

Original Research Article

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Diversity and Characterization of Plant Growth Promoting Rhizobacteria Associated with Wheat (*Triticum aestivum*) Rhizosphere in North-Western Himalayan State of Himachal Pradesh in India

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ABSTRACT

Keeping in view the scarce knowledge of the composition of bacterial diversity associated with roots of wheat, the present study was undertaken to explore its diversity & investigate their role in plant growth promotion. Present study reveals, the diversity of bacterial isolates from soils under wheat cultivation in districts of Solan of Himachal Pradesh. Phenotypic and physiological characteristics of the PGPR isolates were recorded to categorize and identify the bacteria. Sixty different rhizobacteria isolates were recovered from different locations of the district of which some were rainfed and some were irrigated. The characteristics of the bacterial isolates were determined using the colony morphology, gram staining as well as biochemical properties. These isolates were then tested for different PGPR traits, such as production of lytic enzymes, siderophore, P-solubilization, Ammonia and HCN.

Keywords

Wheat,
Rhizosphere,
PGPR, PGP
attributes, Diversity

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Introduction

Wheat

Wheat (*Triticum aestivum*) is the major grain that sustains humanity. Wheat grown in temperate climate and it is staple food for 35% of world's population. On other hand, it

provides more calories and protein in the diet than any other crop (Laegreid *et al.*, 1999). Wheat (*Triticum astivum* L) are members of Graminae family. Currently, India is second largest producer of Wheat in the world after China with about 12% share in total world wheat production. Himachal Pradesh – a north-western Himalayan state of India is one

of the major contributors for wheat production of India (Mishra *et al.*, 2007). In 2016, world production of wheat was 749 million tonnes, making it the second most-produced cereal after maize (1.03 billion tonnes), with more than rice (499 million tonnes) (United Nations, Food and Agriculture Organization. 2016).

PGPR are beneficial for plant growth and also referred as Yield Increasing Bacteria (YIB). They affect plant growth and yield in a number of ways and enhancement reproductive growth is documented in a range of crops like cereals, pulses, ornamentals, vegetables, plantation crops and some trees (van Loon *et al.*, 1998). Since rhizosphere of any crop is found rich in PGP rhizobacteria, therefore it becomes important to isolate potential microorganisms from rhizosphere of wheat from different sites & assess their diversity & role in PGP activities.

The plant growth promoting (PGP) effect of the PGPR is mostly explained by the release of metabolites directly stimulating growth. Several mechanisms have been postulated to explain how PGPR benefit the host plant. These include: (a) the ability to produce plant growth regulators or phytohormones such as indole acetic acid (IAA), cytokinins, and gibberellins (Glick, 1995; Marques *et al.*, 2010); (b) enhancing symbiotic N₂ fixation (Khan *et al.*, 2006); (c) solubilizing inorganic phosphate and mineralization of organic phosphate and/or other nutrients (Glick, 1995); (d) antagonistic effect against phytopathogenic microorganisms by production of siderophores. Interest in the beneficial rhizobacteria associated with cereals has increased recently and several studies clearly demonstrated the positive and beneficial effects of PGPR on growth and yield of different crops especially wheat at different environment under variable ecological conditions. Keeping in mind the

study was planned to isolate the native strains from rhizosphere of wheat grown on different soils of Solan district of Himachal Pradesh. These bacterial isolates were characterized and screened *in vitro* for PGP potentials.

Materials and Methods

Sample collection and isolation of bacteria

Wheat (*Triticum aestivum* L.) plant rhizospheric soil samples were collected from different sites of Solan district of Himachal Pradesh. Samples were collected individually in sterilized polythene bags. Rhizospheric bacteria were isolated from 1 g soil tightly adhering to the root by serial dilution plating on Luria-Bertani agar (LB), King's B agar and Nitrogen-free agar medium (Jenson medium) agar plates as described (Somasegaran and Hoben, 1994). The plates were incubated at $28 \pm 2^{\circ}\text{C}$ till the appearance of bacterial colonies. Individual colonies were picked and streaked on respective plates for further purification.

Biochemical characterization

Colony morphology, size, color, shape, and growth pattern were recorded after 24h of growth on different nutrient agar medium, plates were incubated at $28 \pm 2^{\circ}\text{C}$ as described by Somasegaran and Hoben (1994).

Colony morphology and pigment production

Colony morphology (form, elevation, margin, shape, surface) and the production of pigment was checked on King's B agar at $28 \pm 2^{\circ}\text{C}$ after 24 to 48h.

Gram staining

Four different reagents in the order of Crystal violet, Gram's iodine, alcohol/acetone destaining reagents, and safranin were used to

identify and classifying bacteria.

The gram's reaction was performed as described (Aneja, 2003).

Catalase test

Catalase production was checked by placing a drop of H₂O₂ onto the bacterial colony on a glass slide (Taylor *et al.*, 1972).

Bio assays for Plant Growth Promoting Traits

P- solubilization

Solubilization of insoluble Phosphate. Each bacterial culture was spot inoculated in the center of agar plates containing tricalcium phosphate as insoluble phosphate source (Pikovskaya's, 1948). The plates were incubated at 28 ± 2°C for 7–10 days and observed for the formation of halo zone around the colonies.

Siderophores production

Nutrient agar medium was used for the production of siderophore with slight modification (Meyer and Abdallah, 1978). 0.5 ml of 18 h old inoculums of PGPR's isolates was added to 100 ml of king's B medium in 250 ml flask. The flask were incubated at 28⁰ C for 72 h under shake condition on rotary shaker. The supernatant was harvested by centrifugation at 10,000 rpm for 20 minutes at 4⁰ C and store at 4⁰ C.

Qualitative assay

Siderophores production was detected by chrome azurol-S (CAS) plate assay method with slight modification (Schwyn and Neilands, 1987). 25 ml of CAS dye (prepared by mixing 1.5 ml of 1 mM FeCl₃, 6H₂O in 10 mM HCl + 7.5 ml of 2 mM of CAS stock solution dissolved in 50 ml of HDTMA, add

30 ml piperazine solution (4.307g piperazine was dissolved in 30ml water and 6.75ml of concentrated HCl was added to make pH 5.6) into it and final volume was made to 100 ml with distilled water) was mixed with 250 ml of nutrient agar and mixed well before pouring. 100µl of 72 h old culture supernatant of each test bacteria was placed on prepoured chrome azurol-S agar (CAS) plates. Plates were incubated at 28⁰ C for 72 h. Production of siderophore was expressed in terms of mm diameter of pinkish/orange halo produced around the bit/well at 28⁰C in 72h.

HCN

All the isolates were screened out for the production of hydrogen cyanide (HCN) (Bakker and Schippers, 1987). Cultures were streaked on prepoured plates of nutrient agar medium amended with 1.4 g/l glycine. Whatman No.1 filter paper strip were soaked in 0.5 per cent picric acid followed by 2 per cent sodium carbonate and were placed in the lid of each petriplates. Petriplates were sealed with parafilm and were incubated at 28⁰C for four days. Uninoculated control with picric acid paper strips was kept for comparison of results. Plates observed for change of color of filter paper from yellow (-) to brown (+++) to dark brown (++++). Intensity of color developed indicated as high production of volatile HCN.

Ammonia production

Ammonia production was checked according to Lata and Saxena (2003). All isolates were grown in peptone water (5 ml) in tubes. Tubes were incubated at 28⁰C for 4 days. 1ml of Nessler's reagent was added to each culture tube. Presence of faint yellowish to brown color (+) indicated small amount of ammonia and deep yellow (++) to brown color (++++) indicated large amount of ammonia production.

Lytic enzymes

Protease

All isolates were screened out for production of protease by well plate assay (Mehta and Singh, 2006) on skim milk agar (1% skim milk was used in nutrient agar medium) and autoclaved separately before pouring, content of both the flasks were mixed and then plates were poured. 100µl of 72h old cell free supernatant of each isolates was added to each well cut on the prepoured skim milk agar. Plates were incubated at 28⁰C for 24-48 h and observed the clear zone production around the well. The proteolytic activity expressed in the terms of mm diameter of clear zones produced around the well after incubation at 28⁰C for 48 h.

Cellulase

All the isolates were screened out for the cellulase activity on cellulose agar plates. Add 100µl of 72 h old culture supernatant in the well already cut on the pre poured cellulose agar plates with the help of sterile cork borer. Plates were incubated at 28⁰C for 24-72h cellulase activity expressed in terms of mm diameter of clear zone produced around the well (Pathania *et al.*, 2012).

Chitinase

All the isolates were screened out for production of chitinase and estimated by well plate assay on chitin agar plates 1% of colloidal chitin was added to nutrient agar. 100µl of 72 h old supernatant of bacterial isolates was added to well cut on the chitin plate. Plates were incubated at 28⁰ C for 3-7 days and observed for clear zone (mm dia.) produced around the well (Queiene *et al.*, 2008).

Results and Discussion

Collection of rhizospheric soil samples

The rhizospheric soil samples for PGPR's

isolation were collected from rhizosphere of *Triticum aestivum* crop grown in its natural habitat at different location of Solan district of Mid Himalayan region of Himachal Pradesh, India, located at the different altitude above mean sea level in (Deothi (1502m), Kandaghat (1425m) and Dharja (1502m).

Isolation of PGPR from rhizospheric soil

PGPR isolates were isolated from the rhizospheric soil sample by serial dilution and spread plate method using different media i.e Nutrient agar, Jensen, Luria-Bertani and King's B agar medium at 28⁰C. Nutrient agar media (Lapage *et al.*, 1970) for bacteria present in the rhizosphere, Jensen for nitrogen fixing bacteria (Jensen, 1942; Ranganayaki *et al.*, 1981), Luria-Bertani for *Bacillus* sp. (Lennox, 1955) and King's B agar for *Pseudomonas* sp. (King *et al.*, 1954) specific media. The total viable count of rhizobacteria from *Triticum aestivum* from selected sites of Solan is represented in table 1.

The total rhizobacterial population on nutrient agar medium harboured the maximum (250x10⁵ CFU/g soil) microbial population at Deothi, followed by Dharja (165x10⁵ CFU/g soil) and Kandaghat (70x10⁵ CFU/g soil) of district Solan. However, population of PGPR isolates on Jensen's medium was found highest at site Deothi (200x10⁵ CFU/g soil), followed by Dharja (120x10⁵ CFU/g soil) and Kandaghat (70x10⁵ CFU/g soil). In case of King's B agar, maximum rhizobacterial population was found at site Deothi (230x10⁵ CFU/g soil), followed by Dharja (170x10⁵ CFU/g soil) and Kandaghat (100x10⁵ CFU/g soil) In case of Luria-Bertani agar media, maximum rhizobacterial population was found at site Dharja (180x10⁵ CFU/g soil), followed by Deothi (102x10⁵ CFU/g soil) and Kandaghat (19x10⁵ CFU/g soil) (Table 1).

The variation in the population of rhizobacteria may be attributed to location,

age of plant, variety/cultivar type, time of sampling, physico-chemical and biological properties of soil and environmental conditions of the locations. The rhizosphere, considered to be a hot spot of bacterial diversity, harbours bacterial flora whose diversity is mainly expressed in terms of functions adapted to the root presence, and in particular to favour plant growth. This is in turn beneficial to the whole rhizosphere microbiota through the highly nutritive and energetically rhizodepositions (Aragno, 2005). Tamilarasi *et al.* (2008) reported that the varying degree of population observed in roots of the plant is due to the effect of chemical composition of root exudates of individual plants on the microorganisms.

Morphological and physiological characterization of PGPR's isolated from rhizospheric soil of wheat

In total, 60 PGPR's isolates were isolated on four different nutrient agar medium i.e. Nutrient agar, Jensen's media, King's B medium and Luria-Bertani agar. The morphological characters of bacterial isolates i.e. color, form, elevation and margins of bacterial isolates were noted down and presented in fig. 1, 2& 3.

Deothi site

The color of colonies of bacterial isolates from Deothi varied from white, milky white and yellow. Majority of isolates were white (46 %) in color, 29 % were milky white in color, 21 % isolates were off white and 4% yellow in color. 67% isolates are circular and 33% isolates were found puntiform in form. Out of total isolates, 54 % isolates were raised while 46 % had flat elevation. 62 % bacterial isolates were gram positive and 38 % were found to be gram negative (Fig 1).

Kandaghat site

The color of colonies of rhizobacterial

isolates from Kandaghat varied from white, yellowish green and dark yellow. Majority of isolates were greenish yellow (38%) in color, 37 % were yellowish green in color, while 13% isolates were white and 12% were dark yellow in color. 63% isolates were circular and 37% isolates were puntiform in form. Out of total isolates, 50 % isolates were raised while 50 % had flat in elevation. 88% isolates were gram negative and 12% were found to be gram positive (Fig. 2).

Dharja site

The color of colonies of rhizobacterial isolates from Dharja varied from cream, yellow and white. Majority of isolates were cream (67%) in color, 17 % were found yellow in color and 16% isolates were white in color. 75% isolates were circular while 25% isolates were puntiform in form. Out of total isolates, 50% isolates were raised while 50% had flat elevation. 92% bacterial isolates were gram negative and 8 % were found to be gram positive in nature (Fig 3).

Deothi site

Phosphate solubilization

Phosphate solubilization activity shown by bacterial isolates from Deothi was expressed in the range of 6 to 25 mm diameter of yellow zone on PVK agar plate. Out of twenty four isolates, only nineteen isolates showed Phosphate solubilization. Maximum P-solubilization was shown by the isolates De-5 and De-21 (25 mm) followed by the isolate De-23 (24mm) and De-12 (20mm). Other isolates showed slightly less P-solubilization in the range of 6 to 18 mm dia. From Deothi site majority of the isolates were found positive for P- solubilization *in-vitro* conditions on PVK agar and some isolates were shown negative results for P-solubilization.

Siderophore production

The siderophore production of the all isolates from Deothi site was tested by qualitative method on CAS agar media. All the isolates were screened out for the siderophore production on chrome azurol-S (CAS) agar. The results were obtained qualitatively in the form of yellow colored zone (mm dia) produced around the well on chromazurol-S (CAS) agar plate at 28°C after 48 h. The PGPR isolates from Deothi site showed the siderophore production in range of 9 to 14 mm diameter of zone produced on CAS agar. Out of twenty four isolates, twenty three isolates were found to produce the siderophore in plate assay.

Maximum siderophore production was shown by isolate De-4 (14 mm) followed by De-23 and De-20 (13 mm). From Deothi site majority of the isolates were found positive for siderophore production and some few isolates were shown negative results for siderophore production.

HCN

Isolates of PGPR from Deothi showed HCN production on nutrient agar medium amended with glycine. Out of twenty four isolates, maximum production of HCN (++++) was shown by De-21 to change the colour of filter paper strip yellow to brown. No HCN production was shown by other isolates from Deothi site. In case of HCN production majority of the isolates from Deothi site was shown results negative for HCN.

Ammonia

The production of ammonia in peptone water after inoculation with test isolates and incubation for four days at 28°C was observed by using Nessler's reagent. Color change in the broth tubes after growth from yellow to brown after addition of Nessler's reagent

showed presence of ammonia that was soluble in the media. Out of twenty four isolates, fourteen isolates showed maximum production of ammonia colour change from yellow to orange (+++) while other isolates showed slightly less production of ammonia i.e colour change from yellow to light orange (++)). All the isolates from Deothi site were shown positive results for ammonia in different ranges (++) to (+++).

Lytic enzymes

Protease

Out of total 24 isolates, 21 isolates were found to be positive for protease production. Protease production was in the range of 18-38mm diameter of clear zone. Maximum production was shown by De-8 (38.0 mm) followed by De-5, De-7 and De-21, De-6 (35.0 mm) and De-10 (34.0mm) of clear zone. Other isolates showed the zone size in the range of 18-33mm.

Chitinase

Out of 24 isolates, only 7 isolates were found to be positive for chitinase production. Chitinase production was observed in the range of 8-20 mm diameter of clear zone. Maximum production was shown by isolates De-14, De-15 (20.0 mm) followed by De-13 (18.0 mm) and De-3, De-17 (15.0 mm). Other isolates showed the zone size in the range of 6-8 mm. Most of the isolates were found negative results for chitinase and some were found positive for chitinase production.

Cellulase

Out of total 24 isolates of PGPR, only 3 isolates were found to be positive for cellulase production. Cellulase production was shown in the range of 11-14 mm diameter of clear zone. Maximum production was shown by De-21 (14.0 mm) followed by De-5 (12.0

mm) and De-24 (11.0 mm). From Deothi site Majority of the isolates were found negative results for cellulase production, only three isolates were shown positive for cellulase production.

Phosphate solubilization

Phosphate solubilization activity shown by bacterial isolates from Kandaghat was expressed in the range of 11 to 32 mm diameter of yellow zone on PVK agar . Out of twenty four isolates, only seventeen isolates were shown phosphate solubilization. Maximum P- solubilization was shown by the isolates Kn-5 (32 mm) and followed by the isolate Kn-17 (30 mm), Kn-20 (28 mm) and Kn-19, Kn-4 (27 mm). Other isolates showed slightly less P-solubilization in the range of 11 to 26 mm. From Kandaghat site most of the isolates were found positive for P-solubilization.

Siderophore production

The isolates from Kandaghat site showed the siderophore production in range of 15 to 20 mm diameter of pink zone produced on CAS agar by qualitative method. Out of total twenty four isolates, twenty one isolates were found to produce the siderophore production. Maximum siderophore production was recorded for isolate Kn-14 (20 mm) followed by Kn-5 (18mm) and Kn-9 (17 mm). So from kandaghat site majority of the isolates were shown positive results for siderophore production.

Isolates of PGPR from Kandaghat showed HCN production on nutrient agar medium amended with glycine. Out of twenty four isolates, maximum production of HCN (++++) was shown by Kn-7 to change the color of filter paper strip yellow to brown. No HCN production was shown by other isolates from Kandaghat site.

Lytic enzymes

Protease

Out of 24 isolates, 20 isolates were found to be positive for protease production. Protease production was in the range of 10-24 mm diameter of clear zone. Maximum production was recorded for Kn-2 (24.0 mm) followed by isolates Kn-5 (21.0 mm) and Kn-1 and Kn-7 (20.0mm) of clear zone. Other isolates showed the zone size in the range of 10-18 mm of clear zone. From Kandaghat site most of the isolates were found positive for protease production.

Chitinase

All the isolates from Kandaghat site were found to be negative for chitinase production, because chitinase enzyme was absent in these isolates.

Cellulase

All the isolates from Kandaghat were found to be negative for cellulase production, because cellulose enzyme was absent in these isolates.

Phosphate solubilization

Phosphate solubilization activity shown by bacterial isolates from Dharja was expressed in the range of 9 to 23 mm diameter of yellow zone on PVK agar. Out of twelve isolates, eleven isolates showed positive results for phosphate solubilization. Maximum P-solubilization was shown by the isolates Dh-12 (23mm) followed by the isolate Dh-7, Dh-11 (22mm) and Dh-10 (21 mm). Other isolates showed slightly less P-solubilization in the range of 9 to 20 mm diameter. Majority of the isolates from Dharja site showed P-solubilization *in-vitro* conditions.

Siderophore production

The PGPR isolates from Dharja site showed the siderophore production in the range of 9 to 16 mm diameter of pink zone produced on CAS agar by qualitative method. Out of twelve isolates, eleven isolates were found to produce the siderophore. Maximum siderophore production was shown by isolate Dh-9 (16 mm) followed by Dh-8 (15 mm) and Dh-7 (14 mm). Most of the isolates were recorded positive for siderophore production by qualitatively.

HCN

Isolates of PGPR from Dharja showed HCN production on nutrient agar medium amended with glycine. Out of twelve isolates, nine isolates showed HCN production. Maximum production of HCN (++++) was shown by six isolates to change the color of filter paper strip yellow to brown. Three isolates produced in the range of (+++). Majority of the isolates were recorded positive for HCN production in the range of light brown (+++) to dark brown (++++).

Ammonia

The production of ammonia in peptone water after inoculation with test isolates and incubation for four days at 28⁰C was observed by using Nessler's reagent. Color change in the broth tubes after growth from yellow to brown after addition of Nessler's reagent showed presence of ammonia that was soluble in the media. Out of twelve isolates, six isolates showed maximum production of ammonia (++++) while other six isolates showed slightly less production of ammonia (++) . All the isolates from Dharja site were found positive for ammonia production.

Lytic enzymes

Protease

All the isolates were found to be positive for protease production. Protease production was found in the range of 25-35mm diameter of clear zone. Maximum production was shown by Dh-7 (35.0 mm) followed by Dh-2, Dh-7, Dh-6 (33.0 mm) and Dh-1, Dh-5 and Dh-11 (32.0 mm) of clear zone.

All the isolates from Dharja site was found positive for protease production on skim milk agar.

Chitinase

Out of total 12 isolates, only 9 isolates were found to be positive for chitinase production. Chitinase production was recorded in the range of 8-20 mm diameter of clear zone. Maximum production was shown by Dh-4, Dh-11 (20.0 mm) followed by Dh-3 (18.0 mm). Majority of the isolates were recorded for chitinase production.

Cellulase

Out of 12 isolates only 3 isolates were found to be positive for cellulase production. Cellulase production was in the range of 10-13 mm diameter of clear zone. Maximum production showed by Dh-3 (13.0 mm) followed by Dh-7 (12.0 mm) and Dh-8 (10.0 mm).

Most of the isolates were found negative for cellulase production on nutrient medium containing cellulose as a substrate.

Table.1 Population density of rhizobacteria on different isolation media from sites Deothi, Kandaghat and Dharja of Solan District of Himachal Pradesh

| Site of rhizospheric soil sample collection | Total viable count on different nutrient medium | | | | | | | | | | | |
|---|---|------------------|----------------------------|------------------|------------------|----------------------------|------------------|------------------|----------------------------|---------------------|------------------|----------------------------|
| | Nutrient agar | | | King's B agar | | | Jensen media | | | Luria Bertani agar\ | | |
| | Dilutions | | cfu/g soil×10 ⁵ | Dilutions | | cfu/g soil×10 ⁵ | Dilutions | | cfu/g soil×10 ⁵ | Dilutions | | cfu/g soil×10 ⁵ |
| | 10 ⁻⁴ | 10 ⁻⁶ | | 10 ⁻⁴ | 10 ⁻⁶ | | 10 ⁻⁴ | 10 ⁻⁶ | | 10 ⁻⁴ | 10 ⁻⁶ | |
| Deothi | 250 | 40 | 250 x 10 ⁵ | 230 | 110 | 230 x 10 ⁵ | 200 | 60 | 200 x 10 ⁵ | 102 | 69 | 102 x 10 ⁵ |
| Kandaghat | 70 | 15 | 70 x 10 ⁵ | 100 | 65 | 100 x 10 ⁵ | 70 | 36 | 70 x 10 ⁵ | 19 | 7 | 19 x 10 ⁵ |
| Dharja | 165 | 53 | 165 x 10 ⁵ | 170 | 68 | 170 x 10 ⁵ | 120 | 59 | 120 x 10 ⁵ | 180 | 78 | 180 x 10 ⁵ |

Table.2 Potential of bacterial isolates for different PGP attributes of site Deothi

| Sr. no | Isolate | Siderophore production | Phosphate - solubilization | HCN* | Ammonia** | Protease Clear zone (mm dia.) | Chitinase Clear zone (mm dia.) | Cellulase Clear zone (mm dia.) |
|----------------------------|---------|------------------------|----------------------------|-----------------------------------|-----------------|-------------------------------|--------------------------------|--------------------------------|
| | | Plate (mm dia) | Plate (mm dia) | Change of color (yellow to brown) | | | | |
| | | | | (Paper strip) | (Culture broth) | | | |
| 1 | De-1 | 9 | 0 | - | ++ | 20.0 | 0.0 | 0.0 |
| 2 | De-2 | 11 | 15 | - | +++ | 0.0 | 0.0 | 0.0 |
| 3 | De-3 | 12 | 0 | - | ++ | 29.0 | 15.0 | 0.0 |
| 4 | De-4 | 14 | 15 | ++ | ++ | 22.0 | 0.0 | 0.0 |
| 5 | De-5 | 12 | 25 | - | +++ | 35.0 | 8.0 | 12.0 |
| 6 | De-6 | 12 | 18 | + | ++ | 34.0 | 6.0 | 0.0 |
| 7 | De-7 | 11 | 10 | - | +++ | 35.0 | 0.0 | 0.0 |
| 8 | De-8 | 10 | 0 | - | +++ | 38.0 | 0.0 | 0.0 |
| 9 | De-9 | 0 | 6 | - | ++ | 26.0 | 0.0 | 0.0 |
| 10 | De-10 | 9 | 8 | - | +++ | 33.0 | 0.0 | 0.0 |
| 11 | De-11 | 9 | 10 | - | ++ | 34.0 | 0.0 | 0.0 |
| 12 | De-12 | 9 | 20 | - | +++ | 27.0 | 0.0 | 0.0 |
| 13 | De-13 | 10 | 15 | - | +++ | 20.0 | 18.0 | 0.0 |
| 14 | De-14 | 12 | 0 | - | +++ | 22.0 | 20.0 | 0.0 |
| 15 | De-15 | 9 | 10 | - | +++ | 30.0 | 20.0 | 0.0 |
| 16 | De-16 | 8 | 12 | - | +++ | 26.0 | 0.0 | 0.0 |
| 17 | De-17 | 10 | 8 | - | +++ | 25.0 | 15.0 | 0.0 |
| 18 | De-18 | 9 | 0 | - | ++ | 0.0 | 0.0 | 0.0 |
| 19 | De-19 | 11 | 12 | - | ++ | 18.0 | 0.0 | 0.0 |
| 20 | De-20 | 13 | 13 | - | ++ | 0.0 | 0.0 | 0.0 |
| 21 | De-21 | 12 | 25 | +++ | +++ | 35.0 | 0.0 | 14.0 |
| 22 | De-22 | 10 | 10 | - | +++ | 30.0 | 0.0 | 0.0 |
| 23 | De-23 | 13 | 24 | - | ++ | 29.0 | 0.0 | 0.0 |
| 24 | De-24 | 12 | 15 | - | +++ | 26.0 | 0.0 | 11.0 |
| C D _{0.05} | | 2.72 | 1.46 | - | - | 1.54 | 0.88 | 0.58 |

Table.3 Potential of bacterial isolates for different PGP attributes of site Kandaghat

| Sr. no | Isolate | Siderophore production | Phosphate solubilization | HCN* | Ammonia** | Protease Clear zone (mm dia.) | Chitinase Clear zone (mm dia.) | Cellulase Clear zone (mm dia.) |
|------------|---------|------------------------|--------------------------|-----------------------------------|-----------------|-------------------------------|--------------------------------|--------------------------------|
| | | Plate (mm dia) | Plate (mm dia) | Change of color (yellow to brown) | | | | |
| | | | | (Paper strip) | (Culture broth) | | | |
| 1 | Kn-1 | 0 | 0 | - | ++ | 20.0 | 0.0 | 0.0 |
| 2 | Kn-2 | 0 | 25 | - | ++++ | 24.0 | 0.0 | 0.0 |
| 3 | Kn-3 | 16 | 0 | - | ++ | 0.0 | 0.0 | 0.0 |
| 4 | Kn-4 | 15 | 27 | - | ++ | 15.0 | 0.0 | 0.0 |
| 5 | Kn-5 | 18 | 32 | - | ++ | 21.0 | 0.0 | 0.0 |
| 6 | Kn-6 | 0 | 24 | - | +++ | 16.0 | 0.0 | 0.0 |
| 7 | Kn-7 | 15 | 25 | +++ | ++++ | 12.0 | 0.0 | 0.0 |
| 8 | Kn-8 | 16 | 25 | - | ++ | 20.0 | 0.0 | 0.0 |
| 9 | Kn-9 | 17 | 26 | - | ++++ | 10.0 | 0.0 | 0.0 |
| 10 | Kn-10 | 15 | 23 | - | ++++ | 12.0 | 0.0 | 0.0 |
| 11 | Kn-11 | 16 | 25 | - | ++++ | 16.0 | 0.0 | 0.0 |
| 12 | Kn-12 | 15 | 16 | - | +++ | 0.0 | 0.0 | 0.0 |
| 13 | Kn-13 | 16 | 0 | - | ++++ | 14.0 | 0.0 | 0.0 |
| 14 | Kn-14 | 20 | 0 | - | +++ | 16.0 | 0.0 | 0.0 |
| 15 | Kn-15 | 16 | 0 | - | ++++ | 18.0 | 0.0 | 0.0 |
| 16 | Kn-16 | 15 | 11 | - | ++ | 12.0 | 0.0 | 0.0 |
| 17 | Kn-17 | 16 | 30 | - | ++++ | 14.0 | 0.0 | 0.0 |
| 18 | Kn-18 | 16 | 0 | - | ++++ | 17.0 | 0.0 | 0.0 |
| 19 | Kn-19 | 15 | 27 | - | ++++ | 12.0 | 0.0 | 0.0 |
| 20 | Kn20 | 16 | 28 | - | +++ | 0.0 | 0.0 | 0.0 |
| 21 | Kn-21 | 16 | 25 | - | ++++ | 15.0 | 0.0 | 0.0 |
| 22 | Kn-22 | 15 | 22 | - | +++ | 0.0 | 0.0 | 0.0 |
| 23 | Kn-23 | 16 | 26 | - | ++++ | 18.0 | 0.0 | 0.0 |
| 24 | Kn-24 | 16 | 16 | - | ++ | 12.0 | 0.0 | 0.0 |
| CD 0.05 | | 1.54 | 1.43 | - | - | 1.50 | - | - |

Table.4 Potential of bacterial isolates for different PGP attributes of site Dharja

| Sr. no | Isolate | Siderophore production | Phosphate solubilization | HCN* | Ammonia** | Protease Clear zone (mm dia.) | Cellulase Clear zone (mm dia.) | Chitinase Clear zone (mm dia.) |
|--------------------|---------|------------------------|--------------------------|-----------------------------------|-----------------|-------------------------------|--------------------------------|--------------------------------|
| | | Plate (mm dia) | Plate (mm dia) | Change of color (yellow to brown) | | | | |
| | | | | (Paper strip) | (Culture broth) | | | |
| 1 | Dh-1 | 12 | 12 | ++++ | ++ | 32.0 | 0.0 | 0.0 |
| 2 | Dh-2 | 11 | 10 | ++++ | +++ | 33.0 | 0.0 | 12.0 |
| 3 | Dh-3 | 9 | 20 | - | +++ | 25.0 | 13.0 | 18.0 |
| 4 | Dh-4 | 0 | 20 | - | ++ | 31.0 | 0.0 | 20.0 |
| 5 | Dh-5 | 14 | 9 | +++ | ++ | 32.0 | 0.0 | 15.0 |
| 6 | Dh-6 | 11 | 0 | - | ++ | 33.0 | 0.0 | 0.0 |
| 7 | Dh-7 | 14 | 22 | ++++ | +++ | 35.0 | 12.0 | 11.0 |
| 8 | Dh-8 | 15 | 16 | ++++ | +++ | 32.0 | 10.0 | 8.0 |
| 9 | Dh-9 | 16 | 19 | ++++ | ++ | 30.0 | 0.0 | 15.0 |
| 10 | Dh-10 | 10 | 21 | +++ | +++ | 30.0 | 0.0 | 20.0 |
| 11 | Dh-11 | 11 | 22 | +++ | ++ | 32.0 | 0.0 | 0.0 |
| 12 | Dh-12 | 12 | 23 | ++++ | +++ | 29.0 | 0.0 | 13.0 |
| CD _{0.05} | | 1.62 | 1.62 | - | - | 1.65 | 0.74 | 1.47 |

Plate.1 Collection of rhizospheric soil samples from *Triticum aestivum* rhizosphere from different sites of Solan district of Himachal Pradesh



Plate.2 Total viable count of rhizobacteria from the rhizospheric soil of wheat on four different medium from Solan district

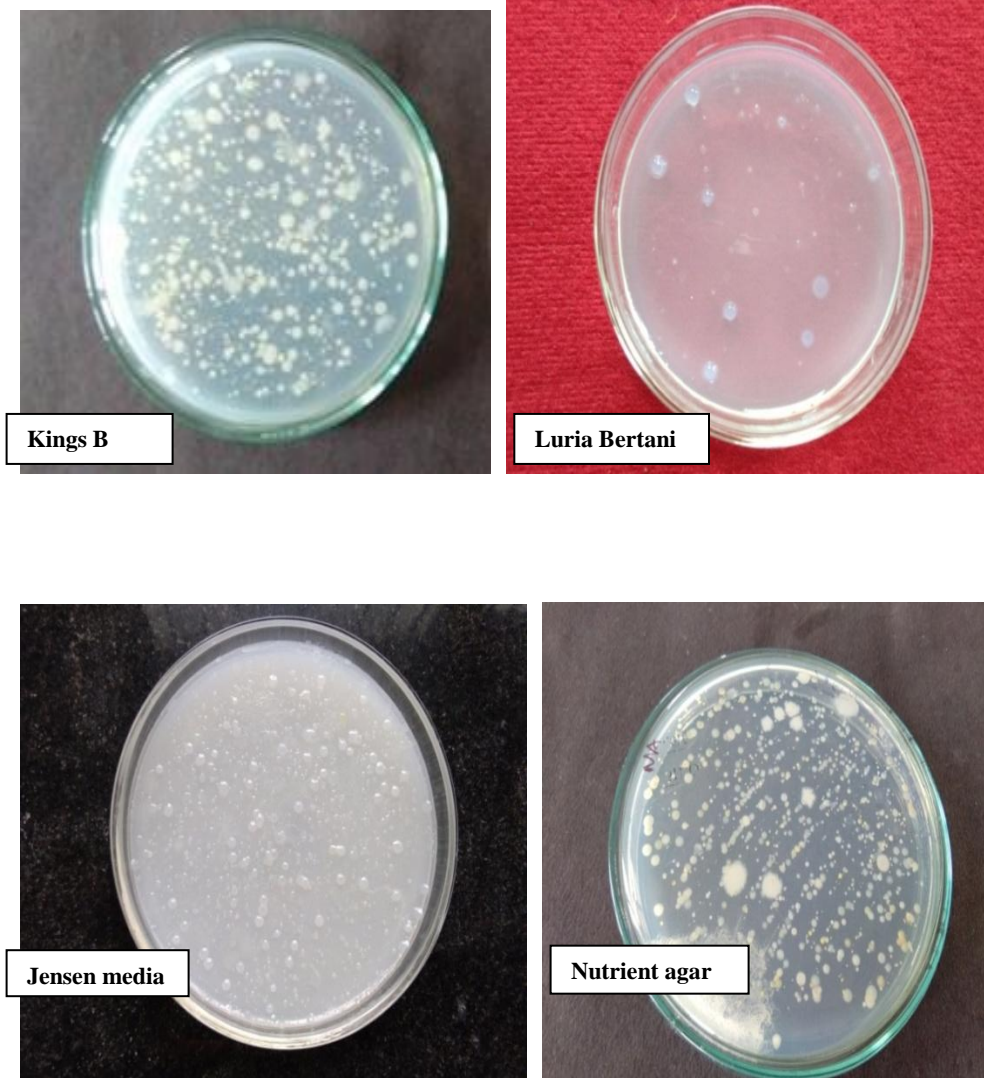


Fig.1 Morphological characteristics of rhizobacterial isolates from site Deothi

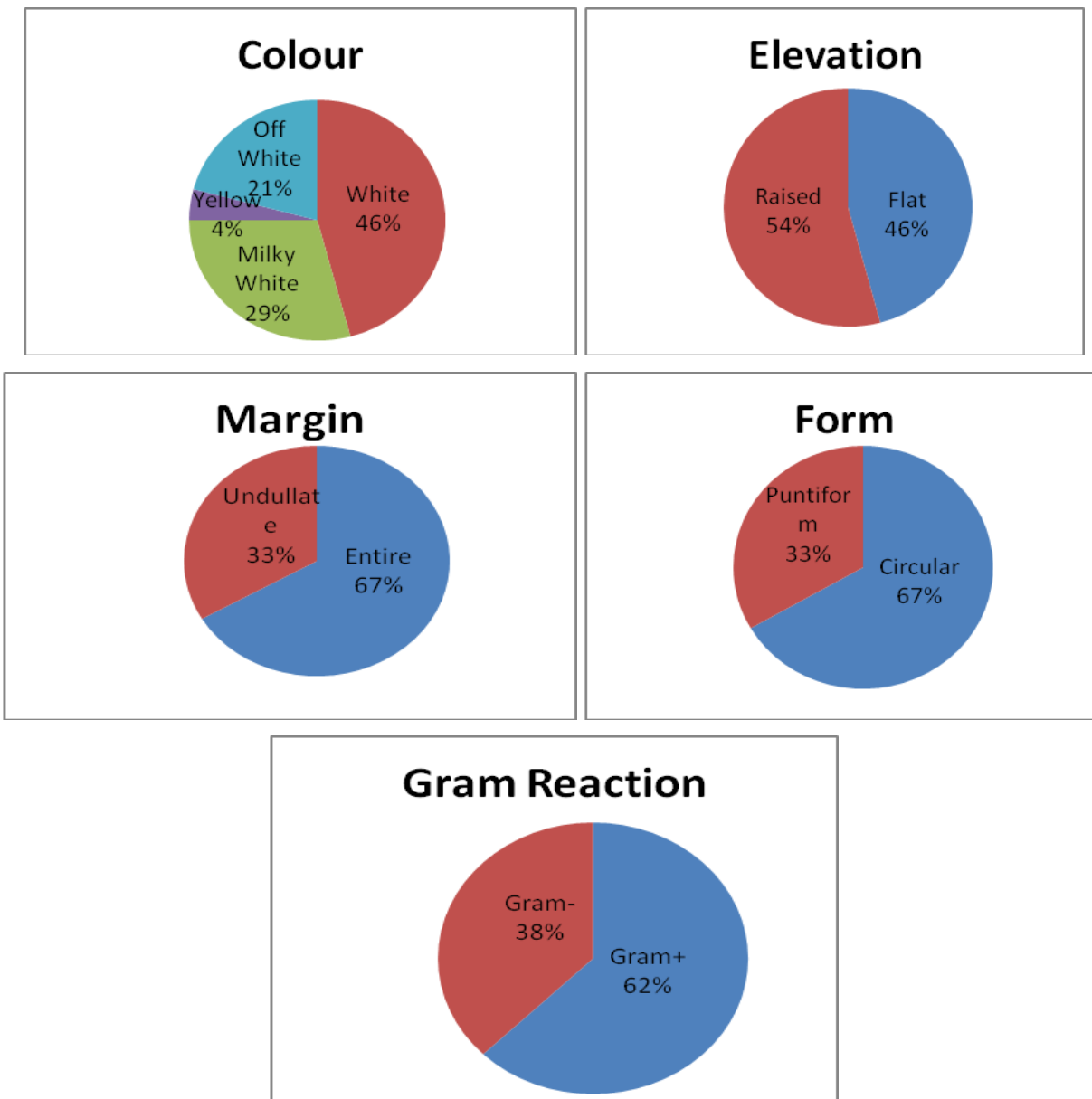


Fig.2 Morphological characteristics of bacterial isolates from site Kandaghat

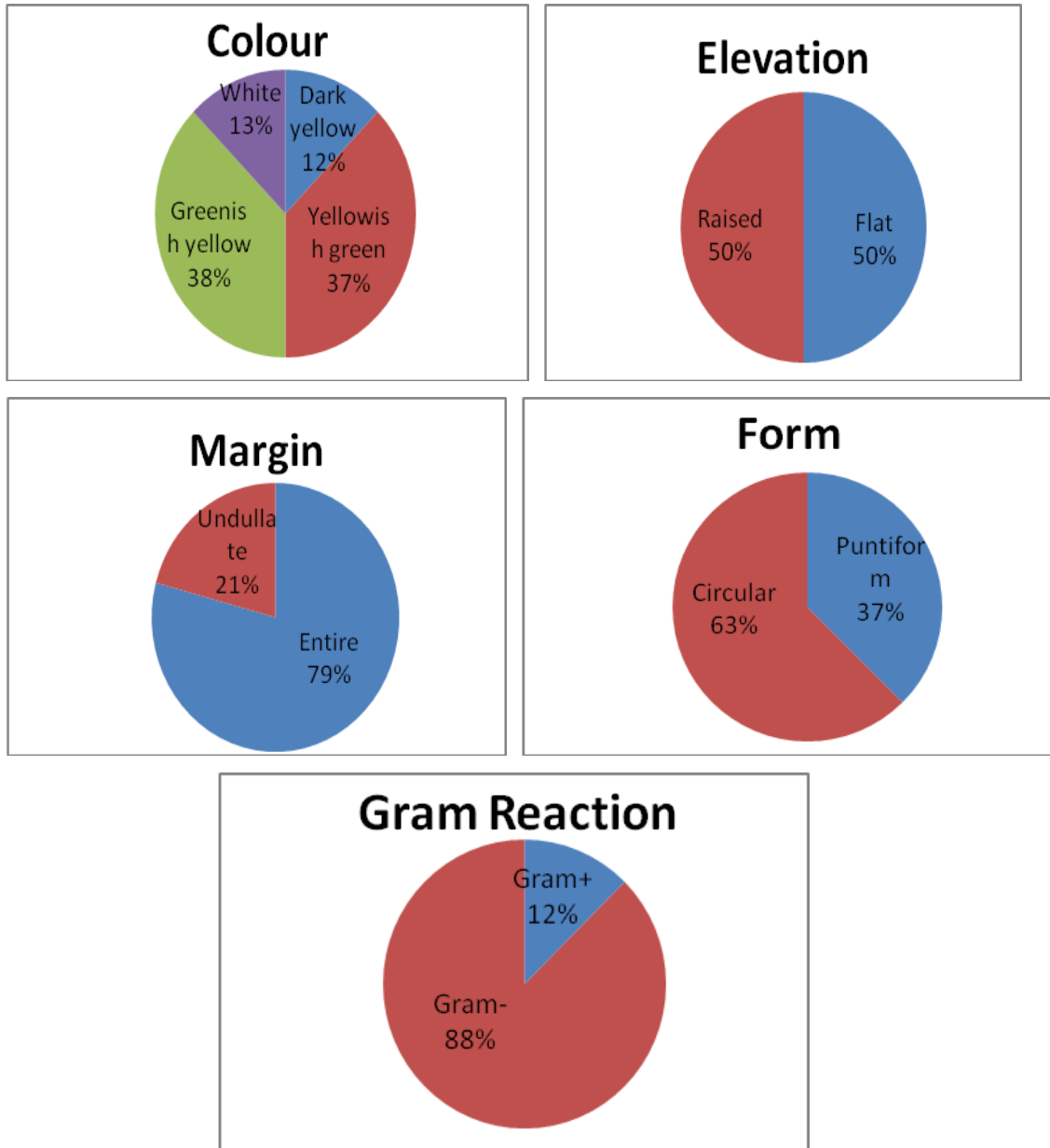
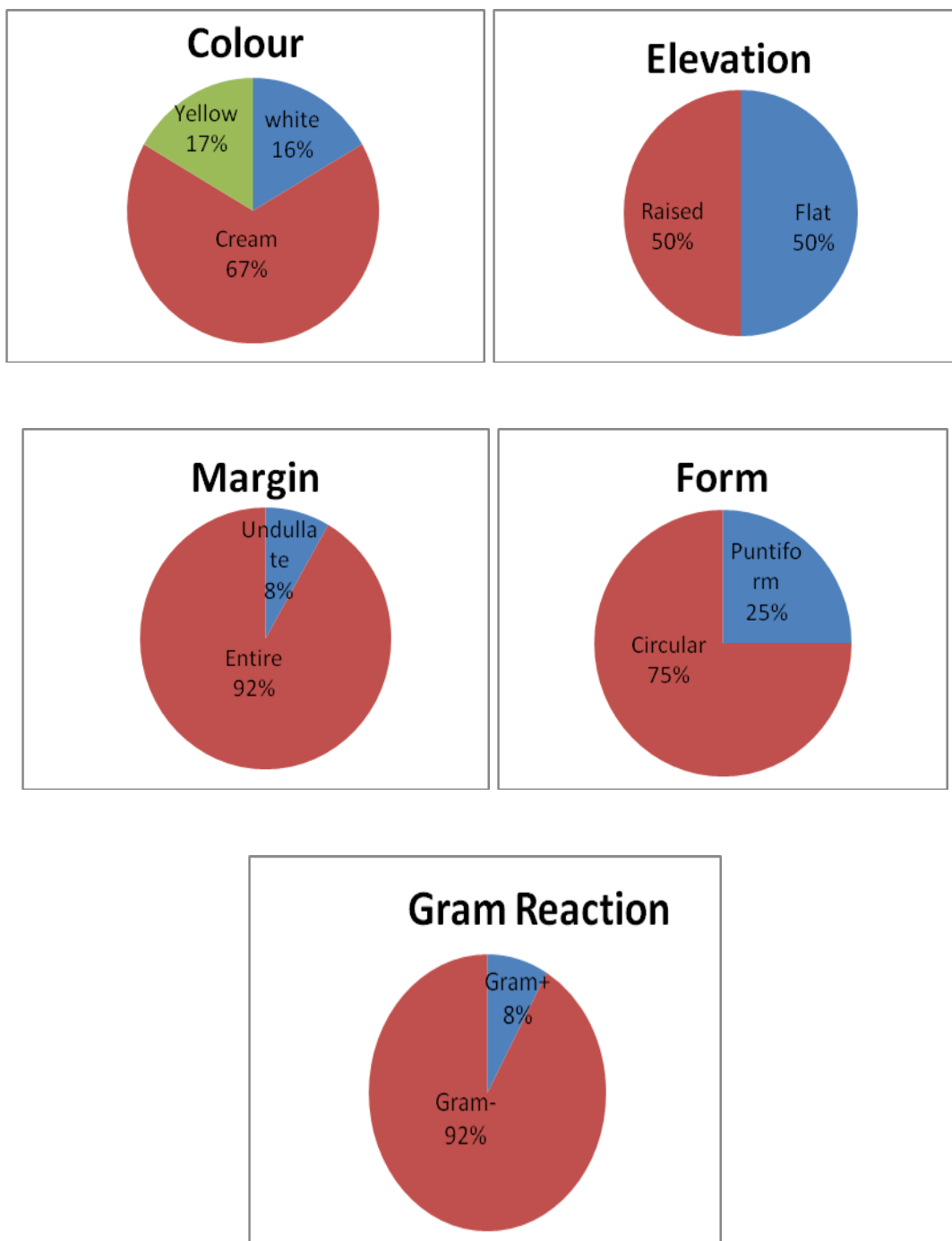


Fig.3 Morphological characteristics of bacterial isolates from site Dharja



Total sixty isolates were recovered from the different three sites of Solan district (Deothi, Kandaghat and Dharja) of Himachal Pradesh. These isolates were isolated on the four different nutrient media. In case of elevation

some were flat and few isolates were raised. Majority of isolates were entire and few were observed undulate in their margin. Most of the isolates were found circular and some were puntiform in their form. Out of total

sixty isolates, majority of isolates were gram negative and few isolates were gram positive in nature. Majority of the isolates were found bacilli and fewer were cocci in their shape. Our results are in agreement with those from other investigations that found gram negative bacteria as main composition of rhizosphere and root associated microbial communities in many plant species (Joshi *et al.*, 2006).

Thirty seven isolates were found positive for P- solubilization from these three sites. Phosphorus (P) is one of the major plant nutrients, that promote shoot and root growth of plants. Chemical fertilizers are main source for phosphorus supplying in agricultural systems but about 75-90 % of added phosphorus to the soil is being fixed by Fe, Al³⁺ and Ca²⁺ complexes (Tarun *et al.*, 2006). Gupta (2012) also reported that the population of phosphate solubilizing microorganisms, in general, varied from 20-24% of the total population, however in some soils it may be 85% of the total population. In another studies conducted by Kundu *et al.*, (2002) reported that about 16% of the total bacterial population in rhizosphere of wheat was P-solubilizer.

Out of total sixty isolates, fifty five isolates were recorded for siderophore production. Similar study was demonstrated that *Acinetobacter calcoaceticus* isolated from wheat rhizosphere produces catechol type of siderophores during exponential phase, which is influenced by iron content of medium (Sarode *et al.*, 2009). The organisms used were siderophoregenic pyoverdinin-producing *Pseudomonas putida* and *Pseudomonas aeruginosa* strains from two diverse habitats. Inoculation with siderophoregenic PGPR increased percentage germination, shoot height, shoot and root length, weight of spikelets, chlorophyll content, grain yield and iron content in wheat crop (Sarode *et al.*, 2013; Mishra *et al.*, 2013).

In case of ammonia all the isolates were showed positive results for ammonia production. Previous studies Chaiharn *et al.*, (2008) reported the production of ammonia by phosphate solubilizing microorganisms, more than 64% of the isolates were found to produced ammonia. Another study by Ahmad *et al.*, (2006) reported that the several plant growth promoting rhizobacteria were found to produce ammonia in peptone water amended with Nessler's reagent. Only eleven isolates were showed positive results for HCN production. Although Hydrogen Cyanide acts as a general metabolic inhibitor, it is synthesized, excreted and metabolized by hundreds of organisms present in the rhizosphere mainly Plant Growth Promoting Rhizobacteria (PGPR) (Zeller *et al.*, 2009). The similar study demonstrated that the HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soil of wheat a biocontrol metabolite in *Pseudomonas* species (Saharan and Nehra, 2011). Fifty three isolates were found positive for protease production. Protease a hydrolytic enzymes which suppress/ inhibit the growth of the bacterial and fungal pathogens present in the rhizosphere of the plant. So the protease production by rhizobacteria is important attribute, all the isolates produced protease in good quantity. Similar study was reported by (Upadyay *et al.*, 2013) some proteolytic enzymes especially elastase, subtilisin, pronase also possess bacteriolytic properties against different gram positive and gram negative bacteria. Cell wall-degrading enzyme protease secreted by biocontrol strains of PGPR exert a direct inhibitory effect on the hyphal growth of fungal pathogens by degrading their cell wall. In a similar manner, *Bacillus cepacia* synthesizes β -1,3-glucanase, which destroys the cell walls of the soil borne pathogens *Rhizoctonia solani*, *Pythium ultimum*, and *Sclerotium rolfsii* (Sadfi *et al.*, 2005).

Out of sixty isolates only sixteen isolates were found positive for chitinase production. Chitinase degrades chitin, an insoluble linear polymer of β -1, 4-N-acetyl-glucosamine, which is the major component of the fungal cell wall. So the chitinase production by rhizobacteria is important attribute only seven isolates were produced chitinase. Those PGPR produce chitinase is a novel biological control agents because the cell wall of most of the fungal pathogens made up of chitin thus the cell wall of these pathogens is degraded by chitinase producer biological agents, so these isolates of PGPR were also act as biological control agents. Similar study was reported by (Jha and Saraf, 2015) potential biocontrol agents with chitinolytic activities include *B. licheniformis*, *B. cereus*, *B. circulans*, *B. subtilis*, and *B. thuringiensis* among the Gram-negative bacteria, *Serratia marcescens*, *Enterobacter agglomerans*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens* have been found to possess chitinolytic activities. Only six isolates were recorded positive for cellulase production. Cellulase is also a hydrolytic enzymes which can degrade the cell wall of the lower fungal pathogens present in the rhizospheric region of plants. Most of the lower fungi cell wall is made up of cellulose a major constituent, thus these isolates were able to degrade the cell wall of plant pathogens having cellulose a major cell wall constituents. These isolates produced cellulase in appropriate quantity, showed different ranges of zone on the plate. The similar previous study was demonstrated that the cellulase synthesized by strains of *Paenibacillus* and *Streptomyces* spp. can easily degrade fungal cell walls of pathogenic *F. oxysporum* (Compant *et al.*, 2005). In other crops the similar study was reported Viveknathan *et al.*, (2004) studied that various kinds of enzymes (Cellulase, Chitinase and Protease) were produced by microorganisms. Lytic enzymes induced by *Pseudomonas fluorescens* and other

biocontrol organisms mediated defence against the *Anthraco*se pathogen in mango leading to improved yield attributes.

It is concluded that, this is a basic study that has provided an insight into the bacterial community present in the mid hill zone of Himachal Pradesh, India .We have demonstrated efficient P-solubilizing, siderophore, ammonia, HCN and lytic enzymes producing bacteria present among the natural population. These characteristics are considered as important PGP traits and have been found effective in positively improving the growth and N contents of tested wheat plants. These isolates offer potentially in field applications as PGP agents in wheat.

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