

Original Research Article

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Characterization of *Pseudomonas aeruginosa* MCC 3198 and its Potential for Growth Promotion of Seedlings of the Medicinal Plant *Celosia cristata* L.

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ABSTRACT

Conservative methods of harvest propagation to develop expansion and yield of vegetation underneath in diverse ecological threats are lengthy and not triumphant in many cases. Use of expensive injurious agrochemicals and pesticides causes stern danger to environment and renders growth of challenging pathogens. At present time, more and more attention has been made towards cost-effective and environment-friendly alternatives to improve natural wealth and assist plant development. Beneficial bacteria, particularly in the rhizosphere of plants, are deliberated and established to exert growth-promoting activities. These important rhizobacteria consist of the symbiotic *Rhizobium* species, few specific actinomycetes, mycorrhizal fungi and some free-living bacteria. Plant growth promoting rhizobacteria (PGPR) are a cluster of favorable bacteria which have the prospective for improving plant growth, development and yield. Research works on the effect of PGPR on medicinal plants are very limited. Accordingly, the present communication is dealt with the characterization of PGPR bacteria isolated from the rhizosphere of little explored medicinal herb, *Celosia cristata* L. The bacterium was identified as *Pseudomonas aeruginosa* (MCC 3198). Seed germination, seedling vigor, root length, shoot length, leaf count, dry and fresh weight, chlorophyll content and some defense enzymes of the plant *C. cristata* have significantly been improved as a result of application of this bacteria.

Keywords

Celosia cristata,
PGPR,
Pseudomonas
aeruginosa

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Introduction

Plant growth-promoting rhizobacteria (PGPRs) is a specific group of soil bacteria that aggressively colonize the rhizosphere and rhizoplane, and substantially improve plant growth and productivity. PGPR work as plant growth promoters and biological control agents *via* direct or indirect mechanisms.

Direct mechanisms by PGPRs include the provision of bioavailable phosphorus and nitrogen for plant uptake, sequestration of iron by siderophores, production of plant hormones like, auxins, cytokinins, and gibberellins, and lowering ethylene levels inside plants using ACC deaminase that accumulate in plants subjected to biotic and abiotic stresses (Glick, 1995; Glick *et*

al., 1999; Mayak *et al.*, 2004). The indirect mechanisms include the production of antibiotics, reducing iron availability for phytopathogens in the rhizosphere, enzymatic lysis of fungal cell wall and insect-gut membrane secreting chitinase enzyme for the hydrolysis of chitin layer of the eggshell of nematodes, competition with detrimental microorganisms for sites on plant roots, and induction of systemic resistance in plants against various pathogens and pests (Ramamoorthy *et al.*, 2001). Bacterial strains showing PGPR activity have been reported for diverse bacterial taxa including *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, and *Serratia* (Gray and Smith, 2005).

To date, PGPRs have been shown to promote the growth of cereals, ornamentals, vegetables, and food crops (Vessey, 2003; Lugtenberg and Kamilova, 2009; Mishra *et al.*, 2010). However, a limited number of studies have been undertaken regarding the interactions between PGPRs and medicinal or aromatic plants. This communication, therefore, aims to bring in-verified or putative mechanisms by which PGPRs promote seed germination, intensification, nutrient acquisition, and defense response in aromatic and medicinal plants.

Materials and Methods

Isolation and screening of Plant Growth Promoting Rhizobacteria (PGPR)

The plant *Celosia cristata* L. were collected from the garden of the department of Botany, The University of Burdwan, Burdwan (23.2565951, 87.8434078) to isolate PGPR. The plant was maintained with proper care in the garden. The Rhizobacteria were isolated from the root adhering soil of *C. cristata* L.

by serial dilution technique and plated on Pikovskaya's agar medium (Pikovskaya, 1948) [Yeast extract-0.50 g; Dextrose-10.00 g; Calcium phosphate-5.00 g; Ammonium phosphate-0.50 g; Potassium chloride-0.20 g; Magnesium sulphate-0.10 g; Manganese sulphate-0.0001 g; Ferrous sulphate-0.0001 g; Agar-15.00 g; Water – 1000 ml; pH – 6.5].

Scattered colonies appeared in the plate after 3 days of incubation at $30 \pm 1^\circ$ C were observed and one colony (SD/B) was selected as it exhibited the highest diameter of halo zone around the colony. Pure cultured of the isolate SD/B was then made by dilution streak method and maintained in the same medium with a sub-culturing period of 15 days and used for further studies.

Identification and phylogeny of the selected isolate SD/B

Phenotype based identification

The selected phenotypic characterization such as cell size, colony morphology, gram staining behaviour and oxygen requirement was studied following the methods of Benson (2002).

Molecular identification

The molecular identification of the selected SD/B isolate was done by 16S rDNA sequencing. For this, the genomic DNA was isolated by the standard chloroform extraction method (Sambrook *et al.*, 1989) followed by PCR amplification of 16S rDNA. The universal primers 5'-GAG TTT GAT CCT GGC TCA G-3' was used as forward primer and 5'-AGA AAG GAG GTG ATC CAG CC-3' was used as reverse primer. The amplified PCR product was purified by PEG-NaCl precipitation and sequenced in an automated DNA Sequencer (Applied Biosystems Inc., Foster city, CA). Sequence

assembly was carried out by the Lasergene package software and identified by EZ-Taxon search considering the 16S rDNA sequence of SD/B as query sequence. The 16S rDNA sequence was deposited to NCBI to obtain sequence accession number.

Construction of phylogenetic tree

The phylogenetic tree of the selected isolate SD/B was built using the 16S rDNA sequences of valid strains obtained from EZ-taxon, National Centre for Biotechnology Identification (NCBI) and Ribosomal Database Project (RDP) sequence database searching. MEGA7 Software (Kumar *et. al.*, 2016) was used to construct the phylogenetic tree.

Characterization of plant growth promoting traits of SD/B strain

Phosphate solubilization

The phosphate solubilizing ability of the bacteria SD/B was detected using Pikovskaya's agar plates. The phosphate solubilizing ability was observed by using di-calcium phosphate (DCP), tri-calcium phosphate (TCP) and zinc phosphate (ZP) separately in Pikovskaya's agar medium for detecting a clear zone around the colonies.

Quantitative estimation of phosphates solubilization was determined following ammonium molybdate method (Yoon SJ *et al.*, 1996).

Test for nitrogen fixation

The ability to fix atmospheric nitrogen was tested by inoculating the isolate SD/B in the Asbay's Mannitol Agar media without ready source of nitrogen i.e. Ammonium sulphate and they are allowed to incubate at $30\pm 2^\circ$ C temperature for 3 days. Occurrence of

bacterial growth indicates its ability to fix atmospheric nitrogen.

Indole-3-acetic acid (IAA) production

Qualitative and quantitative Indole-3-acetic acid (IAA) Production by the bacterial strain (SD/B) was done by the method of Brick *et.al.* (1991).

Hydrocyanic acid (HCN) production

HCN production was determined by the picrate assay (Lorck, 1948).

Ammonia production

The ammonia production ability of the isolate was tested using the method of Cappuccino and Sherman (1992).

Assay of siderophore production

The Fe(III) specific ligand as deterrent of soil borne phytopathogens was assayed following the method of Schwyn and Neilands (1987).

Protease activity

Protease activity was determined following Chaiharn (2008) [Composition of protein precipitating reagent was- Mercuric chloride – 3g, Concentrated HCl – 4ml, Distilled water – 20ml]

In vitro plant growth promotion experiment

Mature *C. cristata* L. seeds were surface sterilized with 0.1% HgCl₂ for 3 min followed by successive washing with sterile distilled water and then the water was decanted. The seeds were kept for 10 minutes in the broth culture of bacteria on the log phase containing 4.9×10^6 colony forming unit (CFU)/ml. For germination, seeds were then placed in

sterilized Petri plates containing moist filter paper at 25 ± 2 °C for 3 days in the dark and then transferred to a growth room for 11 days [28 ± 2 °C temperature, RH 70-80%, illumination $270 \text{ mE m}^{-2} \text{ s}^{-1}$ (for 12 h)]. Seeds treated with sterilized medium without bacterial culture were considered as control. After germination the seedlings were examined for following parameters like-

Study of the effect of SD/B on seed germination and morphological parameters of the seedlings

After completion of 14 days of growth, various growth parameters like, Germination percentage, root length, shoot length and seedling vigor index were recorded.

Germination percentage

The germination percentage of both the control and PGPR treated sets were calculated by using the following formula

$$\text{Germination Percentage} = \frac{\text{Total no. of seed germinated}}{\text{total no of seeds in all replicates}} \times 100$$

Seedling vigor index

Seedling vigor index (SVI) was calculated according to Abdul-Baki and Anderson (1973) by using following equation-

$$\text{SVI} = \text{SDW (or SL)} \times \text{GP}$$

Where, GP- germination percentages, SDW- seedling dry weight and SL- seedling length (shoot length + root length) in mm, respectively.

Estimation of total chlorophyll

Estimation of total chlorophyll of seedlings was done following Acetone extraction method of Arnon (1949).

Study of the effect of SD/B on biochemical parameters of seedlings

Estimation of proline content: Proline content of control and PGPR treated seedlings was done following the method of Bates *et al.*, (1973).

Enzyme extraction from seedlings: 1g tissue of freshly grown seedling from each set (control as well as PGPR treated sets) were taken and homogenized in pre-chilled mortar pestle containing 10ml of ice cold 50mM Tris – Acetate buffer of pH-6.0. Then the homogenate were centrifuged at 14000 rpm in 4°C temperature for 20 minutes. The supernatant were then filtered through Sephadex G-25 column, and was ready to estimate the activities of defense enzymes like, SOD, Catalase, Polyphenol oxidase and Peroxidase.

Estimation of SOD activity of seedlings

It was measured using the method of Beauchamp and Fridovich, (1973) and expressed as Unit/g.fw.

Estimation of catalase activity of seedlings

It was measured using the method of Aebi, (1984) and expressed as EU/min/g fresh weight.

Estimation of peroxidase activity of seedling

It was done using the method of Addy and Goodman, (1972) and expressed as Unit/ml.

Estimation of ascorbate oxidase activity of seedling

It was done using the method of Vines and Oberbacher (1965) and expressed as Units/ml enzyme.

Results and Discussion

The plant growth promoting rhizobacteria possess important PGP traits that enhance plant growth and maintain plant health essential for sustainable agriculture. Moreover, it also possesses the ability to improve the growth of medicinal plant correspondingly increase secondary metabolite production. Considering this PGPR was isolated from *Celosia cristata* L, followed by its characterization and testing for the plant growth promotion ability.

Isolation and screening of Plant Growth Promoting Rhizobacteria (PGPR)

The Rhizobacteria were isolated from the root adhering soil of *C. cristata* L. by serial dilution technique and plated on Pikovskaya's agar medium. The isolated bacteria were selected based on the diameter of the halo zone which indicated their phosphate solubilizing ability and accordingly characterised for identification.

Identification and phylogeny of the selected isolate SD/B

Phenotype based identification

The phenotypic characterization of the selected SD/B was made as per standard method which are given in Table 1 and Figure 1a,b.

Molecular identification

Based on 16S rDNA sequence homology, the strain SD/B showed 100% pair wise similarity with *Pseudomonas aeruginosa* [JCM 10591] (Table 1-a). The phylogenetic tree revealed that the SD/B strain formed the cluster with *Pseudomonas aeruginosa* JCM 5962(T). The 16 S rDNA of SD/B strain is given below in FASTA format and strain accession number

of this strain obtained from MCC is MCC3198.

Information about other close homologs for the microbes can be obtained from the Alignment View table (Table 1a).

FASTA Sequence of SD/B (1421 bp)

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AGAGTTTGATCATGGCTCAGATTGAAC
GCTGGCGGCAGGCCTAACACATGCAA
GTCGAGCGGATGAAGGGAGCTTGCTC
CTGGATTCAGCGGCGGACGGGTGAGT
AATGCCTAGGAATCTGCCTGGTAGTGG
GGGATAACGTCCGGAAACGGGCGCTA
ATACCGCATAACGTCCTGAGGGAGAAA
GTGGGGGATCTTCGGACCTCACGCTAT
CAGATGAGCCTAGGTCGGATTAGCTAG
TTGGTGGGGTAAAGGCCTACCAAGGC
GACGATCCGTAACCTGGTCTGAGAGGAT
GATCAGTCACACTGGAAGTGAACAC
GGTCCAGACTCCTACGGGAGGCAGCA
GTGGGGAATATTGGACAATGGGCGAA
AGCCTGATCCAGCCATGCCGCGTGTGT
GAAGAAGGTCTTCGGATTGTAAAGCA
CTTTAAGTTGGGAGGAAGGGCAGTAA
GTAAATACCTTGCTGTTTTGACGTTACC
AACAGAATAAGCACCGGCTAACTTCGT
GCCAGCAGCCGCGGTAATACGAAGGG
TGCAAGCGTTAATCGGAATTACTGGGC
GTAAAGCGCGCGTAGGTTGGTTCAGCA
AGTTGGATGTGAAATCCCCGGGCTCAA
CCTGGGAACTGCATCCAAAATACTGA
GCTAGAGTACGGTAGAGGGTGGTGGGA
ATTTCTGTGTAGCGGTGAAATGCGTA
GATATAGGAAGGAACACCAGTGGCGA
AGGCGACCACCTGGACTGATACTGAC
ACTGAGGTGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCA
CGCCGTAAACGATGTGACTAGCCGTT
GGGATCCTTGAGATCTTAGTGGCGCAG
CTAACGCGATAAGTCGACCGCCTGGG
GAGTACGGCCGCAAGGTTAAAACCTCA
AATGAATTGACGGGGGCCCGCACAAAG
CGGTGGAGCATGTGGTTTAAATTCGAAG
CAACGCGAAGAACCTTACCTGGCCTTG
```

ACATGCTGAGAACTTTCCAGAGATGGA
TTGGTGCCTTCGGGAACTCAGACACAG
GTGCTGCATGGCTGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTAAAGTCCCGTA
ACGAGCGCAACCCTTGTCCTTAGTTAC
CAGCACCTCGGGTGGGCACTCTAAGG
AGACTGCCGGTGACAAACCGGAGGAA
GGTGGGGATGACGTCAAGTCATCATG
GCCCTTACGGCCAGGGCTACACACGTG
CTACAATGGTCGGTACAAAGGGTTGCC
AAGCCGCGAGGTGGAGCTAATCCCAT
AAAACCGATCGTAGTCCGGATCGCAGT
CTGCAACTCGACTGCGTGAAGTCGGAA
TCGCTAGTAATCGTGAATCAGAATGTC
ACGGTGAATACGTTCCCGGGCCTTGTA
CACACCGCCCGTACACCATGGGAGTG
GGTTGCTCCAGAA

Construction of phylogenetic tree

Based on 16S rDNA sequences of valid strains obtained from EZ-taxon, National Centre for Biotechnology Identification (NCBI) and Ribosomal Database Project (RDP) sequence database a phylogenetic tree of SD/B strain was made (Fig. 2). The evolutionary history was inferred using the Maximum Parsimony method. Tree #1 out of 2 most parsimonious trees (length = 479) is shown. The consistency index is (0.697674), the retention index is (0.666667), and the composite index is 0.648573 (0.465116) for all sites and parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches (Felsenstein, 1985). The MP tree was obtained using the Sub-tree-Pruning-Regrafting (SPR) algorithm (Nei and Kumar, 2000) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). The analysis involved 8 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1329 positions in the final dataset.

Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016).

Characterization of plant growth promoting traits of SD/B strain

The plant growth promoting (PGP) traits are essential for plant growth promotion. Following PGP traits of the selected SD/B strain are detected and these are given in Table 2 and 3 (Fig. 3; Plate a, b, c, e, f).

This table shows the results of the quantitative tests done for the isolated PGPR where it has been found that the PGPR of our interest solubilize 24 ppm of phosphate per ml; produce 35 ppm of IAA/ml; The OD values of P₄ and IAA has been put to respective standard curve to determine the concentration.

Study of the effect of SD/B on seed germination, morphological and biochemical parameters of the seedlings

The effect of the selected SD/B isolate on Seed Germination, Morphological and Biochemical Parameters of the Seedlings was made as per standard method which are given in Table 4 (Fig. 3; Plate g, h, i, j, k, l, m).

Table 4 shows that the PGPR treatment displayed higher seedling vigor index than control or untreated seedlings which were 24.699 and 5.64 respectively. The chlorophyll-a, b, total chlorophyll content of treated plants have been recorded to be 125 mg/ml, 325 mg/ml, 450 mg/ml respectively which were also appeared to be higher than control. In cases of the stress, Proline and defense enzyme like, Peroxidase activity of the non-treated plants were found to be 0.228 $\mu\text{mol g}^{-1}$ FW and 94 Unit/ml/min respectively in contrast to 0.138 $\mu\text{mol g}^{-1}$ FW and 243 Unit/ml/min of the treated seedlings. Correspondingly, the defense enzymes like, Catalase (0.185 EU/min/g fresh weight),

Superoxide Dismutase (SOD) (231 Unit/g fresh weight/min) and Ascorbate oxidase (0.265 units/min/g fw) were higher in treated seedlings than that of control ones (0.120 EU/min/g fresh weight; 188 Unit/min/g.fw, 0.250 units/min/g fw of the enzymes like, Catalase, SOD and Ascorbate oxidase respectively).

The rhizosphere represent one of the most intricate ecosystem on Earth by means of about every root on the world anticipated to have a chemically, physically, and biologically exclusive rhizosphere. Regardless of its inherent complication, consideration of the rhizosphere is vital if we are to resolve some of the world's mainly imminent ecological crises, like, sustainable foodstuff, yarn and energy fabrication and

upliftment of active secondary metabolite production of medicinally important herbs.

This study gave us an insight into the bacterial community present in the rhizospheric soil of a medicinally important plant *C. cristata* L. from where we have isolated a bacteria exhibiting the highest diameter of halo zone around the colony on Pikovskaya's agar medium (Pikovskaya, 1948) and designated it as SD/B. In our study we found that the isolated bacterial strain SD/B to be *Pseudomonas aeruginosa*, which is a gram negative, aerobic bacteria of 0.8 to 1.2 μ M in length. *Pseudomonas aeruginosa* JCM 5962(T) was first isolated from soil and described by Schroeter, 1872; Migula, 1900; though, the plant growth promoting activity of this bacteria has not been reported earlier.

Table.1 Phenotypic characteristics of the isolate SD/B

Parameters	Result
Colony Morphology	Round, Raised
Cell Size	0.8 – 1.2 μ M
Shape	Small, Rod shaped
Gram Staining Behavior	Gram-negative
Oxygen Requirement	Aerobic

Table.1a Significant alignments produced by the sequence

Rank	Name	Strain	Authors	Accession	Pair wise Similarity(%)
1	<i>Pseudomonas aeruginosa</i>	JCM 5962(T)	(Schroeter 1872) Migula 1900	BAMA01000 316	100
2	<i>Pseudomonas aeruginosa</i>	paerg010	Noll B,N.2018	LR130536.1	100
3	<i>Pseudomonas aeruginosa</i>	paerg011	Noll B,N.2018	LR130535	100
4	<i>Pseudomonas aeruginosa</i>	paerg012	Noll B,N.2018	LR130537.1	100
5	<i>Pseudomonas aeruginosa</i>	paerg005	Noll B,N.2018	LR130534.1	100
6	<i>Pseudomonas aeruginosa</i>	paerg009	Noll B,N.2018	LR130533.1	100
7	<i>Pseudomonas aeruginosa</i>	paerg004	Noll B,N.2018	LR130531.1	100
8	<i>Pseudomonas aeruginosa</i>	paerg003	Noll B,N.2018	LR130530.1	100
9	<i>Pseudomonas aeruginosa</i>	paerg000	Noll B,N.2018	LR130528.1	100
10	<i>Pseudomonas aeruginosa</i>	paerg002	Noll B,N.2018	LR130527.1	100

Table.2 Qualitative estimation of PGPR traits

Characters	Bacterial strains
	SD/B
Phosphate solublization	++++
N ₂ fixation	++++
IAA production	+++
NH ₃ production	+++
HCN production	++++
Protease activity	++++
Siderophore activity	-

* (Data's are mean value of three replicates) [Values are mean ± SD; (n = 5) **P< 0.05, significant, compared to control]

The above table shows the results of the qualitative tests done for the isolated PGPR. '+' sign represented the test is positive, more '+' signs means more positive. '-' sign indicated the test is negative.

Table.3 Quantitative Estimation of PGPR Traits

Characters	Bacterial strains
	SD/B ± SD
Phosphate solublization	24 ± 0.026 ppm/ml
IAA production	35 ± 0.016 ppm/ ml

* (Data's are mean value of three replicates) [Values are mean ± SD; (n = 5) **P< 0.05, significant, compared to control]

This table shows the results of the quantitative tests done for the isolated PGPR where it has been found that the PGPR of our interest solubilize 24 ppm of phosphate per ml; produce 35 ppm of IAA/ml; The OD values of P₄ and IAA has been put to respective standard curve to determine the concentration.

Table.4 Effect on seed germination, seedling growth and defense

Estimation	Treatment	
	Control ± SD	Treated with PGPR ± SD
Germination Percentage	60 ± 0.25%	78.66 ± 0.34%
Root Length	1.0 ± 0.4 cm.	2.0 ± 0.19 cm.
Shoot Length	2.4 ± 0.03 cm.	4.0 ± 0.09cm.
Root Dry-weight	5 ± 0.036mg	37 ± 0.041mg
Shoot Dry-weight	89 ± 0.027mg	211 ± 0.059mg
Seedling vigor Index	5.64	24.699
Chlorophyll - a	20 ± 0.01 mg/ml	125 ± 0.07mg/ml
Chlorophyll - b	180 ± 0.07 mg/ml	325 ± 0.03 mg/ml
Total Chlorophyll	200 ± 0.08 mg/ml	450 ± 0.1 mg/ml
Proline content	0.228 ± 0.007 μmol g ⁻¹ FW	0.138 ± 0.004 μmol g ⁻¹ FW
Catalase activity	0.120 ± 0.006 EU/min/g fresh weight	0.185 ± 0.005 EU/min/g fresh weight
Superoxide Dismutase activity	188 ± 0.2 Unit/g.fw/min	231 ± 0.7 Unit/g.fw/min
Peroxidase activity	94 ± 0.74Unit/ml/min	243 ± 0.36 Unit/ml/min
Ascorbate oxidase activity	0.250 ± 0.001 units/min/ml enzyme	0.265 ± 0.005units/min/ml enzyme

* (Data's are mean value of three replicates) [Values are mean ± SD; (n = 5) **P< 0.05, significant, compared to control]

Fig.1 Microscopic view of bacterial slide (a) and SEM view of SD/B (b)

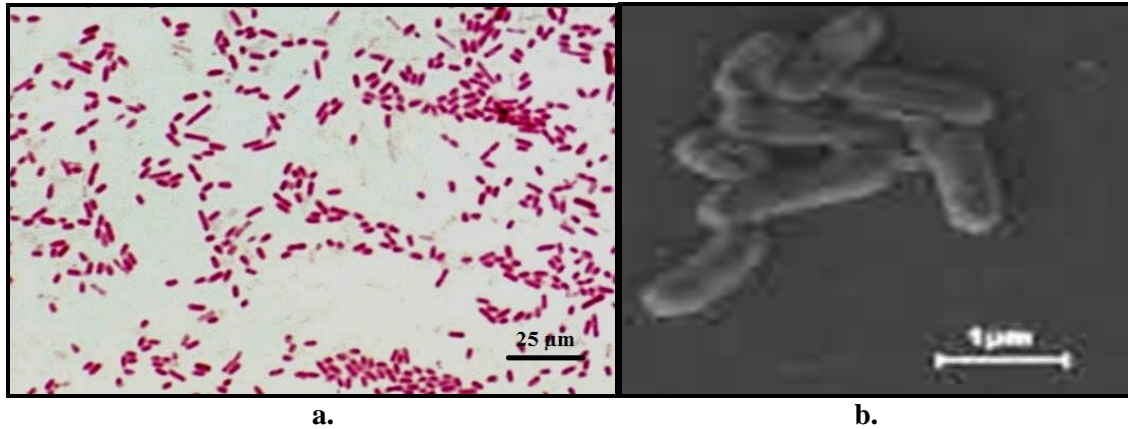


Fig.2 Phylogenetic tree of SD/B strain with other strains of homologous sequences

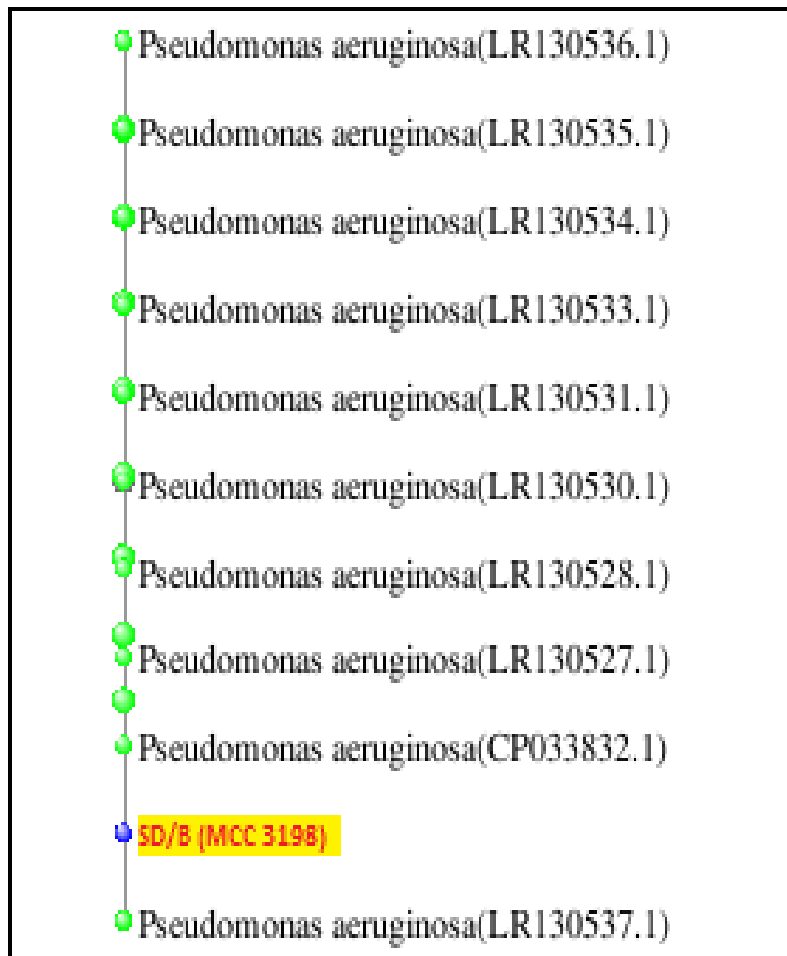
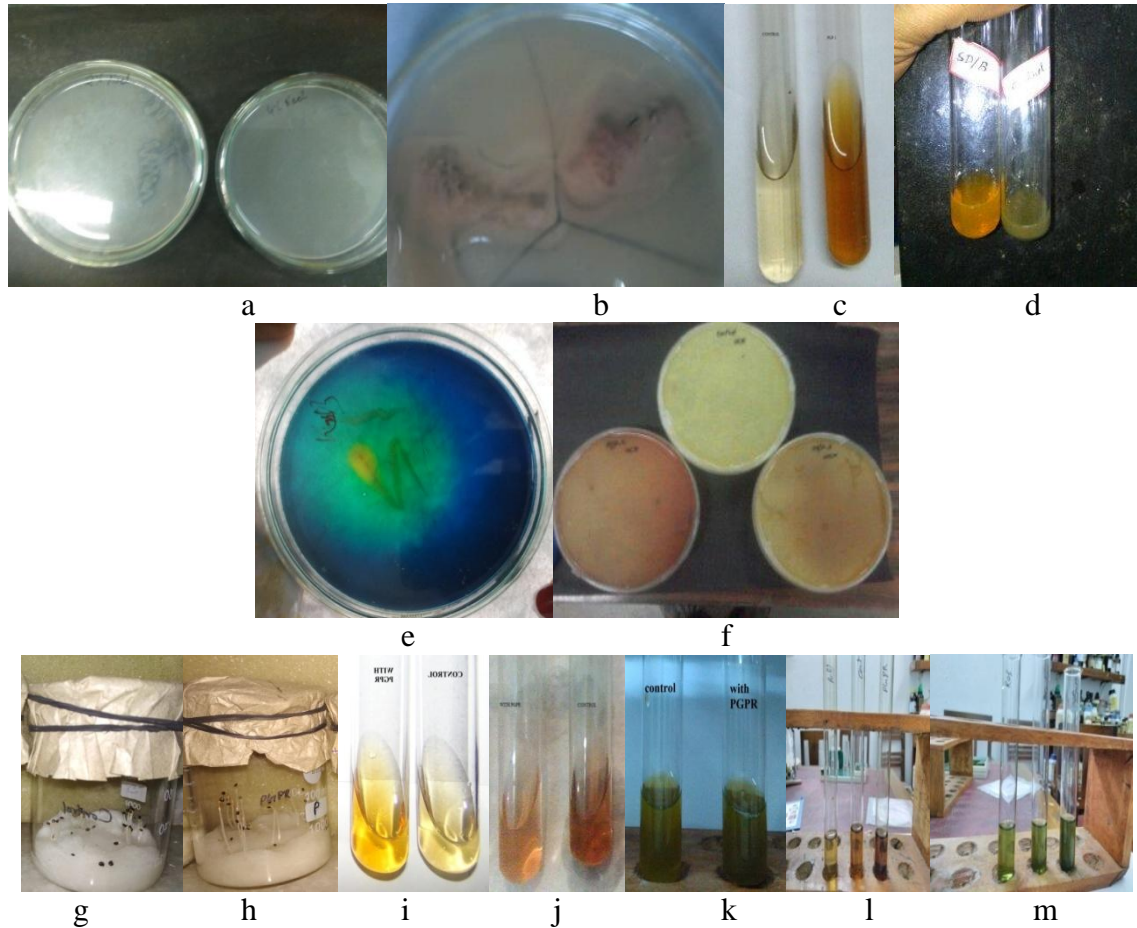


Fig.3 Plate(a) phosphate solublization; (b)- IAA qualitative assay; (c) - IAA quantitative assay; (d)- Ammonia productivity; (e)- Siderophore productivity; (f)- HCN productivity (g) – Control set of seed germination; (h) – PGPR treated set of seed germination; (i) - POX activity of seedling; (j) - Prolin content of treated seedling; (k) - Total chlorophyll of seedling;(l) - SOD activity of seedling; (m) - Peroxidase activity of seedling



The isolate SD/B exhibited some important PGP traits viz. phosphate solubilization ability, atmospheric N₂ fixing ability as well as IAA productivity, HCN production, ammonia production ability and protease activity in laboratory condition, which were the main criteria of a rhizobacteria to be designated as PGPR (Majeed *et al.*, 2015). These characteristics are considered as important plant growth promoting traits and have been found certainly useful in improving the growth and nitrogen contents of the tested plants by direct mechanism. As this strain was found to fix atmospheric nitrogen, produce

indole-3-acetic acid (IAA), siderophores and solubilize inorganic phosphate and HCN that are capable of stimulating plant growth and help plants to acquire sufficient iron, phosphate, and other essential nutrients for optimum growth (Glick, 1995; Chabot *et al.*, 1996; Rajkumar *et al.*, 2006; Idris *et al.*, 2007).

These important PGP traits found to enhance the plant growth when tested the effect of the bacteria on the seed germination of the ethno medicinal plant *C. cristata* L. Bacterial inoculant was able to increase plant growth

and germination rate, improve seedling emergence. It exposed that under in vitro conditions, seed treatment with PGPR strains improved seed germination, seedling vigor, seedling emergence, seedling stand, chlorophyll a, b and total chlorophyll content of the PGPR treated seedlings over the control. Comparable enhancement of seed germination parameters by rhizobacteria has been reported in other foliage such as sorghum (Raju *et al.*, 1999) and Lenin and Jayanthi (2012) showed that a formulation of PGPR including *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Pseudomonas fluorescens* and *Bacillus megaterium* significantly enhanced germination rate, vigour index and chlorophyll content of medicinal plant *Catharanthus roseus*.

Besides, this strain also found to enhance the defense related enzymes like superoxide dismutase, peroxidase, ascorbate oxidase, etc. of the treated plant. Which got support from the works of Gururani *et al.*, (2012) and Kohler *et al.*, (2010) PGPR enhances ROS-scavenging enzymes such as catalase and ascorbate peroxidase which may help the plants under salinity and drought pressure to stabilize the damaging effects of ROS. Similarly, Ramamoorthy *et al.*, (2002) and Dutta *et al.*, (2008) showed that, the treatment of pathogen-challenged tomato, hot pepper and pigeon pea with luminous *Pseudomonads* increased activities of POX and PPO. Similarly, Attia and Saad (2001) have showed that vegetative expansion and chemical concerto in medicinal plant *Catharanthus roseus* were promoted by the mutual management of *Azotobacter* and phosphate-solubilizing bacteria.

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