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# Isolation and identification of rhizospheric, endophytic and epiphytic bacteria from chilli cultivars of telangana and its functional characterization

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**Abstract**
Plant and soil microbiome research is becoming significant for understanding how microorganisms affect agricultural productivity. The utility of microorganisms in improving soil fertility, plant nutrition, and crop productivity has continued to draw attention. In present study, microbial communities from rhizophere, phyllosphere and spermosphere have been isolated from chilli cultivars of Telangana. The total 43 isolates which were unique in their shapes, colour and margin has isolated and evaluated for stress tolerance, PGP traitsand bio-control activities. The total 10 multi PGP isolates identified through 16SrRNA and results revealed that 6 were belongs to Bacillus, one identifies as Pseudomonas and 3 were belongs to Priestia genera. Bacterial isolates were observed for phosphate solubilization ranges (0.5 mm -2 mm), Zinc solubilization (0.5-2.8 mm), potassium solubilization (0.9-1.9 mm) and further analyzed for the synthesis of IAA which was positive in 27 isolates, siderophore (24), HCN production (41). In hydrolytic enzyme production, 21 isolates were positive in amylase, 15 in cellulase, 9 in protease, 16 isolates in lipase and 6 were positive in pectinase enzymes out of 43 isolates. When antagonistic activity carried out, total 10 isolates inhibited the fungal pathogen i.e. *Fusarium Oxysporum in vitro* with various degree of inhibition. Out of 10 identified multi PGP bacterial isolates *Pseudomonas parafulva* W2EPC3 and *Bacillus subtillis* MB2EPC1 were found as best cultures showed maximum activities which belong to epiphytic category. This indicated the initial potential to develop as PGP organisms and bio-control agentused as a bio-fertilizers for chilli and other crops which can be helpful for sustainable agriculture practices.

**Keywords:** Rhizospheric, endophytic and epiphytic bacteria, plant growth promotion and bio-control

## Introduction
Chilli (*Capsicum annuum* L.) belongs to the Solanaceae plant family, is abundant in minerals, vitamins, capsaicin, phenolic acids, and flavonoids, among other nutrients (Ananthan, Subhash, & Longvah, 2018) [1]. According to reports, chili has a major impact on pharmaceutical applications, including weight control, cholesterol level reduction, antioxidant, antibacterial, and fat buildup prevention. (Cervantes-Paz *et al*., 2012) [9]. Reducing the use of inorganic fertilizers is the main goal of recent agricultural advancements; biofertilizers provide an alternate method for increasing crop production in sustainable agriculture (Zaidi *et al*., 2009) [36]. Biological control agents provide a number of challenges in the field, including variable performance, regardless of the mode of action. Combining a few biological control agents has been suggested as a way to improve robustness by offering complementarity in the modes of action and functional redundancy (De Vrieze *et al*., 2018) [10]; however, some research has demonstrated that single strains can also perform in similar to mixed consortia (Pertot *et al*., 2017) [27].
In the present scenario, the quest of more sustainable agricultural practices has drawn the attention to the biological potential of the interactions between crops and microorganisms. (Pii *et al*., 2015) [28]. Microorganisms are beneficial by promoting growth and controlling plant diseases, as well as reducing environmental pollution. The aerial part of the plant or all the aboveground surfaces of a plant usually leaf surfaces that represent microbial habitats are referred to as the phyllosphere. In particular, leaf surfaces host a dense population of bacteria (i.e., epiphytes) leaves host bacterial communities that are uniform in composition within a plant species.

The functions of most non-pathogenic leaf-colonizing bacteria, their dynamics at the community level, and their interactions with the plant host remain largely unknown. Although there is evidence that these bacteria may promote plant growth and provide protection against various stresses. Seed-borne endophytes are of particular importance because they can vertically transmit from one generation to the next via the seed, assuring their presence in the next generation of plants. Endophytes promote the improving of availability and absorption of nutrients, minerals and water; induce the tolerance of the host to stress caused by abiotic factors, including osmotic stress, exposure to heavy metals and xenobiotic molecules and act in the biological control of plant pathogens. The dynamic community of microorganisms associated with plant roots is known as the rhizospheric microbiome, or root microbe. Microorganisms are widely recognized for their crucial involvement in numerous biotic systems that maintain the biological and physiochemical equilibrium in soil. In particular, group of bacteria commonly termed Plant Growth-Promoting Rhizobacteria (PGPR) that naturally inhabits in close association with roots and benefits their host improving their growth by several mechanisms.

Biocontrol agents are advantageous as they can self-sustain after initial establishment and are capable of spreading on their own. The highly diverse nature of plant microbiota suggests that these microorganisms play a variety of ecological roles, and the bacterial communities they are linked with may have a wide range of functional characteristics. In order to comprehend their possible functional diversity we have isolatedndophytic bacteria from chilli seeds, rhizospheric bacteria from the soil of chilli cultivars, and epiphytic bacteria from the leaves of chilli plants.

In this work, bacteria that fix nitrogen and solubilize phosphate, and potassium are isolated from the rhizospheric soil, leaves, and seeds of the chilli plant, and then these microbes are used as a microbial consortium to aid in crop growth and the bacterial isolates were examined for their tolerance at different temperatures, salt concentration and pH on both the survival and growth of bacterial communities.

## Materials and Methods

### Sample collection

Chilli plant samples along with soil were collected from the districts of Telangana viz., Warangal and Mehaboobabad soil, leaves and fruits of chilli were separated and refrigerated.

### Isolation of Rhizospheric, Epiphytic and Endophyticbacteria

Sample of 2 gms of soil from the root zone was taken and mixed the sample into the 9ml of saline solution. Serial dilutions of sample were made upto the concentration from 10-1 to 10-9 and spread plating was doneon the nutrient agar plates and incubated the plates at 28 °C for 24 hrs (Veerapagu M *et al.*, 2018) [34].

The epiphytic bacteria were isolated in a method given by (Banoo A, *et al.*, 2020) [5]. Dorsal and ventral surface of each leaf was pressed against the surface of nutrient agar plates at three places separately. The plates were properly labeled and incubated in an inverted position at 28 ± 2 °C for bacteria for 2 days.

Endophytic bacteria isolated by following the method given by Ripendchilli fruits were selected and seeds were taken out from them. 2 gms of Surface sterilized seeds was taken and ground them into a homogenous mixture using sterile mortar & pistil mix them into 9ml of pre-prepared saline test tube.Spread plating was done on nutrient agar plates and incubated the plates at 28 °C for 24 hrs.

### Evaluation of bacterial isolatesforstress tolerance

To check the effect of temperature, Sharma, S *et al.* (2018) [33] bacterial isolates were studied by streaking on nutrient broth medium containing 2% agar respectively. The cultures were grown at different temperature in the range of -20 to 45 °C and observed the plates for growth of the bacterial isolates. To check the effect of PH, bacterial isolates were studied by streaking on nutrient broth medium containing 2% agar and plates were observed for growth of the bacterial isolates as per Sharma, S *et al.* (2018) [33]. To examine the salt tolerance of isolated bacterial cultures was performed according to Kumar A *et al.* (2021) [21] and bacterial strains inoculated on nutrient agar (NA) media supplemented with 0, 4, 6, 8% w/v NaCl. To examine the osmotic stress tolerance of bacterial isolates bacterial cultures inoculated in tryptone soy broth amended with polyethylene glycol-6000 - 5, 10, 15g/L concentration. The growth of the bacterial isolates was observed by taking spectrophotometer readings at 600 nm.

### *In vitro* screening of bacterial isolates for their functional characterization

### Nitrogen fixation efficacy

Nitrogenase activity (acetylene reduction assay) of isolated and purified culture was determined by using Gas Chromatography method (Bradford 1976) [7]. Purified cultures were inoculated on Rennie slant and incubated at 28 ± 20 °C for 3 days in an incubator for 3 days. Acetyene gas (10%v/v) was injected into the tubes and incubated for 24 hrs. After incubation, 1 ml air sample was removed from the tubes and injected into Gas Chromatography for analysis. The ethylene produced by reduction of acetylene was assayed using a Gas Chromatography as per Bradford and Williams method (1977) [7]. The Acetylene Reduction Assay of the isolates was calculated using the formula Nanomoles of $C_2H_4$ mg protein hr-1 $= C * Ps * V / T * P * P_{STD}$.

### Phosphorus solubilization

The phosphorus solubility was done on sterilized pikovskaya, agar medium and spot inoculated with bacterial cultures and incubated at 28 ±10 °C for 4-5 days. Formation of clear zone around the colonies was considered as positive results for phosphorus solubilization (Pikovskaya, 1948) [29].

PSE (Phosphate solubilization Efficiency) = Z/C × 100

Z - Clearance zone including bacterial growth

C - Colony diameter

### Potassium solubilization

The bacterial isolates were spot inoculated on Aleksandrov agar media and after incubation the diameters of clearing zone around the colonies were recorded. Solubilization index was calculated and scores were assigned as described for phosphate solubilization Hu *et al.* (2006) [19].

### Zinc solubilization

The bacterial isolates were inoculated on tris minimal media supplemented with ZnO and incubated at 28 ºC for 72 hrs. The diameter of the clearing zone around the colonies was measured Saravanan *et al.* (2004) [30].

### Ammonia production

The isolates were inoculated in peptone water and incubated for 4 days at 28 °C and centrifuged at 10,000 rpm for 15 min at 4 °C. To the supernatant, 0.5 ml of Nessler's reagent was added for development of brown to yellow colour which is indicator of ammonia production as per the Cappucino and Sherman (1992).

### IAA production

The nutrient broth was prepared with supplemented with 5 mM of tryptophan. Cultures were inoculated in nutrient broth and incubated in the tubes for 4 to 6 days. After the incubation period, centrifuge the tubes at 10,000 rpm for 20 min, at 4 °C. Take 1 ml of supernatant from each tube and two drops of orthophosphoric acid, followed by 4 ml of Salkowski reagent. Moreover, incubated these tubes for 25 mins, at room temperature. Development of light pink colour indicated positive for IAA production. After which readings were taken spectrophotometrically at 530 nm according to Glickmann and Dessaux (1995) [15].

### Hydrolytic enzyme production

For amylase activity bacterial cultures were spot inoculated on starch agar plates and a clear zone around bacterial colonies indicated positive for starch hydrolysis. Lipase activity can be determined by spot inoculating the bacteria cultures on media composed of peptone, 1%; Nacl, 0.5%; cacl2, 0.01%; Tween 20, 1%; agar, 1.5%; PH,7 and observed for the presence of white precipitate around the colony were positive for lipase activity (Feng *et al*., 2011) [14].

The proteolytic activity was done on skim milk agar plates and clear zones of hydrolysis around the bacterial cultures indicated positive for casein hydrolysis. Cellulase activity was determined according to method described by Emmyrafedziawati and Stella (2015) [12]. The bacterial cultures were spot inoculated on media containing carboxy methyl cellulose (CMC), after incubation observed for development clear zone around the colony. Pectinase activity of bacterial cultures were determined by spot inoculating cultures on media containing pectin (1%) and observed for development of halo zone around the colony were positive for pectinase activity (Hankin *et al*. 1917) [18].

### Bio control activity

### Production of siderophore

Bacterial isolates were tested qualitatively using aqueous ferric chloride solution. 0.06 g of Chrome Azurol Sulfonate (CAS) was dissolved in 5 ml of double-distilled water (solution I) and mixed with 9 ml of solution II containing 0.0027 g of $FeCl_3.6H_2O$ in 10 ml of 10 mMHCl. This was added to 0.073 g of hexadecyltrimethyl ammonium bromide (HDTMA) dissolved in 40 ml of double-distilled water. The dark blue colour CAS reagent was autoclaved for 15 min. king's B media was prepared separately and to this CAS reagent was added, which gives blue colour to the media. The isolates were spot inoculated on the plates under aseptic conditions and observed the orange colour zones around the colonies were considered positive according to Schwyn and Neilands (1987) [32].

### Production of hydrogen cyanide

Bacterial isolates were spot inoculated on nutrient agar plates under aseptic conditions containing 4.4 g per litre of glycine. A disc of whatman filter paper No.1 of the diameter equal to the petri plate size was soaked in a solution of HCN reagent (0.5% picric acid (w/v) in 1% sodium carbonate) for 1 min and placed on the surface of the plate. The change in colour from yellow to light brown, moderate or strong reddish brown of the filter paper considered positive for HCN production. (Bakker and Schippers, 1987) [4].

### Dual culture activity against pathogen

Bacterial isolates were streaked on one corner of the plates, the PDA plates without isolate were considered as negative control and in another plate, the strains were streaked against the pathogen on PDA medium and plates were incubated at 28±2 °C for 4-5 days. After incubation, colony diameter were measured and converted to the percentage of pathogen inhibition (Paul, N.C *et al*., 2013) with the following formula.

Percentage of inhibition =R1-R2 / R2 X 100

Where R1 represents the colony radii of Fusarium wilt in the control plate and R2 represents the colony radii of Fusarium wilt in the tested plates.

### Molecular characterization

Microbial genomic DNA was extracted from fully grown cuturesas given by Ausubel *et al*. (1999) [3]. Agarose gel (0.8%) electrophoresis was used for quantification of extracted DNA. Pure DNA was extracted from the bacterial cultures visualization was done by using a UV transilluminator and documented in a gel documentation system. The reaction volume for the PCR was 10 µl with 16s rRNA forward and reverse primers. The 16s rRNA gene of target bacterial isolates was amplified by the method of Heddi *et al*. (1998). The amplified PCR products were resolved on agarose electrophoresis using 1% of agarose gel in 1 X Tris-acetate EDTA buffer The 16S rRNA sequences (Barcode Bioscience, Bangalore) were aligned using BioEdit and contigs were created using Cap3 Software. The sequences obtained for different bacterial isolates were searched against the sequences of 16S rRNA of bacterial isolates available in the Gene bank Nucleotide Database (http://www.ncbi.nih.gov/blast) and based on the maximum identity of the sequences, the identity of the isolated bacteria was determined and sequence data was submitted to Gen Bank NCBI.

## Results and Discussion

### Isolation and purification of bacterial communities

Bacterial isolates were isolated by using nutrient agar and depending on the colony morphology and gram staining the unique and individual isolates were selected. Total 43 bacterial culture were isolated from different samples of chilli, out of them 12 were ephiphytic bacteria, 14 were endophytic bacteria, and 17 were belongs to rhizhospheric bacteria.

### Screening of bacterial isolates fortemperature, pH and salt stress

The bacterial isolates were screened at different temperatures 40 °C, 30 °C and -20 °C incubating the bacterial cultures in nutrient broth. Out of 43 isolates25 bacterial isolates growth was observed at 40 °C and remaining cultures didn't grow. Whereas -20 °C, 30 °C temperature, 24 and 28 isolates was shown the growth respectively. A total of 43 bacterial isolates were examined at different pH values. Out of them, 19 isolates has grown at pH 5, each of 23 isolates growth was observed at pH 7 and pH 9respectively. When bacterial culture tested under different salt concentration, Among 43 isolates 26 was shown growth at 0.01% of salt concentration. Similarly 20, 21 isolates was shown positive results at concentration of 0.04%, 0.06% respectively. While evaluation of osmotic stress tolerance of bacterial cultures at different concentration from 5-15% of PEG. Out of 43 bacterial isolates examined for stress tolerance 25 isolates has shown the tolerance of stress at 5%. The 23, 2 isolates was shown growth at 10%, 15% concentration respectively.

**_In vitro_ screening of the isolates for Plant Growth Promoting activities**

Among the 43 isolates screened for nitrogen fixation efficiency, only 6 isolates has shown Nitrogen fixation which includes *Priestiaaryabattai* W2RC2 (0.237 nmol C2H4/mg protein/hr) shown significantly highest N fixation followed by *Bacillus subtillis* MB2EPC1 (0.088 nmol C2H4/mg protein/hr), and *Priestiaaryabattai* W3RC1 (0.035 nmol C2H4/mg protein/hr), *Priestiamegatarium*W2RC3 (0.026nmol $C_2H_4$/mg protein/hr) and the least was recorded by *Pseudomonas parafulva* W2EPC3 (0.010 nmol C2H4/mg protein/hr). The examination that acetylene is an inhibitor of dintrogen ($N_2$) fixation and it is converted to ethylene by nitrogen fixing enzyme nitrogenase.

All the bacterial isolates were examined for potassium solubilization. Among 43 isolates 9 isolates were shown clear zone of solubilization and which ranges from 0.8-2 mm. The highest zone of solubilization was shown by W3RC2 followed by *Bacillus amyloliquifaciens* MB1EPC1(1.4 mm), *Bacillus altitudinis* W2ENC4 (1.2 mm) as mentioned in the (Table 1 & Fig.1A). Comparable results with present research work were obtained by Khanghahi *et al*. (2017) [20]. When isolated PSB from rhizosphericsoils of paddy fields and screened for their K-solubilizing ability on modified Aleksandrov agar medium.

Total of 43 isolates were tested for the phosphorus solubilization. Among them 12 isolates were shown zone of solubilization around the colonies and the range of inhibition were observed from 2 mm to 0.5 mm. The highest (+++) zone of solubilization was shown by *Bacillus altitudinis* W2ENC4 (2 mm), W1ENC1 (2 mm), followed by *Bacillus altitudinis* W2ENC2 (1.5 mm) and the least zone of solubilization was observed by *Bacillus tequilensis* W1ENC2 (0.4 mm), MB2EPC2 (0.5 mm) (Table.1& Fig.1B). Related results with the current work were obtained by (Suchitra Aphimeteetamrong and Chokchai Kittiwongwattana, 2017) [2] when they isolated epiphytic bacteria were from surfaces of roots, all 113 isolates were assessed for their phosphate solubilizing ability.

While zinc solubilization, the 24 isolates were shown clear zone around the colonies and ranges was observed from 2.8 mm to 0.5mm. The highest zone of solubilization was shown by W1EPC1 (2.8 mm), followed by *Bacillus velezensis* MB1EPC2 (2.7 mm), W3EPC2 (2 mm) and the least has shown by MB3ENC3 (0.6mm), *Priestiamegatarium* W2RC3(0.6 mm), W2RC4 (0.6 mm) (Table 1& Fig.3A&1C). In a similar way Saravanan, V.S *et al*., (2004) [30] was screened zinc solubilizing ability of *Bacillus sp*. and *Pseudomonas sp*. using zinc oxide and zinc sulphide (sphalerite) in plate assay.

All the 43 bacterial isolates were screened for production of ammonia, out of them 26 isolates were developed brown to yellow colour, where 18 isolates were shown high (+++) brown to yellow colour which includes *Pseudomonas parafulva* W2EPC3, followed by W2EPC1, W3EPC2, and *Bacillus velezensis* MB1EPC2 shown less (+) colour change (Table.1& Fig.1E). One of the characteristics of rhizobacteria that contributes to the indirect encouragement of plant growth is the production of ammonia, which inhibits the pathogen (Nain *et al*., 2012) [24].

In IAA production of bacterial isolates, a total 27 isolates were shown the development of pink colour. The highest colour change was shown by two isolates in quantification viz., MB3EPC1 (0.955 mg) followed by *Pseudomonas parafulva* W2EPC3 (0.309), W2RC4 (0.282 mg) and Less (+) IAA production was observed in W1RC3 (0.043 mg) (Table 1& Fig.1D). Indole-3-acetic acid (IAA) production by endophytic bacteria is one of the major contributions towards plants.

**Hydrolytic enzyme production**

In hydrolytic enzyme production, a total 21 isolates were shown positive in amylase enzyme production and 6 isolates has shown more hydrolysis (+++) include *Bacillus altitudinis*W2ENC2, W3ENC2, W1RC1, W1RC2, W1RC3, W2RC4. Bacteria produce various kinds of extracellular enzymes, which are hydrolases, lyases, amylases. Hallmann *et al*. (1997) [17] showed that endophyte-producing enzymes could help to initiate the host symbiosis process.

Cellulase is the most abundant biomass on earth. This enzyme is produced by several microorganisms, commonly by bacteria and fungi. Among 43 isolates, 15 isolates has shown cellulase activity.For protease enzyme activity nine isolates were shown positive results with strong producers such *Priestiamegatarium* W2RC3, W2RC4, *Priestiaaryabattai* W3RC1, W3RC2, MB2ENC1, MB2ENC2. The results of the present research work shows similarity with the results which were reported by R. Vijayalakshmi *et al*., (2016) [35] who have isolated six bacterial endophytes from the leaves of the three traditionally practiced medicinal plants and were screened for enzyme activity. Results found that endophytic microbial isolates exhibited amylase, protease and cellulase activities.

Out of 43 bacterial isolates examined for lipase production, 16 isolates has shown positive and MB3RC2 shows highly positive followed by W2EPC1, MB3ENC1. The 6 isolates has shown positive in pectinase activity and W2EPC4 isolate shown moderate (++) results followed by W1RC1, *Priestiaaryabattai* W3RC1data recorded are mentioned in the Table 1. In a similar way (Bhutani N *et al*., 2021) [6] isolated endophytic bacteria from *Vignaradiata* and *Cajanuscajan* and studied the production of hydrolytic enzymes.

**Bio Control Activity**

**Siderophore Production**

All the bacterial isolates were analysed for siderophore production, among 43 isolates, 24 isolates were shown positive and highest (+++) production recorded by MB1RC2, followed by MB2RC4, *Bacillus velezensis* MB1EPC2 (Table.1& Fig.2B). Endophytes reduces the bacterial, fungal, and viral diseases by producing siderophores.

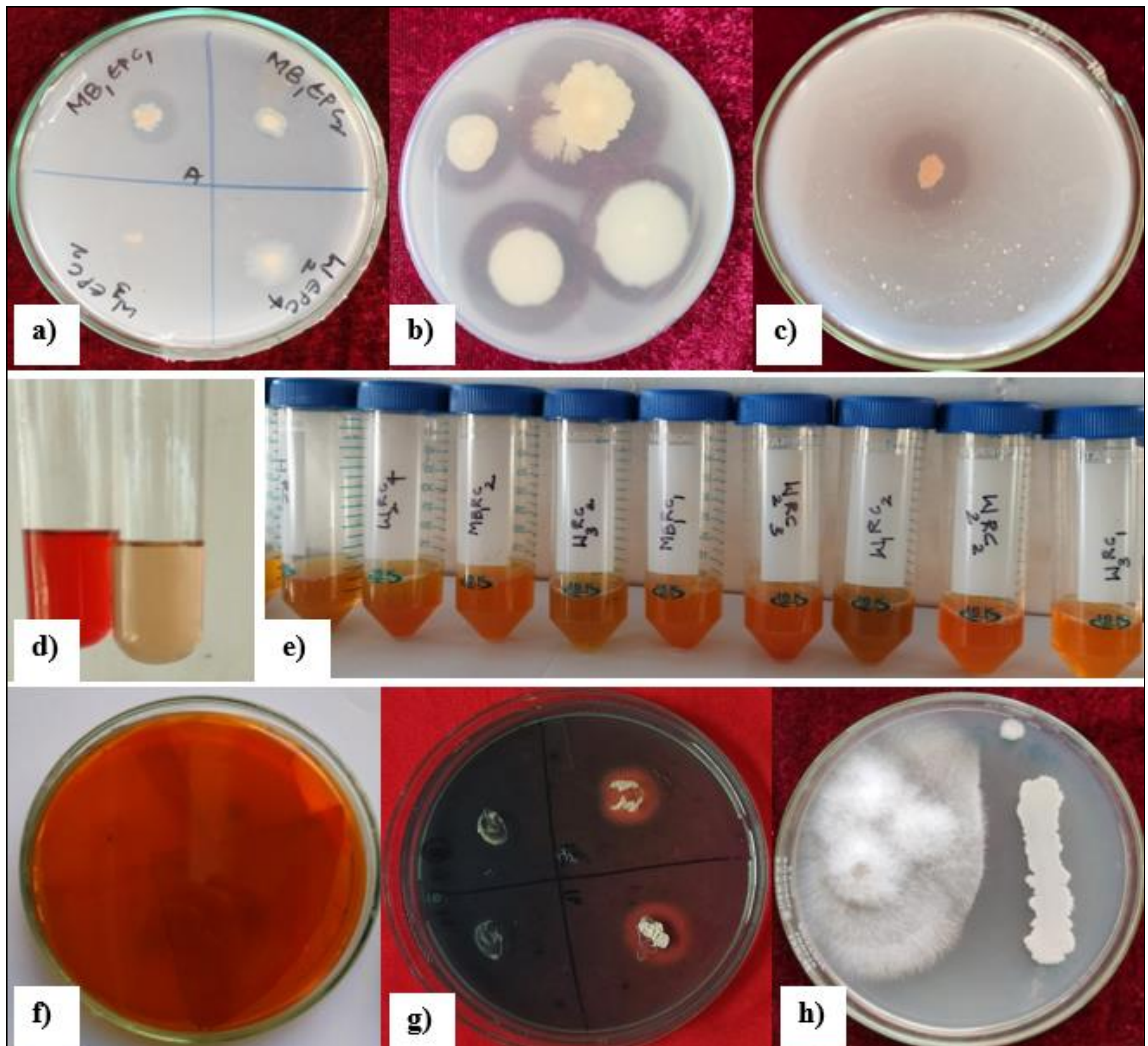

**Fig 1:** Plant growth promoting activities and Biocontrol activities: a) Potassium b)Phosphorus c)Zinc d) IAA e) Ammonia f) HCN production g) Siderophore production and h)Antagonistic activity against *Fusarium oxysporum*

**Hydrogen Cyanide Production**

Among 43 bacterial isolates which were screened for HCN production, a total of 41 isolates has shown positive results. The highest (+++) HCN production was shown by the isolates W1RC1, followed by W1RC2, W1EPC1. The growth inhibition and supression of other living things have been associated to bacterial production of HCN (Zdor, 2015) (Table 1&Fig 2A).

**Dual culture assay of Bacterial isolates against fungal pathogen *Fusarium oxysporum*:** Out of 43 bacterial isolates evaluated for potentiality, only 10 cultures MB1EPC1 (33.3 mm), MB1EPC2 (35.7 mm), MB2EPC1 (28.5 mm), W2EPC3 (42.8 mm), W1ENC2 (30.58 mm), W2ENC2 (14.28 m), W2ENC4 (22.68 mm), W2RC2 (36.72 mm), W2RC3 (24.48 mm), W3RC1 (22.68 mm) were inhibited the growth of fungal inoculum and selected for molecular studies. Similarly Al-Nadabi, H.H., (2021) isolated endophytic bacteria from date palm leaves of 3 different cultivars viz., Nighal, Khalas and Khinaizi and examined for their inhibitory activity against leaf spot pathogens of date palm *viz*., *Fusariumsolani, Alternaria sp., Nigrospora sp.,* using an *in vitro* dual culture assay.

**Molecular characterization of the prominent isolates:** In the present study, ten isolates were selected based on the multi PGP studies and identified by 16S rRNA gene sequencing. The sequenced PCR product of the bacterial isolates matched with the gene bank database. BLAST search results through NCBI showed similarity of MBEPC1 with Bacillus *amyloliquifaciens*, MB1EPC2 with *Bacillus velezensis*, MB2EPC1 with *Bacillus subtilis*, W2EPC3 with *Pseudomonas parafulva*, W1ENC2 with *Bacillustequilensis*, W2ENC2 with *Bacillus altitudinis*, W2ENC4 with *Bacillus altitudinis*, W2RC2 with *Priestiaaryabhattai*, W2RC3 with *Priestiamegatarium*, W3RC1 with *Priestiaaryabhattai*. These cultures were deposited at NCBI and accession numbers were obtained are presented in the table below. Out of 10 cultures 6 isolates belongs to *Bacillus* genera, one isolate was identified as *Pseudomonas* generaand 3 isolates belongs *to Priesta*genera.

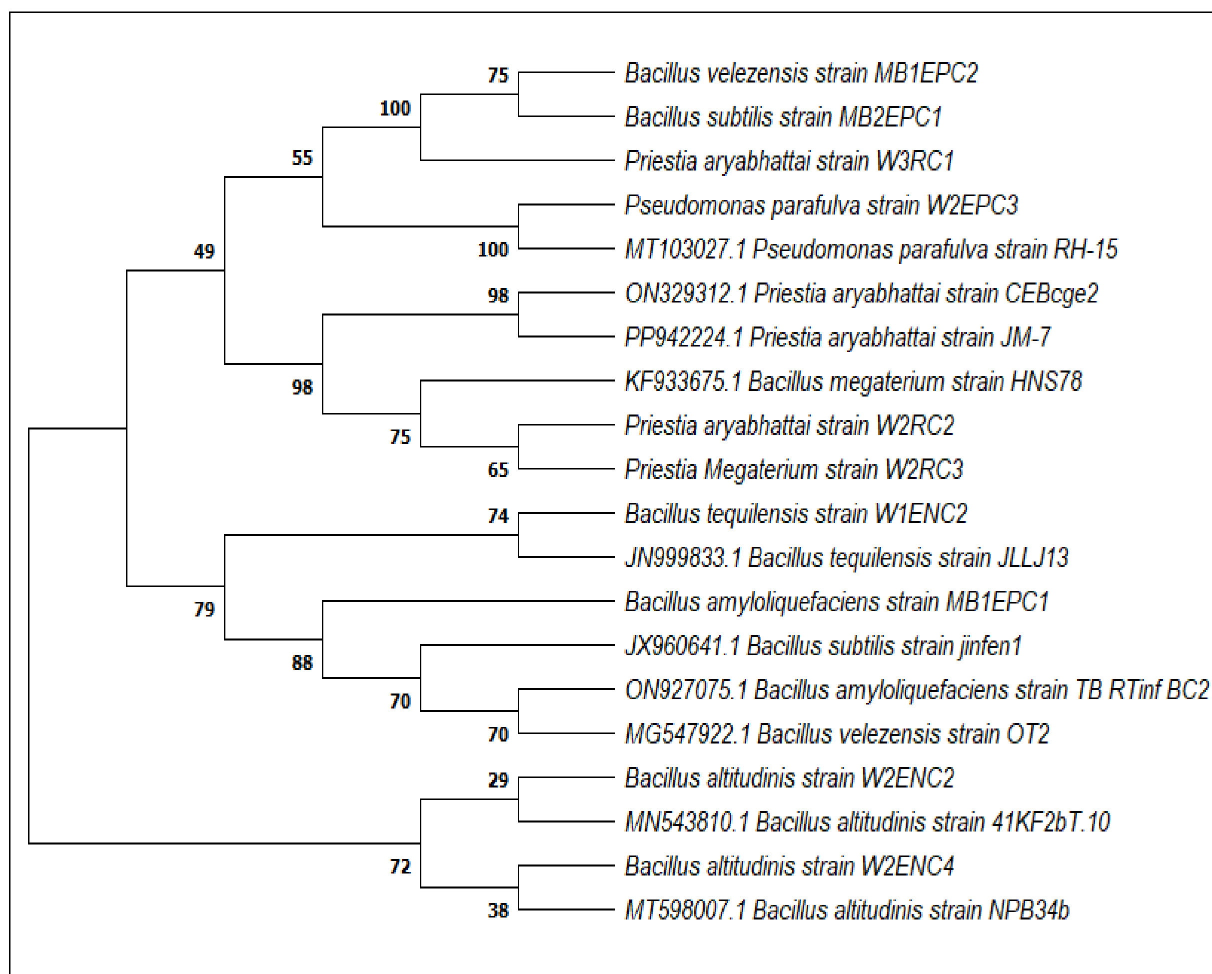


**Fig 2:** Phylogenetic tree based on 16s rRNA gene sequences and their closest phylogenetic neighbors

**Table 1:** *In vitro* screening of the bacterial isolates for plant growth promoting, bio-control activities and hydrolytic enzyme production

| Isolate name | Plant growth promotion | | | | | | | Bio-control activity | | | Hydrolytic enzyme production | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | K | P | Zn | Con of Zn in mg/ml | IAA | $NH_3$ | Siderophore | HCN | (%) Inhibition of *FO* pathogen | Amy | Cellu | Prot | Pecti | Lip |
| W1EPC1 | - | - | - | 2.8 | 0.040 | 0.092 | - | - | +++ | - | - | - | - | - | - |
| W1EPC2 | - | - | - | 1.5 | 0.046 | 0.077 | - | - | ++ | - | ++ | - | - | - | + |
| W2EPC1 | - | - | - | 0.5 | 0.070 | - | +++ | +++ | ++ | - | - | - | - | +++ | - |
| W2EPC3 | 0.010 | 0.9 | - | 0.7 | 0.076 | 0.309 | +++ | + | +++ | 42.8 | - | - | - | - | + |
| W2EPC4 | - | - | - | 1.1 | 0.056 | - | - | - | +++ | - | + | - | - | - | ++ |
| W3EPC2 | - | - | - | 2 | 0.051 | - | +++ | - | +++ | - | - | - | - | - | - |
| MB1EPC1 | - | 1.4 | - | 1.2 | 0.367 | - | +++ | + | +++ | 33.3 | + | - | - | - | - |
| MB1EPC2 | - | - | 1 | 2.7 | 0.063 | 0.074 | + | +++ | +++ | 35.7 | + | + | - | + | - |
| MB2EPC1 | 0.088 | 0.9 | 1.5 | - | - | 0.087 | ++ | - | + | 28.5 | ++ | - | - | ++ | - |
| MB2EPC2 | - | 1 | 0.5 | 1 | 0.165 | 0.061 | +++ | +++ | + | - | - | - | - | ++ | - |
| MB3EPC1 | - | - | - | 1.9 | 0.052 | 0.955 | - | - | ++ | - | - | - | - | - | - |
| MB3EPC2 | - | 1 | - | 1.9 | 0.054 | - | - | +++ | ++ | - | - | - | - | - | - |
| W1ENC1 | - | - | 2 | 2 | 0.659 | - | + | +++ | +++ | - | - | + | - | - | - |
| W1ENC2 | - | 1 | 0.4 | 2 | 0.362 | - | - | +++ | +++ | 30.58 | - | + | - | - | - |
| W2ENC2 | 0.022 | - | 1.5 | 1 | 0.062 | - | +++ | ++ | - | 14.28 | +++ | - | - | ++ | - |
| W2ENC4 | - | 1.2 | 2 | - | - | - | - | - | +++ | 22.68 | ++ | + | - | - | - |
| W3ENC2 | - | - | - | - | - | 0.046 | +++ | ++ | +++ | - | +++ | ++ | - | ++ | - |
| MB1ENC1 | - | - | - | - | - | - | + | + | +++ | - | + | + | - | - | - |
| MB1ENC2 | - | - | - | - | - | 0.259 | - | - | +++ | - | - | + | - | ++ | - |
| MB1ENC3 | - | - | - | - | - | 0.076 | - | +++ | +++ | - | + | + | - | - | - |
| MB2ENC1 | - | - | - | - | - | - | +++ | - | +++ | - | ++ | - | +++ | + | - |
| MB2ENC2 | - | - | - | - | - | - | - | - | + | - | - | - | +++ | - | - |
| MB2ENC3 | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| MB3ENC1 | - | - | - | 1.1 | 0.280 | - | +++ | - | ++ | - | - | - | + | +++ | - |
| MB3ENC2 | - | - | - | 1 | 0.199 | - | ++ | + | ++ | - | ++ | - | - | - | - |
| MB3ENC3 | - | - | - | 0.6 | 0.599 | - | +++ | +++ | - | - | - | - | - | ++ | - |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| W1RC1 | - | - | 1.5 | - | - | - | + | - | +++ | - | +++ | + | + | - | - |
| W1RC2 | - | - | 1 | - | - | 0.083 | ++ | - | +++ | - | +++ | - | - | - | + |
| W1RC3 | - | - | 1 | - | - | 0.043 | +++ | + | ++ | - | +++ | - | - | - | - |
| W2RC2 | 0.237 | - | 1 | - | - | 0.28 | +++ | + | ++ | 36.72 | + | - | ++ | - | - |
| W2RC3 | 0.026 | - | - | 0.6 | 0.161 | 0.061 | +++ | ++ | ++ | 24.48 | + | + | +++ | ++ | - |
| W2RC4 | - | - | - | 0.6 | 0.076 | 0.282 | +++ | ++ | ++ | - | +++ | - | +++ | + | - |
| W3RC1 | 0.035 | - | - | 1.8 | 0.088 | 0.067 | ++ | - | ++ | 22..68 | - | - | +++ | ++ | + |
| W3RC2 | - | 1.9 | 1 | - | - | 0.138 | ++ | ++ | ++ | - | - | + | +++ | + | + |
| W3RC3 | - | - | - | - | - | 0.148 | - | - | ++ | - | + | + | - | - | - |
| MB1RC1 | - | - | - | - | - | 0.106 | +++ | - | ++ | - | - | - | - | - | - |
| MB1RC2 | - | - | - | - | - | - | +++ | +++ | ++ | - | + | + | - | ++ | - |
| MB1RC3 | - | 1 | - | - | - | 0.076 | - | ++ | ++ | - | - | - | - | - | - |
| MB2RC1 | - | - | - | 1 | 0.391 | 0.074 | - | ++ | ++ | - | - | + | - | - | - |
| MB2RC3 | - | - | - | 1 | 0.370 | 0.094 | - | ++ | + | - | - | + | - | - | - |
| MB2RC4 | - | - | - | 1.9 | 0.055 | - | - | +++ | + | - | - | - | - | - | - |
| MB3RC1 | - | - | - | 1 | 0.139 | 0.123 | - | +++ | ++ | - | + | - | - | - | - |
| MB3RC2 | - | - | - | - | - | 0.050 | +++ | - | + | - | - | - | - | +++ | - |

+++strong producer, ++moderate producer, + weak producer, FO - *Fusarium Oxysporum*, Amy-Amylase, Cellu-Cellulase, Prot-Protea, Pect-Pectinase, andLip-Lipase

**Table 2:** Molecular identification of the potential isolates by 16s rRNA

| Sl. No. | Isolate Name | Molecular Identification | Percent Similarity | Accession Number |
|---|---|---|---|---|
| 1. | MB1EPC1 | *Bacillus amyloliquifaciens* | 100% | PQ192691 |
| 2. | MB1EPC2 | *Bacillus velezensis* | 99% | PQ192692 |
| 3. | MB2EPC1 | *Bacillus subtilis* | 99.25% | PQ192693 |
| 4. | W2EPC3 | *Pseudomonas parafulva* | 99.54% | PQ192694 |
| 5. | W1ENC2 | *Bacillus tequilensis* | 99.85% | PQ192695 |
| 6. | W2ENC2 | *Bacillus altitudinis* | 99.85% | PQ192696 |
| 7. | W2ENC4 | *Bacillus altitudinis* | 100% | PQ192697 |
| 8. | W2RC2 | *Priestiaaryabhattai* | 99.77% | PQ192698 |
| 9. | W2RC3 | *Priestiamegatarium* | 99.77% | PQ192699 |
| 10. | W3RC1 | *Priestiaaryabhattai* | 99.23% | PQ192700 |

## Conclusions

In the present study the rhizospheric, epiphytic, endophytic microbial communities from chilli cultivars successfully isolated and examined for different growth conditions, multi PGP traits and bio-control activities. Among of 43 isolates, 10 multi plant growth promoting bacterial isolates were selected and identified by 16s rRNA gene sequencing and 6 isolates belongs to *Bacillus*genera, 3 isolates identified as *Priesta* genera and one was *Pseudomonas*. This study concludes that the microbial communities with PGP,andbiocontrol activities against pathogen can be used as consortia which can enhance the crop productivity meanwhile the use of chemical fertilizers can be reduced especially in Chilli crop.

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