International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(5): 790-795

Received: 17-07-2020
Accepted: 23-08-2020

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Isolation and characterization of phosphorus solubilizing bacteria, potassium releasing bacteria and sulphur oxidizing bacteria in maize (*Zea mays* L.) rhizosphere soils of Andhra Pradesh

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DOI: https://doi.org/10.22271/chemi.2020.v8.i5k.10396

Abstract
In this study, the Phosphorus Solubilizing Bacteria (PSB), Potassium Releasing Bacteria (KRB) and Sulphur Oxidizing Bacteria (SOB) strains were acquired from maize rhizosphere soils from Guntur, Kurnool and Viziangaram districs of Andhra Pradesh state. Those isolates were identified on different medias like, Pikovskaya's agar, Aleksandrow's agar and Thiosulphate agar medias followed by PSB, KRB and SOB isolates respectively. Those isolates were studied by their gram reaction, cultural, morphological, biochemical and PGPR characteristics.

Keywords: Rhizosphere, biochemical, PSB, KRB, SOB, PGPR

1. Introduction
The continues use of inorganic fertilizers to buildup crop productivity and soil fertility after results in unexpected harmful environmental effects, including leaching of nitrate into ground water, surface runoff of phosphorus and nitrogen run-off, and eutrophication of aquatic ecosystems (Adesemoye and Kloepper, 2009) [1]. Bio-inoculants is a substance, which contains the living microorganisms that colonizes the rhizosphere and encourages production by increasing the supply of plant nutrients (Vessey, 2003) [23]. Bio-fertilizers promotes plant growth by fixing nitrogen, phytohormones production, phosphate solubilization and potassium releasing (Bashan *et al*., 2004) [3].

Phosphorus (P) is one of the essential macronutrients for biological growth and development. The simultaneous use of PSB as inoculants increases phosphorus intake resulted in enhanced plant growth and crop yield (Rodriguez and Fraga, 1999) [18]. Potassium (K) is essential in all cell metabolic processes and involves with photosynthesis, carbohydrate translocation, protein synthesis, disease resistance and drought tolerance. Consequently, K deficiency becomes a problem because K decreases easily in soils due to crop uptake, runoff, leaching and erosion (Sheng and Huang, 2002) [21]. Sulphur (S) requirement as it is an important component of plant proteins (amino acid synthesis) and chlorophyll formation. The Sulphur deficiency was characterized by a yellowing of the younger leaves followed by reddening of stems and leaves starting from leaf edges and gradually spreading to midrib and older leaves remains green (Rahul *et al*., 2017) [16].

The fertilization of the soil is very important to increased yield. However, commercial fertilizers are expensive means of supplementing essential nutrients to soil for plant growth. Inoculation of rhizobacteria increases plant growth parameters. Keeping this in view the present study is proposed to isolate and characterize the *in vitro* phosphate solubilising, potassium releasing and sulphur oxidizing bacteria from different soils and selected efficient isolates based on their PGPR characteristics.

2. Materials and Methods
2.1 Soil Samples Collection
The rhizosphere soil samples were collected from different districts, Guntur, Kurnool and Vizianagaram for the isolation of PSB, KRB and SOB bacterial isolates.

The soil samples were mainly collected from maize rhizosphere. Crop plants were selected randomly in the field and the intact root system was dug out, carefully taken in plastic bags, labeled well and stored at 4 ºC.

2.2 Isolation Technique

2.2.1 Serial dilution technique

One gram sample of soil serially diluted upto 10^{-6} dilutions and 0.1 ml each of the aliquots from 10^{-5}, 10^{-3} and 10^{-4} dilutions was transferred to already solidified Pikovskaya's agar, Aleksandrow's agar and Thiosulphate agar medium. After plating with Pikovskaya's agar, Aleksandrow's agar and Thiosulphate agar medium, the plates were incubated in an inverted position for 3-5 days at 30 ℃. Characteristic Solubilization zones around colonies growing over the medium in PSB, KRB isolates followed Pikovskaya's agar, Aleksandrow's agar medium but yellow colour colonies in SOB on Thiosulphate agar medium. Further, the PSB, KRB and SOB isolates were purified by the streak plate method and well isolated colonies on the plates were preserved on their respective agar slants. Isolates were maintained on slants at 4 ℃ in a refrigerator for further use.

2.3 Morphological Characterization

All the isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950) [2].

2.4 Colony Morphology

The morphological characteristics of the colony of each isolate were examined on Nutrient agar medium by incubating for specific period. Cultural characterization of isolates such as shape, size, elevation, surface, margin and colour of the colony were recorded.

2.5 Biochemical characterization of PSB, KRB and SOB isolates

2.5.1 Indole Production

Sterilized Hydrogen Sulfide-Indole-Motility agar (SIM agar) slants or Tryptophan broth tubes were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28 ± 2 ºC. Following incubation, 10 drops of Kovac's indole reagent was added to each tube. The isolates showing the production of red color was recorded as positive for indole production (Cheesbrough, 2006) [7].

2.5.2 Methyl Red Test

Sterilized glucose-phosphate broth tubes were inoculated with the test culture and incubated at 28 ± 2 ºC for 48 h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Red color production was taken as positive and yellow color production was taken as negative for the test (Olutiola *et al*., 2000) [11].

2.5.3 Voges Prausker's Test

To the pre sterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37 ºC for 48h. After incubation ten drops of Baritt's reagent, A was added and gently shaken followed by addition of 10 drops of Baritt's reagent B. Development of pink color in the broth was taken as positive for the test (MacFaddin, 2000) [10].

2.5.4 Citrate Utilization

Isolates were streaked on Simmon's citrate agar slants and incubated at 28 ±2 ºC for 24 h. Change in color from green to blue indicates the positive reaction for citrate utilization (Cappucino, 1983).

2.6 Screening of bacterial isolates for plant growth promoting traits

2.6.1 Siderophore Production

Siderophore production was estimated at qualitatively. Chrome azurol sulphonate (CAS) agar medium (Schwyn and Neilands, 1987) was used for the detection of siderophores, isolates were grown in synthetic medium, containing 0.5 µM of iron and incubated for 24 hrs on a rotary shaker at room temperature. CAS assay is used to detect the siderophores. The CAS plates were used to check the culture supernatant for the presence of siderophores. Culture supernatant was added to the wells made on the CAS agar and incubated at room temperature for 24 hrs. Formation of yellow to the orange colored zone around the wells indicates siderophore production.

2.6.2 In-vitro assay for phosphate solubilizing activity

Phosphate solubilization activity was determined using Pikovskaya's agar medium containing 0.5% (W/V) $Ca_3(PO_4)_2$ (Pikovskaya, 1948) [15]. Pikovskayas agar plates were prepared and sterilized. The inoculums were spot inoculated on the pikovskayas plate. 24 hrs old culture was used for the inoculation. The plates were incubated for 72-96 hrs at room temperature. The clear zone was observed around the spotted area after the incubation period.

2.6.3 HCN Production

The HCN production was tested by the method of Castric and Castric (1983) [6]. First respective media plates i.e., Pikovskaya's broth (PSB), modified Aleksandrow's agar media (KRB), thiosulphate agar was prepared separately and incubated for 24 hrs. After that, 1 ml of culture of each test isolate was inoculated on respective media plates separately. A disc of Whatman filter paper no.1 of the diameter equal to the petri plate size, impregnated with alkaline picric acid solution (0.5% picric acid (w/v) in 1% sodium carbonate) was placed in the upper lid of the inoculated petri plates under aseptic condition. The control plate did not receive the inoculum. The plates were incubated upside up at 28 ^{0}C±2 ^{0}C for 48-72 hrs. Change in colour from yellow to light brown, moderate or strong reddish brown was taken as indication of HCN production.

3. Results and Discussion

3.1 Collection of soil samples

The rhizosphere soil samples were drawn from Guntur, Kurnool and Vizianagaram districts of Andhra Pradesh, where maize is grown and geographical indications were recorded at sampling sites (Table 1).

3.2 Isolation of phosphorous solubilizing bacteria (PSB), potassium releasing bacteria (KRB) and sulphur oxidizing bacteria (SOB) from rhizosphere soil sample

The phosphorus solubilising bacteria (PSB), potassium releasing bacteria (KRB) and sulphur oxidizing bacteria (SOB) populations in the rhizosphere soils were determined in (Table 2 and plate 1) and coding of isolates according to district, mandal, village and isolate identified were shown in (table 3). PSB population ranges between 3.82-12.46 × 10^6 CFU g^1 soil. Maximum PSB population were recorded in the village Nelapadu, Guntur district (12.46 × 10^6 CFU g^{-1} soil) and least population was attained in the soils of Kolakaluru,

Guntur district (3.48 × 10^6 CFU g^{-1} soil). Similar results were shown by Pandey and Chayanika (2018) who obtained 10 PSB isolates from the soil sample of rhizosphere on Pikovskaya's agar medium.

The KRB population ranges between 2.42 - 9.80 × 10^6 CFU g^{-1} soil. Maximum KRB population was recorded in soil sample from Bethapudi, Guntur district (9.80 × 10^6 CFU g^{-1} soil) and minimum KRB population was obtained in soil sample of Singupalem, Guntur district (2.42 × 10^6 CFU g^{-1} soil). Similar results was observed by Parmar *et al*. (2016) [13] isolated potassium solubilizers from rhizosphere soils, using Aleksandrow's media.

SOB population ranges between 1.66 - 4.28 × 10^6 CFU g^{-1} soil. Maximum SOB population was recorded in soil sample from Borabanda, Vizianagaram district (4.28 × 10^6 CFU g^{-1} soil) and minimum SOB population was recorded at rhizosphere soils of Rajupeta, Vizianagaram district (1.34 × 10^6 CFU g^{-1} soil). Similar results was observed by Reddy *et al*. (2018) [17] to isolated sulphur oxidising bacteria on thiosulphate agar media from saline soils of groundnut growing places in Andhra Pradesh, India.

3.3 Cultural and morphological traits of PSB, KRB and SOB isolates

After isolation of PSB, KRB and SOB isolates were studied for morphological and cultural traits (Table 4). Among the 4 PSB isolates were mostly irregular shaped, light yellow colour and rough colonies were observed. All PSB isolates was gram -ve reaction and rod shaped. Comparable results was reported by Paul and Sinha (2016) [14] the phosphate solubilising bacterium was identified using physiological, morphological characters and these isolates were showed gram -ve, rod shaped and motility.

The KRB isolates were mostly round shaped, creamy colour and smooth colonies were observed. All KRB isolates was gram +ve reaction and rod shaped. Similar results was reported by Kavya *et al*. (2020) [9] studied characteristics of six isolates of K releasing bacteria from different places of Andhra Pradesh. Majority of the isolates were identified as entire smooth margin, raised, translucent, gram +ve rods and whitish to creamy in appearance. Among all SOB isolates were mostly round shaped and creamy colour, smooth colonies, gram -ve reaction and rod shaped were observed. Similar results was observed by Veerender *et al*. (2014) [22] total of 16 bacterial isolates are drawn from different ecosystems. The screened isolates were resulted as Gram negative, rod shaped; smooth, concentric and white colour colonies.

3.4 Biochemical and physiological characterization

The study of phosphorus solubilising bacteria, potassium releasing bacteria and sulphur oxidizing bacteria were checked against biochemical tests *viz*., methyl red test, voges proskauer's test, citrate utilization test and indole production (Table 5).

All four isolates of (PSB), 3 isolates are positive for citrate utilization and methyl red tests. All the isolates were positive for voges proskauer's and indole production. From three of potassium releasing bacteria, one isolate (GRBK) showed negative and remaining positive for citrate utilization test. For voges proskauer's, methyl red and Indole production all isolates showed positive results. Among all the isolates of Sulphur oxidizing bacteria (SOB) were showed positive results for voges proskauer's and methyl red test. For Citrate utilization test isolate VBKS and indole production test isolate VSBS showed negative results and remaining all isolates were showed positive.

Similar results were observed by Bashir *et al*. (2018) [4] isolated PSBs from various soil samples of rhizosphere and performed various tests *viz*., citrate utilization test, indole production test and gas production. Saha *et al*. (2016) [14] characterized potassium releasing bacteria based upon their morphological and biochemical characteristics. Reddy *et al*. (2018) [17] to isolated sulphur oxidising bacteria on thiosulphate agar media and those isolates were characteristics based upon their biochemical characteristics.

3.5 Screening of bacterial strians for plant growth promoting (PGPR) properties

Among all isolates were screened by PGP activities viz., phosphate solubilization, siderophore and HCN production (Table 6 and plate 2).

Among all PSB isolates GTAP recorded the phosphate solubilization zone 6.2 mm diameter was heighest. For HCN isolates *i.e*., GTAP, GTCP, GTKP and siderophore production GTAP and GTCP isolates were showed positive results. The KRB isolate GRPK isolate was formed a clear zone of phosphate solubilization which ranged 4.1mm. For HCN production GRPK and GRSK isolates were shown positive results. For siderophore production GRPK isolate give positive result. Out of all SOB isolates, only one isolate VBRS were formed clear zone of phosphate solubilization zone and HCN production. For siderophore production VBRS and VSBS isolates give positive results.

Similar results were observed with Habibi *et al*. (2019) [8] were isolated 98 bacterial strains and their cultural, morphological and PGP traits, such as indole-3-acetic acid production, acetylene reduction, phosphate and potassium solubilization, and siderophore production were evaluated.

Conclusion

The present study revealed that that isolation, cultural, morphological, biochemical and PGPR characterization of phosphorus solubilising, potassium releasing and sulphur oxidizing bacteria are not just necessary to understand their ecological function in the rhizosphere rather utilization in eco-friendly and improves the soil health, fertility conditions. Out of 10 isolates, for PSB (GTAP), KRB (GRPK) and SOB (VBRS) were selected as efficient isolates by their PGPR activities. These efficient isolates were used for further field application as microbial inoculants to enhance plant growth parameters and yield attributes.

Table 1: Details of maize rhizosphere soil samples collected from different places of Andhra Pradesh

S.No	Latitude No	Longitude No	District	Mandal	Village	Soil Type
1	16^0 24' 85.3' N	80^0 62' 90.2' E	Guntur	Tenali	Angalakuduru	Black
2	16^0 21'53.5' N	80^0 67' 22.1' E	Guntur	Tenali	Chinna ravuru	Black
3	16^0 26' 44.2' N	80^0 68' 54.7' E	Guntur	Tenali	Nelapadu	Black
4	16^0 30' 00.4' N	80^0 61' 69.9' E	Guntur	Tenali	Kolakaluru	Black
5	16^0 29' 51.7' N	80^0 65' 25.2' E	Guntur	Tenali	Nandivelugu	Black
6	16^0 02' 34.0' N	80^0 81' 42.9' E	Guntur	Repalle	Bethapudi	Black

7	16^0 00' 20.1' N	80^0 83' 18.4' E	Guntur	Repalle	Uppudi	Black
8	15^0 95' 60.5' N	80^0 86' 72.1' E	Guntur	Repalle	Singupalem	Black
9	15^0 93' 06.3' N	80^0 84' 30.4' E	Guntur	Repalle	Potumeraka	Black
10	15^0 95' 79.0' N	80^0 87' 69.2' E	Guntur	Repalle	Visweswaram	Black
11	15^0 49' 24.2' N	78^0 51' 88.8' E	Kurnool	Nandhyala	Billalapuram	Red
12	15^0 43' 58.4' N	78^0 49' 23.9' E	Kurnool	Nandhyala	Chabolu	Red Sandy
13	15^0 54' 09.3' N	78^0 45' 36.8' E	Kurnool	Nandhyala	Munagala	Red
14	18^0 59' 56.3' N	83^0 36' 84.0' E	Vizianagaram	Bobbili	Patha bobbili	Black
15	18^0 61' 27.3' N	83^0 33' 91.9' E	Vizianagaram	Bobbili	Rajupeta	Black
16	18^0 57' 72.5' N	83^0 39' 09.8' E	Vizianagaram	Bobbili	Krishnapuram	Black
17	18^0 58' 30.4' N	83^0 37' 42.6' E	Vizianagaram	Bobbili	Jagannadhapuram	Red
18	18^0 54' 16.6' N	83^0 26' 45.4' E	Vizianagaram	Saluru	Borabanda	Black
19	18^0 52' 65.8' N	83^0 23' 57.0' E	Vizianagaram	Saluru	Sivarampuram	Black
20	18^0 66' 76.0' N	83^0 16' 06.5' E	Vizianagaram	Saluru	Pattuchennuru	Red Sandy
21	18^0 64' 72.7' N	83^0 14' 29.5' E	Vizianagaram	Saluru	Nimmalapadu	Black
22	18^0 20' 91.6' N	83^0 59' 51.9' E	Vizianagaram	Garividi	Konuru	Black
23	18^0 34' 41.7' N	83^0 55' 14.7' E	Vizianagaram	Garividi	Mandiravalasa	Black
24	18^0 33' 34.0' N	83^0 57' 27.1' E	Vizianagaram	Garividi	Chandapuram	Black
25	18^0 34' 58.7' N	83^0 52' 99.2' E	Vizianagaram	Garividi	Yenuguvalasa	Red

Table 2: Microbial population (CFU g^{-1} soil) of PSB, KRB and SOB isolates

S. No	Village	Mandal	District	PSB population (10^6 CFU g^{-1} soil)	KRB population (10^6 CFU g^{-1} soil)	SOB population (10^6 CFU g^{-1} soil)
1	Angalakuduru	Tenali	Guntur	9.46	-	-
2	Chinna ravuru	Tenali	Guntur	8.51	-	-
3	Nelapadu	Tenali	Guntur	12.46	-	-
4	Kolakaluru	Tenali	Guntur	3.82	-	-
5	Bethapudi	Repalle	Guntur	-	9.80	-
6	Singupalem	Repalle	Guntur	-	2.42	-
7	Potumeraka	Repalle	Guntur	-	3.60	-
8	Rajupeta	Bobbili	Vizianagaram	-	-	1.66
9	Krishnapuram	Bobbili	Vizianagaram	-	-	2.84
10	Borabanda	Saluru	Vizianagaram	-	-	4.28

Table 3: The isolated bacterial codes according to the district, mandal, village and isolate identified

S.No	District	Mandal	Village	PSB/KRB/SOB	Isolate code
1	Guntur	Tenali	Angalakuduru	PSB	GTAP
2	Guntur	Tenali	Nelapadu	PSB	GTNP
3	Guntur	Tenali	Chinna ravuru	PSB	GTCP
4	Guntur	Tenali	Kolakaluru	PSB	GTKP
5	Guntur	Repalle	Bethapudi	KRB	GRBK
6	Guntur	Repalle	Singupalem	KRB	GRSK
7	Guntur	Repalle	Potumeraka	KRB	GRPK
8	Vizianagaram	Bobbili	Krishnapuram	SOB	VBKS
9	Vizianagaram	Bobbili	Rajupeta	SOB	VBRS
10	Vizianagaram	Saluru	Borabanda	SOB	VSBS

Table 4: Morphological characteristics of PSB, KRB and SOB isolates

S.No	Isolate name	Cultural characters						Morphological characters	
		Size	Shape	Colour	Elevation	Surface	Margin	Gram reaction	Shape
1	GTAP	Medium	Round	Light yellow	Convex	Smooth	Entire	Gram –ve	Rod
2	GTNP	Small	Irregular	Whitish	Raised	Rough	Entire	Gram –ve	Rod
3	GTCP	Medium	Irregular	Light yellow	Convex	Rough	Irregular	Gram –ve	Rod
4	GTKP	Small	Irregular	Light yellow	Flat	Rough	Irregular	Gram –ve	Rod
5	GRBK	Medium	Round	Creamy	Convex	Smooth	Irregular	Gram +ve	Rod
6	GRSK	Small	Round	Creamy	Convex	Smooth	Entire	Gram +ve	Rod
7	GRPK	Small	Round	Whitish	Raised	Smooth	Entire	Gram +ve	Rod
8	VBKS	Small	Round	Creamy	Raised	Smooth	Irregular	Gram –ve	Rod
9	VBRS	Medium	Round	Whitish	Convex	Smooth	Regular	Gram –ve	Rod
10	VSBS	Small	Irregular	Creamy	Convex	Smooth	Irregular	Gram –ve	Rod

Table 5: Biochemical characterization of PSB, KRB and SOB isolates

S.no	Isolate name	Methyl red test	Voges -proskauer's	Citrate utilization	Indole Production
1	GTAP	+	+	+	+
2	GTNP	+	+	-	+
3	GTCP	-	+	+	+
4	GTKP	+	+	+	+

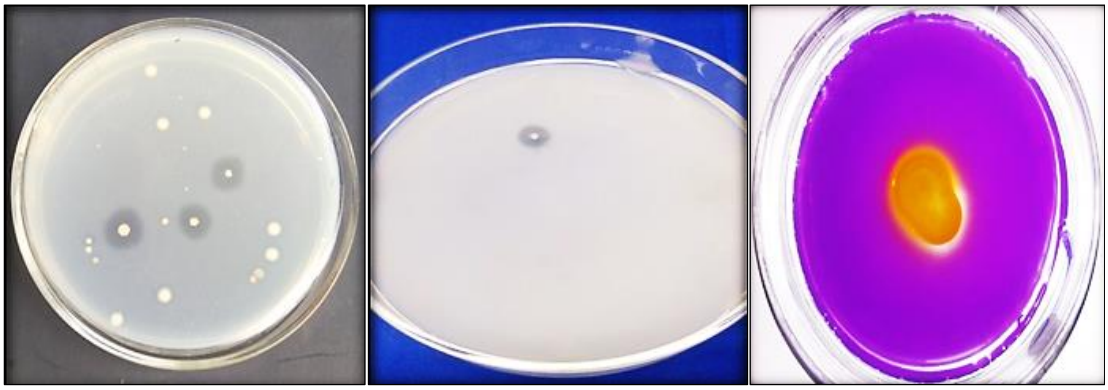
5	GRBK	+	+	-	+
6	GRSK	+	+	+	+
7	GRPK	+	+	+	+
8	VBKS	+	+	-	+
9	VBRS	+	+	+	+
10	VSBS	+	+	+	-

(+Ve Positive) (-Ve Negative)

Table 6: PGPR characteristics of PSB, KRB and SOB isolates

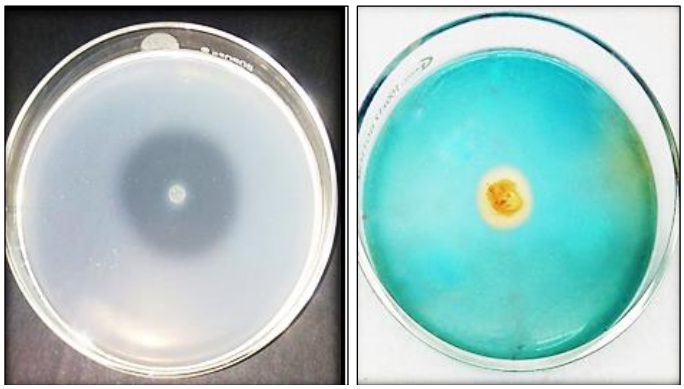
S.no	Isolate name	Phosphate solubilisation			Siderophrore production	HCN production
		Zone diameter (mm)		Phosphate Solubilization Index (PSI)		
		Solubilization zone	Culture diameter			
1	GTAP	6.2	4.8	2.29	+	+
2	GTNP	1.6	2.5	1.64	-	-
3	GTCP	2.8	6.1	1.45	+	+
4	GTKP	1.7	3.8	1.44	-	+
5	GRBK	-	1.6	-	-	-
6	GRSK	-	2.1	-	-	+
7	GRPK	4.1	4.3	1.95	+	+
8	VBKS	-	4.1	-	-	-
9	VBRS	3.2	3.4	1.94	+	+
10	VSBS	-	1.8	-	+	-

(+Ve Positive) (-Ve Negative)

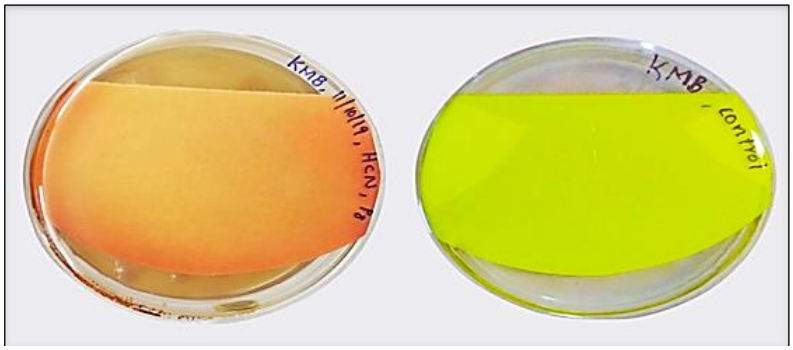


a) Isolation of PSB **b)** Isolation of KRB **c)** Isolation of SOB

Plate 1: Isolation of PSB, KRB and SOB (a, b and c) isolates



a) P-Solubilization zone **b)** Siderophore production



c) HCN production

Plate 2: PGPR characteristics of PSB, KRB and SOB (a, b and c) isolates

References

1. Adesemoye AO, Kloepper JW. Plant–microbes interactions in enhanced fertilizer-use efficiency. Applied Microbiology and Biotechnology. 2009; 85:1-12.
2. Barthalomew JW, Mittewer T. A simplified bacterial spore strain. Stain Technology. 1950; 25(3):153-156.
3. Bashan Y, Holguin G, De-Bashan LE. *Azospirillum*-plant relationships: physiological, molecular, agricultural and environmental advances. Canadian Journal of Microbiology. 2004; 50: 521-577.
4. Bashir Z, Zargar MY, Baba ZA, Mohiddin FA, Peer FA, Mir SA, Najar GR. Isolation and characterization of phosphorus solubilizing bacteria (PSB) from rhizospheric soils of apple (*Malus Domestica*). Journal of Pharmacognosy and Phytochemistry. 2018; 7(2):2997-3000.
5. Cappuccino JC, Sherman N. In: Microbiology. A Laboratory Manual, New York, 1992, 125-179.
6. Castric KF, Castric PA. Method for rapid detection of cyanogenic bacteria. Applied and Environmental Microbiology. 1983; 45(2):700-702.
7. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd Edn., 2006. Cambridge University Press, Cambridge, UK., ISBN-13: 9781139449298.
8. Habibi S, Djedidi S, Ohtsu NO, Sarhadi WA, Kojima K, Rallos RV *et al*. Isolation and screening of indigenous plant growth rhizobacteria from different rice cultivars in Afghanistan soils. Microbes and Environments. 2019; 34 (4):347-355.
9. Kavya Y, Trimurtulu N, Gopal AV, Vani PM, Prasad NVVSD. Effect of inoculation of microbial consortia on soil physiochemical and nutrient status. Current Journal of Applied Science and Technology. 2020; 39(4):1-8.
10. MacFaddin JF. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA, 2000.
11. Olutiola PO, Famurewa O, Sonntag HG. Introduction to General Microbiology: A Practical Approach. 2nd Edn. Bolabay Publications, Ikeja, Nigeria, 2000.
12. Pandey D, Chayanika P. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of potato plant. International Journal of Current Microbiology and Applied Sciences. 2018; 7(1):967-975.
13. Parmar KB, Mehta BP, Kunt MD. Isolation, characterization and identification of potassium solubilizing bacteria from rhizosphere soil of maize (*Zea mays*). International Journal of Science, Environment and Technology. 2016; 5(5):3030-3037.
14. Paul D, Sinha SN. Isolation and characterization of phosphate solubilizing bacterium *Pseudomonas aeruginosa* KUPSB12 with antibacterial potential from river Ganga, India. Annals of Agrarian Science. 2016; 15(1):130-136.
15. Pikovskaya RI. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiologia*. 1948; 17:362-370.
16. Rahul KS, Amit MP, Aravinda Kumar BN, Hebsur NS. Sulphur nutrition in maize-a critical review. International Journal of Pure and Applied Bioscience. 2017; 5(6):1582-1596.
17. Reddy NA, Gopal AV, Lakshmipathy R, Padma V. Isolation, characterization and screening of sulphur oxidizing bacteria from rhizosphere soils of groundnut. International Journal of Currrent Microbiology and Applied Sciences. 2018; 7(8):2639-2644.
18. Rodriguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances. 1999; 17(4-5):319-339.
19. Saha M, Maurya BR, Meena VS, Bahadur I, Kumar A.. Identification and characterization of potassium solubilizing bacteria (KSB) from Indo-Gangetic plains of India. Biocatalysis and Agricultural Biotechnology. 2016; 7:202-209.
20. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry, 2003, 160:47-56.
21. Sheng XF, Huang WY. Physiological characteristics of strain NBT of silicate bacterium. *Acta pedologica Sinica*. 2002; 39:863-871.
22. Veerender K, Sridar R, Sivaji M. Isolation and characterization of sulphur oxidizing bacteria from different ecosystems. Society for Scientific Development in Agriculture and Technology. 2014; 9:104-107.
23. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil. 2003; 255:571-586.