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Efficiency of plant growth promoting bacteria for growth and yield enhancement of maize (*Zea mays*) isolated from rock phosphate reserve area Hazara Khyber Pakhtunkhwa, Pakistan



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ABSTRACT

The usage of novel Plant Growth-Promoting Rhizobacteria (PGPR) as bioinoculant is a good opportunity for ecological farming practices to improve soil condition, quality of grain, crops' yield and biodiversity conservation. The purpose of recent research was focused to examine, isolate and characterize PGP bacteria that colonize the rhizosphere for the duration of the maize plant's seedling. For this purpose, 14 samples of soils and roots in the maize rhizosphere were collected from rock phosphate area of Hazara, Pakistan. Forty morphologically natural bacterial colonies have been extracted and tested for their PGP innovations and biocontrol residences and further recognized as plant production advancing rhizobacteria. To find the effective PGPR strains with numerous activities, an aggregate of 150 bacterial colonies were sequestered from different rhizospheric soils of the Hazara Pakistan rock phosphate area. These tested bacterial strains were subjected to biochemical description and in vitro screening for their plant growth-promoting qualities like generation of indole acetic acid (IAA), alkali (NH₃), hydrogen cyanide (HCN), siderophores, catalases, proteases and pectinases. All the isolates of rhizobacteria showed IAA producing capacity, as well as found positive for catalase and HCN. The above results suggested that, in addition to biocontrol marketers, PGPR could be used as biofertilizers to substitute agro-chemicals in order to increase crop production. These microorganisms can therefore be further developed and used for greenhouse and discipline packages.

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1. Introduction

Application of chemical fertilizer, herbicide, and pesticide made by agrochemicals although produces higher grain yield, yet its continuous use have been shown to adversely affect the whole environment and animals (Meissle et al., 2010). Subsequently in long term complications it also effect rhizosphere, human health, biodiversity and beneficial microorganism flora (Pérez-Jaramillo et al., 2016). Current research reports indicated that the constant usage of different dangerous fertilizers causes groundwater infection, which in turn causes the majority of cancers, goitre, birth defects,

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hypertension, testicular cancer and the majority of cancers in the stomach. These fertilizers are also not available on low cost for farmers (Culliney et al., 1992). The practice of PGP rhizobacteria as a proven bio-inoculant for sustainable agricultural practices had been demonstrated in numerous researches to improve soil fitness, induce crop productiveness, grain size and in addition conserve biodiversity (Bergottini, 2015; Saharan and Nehra, 2011; Yadav et al., 2018). These PGPRs grows inside or around root tissues to promote plant growth and provide protection against plant pathogens and abiotic pressure (Backer et al., 2018; Compant et al., 2005; Nadeem et al., 2014).

Rhizobacteria also known as rhizosphere competent bacteria are capable of multiplying and inhibiting the ecological positions at multiple stages of plant growth in roots (Ahmad and Kibret, 2014; Lugtenberg and Kamilova, 2009). Through many mechanisms rhizobacteria exert beneficial effects on the host plants. For example (i) Synthesis of phyto-hormones that may be absorbed through vegetation, (ii) Fixing of atmospheric nitrogen (iii) Mobilization of soil compounds and synthesis of useful vitamins, (Bevivino et al.) Protection of plants under demanding situations in that way counteracting the terrible impacts of strain, (v) Protection in opposition to plant pathogens reducing plant sicknesses or death (Awasthi et al., 2011; Di Benedetto et al., 2017; Meena et al., 2016). The owning one or may be more of these mention characters are known as plant growth-promoting rhizobacteria (Husen, 2016). Many PGPR have been used globally for decades as bio-inoculants for the improvement of crop yields, quality and fertility of soil (Itelima et al., 2018).

The promising effects of PGPRs on plant growth and development provides a suitable alternative to traditional use of harmful agrochemicals (Bhattacharyya and Jha, 2012). Developing agriculture countries like Pakistan and India can successfully reduce the use of synthetic chemicals in cultivated fields by incorporating these PGPRs and substantially increase the economic yield and decrease harmful ecological impact (Singh et al., 2011). Therefore it is important to investigate, isolate, and identify these native soil bacterial communities and characterize their potential as prospective PGPRs. One conceivable methodology is to investigate soil microbial population assorted variety for PGPRs exhibiting various PGP activities and very much adjusted to specific soil condition (Parray et al., 2019).

The purpose of recent study was to examine isolated and characterized PGP bacteria that colonize the rhizosphere for the duration of the maize plant. 40 Morphologically extraordinary natural bacterial colonies have been extracted and tested for their PGP innovations and biocontrol residences are recognized as plant production advancing PGPR (plant growth promoting rhizobacteria). Isolation of effective PGPR strains with numerous exercises, an aggregate of 150 bacterial colonies were isolated from diverse rhizospheric soils. The studied test segregates were described biochemically and subjected to in vitro screening for their plant development promoting qualities like generation of indole-acetic-acid (IAA), alkali (NH₃), hydrogen cyanide (HCN), siderophores, catalases, proteases and pectinases. PGPR could be used as bio-fertilizers to replace agrochemicals in order to increase crop productivity.

2. Materials and methods

2.1. Soil sample collection

The samples were collected from different locations of rock phosphate mine area of Hazara division for each sample, several sub-samples were taken.

2.2. Isolation, characterization and identification of rhizobacteria

Soil samples were collected from sympathetic resources, rock phosphate mines, maize rhizosphere and maize endorhizosphere. For isolation cause, Luria-bertani media was for isolation purpose. LB media was composed of organized yeast extract 5 g, sodium chloride 10 g, tryptone 10 g, agar 18 g, distilled water 1L. pH was adjusted at eight and the media was autoclaved and then poured into petri plates. When media solidified 0.1 mL suspension turned into taken from 10 to 3, 10-four and 10–5 and spread on agar plates by sterile L-fashioned glass rod. After spreading, plates had been incubated for 24 to 48 h at 28 °C (Anderson & Habiger, 2012).

2.3. Purification of bacterial colonies

For purification of bacterial colonies new agar plates were prepared and specific colony of bacteria was taken on the basis of color and shape and streaked on the plates and incubated at 28 °C for 24 to 48 h. Different isolated colonies were preserved in the form of slants and stored at 4 °C for further experiment (Klopper et al., 1988).

2.4. Biochemical portrayal of rhizobacteria

The selected confines were biochemically defined with the aid of Gram's reaction, oxidase control, H₂S formation, NO₂ reduction, in line with the standard techniques (Cappuccino and Sherman, 1992). The portrayal of rhizobacteria creating Indole acetic acid (IAA) advent for PGP qualities has changed into recognized as depicted by (Okon and Labandera-Gonzalez, 1994). In LB broth, bacterial isolates containing L-Tryptophan were inoculated as a hundred mg/L precursor of IAA. Bacterial strains has been incubated a 28 ± 2 °C for seven days. A few drops of Kovac's reagent added into tubes after incubation. The improvement on the medium pinnacle of cherry pink color undoubtedly suggests results. A loop full of bacterial isolates inoculated in sterilized 50 mL LB broth containing 0.05 g of L-Tryptophan are incubated in 180 rpm incubator shaker for 7 days for quantitative assay. In supernatants, after incubation cultures were placed in falcon tubes centrifuged for 10 min at an estimate of 10,000 rpm of Indole acetic acid (IAA). After centrifugation 1 mL of supernatant has been converted and delivered along 4 mL Salkowski reagent. Purple colour appearance in test tubes led to IAA synthesis. The absorbance of purple shade changed was observed after 30 min at 535 nm in a visible spectrophotometer.

2.5. Screen for hydrogen cyanide (HCN) production

Bacterial strains were subjected to screening for biosynthesis of HCN using the previously available protocol by (Castric, 1975). The isolates were inoculated over the nutrient media plates containing 4 g glycine consistent with 1 L. To the pinnacle of the plate, Whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed and sealed with parafilm. Then plates were subjected to incubation at 30 °C for four days and discovered with a color trade of the clear out paper from deep yellow to reddish-brown indicated the formation of HCN (Bakker and Schippers, 1987).

2.6. Screening bacterial isolates for hydrolytic enzyme manufacturing

Bacterial strains of this study were subjected to screening for the synthesis of hydrolytic enzymes including protease, cellulose and amylase.

2.7. Protease production activity

In this activity test isolates of bacteria were analysed for proteolytic enzymes producing capability on skim milk agar (3% v/v) medium (Chang et al., 2009). After a brief incubation of 48 h at 30 °C, the diameter of the clear zone established round the bacterial colonies was measured.

2.7.1. Amylase production (starch hydrolysis)

The strains of bacteria were inoculated on medium plates of starch agar Yeast extracts 1 g, MgSO4·7H2O 0.1 g, K2HPO4, 7g Agar 18 g, K2HPO4 2 g, (NH4)2SO4 1 g, NaCl 5 g distilled water 1 L) and subjected to a brief incubation of 48 h at 30 °C. After the incubation test plates were flooded using iodine solution at the giving up of the incubation duration, left for one minute and poured off after that. A blue coloured product is obtained after the reaction of Iodine with starch. This blue coloration disappears unexpectedly. Subsequently the colourless region around bacterial colonies suggests the amylase synthesis (Gupta et al., 2003).

2.7.2. Siderophore production

Siderophore advent become recognized through the all-inclusive technique (Schwyn and Neilands, 1987) utilising blue agar plates containing the color chrom azurol S (CAS). Orange radiance around the circles on blue have been demonstrative for siderophore advent.

3. Results

Gram recoloring was accomplished for 14 strains RM7, RM39, RM69, RM57, RM4, RM15, RM25, RM10, RM35, RM8, RM34,

RM64, RM38 and RM59. The outcome demonstrates that the 11 strains were Gram negative while the 3 strains RM57, RM38 and RM4 were Gram positive (Table 1). Plenitude of rhizobacterial populace in the rhizosphere of maize is surrendered. In the present examination 40 detaches of rhizobacteria, were checked for in vitro PGP exercises. Screening after effects of PGP attributes are portrayed in Table 2. IAA generation was appeared in 14 strains out of 40. Creation of siderophore was distinguished less every now and again than other PGP attributes (Fig. 1; Table 3). Creation of catalase was shown by all detaches of rhizobacteria. Creation of siderophore was shown by all the detached of rhizobacteria except three. Be that as it may, creation of HCN was not distinguished in rhizobacterial secludes under investigation (information not appeared) (Fig. 4). Creation of protease was shown by all detaches of rhizobacteria. Creation of pectinase was shown by all detaches of rhizobacteria except four. Creation of amylase shown by all detaches of rhizobacteria except four (Fig. 5). Catalase action was identified in all the bacterial strains that might be conceivably extremely invaluable (see Figs. 2, 3 and Fig. 6).

Siderophores may straightforwardly animate the production of additional anti-microbial combinations by expanding the accessibility of the minerals in focus to microscopic organisms. Anti-infection and siderophores may further work as pressure factors or singles including nearby and deliberate host obstruction it has been accepted that vaccination with microscopic organisms for example, Bacillus, Pseudomonas, Rhizobium, and Azotobacter may upgrade the plant development because of their nitrogen fixation ability. The bacterial samples studied in this work had the option to deliver catalase. Strains of bacteria indicating catalase movement must be profoundly impervious to ecological, mechanical, and compound pressure. A portion of the above-tried discon-

Table 1
Morphological characterization of isolated strains of MAIZE.

Sr.No	Bacterial isolates	Gram reaction	bacteria shape	cell grouping	Form	Elevation	Color	Margin
1	RM7	+	Coccus	Streptococcus	Circular	Flat	Red	Undulate
2	RM39	+	Coccus	Streptococcus	Spindle	Flat	Off white	Entire
3	RM69	+	Coccus	Streptococcus	Punctiform	Raised	Off white	Entire
4	RM34	+	Coccobacillus	Streptococcus	Spindle	Flat	Off white	Entire
5	RM57	+	Coccus	Streptococcus	Circular	Umbonate	Off white	Erose
6	RM38	+	Coccobacillus	Streptococcus	Circular	Raised	Off white	Entire
7	RM4	+	Coccus	Streptococcus	Punctiform	Flat	Peach	Erose
8	RM15	+	Coccus	Monococcus	Punctiform	Umbonate	Off white	Entire
9	RM25	-	Coccus	Streptococcus	Circular	Flat	White	Erose
10	RM10	+	Coccus	Streptococcus	Spindle	Flat	Off white	Undulate
11	RM8	+	Coccus	Streptococcus	Circular	Flat	Peach	Erose
12	RM64	-	Coccus	Streptococcus	Spindle	Flat	Off white	Entire
13	RM59	-	Coccobacillus	Streptococcus	Circular	Flat	Off white	Undulate
14	RM35	-	Coccus	Monococcus	Rhizoid	Flat	Off white	Filamentous

Table 2
Biochemical characterization of isolated strains of MAIZE.

Sr.No	Strain id	IAA	PSB	Ammonia	HCN	Siderophore	Catalase	Protease	Pectinase	Amylase
1	RM7	+	++	++	-	-	+	+	+	+++
2	RM39	++	+	++	-	-	+	+	-	+++
3	RM69	-	-	++	-	-	+	+	-	++
4	RM34	-	-	-	-	-	+	+	-	++
5	RM57	-	-	+	-	-	+	+	-	+
6	RM38	-	-	++	-	-	+	-	-	+
7	RM4	++	-	-	-	-	+	+	-	-
8	RM15	++	-	-	-	-	+	+	-	-
9	RM25	-	-	-	-	-	+	+	-	+
10	RM10	-	-	-	-	-	+	+	+	-
11	RM8	++	-	++	-	-	+	+	-	+
12	RM64	-	++	++	-	-	+	-	-	+
13	RM59	++	++	++	+	-	+	-	-	+
14	RM35	-	++	++	-	-	+	+	-	-

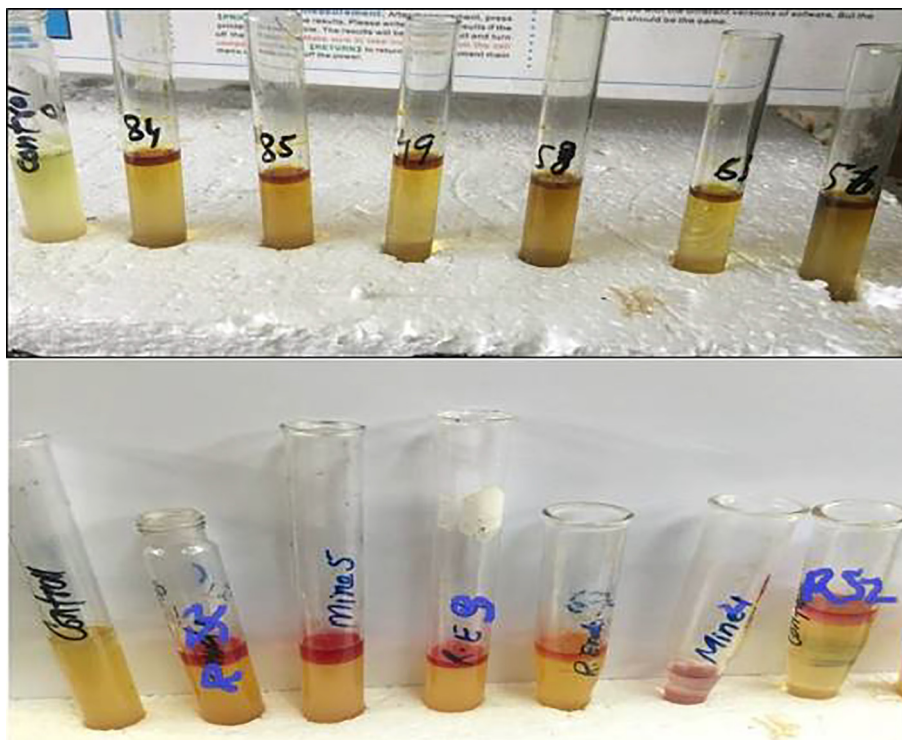


Fig. 1. Indole acetic acid production in LB broth containing L-tryptone.

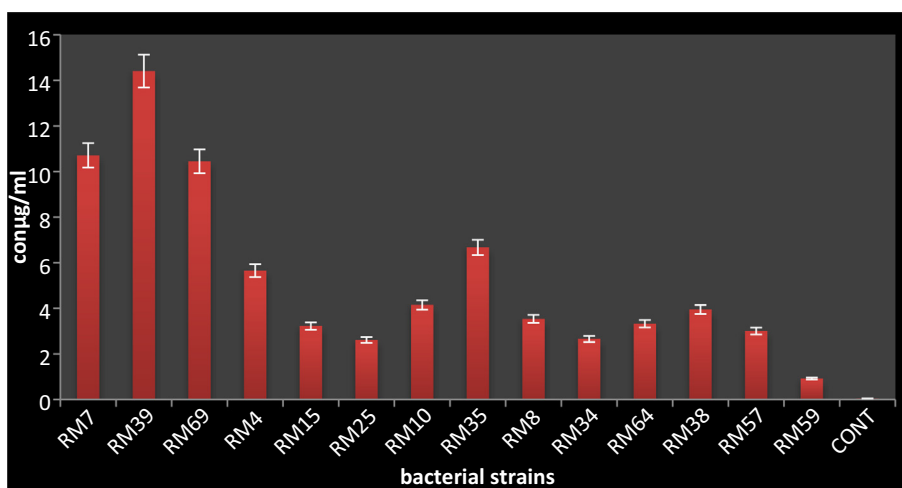


Fig. 2. Indole acetic acid production with the application of bacterial strains.

nects could show more than a few PGP characteristics, which may advance plant development legitimately or in a roundabout way or synergistically. In any case, writes about plant-subordinate rhizosphere impact on microbial network capacities are constrained. Plant roots impact soil borne microbial networks by means of a few systems, including discharge of natural mixes, rivalry for supplements, and giving a strong surface to connection.

Besides, it is realized that some PGPR strains can express numerous gainful capacities. Microorganisms have built up the components to adapt to an assortment of toxic metals for their existence on the earth contaminated with these metals. Some rhizobacteria resistant to different overwhelming metals were observed displaying two or three PGP exercises. Substantial metals, at a higher focus, are lethal to cells and can cause cell demise

through association with dynamic site nucleic acids and compounds. *Azotobacter* spp, when vaccinated into substantial metal tainted soil, repressed N₂-obsession.

However these attributes, plant improvement advancing bacterial isolates must have rhizospheric capability, equipped to bear and colonize in the rhizospheric soil. Shockingly, the collaboration among affiliated PGPR and plant life can be insecure. The brilliant results were given in vitro cannot usually be reliably duplicated under discipline situations. Therefore it is normal for vaccination with rhizobacteria containing PGP attributes to raise root and shoot improvement just like nodulation. Furthermore, assessment of the segregates showing several advancing plant development (PGP) attributes on the soil–plant framework is predicted to demonstrate their adequacy as compelling PGPR.

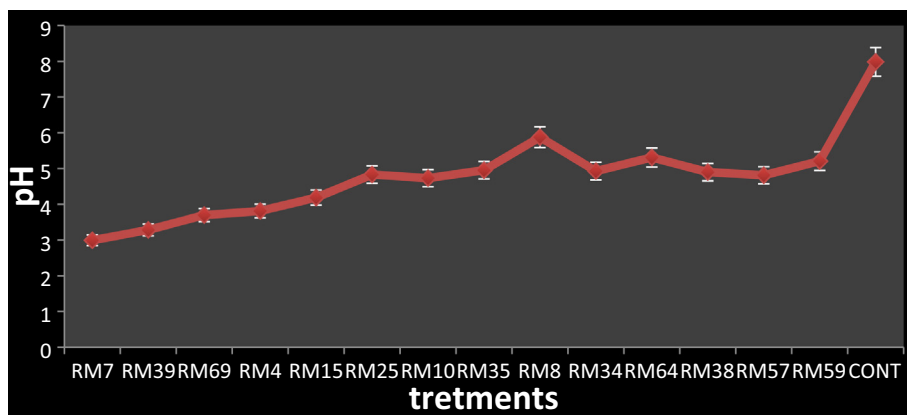


Fig. 3. The effect of pH with the application of bacterial strains.

Table 3
Production and quantification of IAA in isolated bacterial strain.

Sr.no	Strain I.D	IAA production	IAA (µg/ml)
1	RM7	++++	84.113
2	RM39	+++	83.528
3	RM69	+++	68.799
4	RM34	+	22.183
5	RM57	+	20.887
6	RM38	+	20.912
7	RM4	++	33.786
8	RM15	++	31.373
9	RM25	++	27.854
10	RM10	+	26.133
11	RM8	+	23.92
12	RM64	+	21.45
13	RM59	+	14.525
14	RM35	+	24.849

4. Discussion

Maize is one of the three most important cereal crop species providing about 50% of the daily energy to human and animals in Americas and Africas (Palacios-Rojas et al., 2020) but the ever increasing populace in the world demands more production, sustainability and vital agricultural breeding programs of maize (Shiferaw et al., 2011). To meet this demand, fertilizers application is increasing day by day resulting in high cost of production and adverse effects on the environment. Alternatively plant growth-promoting rhizobacteria have positively affected the growth and yield of crops like according to (Breedt et al., 2017) some species

have increased the yield of maize from 24 to 34%. According to (Di Salvo et al., 2018) using PGPR as inoculants of cereal crops like maize can enhance the growth and grain yield. Furthermore, plant growth-promoting bacteria can also may fix atmospheric Nitrogen and delay Nitrogen remobilization in maize plant to increase its yield (Kaur et al., 2016). Similarly a number of PGPR have been reported to promote plant growth by working as biofertilizers and biocontrol agents at the same time (Bevivino et al., 1998).

From the outcome, the greater part of the segregates was Gram negative and this is pair with past reports that the rhizosphere of numerous plants gives helpful and positive condition to Gram negative microscopic organisms (Johansen and Olsson, 2005). A large

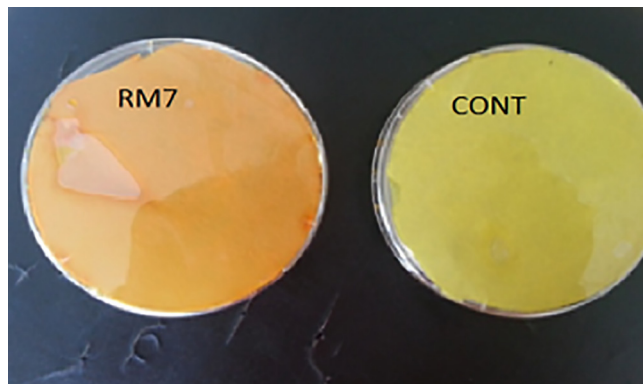


Fig. 5. HCN production with the application of bacterial stains.

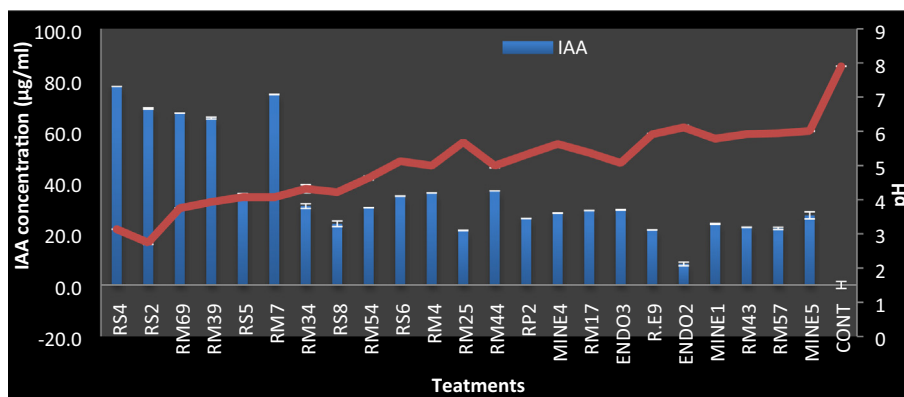


Fig. 4. The effect of IAA with the application of bacterial strains.

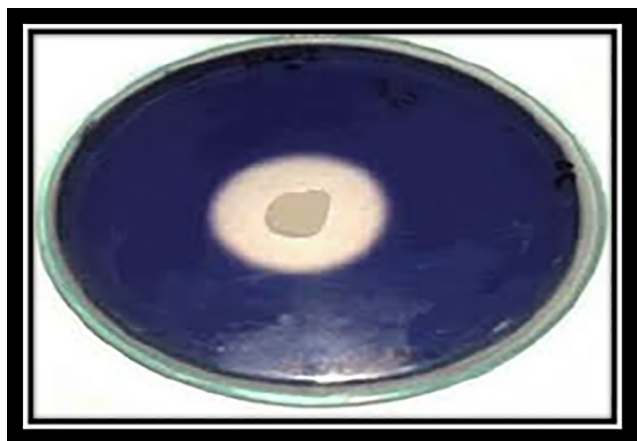


Fig. 6. Amylase production with the application of bacterial stains.

portion of these Gram negative microbes are motile and as indicated by (Johansen and Olsson, 2005), are invigorated by rhizodeposition while Gram positive microscopic organisms are hindered.

Plant rhizosphere is recognised as a favoured biological speciality for soil microbes because of rich supplement accessibility. Numerous research reports can be accessed about *Azotobacter* spp. acquired from various sources indicated IAA generation (Gonzalez-Lopez et al., 1986; Jagnow, 1987). During the course of this study IAA generation in *Azotobacter* samples are in concurrence with prior reported research investigations. The capacity of microscopic organisms to deliver IAA in the rhizosphere relies upon the accessibility of antecedents and take-up of microbial IAA by plant. Development advancement might be ascribed to different instruments, for example, creation of plant development advancing hormones in the rhizosphere and related PGP activities (Abd El-Azeem et al., 2007). Synthesis of IAA by *Bacillus* and *Azotobacter* is a common standard for our test samples of bacteria. More elevated amount of IAA generation by rhizobacteria was recorded by different specialists. Another significant characteristic of PGPR, that may by implication impact the plant development, is the generation of smelling salts.

Siderophore chelates iron and different metals add to ailment concealment by giving an upper hand to biocontrol operators for the constrained supply of fundamental follow minerals in common environments (Höfte et al., 1992). Like our discoveries of various PGP exercises among PGPR have been accounted for by some different specialists while such discoveries on locally available microbial isolates in India are less generally investigated. The natural substance in soil tests was seen one among the important factors controlling the bacterial network structure (Zhou et al., 2002).

The nature of this impact is exceptionally factor and relies on both the sum and creation of natural materials discharged by the plants (Griffiths et al., 1998). Any microbial use in horticulture requires an assessment of the ecological dangers related with the presentation of indigenous or non-indigenous microorganisms into the plant rhizosphere just as an evaluation of the most reasonable conditions for viable and fruitful foundation of the PGPR vaccination in the rhizosphere of the host plant (De Leij et al., 1995).

It was additionally obvious that more societies of PGPR incurred from chickpea rhizosphere were resistant to raised levels substantial metals. Researchers found that by diminishing substantial metal harmfulness, PGPR expands plant development (Burd et al., 1998). Choosing metal resistant and vigorous microorganisms to build PGP mixes could be helpful for accelerating plant rhizosphere recolonization in contaminated soils (Carlot et al., 2002).

Rother and associates discovered a lower in knob and plant length and in nitrogenase movement of clover at locations vigor-

ously defiled with Cd and Pb (Rother et al., 1983). Chromium-secure pseudomonads secluded from paint industry effluents had the option to invigorate seed germination and development of *Triticum aestivum* in the sight of potassium dichromate.

5. Conclusion

This examination shows the nearness of Rhizobia in the foundations of MAIZE from the developing locale of rock phosphate area Hazara, Pakistan. The morphological qualities likewise show the nearness of conditions in the rhizospheres that can empower the plant use a lot of nitrogen for legitimate usage of nitrogen mixes. In a creating nation like Pakistan it is critical to expand the yield of heartbeats and numerous different harvests by using the consequences of such sorts of research. Thus, further investigations including recognizable proof of strains to species level and field studies are prescribed before selection by ranchers in farming practices.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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