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Characterization of efficient plant growth promoting rhizobacteria associated with chrysanthemum (*Dendranthema grandiflora* Tzvelev)

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Abstract
Chrysanthemum (*Dendranthema grandiflora* Tzvelev) belongs to family Asteraceae, is a popular flower crop suitable for both pot culture and bedding purposes. The quality of flowers is greatly influenced by the quantity as well as sources of nutrients. Presently, these nutrients are supplied through chemical fertilizers that not only adversely affects the soil health and environment but also reduces the productivity of crops. The situation emphasized the need for developing alternate production system that is eco-friendly and is more judicious in maintaining soil health. So, the present investigations were carried out to screen the potential PGPR isolates from rhizosphere and roots of chrysanthemum collected from different sites of Solan (Nauni, Kandaghat and Chail) and Sirmour (Rajgarh, Sargaon and Narag) districts of Himachal Pradesh. Out of 49 purified isolates (31 rhizospheric and 18 endophytic), eight isolates viz. SN_1, SN_2, SN_{11}, SJ_6, SJ_8, RJ_1, SR_1, SR_5 were selected on the basis of their efficacy to have maximum plant growth promoting traits like P-solubilization, growth on nitrogen free medium, siderophore, auxin, HCN production and antagonism against *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium oxysporum* under laboratory conditions. The optimum pH and temperature for the growth of the isolates was 7.0 and 35 ^{o}C, respectively. On the basis of the maximum PGP traits, bacterial isolates SN_1 and SN_{11} were selected and identified as *Bacillus subtilis* using 16S rRNA gene sequencing and deposited in NCBI Gen Bank vide accession number KU200358 and KY200357, respectively.

Keywords: Chrysanthemum, Plant growth promoting rhizobacteria (PGPR),

Introduction
Chrysanthemum (*Dendranthema grandiflora* Tzvelev) popularly known as 'Guldaudi' or 'mums' a member of the family Asteraceae (Anderson, 1987) [1], are herbaceous perennial plants or subshrubs, occupies a prominent place in ornamental horticulture is one of the commercially exploited flower crops (Kumari *et al.*, 2015) [2]. Chrysanthemums are one of the prettiest varieties of perennials and also known as favorite flower for the month of November. It is mainly grown for cut and loose flowers used for decoration, hair adornments, making garlands and religious function. Chrysanthemum is not only being used for its flowers but also for essential oils, sesquiterpenoids, medicinal herb (i.e. powerful anti-microbial, anti-inflammatory, immuno-modulatory, and neuro-protective effects), insecticides, etc. The quality of flowers is greatly influenced by the quantity as well as sources of nutrients. Presently, these nutrients are supplied through chemical fertilizers. The escalating prices of chemical fertilizers and their indiscriminate use has not only adversely affects the soil health and environment but also reduces the productivity of crops. The situation emphasized the need for developing alternate production system that is eco-friendly and is more judicious in maintaining soil health. So, the present investigations were carried out to characterize and evaluate the effects of plant growth-promoting rhizobacteria (PGPR) isolated from rhizosphere and roots of chrysanthemum. Plant growth-promoting rhizobacteria (PGPR) are free-living soil bacteria that aggressively colonize the rhizosphere/endo rhizosphere, enhance the growth and yield of plants when applied to seed or crops (Kumar *et al.*, 2014) [3]. In recent years, much attention has been paid to natural methods of crop growing in expectation n of moving toward agriculturally and environmentally sustainable development. Plant Growth Promoting Rhizobacteria (PGPR) are considered as a biological fertilizer, one of the most important requirements to protect environment from pollution, a cheap alternative that replaces expensive chemical fertilizers as they can contribute to mobilization, mineralization and recycling of nutrients in an effective manner (Prasanna *et al.*, 2016) [4] and provides a safe and clean product (Barea, 2015) [5]. The use of microbial technologies is increasing day by day in agriculture (Rascovan *et al.*, 2016) [6] to reduce the impacts on

human health and environment, development of resistance in plant pests, etc. A number of soil bacteria which flourish in plant rhizosphere and roots stimulate plant growth by different mechanisms and are collectively known as plant growth promoting rhizobacteria (PGPR). The direct mechanisms include atmospheric nitrogen fixation, phosphate solubilization, siderophore production and secretion of plant growth promoting hormones (Bhardwaj *et al.*, 2017) [7]. The indirect mechanisms include biological control of phytopathogens/deleterious microbes through antibiotic production, lytic enzymes, siderophore and HCN secretion. These mechanisms remarkably improve plant health and promotes growth and yield of the crop (Gholami *et al*., 2009; Kaushal and Kaushal, 2013) [8, 9]. PGPR includes the genera *Acinetobacter, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Rhizobium* and *Serratia* (Dursan *et al.*, 2008) [10]. The predominant PGPR's belong to genera *Pseudomonas* and *Bacillus* because of their association with soil organic matter, nutritional diversity and rapid growth rate (Egamberdiyeva, 2007) [11]. It have been reported that specific micro-organisms improve growth and yield of crop. Thus, inoculation with specific bacteria (PGPR) may enhance the health and fertility of the soil that contributes and leads to the production of higher value sustainable products with good quality. The proposed research work was aimed towards development of efficient biofertilizer/PGPR with multiple plant growth promoting (PGP) traits.

Materials and Methods

Collection of soil and root samples

The rhizospheric soil and root samples of chrysanthemum plants were collected from two districts i.e. Solan and Sirmour of Himachal Pradesh. In each district, three locations viz. Kandaghat, Nauni, Chail of district Solan and Rajgarh, Narag, Sargaon of district Sirmour were selected and under each location two sites were selected randomly for collection of samples. From each site two samples were collected. A total of forty eight samples viz. twenty four rhizospheric soil and twenty four root samples were collected. The samples were placed in plastic bags and brought to Soil Microbiology Laboratory, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan for further isolation and characterization work.

Isolation and enumeration of microbial population

Bacterial isolates insistent in the rhizospheric soil samples and roots were obtained by serial dilution and plate count technique by using nutrient agar medium. Enumeration on Pikovskaya's agar (PVK), Jensen medium and King's medium was done using replica plate technique. The microbial count was expressed as colony forming unit per gram of soil (cfu g^{-1}).

Maintenance of the cultures

The isolated cultures were purified by streak plate method and maintained on the slants of respective medium at 4°C in refrigerator. Morphological and biochemical characterization of the isolates was performed as per the criteria of Bergey's Manual of Systematic Bacteriology (Claus and Berkley, 1986) [12].

Screening for multifarious plant growth promoting traits

1. **Mineral phosphate solubilization**
   Phosphate solubilizing activity of each bacterial isolate was done on Pikovskaya's (PVK) agar plate as per the method of Pikovskaya (1948) [13] and noted for clear yellow zone around the colony. Phosphate solubilization index (PSI) was measured using the formula (Edi-Premono *et al*., 1996) [14]. Further, quantitative estimation of P was done in PVK broth amended with 5.0 g/l tri-calcium phosphate (TCP) by the vanadomolybdate method (Sundara Rao and Sinha, 1963) [15].
2. **IAA production**
   Each bacterial isolate was grown in Luria Bertani broth (amended with 5 mM L-tryptophan, 0.065% sodium dodecyl sulfate and 1% glycerol) for 72 h at 35±2 ºC under shake conditions. Quantitative estimations were done using Salkowski reagent spectrometric ally (Glick, 2012) [16].
3. **Nitrogen fixing activity**
   Each of the purified isolate was streaked on Jensen's medium and was incubated for 72 to 120 h and the plates showing growth of bacteria in the form of bacterial colony were selected (Jensen, 1987) [17].
4. **Hydrogen cyanide production**
   Bacterial isolates were streaked on King's B agar medium with 4.4 g glycine/l. Whatman no. 1 filter paper, was cut into uniform strips, 8 cm long and 0.5 cm wide; saturated with an alkaline picrate solution (0.5% picric acid + 0.2% sodium carbonate; pH 13); and placed inside the lid of a petri dish. The plates were then sealed air tight with parafilm and incubated at 35±2ºC for 48 h. Thereafter, a colour change in the sodium picrate present in the filter paper from yellowish to reddish brown was considered to be an indication of HCN production (Bakker and Schippers, 1987) [18].
5. **Siderophore production**
   The ability of the isolates to produce siderophore was determined using blue agar plates containing chrome azurol S (Schwyn and Neilands, 1987) [19]. Each isolate was inoculated on to the plate and incubated at 35±2 ºC for 48 h. Orange halos around the isolate on the blue agar served as indicators of siderophores excretion.
6. **Antifungal activity**
   A dual plate method was used for *in vitro* screening of bacterial strain against different fungal pathogens *viz.*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Pythium ultimum*. Percent growth inhibition was calculated using the formula proposed by Vincent (1947) [20].

$$I = \frac{C - T}{C} \times 100$$

Where,
I is the percentage of growth inhibition,
C is the growth of fungus in control and
T is the growth of fungus in treatment

Growth under different temperature, pH and salinity conditions

3 ml of nutrient broth was taken in 5 ml test tubes and inoculated with 0.1 ml of 48 h old bacterial cell suspension (O.D. 1.0 at 540 nm). Growth curves were drawn by growing the culture at different temperature (20, 25, 30, 35, 40 and 45 ºC), pH (5, 6, 7, 8 and 9). The optimum temperature and pH suitable for the growth were selected on the basis of turbidity caused by the bacterial growth in test tube.

Biochemical and molecular identification of bacterial isolates

Morphological characteristics of isolates including colony morphology, Gram's reaction, cell shape and presence of spores were investigated. Colony morphology and cell morphology were observed on NA medium and nutrient broth, respectively. The biochemical characterization of the isolate was done using commercial kits (KB009 Hi carbohydrate TM kit) (Holt *et al.*, 1994) [21].

PCR amplification of bacterial 16S rRNA, sequencing and phylogenetic analysis

PCR reaction was carried out using universal 16S rRNA gene primers in 20 µl reaction mixture. It contained ~50ng of template DNA, 20 pmoles of each primer, 0.2 mm dNTPs and 1 U Taq polymerase (Genei, Banglore) in 1xPCR buffer. Reaction were cycled 35 times at 94 ºC for 30 s, 58ºC for 30 s, 72 ºC for 1 min 30 s followed by final extension at 72ºC for 10 min. The PCR products were analyzed on 1% agarose gel in 1xTAE buffer, run at 100V for 1 h. Gel was stained with ethidium bromide and photographed. The amplified PCR product was excised from the gel and purified using gel/PCR extraction kit (RBC's Real genomics). The comparison of sequence was performed via the internet at National Center for Biotechnology Information (NCBI) database by employing BLAST algorithm (Altschul *et al.*, 1997) [22]. Multiple alignments were generated by the MULTALIN program from the web site: http://prodes.toulouse.inra.fr/multialin/multialin.html (Corpet, 1988) [23]. Phylogenetic relatedness of isolates was drawn using neighbour joining phylogenetic tree using Mega 6 software. The gene sequence has been submitted under Accession No.-KF560310 in NCBI Gen Bank database.

Statistical analysis

The data were statistically analyzed as described by Gomez and Gomez (1984) [24].

Results and Discussion

Isolation and enumeration of microbial population associated with Rhizospheric and roots of chrysanthemum plants

Isolation of microorganisms was carried out from the rhizosphere and roots of the chrysanthemum (*Dendranthema grandiflora* Tzvelev) collected from different locations/sites/subsites of Solan (Nauni, Kandaghat and Chail) and Sirmour (Rajgarh, Sargaon and Narag) districts of Himachal Pradesh. The population capable of growth on different media was counted and reported as cfu/g soil or cfu/g root.

Microorganisms colonizing the rhizosphere and roots of chrysanthemum at different districts located in mid hills of Himachal Pradesh is presented in Table 1. The microbial counts in rhizosphere and endorhizosphere varied to great extent i.e. (114.33×10^4 to 179.67×10^4 cfu/g soil), (68.33×10^4 to 75×10^4 cfu/g soil), (69.33×10^4 to 74.33×10^4 cfu/g soil) and (69.00×10^2 to 99.33×10^2 cfu/g roots), (47.67×10^2 to 58.00×10^2 cfu/g roots), (44.00×10^2 to 61.00×10^2 cfu/g roots) on nutrient agar medium, Pikovskaya's medium (P-solubilizers) and Jensen's medium (asymbiotic nitrogen-fixers), respectively.

Table 1: Enumeration of rhizospheric and endophytic bacterial population associated with chrysanthemum plant

Location	Site	Rhizospheric count (10^4 cfu/g soil)			Endophytic count (10^2 cfu/g roots)		
		Nutrient agar	Pikovskaya's agar	Jensen's medium	Nutrient agar	Pikovskaya's agar	Jensen's medium
Solan	Nauni	179.67	73.67	73.00	99.33	53.00	61.00
		171.67	73.33	70.67	96.33	55.33	54.33
	Kandaghat	155.67	75.00	72.00	72.00	47.67	52.00
		144.00	69.00	72.00	73.00	50.33	55.00
	Chail	140.67	73.00	74.33	72.67	53.33	48.33
		148.33	72.33	72.00	73.00	49.67	55.00
Sirmour	Rajgarh	141.00	73.33	70.67	69.00	48.67	51.00
		136.00	70.33	71.00	73.33	53.67	49.33
	Sargaon	130.33	74.33	70.33	73.00	52.33	48.67
		125.00	72.00	74.00	73.00	58.00	51.67
	Narag	115.00	71.67	69.33	72.33	49.00	46.67
		114.33	68.33	69.67	73.67	49.67	44.00

The variation in the population of both rhizosphere soil bacteria and endophytes may be attributed to location, age of plant, variety/cultivar type, time of sampling, physico-chemical properties of soil and environment conditions of the locations. The results are in conformation with those of Kaushal *et al.* (2011) [25] and Mehta *et al.* (2014) [26] who has also reported greatest variation in microbial population with respect to location/plant parts used for isolation purpose. Hallmann *et al.* (1997) [27] and Mandyal *et al.* (2012) [28] also reported that under natural conditions, the rhizosphere and phyllosphere of the plants harbour a large and varied population of the microorganisms.

Screening of bacterial isolates for multifarious plant growth promoting traits

On the basis of predominant growth and activities on different media, a total of forty nine bacterial isolates (thirty one rhizospheric and eighteen endophytic) were selected. These isolates were screened for their ability to perform multifarious plant growth promoting activities i.e. P-solubilization, growth on nitrogen free medium, siderphore production, auxin, HCN production and antagonism against major fungal pathogens i.e. *Fusarium oxysporum* (causal organism of stem rot and wilt), *Pythium ultimum* (causal organism of root rot) and *Rhizoctonia solani* (causal organism of foot rot). All of the bacterial isolates exhibited variation in performance of different plant growth promoting traits. All the forty nine isolates were phosphate solubilizer and nitrogen-fixer, twenty eight were siderophore producers and only seven isolates were HCN producer. Eight isolates showed antagonism to *Fusarium oxysporum*, twelve isolates to *Pythium ultimum* and twenty six isolates to *Rhizoctonia solani* (Table 2). Bacteria that inhabit the rhizosphere may influence plant growth by contributing to a host plant's endogenous pool of bioactive

compounds such as phytohormones, antibiotics, siderophores (Mubarik *et al*., 2010) [29]. PGPR can exhibit a variety of characteristics i.e. indirect and direct mechanisms, responsible for influencing plant growth. The indirect effects are related to production of metabolites, such as antibiotics, siderophores, or HCN, that decrease the growth of phytopathogens and other deleterious microorganisms, whereas, the direct effects are dependent on production of plant growth regulators or improvements in plant nutrients uptake (Wahyudi *et al*., 2011 and Sharma *et al.,* 2015) [30, 31].

Table 2: Screening of bacterial isolates for multifarious plant growth promoting traits isolated from chrysanthemum

Isolates	P-solubilization	N-free medium	HCN production	Siderophore production	Antagonism against		
					Fusarium oxysporum	*Pythium ultimum*	*Rhizoctonia solani*
NA_1	+	++	-	+++	-	-	-
NA_2	++	+	-	+++	-	-	-
NA_3	++	+	+	+++	-	-	-
NA_4	+++	++	+	-	-	++	-
KT_1	+	++	-	+++	++	-	-
KT_2	+	++	-	-	-	+	-
KT_3	++	++	-	+++	+	-	-
SN_1	++	+++	+	+++	++	-	+++
SN_2	++	++	-	+++	++	-	++
SN_3	+	++	-	-	+	++	+
SN_4	+	+	-	-	+	-	-
SN_5	+	+	-	+++	-	-	-
SN_6	++	+	-	-	++	-	+
SN_7	++	++	-	-	+	-	+
SN_8	+++	++	-	-	-	-	-
SN_9	++	+	-	-	++	-	-
SN_{10}	+++	+	-	-	+	-	-
SN_{11}	++	+++	+	+++	++	++	-
SN_{12}	+	+	-	-	-	+	-
SJ_1	+	++	+	-	++	-	-
SJ_2	++	++	-	-	-	-	-
SJ_3	++	++	+	-	-	+	-
SJ_4	+++	++	-	-	+	-	-
SJ_5	+	++	-	+++	-	-	-
SJ_6	++	+	-	++	++	+++	-
SJ_7	+	+++	+	-	-	-	-
SJ_8	++	++	-	++	++	++	-
SJ_9	+	+	-	-	++	+	-
SJ_{10}	+	++	-	-	+	++	-
CH_1	++	++	-	-	++	-	-
CH_2	++	+	-	++	+	-	-
CH_3	+++	++	-	-	-	-	++
RJ_1	++	++	-	+++	++	++	-
RJ_2	+	+++	-	++	++	-	-
RJ_3	++	++	-	+++	+	-	-
RJ_4	++	++	-	+++	-	-	-
RJ_5	+	+++	-	++	+	-	-
RJ_6	+	+++	-	+++	-	-	-
RJ_7	++	+	-	+	++	-	-
RJ_8	+	++	-	++	-	-	-
RJ_9	+	++	-	+++	-	-	-
SR_1	++	++	-	+++	-	++	++
SR_2	+	+++	-	+++	-	-	-
SR_3	++	+++	-	++	+	-	-
SR_4	++	+	-	++	-	-	-
SR_5	++	+++	-	+++	++	-	++
SR_6	++	+	-	-	-	-	-
SR_7	+++	++	-	-	-	-	-
SR_8	++	++	-	+++	-	-	-

P-solubilization: No P-solubilization (-), ≤ 50% (+), 50-100% (+ +), ≥ 100% (+ + +)
Siderophore units: No activity (-), ≤ 50% (+), 50-75% (+ +), ≥ 75% (+ + +)
Antifungal activity: No activity (-), ≤ 30% (+), 30-60% (+ +), ≥ 60% (+ + +)
Growth on N-free medium: 30-60% (+ +) and 60-100% (+ + +)

Out of total 49 isolates, only eight best isolates were selected on the basis of PGP traits for further experimentation.

Mineral phosphate solubilization: The P-solubilizing activities of selected bacterial isolates were compared on the basis of per cent P-solubilization efficiency (%SE) on PVK agar medium and P-solubilization in PVK broth**.** The results revealed that the SN_1 isolate had highest (87.51%) P-solubilization efficiency, however, the lowest (64.73%) phosphate solubilizing efficiency (%SE) was recorded with SJ_8 isolate. The quantitative results revealed significant variation amoung the isolates to solubilize the insoluble tricalcium phosphate in liquid medium (Table 3, Fig1.A.). The maximum (438.23 µg/ml) P-solubilization was recorded with SN_1 isolate, whereas, isolate SJ_8 solubilised minimum (369.24 µg/ml) TCP in liquid medium. The maximum decrease in final pH of supernatant i.e. from 7.0 to 3.59 was

recorded in case of SN_1 isolate, whereas the minimum decrease in final pH of supernatant was recorded (4.38) with the SJ_8 isolate. Phosphate solubilizing bacteria convert the insoluble form of phosphorus to soluble form through acidification, secretion of organic acids or protons (Richardson *et al.,* 2009) [32]. Thus, P-solubilization is considered as one of the most important attribute of the PGPR (Patel *et al.,* 2008; Joseph and Jisha, 2009; Dutta *et al.,* 2014) [33, 34, 35].

Siderophore production: The siderophore production efficiency of selected bacterial isolates was confirmed using the Chromo Azuerol Sulphate (CAS) assay. The data presented in Table 3, (Fig 1.B.) revealed that all the isolates were positive for siderophore production. Maximum (220.00 %) siderophore production efficiency was recorded with SR_5 isolate, and the minimum (42.96 %) siderophore production efficiency was recorded with SN_2 isolate. The potential to produce siderophore by microorganisms in improving iron availability to plants and sequestering it from pathogens has been reported by many workers (Wani *et al*., 2007) [36]. Siderophore producing microorganisms protects plants at two levels: first, limiting growth of plant pathogens and secondly triggering plants defensive mechanism (Ramos *et al.,* 2010) [37].

IAA Production: The IAA production by the selected bacterial isolates varied from 35.13 to 69.32µg/ml (Table 3, Fig 1.D.). Isolate SJ_8 produced a significantly higher amounts of IAA i.e. 69.32µg/ml after 72 h of incubation at 35 ^{0}C and the minimum IAA (35.13µg/ml) production was recorded with SN_2 isolate. Gracia *et al*. (2001) [38] reported that IAA is one of the physiologically most active auxins. The bacterial IAA stimulates the root development of host plant, which results in better absorption of water and nutrients from the soil (Ahemad and Khan, 2011; Dutta *et al.,* 2014) [34, 35].

Hydrogen cyanide production: All the bacterial isolates were screened for HCN production on King's B medium. The isolate SN_1 and SN_{11} showed complete change in colour of filter paper from yellow to dark brown and in other six isolates the change in colour was observed at the edge of filter paper. The HCN production may help in disease suppression (Voisard *et al.,* 1989) [40].

Table 3: % P-solubilization efficiency, % Siderophore production efficiency and IAA production (µg/ml) by selected bacterial isolates of chrysanthemum (*Dendranthema grandiflora* Tzvelev)

Isolates	% P-solubilization Efficiency in solid medium	P-solubilization in liquid medium (µg/ml)	% Siderophore Production Efficiency	IAA Production (µg/ml)
SR_5	87.51 (69.36)*	438.23	220.00	42.67
SJ_6	65.61 (54.08) *	389.26	126.25	46.84
SN_{11}	80.00 (63.42) *	416.12	104.82	40.83
SN_1	75.29 (60.22) *	413.21	92.77	50.70
SJ_8	64.73 (53.55) *	369.24	88.24	69.32
SR_1	74.08 (59.37) *	409.16	80.42	54.26
RJ_1	83.21 (65.83) *	426.02	61.33	39.68
SN_2	74.11 (59.39) *	398.62	42.96	35.13
$CD_{0.05}$	4.05	7.21	7.62	1.59

()*Figures in parentheses are arc sin transformed value

Antifungal activities: Bacterial isolates exhibited variation in antifungal activity against the tested fungal pathogens. Isolates SN_{11}, SJ_6, SJ_8, RJ_1, SR_1 showed inhibition against *Pythium ultimum* (Fig 1.C (a).). The maximum (42.22 %) growth inhibition was recorded with SR_5 isolate and minimum (30.44 %) growth inhibition was recorded for isolate SN_2. All isolates except SR_1 showed inhibition against *Rhizoctonia solani* (Fig 1.C (b).), however, maximum (76.12 %) growth inhibition was recorded with SJ_6 isolate and minimum (37.78 %) growth inhibition was recorded with isolate RJ_1 against *Pythium ultimum.* Similarly, only four isolates i.e. SN_1, SN_2, SR_1 and SR_5 showed inhibition against *Fusarium oxysporum* (Fig 1.C(c).). The maximum (75.11 %) inhibition was recorded for SN_1 isolate and the minimum (41.56 %) was noted for SN_2 isolate (Table 4). Our results are in conformation with those of Sharma *et al*. (2014) [41] who have reported per cent inhibition against *Rhizoctonia* sp. in the range of 7.27-53.84 by *Pseudomonas* strain. Biological control using microorganisms has been studied intensively by many researchers as an effective alternative to control pests/diseases (Duffy *et al.,* 2004 and Wen *et al.,* 2010) [42, 43]. The formation of zone may be due to secretion of antifungal substances that might have diffused in the medium and inhibited the fungal growth.

Table 4: Growth inhibition (%) of test fungus by selected bacterial isolates of chrysanthemum (*Dendranthema grandiflora* Tzvelev)

Isolates	Per cent inhibition against		
	Pythium ultimum	***Rhizoctonia solani***	***Fusarium oxysporum***
SN_1	0.00 (0.00)*	33.33	75.44 (60.29)*
SN_2	0.00 (0.00)	30.44	41.56 (40.13)
SN_{11}	40.00 (39.21)	35.56	0.00 (0.00)
SJ_6	76.12 (60.73)	34.22	0.00 (0.00)
SJ_8	39.33 (38.82)	35.56	0.00 (0.00)
RJ_1	37.78 (37.90)	40.67	0.00 (0.00)
SR_1	50.20 (45.10)	0.00	51.11 (45.62)
SR_5	0.00 (0.00)	42.22	51.78 (46.00)
$CD_{0.05}$	3.49	3.89	2.44

Figures in ()* parentheses are arc sine transformed values

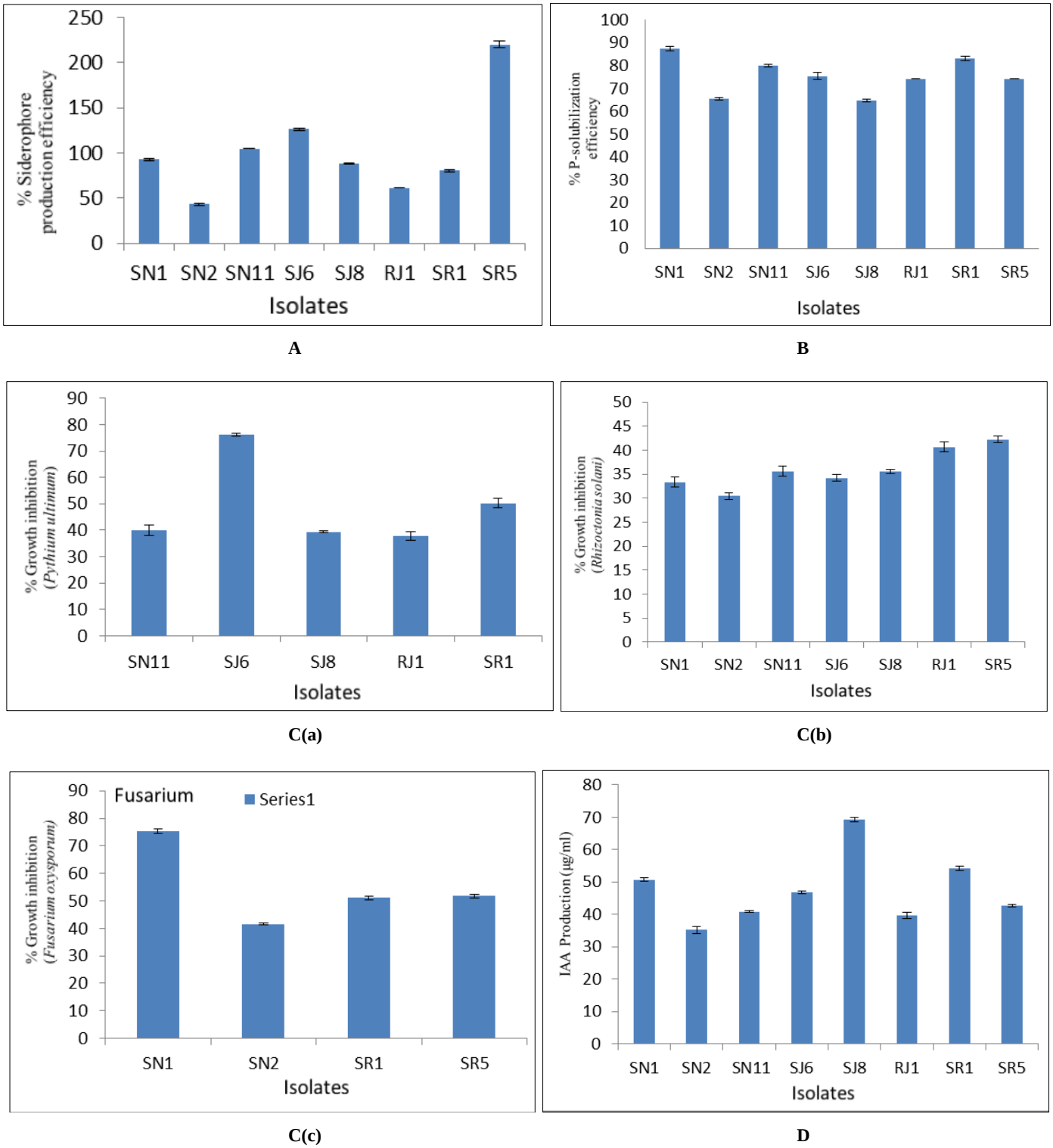

Fig 1: A. % P-solubilization efficiency; B. % Siderophore production efficiency; C. % Growth inhibition against (a). Pythium ultimum, (b). Rhizoctonia solani, (c). Fusarium oxysporum; D. IAA production (µg/ml) by selected bacterial isolates of chrysanthemum (*Dendranthema grandiflora* Tzvelev)

Morphological, physiological and biochemical characteristics of selected bacterial isolates

On the basis of multifarious plant growth promoting traits only eight efficient PGPR isolates (SN_1, SN_2, SN_{11}, SJ_6, SJ_8, RJ_1, SR_1 and SR_5) were selected and subjected to morphological, physiological and biochemical tests. The selected eight isolates showed variation in colony, elevation, margin, surface, pigment, shape and Gram's test (Table 5). The isolates grew at a wider pH (5-8) and temperature (20-40°C) range, respectively, however all the isolates showed optimum growth at 35°C and 7.0 pH (Kaushal *et al*., 2011) [25]. All the isolates were positive for Indole test, Starch hydrolysis, Caesin hydrolysis, Catalase test, Carbohydrate (glucose, sucrose, fructose and lactose) fermentation, Urease test, Gelatin hydrolysis. Isolates RJ_1, SR_1 and SR_5 was negative for Voges Proskauers test. Isolates SN_1, SN_2, SN_{11} and SJ_8 were positive for citrate utilization. Only SN_{11} isolate was positive for Methyl-red test. However, all isolates were negative for Hydrogen sulphide (H_2S) production (Table 5).

Table 5: Morphological and biochemical characterization of selected bacterial isolates of chrysanthemum

Characteristics	Bacterial Isolates							
	SN_1	SN_2	SN_{11}	SJ_6	SJ_8	RJ_1	SR_1	SR_5
Morphological								
Form	Irregular	Circular	Circular	Irregular	Punctiform	Irregular	Punctiform	Circular
Elevation	Flat	Flat	Flat	Flat	Flat	Flat	Convex	Flat
Margin	Undulate	Entire	Entire	Undulate	Entire	Undulate	Entire	Entire
Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Pigment	Cream	Cream	Cream	Cream	Grey	Cream	Cream	Cream
Gram's test	+	+	-	-	-	+	-	-
Shape	Rods	Rods	Rods	Rods	Rods	Rods	Rods	Rods
Biochemical								
Catalase test	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+
Indole test	+	+	+	+	+	+	+	+
Carbohydrate fermentation	+	+	+	+	+	+	+	+
Urease test	+	+	+	+	+	+	+	+
Citrate utilization test	+	+	+	-	+	-	-	-
Hydrogen sulphide production	-	-	-	-	-	-	-	-
Methyl red test	-	-	+	-	-	-	-	-
Voges Proskauer test	+	+	+	+	+	-	-	-

(+) indicates positivity of test; (-) indicates negativity of test

Molecular characterization (16s rRna sequencing) of selected bacterial isolates (SN_1 and SN_{11})

In order to place the isolates SN_1 and SN_{11} in an appropriate taxonomic position, the DNA sequence of the rRNA gene was determined. Primers 25 F and 1315 R were successfully used for the amplification of 16S rRNA from bacterial isolates of SN_1 and SN_{11} (Plate 1). As a result of amplification, an amplicon of expected size i.e. 1400 bp was obtained. The isolate were identified as *Bacillus subtilis* with accession number KU200357 and KU200358 for SN_1 and SN_{11}, respectively.

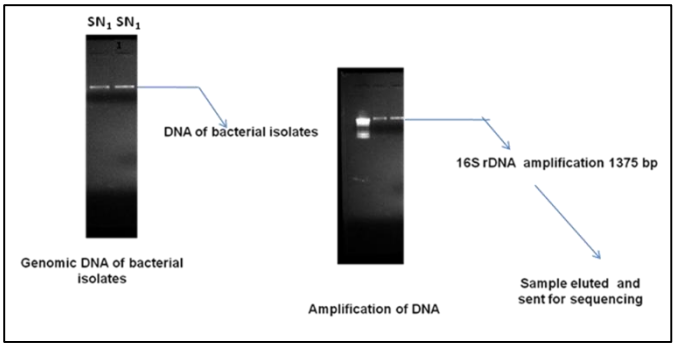

Fig 2: Molecular identification of bacterial isolate by 16s rRNA gene amplification

Conclusion

Our research effort are towards helping the poor farmers as the focus of this study is on isolation, screening and characterization of rhizobacteria and their role in plant growth promotion. A pool of promising rhizobacteria was screened for their plant growth promoting properties. The differences in plant growth promotion among the isolates were attributed to their individual competencies. On the basis of results of different PGP activities and their biocontrol ability, we suggested that these strains of PGPR have potential to be used as biofertilizers as well as bio protectant agents having the potential to supplement the chemical fertilizers and pesticides.

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