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Phenotypic characterization of root-associated Rhizomicroflora of (*Phaseolus vulgaris)* French Yellow Bean under temperate ecologies

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Abstract
The present study was aimed to assess the diversity of rhizospheric micro flora of *Phaseolus vulgaris* (French yellow) as well as their efficacy as potential bioinoculants in organic agriculture under temperate conditions of Kashmir. Isolation and characterization of rhizobacteria are an effort to determine the ability of root colony bacteria to produce various compounds that can be used for various purposes of bio-fertilizer formulations and microbial-based industrial interests. The morphological characters like colony shape, size, texture, colour was recorded along with cell characters like gram's staining and endospore staining. Also, cultural characters like citrate utilization, oxidase test, methyl red test and catalase test was also evaluated. Apart from these, biochemical characterization was also done to evaluate the PGP production ability along with antifungal activities like Ammonia Production, ACC deaminase activity, Phosphorous Solubilization, HCN production, Chitinase Activity, Phytopathogen inhibition using dual culture technique against *Rhizoctonia solani* and *Fusarium oxysporum.* The characterization revealed a high varsity among the rhizobacterial isolates that were isolated from the Rhizosphere depicting a plethora of diversity among the root Rhizosphere of *Phaseolus vulgaris* (French Bean).

Keywords: Phenotypic, characterization, micro flora, French yellow, Temperate, ecology

1. Introduction
Rhizosphere refers to the tiny patch of soil that surrounds and influences plant roots because it contains more nutrients than the majority of the soil, it has more vigorous biological and chemical activity. (Ahmed *et al.*, 2019) [5]. A wide range of macro and microorganisms including bacteria, fungi, virus, protozoa, algae, nematodes and micro-arthropods co-exist in rhizosphere and show a variety of interactions between themselves as well as with the plant. (Bahadur *et al*., 2017) [8]. Rhizomicroflora is made up of a variety of symbiotic and non-symbiotic bacteria, and research has proven that these bacteria are among the best for the soil, plants, and the sustainability of the ecosystem as a whole. (Papik *et al*., 2020) [36] These rhizobacteria have a variety of direct and indirect strategies that they use to enhance plant development in various agricultural environments. (Goudaa S., *et al*., 2018 and Umar W. *et al.*, 2020) [14, 48, 15]. When it comes to enhancing plant growth and stress tolerance as well as achieving sustainable agriculture, plant growth promoting rhizobacteria (PGPR), which are a collection of helpful bacteria found in the rhizosphere and rhizoplane of the plants, have a plethora of PGP activities as well as phytopathogen inhibition activity. (Shrivastava and Kumar, 2015; Turan *et al*., 2017; Gouda *et al*., 2018; Grobelak *et al*., 2018; Nagargade *et al*., 2018) [45, 47, 15, 16]. Through a number of mechanisms, including nitrogen fixation, the production of plant growth hormones (Auxins, cytokinins, and gibberellins), the solubilization of phosphates, and the sequestration of iron through the production of siderophores, they have an indirect and direct impact on plant growth and phytopathogen management. (Bhattacharyya and Jha, 2012; Egamberdieva and Lugtenberg, 2014; Shameer and Prasad, 2018) [9, 12, 44]. Rhizobacteria, which live in the rhizosphere, employ a variety of strategies to promote plant development, such as metabolic changes, modifications to phytohormone levels, the generation of exopolysaccharides, root colonization, and improved nutrient availability. (Ali M.A.*et al*., 2017; Ismail M.A. *et al*., 2021; Khan N. *et al*., 2021) [6, 20, 23]
The production of antimicrobials, induction of induced systemic resistance, rhizosphere competence, and production of antagonistic stimulants for biological control are some of the

mechanisms these rhizobacteria use to induce plant resistance to various biotic and abiotic stresses, such as pathogen attack and heavy metal contamination, and indirectly improve plant growth. (Ali M.A. *et al*., 2017; Mustafa A. *et al*., 2019a; Mustafa A. *et al*., 2019b; Ray P. *et al*., 2020; Abbas T. *et al*., 2020) [6, 27-28, 40, 2]. Additionally, rhizobacteria employ the synthesis of bio surfactants and siderophores, biosorption, and the formation of polymeric materials as their bioremediation processes for polluted soils. (Ahmad M. *et al*., 2019; Nazli F. *et al*., 2020; Haider F.U. *et al*., 2021) [4, 32, 18]. Additionally, it has recently been shown that certain rhizobacteria have the ACC-deaminase enzyme, which hydrolyzes ACC into ammonia and alpha-keto butyric acid. It is recognized that ethylene has an impact on various aspects of nodule and root growth. The ACC-deaminase activity of rhizobacteria that promote plant development is crucial for the host nodulation response. (Remans *et al.,* 2007) [42].

2. Sampling and isolation

2.1. Sampling and sources of rhizobacteria

The bean rhizospheric soil samples was collected from healthy bean fields in North Kashmir's three districts *viz*; Bandipora, Baramulla and Kupwara. Two blocks from each district were selected and from these blocks, two villages per block will be selected, leading to the number of locations in each district to be four and samples collected to be 96. The rhizosphere soil samples were collected at flowering stage by quartering and coning method in order to get a homogenous soil sample or a representative sample for each location. The samples were then immediately brought to the laboratory and preserved at 4 ºC for further studies.

2.2. Sample preparation and isolation of bacteria

The microflora was isolated from the collected rhizospheric soil by employing serial dilution method. The serial dilution (upto 10^{-7}) were made in 9.0 ml sterilized water blank and 0.1 ml of diluted soil suspension was plated on medium plates including Luria Bertani Media (LB), Yeast Extract Mannitol Agar (YEMA) and King's B Agar Media. The plated were incubated at 28±2 ºC in BOD incubator for 3-4 days. The population of microflora was observed on each media and was isolated for further screening procedures. Bacterial isolates that showed different colony morphology were taken and purified using nutrient agar media.

3. Characterization and screening of isolated Micro flora

3.1 Study of morphological characters of Rhizosphere bacterial isolates:

Different morphological characters like colony morphology, cell morphology, Gram's reaction and endospore staining in all the isolates were studied.

3.1.1. Study of colony morphology

All the bacterial isolates were observed for colony morphology during purification process. The colony morphology was studied on plates after streaking a loopfull of isolated colony and colony characters like shape, elevation, size and color were observed.

3.1.2. Study of cell shape, Gram's Reaction and Endosopre Staining

All the bacterial isolates were subjected to gram and spore staining. A smear was prepared from isolated colonies and stained with Gram's Stain and Endospore stain (Malachite Green). Slides were observed under microscope at 40X. Cell shape, Gram's Reaction and spores were observed and were photographed.

3.2. Study of cultural characters of Rhizosphere bacterial isolates

3.2.1. Catalase test

This test is carried out to determine the ability of isolated bacteria to degrade hydrogen peroxide by producing enzymes, catalase or peroxidase. A drop of 3% hydrogen peroxide is added to the bacterial colony on a sterile glass slide and mixed well. The production of air bubbles was observed for one minute. The production of air bubbles indicates positive catalase and no bubbles indicates negative catalase.

3.2.2. Oxidase test

The oxidase test is carried out to determine the presence of the oxidase permease enzyme in bacteria. Oxidase reagents contain reducing agents that change color when oxidized. One loop of bacterial colony was blotted on oxidase paper. Observations were made by looking at the reaction caused, if the results showed blue streaks on oxidase paper it indicate that the tested bacteria have a positive oxidase enzyme and if there is no change in color, it shows a negative oxidase result.

3.3.3. Methyl Red Test

The methyl red test is employed to detect the ability of microorganisms to oxidize glucose with the production of the high concentrations of acid products. Although all enteric forms ferment glucose with organic acid as the end product, the MR test is used in differentiate particularly between Escherichia coli and Enterobacter aerogenes which can utilize glucose and produce organic acids as end product during early incubation period. A bright red color indicating a pH of 4.2 or less is a positive test. Yellow or orange color indicates negative reaction. A weakly positive test will be red orange.

3.3.4 Citrate Utilization Test

This test was done in order to evaluate the ability of the bacterial isolates to utilize citrate as a source of carbon. In the absence of fermentable glucose or lactose, some microorganisms use citrate as the carbon source for their energy. Simmon's citrate medium (a modification of Koser's medium is used to test the organism for citrate utilization. Bacteria is grown in the slants and kept for 96 hrs for 28± 2˚C. A positive test medium shows color changes from green to deep Prussian blue while as retention of original green colour and no growth on the line of streak indicates a negative reaction.

3.3. Study of biochemical characters of rhizospheric bacterial isolates.

3.3.1. Antifungal behavior using Phytopathogen inhibition against various pathogens. (Dual culture)

The assay for antagonism was performed on Petri-dishes containing PDA by dual culture method (Naik *et al*., 2009) [33]. The seven day old mycelial plugs (5 mm diameter) of pathogens (*Fusarium oxysporum* and *Rhizoctonia solani*) and bacterial antagonists were placed on the same Petri-dish 6 cm apart from each other. The bacterial isolates and fungal mycelial discs were placed simultaneously on Petri-dishes. The dishes were incubated at 25+1˚C for seven days. The

percent growth inhibition (PGI) was calculated using the formula:

$$PGI = \frac{T-C}{C} \times 100$$

Where "PGI" is the percent growth inhibition, "T" is the growth of the pathogen in treatment and C is its growth control. The growth of fungal pathogen was measured using a measuring tape.

3.3.2. 1-aminocyclopropane-1-carboxylate deaminase activity. (ACC deaminase)

ACC deaminase assay was done by the method described by, Govindasamy *et al*. (2009) [17]. Bacterial isolates were cultured in 5 ml of LB medium for 24 hours at 28 °C. The cell pellet was collected by centrifugation at 8000 rpm for 5 min and washed with sterile distilled water then re-suspended in 1 ml of sterile water and spot inoculated on Petri plates containing DF salt minimal medium supplemented with 3 mM ACC. Plates containing DF minimal medium without ACC served as a negative control and with $(NH)_2SO_4$, as N-source served as a positive control. The plates were incubated for 3 to 4 days at 28°C. Growth of isolates on ACC supplemented plates was compared to positive and negative control plates.

3.3.3. HCN production (Bakker and Schipper, 1987) [10].

For the estimation hydrogen cyanide produced by bacterial isolates the method of Baker and Schippers (1987) [10] was followed. The bacterial cultures were streaked on pre-poured plates of King's medium (Annexure I amended with 4.4g/l glycine. Whatman No.1 paper strips were first soaked in reagent A (0.5% picric acid in 0.2% sodium carbonate) then placed on the medium containing Petri-plates. The Petri-plates were sealed with parafilm and incubated at 37 °C for 1-4 days. Uninoculated control was also maintained for comparison. The Petri plates were monitored for change in color of filter paper (from yellow to light brown to dark brown).

3.3.4. Chitinase activity

The chitinase activity of bacterial isolates was estimated as per Reissig *et al*. (1955) [41] and for preparation of colloidal chitin the method of Berger and Reynolds (1958) [25] was adopted. For colloidal chitin preparation, the powdered chitin (10 g) was digested overnight with concentrated hydrochloric acid (500 ml) at 4 °C. Then contents were centrifuged (10,000 rpm) for 20 minutes and supernatant removed by filtration. The collected contents were washed three times to remove all acid traces till a pH of 4 was achieved. The pH was adjusted by using 2N NaOH and 1 N HCl. The collected contents were dried in oven at 45 °C for 20 hours and later added to minimal media. (0.3% w/v). All the selected cultures were grown in LB broth. The log phase growing cells (72 h old) of each culture (15 µl) were spotted on the already prepared minimal media Petri-plates amended with 0.3 per cent colloidal chitin; and the plates were incubated at 30°C for 7 days, then iodine was added to these plates. Development of hallow zone around the colony after the addition of iodine was considered as positive for chitinase enzyme production. The halo zone "CZ" (the clear visible zone around bacterial colonies indicating chitin hydrolysis) and colony diameter (CS) of the isolates was measured with a measuring tape. Then the ratio of both the parameters was calculated (CS:CZ).

3.3.5. Phosphate solubilization (Pikovaskaya, 1948)

All the selected cultures were grown in TSA broth. Log phase growing cells of each culture (15 all were spotted on Pikovskaya's medium plates. These plates were incubated at 28-2 °C for 3-4 days. Zone of solubilization and colony size were measured using a measuring scale and these values were used to calculate solubilization index as per the following formula of Edi Premono *et al*. (1996) [13].

SI = Colony diameter + Halozone diameter/colony diameter

3.3.6. Ammonia production (Cappuccino and Sherman 1992).

Rhizobacterial bacterial isolates were screened for the production of ammonia in peptone water as per the method adopted by Cappucino and Sherman (1992). Freshly grown cultures were inoculated in 8-10 ml peptone water each tube and incubated at 28±12 °C for 4 days; then centrifuged for 15 minutes at 10,000 rpm. The supernatant was collected. Then 1 ml Nessler's reagent was added to 1ml supernatant and the volume made up to 10 ml with distilled water. Brown to yellow color was developed which indicated presence of ammonia.

4. Results and discussion

4.1. Isolation of rhizobacteria from the Rhizosphere of *phaseolus vulgaris* (French Yellow).

A total of 100 rhizospheric bacteria were isolated from the contrasting soils samples from different latitudes of North block of Kashmir including Baramulla, Bandipora and Kupwara. A total of 32 samples from each 4 selected locations of these districts that sums up to a total of 96 samples from 3 districts were collected from the rhizosphere of Phaseolus vulgaris (French Yellow) for the purpose of isolating variety of bacterial population. The bacterial isolation was done on 3 contrasting media for getting a vast range of bacterial population that included LB media, YEMA media and King's B media. Serial dilution method of elucidating bacterial population on different media was used. Nomenclature to each isolated bacteria was given based on the place from where they were isolated and on the specificity of the media. (Table 1).

Table 1: Nomenclature to each isolated bacteria based on the specificity of the media.

S.no	Bacterial Isolates		
	King's B Media	YEMA	LB Media
1	KB1A	KL1*1	LBM1
2	KB2B	KL1*2	LBM2
3	KB3C	KL1*3	LBM3
4	KB4D	KL1*4	LBM4
5	KB5E	KL1*5	LBM5
6	KB6F	KL1*6	LBM6

7	KB7G	KL1*7	LBM7
8	KB8H	KL1*8	LBM8
9	KB9I	KL1*9	LBM9
10	KB1J	KL1*10	LBM10
11	KB2K	KL1*11	LBM11
12	KB3L	KL1*12	LBM12
13	KB4M	YEMA1	LBM13
14	KB5N	YEMA2	LBM14
15	KB6O	YEMA3	LBM15
16	KB7P	YEMA4	LBM16
17	KB8Q	YEMA5	LBM17
18	KB9R	YEMA6	LBM18
19	KB1S	YEMA7	LBM19
20	KB3T	YEMA8	LBM20
21	KB2U	YEMA9	LBM21
22	KB4V	YEMA10	LBM22
23	KB5W	YEMA11	LBM23
24	KB6X	YEMA12	LBM24
25	KB7Y	YEMA13	LBM25
26	KB8Z		LBM26
27	KBB1		LBM27
28	KBB2		LBM28
29	KBB3		LBM29
30	KBB4		LBM30
31	KBB5		
32	KBB6		
33	KBB7		
34	KBB8		
35	KBB9		
36	KBB10		
37	KSS1		
38	KSS2		
39	KSS3		
40	KSS4		
41	KSS5		
42	KSS6		
43	KSS7		
44	KSS8		
45	KSS9		

4.2. Morphological characterization of the isolated rhizobacterial isolates.

A more detailed oriented morphological examination of the isolated micro flora was done in order to get an overview of the morphological characters including, the colony characters, and the staining properties of the bacterial population. At the purification stage of bacterial isolates, the colony characters were studied for all the isolates simultaneously. The colony characters showed a distinct variation for all the isolates ranging from round to irregular wherein the majority of the isolates were round (53%), being slimy to being non slimy or gummy out of which 65% were non slimy, from being raised to being flat and uneven, wherein the majority of the isolates were raised (61%). Not only there was a variation in the colony shape but the isolated bacteria showed a vast range in coloration ranging from being whitish to slightly creamish, yellowish, pinkish white, red, yellowish brown and to yellowish orange. The majority 29% were white colored followed by cream colored i.e. 24%. Some of the colonies were larger in size while some were minute. The majority of bacterial colonies were medium in size (44%) followed by 31% being small. (Graph 1).

Cell Size varied from being oval, rod and even cocci. But the majority of bacterial cells i.e 76% showed rod shaped cells. Staining properties of the isolates were also studies based on gram staining and as well as endospore staining to see if the bacteria are Gram Positive or negative and whether there is a presence of endospore or not and if it persists then at what position it was found. Out of the 100 isolates, the majority which is 77% isolates were found to be Gram negative while the rest 23% were gram positive bacteria. Majority of the isolates were found to be non- endospore formers, but only 19% isolates were found to be endospore positive with the spore position varying from central to terminal. (Plate. 1). Many researchers have shown that majority of the rhizobacterial isolates are gram negative. Megersa and aseefa in 2011. Similarly, Rani 2012 also isolated bacterial isolates on the basis of their morphological, cultural characters. Rawat 2011 also in the same way selected rhizobacterial isolates on the basis of color of their colonies and morphological characters. Joshi *et al*. 2011, also in the same way selected 93 isolates from the Rhizosphere on the basis of their colony morphology.

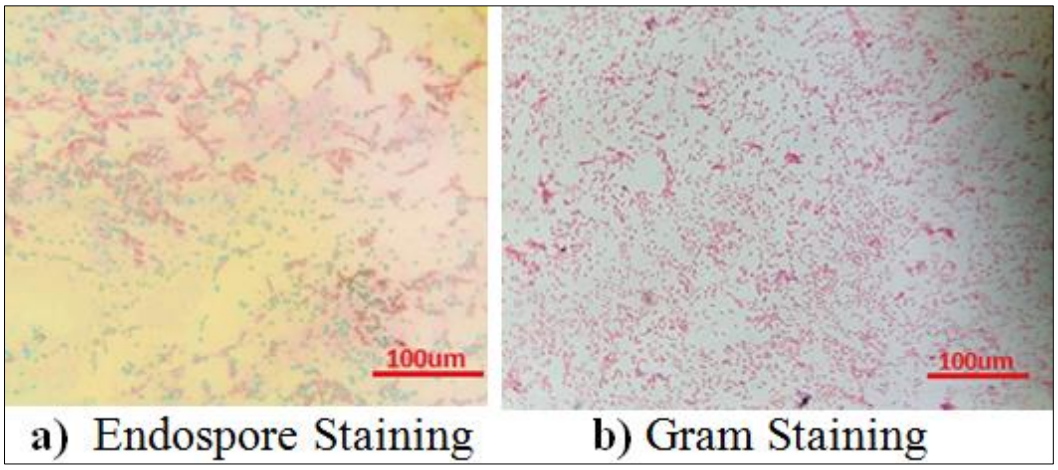
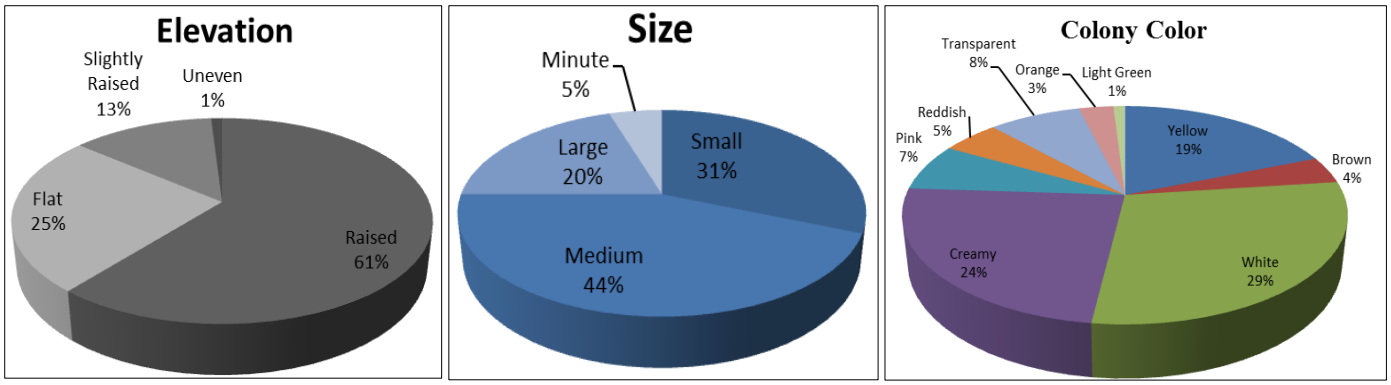


Plate 1: Staining of the bacterial isolates for a) Endospore Staining b) Gram Staining.



Graph: 1 Percentage Distribution of isolates on basis of Morphological Characters

4.3. Cultural Characterization of the isolated rhizobacterial isolates

4.3.1. Catalase test

This test demonstrates the presence of catalase, an enzyme which catalyzes the release of oxygen from hydrogen peroxide. It is used to differentiate the bacteria which are capable to synthesize an enzyme catalase from the non-catalase synthesizing bacteria Catalase synthesizing bacteria possess an ability to protect the cell against reactive oxygen species. The enzyme catalase catalyzes the decomposition of hydrogen peroxide into water and oxygen. Out of the100 French bean rhizobacterial isolates only 62% isolates were able to show catalase activity by producing bubbles after addition of few drops of H_2O_2 on slides containing 24 h fresh broth culture while 16% did not produced bubbles indicating negative reaction. (Plate 2).

Plate 2: The formation of bubbles on the slides shows presence of enzyme catalase activity

4.3.2. Methyl red test

All the French bean rhizobacterial isolates were grown in MR-VP broth and then using methyl red indicator was used for assessment of methyl red production. Out of the 100 isolates only 67% rhizospheric isolates were tested positive for methyl red test and the rest 33% were negative for the test (Plate 3).

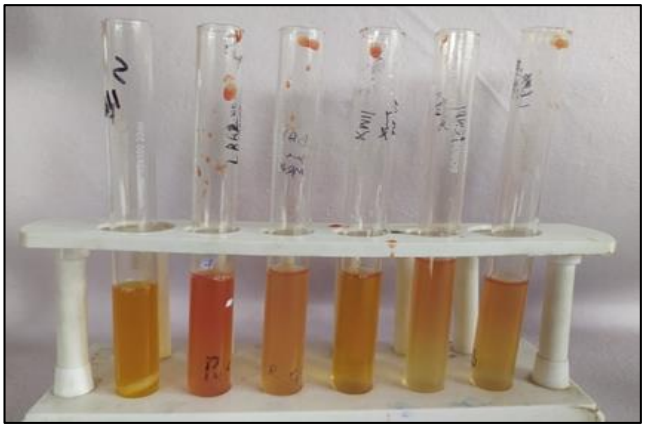


Plate 3: The presence of reddish to dark yellow color indicates a positive methyl red test.

4.3.3. Oxidase test

Oxidase test is the test which is used to determine the ability of bacteria to produce certain cytochrome c oxidase enzyme of bacterial electron transport chain. Among 100 French bean rhizobial isolates70% isolates exhibited positive oxidase activity by developing dark purple color on the filter paper exposed to bacterial cultures while 30% isolates did not produce any such color indicating negative test. (Plate 4).

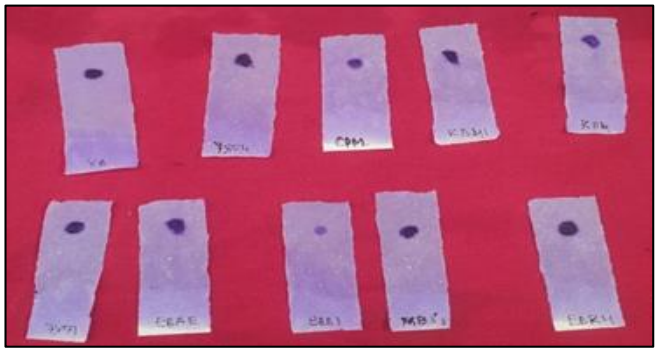


Plate 4: The formation of dark blue color on the filter paper indicates a positive oxidase test.

4.3.4. Citrate Utilization test

Citrate utilization test is done in order to determine the ability of the bacteria to utilize citrate as a carbon source in absence of glucose as a source of their energy. Among the isolates only 51% were able to produce a prussian blue color indicating the positive results. The remaining 49% isolates remained green which indicated a negative response to the test. (Plate 5)

Plate 5: The formation of Prussian blue color in the test tube indicates a positive citrate test.

4.4. Biochemical Characterization of the rhizobacterial isolates

4.4.1. Phytopathogen Inhibition using Dual culture technique

All the isolated bacterial root isolates were assessed for their antifungal activity against two soil borne pathogens viz., *Fusarium oxysporum* and *Rhzioctonia solani*. The study revealed that only 15 isolates among the 100 inflicted growth inhibition of *F. oxysporum*. While as in case of Rhizoctonia solani only 11 isolates showed anti-fungal activity against the pathogen. The percent growth inhibition (PGI%) of *Rhizoctonia solani* bacteraia inflicted growth inhibition percentage of 9.73 to 29.40 per cent (Table 2), while in case of *Fusarium oxysporum* by potential isolates was in the range of 11.48 to 30.80 per cent. (Table 3). Idris *et al* 2007 [19], demonstrated effective biological control by the rhizobacterial isolates against Fusarium oxysporum.

Table 2: Assessment of Rhizobacterial Isolates for Antifungal Activity against *Rhizoctonia solani* by Phytopathogen Inhibition using dual culture technique (Plate Assay).

S. No.	Rhizobacterial Isolates	Phytopathogen Inhibition %
1	KB3C	10.41^{fg}
2	KBB6	15.82^{d}
3	KB3T	25.09^{b}
4	KB3L	15.61^{d}
5	KB7Y	14.63^{e}
6	KL1*11	13.93^{e}
7	YEMA10	29.40^{a}
8	KSS2	21.73^{c}
9	KB5W	21.11^{c}
10	KL1*3	10.75^{f}
11	YEMA11	9.73^{g}

Overall Mean: 17.1
CD ($p \leq 0.05\%$): 0.974
CV%: 4.450
SE(m): 0.341
*Only those bacterial isolates that showed a positive antagonism were evaluated
*a, b, c…… ranking of the isolates based on their efficacy

Table 3: Assessment of Rhizobacterial Isolates for Antifungal Activity against *Fusarium oxysporum* by Phytopathogen Inhibition using dual culture technique (Plate Assay).

S. No.	Rhizobacterial Isolates	Phytopathogen Inhibition %
1	KB3C	12.28^{fg}
2	KB3L	18.08^{d}
3	KB3T	27.15^{b}
4	KB5W	18.05^{d}
5	KB7Y	17.55^{d}
6	KBB4	17.83^{d}
7	KBB6	17.18^{d}
8	KSS2	23.98^{c}
9	KL1*3	25.48^{c}
10	KL1*4	13.05^{f}
11	KL1*11	11.65^{g}
12	YEMA10	30.80^{a}
13	YEMA11	14.35^{e}
14	LBM1	11.85^{fg}
15	LBM5	11.48^{g}

Overall Mean: 18.2
CD ($p \leq 0.05\%$): 1.112
CV%: 4.802
SE(m): 0.392
*Only those bacterial isolates that showed a positive antagonism were evaluated

*a, b, c…… ranking of the isolates based on their efficacy

4.4.2. 1-Amino-Cyclopropane 1 Carboxylate (ACC) deaminase Activity

All the isolates were found to grow luxuriously on control MS agar containing $(NH_4)_2SO_4$ as nitrogen source, whereas, only 20 isolates showed luxurious growth on plates having ACC as sole source of nitrogen showing their ability to produce enzyme ACC deaminase. While the rest of the isolates were negative for ACC deaminase activity. (Plate 6). Many reports suggests that rhizobacterial population have the ability to produce ACC deaminase which helps them to survive in stress condition. Likewise research have been recorded by many researchers. Santoyo *et al*. 2019; Ali *et al*. (2018) [7], Maxton *et al*., 2018; Safari *et al*., 2018) [43, 31, 46].

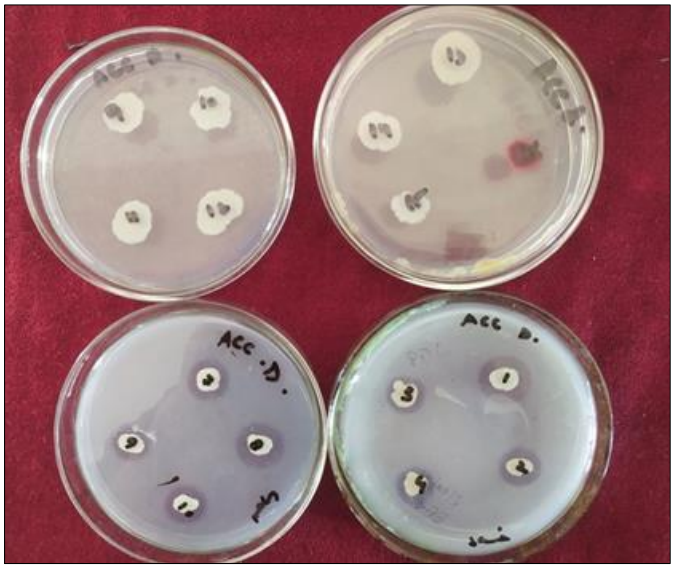


Plate 6: The presence of luxurious growth on the ACC amended media showed the presence of ACC deaminase activity.

4.4.3. Hydrogen Cyanide (HCN) production

Among the different beneficial chemical compounds produced by rhizobacteria, HCN is recognized as a biocontrol agent based on its toxic effect against various plant pathogens and thus HCN helps in improving the plant growth by inhibiting or reducing the pathogenic effect of plant pathogens. Total 100 isolates were examined under controlled conditions for HCN production out of which 19 rhizobacterial isolates tested positive for HCN production while 81 did not change the color of filter paper impregnated with picric acid, as yellow indicating no ability to produce HCN (Plate 7). Many researchers have reported that Hydrogen cyanide is produced by *Pseudomonas*, *Rhizobium*, *Bacillus*, *Alcaligenes*, and *Aeromonas* and improves the antifungal activity of these bacteria. (Abbas, *et al*. 2020; Olanrewaju*et al* 2017; Liu D *et al* 2019 and Abbas *et al* 2018 [2].

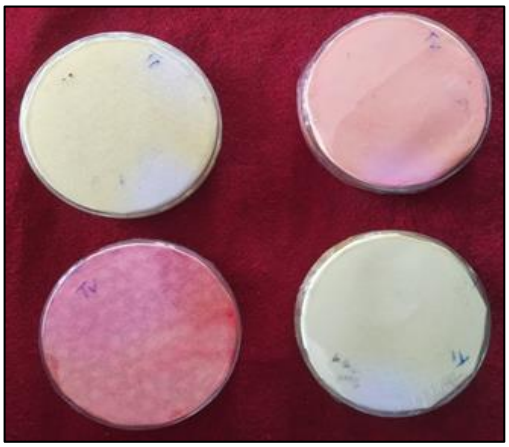


Plate 7: The change in the color of filter paper indicates the presence of HCN activity.

4.4.4. Chitinase Activity

The screening of bacterial isolates for chitinase activity revealed that only 9 bacterial isolates out of 100 were capable of producing chitinase enzyme in chitin amended plate assay, which depicted the ability these isolates to produce enzyme chitinase having potential to protect the plant against fungus and insects. The mean of the ratio of colony size to the clearance zone in these isolates i.e. CS:CZ was observed in the range of 0.801 to 2.00. (Table. 4). Many researchers have showed that many bacterial species including *Serratia marcescens*, *Enterobacter agglomerans*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens* have been found to have chitinolytic activities among Gram-negative bacteria reported by Budi S. W. *et al* 2019. Also many bacterial strains like *B. Licheniformis*, *B. cereus*, *B. Circulans*, and *B. thuringiensis* have reported to show chinolytic activity as reported by Liu *et al*. 2019.

Table 4: Chitinase Activity of different Rhizobacterial Isolates

	Rhizobacterial Isolates	Chitinase activity CS:CZ
1	KB3T	1.89^{b}
2	KSS2	1.77^{c}
3	KB7Y	1.49^{e}
4	YEMA10	2.00^{a}
5	KBB6	1.10^{f}
6	KB3L	1.13^{f}
7	KL1*3	1.65^{d}
8	KBB4	1.57^{de}
9.	YEMA11	0.81^{g}

Overall Mean: 1.50
CD ($p \leq 0.05\%$): 0.092
CV%: 4.803
SE(m): 0.032
*Only those bacterial isolates that showed a positive halo zone

formation were evaluated
*a, b, c…… ranking of the isolates based on their efficacy

4.4.5. Phosphorous Solubilization

All of 100 bacterial isolates were studied invitro for phosphorus solubilizing characteristics out of which only 21 isolates possessed the ability to solubilize phosphorus in Pikovaskaya's media containing tri calcium phosphate as insoluble source of phosphorus, while 79 per cent did not produced any halo zone around the colonies, indicating no ability of rhizobacteria to solubilize phosphorus. (Table 5).Likewise reports have been suggested by Kumar *et al*. 2018 who observed that 40% of the isolates from the Rhizosphere soils possess phosphorus solubilization activities. Also research done by Umar *et al*. 2020 [48] reported that Certain *Rhizobium*, *Pseudomonas*, and *Bacillus* species are considered phosphate-solubilizing bacteria (PSB). *Bacillus*, *Rhizobium*, *Pseudomonas*, *Azotobacter*, *Burkholderia*, *Enterobacter*, *Microbacterium*, *Serratia*, *Burkholderia*, and *Beijerinckia* are the most significant PSB which are isolated from the rhizopsheric soils as reported by Kalayu G. in 2019.

Table 5: Phosphorus Solubilization Index (%) of the isolated rhizobacteria.

S. No.	Rhizobacterial Isolates	Phosphorous Solubilization Index %
1	KL1*6	1.43 ^{k}
2	KB3L	1.78 ^{cde}
3	YEMA10	1.85 ^{a}
4	KB3T	1.85 ^{a}
5	KB7Y	1.83 ^{ab}
6	KBB4	1.78 ^{cde}
7	KBB6	1.75 ^{e}
8	KSS2	1.83 ^{ab}
9	KB1A	1.65 ^{g}
10	KL1*3	1.77 ^{de}
11	LBM21	1.70 ^{f}
12	KL1*11	1.57 ^{ij}
13	KB5W	1.80 ^{bcd}
14	YEMA11	1.65 ^{g}
15	LBM1	1.82 ^{abc}
16	LBM16	1.61 ^{abc}
17	KL1*2	1.60 ^{gh}
18	KB5N	1.57 ^{hij}
19	YEMA1	1.54 ^{j}
20	LBM3	1.46 ^{k}
21	KB8Q	1.46 ^{k}
Overall Mean		1.68
CD ($p \leq 0.05\%$)		0.040
CV%		1.874
SE (m)		0.014

*Only those bacterial isolates that showed a positive halo zone formation were evaluated
*a, b, c…… ranking of the isolates based on their efficacy

4.4.6. Ammonia Production

An ability of ammonia production of rhizobacterial isolates were assessed under invitro condition and observed that out of 100 isolates, 14 per cent isolates produced ammonia by changing the colour of peptone water to deep yellow to brown while 86 per cent isolates did not produce yellow to brown colour in peptone water after adding Nessler's reagent indicating that they did not produce ammonia (Plate: 8). Many researches have reported that rhizobacteria from French bean Rhizosphere are strong ammonia producers. Many researchers have reported the ability of rhizobacterial isolates to produce ammonia (Kumar *et al* 2012) [24]. In similar studies Dastager *et al*. (2010) [11] found Micrococcus sp (N11-0909) isolated from cowpea Rhizosphere to be a strong ammonia producers and Meenu Saraf *et al*. (2014) [29] reported ten Pseudomonas strains from chickpea rhizosphere as resilient ammonia producers.

Plate 8: The change in color to brown, to red or dark yellow shows a positive ammonia production test

5. Conclusion

On assessing the diversity of the bean Rhizosphere, it was observed that there was quite a varsity in the diversity of rhizobacterial isolates. The similarity in shape, margin, elevation and colony color of bacteria does not always show the same gram reaction or similarity in gram reaction does not always show the same morphology of the isolates. Furthermore, it can be assumed that 100 bacterial isolates that have various morphological variations and gram reactions have the potential to also have various different biochemical characters that can potentially be exploited as PGP's and Antifungal compounds.

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