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# 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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**Abstract**

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; Genectic 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: Pikovskaya; 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




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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 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 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, Banglore) 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/multialin/multialin.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

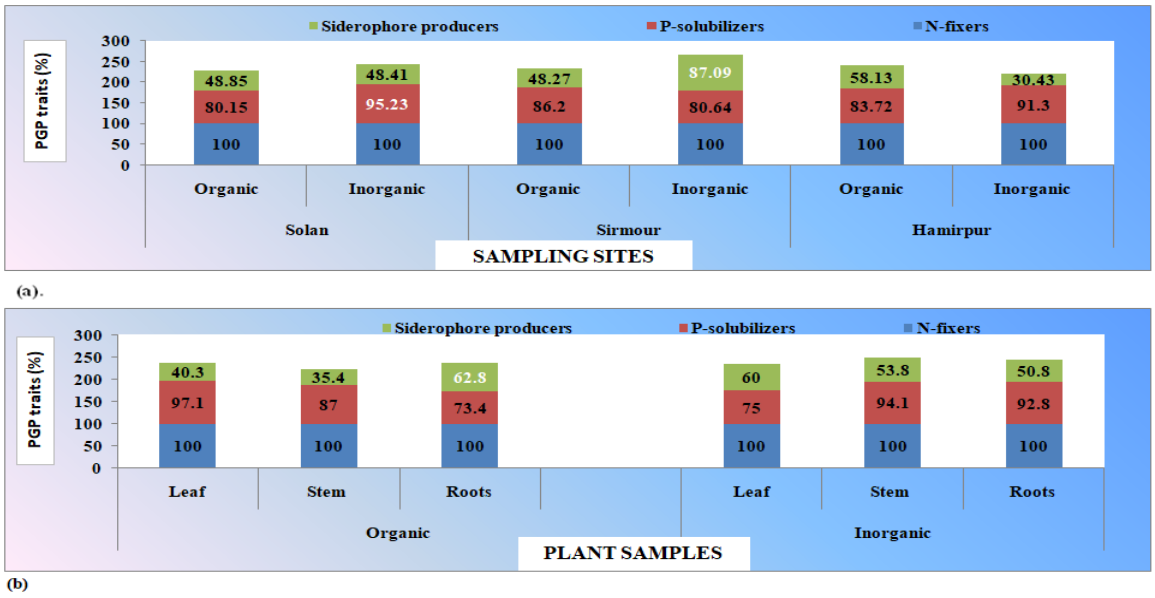


**Figure 1:** Characterization of bacterial endophytes isolated from (a). Different sites of sampling and (b). Different plant parts of organic and inorganic samples for phosphate solubilization, siderophore production and ability to fix nitrogen.

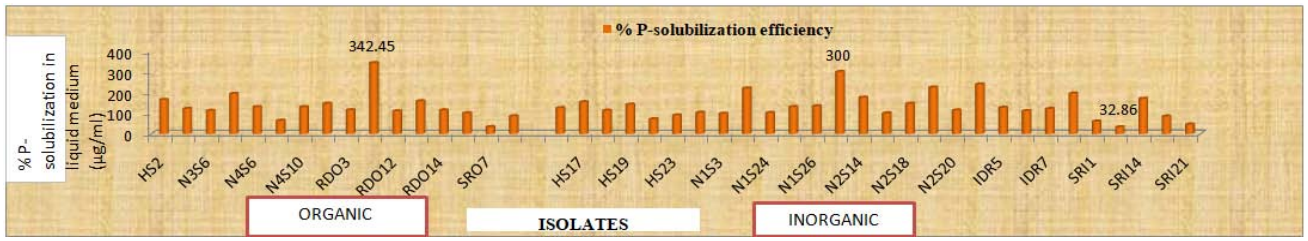


**Figure 2:** Percent P-solubilization efficiency (% SE) of selected bacterial endophytes on solid medium.

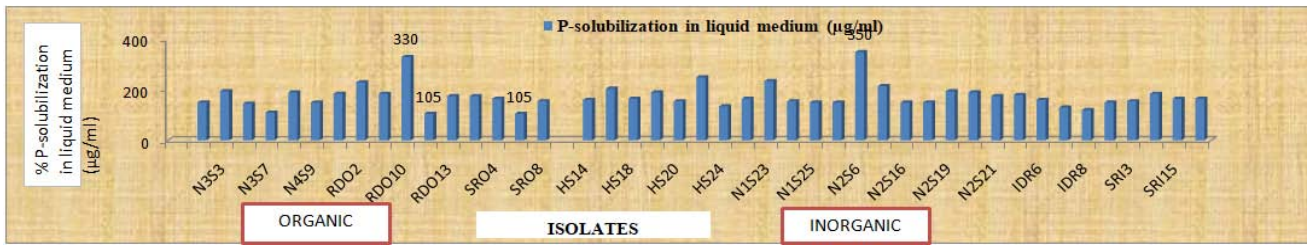


**Figure 3:** P-solubilization efficiency (µg/ml) of selected bacterial endophytes in liquid medium.

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 $MgSO_4$, 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

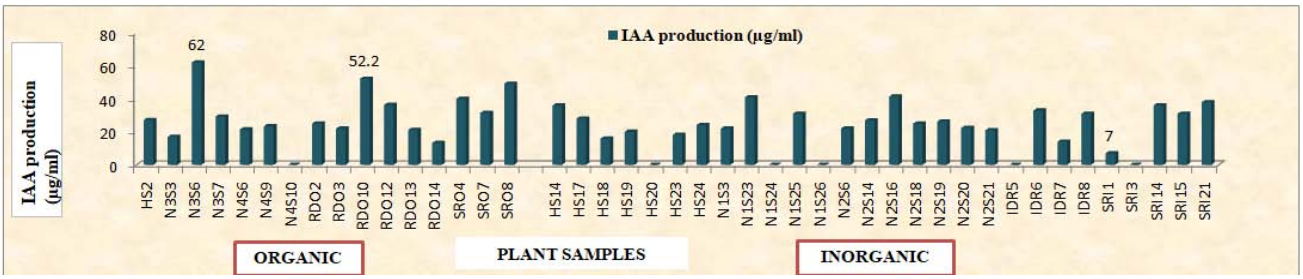


**Figure 4:** IAA production by selected bacterial endophytic isolates in Luria Bertani broth.

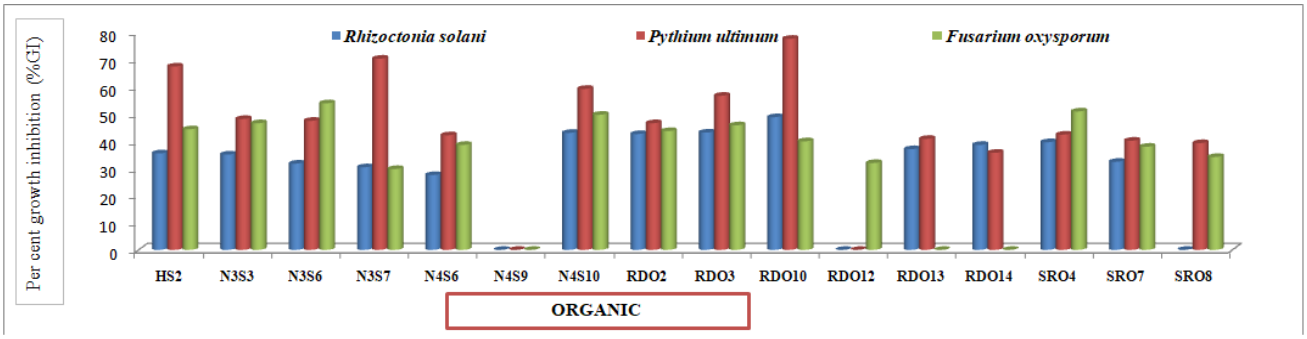


**Figure 5(a):** Percent growth inhibition by selected bacterial isolates isolated from organic plant samples of Chrysanthemum against *Rhizoctonia solani, Pythium ultimum and Fusarium oxysporum.*

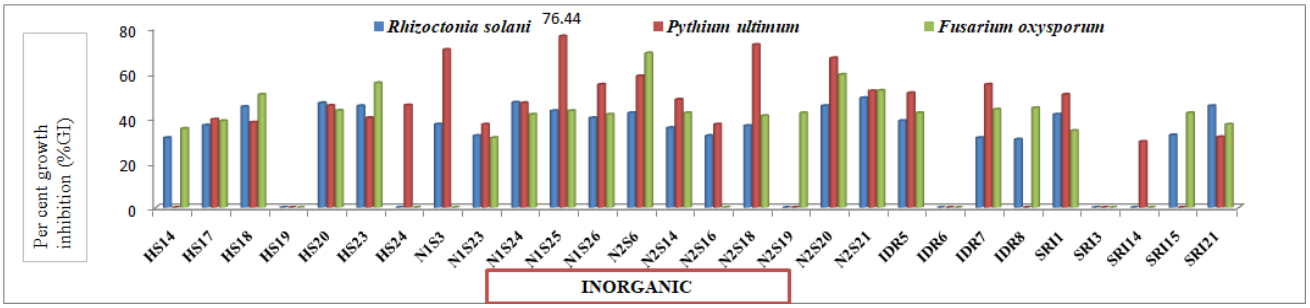


**Figure 5(b):** Percent growth inhibition by selected bacterial isolates isolated from inorganic plant samples of Chrysanthemum against *Rhizoctonia solani, Pythium ultimum* and *Fusarium oxysporum.*

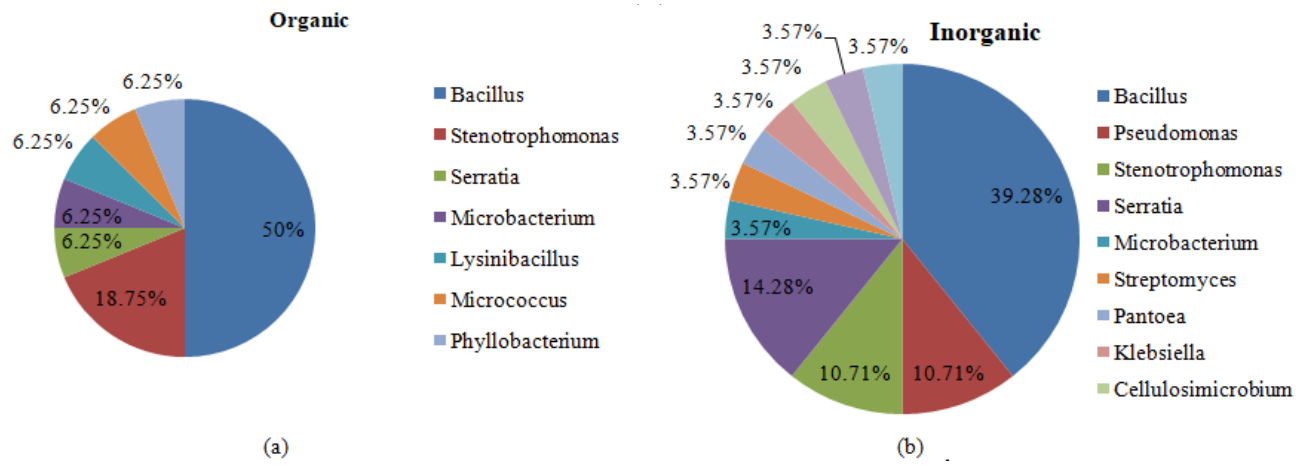


**Figure 6(a):** Relative abundance of endophytic bacteria at genus level associated with (a) organic plant samples and (b) inorganic plant samples.

**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 ($74.05\times10^2$ cfu/g roots) viable count was recorded for root samples, which was found to be significantly more than stem ($57.59\times10^2$ cfu/g stem) and leaf samples ($52.75\times10^2$ cfu/g leaf). However, the maximum ($68.97\times10^2$ cfu/g sample) count was recorded for Nauni (Solan) location which was statistically at par with ($68.34\times10^2$ cfu/g sample) Deothi (Solan) location, whereas, minimum ($55.02\times10^2$ cfu/g sample) for Neri (Hamirpur) location. Among different media used for isolation of bacterial endophytes, maximum ($72.45\times10^2$ cfu/g sample) viable count was registered for tryptic soya agar medium, which was statistically at par with nutrient agar medium ($72.13\times10^2$ cfu/g sample) while minimum ($39.49\times10^2$ 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 ($65.87\times10^2$ cfu/g roots) viable count was recorded for root samples and minimum ($50.11\times10^2$ cfu/g leaf) for leaf samples. For different sites, the maximum ($66.66\times10^2$ cfu/g sample) count was recorded for Nauni (Solan) location which was at par with ($65.64\times10^2$ cfu/g sample) Deothi (Solan) location and minimum ($49.06\times10^2$ cfu/g sample) for Neri (Hamirpur) location. Among different media used for isolation of bacterial endophytes, maximum ($6.315\times10^2$ cfu/g sample) viable count was registered for both nutrient agar and tryptic soya agar medium and minimum ($39.49\times10^2$ cfu/g sample) was recorded for King's B medium. The endophytic bacterium actually resides within apoplastic 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, physic-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 ($10^6$ × 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 $RDO_{10}$ and minimum (1.33) PSI was recorded with isolate $SRO_7$. While, within isolates of inorganic samples, the maximum (4.00) Phosphate Solubilizing Index (PSI) was recorded with isolate $N2S_6$ and minimum (1.32) PSI was recorded with isolate $SRI_3$. 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 $RDO_{10}$ had highest (342.45 per cent) P-solubilization efficiency, however, the lowest (33.33 per cent) phosphate Solubilizing Efficiency (%SE) was recorded with isolate $SRO_7$. Whereas, within isolates of inorganic samples, the isolate $N2S_6$ had highest (300.00 per cent) P-solubilization efficiency, however, the lowest (32.86 per cent) phosphate Solubilizing Efficiency (%SE) was recorded with isolate $SRI_3$. 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 (µg/ml) by different bacterial endophytes of chrysanthemum (*Dendranthema grandiflora Tzvelev*).

| Isolates | Viable Count ($10^6$ × cfu/ml) | Indole-3-acetic acid (µg/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 $RDO_{10}$ isolate, whereas minimum (105 µg/ml) was recorded for $RDO_{12}$ and $SRO_7$ isolates. However, within isolates from inorganic samples, the maximum (350.00 µg/ml) P-solubilization was recorded for $N2S_6$ isolate, whereas minimum (120 µg/ml) was recorded for $IDR_8$ isolate. Also the viable count after 72 h of incubation varied from ($44\times10^6$ to $92\times10^6$ cfu/ml) and ($37\times10^6$ to $104\times10^6$ 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 $Mg^{2+}$, $Ca^{2+}$, $Al^{3+}$ and $Fe^{3+}$, 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 Azuerol 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 hydroxymate (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 $RDO_{10}$ 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 $SRI_3$ and maximum (186.23 %SU) siderophore production was recorded with isolate $IDR_5$. 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 $N3S_6$ which is statistically higher than that of all other isolates and minimum (13.30 µg/ml) IAA production was recorded with isolate $RDO_{14}$. Similarly, within isolates from inorganic plant samples, maximum (56.00 µg/ml) IAA production was recorded with isolate $N2S_6$ and minimum (7.00 µg/ml) IAA production was recorded with isolate $SRI_1$. Viable count after 72 h of incubation varies from ($32.00\times10^6$ to $47.80\times10^6$ cfu/ml) and ($23.67\times10^6$ to $55.60\times10^6$ cfu/ml) fror 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 | | |
|---|---|---|---|
| | *Rhizoctonia solani* | *Pythium ultimum* | *Fusarium oxysporum* |
| **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) = $\frac{C-T}{C}\times 100$ , Where, C: growth of fungus in control; T: Growth of fungus in test.

(2.64) EI was recorded for isolate $RDO_{10}$, whereas, minimum (1.19) was recorded with isolate $IDR_8$. 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 $N1S_{25}$ and minimum (1.45) with isolate $HS_{18}$. Similarly, maximum (2.55) EI for amylase enzyme activity was noted with isolate $N1S_{25}$ and minimum (1.17) with isolate $IDR_8$. 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 productionis 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 peoduction 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 $RDO_{10}$ against *Rhizoctonia solani* and *Pythium ultimum* while minimum (27.56 and 35.78 per cent) growth inhibition was shown by isolate $N4S_6$ and $RDO_{14}$ 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 | *Stenotrophomonas maltophilia* strain ATCC 13637 | 97.54 | Gammaproteobacteria | *Stenotrophomonas maltophilia* strain HS2 |
| **$N3S_3$** | 555 | MN186799 | *Bacillus velezensis* strain CBMB205 | 97.10 | Firmicutes | *Bacillus velezensis* strain N3S3 |
| **$N3S_6$** | 856 | MN186803 | *Bacillus amyloliquefaciens* strain MPA 1034 | 99.18 | Firmicutes | *Bacillus amyloliquefaciens* strain N3S6 |
| **$N3S_7$** | 708 | MN242732 | *Lysinibacillus pakistanensis* strain NCCP-54 | 98.15 | Firmicutes | *Lysinibacillus pakistanensis* strain N3S7 |
| **$N4S_6$** | 944 | MN186795 | *Bacillus subtilis* strain IAM 12118 | 98.06 | Firmicutes | *Bacillus subtilis* strain N4S6 |
| **$N4S_9$** | 871 | MN186793 | *Micrococcus luteus* strain NCTC 2665 | 99.66 | Actinobacteria | *Micrococcus luteus* strain N4S9 |
| **$N4S_{10}$** | 922 | MN186783 | *Bacillus licheniformis* strain DSM 13 | 99.56 | Firmicutes | *Bacillus licheniformis* strain N4S10 |
| **$RDO_2$** | 1174 | MN186791 | *Bacillus wiedmannii* strain FSL W8-0169 | 97.08 | Firmicutes | *Bacillus wiedmannii* strain RDO2 |
| **$RDO_3$** | 863 | MN186796 | *Phyllobacterium ifriqiyense* strain STM 370 | 99.77 | Alphaproteobacteria | *Phyllobacterium ifriqiyense* strain RDO3 |
| **$RDO_{10}$** | 916 | MN186774 | *Bacillus subtilis* strain IAM 12118 | 98.91 | Firmicutes | *Bacillus subtilis* strain RDO10 |
| **$RDO_{12}$** | 723 | MN242729 | *Bacillus aryabhattai* B8W22 | 99.17 | Firmicutes | *Bacillus aryabhattai* strain RDO12 |
| **$RDO_{13}$** | 1115 | MN186787 | *Serratia nematodiphila* strain NBRC 102204 | 96.45 | Gammaproteobacteria | *Serratia nematodiphila* strain RDO13 |
| **$RDO_{14}$** | 1058 | MN186808 | *Stenotrophomonas maltophilia* strain IAM 12423 | 99.33 | Gammaproteobacteria | *Stenotrophomonas maltophilia* strain RDO14 |
| **$SRO_4$** | 791 | MN186789 | *Microbacterium testaceum* strain DSM 20166 | 98.48 | Actinobacteria | *Microbacterium testaceum* strain SRO4 |
| **$SRO_7$** | 925 | MN186797 | *Bacillus toyonensis* strain BCT-7112 | 99.67 | Firmicutes | *Bacillus toyonensis* strain SRO7 |
| **$SRO_8$** | 654 | MN242742 | *Stenotrophomonas pavanii* strain LMG25348 | 99.24 | Gammaproteobacteria | *Stenotrophomonas pavanii* strain SRO8 |
| **ISOLATES FROM INORGANIC PLANT SAMPLES** | | | | | | |
| **$HS_{14}$** | 995 | MN186781 | *Bacillus mojavensis* strain ifo 15718 | 99.09 | Firmicutes | *Bacillus mojavensis* strain HS14 |
| **$HS_{17}$** | 797 | MN242733 | *Stenotrophomonas bentonitica* strain BII-R7 | 90.59 | Gammaproteobacteria | *Stenotrophomonas bentonitica* strain HS17 |
| **$HS_{18}$** | 867 | MN186805 | *Stenotrophomonas rhizophila* strain e-p10 | 93.22 | Gammaproteobacteria | *Stenotrophomonas rhizophila* strain HS18 |
| **$HS_{19}$** | 750 | MN186806 | *Stenotrophomonas bentonitica* strain BII-R7 | 95.97 | Gammaproteobacteria | *Stenotrophomonas bentonitica* strain HS19 |
| **$HS_{20}$** | 1024 | MN186802 | *Cellulosimicrobium funkei* strain W6122 | 98.91 | Actinobacteria | *Cellulosimicrobium funkei* strain HS20 |
| **$HS_{23}$** | 926 | MN186786 | *Pseudomonas aeruginosa* strain NRBC 12689 | 99.57 | Gammaproteobacteria | *Pseudomonas aeruginosa* strain HS23 |
| **$HS_{24}$** | 633 | MN186784 | *[Pseudomonas] hibiscicola* strain ATCC 19867 | 95.55 | Gammaproteobacteria | *[Pseudomonas] hibiscicola* strain HS24 |
| **$N1S_3$** | 962 | MN186794 | *Bacillus halotolerans* strain DSM 2802 | 98.86 | Firmicutes | *Bacillus halotolerans* strain N1S3 |
| **$N1S_{23}$** | 900 | MN242728 | *Serratia nematodiphila* DZ0503SBS1 | 99.67 | Gammaproteobacteria | *Serratia nematodiphila* strain N1S23 |
| **$N1S_{24}$** | 787 | MN186780 | *Bacillus tequilensis* strain 10b | 98.05 | Firmicutes | *Bacillus tequilensis* strain N1S24 |
| **$N1S_{25}$** | 1227 | MN186776 | *Bacillus subtilis* strain JCM 1465 | 95.11 | Firmicutes | *Bacillus subtilis* strain N1S25 |
| **$N1S_{26}$** | 1014 | MN186807 | *Streptomyces rubiginosohelvolus* strain NBRC 12912 | 99.01 | Actinobacteria | *Streptomyces rubiginosohelvolus* strain N1S26 |
| **$N2S_6$** | 360 | MN186777 | *Pseudomonas aeruginosa* strain DSM 50071 | 96.30 | Gammaproteobacteria | *Pseudomonas aeruginosa* strain N2S6 |
| **$N2S_{14}$** | 1226 | MN186775 | *Serratia marcescens* strain NBRC 102204 | 97.58 | Gammaproteobacteria | *Serratia marcescens* strain N2S14 |
| **$N2S_{16}$** | 638 | MN186800 | *Serratia nematodiphila* strain DZ0503SBS1 | 99.06 | Gammaproteobacteria | *Serratia nematodiphila* strain N2S16 |
| **$N2S_{18}$** | 994 | MN186778 | *Bacillus subtilis* strain NRBC 13719 | 99.69 | Firmicutes | *Bacillus subtilis* strain N2S18 |
| **$N2S_{19}$** | 999 | MN186798 | *Serratia marcescens* strain NBRC 102204 | 98.15 | Gammaproteobacteria | *Serratia marcescens* strain N2S19 |
| **$N2S_{20}$** | 983 | MN186779 | *Bacillus aryabhattai* B8W22 | 98.88 | Firmicutes | *Bacillus aryabhattai* strain N2S20 |
| **$N2S_{21}$** | 1216 | MN186792 | *Klebsiella grimontii* strain SB73 | 94.22 | Gammaproteobacteria | *Klebsiella grimontii* strain N2S21 |
| **$IDR_5$** | 1003 | MN186801 | *Bacillus subtilis* strain IAM 12118 | 98.60 | Firmicutes | *Bacillus subtilis* strain IDR5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **$IDR_6$** | 189 | MN242731 | *Pantoea ananatis* strain 1846 | 97.27 | Gammaproteobacteria | *Pantoea ananatis* strain IDR6 |
| **$IDR_7$** | 927 | MN186810 | *Arthrobacter globiformis* strain JCM 1332 | 97.51 | Actinobacteria | *Arthrobacter globiformis* strain IDR7 |
| **$IDR_8$** | 922 | MN186804 | *Microbacterium trichothecenolyticum* strain DSM 8608 | 98.80 | Actinobacteria | *Microbacterium trichothecenolyticum* strain IDR8 |
| **$SRI_1$** | 968 | MN186785 | *Bacillus subtilis* strain BRCC 10255 | 97.83 | Firmicutes | *Bacillus subtilis* strain SRI1 |
| **$SRI_3$** | 930 | MN186790 | *Bacillus pseudomycoides* strain NBRC 101232 | 99.68 | Firmicutes | *Bacillus pseudomycoides* strain SRI3 |
| **$SRI_{14}$** | 870 | MN186782 | *Staphylococcus sciuri* strain DSM 20345 | 99.31 | Firmicutes | *Staphylococcus sciuri* strain SRI14 |
| **$SRI_{15}$** | 879 | MN186809 | *Bacillus megaterium* strain ATCC 14581 | 99.54 | Firmicutes | *Bacillus megaterium* strain SRI15 |
| **$SRI_{21}$** | 304 | MN242730 | *Bacillus flexus* strain SBMP3 | 99.34 | Firmicutes | *Bacillus flexus* strain SRI21 |

per cent) growth inhibition was observed with isolates $N3S_6$ against *Fusarium oxysporum,* respectively. The minimum (29.78 per cent) growth inhibition was observed with isolates $N3S_7$ 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 $N2S_{21}$, $N1S_{25}$ and $N2S_6$ 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 $IDR_8$, $SRI_{14}$ and $N1S_{23}$ 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 $SJ_6$ isolate, 42.22 per cent against *Rhizoctonia solani* with $SR_5$ isolate and 75.44 per cent against *Fusarium oxysporum* with $SN_1$ 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 hydolyse 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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