International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 10 Issue: 03 | Mar 2023 www.irjet.net p-ISSN: 2395-0072

ISOLATION AND CHARACTERIZATION OF POTENTIAL PLANT GROWTH PROMOTING MICROBES ISOLATED FROM *TRITICUM AESTIVUM L.* RHIZOSPHERE AND ENDOPHYTES

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Abstract - *In the present study, samples of rhizosphere and not nodules were collected from the farm of Kosad village, Amroli, Surat of Gujarat to isolate plant growth promoting microbial consortium. Plans-bacterial interactions in the rhizosphere are complex and determine the plant health, productivity, and soil fertility. One of the most promising potential isolates exhibiting organic acid was identified as Bacillus cereus strain MWS5 and bacillus flexus strain MWR3 based on the Morphological, biochemical characterization. The isolates were initially screened based on the seed germination assay for their plant growth promoting traits. The results indicated that Bacillus cereus strain MWS5 and bacillus flexus strain MWR3 were significant potential when applied to germinated mungbean seed to uncover its efficacy as effective PGPR.*

Key Words: Wheat field, identification of isolates, PGPR, morphological and biochemical characterization, pot trial

1.INTRODUCTION

Table -1: Taxonomical classification of wheat:

Kingdom	Planate
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Commelinidae
Order	cyperales
Family	Poaceae
Genus	Triticum
Species	*Aestivum L.*

Wheat is a grass widely cultivated for its seed, a cereal grain which is a worldwide staple food. There are many species of wheat which together make up the genus Triticum; the most widely grown is common wheat (*T. aestivum*).

Globally, wheat is the leading source of vegetal protein in human food, having a protein content of about 13%, which is relatively high compared to other major cereals and staple foods. In 100 grams, wheat provides 327 calories and is a rich source of multiple essential nutrients, such as protein, dietary fiber, manganese, phosphorus, and niacin. Several B vitamins and other dietary minerals are in significant content. Wheat is 13% water, 71% carbohydrates, and 1.5% fat. Its 13% protein content is comprised mostly of gluten as 75-80% of total wheat protein, which upon digestion, contributes amino acids for human nutrition.

The cultivation of wheat dates to more than 5000 years back during the era of Indus valley civilization where the original species was Triticum Sphaerococcum popularly known as Indian wheat has now disappeared and replaced by present day species- *Triticum aestivum* or the common Bread Wheat. Triticum durum or the Macaroni wheat and the Triticum dicoccum or the Emmer Wheat.

Currently, India is second largest producer of Wheat in the world after China with about 12% share in total world Wheat production. Now, India is surplus and, in a position, to export Wheat in the International Market and can earn foreign exchange. India has exported about 30 lakh tonnes of Wheat worth Rs.1.490 crore during 2001-02.

Three species of Wheat namely, (i) *T. aestivum*, (ii) *T. durum* and (iii) *T. dicoccum* are being cultivated in the country, as per details given as under:

Table -2: Cultivated area of wheat in india

No.	Species	% Share of Production	Major growing areas
1	*T. aestivum*	95%	Uttar Pradesh, Punjab, Haryana. Rajasthan, Bihar, West Bengal, Assam, Parts of Madhya Pradesh, Himachal Pradesh, Jammu & Kashmir
2	*T. durum*	4%	Madhya Pradesh, Maharashtra, Gujarat, Southern Rajasthan, and few locations in Punjab

3	*T. dicoccum*	1%	Karnataka, Maharashtra & Tamil Nadu

The total area under Wheat in the world is around 225.62 million ha, with a production of 685.6 million tonnes (2009-10). The major Wheat producing countries are China, India, USA, France, Russia, Canada, Australia, Pakistan, Turkey, UK. Argentina, Iran, and Italy. These countries contribute about 76% of the total world Wheat production.

Chemical fertilizers are generally used to supply essential nutrients to the soil- plant system throughout the world. However, the prices, availability, and the environmental concerns of chemical fertilizers especially the N fertilizers are real issues of today's agriculture. Application of chemical fertilizers in slopping landscapes under high annual rainfall normally exist in the mountain ecosystem of the Hindu Kush Himalayan (HKH) region may not be effective because of surface runoff and leaching. Therefore, there is an urgent need to find alternative strategies that can ensure competitive crop yields, provide environmental safety, and protection while maintain long term ecological balance in agro-ecosystem. Use of microbial inoculants or plant growth-promoting rhizobateria (PGPR) for the enhancement of sustainable agricultural production is becoming a more widely accepted practice in intensive agriculture in many parts of the world.

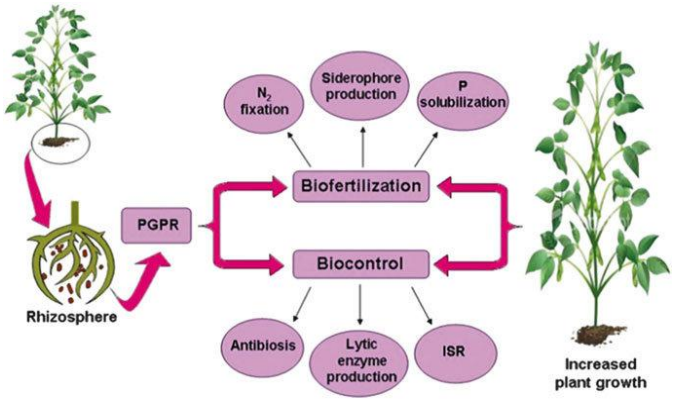


Fig-1 Schematic illustration of important mechanisms known for plant growth promotion by PGPR.

Plant growth-promoting rhizobacteria are free-living soil bacteria that aggressively colonize the rhizosphere/plant roots, and enhance the growth, and yield of plants when applied to seed or crops (Kumar et al., 2014). 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 asymbiotic N2 fixation(Sahin et al., 2004; Khan,2005); (c) solubilizing inorganic phosphate and mineralization of organic phosphate and/or other nutrients (Glick, 1995; Jeon et al., 2003): (d) antagonistic effect against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes, and/or fungicidal compounds, and competition with detrimental microorganisms (Dey et al.,2004; Lucy et al., 2004).

Keeping in mid the study was planned to isolate the native strains from rhizosphere and endo-rhizosphere of wheat grown on different soils. These bacteria were characterized and Interaction of potential organic acid producing microbes (plant growth promoting microbes) with moungbean seed.

1.2 Objective:

1. Isolation and characterization of potential plant growth promoting microbes. isolated from *triticum aestivum L.* rhizosphere and endophytes.
2. Morphological and biochemical characterization of potential plant growth promoting microbes.
3. Interaction of potential organic acid producing microbes (plant growth promoting microbes) with moungbean seed.

2. REVIEW OF LITERATURE

Plants and microbes can have a variety of interactions including pathogenic, symbiotic, and associative- all which impact plant productivity, stress tolerance and disease resistance. Beneficial symbiotic relationships between legumes and nitrogen- fixing microbial symbionts have been recognized by **(Bergersen 1971)** and have the detrimental effects of plant pathogens on crops **(Oerke 2006)**.

Aside from the effects of specific pathogens and symbionts on plant health, recent research done by **(Mendes et al. 2011; Panke-Buisse et al. 2015)** indicated that the composition of microbial communities at roots, the so-called root microbiome, can have significant impacts both on plant development and their stress tolerance.

Further, inoculation by different members of the plant microbiome may differentially alter plant phenotype **(Zamioudis et al., 2013; Timm et al., 2016)**. The presence of unique bacterial strains in legume genotypes explained more variation in shoot biomass root biomass, and plant height than plant genotype did **(Tan & Tan, 1986)**. Inoculation of common endophytes can also inhibit primary root elongation and promote lateral root formation and root hair production **(Zamioudis et al., 2013; Weston et al., 2012)**.

Plant growth-promoting rhizobacteria are free-living soil bacteria that aggressively colonize the rhizosphere/plant roots, and enhance the growth, and yield of plants when applied to seed or crops. **(Kumar et al., 2014)**.

The ability to produce plant growth regulators or phytohormones such as indole acetic acid (IAA), cytokinin, and gibberellins **(Glick, 1995; Marques et al., 2010)**; (b) enhancing asymbiotic N2 fixation **(Sahin et al., 2004; Khan, 2005)**; (c) solubilizing inorganic phosphate and mineralization of organic phosphate and/ or other nutrients **(Glick, 1995; Jeon et al., 2003)**; (d) antagonistic effect against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes, and/or fungicidal compounds, and competition with detrimental microorganisms **(Dey et al., 2004; Lucy et al., 2004)**.

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 **(Ozturk et al., 2003; Marques et al., 2010; Mehnaz et al., 2010; Zhang et al., 2012)**.

Inoculation with Pseudomonas fluorescens showed a significant increase in root weight 19-43%, number of tillers per plant 10-21%, grain yield 15-43%, and straw yield 22-39% of wheat compared to un-inoculated plants **(Shaharoona et al., 2008)**. Moreover, inoculation with PGPR strain Azotobacter saved 25-30 kg N ha-1 chemical fertilizer **(Narula et al., 2005)**.

More recently, **Kumar et al. (2014)** conducted experiments on wheat under pot and field condition to examine the effect of PGPRS on the growth and yield of wheat and found that triple combination of strains B. megaterium, A. chlorophenolicus, and Enterobacter significantly increased 17.5, 79.8, 78.6, and 26.7% plant height, grain yield, straw yield, and test weight under pot condition and also 29.4, 27.5, 29.5, and 17.6% under field condition, respectively. Knowledge of the native bacterial population, their characterization, and identification is required for understanding the distribution and diversity of indigenous bacteria in the rhizosphere of specific crops **(Keating et al., 1995; Chahboune et al., 2011)**.

With increasing awareness about the-chemical-fertilizers based agricultural practices, it is important to search for region specific microbial strains which can be used as a growth promoting/enhancing inoculum to achieve desired crop production **(Deepa et al., 2010)**.

Colony morphology, size, colour, shape, gum production, and growth pattern were recorded after 24 h of growth on LB agar plates at 28 ± 2°C as described by **Somasegaran and Hoben (1994)**. Based on cell and colony morphology, nine different morphotypes were identified from rhizosphere and endosphere of wheat. These morphotypes were subjected to restriction fragment length polymorphism of IGS to distinguish among these genotypes.

Alternatively, **Somers et al. (2004)** classified PGPR based on their functional activities as (i) biofertilizers (increasing the availability of nutrients to plant), (ii) phytostimulators (plant growth promotion, generally through phytohormones), (iii) rhizoremediators (degrading organic pollutants) and (iv) biopesticides (controlling diseases, mainly by the production of antibiotics and antifungal metabolites) **(Antoun and Prévost, 2005)**. Furthermore, in most studied cases, a single PGPR will often reveal multiple modes of action including biological control **(Kloepper, 2003; Vessey, 2003)**. Furthermore, **Gray and Smith (2005)** have recently shown that the PGPR associations range in the degree of bacterial proximity to the root and intimacy of association. In general, these can be separated into extracellular (ePGPR), existing in the rhizosphere, on the rhizoplane, or in the spaces between cells of the root cortex, and intracellular (iPGPR), which exist inside root cells, generally in specialized nodular structures **(Figueiredo et al., 2011)**.

Most of rhizobacteria belonging to this group are Gram-negative rods with a lower proportion being Gram-positive rods, cocci or pleomorphic **(Bhattacharyya and Jha, 2012)**. Moreover, numerous actinomycetes are also one of the major components of rhizosphere microbial communities displaying marvelous plant growth beneficial traits **(Bhattacharyya and Jha, 2012; Merzaeva and Shirokikh, 2006)**. Among them, Micromonospora sp., Streptomyces spp., Streptosporangium sp., and Thermobifida sp., which have shown an enormous potential as biocontrol agents against different root fungal pathogens, are worthy of mention **(Bhattacharyya and Jha, 2012; Franco-Correa et al., 2)**.

Application of PGPR (Plant Growth-Promoting Rhizobacteria) like Bacillus, Pseudomonas, Serratia and Rhizobium spp. have shown to be beneficial to crops and are reported to enhance growth and productivity. They are reported to improve germination rateFurther they are found to secrete antibacterial and bio protective compounds which are effective against plant pathogens and pests, facilitate enhanced uptake of nitrogen and solubilisation of recalcitrant phosphorus and its assimilation and production of siderophores to facilitate the absorption of minerals **(Herman et al., 2008; Ashrafuzzaman et al., 2009; Richardson et al., 2009)**. Sugarcane is sensitive to salinity stress (EC<2 dS m^{-1}) and high salt levels adversely affect plant growth. Higher EC inhibit the roots from absorbing mineral nutrients leading to nutrient deficiency (**Sharp and Davies, 2009; Macedo and Jan 2008)**.

Plant growth promoting rhizobacteria are the soil bacteria inhabiting around/on the root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere. Generally, plant growth promoting rhizobacteria facilitate the plant growth directly by either assisting in resource acquisition

(nitrogen, phosphorus, and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents.

Various studies have documented the increased health and productivity of different plant species by the application of plant growth promoting rhizobacteria under both normal and stressed conditions. The plant-beneficial rhizobacteria may decrease the global dependence on hazardous agricultural chemicals which destabilize the agro - ecosystems.

3. MATERIAL AND METHOD

3.1 Sample Collection

Sampling of root and rhizosphere soil of wheat was collected from the farm of Kosad village. Amroli, Surat having latitude: 21°14'30.1272" N, Longitude: 72°51'30.8952"E. The area was divided into three blocks and in each of them one root sample containing adhered soil were taken around three randomly distributed small plants. All three samples were mixed and filled in plastic bag in an ice box and carried to the laboratory. Sample was kept in refrigerator at 4°C.

3.1.1 Bacterial Isolation:

Material required: sterile distilled water, test tubes, pipettes NA media, Petri plates, Flask, Forceps, scalpel, Laminar-airflow, Incubator, Soil, and root sample of Wheat, Inoculating loop, Alcohol.

Procedure:

- For rhizosphere soil, clump of soil loosely adhering to the roots were removed and one gram of soil sample taken, and sample were added into 10ml of distilled sterile water, then serial dilution done upto 10-6.
- From the 10-6 diluted sample, 0.1ml aliquots were spreaded in petriplates of NA medium. Same 2 sets were prepared. The plates were kept at 37°C for 24 hours.
- Parallely, Roots were washed under tap water followed by other wash of distilled water. Roots were cut into small pieces.
- Area of one Petri plate was divided into eight parts and in each part root sample was inoculated. Like this, two plates were prepared. Plates were kept for incubation at 37 °C for 24 hours.
- After observation, distinct colonies are selected and streaked individually on NA plates for the pure or isolated colonies. And subculture was conducted to avoid cross contamination.
- Plates were stored at 40C after characterization of colonies. Colony morphology was described with special emphasis on pigmentation, colony elevation, opacity, etc.
- For the bacterial characterization, gram staining technique was performed and observed under light microscope.

3.2 Characterization:

3.2.1 Gram Staining:

Materials Required: Clean glass slides, Inoculating loop Bunsen burner, Bibulous paper, Microscope, Lens paper and lens cleaner, Immersion oil Distilled water, 18-to-24-hour cultures of organisms.

Reagents:

Primary Stain	- Crystal Violet
Mordant	- Grams Iodine
Decolourizer	- Ethyl Alcohol
Secondary Stain	- Safranin

Gram Stain Procedure:

- Place slide with heat fixed smear on staining tray.
- Gently flood smear with crystal violet and let stand for 2 minutes.
- Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
- Gently flood the smear with Gram's iodine and let stand for 2 minutes.
- Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. The smear will appear as a purple circle on the slide.
- Decolorize using 95% ethyl alcohol or acetone. Tilt the slide slightly and apply the alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Be careful not to over-decolorize.
- Immediately rinse with water.
- Gently flood with safranin to counterstain and let stand for 2-3 minutes.
- Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
- Blots dry the slide with blotting paper.
- View the smear using a light-microscope under oil-immersion.

3.2.2 Biochemical characterization of root associated soil bacteria and endophytes:

3.2.2.1 Indole production test
3.2.2.2 Methyl red test
3.2.2.3 Voges-Proskauer test
3.2.2.4 Citrate utilization test
3.2.2.5 Hydrogen sulphide production test
3.2.2.6 Catalase Test
3.2.2.7 Oxidase test

3.2.2.8 Starch hydrolysis test
3.2.2.9 Urea Hydrolysis test
3.2.2.10 Nitrate reduction test
3.2.2.11 Casein Hydrolysis test
3.2.2.12 Gelatin Hydrolysis test

Screening of extracellular enzyme producing microorganisms:

3.2.2.13 Amylase producer
3.2.2.14 Protease producer
3.2.2.15 Cellulase producer
3.2.2.16 Lipase producer
3.2.2.17 Organic acid producing microorganisms.

3.3 Pot Trial:

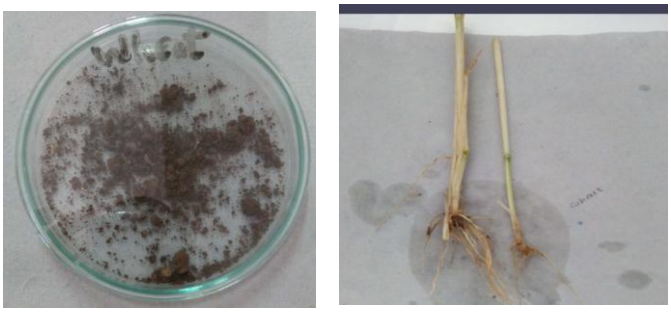
Interaction of different potential organic acid producing microbes were done with germinating mungbean seed. Total three replication were taken along with control for each isolate at different times interval with different growth parameters like root length, Total protein contents and germination percentage.

Germination percentage (GP)= seeds germinated/total seeds x 100.

4. RESULT AND DISCUSSION

4.1 Sample collection:

Sampling of root of size approx. 15-20 cm (plate: 1) and rhizosphere soil (plate: 1) of wheat was collected from the farm of Kosad village, Amroli, Surat.



MWS **MWR**

Plate 1: Collection of Potential isolate from soil rhizophere and root of *Triticum aestivum*

Bacterial Isolation:

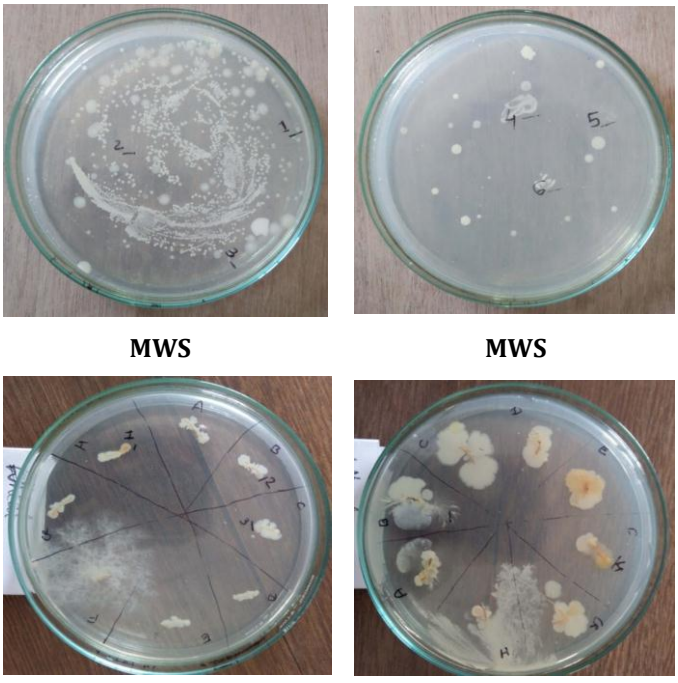
Dilution of collected sample up to 1:1000000 of soil (MWS) on NA plates and parallely inoculated healthy roots on NA plates kept for 24 hours of incubation at 37C.

In case of soil associated microbes, 200-300 colonies have been observed and from the observed colonies. 15-20 colonies showing distinct characteristics has been isolated and streaked further on NA plate and out of 15-20 colonies only 4 colonies were showing significant characteristics [Plate 3: (MWS1, MWS2, MWS5, MWS6)]

Further for the bacterial characterization, gram staining was performed and observed under light microscope (plate: 4) Out of the 4 isolates, MWS-1 was only gram negative remaining three isolates (MWS-2, MWS-5, MWS-6) were found Gram positive (Table: 3). All three Gram positive isolates were rod shaped occurring singly and cluster. Whereas one Gram negative isolate (MWS-1) was short rod. All 4 isolates were motile.

In case of endophyte, four distinct colonies [Plate: 5 (MWR1, MWR2, MWR3, MWR4)] has been observed and further Gram staining was performed on all four isolates by using standard protocol. (Plate:6)

Out of the 4 isolates, MWS-1 was only gram negative remaining three isolates (MWS- 2, MWS-5, MWS-6) were found Gram positive (Table: 4). All three Gram positive isolates were rod shaped occurring singly and cluster. Whereas one Gram negative isolate (MWS-1) was short rod. All 4 isolates were motile.



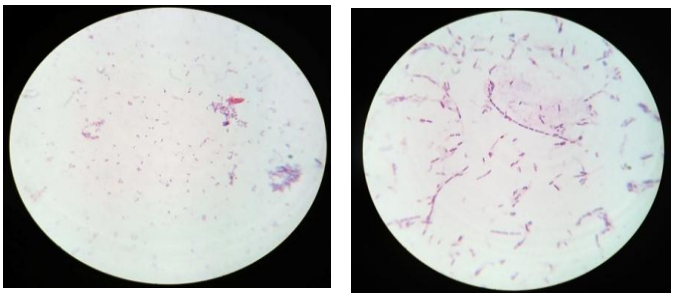
MWS **MWS**

MWR **MWR**

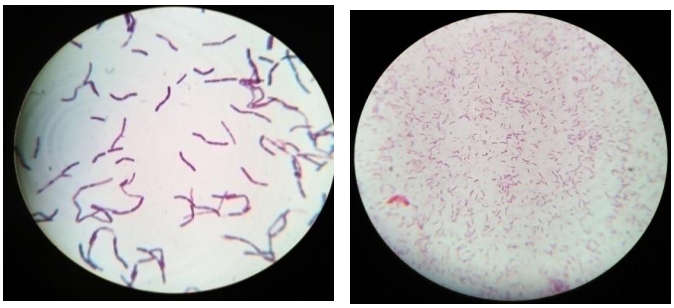
Plate 2: Isolation of different Potential isolate from soil rhizophere and root of *Triticum aestivum*

MSW1 & MSW2 MWS5 & MWS6

Plate 3: Isolated pure culture from soil of *Triticum aestivum*

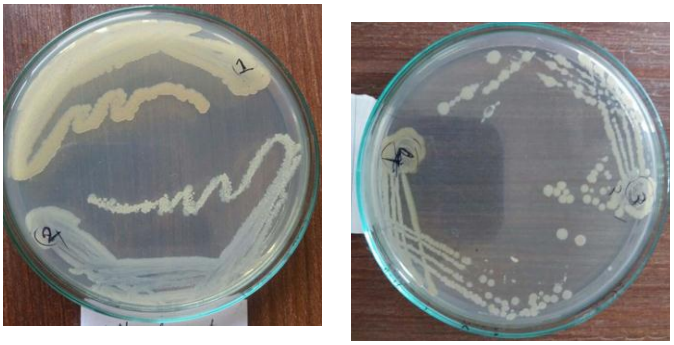


MWS1 MWS2



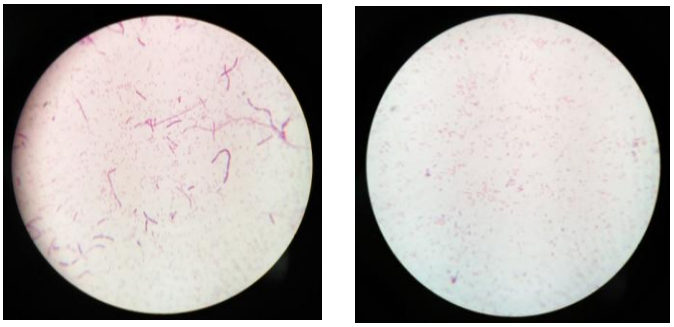
MWS5 MWS6

Plate 4: Gram stain of isolated MWS1, MWS2, MWS5, MWS6

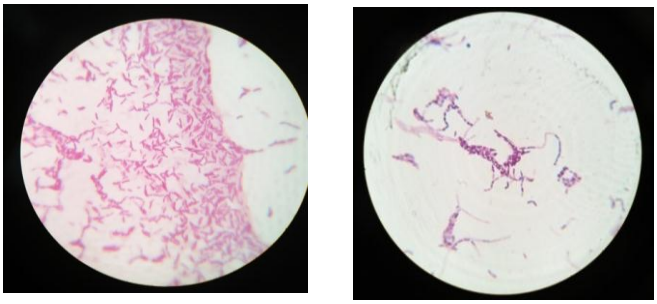


MWR1 & MWR2 MWR3 & MWR4

Plate 5: Isolated pure culture from rootl of *Triticum aestivum*



MWR1 MWR2



MWR3 MWR4

Plate 6: Gram stain of isolated MWR1, MWR2, MWR3, MWR4

Table -3: Analysis of morphological characteristics of soil sample by gram staining

Characteristic	MWS-1	MWS-2	MWS-5	MWS-6
Gram staining	Gram Negative	Gram positive	Gram positive	Gram positive
Shape	Shord rod	Rod	Rod	Rod
Motility	-	-	-	-
Temp (0c)	25-37	22-35	17-63	15-40
pH	6-8	6.5-7.8	5.7-7	4.5-7
NaCl %	1-5	1-2	1-7	1-2
Colony size	Large	Small	Medium	Medium
Colony shape	Circular	Circular	Circular	Circular
Colony edge	Entire	Entire	Entire	Entire
Colony elevation	Umbonate	Flat	Flat	Raise
Colony opacity	Opaque	Translucent	Opaque	Opaque
Colony pigmentation	Brown	No	No	No
Colony consistency	Viscid	Brittle	Brittle	Brittle
Colony surface	Shiny	Dull	Shiny	Shiny

Table -4: Analysis of morphological characteristics of root sample by gram staining

Characteristic	MWR-1	MWR-2	MWR-3	MWR-4
Gram staining	Gram positive	Gram Negative	Gram positive	Gram positive
Shape	Rod	Spherical	Rod	Rod
Motility	+	-	-	-
Temp (^{0}C)	15-50	20-40	5-40	15-40
pH	5.5-8.5	6-7.8	5-8	4.5-7
NaCl %	1-7	2-4	1-2	1-2
Colony size	Medium	Medium	Large	Medium
Colony shape	Wrinkled	Circular	Circular	Circular
Colony edge	Undulate	Entire	Entire	Entire
Colony elevation	Raised	Raised	Flate	Raised
Colony opacity	Opaque	Translucent	Opaque	Opaque
Colony pigmentation	Yellow orange	No	No	No
Colony consistency	Mucoid	Brittle	Brittle	Brittle
Colony surface	Dull	Shiny	Shiny	Shiny

4.2 Biochemical Characteristics of Root Associated Bacteria:

MWS-6, MWR-2 and MWR-4 were showing positive result towards xylose test, which means that microbe will accumulate acidic by-products. In a positive test, the pH indicator in the medium changes colour from its normal red to yellow, indicating acid production. MWS-5, MWR-1, MWR-2, and MWR-3 were showing positive response towards sucrose test which means Bacteria were identified based largely on what organic compounds they can break down. The range of compounds used depends on the collection of enzymes a species of bacteria can make. Only MWS-5, MWR-I and MWR-3 were showing positive result towards mannitol test which means that they can ferment the carbohydrate (sugar) mannitol as a carbon source. MWS-1, MWS-5, MWR-1, MWR-2, and MWR-3 were showing positive result towards maltose test which means that they can ferment the carbohydrate (sugar) maltose as a carbon source. MWS-2. MWS-5, MWR-1 and MWR-2 were showing positive result towards fructose test which means that they can ferment the carbohydrate (sugar) fructose as a carbon source.

Colonies of MWS-1.MWS-2 was showing urea production which means that microbe can use the compound urea as a source of carbon and energy for growth. Use of urea is accomplished by the enzyme urease. MWS-5 and MWR-3 were showing positive results towards gelatine hydrolysis test which means that they have ability to produce gelatinase that liquefy gelatine to polypeptides and amino acid. MWS-1, MWS-2, MWS-5, MWS-6, MWR-1, MWR-2, MWR-3, and MWR-4 were showing positive result toward glucose uptake which is important source for C and N that help in promoting various metabolism.MWS-1 and MWS-2 were showing positive result towards indole test which determine the ability of organism to convert tryptophan into indole. MWS-5, MWR-1 and MWR-2 were showing response towards citrate test which means that they have ability to use citrate as the sole source of carbon and energy. All the isolated colonies were showing positive response towards catalase test, which means that these organisms have ability to produce catalase that detoxicity H2O2 by breaking it into H2O and oxygen. MWS-1, MWS-2 MWS-5 and MWR-1 were showing positive response towards nitrate reduction, which means that bacteria have ability to reduce nitrate which further help them in defence mechanism. MWS-S and MWR-1 were showing positive response towards starch degradation, by which I was able to differentiate species from the genera such as clostridium and bacillus. MWS-6 and MWR-4 were showing positive result towards MR test, which help me to detect PH along with, which fermentation pathway is used to utilize glucose. MWS-1, MWS-5, MWR-1, and MWR-2 were showing positive response towards lactose test, which means that microbes can ferment carbohydrate-lactose as a carbon source. Only MWR-1 was showing positive result towards VP test which means that they were producing acetyl methyl carbinol from glucose fermentation. In which, acetyl methyl carbinol is converted to diacetyl in the presence α-naphthol, strong alkali and atmospheric oxygen(Table -5 & Table -6).

Table -5: Biochemical characterization of root associated soil bacteria and endophyte.

Substrate	MWS1	MWS2	MWS5	MWS6	MWR1	MWR2	MWR3	MWR4
Gelatine	-	-	+	-	-	-	+	-
Urea production	+	+	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-
VP test	-	-	-	-	+	-	-	-
Indole test	+	+	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+
Lactose	+	-	+	-	+	+	-	-

Maltose	+	-	+	-	+	+	+	-
Manitol	-	-	+	-	+	-	+	-
Sucrose	-	-	+	-	+	+	+	-
Xylose	-	-	-	+	-	+	-	+
Fructose	-	+	+	-	+	+	-	-
Citrate	-	-	+	-	+	+	-	-
MR test	-	-	-	+	-	-	-	+
Starch	-	-	+	-	+	-	-	-
Catalase	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	-	+	-	-	-

Table -6: Enzymatic profiling of root associated soil bacteria and endophyte.

Name Of Activity	**MWS 1**	**MWS 2**	**MWS 5**	**MWS 6**	**MWR 1**	**MWR 2**	**MWR 3**	**MWR 4**
Lipase	-	-	-	-	-	-	-	-
Amylase	-	-	+	+	+	-	-	-
Cellulase	-	-	+	-	+	-	+	-
Protease	-	-	-	-	-	-	-	-
Organic activity	+	+	+	+	+	+	+	+
Organism	Nitrobacter sp	Nitrosomonas sp.	Bacillus sp.	Acetobacter	Bacillus sp.	Pseudomonas	Bacillus sp.	Acetobacter

4.3 Pot Trial:

Further Pot studies were carried out to assess the growth and biochemical changes in Vigna radiate (Mung bean) after inoculating it with Isolates MWS5 and MWR3.Treated seeds were allowed to germinate in petri plates at different time interval, (12hr,24hr.48hr,76hr.96hr) while uninoculated mungbean seed serve as control (MWS-5C & MWR-3C). However, it was observed that Vigna radiata plants inoculated with MWS-5 exhibited significant increment of root growth of about 29mm,51.33mm, 53mm, 58mm at time interval of 24 hr., 48hr., 72hr., and at 96 hr. when compared with control MWS-5 C, increment of root length of about 23.66mm, 46.33mm, 50.66mm, 56.33mm. Similarly, MWR3 also exhibited significant increment of root of about 30mm, 54.33mm, 51.66mm, 54.33mm respectively at time interval of 24 hr., 48hr., 76hr. and at 96 hr., when compared with control MWR3 C, show increment of root length of about 24mm, 46.66mm, 46mm, 46mm, 50.33mm (Table -7 & Table -8).

Table -7: Root length of MWS 5 at different time interval.

Isolated Code	**No. of seed**	**24hr**	**48hr**	**72hr**	**96hr**
C-MWS 5	1	20	45	52	59
	2	24	46	50	50
	3	27	48	50	58
Average		23.66	46.33	50.66	56.33
MWS 5	1	28	52	55	55
	2	30	50	51	55
	3	29	52	53	60
Average		29	51.33	53	58

Table -8: Root length of MWS 3 at different time interval.

Isolated Code	**No. of seed**	**24hr**	**48hr**	**72hr**	**96hr**
C-MWR 3	1	24	46	48	50
	2	25	52	45	50
	3	23	42	45	51
Average		24	46.66	46	50.33
MWR 3	1	36	55	55	52
	2	29	58	50	55
	3	25	50	50	56
Average		30	54.33	51.66	54.33

Estimation Of Protein Content:

In case of protein content-on the basis of Fowling lowery method it was observed that Vigna radiata plants inoculated with MWS-5 exhibited significant increment of protein concentration of about 7.449mg/ml, 9.058mg/ml,

11.085mg/ml & 14.354mg/ml at time interval of 24 hr,48hr,72hr and at 96 hr., when compared with control MWS-5C, which show increment of protein concentration of about 5.013mg/ml, 7.2851mg/ml, 10.071mg/ml & 13.831mg/ml. similarly MWR-3 also exhibited significant increment of protein concentration of about 7.765mg/ml, 8.456mg/ml, 12.341mg/ml and 15.927mg/ml respectively at time interval of 24hr., 48hr., 76hr., and at 96hr., when compared with control MWR-3C, show increment of protein content of about 6.675mg/ml, 7.847mg/ml, 10.157mg/ml and 14.907mg/ml (Table -9).

Table -9: Estimation of protein activity of Mungbean at different time interval.

Isolated Code	protein concentration (mg/ml) at different time interval			
	24hr	48hr	72hr	96hr
C-MWS 5	5.013	7.285	10.071	13.831
MWS 5	7.449	9.058	11.085	14.354
C-MWR 3	6.675	7.847	10.157	14.655
MWR 3	7.765	8.456	12.341	15.927

Germination of mungbean seed:

After 12 hours

Control MWS 5

MWS 5

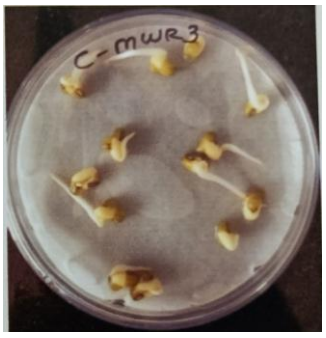
Control MWR 3

MWR 3

After 24 hours

Control MWS 5

MWS 5



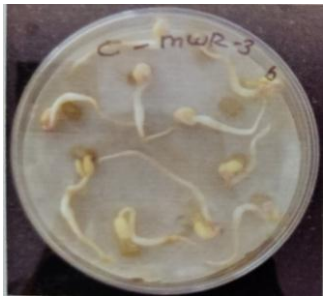
Control MWR 3

MWR 3

After 48 hours

Control MWS 5

MWS 5



Control MWR 3

MWR 3

After 72 hours

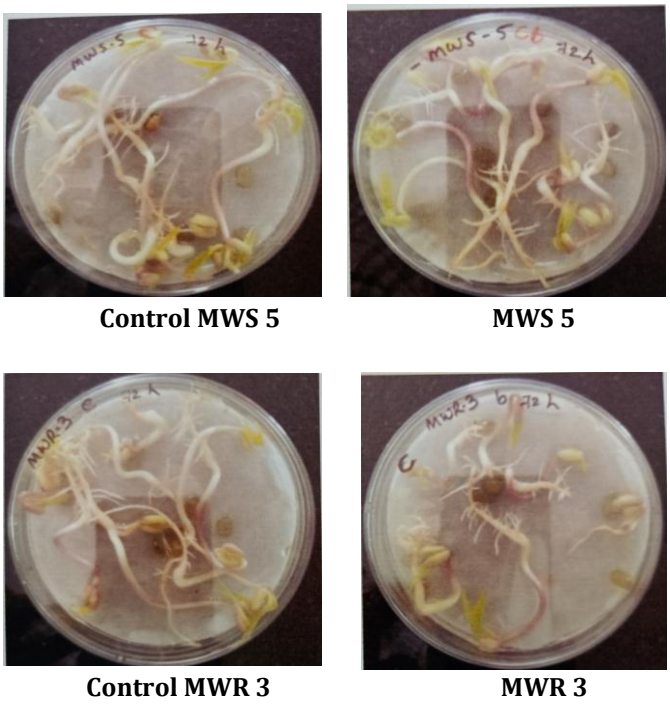


Plate 6: Germination of mungbean seed at different time interval.

Table -10: Germination percentage of mungbean at different time interval.

A. Germination percentage of mungbean after 12 hours

Sr No.	Plates	No. of seed	Germinated seed	Germination percentage
1	C-MWS5	10	7	70%
2	MWS5	10	9	90%
3	C-MWR3	10	8	80%
4	MWR3	10	9	90%

B. Germination percentage of mungbean after 24 hours

Sr No.	Plates	No. of seed	Germinated seed	Germination percentage
1	C-MWS5	10	10	100%
2	MWS5	10	10	100%
3	C-MWR3	10	10	100%
4	MWR3	10	10	100%

C. Germination percentage of mungbean after 48 hours

Sr No.	Plates	No. of seed	Germinated seed	Germination percentage
1	C-MWS5	10	10	100%
2	MWS5	10	10	100%
3	C-MWR3	10	10	100%
4	MWR3	10	10	100%

D. Germination percentage of mungbean after 72 hours

Sr No.	Plates	No. of seed	Germinated seed	Germination percentage
1	C-MWS5	10	10	100%
2	MWS5	10	10	100%
3	C-MWR3	10	10	100%
4	MWR3	10	10	100%

E. Germination percentage of mungbean after 96 hours

Sr No.	Plates	No. of seed	Germinated seed	Germination percentage
1	C-MWS5	10	10	100%
2	MWS5	10	10	100%
3	C-MWR3	10	10	100%
4	MWR3	10	10	100%

5. CONCLUSIONS

Wheat is a grass widely cultivated for its seed, a cereal grain which is a worldwide staple food. Wheat crop has wide adaptability. It can be grown not only in the tropical and sub-tropical zones, but also in the temperate zone and the cold tracts of the far north beyond even the 60-degree north altitude. Microbes in the rhizosphere are involved in many processes that determine agricultural soil productivity, including preservation of soil structure, nutrient recycling, disease control and degradation of pollutants.

On basis of morphological and biochemical characterizations, isolates were identified as MWS1 (nitrobactor sp), MWS2 (nitrosomonas.), MWS5 (Bacillus sp.) and MWS6 (acetobacter) respectively as root associated soil microbes and isolate MWR1, MWR2, MWR3, MWR4 name like as Bacillus subtillis pseudomonas, bacillus sp., acetobater respectiv as endophytes. Further Pot studies were carried out to assess the growth and biochemical components of Vigna radiate. Seeds were treated with two potential isolates viz. as Bacillus cereus strain MWS5 and bacillus flexus MWR3 exhibited significant increment in root growth as well as protein content at different time interval i:e upto 58mm as compared to root growth of control of MWS5-C which exhibit root growth of about 56.33mm and protein content is about 14.354mg/ml of MWS5 as compared to protein content of control (MWS5-C) which was about 13.831. Similarly, mungbean inoculated with MWR3 show Significant increment in root length of approx 54.33mm as compared to MWR3-C which show root growth of about 50.33mm. as well as protein content is about 15.927mg/ml of MWR3 as compared to protein content of control (MWR3-C) which was about 14.831mg/ml.

So, from all the above observation and result obtained, it can be concluded that these potential isolates could be further studied for metabolic profiling for identification and quantification of organic acid and field trail.

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BIOGRAPHIES

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