	+	-	-	*A.beijerinckii*
+	+	Pale color	+	+	+	+	+	+	+	*A.vinelandii*
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
-	+	Yellowish	-	+	+	+	+	-	-	*A.beijerinckii*
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
-	+	Yellowish	-	+	+	+	+	+	-	*Azotobacter sp*
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
-	+	Yellowish	-	+	+	+	+	-	-	*A.beijerinckii*
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
+	+	Pale color	+	+	+	+	+	+	+	*A.vinelandii*
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
-	+	Yellowish	-	+	+	+	+	-	-	*A.beijerinckii*
-	+	Yellowish	-	+	+	+	+	+	-	*Azotobacter sp*
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
-	+	Yellowish	-	+	+	+	+	+	-	*Azotobacter sp*
-	+	Yellowish	-	+	+	+	+	-	-	*A.beijerinckii*
+	+	Brown to black	+	+	+	+	+	+	+	*A.chroococcum*
+	+	Pale color	+	+	+	+	+	+	+	*A.vinelandii*

(+) showed positive growth; (-) showed negative growth

TABLE -5
Characterization of Bacillus isolates obtained from the rhizosphere soil of Gloriosa superba

Motility	Spore staining	Acid production	Hydrolysis of starch	Hydrolysis of gelatin	Casein hydrolysis	Catalase test	Oxidasetest	Indole test	Methyl test	Urease test	Voges-Proskauer test	Utilization of citrate	Tentative identification
+	+	+	+	+	-	+	+	-	+	-	+	+	*B. subtilis*
+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
+	+	+	+	+	+	+	+	-	-	-	-	-	*B. circulans*
+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
+	+	+	+	+	-	+	+	-	+	-	+	+	*B. subtilis*
+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
+	+	+	+	+	+	+	+	-	-	-	-	-	*B. circulans*
+	+	+	+	+	-	+	+	-	+	-	-	-	*B cereus*
+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
+	+	+	+	+	+	+	+	+	-	+	+	-	*B. alvei*
+	+	+	+	+	-	+	+	-	+	-	-	-	*B cereus*
+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
+	+	+	+	+	-	+	+	-	+	-	-	-	*B cereus*
+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
+	+	+	+	+	-	+	+	-	+	-	-	-	*B cereus*
+	+	+	+	+	-	+	+	-	+	-	+	+	*B. subtilis*
+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
+	+	+	+	+	+	+	+	+	-	+	+	-	*B. alvei*

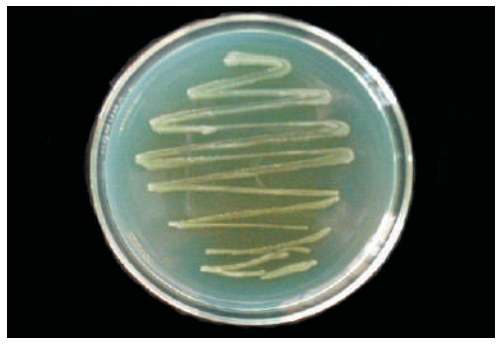
(+) showed positive growth; (-) showed negative growth

TABLE – 6
Characterization of Pseudomonas isolates obtained from the rhizosphere soil of Gloriosa superba

Gram reaction	Motility	Starch hydrolysis	Hydrolysis of gelatin	Egg yolk reaction	Pigment production	Casein hydrolysis	Catalase test	Oxidase test	Indole test	Methyl test	Citrate utilization test	H_2S Production	Nitrate reduction	Tentative identification
-ve	+	-	-	-	+	+	+	+	-	-	+	-	-	*P. putida*
-ve	+	-	-	-	+	+	+	+	-	-	+	-	-	*P. striata*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	+	*P. aeruginosa*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fluorescens*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fluorescens*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	+	*P. aeruginosa*
-ve	+	-	-	-	+	+	+	+	-	-	+	-	-	*P. putida*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. striata*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fluorescens*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fluorescens*
-ve	+	-	-	-	+	+	+	+	-	-	+	-	-	*P. putida*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fluorescens*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fluorescens*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fluorescens*
-ve	+	-	-	-	+	+	+	+	-	-	+	-	-	*P. putida*
-ve	+	-	-	+	-	+	+	+	-	-	+	-	-	*P. fragi*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fluorescens*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. striata*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fluorescens*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fragi*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fluorescens*
-ve	+	-	+	-	+	+	+	+	-	-	+	-	-	*P. fluorescens*
-ve	+	-	-	-	+	+	+	+	-	-	+	-	-	*P. striata*
-ve	+	-	+	+	+	+	+	+	-	-	+	-	+	*P. aeruginosa*

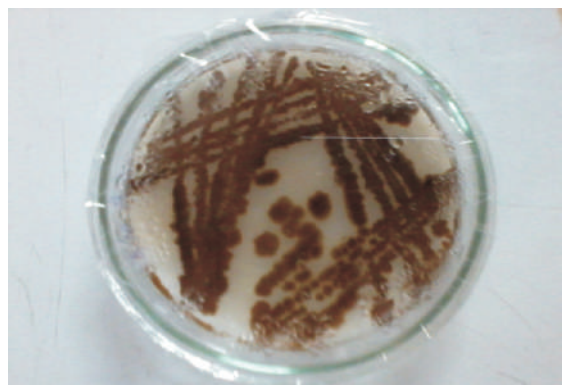
(+) showed positive growth ; (-) showed negative growth

PLATE –1



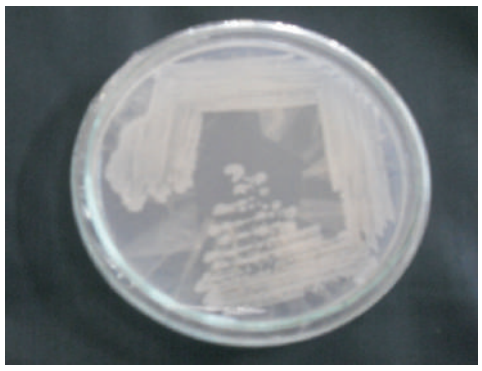
Azospirillum lipoferum* (GAz-13) isolated from *Gloriosa superba

PLATE -2



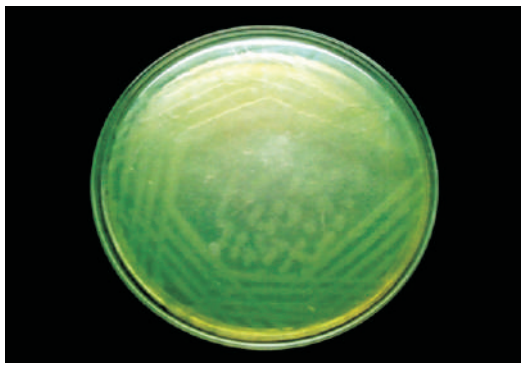
Azotobacter chroococcum* (GAt -1) isolated from *Gloriosa superba

PLATE -3



Bacillus megaterium* (GBm-18) isolated from *Gloriosa superba

PLATE -4



Pseudomonas fluorescens* (GPf-21) isolated from *Gloriosa superba

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