

Research Article

To Study the Culturable Bacterial Endophytes Community Diversity and Abundance Associated with Chrysanthemum (*Dendranthema Grandiflora Tzvelev*) Plant Grown Under Organic and Commercial Practices

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Chrysanthemum (*Dendranthema grandiflora Tzvelev*) belongs to family Asteraceae and 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. 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 PGPB isolated from chrysanthemum plant (roots, stem and leaf) samples. Out of 143 purified isolates, a total of forty four (16 organic and 28 inorganic) morphologically distinct isolates with dominant PGP traits, isolated from different plant samples collected from different districts of Himachal Pradesh were selected for further screening for P-solubilization efficiency, siderophore, IAA, HCN, ammonia, lytic enzyme production and antagonism against *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium oxysporum* under laboratory conditions. These selected forty four isolates were then assessed and compared to study the genetic diversity of culturable bacterial endophytes of chrysanthemum.

Keywords: Chrysanthemum; Plant Growth Promoting Bacteria (PGPB); P-solubilization; Siderophore; IAA; Biocontrol; Genetic diversity**Abbreviations**

PGPB: Plant Growth Promoting Bacteria; PGP: Plant Growth Promoting; IAA: Indole Acetic Acid; HCN: Hydrogen Cyanide; PGPR: Plant Growth Promoting Rhizobacteria; cfug⁻¹: Colony Forming Unit Per Gram; PVK: Pivovskaya; PCR: Polymerase Chain Reaction; dNTPs: Deoxynucleotide Triphosphates; DNA: Deoxyribonucleic Acid; TAE: Tris Acetate; EDTA: Ethylenediamine tetra-acetic acid

Introduction

Chrysanthemum (*Dendranthema grandiflora Tzvelev*) popularly known as 'Guldaudi' or 'mums' a member of the family Asteraceae [1], are herbaceous perennial plants or subshrubs, occupies a prominent place in ornamental horticulture is one of the commercially exploited flower crops [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/endorhizosphere, enhance the growth and yield of plants when applied to seed or crops [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 [4] and provides a safe and clean product [5]. The use of microbial technologies is increasing day by day in agriculture [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). Endophytic bacteria from leaf, stem and root are known to enhance plant growth in non-leguminous crops and improve their nutrition through nitrogen fixation, phosphate solubilisation or siderophore production (iron chelation). Besides biofertilization, endophytic bacteria are also reported to promote plant growth and yield through direct production of phytohormones, or enzymes, or indirectly through biological control of plant pests and diseases or induced resistance response (biotization). In return, the plant protects endophytes and provides them with nutrients in form of photosynthates. Endophytes are increasingly gaining scientific and commercial interest because of this potential to improve plant quality and growth and their close association with internal tissues of host plant. The direct mechanisms include atmospheric nitrogen fixation, phosphate solubilization, siderophore production and secretion of plant growth promoting hormones [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 [8,9]. PGPR includes the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* (Dursan *et al.* 2008). The predominant PGPR's belong to genera *Pseudomonas* and *Bacillus* because of their association with soil organic matter, nutritional diversity and rapid growth rate [11]. It has 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 to study culturable endophytes community diversity and abundance associated with chrysanthemum plant grown under organic and commercial practices and development of efficient biofertilizer/plant growth promoting bacteria with multiple Plant Growth Promoting (PGP) traits.

Materials and Methods

Collection of Plant Samples

The plant samples (root, stem and leaf) of chrysanthemum (*Dendranthema grandiflora* Tzvelev) were collected from Solan, Sirmour and Hamirpur districts of Himachal Pradesh. A total of 48 samples i.e. 24 organic and 24 inorganic plant (leaf, stem and roots) samples were collected from selected locations. In each district, two locations were selected and under each location two sites were selected for collection of samples. From each site two samples were collected i.e. one organic and one inorganic. The samples were placed in plastic bags and stored in Soil Microbiology Laboratory for further isolation and analysis work.

Isolation and Enumeration of Microbial Population

The plant (leaf, stem and root) samples were washed under running tap water, surface sterilized with 70 per cent ethanol for 45 seconds and 2.0 per cent sodium hypochlorite for 4-5 minutes followed by repeated 5-6 times washing in sterilized distilled water. The surface sterility of plant samples was cross checked by incubating

the sterilized nutrient agar medium plates containing 0.1ml of final wash as control for 48 h at 28±2°C. One gram of surface sterilized plant sample was crushed in 9 ml of sterilized distilled water to produce slurry using pestle and mortar under aseptic conditions. A known amount (0.1ml) of serially diluted suspension was spread on pre-poured solid agar medium *viz.*, nutrient agar medium [12], tryptic soy agar and King's B medium with the help of glass spreader under aseptic conditions. Plates were incubated in inverted position at 28±2°C for 24 to 48 h. After the incubation period, the microbial count was expressed as colony forming unit per gram of plant sample (cfug⁻¹ plant sample).

Screening for Multifarious Plant Growth Promoting Traits

Selected bacterial endophytes were screened for Phosphate solubilizing Pikovskaya's (PVK) agar plate as per the method of Pikovskaya [13] and noted for clear yellow zone around the colony, Nitrogen fixing activity on Jensen's medium [14], Siderophore production using blue agar plates containing chrome azurol S [15], IAA production in Luria Bertani broth (amended with 5 mM L-tryptophan, 0.065% sodium dodecyl sulfate and 1% glycerol), Hydrogen cyanide production on King's B agar medium with 4.4 g glycine/l [16], lytic enzyme production and antifungal activity against different fungal pathogens *viz.*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Pythium ultimum* on potato dextrose agar medium and percent growth inhibition was calculated [17].

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 nutrient agar medium and nutrient broth, respectively. The biochemical characterization of the isolate was done using commercial kits (KB009 Hi carbohydrate TM kit) [18].

PCR Amplification of Bacterial 16S rDNA, 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, Bangalore) in 1xPCR buffer. Reaction were cycled 35 times at 94°C for 30 s, 58°C for 30 s, 72°C for 1 min 30 s followed by final extension at 72°C 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 [19]. Multiple alignments were generated by the MULTALIN program from the web site: <http://prodes.toulouse.inra.fr/multalin/multalin.html> [20]. 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 GenBank database.

Genetic Diversity of Selected Bacterial Endophytes

To assess and compare the genetic diversity of predominant bacterial endophyte isolates from roots, stem and leaves of

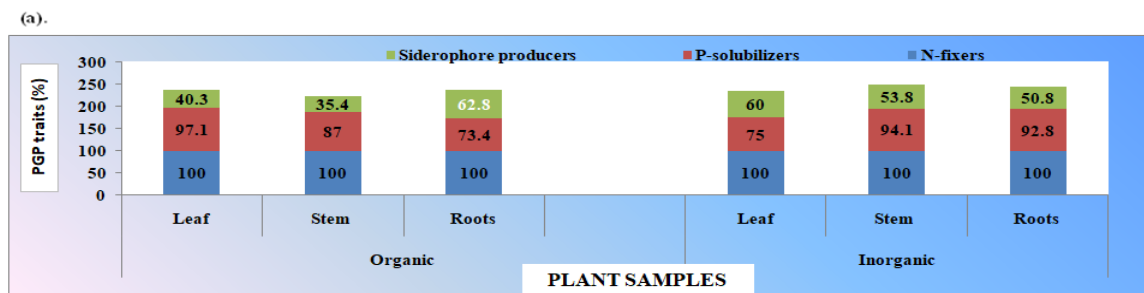
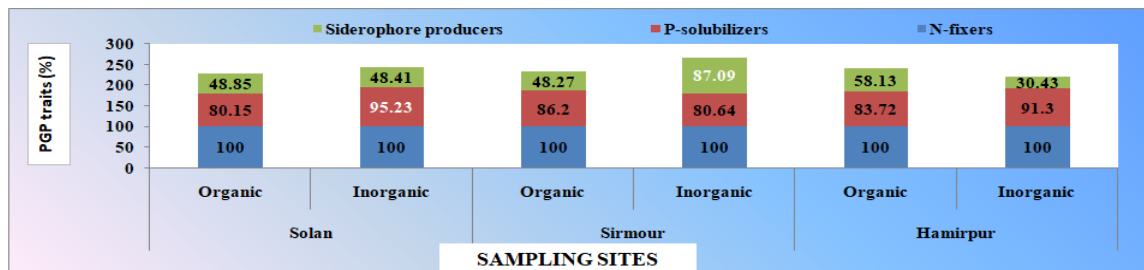


Figure 1: Characterization of bacterial endophytes isolated from (a). Different sites of sampling and (b). Different plant parts of organic and inorganic samples for phosphate solubilization, siderophore production and ability to fix nitrogen.

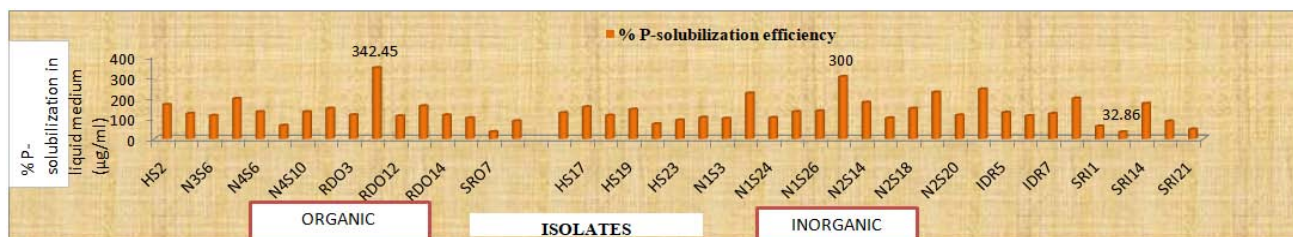


Figure 2: Percent P-solubilization efficiency (% SE) of selected bacterial endophytes on solid medium.

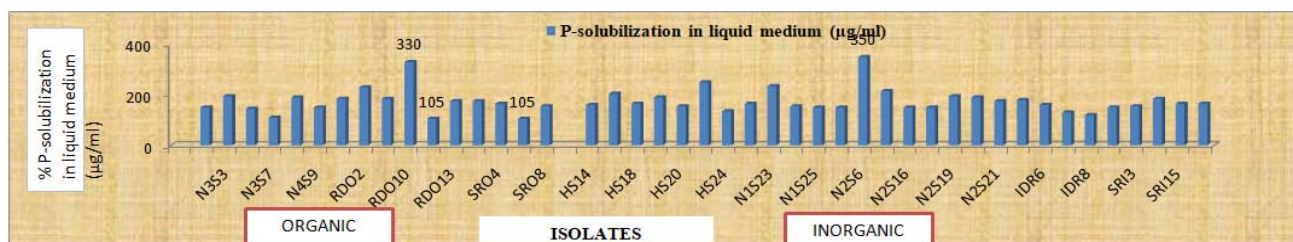


Figure 3: P-solubilization efficiency (µg/ml) of selected bacterial endophytes in liquid medium.

chrysanthemum, DNA sequence analysis of 16S rRNA gene was conducted. The amplification of gene encoding 16S rDNA of bacterial endophyte isolates was done using standard PCR reaction employing universal primer set ‘16S-1375’ (16S-1375F: 5’GCAAGTCGAGCGGACAGATGGGAGC3’ and 16S-1375R: 5’AACTCTCGTGGTGTGACGGGCGGTG3’). PCR reactions were performed in a 25 µL volume containin 2 µL MgSO₄, 2 µL dNTPs (10mM each), 0.3 µL Taq polymerase and 1 µL each of forward and reverse primers. Amplifications were run under the following cycling conditions: initial denaturation at 95°C, followed by 30 cycles of denaturing at 94°C for 30 seconds, annealing at 54°C for 30 seconds, extension at 72°C for 1 min 30 seconds followed by final extension at 72°C for 10 min.

Statistical Analysis

The data were statistically analyzed as described by Gomez and Gomez [21].

Results and Discussion

Isolation and Enumeration of Bacterial Endophytes

Isolation of microorganisms was carried out from the leaf, stem and roots of the chrysanthemum (*Dendranthema grandiflora Tzvelev*) collected from different locations/sites/subsites of Solan (Nauni and Deothi), Sirmour (Rajgarh and Sargaon) and Hamirpur (Neri and Didwi Tikker) districts of Himachal Pradesh. The population capable of growth on different media was counted and reported as cfu/g

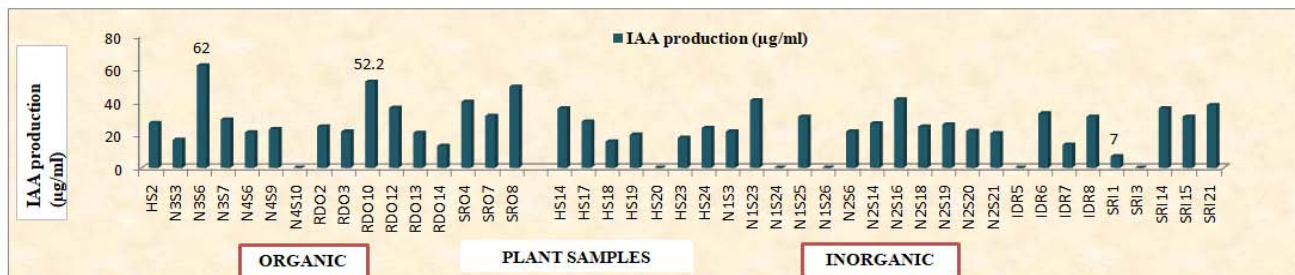


Figure 4: IAA production by selected bacterial endophytic isolates in Luria Bertani broth.

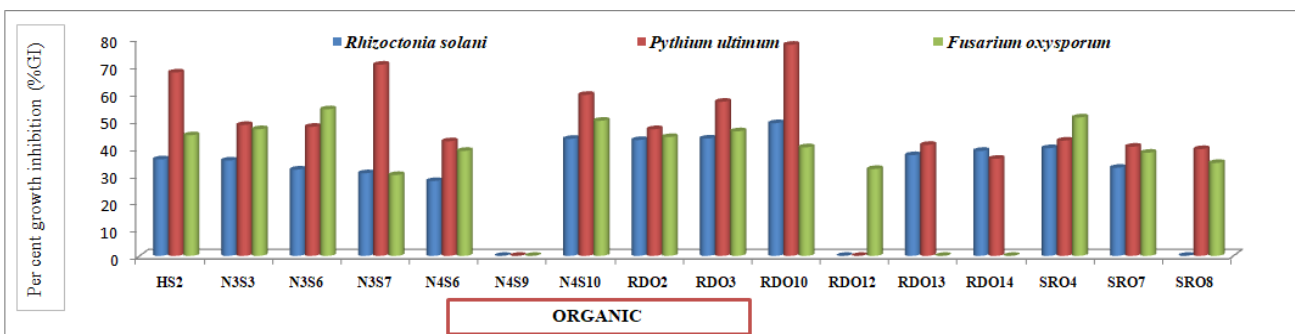


Figure 5(a): Percent growth inhibition by selected bacterial isolates isolated from organic plant samples of Chrysanthemum against *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium oxysporum*.

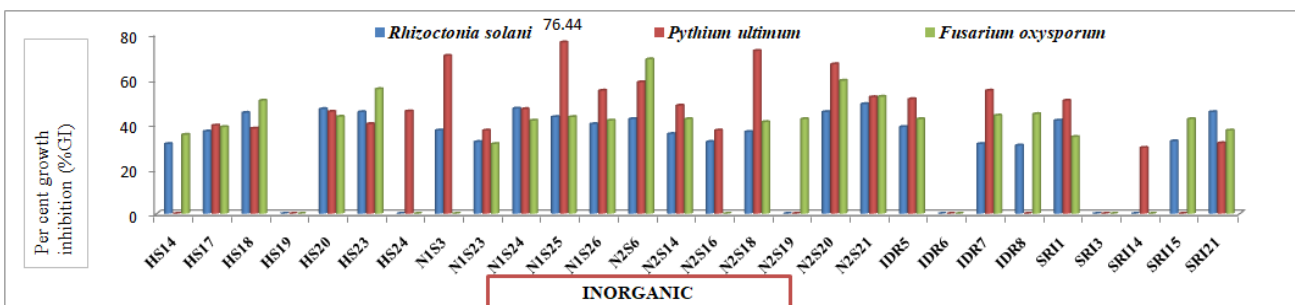


Figure 5(b): Percent growth inhibition by selected bacterial isolates isolated from inorganic plant samples of Chrysanthemum against *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium oxysporum*.

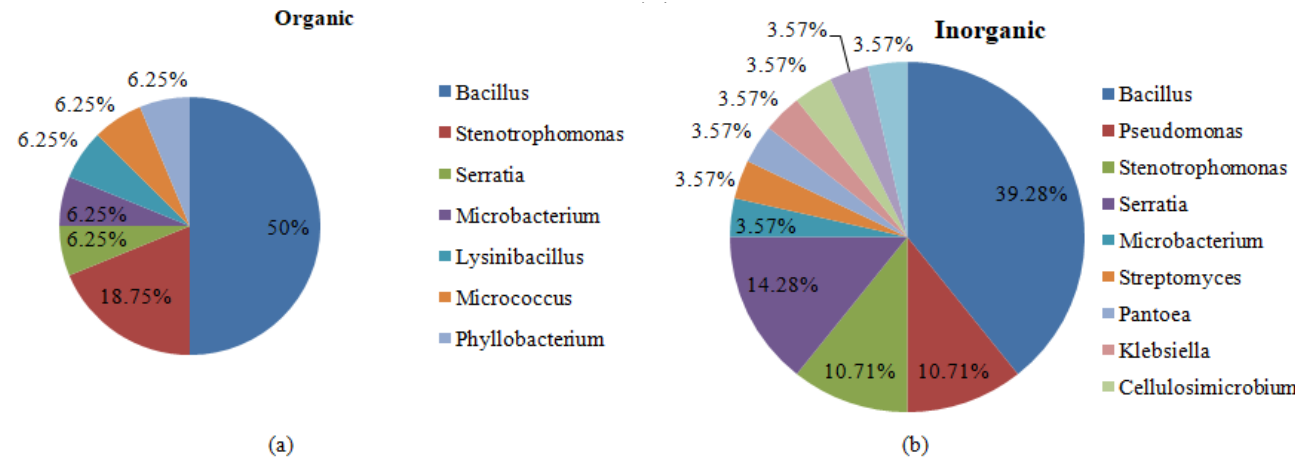


Figure 6(a): Relative abundance of endophytic bacteria at genus level associated with (a) organic plant samples and (b) inorganic plant samples.

Table 1: Enumeration of bacterial endophytes associated with chrysanthemum under organic cultivation.

Location	Sites	Endophytic count (10 ² cfu/g plant sample)															
		Nutrient Agar				Tryptic Soy Agar				King's Medium				Mean			
		Roots	Stem	Leaf	Mean	Roots	Stem	Leaf	Mean	Roots	Stem	Leaf	Mean	Roots	Stem	Leaf	Mean
Solan	Nauni	93.33	80.33	72.33	81.99	94.00	77.56	69.23	80.26	58.67	41.67	33.67	43.00	82.00	66.52	58.41	68.97
	Deothi	96.33	83.33	75.33	84.99	98.67	75.67	63.67	79.33	51.00	34.67	36.44	39.07	82.00	64.55	58.48	68.34
Sirmour	Rajgarh	83.00	62.21	67.00	70.73	89.67	72.67	63.67	75.33	47.67	30.67	32.67	40.33	73.44	55.18	54.44	61.02
	Sargaon	77.67	54.67	56.67	63.00	88.00	71.00	61.00	73.33	51.67	34.67	32.27	39.77	72.44	53.44	49.98	58.62
Hamirpur	Neri	75.00	62.21	54.00	63.73	78.67	61.67	54.67	65.00	44.00	33.45	31.56	34.97	65.89	52.44	46.74	55.02
	Didwi Tikker	79.67	66.67	58.67	68.33	75.00	58.67	50.67	61.44	51.00	34.98	36.00	39.81	68.55	53.44	48.44	56.81
	Mean	84.16	68.23	64.00	72.13	87.33	69.54	60.48	72.45	50.66	35.01	33.76	39.49	74.05	57.59	52.75	

CD_{0.05} for Plant parts (P)=1.76; Media(M)= 1.75; Interaction P X M=3.04; Intraction P X S X M=NS; Sites (S)=2.48; Interaction P X S=NS; Interaction S X M=4.30

Table 2: Enumeration of bacterial endophytes associated with chrysanthemum under inorganic cultivation.

Location	Sites	Endophytic count (10 ² cfu/g plant sample)															
		Nutrient Agar				Tryptic Soy Agar				King's Medium				Mean			
		Roots	Stem	Leaf	Mean	Roots	Stem	Leaf	Mean	Roots	Stem	Leaf	Mean	Roots	Stem	Leaf	Mean
Solan	Nauni	90.33	79.33	72.33	80.66	86.67	74.67	67.67	76.33	51.00	40.45	37.56	43.00	76.00	64.81	59.18	66.66
	Deothi	87.00	77.56	69.00	77.85	89.67	78.67	71.67	80.00	45.00	34.78	37.44	39.07	73.89	63.67	59.37	65.64
Sirmour	Rajgarh	67.67	61.66	49.67	59.66	70.00	59.67	52.45	60.70	46.00	36.78	38.21	40.33	61.22	52.70	46.77	53.56
	Sargaon	69.67	58.67	51.67	60.00	72.00	61.56	54.87	62.81	45.66	37.67	36.00	39.77	62.44	52.63	47.51	54.19
Hamirpur	Neri	66.00	57.67	48.00	57.22	64.67	53.67	46.67	55.00	42.67	31.66	30.60	34.97	57.78	47.66	41.75	49.06
	Didwi Tikker	71.00	63.29	53.21	62.50	72.67	61.67	54.67	63.00	48.00	41.00	30.45	39.81	63.89	55.32	46.11	55.10
	Mean	75.27	66.36	57.31	66.31	75.94	64.98	58.00	66.31	46.38	37.05	35.04	39.49	65.87	56.13	50.11	

CD_{0.05} for Plant parts (P)=1.31; Media (M)=1.32; Interaction P X M=2.27; Intraction P X S X M=NS; Sites (S)=1.85; Interaction P X S=NS; Interaction S X M=3.21

sample.

Microbial population in the organic samples of chrysanthemum plants

A summary of endophytic microorganisms in organic plant sample (roots, stem and leaf) of chrysanthemum at different districts located in Himachal Pradesh is presented in (Table 1) and Plate 1. Among different plant samples, maximum (74.05×10² cfu/g roots) viable count was recorded for root samples, which was found to be significantly more than stem (57.59×10² cfu/g stem) and leaf samples (52.75×10² cfu/g leaf). However, the maximum (68.97×10² cfu/g sample) count was recorded for Nauni (Solan) location which was statistically at par with (68.34×10² cfu/g sample) Deothi (Solan) location, whereas, minimum (55.02×10² cfu/g sample) for Neri (Hamirpur) location. Among different media used for isolation of bacterial endophytes, maximum (72.45×10² cfu/g sample) viable count was registered for tryptic soya agar medium, which was statistically at par with nutrient agar medium (72.13×10² cfu/g sample) while minimum (39.49×10² cfu/g sample) was recorded for King's B medium.

Data presented in (Table 2) revealed that inorganic plant sample (roots, stem and leaf) of chrysanthemum collected from different locations harboured variable number of bacteria. Among different plant samples, maximum (65.87×10² cfu/g roots) viable count was recorded for root samples and minimum (50.11×10² cfu/g leaf) for leaf samples. For different sites, the maximum (66.66×10² cfu/g sample) count was recorded for Nauni (Solan) location which was at par with (65.64×10² cfu/g sample) Deothi (Solan) location and minimum

(49.06×10² cfu/g sample) for Neri (Hamirpur) location. Among different media used for isolation of bacterial endophytes, maximum (6.315×10² cfu/g sample) viable count was registered for both nutrient agar and tryptic soya agar medium and minimum (39.49×10² cfu/g sample) was recorded for King's B medium. The endophytic bacterium actually resides within apoplasmic spaces inside the host plant and there is only some evidence of endophytes occupying intracellular spaces [22]. The internal tissues of plants provide relatively uniform and protected environment when compared with rhizosphere and rhizoplane [23,24]. Reported that variation of microbial diversity depends much on soil chemical, physical and biological properties. Gupta [25] also reported that the population of phosphate solubilizing microorganisms varied from 20-24 per cent of the total population and in some soils it may be up to 85 per cent of the total population. The solubilization of phosphorus in the rhizosphere and endorhizosphere is the most common mode of action implicated in PGPB that increase nutrient availability to host plants [26,27]. The variation in the endophytic bacterial population may be attributed to location, variety, time of sampling, physico-chemical properties of soil and environmental conditions of the location. The results are in confirmation with those of Sharma [28] and Kaushal (2011) who has also reported significant variation in microbial population with respect to location/plant parts used for the isolation.

Screening of Bacterial Endophytes on The Basis of Phenotypic Characterization and Multifarious Plant Growth Promoting Traits

All the bacterial endophytes isolated from organic and inorganic

Table 3: Qualitative and Quantitative estimation of tri calcium phosphate solubilization by selected bacterial endophytes.

Isolates	P-solubilization in solid medium		Viable Count (10 ⁶ x cfu /ml)	P-solubilization in liquid medium (µg/ ml)	Final pH of supernatant
	Phosphate solubilization index (PSI)	(%) P-solubilization efficiency (%SE)			
ISOLATES FROM ORGANIC PLANT SAMPLES					
HS ₂	2.65	165.00	82.00	150	5.62
N3S ₃	2.21	121.67	57.00	195	5.31
N3S ₆	2.01	112.33	44.00	145	5.84
N3S ₇	2.93	193.94	92.00	110	5.74
N4S ₆	2.29	129.88	49.00	190	5.99
N4S ₉	1.64	64.15	67.00	150	5.53
N4S ₁₀	1.98	129.85	71.00	185	5.71
RDO ₂	2.46	146.57	74.00	230	5.37
RDO ₃	2.15	115.05	65.00	185	5.42
RDO ₁₀	4.45	342.45	92.00	330	4.34
RDO ₁₂	2.09	109.59	47.00	105	5.50
RDO ₁₃	2.58	158.21	71.00	175	5.99
RDO ₁₄	2.14	114.28	82.00	175	5.31
SRO ₄	2.00	100.00	65.00	165	5.32
SRO ₇	1.33	33.33	64.00	105	5.56
SRO ₈	1.86	86.00	55.00	155	5.32
Mean	2.29	132.64	67.31	171.87	5.49
ISOLATES FROM INORGANIC PLANT SAMPLES					
HS ₁₄	2.25	125.00	80.00	160	4.79
HS ₁₇	2.53	153.97	82.00	205	5.12
HS ₁₈	2.13	113.21	104.00	165	5.41
HS ₁₉	2.41	141.67	86.00	190	5.23
HS ₂₀	1.71	71.67	58.00	155	5.03
HS ₂₃	1.90	90.00	65.00	250	4.65
HS ₂₄	2.03	103.03	69.00	135	3.65
N1S ₃	1.97	97.87	54.00	165	4.02
N1S ₂₃	3.20	220.00	79.00	235	5.41
N1S ₂₄	2.01	101.89	45.00	155	5.41
N1S ₂₅	2.30	130.43	87.00	150	4.09
N1S ₂₆	2.33	133.77	57.00	150	4.79
N2S ₆	4.00	300.00	71.00	350	4.19
N2S ₁₄	2.63	175.44	65.00	215	5.52
N2S ₁₆	2.00	100.00	37.00	150	4.79
N2S ₁₈	1.79	145.61	40.00	150	3.35
N2S ₁₉	3.24	224.24	42.00	195	5.41
N2S ₂₀	2.14	114.00	71.00	190	5.02
N2S ₂₁	3.40	240.00	82.00	175	5.11
IDR ₅	2.17	127.00	68.00	180	5.01
IDR ₆	2.10	110.83	70.00	160	5.11
IDR ₇	2.21	121.21	81.00	130	4.93
IDR ₈	2.95	195.35	45.00	120	4.80
SRI ₁	1.60	60.00	85.00	150	5.03

SRI ₃	1.32	32.86	80.00	155	4.71
SRI ₁₄	2.70	170.00	47.00	185	4.93
SRI ₁₅	1.85	85.00	65.00	165	5.71
SRI ₂₁	1.45	45.98	54.00	165	5.21
Mean	2.29	133.21	66.75	176.78	4.87
CD_{0.05}					
OvsINO	NS	NS	NS	3.00	0.13
WOR	0.92	13.08	12.59	13.54	0.60
WINO	0.92	13.08	12.59	13.54	0.60

Table 4: Qualitative and Quantitative estimation of siderophore production efficiency by selected bacterial endophytes of chrysanthemum (*Dendranthema grandiflora* Tzvelev).

Isolates	Siderophore estimation on solid medium				Final pH of supernatant	Quantitative estimation (Per cent siderophore unit)
	Colony size (mm)	Zone size (mm)	Siderophore production efficiency (%SE)	Siderophore type		
ISOLATES FROM ORGANIC PLANT SAMPLES						
HS ₂	0.60	0.97	61.67	Hydroxymate	5.21	57.84
N3S ₃	0.47	0.83	76.59	Hydroxymate	5.87	69.21
N3S ₆	0.33	0.53	60.60	Carboxylate	5.67	51.23
N3S ₇	1.23	1.53	24.39	Hydroxymate	5.01	51.22
N4S ₆	1.10	1.53	39.09	Carboxylate	5.21	61.37
N4S ₉	0.27	0.47	74.07	Hydroxymate	4.99	58.82
N4S ₁₀	0.27	0.53	96.29	Carboxylate	5.04	146.08
RDO ₂	1.20	1.60	33.33	Hydroxymate	6.02	34.50
RDO ₃	1.23	1.55	26.01	Hydroxymate	5.23	58.82
RDO ₁₀	0.50	1.07	114.00	Hydroxymate	5.02	210.08
RDO ₁₂	0.75	1.15	53.33	Hydroxymate	5.34	62.35
RDO ₁₃	0.95	1.25	31.57	Hydroxymate	4.89	75.49
RDO ₁₄	0.67	1.13	68.66	Hydroxymate	5.45	76.78
SRO ₄	1.20	1.57	30.83	Hydroxymate	5.96	51.37
SRO ₇	1.00	1.27	27.00	Hydroxymate	5.34	35.49
SRO ₈	0.60	1.00	66.67	Carboxylate	5.82	78.82
Mean	0.77	1.12	55.25		5.37	73.71
ISOLATES FROM INORGANIC PLANT SAMPLES						
HS ₁₄	0.67	1.13	68.66	Hydroxymate	5.45	76.78
HS ₁₇	0.77	1.47	90.90	Hydroxymate	5.34	97.98
HS ₁₈	0.73	1.17	60.27	Carboxylate	5.34	66.86
HS ₁₉	0.70	1.40	100.00	Hydroxymate	5.56	121.21
HS ₂₀	0.53	1.13	113.21	Carboxylate	5.84	83.72
HS ₂₃	0.60	1.33	121.67	Hydroxymate	5.35	116.27
HS ₂₄	1.89	2.35	24.59	Carboxylate	5.45	41.18
N1S ₃	0.23	0.53	130.43	Hydroxymate	5.54	136.27
N1S ₂₃	0.40	0.83	107.50	Carboxylate	5.45	101.37
N1S ₂₄	0.60	1.20	100.00	Carboxylate	5.12	86.47
N1S ₂₅	0.47	0.90	91.49	Hydroxymate	5.35	82.75
N1S ₂₆	1.87	2.33	24.59	Carboxylate	5.45	41.18
N2S ₆	0.60	1.40	133.33	Carboxylate	5.45	140.18

N2S ₁₄	1.40	2.10	50.00	Hydroxymate	5.67	56.09
N2S ₁₆	0.95	1.50	57.89	Hydroxymate	5.43	49.67
N2S ₁₈	0.76	1.46	90.90	Hydroxymate	5.34	97.98
N2S ₁₉	0.60	1.40	133.33	Carboxylate	5.34	140.18
N2S ₂₀	0.71	1.41	100.00	Hydroxymate	5.56	121.21
N2S ₂₁	0.53	1.13	113.21	Carboxylate	5.84	83.72
IDR ₅	0.20	0.47	135.00	Carboxylate	5.46	186.23
IDR ₆	1.00	1.27	27.00	Hydroxymate	5.67	36.47
IDR ₇	0.31	0.54	76.67	Carboxylate	5.67	83.33
IDR ₈	0.52	0.75	46.00	Hydroxymate	5.34	50.98
SRI ₁	1.30	1.87	43.85	Carboxylate	5.13	46.37
SRI ₃	0.20	0.60	200.00	Hydroxymate	5.05	178.82
SRI ₁₄	0.30	0.53	76.67	Carboxylate	5.67	83.33
SRI ₁₅	0.50	0.73	46.00	Hydroxymate	5.34	50.98
SRI ₂₁	1.87	2.33	24.59	Carboxylate	5.45	41.18
Mean	0.75	1.25	85.27		5.45	89.24
CD _{0.05}						
OvsINO	NS	0.03	1.51		NS	1.55
WOR	0.20	0.15	6.81		0.33	7.01
WINO	0.20	0.15	6.81		0.33	7.01

ND= not detected

****Initial pH =7.0; ***Per cent Siderophore unit (%SU)** = $\frac{Ar-As}{Ar} \times 100$ where, Ar= Absorbance of reference (control) at 630 nm As= Absorbance of reference test at 630 nm.

plant sample of chrysanthemum collected from different locations were nitrogen fixers. Maximum siderophore producers (87.09 per cent) were recorded for inorganic samples collected from Sirmour district, whereas, minimum (30.43 per cent) were recorded for inorganic samples collected from Hamirpur district. Maximum P-solubilizers (95.23 per cent) were observed for inorganic plant samples collected from district Solan and minimum (80.15 per cent) for organic plant samples collected from district Solan. For organic plant samples, (97.12, 87.09 and 73.45) per cent isolates were P-solubilizers and (40.30, 35.48 and 62.83) per cent isolates were siderophore producers isolated from leaf, stem and roots, respectively. Similarly, for inorganic plant samples (75.00, 94.15 and 92.85) per cent isolates were P-solubilizers and (60.00, 53.84 and 50.89) per cent isolates were siderophore producers isolated from leaf, stem and roots, respectively. Out of total isolated bacterial endophytes, 143 bacterial endophytes (51 organic and 92 inorganic) were selected on the basis of predominant growth, phenotypic characterization and possessing triple plant growth promoting traits viz. P-solubilization, ability to fix nitrogen and siderophore production efficiency on different media. All the isolates exhibited variation in performance of different plant growth promoting traits. All the 143 selected bacterial isolates were P-solubilizers, nitrogen fixers and siderophore producers. Also the data in the tables depicts the colony morphology, Gram's reaction and cell shape of selected isolates. The isolates showed variation w.r.t. Gram's reaction (+ve and -ve) and were rods, cocci and coccobacilli in shape. From the tables, it is revealed that all the isolates possess variable morphological features with respect to their form, elevation, margin, pigment. All the selected isolates from organic and inorganic plant samples showed morphologically different colonies. Out of total

56.86 (29/51) per cent and 51.08 (47/92) per cent endophytic bacteria were Gram's negative for organic and inorganic samples, respectively.

Characterization of Selected Bacterial Endophytes

A total of 44 (16 organic and 28 inorganic) morphologically distinct isolates with dominant PGP traits, isolated from different plant samples collected from different districts of Himachal Pradesh, were selected for further characterization. All the 44 bacterial endophytic isolates were screened for the solubilization of Tri-Calcium Phosphate (TCP) and were able to solubilize TCP in Pikovskaya's agar. Data presented in (Table 3) revealed that within isolates of organic samples, the maximum (4.45) Phosphate Solubilizing Index (PSI) was recorded with isolate RDO₁₀ and minimum (1.33) PSI was recorded with isolate SRO₇. While, within isolates of inorganic samples, the maximum (4.00) Phosphate Solubilizing Index (PSI) was recorded with isolate N2S₆ and minimum (1.32) PSI was recorded with isolate SRI₃. The P-solubilizing activities of selected bacterial endophytes 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 within isolates of organic samples, the isolate RDO₁₀ had highest (342.45 per cent) P-solubilization efficiency, however, the lowest (33.33 per cent) phosphate Solubilizing Efficiency (%SE) was recorded with isolate SRO₇. Whereas, within isolates of inorganic samples, the isolate N2S₆ had highest (300.00 per cent) P-solubilization efficiency, however, the lowest (32.86 per cent) phosphate Solubilizing Efficiency (%SE) was recorded with isolate SRI₃. Whereas, no significant difference was found in PSI and %SE between isolates from organic and inorganic plant samples. The quantitative results revealed significant variation among the

Table 5: Quantification of IAA production ($\mu\text{g/ml}$) by different bacterial endophytes of chrysanthemum (*Dendranthema grandiflora* Tzvelev).

Isolates	Viable Count ($10^6 \times \text{cfu/ml}$)	Indole-3-acetic acid ($\mu\text{g/ml}$)	Final pH of supernatant
ISOLATES FROM ORGANIC PLANT SAMPLES			
HS ₂	35.60	27.20	5.43
N3S ₃	39.30	17.00	5.38
N3S ₆	39.40	62.00	5.84
N3S ₇	47.80	29.30	5.46
N4S ₆	47.50	21.50	5.13
N4S ₉	39.50	23.40	5.67
N4S ₁₀	44.50	ND	5.56
RDO ₂	45.80	25.00	5.87
RDO ₃	35.00	22.00	5.46
RDO ₁₀	41.00	52.20	5.05
RDO ₁₂	45.20	36.30	5.67
RDO ₁₃	43.40	21.10	5.34
RDO ₁₄	33.50	13.30	5.45
SRO ₄	35.00	40.00	5.52
SRO ₇	32.00	31.50	5.31
SRO ₈	47.10	49.20	5.13
Mean	40.72	29.43	5.45
ISOLATES FROM INORGANIC PLANT SAMPLES			
HS ₁₄	41.33	36.00	5.32
HS ₁₇	52.33	28.00	5.67
HS ₁₈	55.60	16.00	5.34
HS ₁₉	46.79	20.10	5.37
HS ₂₀	38.90	ND	5.24
HS ₂₃	47.89	18.20	5.78
HS ₂₄	34.50	24.20	5.38
N1S ₃	42.30	22.00	5.16
N1S ₂₃	33.67	41.00	5.43
N1S ₂₄	36.79	ND	5.95
N1S ₂₅	32.90	31.00	5.77
N1S ₂₆	34.90	ND	5.94
N2S ₆	42.32	56.00	5.52
N2S ₁₄	43.45	27.00	5.21
N2S ₁₆	31.34	41.50	6.01
N2S ₁₈	47.89	25.00	5.53
N2S ₁₉	43.40	26.30	5.93
N2S ₂₀	39.90	22.40	5.44
N2S ₂₁	45.60	21.00	5.84
IDR ₅	44.67	ND	5.92
IDR ₆	38.78	33.00	5.37
IDR ₇	43.40	14.00	5.95
IDR ₈	46.78	31.00	5.61
SRI ₁	45.67	7.00	5.62
SRI ₃	23.67	ND	5.47

SRI ₁₄	33.40	36.00	5.31
SRI ₁₅	39.20	31.00	4.99
SRI ₂₁	38.90	38.00	5.84
Mean	40.93	23.06	5.56
CD _{0.05}			
OvsINO	NS	1.29	0.08
WOR	7.83	5.84	0.37
WINO	7.83	5.84	0.37

ND= not detected

Table 6: *In vitro* screening of selected bacterial endophytes for antagonistic traits of plant growth promotion.

Isolates	Chitinase		Protease		Amylase		HCN production	Ammonia production
	Zone size (mm)	*E.I.	Zone size (mm)	*E.I.	Zone size (mm)	*E.I.		
ISOLATES FROM ORGANIC PLANT SAMPLES								
HS ₂	9.00	1.50	5.00	2.50	3.00	1.50	-	++
N3S ₃	9.50	1.90	5.50	1.89	-	-	+	-
N3S ₆	-	-	8.00	1.86	6.00	1.39	-	+++
N3S ₇	19.00	1.65	-	-	13.00	1.73	+	+++
N4S ₆	16.00	2.05	9.00	2.36	-	-	-	+
N4S ₉	18.50	1.85	-	-	11.00	1.83	+	+
N4S ₁₀	8.50	2.12	3.50	1.67	2.50	1.66	-	++
RDO ₂	-	-	6.00	2.22	4.00	1.48	-	+++
RDO ₃	-	-	7.00	3.04	5.00	2.17	-	-
RDO ₁₀	17.50	2.64	4.50	3.00	1.50	2.14	+	+++
RDO ₁₂	18.00	2.50	4.40	2.44	2.40	1.33	-	-
RDO ₁₃	-	-	5.50	2.50	3.50	1.59	+	++
RDO ₁₄	25.00	2.27	9.50	1.90	7.50	1.50	-	+
SRO ₄	27.00	1.80	-	-	1.70	2.42	-	+++
SRO ₇	17.00	1.54	-	-	1.10	1.37	+	-
SRO ₈	21.00	1.40	-	-	1.50	1.36	-	-
ISOLATES FROM INORGANIC PLANT SAMPLES								
HS ₁₄	14.50	1.76	4.50	3.75	2.50	2.08	+	+++
HS ₁₇	12.00	1.93	-	-	2.60	2.36	-	+
HS ₁₈	6.50	1.71	3.00	1.45	-	-	-	+
HS ₁₉	8.00	2.05	4.00	3.07	2.00	1.53	-	+++
HS ₂₀	10.00	1.66	6.00	3.00	4.00	2.00	+	-
HS ₂₃	27.00	1.58	13.50	1.60	11.50	1.36	-	+
HS ₂₄	20.00	1.33	-	-	4.00	1.81	-	-
N1S ₃	12.00	1.34	8.00	3.07	6.00	2.30	-	++
N1S ₂₃	13.00	1.30	-	-	7.00	2.33	+	+++
N1S ₂₄	-	-	4.50	2.09	2.50	2.27	-	-
N1S ₂₅	17.50	1.40	6.60	3.66	4.60	2.55	-	++
N1S ₂₆	-	-	10.00	2.70	8.00	2.16	-	-
N2S ₆	15.00	1.97	11.00	1.83	9.00	1.50	+	+++
N2S ₁₄	16.50	1.43	-	-	10.50	1.40	-	++
N2S ₁₆	29.00	1.28	4.40	2.93	2.40	1.60	-	++
N2S ₁₈	11.00	1.61	7.00	2.50	-	-	+	-

N2S ₁₉	10.00	1.33	-	-	2.70	1.20	-	+
N2S ₂₀	7.00	1.40	-	-	1.70	2.42	-	++
N2S ₂₁	13.00	1.32	9.00	1.55	7.00	1.20	+	-
IDR ₅	10.50	1.34	6.50	1.71	4.50	1.18	+	-
IDR ₆	22.00	1.18	3.80	2.71	1.80	1.28	-	+++
IDR ₇	12.00	1.30	4.20	3.00	-	-	-	+
IDR ₈	26.00	1.19	4.00	2.35	2.00	1.17	-	-
SRI ₁	8.00	1.29	4.00	2.50	2.00	1.25	-	-
SRI ₃	-	-	3.90	3.54	1.90	1.72	+	+++
SRI ₁₄	9.50	1.37	5.50	1.89	-	-	+	-
SRI ₁₅	14.00	1.41	10.00	1.69	8.00	1.35	+	++
SRI ₂₁	11.00	1.44	-	-	5.00	1.66	-	++

ND= not detected

*Enzyme index (E.I.) = A/B Where, A= Halozone diameter+Colony diameter; B= Colony diameter;

**HCN = Change in colour of filter paper from yellow to brown (+) and (-) no change

***Ammonia production= fair (+); Good (++); Very good (+++) ammonia producers; no activity (-)

isolates to solubilize the insoluble Tri-Calcium Phosphate (TCP) in liquid medium (Table 3). Within isolates from organic samples, the maximum (330.00 µg/ml) P-solubilization was recorded for RDO₁₀ isolate, whereas minimum (105 µg/ml) was recorded for RDO₁₂ and SRO₇ isolates. However, within isolates from inorganic samples, the maximum (350.00 µg/ml) P-solubilization was recorded for N2S₆ isolate, whereas minimum (120 µg/ml) was recorded for IDR₈ isolate. Also the viable count after 72 h of incubation varied from (44×10⁶ to 92×10⁶ cfu/ml) and (37×10⁶ to 104×10⁶ cfu/ml) for isolates from organic and inorganic plant samples, respectively. Phosphorus and nitrogen are among the essential nutrients of the plants. Phosphorus is available to plants in the form of phosphate anions, which are mostly trapped by precipitation with cations such as Mg²⁺, Ca²⁺, Al³⁺ and Fe³⁺, so become insoluble and unavailable to plants in these forms. Bhattacharya and Jha [29] reported that endophytes have the capacity to mineralize and solubilize the inorganic as well as organic insoluble complex forms of phosphorus by releasing organic acids or extracellular hydrolytic enzymes and hence improve the accessibility of nutrients to plants. Phosphorus is one of the essential macronutrient required for biological growth and development of the plants [30,31]. Most of the phosphorus present in the soil is in the form of insoluble phosphates and hence unavailable to plants. Plant growth promoting bacteria are able to solubilize and make them available to the plants. Thus, P-solubilization is considered as one of the most important attribute of the PGPB [32]. The siderophore production efficiency of selected isolates was confirmed using the Chrome Azuero Sulphate (CAS) assay. Table 4 revealed that great variation was observed in colony size (0.27 to 1.23 mm and 0.20 to 1.89 mm), zone size (0.47 to 1.60 mm and 0.47 to 2.35 mm) for isolates from organic and inorganic plant samples, respectively. Only two types of siderophores were produced. i.e. carboxylate (40.90 percent) and hydroxamate (59.09 per cent), whereas, catecholate type of siderophores were not observed for any of the selected isolates. Within isolates of organic samples, the isolate RDO₁₀ had highest (114 per cent) siderophore production efficiency and (210.08 %SU) siderophore production. Whereas, within isolates of inorganic samples, the highest (200.00 per cent) siderophore production efficiency was recorded with isolate SRI₃ and maximum (186.23 %SU) siderophore production was recorded

with isolate IDR₅. Whereas, isolates from inorganic plant samples showed maximum siderophore production efficiency (85.27 per cent) and (89.24 %SU) siderophore production than isolates from organic samples. Significant difference was found in PSI and %SE between isolates from organic and inorganic plant samples. The present results are in confirmation with [33]. Variation in final pH of supernatant ranged from 4.89 to 5.96. The present findings are in line with those of Shyam [34] who reported a wide range (19.42 to 68.07 %SU) with CAS liquid assay. The results are also in confirmation with Kirti [35].

Table 5 revealed that 86.36 (38/44) per cent isolates had the ability to produce IAA from tryptophan. IAA production by selected bacterial endophytes from organic and inorganic plant samples ranged from (13.30 to 62.00 µg/ml) and (7.00 to 56.00 µg/ml). Within isolates from organic plant samples, maximum (62.00 µg/ml) IAA production was recorded with isolate N3S₆ which is statistically higher than that of all other isolates and minimum (13.30 µg/ml) IAA production was recorded with isolate RDO₁₄. Similarly, within isolates from inorganic plant samples, maximum (56.00 µg/ml) IAA production was recorded with isolate N2S₆ and minimum (7.00 µg/ml) IAA production was recorded with isolate SRI₁. Viable count after 72 h of incubation varies from (32.00×10⁶ to 47.80×10⁶ cfu/ml) and (23.67×10⁶ to 55.60×10⁶ cfu/ml) for isolates from organic and inorganic plant samples, respectively. Final pH of the supernatant ranges from 4.99 to 6.01. IAA has been implicated in virtually every aspect of plant growth and development, as well as defense responses [36]. IAA is one of the physiologically most active auxins which is a common product of L-tryptophan metabolism of several plant growth promoting microorganisms [37]. Production of HCN and ammonia by microorganisms has been suggested as an important biofertilizer and biocontrol feature to enhance the plant growth. Selected forty four bacterial endophytes were screened for HCN and ammonia production on King's B medium and peptone broth, respectively. Only 65.90 (29/44) per cent isolates were able to produce ammonia and 36.36 (16/44) per cent isolates were HCN producers (Table 6). Data in Table 6 also revealed that selected isolates were screened for chitinase, protease and amylase enzyme activities. Out of total selected isolates, thirty seven (84.09 per cent) showed chitinase activity with Enzyme Index (EI) ranging from 1.19 to 2.64. Maximum

Table 7: Percent Growth inhibition of test fungus by selected bacterial endophytes of chrysanthemum (*Dendranthema grandiflora* Tzvelev).

Solates	Per cent growth inhibition (%GI) against		
	<i>Rhizoctonia solani</i>	<i>Pythium ultimum</i>	<i>Fusarium oxysporum</i>
ISOLATES FROM ORGANIC PLANT SAMPLES			
HS ₂	35.56	67.56	44.44
N3S ₃	35.12	48.22	46.67
N3S ₆	31.78	47.56	54.00
N3S ₇	30.44	70.44	29.78
N4S ₆	27.56	42.22	38.67
N4S ₉	ND	ND	ND
N4S ₁₀	43.11	59.33	49.78
RDO ₂	42.67	46.67	43.78
RDO ₃	43.23	56.78	45.89
RDO ₁₀	48.89	77.78	40.00
RDO ₁₂	CI	ND	32.00
RDO ₁₃	37.11	40.89	ND
RDO ₁₄	38.67	35.78	ND
SRO ₄	39.67	42.45	51.00
SRO ₇	32.45	40.21	38.00
SRO ₈	ND	39.33	34.22
Mean	30.39	44.70	34.26
ISOLATES FROM INORGANIC PLANT SAMPLES			
HS ₁₄	31.11	ND	35.22
HS ₁₇	36.67	39.33	38.67
HS ₁₈	45.00	38.00	50.44
HS ₁₉	ND	ND	ND
HS ₂₀	46.67	45.50	43.25
HS ₂₃	45.33	40.00	55.56
HS ₂₄	ND	45.67	ND
N1S ₃	37.11	70.44	ND
N1S ₂₃	32.00	37.11	31.11
N1S ₂₄	46.89	46.67	41.56
N1S ₂₅	43.11	76.44	43.11
N1S ₂₆	40.00	54.88	41.56
N2S ₆	42.22	58.67	68.89
N2S ₁₄	35.56	48.22	42.22
N2S ₁₆	32.00	37.11	ND
N2S ₁₈	36.44	72.66	40.89
N2S ₁₉	ND	ND	42.22
N2S ₂₀	45.33	66.67	59.33
N2S ₂₁	48.89	52.00	52.19
IDR ₅	38.67	51.11	42.22
IDR ₆	ND	ND	ND
IDR ₇	31.11	54.88	43.78
IDR ₈	30.44	CI	44.46

SRI ₁	41.56	50.44	34.22
SRI ₃	ND	ND	ND
SRI ₁₄	ND	29.38	ND
SRI ₁₅	32.33	CI	42.22
SRI ₂₁	45.33	31.45	37.11
Mean	30.84	37.37	33.22
CD_{0.05}			
OvsINO	NS	0.81	NS
WOR	3.45	3.68	10.44
WINO	3.45	3.68	10.44

ND= not detected, CI= contact inhibition

*Per cent growth inhibition (%GI) = $\frac{T-C}{C} \times 100$, Where, C: growth of fungus in control; T: Growth of fungus in test.

(2.64) EI was recorded for isolate RDO₁₀, whereas, minimum (1.19) was recorded with isolate IDR₈. Thirty two (72.72 per cent) and thirty eight (86.36 per cent) isolates exhibited protease and amylase activity with EI ranging from (1.45 to 3.66) and (1.17 to 2.55), respectively. Maximum (3.66) EI for protease enzyme activity was noted with isolate N1S₂₅ and minimum (1.45) with isolate HS₁₈. Similarly, maximum (2.55) EI for amylase enzyme activity was noted with isolate N1S₂₅ and minimum (1.17) with isolate IDR₈. Bacterial endophytes protects the plants from the fungal cell wall or cell membrane degradation caused by fungi and insects, by degrading cell membrane proteins or extracellular virulence factors, or by stimulating systemic resistance in plants [38]. HCN is recognized as a biocontrol agent, based on its ascribed toxicity against plant pathogens [39]. The level of HCN produced by bacteria *in vitro* is not only correlated with biocontrol activity but also indirectly increase the availability of phosphate. Ammonia production is responsible for the indirect plant growth promotion and can serve as a triggering factor by suppressing plant pathogens [40]. The production of lytic enzyme has been considered with defence related mechanisms which has been documented by Jetiyanon [41] who found that a mixture of *B. amyloliquefaciens* strain IN937a and *B. pumilus* strain IN937b induced the production of defence related enzymes against the pathogen. Extracellular enzyme production like chitinase, lipase, protease, amylase contributed to the ability of bacteria isolated from *Valeriana officinalis* to suppress the fungal diseases and thus demonstrated the potential of PGPR for biological control [42].

In vitro antifungal activity (Table 7) of all the selected forty four endophytic isolates was tested against phytopathogenic fungi viz. *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium oxysporum*. Bacterial isolates showed variation in antifungal activity against the tested fungal pathogens. Data in present table revealed that thirty six (81.81 per cent), thirty seven (84.09 per cent) and thirty four (77.27 per cent) isolates showed percent Growth Inhibition (%GI) against *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium oxysporum*, respectively. Among isolates obtained from organic plant samples, maximum (48.89 and 77.78 per cent) growth inhibition was recorded with isolates RDO₁₀ against *Rhizoctonia solani* and *Pythium ultimum* while minimum (27.56 and 35.78 per cent) growth inhibition was shown by isolate N4S₆ and RDO₁₄ against *Rhizoctonia solani* and *Pythium ultimum*, respectively. Also, maximum (54.00

Table 8: Genetic diversity of selected bacterial isolates on the basis of phylogenetic analysis.

Endophytes	Base pairs	Accession number	Closest relative	Per cent similarity BLASTn	Phylogenetic group	Strain designation
ISOLATES FROM ORGANIC PLANT SAMPLES						
HS ₂	1025	MN186788	<i>Stenotrophomonas maltophilia</i> strain ATCC 13637	97.54	Gammaproteobacteria	<i>Stenotrophomonas maltophilia</i> strain HS2
N3S ₃	555	MN186799	<i>Bacillus velezensis</i> strain CBMB205	97.10	Firmicutes	<i>Bacillus velezensis</i> strain N3S3
N3S ₆	856	MN186803	<i>Bacillus amyloliquefaciens</i> strain MPA 1034	99.18	Firmicutes	<i>Bacillus amyloliquefaciens</i> strain N3S6
N3S ₇	708	MN242732	<i>Lysinibacillus pakistanensis</i> strain NCCP-54	98.15	Firmicutes	<i>Lysinibacillus pakistanensis</i> strain N3S7
N4S ₆	944	MN186795	<i>Bacillus subtilis</i> strain IAM 12118	98.06	Firmicutes	<i>Bacillus subtilis</i> strain N4S6
N4S ₉	871	MN186793	<i>Micrococcus luteus</i> strain NCTC 2665	99.66	Actinobacteria	<i>Micrococcus luteus</i> strain N4S9
N4S ₁₀	922	MN186783	<i>Bacillus licheniformis</i> strain DSM 13	99.56	Firmicutes	<i>Bacillus licheniformis</i> strain N4S10
RDO ₂	1174	MN186791	<i>Bacillus wiedmannii</i> strain FSL W8-0169	97.08	Firmicutes	<i>Bacillus wiedmannii</i> strain RDO2
RDO ₃	863	MN186796	<i>Phyllobacterium ifriqiyense</i> strain STM 370	99.77	Alphaproteobacteria	<i>Phyllobacterium ifriqiyense</i> strain RDO3
RDO ₁₀	916	MN186774	<i>Bacillus subtilis</i> strain IAM 12118	98.91	Firmicutes	<i>Bacillus subtilis</i> strain RDO10
RDO ₁₂	723	MN242729	<i>Bacillus aryabhatai</i> B8W22	99.17	Firmicutes	<i>Bacillus aryabhatai</i> strain RDO12
RDO ₁₃	1115	MN186787	<i>Serratia nematodiphila</i> strain NBRC 102204	96.45	Gammaproteobacteria	<i>Serratia nematodiphila</i> strain RDO13
RDO ₁₄	1058	MN186808	<i>Stenotrophomonas maltophilia</i> strain IAM 12423	99.33	Gammaproteobacteria	<i>Stenotrophomonas maltophilia</i> strain RDO14
SRO ₄	791	MN186789	<i>Microbacterium testaceum</i> strain DSM 20166	98.48	Actinobacteria	<i>Microbacterium testaceum</i> strain SRO4
SRO ₇	925	MN186797	<i>Bacillus toyonensis</i> strain BCT-7112	99.67	Firmicutes	<i>Bacillus toyonensis</i> strain SRO7
SRO ₈	654	MN242742	<i>Stenotrophomonas pavanii</i> strain LMG25348	99.24	Gammaproteobacteria	<i>Stenotrophomonas pavanii</i> strain SRO8
ISOLATES FROM INORGANIC PLANT SAMPLES						
HS ₁₄	995	MN186781	<i>Bacillus mojavensis</i> strain ifo 15718	99.09	Firmicutes	<i>Bacillus mojavensis</i> strain HS14
HS ₁₇	797	MN242733	<i>Stenotrophomonas bentonitica</i> strain BII-R7	90.59	Gammaproteobacteria	<i>Stenotrophomonas bentonitica</i> strain HS17
HS ₁₈	867	MN186805	<i>Stenotrophomonas rhizophila</i> strain e-p10	93.22	Gammaproteobacteria	<i>Stenotrophomonas rhizophila</i> strain HS18
HS ₁₉	750	MN186806	<i>Stenotrophomonas bentonitica</i> strain BII-R7	95.97	Gammaproteobacteria	<i>Stenotrophomonas bentonitica</i> strain HS19
HS ₂₀	1024	MN186802	<i>Cellulosimicrobium funkei</i> strain W6122	98.91	Actinobacteria	<i>Cellulosimicrobium funkei</i> strain HS20
HS ₂₃	926	MN186786	<i>Pseudomonas aeruginosa</i> strain NRBC 12689	99.57	Gammaproteobacteria	<i>Pseudomonas aeruginosa</i> strain HS23
HS ₂₄	633	MN186784	[<i>Pseudomonas</i>] <i>hibiscicola</i> strain ATCC 19867	95.55	Gammaproteobacteria	[<i>Pseudomonas</i>] <i>hibiscicola</i> strain HS24
N1S ₃	962	MN186794	<i>Bacillus halotolerans</i> strain DSM 2802	98.86	Firmicutes	<i>Bacillus halotolerans</i> strain N1S3
N1S ₂₃	900	MN242728	<i>Serratia nematodiphila</i> strain DZ0503SBS1	99.67	Gammaproteobacteria	<i>Serratia nematodiphila</i> strain N1S23
N1S ₂₄	787	MN186780	<i>Bacillus tequilensis</i> strain 10b	98.05	Firmicutes	<i>Bacillus tequilensis</i> strain N1S24
N1S ₂₅	1227	MN186776	<i>Bacillus subtilis</i> strain JCM 1465	95.11	Firmicutes	<i>Bacillus subtilis</i> strain N1S25
N1S ₂₆	1014	MN186807	<i>Streptomyces rubiginosohelvolus</i> strain NBRC 12912	99.01	Actinobacteria	<i>Streptomyces rubiginosohelvolus</i> strain N1S26
N2S ₆	360	MN186777	<i>Pseudomonas aeruginosa</i> strain DSM 50071	96.30	Gammaproteobacteria	<i>Pseudomonas aeruginosa</i> strain N2S6
N2S ₁₄	1226	MN186775	<i>Serratia marcescens</i> strain NBRC 102204	97.58	Gammaproteobacteria	<i>Serratia marcescens</i> strain N2S14
N2S ₁₆	638	MN186800	<i>Serratia nematodiphila</i> strain DZ0503SBS1	99.06	Gammaproteobacteria	<i>Serratia nematodiphila</i> strain N2S16
N2S ₁₈	994	MN186778	<i>Bacillus subtilis</i> strain NRBC 13719	99.69	Firmicutes	<i>Bacillus subtilis</i> strain N2S18
N2S ₁₉	999	MN186798	<i>Serratia marcescens</i> strain NBRC 102204	98.15	Gammaproteobacteria	<i>Serratia marcescens</i> strain N2S19
N2S ₂₀	983	MN186779	<i>Bacillus aryabhatai</i> B8W22	98.88	Firmicutes	<i>Bacillus aryabhatai</i> strain N2S20
N2S ₂₁	1216	MN186792	<i>Klebsiella grimontii</i> strain SB73	94.22	Gammaproteobacteria	<i>Klebsiella grimontii</i> strain N2S21
IDR ₅	1003	MN186801	<i>Bacillus subtilis</i> strain IAM 12118	98.60	Firmicutes	<i>Bacillus subtilis</i> strain IDR5

IDR ₆	189	MN242731	<i>Pantoea ananatis</i> strain 1846	97.27	Gammaproteobacteria	<i>Pantoea ananatis</i> strain IDR6
IDR ₇	927	MN186810	<i>Arthrobacter globiformis</i> strain JCM 1332	97.51	Actinobacteria	<i>Arthrobacter globiformis</i> strain IDR7
IDR ₈	922	MN186804	<i>Microbacterium trichothecenolyticum</i> strain DSM 8608	98.80	Actinobacteria	<i>Microbacterium trichothecenolyticum</i> strain IDR8
SRI ₁	968	MN186785	<i>Bacillus subtilis</i> strain BRCC 10255	97.83	Firmicutes	<i>Bacillus subtilis</i> strain SRI1
SRI ₃	930	MN186790	<i>Bacillus pseudomycoloides</i> strain NBRC 101232	99.68	Firmicutes	<i>Bacillus pseudomycoloides</i> strain SRI3
SRI ₁₄	870	MN186782	<i>Staphylococcus sciuri</i> strain DSM 20345	99.31	Firmicutes	<i>Staphylococcus sciuri</i> strain SRI14
SRI ₁₅	879	MN186809	<i>Bacillus megaterium</i> strain ATCC 14581	99.54	Firmicutes	<i>Bacillus megaterium</i> strain SRI15
SRI ₂₁	304	MN242730	<i>Bacillus flexus</i> strain SBMP3	99.34	Firmicutes	<i>Bacillus flexus</i> strain SRI21

per cent) growth inhibition was observed with isolates N3S₆ against *Fusarium oxysporum*, respectively. The minimum (29.78 per cent) growth inhibition was observed with isolates N3S₇ against *Fusarium oxysporum*. Similarly, among isolates obtained from inorganic plant samples, maximum (48.89, 76.44 and 68.89 per cent) growth inhibition was recorded with isolates N2S₂₁, N1S₂₅ and N2S₆ against *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium oxysporum*, respectively, while minimum (30.44, 29.38 and 31.11 per cent) growth inhibition was shown by isolate IDR₈, SRI₁₄ and N1S₂₃ against *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium oxysporum*, respectively. Whereas, between isolates from organic and inorganic plant samples, maximum (44.70) per cent growth inhibition against *Pythium ultimum* was shown by isolates from organic plant samples which is statistically higher than isolates from inorganic plant samples (37.37 per cent). However, no significant difference was recorded in per cent growth inhibition against *Rhizoctonia solani* and *Fusarium oxysporum* by isolates from organic and inorganic plant samples. The results are in line with Sharma *et al.* [36] who reported maximum per cent growth inhibition i.e. 76.12 per cent against *Pythium ultimum* with S₆ isolate, 42.22 per cent against *Rhizoctonia solani* with S₅ isolate and 75.44 per cent against *Fusarium oxysporum* with S₁ isolate. Biological control using microorganisms has been studied intensively by many researchers as an effective alternative to control pests/diseases [43,44]. The formation of zone is due to the secretion of antifungal substances that might have diffused in the medium and resulted in the fungal growth inhibition.

Biochemical Characterization of Selected Bacterial Endophytes

Morphological and biochemical characterization were used to identify the isolated bacterial endophytes upto genus level as per Bergey's Manual of Determinative Bacteriology. (Table 8) revealed that out of total forty four isolates, only nineteen (43.18 per cent) isolates were positive for indole test, fourteen (31.81 per cent) isolates showed positive response for methyl red test, twenty six (59.09 per cent) isolates were positive for Voges Proskauer test, twenty three (52.27 per cent) were able to utilize citrate. Hydrogen sulfide production was observed with only twelve (27.27 per cent) isolates. Number of bacterial isolates that showed positive results for different biochemical tests varied as thirty two (72.72 per cent) for catalase, twenty three (52.27 per cent) for oxidase, twenty eight (63.63 per cent) for lipase production. Twenty four (54.54 per cent) isolates were able to hydrolyse gelatin, while twenty eight (63.63 per cent) were able to hydrolyse starch. Whereas, twenty seven (61.36 per cent), thirty three

(75.00 per cent) and twenty nine (65.90 per cent) isolates were able to ferment dextrose, lactose and sucrose, respectively. The results of the present study are in line with that of Ghani *et al.* and Sharma [45,46].

Genetic Diversity of The Selected Bacterial Endophytic Isolate(S) Associated With Chrysanthemum (*Dendranthema Grandiflora Tzvelev*) By 16S rDNA Sequencing

To assess and compare the genetic diversity of culturable bacterial endophytes of chrysanthemum (*Dendranthema grandiflora Tzvelev*) isolated from organic and inorganic samples collected from different districts of Himachal Pradesh, sequence analysis of 16S rDNA gene was conducted. Sequence analysis of forty four isolates, based on BLASTn search revealed the presence of bacteria belonging to 14 different genus *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Lysinibacillus*, *Micrococcus*, *Streptomyces*, *Pantoea*, *Klebsiella*, *Phyllobacterium*, *Serratia*, *Microbacterium*, *Cellulosimicrobium*, *Arthrobacter* and *Staphylococcus*. The isolates exhibited nucleotide similarity with the nearest relatives in the NCBI GenBank database ranging from 90.59 to 99.77 per cent. Among endophytic bacteria *Bacillus* has been reported as most dominant genera [47,48] which support our findings. In general, the Phylum Proteobacteria, including the Classes α , β and γ -Proteobacteria, were reported to be dominant in diversity analysis of endophytes, although members of the Firmicutes are also among the classes most consistently found as endophytes.

Conclusion

Our research effort are towards helping the poor farmers as the focus of this study is on isolation, screening and characterization of plant growth promoting bacteria and their role in plant growth promotion. A pool of promising PGPB 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 PGPB have potential to be used as biofertilizers as well as bioprotectant agents having the potential to supplement the chemical fertilizers and pesticides. From the present investigation it is clear that selected isolates have potential to act as biofertilizer, biostimulant and bioprotectant.

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