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Detection of Plant Growth Promoting Traits in *Pseudomonas lurida* AH1 Isolated from Rhizosphere of *Aconitum Heterophyllum* wall. ex royle

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Abstract: Psychrotrophic microbial inoculants are vital for temperate agriculture and horticulture, as major part of the crop season is characterized by cold. In the present study, an attempt has been made to isolate psychrotrophic *Pseudomonas lurida* from rhizosphere of Himalayan medicinal herb *Aconitum heterophyllum* Wall ex. Royale. The study suggests the possibility to explore potential microflora associated with rarely explored plant wealth of Himalaya. Psychrotrophic *Pseudomonas lurida* AH1 isolated by using traditional microbiological techniques from rhizosphere of *Aconitum heterophyllum* wall. Ex royle- a medicinal plant grown on high altitude of Northwest Himalaya. Bacterial isolate *Pseudomonas lurida* AH1 was identified by 16SrRNA gene sequence and has been registered in NCBI under the accession number MG74660. The strain was characterized for plant growth promoting traits viz., Phosphate Solubilization, *Ammonia Production, HCN production, Protease production*. Antagonistic activity of isolated strain against five soil-borne plant pathogens was also studied by using a dual culture ***in vitro*** assay. The present study proposes the use of *Pseudomonas lurida* AH1 as a potential plant growth promoting rhizobacteria in the context of cold agro-ecosystems. *Pseudomonas lurida* AH1 is capable to produce siderophore, HCN, ammonia, protease and has potential to solubilize phosphates. Also in the present investigation, *Pseudomonas lurida* AH1 was tested against deadly plant pathogens viz., *Alternaria solani, Botrytis cinera, Fusarium oxysporum, Rhizoctonia solani* and *Rosellina* sp.

Keywords: Biofertilizers; Psychrotrophs; *Pseudomonas*; PGPR; Proteolysis; Siderophores

1. Introduction

Extreme environmental conditions like drought, high salt and low temperature affect productivity of several agricultural and horticultural crops. Inoculation with efficient microbes exhibiting multiple plant growth promoting traits at low temperature could be a solution of enhancing the crop production. The genus *Pseudomonas* represents a physiologically and genetically diverse group with a great ecological significance. *Pseudomonas* strains and their products have been used in large-scale biotechnological applications. Fluorescent *Pseudomonads* form a considerable part of the microbial community in the rhizosphere and phyllosphere of plants. They are considered to be most promising group of plant growth promoting rhizobacteria (PGPR) involved in biocontrol of plant diseases.[1]

PGPR promote plant growth by several mechanisms which include induce systematic resistance, produce plant growth regulators like indole acetic acid, gibberellic acid, cytokinins and ethylene, asymbiotic Nitrogen fixation, antagonism against phytopathogenic microorganisms by production of siderophores, antibiotics and cyanide, fungal cell wall lysing enzymes which suppress the growth of fungal pathogens, solubilization of mineral phosphates and other nutrients.[2] Though, a number of PGPR have been reported but still very little is known about psychrotrophic PGPR.[3]

In the context of hill agriculture, psychrotrophic microbial inoculants are required as a major part of the crop season is characterized by cold.[4] In the present study, an attempt has been made to isolate psychrotrophic *Pseudomonas lurida* from rhizosphere of rarely explored high value threatened Himalayan medicinal herb *Aconitum heterophyllum* Wall ex. Royale. *Aconitum heterophyllum* is an *Ayurvedic* herb, roots of which are antibacterial, antitussive and anti-inflammatory especially used in diseases of children and also known to have digestive, carminative and *Tridosha* balancing properties. This is useful in viral fevers, diarrhea, dysentery etc. The species is endemic to the Himalayan region extending from northern Pakistan, the Indian State of Jammu and Kashmir, Himachal Pradesh, Uttarakhand (India) and further up to Nepal. Keeping in mind the indispensable properties of psychrotrophic rhizobacteria and high medicinal value of *Aconitum heterophyllum*, the objectives

of the present study is to isolate psychrotrophic rhizobacteria from rhizosphere of *Aconitum heterophyllum* and to evaluate its growth promoting traits to select and develop more efficient indigenous plant growth promoting and disease suppressing bioagents for cold loving crops.

2. Material and Methods

2.1. Samples Collection

Sangla valley of Himachal Pradesh, India (alpine and sub-alpine regions) was explored for high value medicinal plants, among which one of the most potent, *Aconitum heterophyllum* population was assessed by various ecological tools at an altitude of 3885m to 4205m. Five of the healthiest plants at their flowering stage which were more than 10m distant apart, were selected, and their rhizospheric soil was collected. For isolation process, one composite sample (~10g) was prepared by mixing different soil samples in an equal proportion in a sterilized zipped plastic bag and kept at 20°C in National Bureau of Plant Genetic Resource, Phagli, Regional Station, Shimla, and Himachal Pradesh, India.

2.2. Isolation, Purification and Screening of Bacterial Culture

Isolation of bacteria was performed by standard microbiological methods. Soil sample was diluted in a series of 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}, 10^{-10} and were spread on nutrient agar medium following incubation at 20°C. Bacterial colonies obtained were streaked on nutrient agar to obtain pure culture. Pure cultures were preserved on slants in refrigerator and 30% glycerol (-20°C) in deep freezer. Isolated bacteria were screened for their antagonistic potential against plant pathogens.

2.3. Identification of Screened Bacterial Isolate by 16SrRNA Gene Technique

2.3.1. Isolation of Genomic DNA

Potent screened isolate AH1 was subjected to molecular identification by 16S rRNA gene sequencing. Genomic DNA of AH1 was isolated by using standard protocol of DNA prepkit (*ZymoBIOMICS™* Make). DNA was quantified by using nanodrop spectrophotometer (*Thermo Fisher Scientific* make).

2.3.2. Amplification and Sequencing of Genomic DNA

PCR amplification of 16S rRNA region was carried out with following concentration of reagents i.e. Taq buffer (10 X)—5.0 µL; dNTP 2 mM—2.5 µL; primer (F)—1.0 µL; primer(R)—1.0 µL; Taq polymerase—0.2 µL; glycerol—0.5 µL; water— 12.8 µL; DNA—1 µL; MgCl2—1 µL. The procedure consisted of 35 cycles of 92°C for 1 min, 55°C for 1 min, 72 for 1 min. Samples were amplified by universal primers of 16S rRNA (8F 5'AGAGTTTGATCCTGGCTCAG3) and (1492 R 5'GGTTACCTTGTTACGACTT3). The amplified PCR product was cleaned up using PCR clean up kit (Real Genomics Hi Yield TM Make). Eluted PCR product was submitted to Xcelaris, Ahmedabad, India for sequencing process. Sequences were edited with the Sequence Navigator program (Applied Biosystems) and aligned using ClustalW (version 1.8; Infobiogene). For further analysis, sequence was compared with 16S rDNA sequences from appropriate reference strains registered in the GenBank database. After comparison it was submitted to NCBI to obtain accession no.

2.4. Plant Growth Promoting Traits of Isolated Bacterial Strains

2.4.1. Siderophore production[5,6]

Siderophore was detected by chrome azurol S (CAS) plate assays. CAS plates were prepared as follows:

Solution A: Sterilized blue agar was prepared by mixing chrome azurol (CAS) 60.5 mg/50 ml distilled water with 10 ml iron solution 1 mM $FeCl_3.6H_2O$ in 10 mMHCl. This solution was added to hexadecyltriethyl ammonium bromide (HDTMA) (72.9 mg/40ml distilled water).

Solution B: 750 ml nutrient agar was mixed with 1,4 piperazinediaethane sulphonic acid (30.24 g) pH was adjusted to 6.8 with NaOH (6.0 g) and autoclaved. To prepare CAS agar plates Solution A and Solution B were mixed and poured in Petri dishes. Bit of 72 h old isolate AH1 was placed on CAS agar plate which was incubated at 20°C for 72 h. Siderophore production was observed by formation of yellow or orange halo around the bit.

2.4.2. Phosphate Solubilization[7]

Bacterial isolates AH1 was screened for its tri-calcium phosphate (TCP) solubilizing activity on Pikovoskaya's agar plate. A Bit of 72 h old Pseudomonas lurida AH1 was placed on Pikovoskaya's agar plate (Hi Media make) followed by incubation at 20°C for 5 days. Phosphate solubilization was observed with a zone formation around bit. % Solubilization efficiency (SE) was calculated as follows:

% Solubilization efficiency (SE) = Z-C/100*10

2.4.3. HCN Production[8]

Pseudomonas lurida AH1 was streaked on King's medium (Hi Media Make) amended with glycine (4g/L). Whatman filter paper No 1 soaked in 2% sodium carbonate and then in 0.5% picric acid inside the top of the plate. Plate was sealed with parafilm and incubated at 20°C for 5 days. Production of HCN was observed in terms of change of colour of paper from deep yellow to orange brown to dark brown (++ to +++ to ++++). Control was kept un-inoculated for comparison of results.

2.4.4. Ammonia Production[9]

Bacterial isolate *Pseudomonas lurida* AH1 was inoculated in 5 ml peptone water in test tubes followed by incubation at 20°C for 5 days. 1 ml Nessler's reagent was added to each test tube to check presence of ammonia. Ammonia production was expressed in terms of change of colour of culture from faint yellow to brown (++ to +++++).

2.4.5. Protease Production[10]

Skim milk agar plates were prepared by adding sterilized skim milk to nutrient agar. A bit of 72 h old *Pseuodomonas lurida* AH1 was

cut with the help of sterilized borer and was placed on skim agar plate followed by incubation at 20°C for 72 h. Production of proteolytic activity expressed in terms of mm diameter of clear zone produced around the well.

2.4.6. Antibacterial Activities of Bacterial Isolates against Serious Plant Pathogens

Antimicrobial activity was checked by Bit/disk method as given below:

Indicators: The names of indicators selected for present study are serious plant pathogens viz: *Alternaria solani* MTCC 2101, *Botrytis cinerea* MTCC 2350, *Rhizoctonia solani* MTCC 4633, *Fusarium oxysporum* MTCC 7677, *Colletotrichum gloeosporioides* MTCC 9664, *Rosellinia* sp. MTCC 3878, *Sclerotinia sclerotiorum* MTCC 3878. All these indicators can grow with in a temperature range 20 to 25°C. These indicators were procured from CSIR-Microbial Type Culture Collection, Institute of Microbial Culture Collection, and Chandigarh, India.

Antagonistic test for bacterial isolates against pathogenic fungus: Antagonistic activity of isolated bacterial strains against plant pathogens were studied by using a dual culture ***in vitro*** assay.[11] For testing the antagonistic activity, bit of bacterial isolate AH1 was placed one side of Petri dish and mycelial disc from seven days old PDA culture of selected fungus was placed on the other side of the Petri dish i.e., both the bits were placed on an equal distance from the center of the Petri plate and incubated at 25±2°C for 5-7 days. Petri dishes inoculated with fungal discs alone served as control. This experiment was conducted in 3 replicates for each bacterial species. Percentage inhibition was calculated using the following formula:

Per cent inhibition (I) = C-T/C ×100

C- mycelial growth of pathogen in control.
T- mycelial growth of pathogen in dual culture plate.

Based on antagonistic potential of bacterial isolate AH1 it was selected for the present study and was identified using 16SrRNA gene technique.

3. Results

3.1. Isolation of Bacterial Isolates

Psychrotrophic bacterial isolate AH1 was isolated from the composite soil sample of the rhizospheres of *Aconitum heterophyllum* of Sangla valley (from altitude of 3885m to 4205m amsl) in District Kinnaur of Himachal Pradesh, India which lies in northwestern Himalaya (Fig. 1). *Aconitum heterophyllum* is a threatened high value Ayurvedic medicinal herb found in Himalaya and especially used to cure several types of diseases. In total seven numbers of bacterial isolates were isolated by standard microbiological techniques on nutrient agar medium followed by incubation at 20°C.

3.2. Screening and identification of bacterial isolate AH1

Screening of potential bacterial isolate out of 7 isolated bacterial isolates was done on the basis of its antagonistic behavior against serious plant pathogens. Antagonism was checked by Bit/disk method. Plant pathogens were procured from Microbial Type Culture Collection (IMTECH, Chandigarh). Selected plant pathogens can grow with in a temperature range 20°C to 25°C. These plant pathogens have been selected for the present study after reviewing the published literature. Bacterial isolate AH1 was found to inhibit 6 selected pathogens out of 7 (Table 1).

The most promising isolate AH1 was selected for further study as it exhibited maximum percent inhibition. The isolate AH1 was identified by 16SrRNA gene technique and it was identified as Pseudomonas lurida AH1 (Fig. 2) and has been submitted to Genbank databases. It is registered under the accession number MG746601.

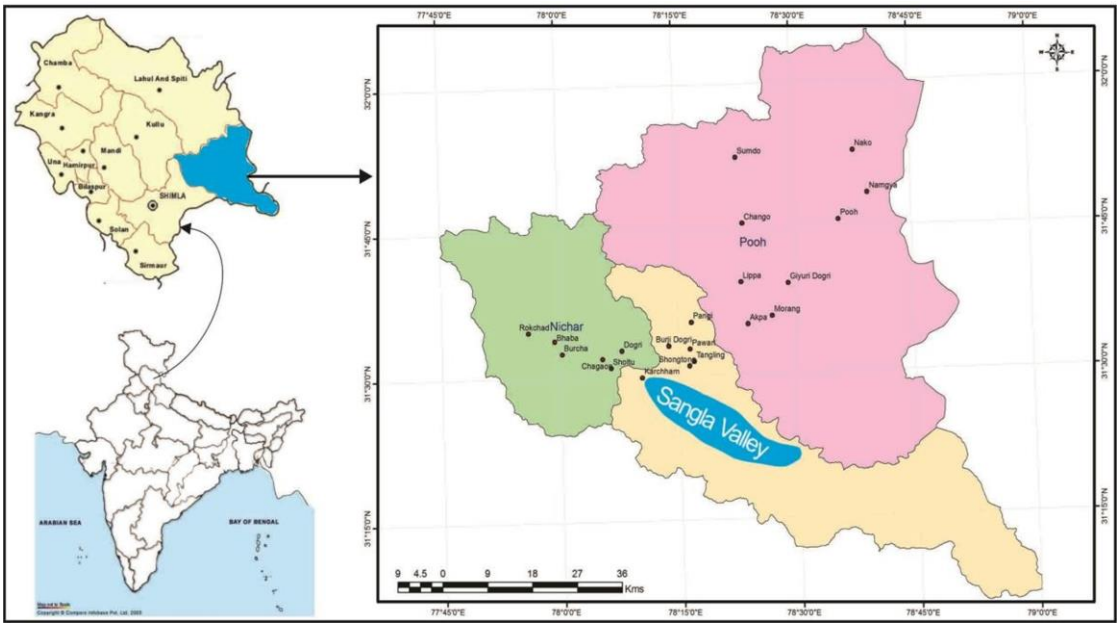

Fig. 1. Map showing the location of sample collection sites.

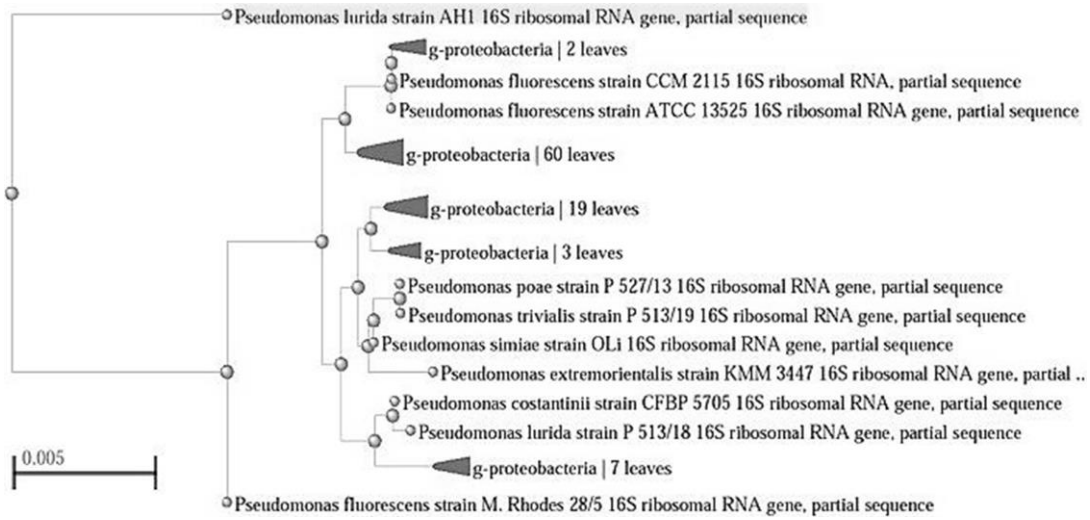

Fig. 2. Phylogenetic relationship between AH1 and representative species based on 16S rRNA sequences

Table 1. Antagonistic activity of isolate AH1 in terms of inhibition percentage against serious plant pathogens

Name of plant pathogen	MTCC No.	Inhibition Percentage
Alternaria solani	MTCC 2101	37.5 %
Botrytis cinerea	MTCC 2350	50 %
Fusarium oxysporum,	MTCC 7677	44.4%
Colletotrichum gloesporioides	MTCC 9664	-
Rhizoctonia solani	MTCC 4633	37.5 %
Sclerotinia sclerotiorum	MTCC 8785	43.7 %
Rosellinia sp.	MTCC 3878	44.4 %

3.3. Plant Growth Promoting Traits of Pseudomonas lurida AH1

3.3.1. Antagonistic Activity

As already mentioned Pseudomonas lurida AH1 inhibited six deadly plant pathogens out of seven tested (Fig. 3). The antagonisticpotential of this strain could be useful as biological control of plant disease which may be an alternative approach to the use of hazardous chemical fungicides. Similar study has been reported for Pseudomonas *fluorescens against Fusarium moniliforme Rhizoctonia solani and Alternaria alternata* using dual culture technique.[11]

3.3.2. Siderophore Production

Siderophore production by *Pseudomonas lurida* AH1 was confirmed by incubating it on CAS agar plates. A yellow to orange colored zone of 5 mm was developed on CAS agar plate indicating siderophore production by AH1 (Fig. 4). The color changed from blue to orange resulting from siderophoral removal of Fe from the dye. Similar finding have been reported in the literature that siderophores are organic compounds whose main function is to chelate the ferric iron (Fe (III)) from the environment.[12] Microbial siderophores also provide plants with Fe, enhancing their growth.

When Fe is limiting.[13] Rhizobial strains able to produce siderophores have been reported to be potential biofertilizers, improving the production of carrots, lettuce, peppers and tomatoes.[14,15]

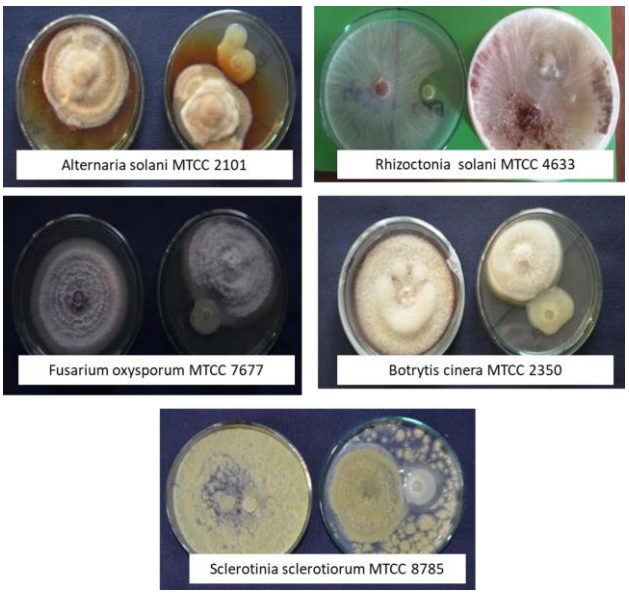

Fig. 3. Antagonistic activities of *Pseudomonas lurida* AH1 against plant pathogen.

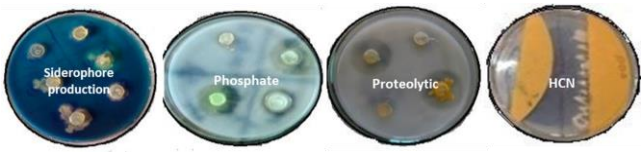

Fig. 4. Plant Growth Promoting Traits of *Pseudomonas lurida* AH1

3.3.3. Phosphate Solubilization

Pseudomonas lurida AH1 was found to be a phosphate solubilizer as it is forming yellow zone around the bit after incubation on Pikovoskaya's agar medium (Fig. 4). Phosphate solubilization was measured in terms of % solubilization efficiency which was found to be 45%. The zone formation could be due to the activity of phosphatase enzyme in bacterial isolates.[16] Phosphorus is the second most important nutrient for plants, after nitrogen. This element is fairly insoluble in soils. It exists in soil as mineral salts or

incorporated into organic compounds. Despite these phosphorus compounds being abundant in agricultural soils, the majority of them occur in an insoluble form.[17] Different bacterial species, particularly rhizosphere colonizing bacteria, have the ability to liberate organic phosphates or to solubilize insoluble inorganic phosphate compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. These bacteria make available the soluble phosphates to the plants, and in return gain root borne carbon compounds, mainly sugars and organic acids, necessary for bacterial growth.[18]

3.3.4. HCN Production

In the present study *Pseudomonas lurid*a AH1 was depicted to produce strong HCN. Production of HCN was observed in terms of change of colour of Whatman filter paper no. 1 from deep yellow toorange brown to dark brown (Fig. 4). It is reported in the literature by many authors that HCN is the common secondary metabolite produced by *rhizosphere Pseudomonas*.[19] In one of the report published in literature the bacterial strains Pf1 and CPf5 were found positive for cyanide production.[20]

3.3.5. Ammonia Production

Ammonia was suspected to be responsible for this alkalization. In a time course experiment, bacterial ammonia and amine emissions were determined using Nessler's reaction. *Pseudomonas lurida* AH1 was found to emit ammonia at substantial levels. Ammonia production is an important characteristic of PGPR, which indirectly influences plants growth. The high ammonia release generated a pH shift in the plant medium. These results substantiated the observation that bacteria growing on peptone-rich media released ammonia in concentrations that were sufficient to alkalize the MS medium which in turn retarded plant growth.[21] Likewise, all *Bacillus* and *Pseudomonas* isolated from chickpea rhizosphere in India have been found to produce ammonia.[22]

3.3.6. Protease Production

Pseudomonas lurida AH1 was observed positive for protease production which was expressed in terms of clear zone (20 mm) around the bit placed on skim milk agar plates (Fig. 4). The enzyme proteases hydrolyze proteins. Protease producing microorganisms has their role in eradication of some fungal and bacterial pathogens thus come under the category of biocontrol agent. Similarly, Bacillus PCSIR EA-3 also observed positive for proteolytic activity.[23]

4. Conclusions

In the current study bacterial isolate *Pseudomonas lurida* AH1 isolated from rarely explored rhizosphere of *Aconitum heterophyllum*- a medicinal plant grown in District Kinnaur Himachal Pradesh, India. This strain was selected on the basis of invitro antifungal assay and was identified by 16SrRNA gene technique. This isolate exhibited multifarious Plant growth promoting traits viz., siderophore production, phosphate solubilization, HCN production, Ammonia and protease production. Thus, the study reveals the *Pseudomonas lurida* AH1 as a rhizobacteria in the context of cold agro-ecosystems, where introduced mesophilic inoculants would fail. Hence, it is concluded that *Pseudomonas lurida* AH1 can be deployed as an inoculant to attain the preferred results of bacterization. However, different field trials and the interaction of this potential plant growth promoting PGPR with other native soil microflora have to be evaluated in future.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1 Sivasakthi S.; Usharani G.; Saranraj P. Biocontrol Potentiality of Plant Growth Promoting Bacteria (PGPR)-Pseudomonas Fluorescens and Bacillus Subtilis: A Review. *Afr. J. Agric. Res*., 2014, **9**, 1265-1277. [CrossRef]
2 Modi K.; Patel P.; Parmar K. Isolation, Screening and Characterization of PGPR from Rhizosphere of Rice. *Int. J. Pure. Appl. Biosci*., 2017, **5**, 264-270. [Link]
3 Podile A.R.; Kishore G.K. Plant Growth-Promoting Rhizobacteria. In Plant-associated bacteria, 2007, 195-230. Springer, Dordrecht. [Link]
4 Selvakumar G.; Mohan M.; Kundu S.; Gupta A.D.; Joshi P.; Nazim S.; Gupta H.S. Cold Tolerance and Plant Growth Promotion Potential of Serratia Marcescens Strain SRM (MTCC 8708) Isolated from Flowers of Summer Squash (Cucurbita pepo). *Lett. Appl. Microbiol*., 2008, **46**, 171-175. [CrossRef]
5 Schwyn B.; Neilands J.B. Universal Chemical Assay for the Detection and Determination of Siderophores. *Anal. Biochem*., 1987, **160**, 47-56. [CrossRef]
6 Louden B.C.; Haarmann D.; Lynne A.M. Use of Blue Agar CAS Assay for Siderophore Detection. *J. Microbiol. Biol. Educ*., 2011, **12**, 51-53. [CrossRef]
7 Pikovskaya R.I. Mobilization of Phosphorus in Soil in Connection with Vital Activity of some Microbial Species. *Mikrobiol*., 1948, **17**, 362-370. [Link]
8 Bakker A.W.; Schippers B.O.B. Microbial Cyanide Production in the Rhizosphere in Relation to Potato Yield Reduction and Pseudomonas Spp-Mediated Plant Growth-Stimulation. *Soil Biol. Biochem*., 1987, **19**, 451-457. [CrossRef]
9 Joseph B.; Patra R.R.; Lawrence R. Characterization of Plant Growth Promoting Rhizobacteria Associated with Chickpea (*Cicer arietinum* L.). *Int. J. Plant Prod.*, 2007, **1**, 141-152. [CrossRef]
10 Aneja K.R. Experiments in Microbiology. *Plant Pathol. Biotechnol*. New Age International. 2007. [Link]
11 Maurya M.K.; Singh R.; Tomer A. In Vitro Evaluation of Antagonistic Activity of Pseudomonas Fluorescens against Fungal Pathogen. *J. Biopestic*., 2014, **7**, 43. [Link]
12 Ali S.S.; Vidhale N.N. Evaluation of Siderophore Produced by Different Clinical Isolate Pseudomonas Aeruginosa. *Int. J. Microbiol. Res*., 2011, **3**, 131. [Link]

13 Garcia-Fraile P.; Carro L.; Robledo M.; Ramirez-Bahena M.H.; Flores-Felix J.D.; Fernandez M.T.; Mateos P.F.; Rivas R.; Igual J.M.; Martínez-Molina E.; Peix A. Rhizobium Promotes Non-legumes Growth and Quality in Several Production Steps: Towards a Biofertilization of Edible Raw Vegetables Healthy for Humans. *PLoS One*, 2012, **7**, 38122. [CrossRef]
14 Garcia-Fraile P.; Menendez E.; Rivas R. Role of Bacterial Biofertilizers in Agriculture and Forestry. *AIMS Bioeng*., 2015, **2**, 183-205. [CrossRef]
15 Flores-Felix J.D.; Menendez E.; Rivera L.P.; Marcos-Garcia M.; Martinez-Hidalgo P.; Mateos P.F.; Martinez-Molina E.; Velazquez M.D.L.E.; Garcia-Fraile P.; Rivas R. Use of Rhizobium leguminosarum as a Potential Biofertilizer for Lactuca sativa and Daucus carota Crops. *J. Plant Nutr. Soil Sci*., 2013, **176**, 876-882. [CrossRef]
16 Tripti; Vipin K.; Anshumali. Phosphate Solubilizing Activity of Some Bacterial Strains Isolated from Chemical Pesticide Exposed Agriculture Soil. *Int. J. Eng. Res. Dev*., 2012, **3**, 1-6. [Link]
17 Otieno N.; Lally R.D.; Kiwanuka S.; Lloyd A.; Ryan D.; Germaine K.J.; Dowling D.N. Plant Growth Promotion Induced by Phosphate Solubilizing Endophytic Pseudomonas Isolates. *Front. Microbiol*., 2015, **6**, 745. [CrossRef]
18 Khan M.S.; Zaidi A.; Ahmad E. Mechanism of Phosphate Solubilization and Physiological Functions of Phosphate-Solubilizing Microorganisms. In: Khan M., Zaidi A., Musarrat J. (eds) Phosphate Solubilizing Microorganisms. Springer, Cham, 2014. [CrossRef]
19 Schippers B.; Bakker A.W.; Bakker P.A.H.M.; Van Peer R. Beneficial and Deleterious Effects of HCN-Producing Pseudomonads on Rhizosphere Interactions. *Plant Soil*, 1990, **129**, 75-83. [CrossRef]
20 Reetha A.K.; Pavani S.L.; Mohan S. Hydrogen Cyanide Production Ability by Bacterial Antagonist and their Antibiotics Inhibition Potential on Macrophomina Phaseolina (Tassi.) Goid. *Int. J. Curr. Microbiol. Appl. Sci*., 2014, **3**, 172-178. [Link]
21 Weise T.; Kai M.; Piechulla B. Bacterial Ammonia Causes Significant Plant Growth Inhibition. *PloS One*, 2013, **8**, 63538. [CrossRef]
22 Yadav J.; Verma J.P.; Tiwari K.N. Effect of Plant Growth Promoting Rhizobacteria on Seed Germination and Plant Growth Chickpea (Cicer Arietinum L.) under in Vitro Conditions. *Biol. Forum*, 2010, **2**, 15-18. [Link]
23 Qadar S.A.U.; Shireen E.; Iqbal S.; Anwar A. Optimization of Protease Production from Newly Isolated Strain of Bacillus sp. PCSIR EA-3.2009. [Link]