

Research Article

ISOLATION AND CHARACTERIZATION OF NATIVE POTASH MOBILIZING PLANT GROWTH PROMOTING RHIZOSPHERIC BACTERIA

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Abstract- Research was carried out to explore plant growth promoting abilities of native rhizospheric potash mobilizing bacterial (KMB) isolates. Seventeen prominent cultures were selected on the basis of the zone of potash solubilization on Aleksandrov agar supplemented with mica which exhibited organic acid and capsular polysaccharides production. Fourteen isolates were positive for acidic exo-polysaccharides. Five prominent cultures were selected on the basis of PGP traits i.e. solubilization of phosphate and zinc; production of indole acetic acid and siderophore; showing various enzyme activity viz. ACC-deaminase, lipase, protease and cellulase. Moreover, these isolates manage phytopathogenic fungi viz. *Fusarium oxysporum, Fusarium solani, Pythium aphanidermatum, Macrophomina phaseolina* and *Alternaria alternata*. Isolates were able to tolerate wide range of pH, temperature and NaCl concentrations. On the basis of morphological, physiological, biochemical and molecular techniques, native KMB PGPR isolates were characterized as; *Acinetobacter pittii, A. oleivorans, A. baumannii, A. calcoaceticus* and *A. junii*. The results endow with a basis for understanding the beneficial effects of KMB for deploying the strains and or its consortium in industrial production of bio-fertilizer cum bio-control agents for agricultural crops.

Keywords- Rhizosphere, KMB, Potash, Acinetobacter, PGPR

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Introduction

A diverse group of soil micro-flora is reported to be involved in the solubilization of insoluble and fixed forms of K into available forms of K which is easily absorbed by plants. Microbial inoculants that are able to dissolve K from mineral and rocks that enhanced plant growth and yield are also economically viable and ecofriendly input. The first evidence of microbial involvement in solubilization of rock potassium was shown by Muentz [1]. A wide range of potassium solubilizing microorganisms (KSMs) viz. Bacillus mucilaginosus, Bacillus edaphicus, Bacillus circulans, Paenibacillus sp., Acidothiobacillus ferrooxidans, Pseudomonas, Burkholderia etc. have been reported to release potassium in accessible form from K-bearing minerals in soils [2-5].

The mechanism of potassium solubilization through which insoluble and structural unavailable forms of potassium compounds are solubilized and mobilized due to the production of various organic acids. The main processes attributed to their conversion in soluble form are acidolysis, complexolysis, exchange reactions etc. [6]. The organic and inorganic acids convert insoluble K (mica, muscovite, biotite, feldspar) to the soluble form of K (soil solution form) increasing the availability of the nutrients to the plants. The various organic acids produced by KSMs differed with diverse organisms. Organic acids were detected in the microbial suspension [7]. KSMs have the ability to weather phlogopite (magnesium mica- KMg₃AlSi₃O₁₀ (FeOH)₂) via aluminum chelation and acidic dissolution of the crystal network.

With the introduction of high-yielding crop varieties, progressive intensification and imbalanced fertilizers, the soils are generally getting depleted in the potassium reserve at a faster rate [8-10]. These emphasized the search to find an alternative and effective indigenous source of K for plant uptake and also to maintain K status in soils for sustaining crop production.

Materials and Methods Isolation of KMB

Soil samples from the rhizospheric region of various crops like maize, wheat, potato, pigeon pea, brinjal, tomato, chilli, onion, lucerne, banana, cotton, groundnut, rice, castor and tobacco were collected from farms of Agronomy, Vegetable, Regional Research Station, Horticulture, and Bidi Tobacco Research station of Anand Agricultural University, Anand. Soil was dug from depth of 15 cm around the rhizospheric region without harming the plant and samples were collected in sterile HDPE bags from 5 to 6 sites making a composite sample and were stored at 4°C until further processed. Soil sample, each 10 g was weighed from, serially diluted and streaked on Glucose Yeast Calcium agar medium (GYCa) followed by incubation at 30±2°C and examined up to 3-5 days for the colonies showing clear zones of calcium release. Such colonies were further purified by four sector streak plate method and studied on the Aleksandrov medium supplemented with mineral mica, incubated at 30±2°C for 6-7 days and zone of solubilization and index (SI) noted. Further, liquid assay was done to study potash mobilization. Aleksandrov broth with mica @ 2g I-1 was dispensed 50 ml in 100 ml Erlenmeyer flask and autoclaved at 121°C for 15 min, allowed to cool and inoculated with 1ml of bacterial suspension @ 1x107 CFU ml-1 and incubated at 30±2°C for 10 days and checked for potassium release [11]. Strain AAU09 KMBW1 was used in the study for comparison as standard culture obtained from Department of Agricultural Microbiology, A.A.U., Anand.

Characterization of native KMB isolates

The selected isolates were preliminarily identified on the basis of cultural, morphological and biochemical characteristics using 9th edition of Bergey's Manual of Determinative Bacteriology and The Prokaryotes.

Isolation and Characterization of Native Potash Mobilizing Plant Growth Promoting Rhizospheric Bacteria

SN	Isolates	Solubilization Index Available K (µg/ml)					
		(SI)	2 DAI	4 DAI	6 DAI	8 DAI	10 DAI
1	M- 1	3.6	7.80	8.54	9.01	8.10	7.25
2	M- 2	8.0	19.57	21.41	20.16	18.57	17.23
3	M- 3	7.5	16.21	17.14	18.31	16.20	15.10
4	M- 4	4.5	5.86	6.24	7.05	6.10	5.45
5	W- 1	6.5	18.50	19.45	20.17	19.81	18.20
6	P-1	5.6	2.50	1.58	3.14	3.28	2.86
7	B- 1	7.5	16.47	17.85	19.39	17.90	16.02
8	Ba- 1	6.0	4.65	5.54	4.75	4.25	4.00
9	Ba- 2	4.7	4.54	3.94	4.95	4.68	3.42
10	PP- 1	3.0	4.12	3.76	3.53	4.74	4.54
11	C-1	4.0	4.27	3.54	3.75	4.34	4.15
12	R-1	6.0	5.02	4.66	4.58	4.64	4.24
13	R- 2	4.3	2.00	1.50	1.68	2.36	2.49
14	R- 3	4.7	3.35	4.52	3.72	3.54	4.14
15	R- 4	6.5	5.24	4.94	5.95	4.68	4.32
16	CS- 1	7.5	19.05	19.89	20.30	19.12	18.26
17	CS-2	4.4	4.21	4.00	3.78	4.15	4.00
18	Std.W1	W1 1.85		5.04	5.76	4.64	4.12
	S	.Em.±	0.260	0.318	0.231	0.346	0.289
		lsd	0.746	0.911	0.663	0.994	0.828
	(CV %	5.46	6.45	4.50	7.15	6.44

Table-1 Potash solubilization by isolates in Aleksandrov media

*Values are mean of 3 replicates

Table-2 PGPR traits of KMB isolates

SN	Isolate	TCP solubilization Zone (mm)	Zinc solubilization Zone (mm)	ACC deaminase activity	Siderophore production	IAA concentration (µg/ml)		Cell wall degrading enzyme activity			
						2 DAI	4 DAI	6 DAI	Lipase	Protease	Cellulase
1	M- 1	2.0	4.0	+	+	0.6	1.3	1.8	+ve	-	-
2	M- 2	5.0	15.0	+++	+++	5.2	7.6	12.2	+ve	+ve	+ve
3	M- 3	6.0	13.0	+++	++	2.7	5.9	9.5	+ve	+ve	-
4	M- 4	-	-	+	-	1.2	2.5	3.4	-	-	-
5	W- 1	3.0	14.0	++	+++	3.3	6.6	10.5	+ve	+ve	+ve
6	P-1	2.0	-	+	++	1.7	2.3	4.1	+ve	-	-
7	B- 1	3.0	16.0	+++	+++	3.1	5.4	6.7	+ve	+ve	+ve
8	Ba- 1	-	13.5	++	+	2.1	3.9	5.1	+ve	+ve	+ve
9	Ba- 2	2.0	-	+	+	1.9	3.4	4.2	+ve	+ve	-
10	PP- 1	2.0	-	-	-	0.8	1.9	2.2	+ve	-	-
11	C-1	2.0	-	++	++	2.3	3.4	4.7	-	-	-
12	R-1	3.0	13.0	+	+	2.7	3.9	4.6	+ve	+ve	-
13	R- 2	2.0	13.0	++	++	2.9	4.3	5.2	+ve	+ve	+ve
14	R- 3	2.0	12.0	+	+	1.1	2.6	3.7	+ve	+ve	+ve
15	R- 4	3.0	14.0	+	++	0.5	1.8	2.1	+ve	+ve	-
16	CS-1	4.0	15.0	+++	+++	4.3	6.4	8.0	+ve	+ve	+ve
17	CS- 2	2.0	-	++	++	2.4	3.7	4.3	-	-	-
18	Std W1	3.0	90	_	+	34	41	55	+ve	-	+ve

Note: +++ strong, ++ moderate, + mild and – absent

K solubilization Mechanism

Acid producing ability of selected bacterial isolates was studied by methyl red test [12]. Production of capsular polysaccharide and acidic exopolysaccharides were determined by its binding affinity to aniline blue or calcofluor dye using spot test on modified M9 minimal medium plates and incubated for 3 days at 28+2°C. Binding affinity with aniline blue was detected by appearance of blue colored colonies showing production of capsular slime and production of acidic exopolysaccharides by observation of fluorescence under UV light [13].

PGPR traits of KMB isolates

Phosphate and Zinc solubilization

All the isolates were spot inoculated on Sperber and zinc solubilizing agar plate and were incubated at 30+2°C for 3-5 days and examined for the colonies showing clear zones [14].

Indole acetic acid (IAA)

IAA production was assayed using the Salkowski reagent (1 ml of 0.5 M FeCl3 to 50 ml of 35% perchloric acid). Standard curve was prepared and used to calculate quantity of IAA (μ g ml-1) [15].

ACC-deaminase activity

Cultures were spotted on DF salt minimal medium in agar plate [16] supplemented with 3 mM ACC (1-Aminocyclopropane-1-Carboxylate) substrate and incubated for 3-4 days at 30+2°C. Growth of isolates on ACC supplemented plates compared with plates containing (NH4)2SO4 @ 2.0 g I-1 as positive and without ACC as negative control plates [17].

Siderophore

Overnight grown cultures were spotted on individual Chrome Azurol S (CAS) agar plates and assessed by a change in the color of the medium surrounding the colony from blue to orange after incubation at 28 °C for 5 days [18].

Cell wall degrading enzymes

Various enzymes viz., Lipase on Tributyrin agar [19], Protease on skimmed milk agar [20] and Cellulase on cellulose agar [21] activity detected by spot inoculation and incubation for 3 days at 30+2°C.

Biocontrol potential

KMB isolates were tested in vitro for their biocontrol potential against five fungal plant pathogens viz. Fusarium solani, Pythium aphanidermatum, Aspergillus niger, *Fusarium oxysporum, Macrophomina phaseolina* and *Alternaria alternata* by dual inoculation technique [22].

Physiological characterization

The pH (5.0, 7.0 and 9.0), salt (2.5, 5.0 and 7.5 percent of NaCl) and temperature (37°C, 40°C and 50°C) tolerance of all the isolates was tested in GYC broth keeping un-inoculated control. These tubes in triplicate were inoculated with 50 μ l previously grown active culture of all isolates and were incubated at 30±2°C for 2-3 days. The presence or absence of growth was recorded by comparing it with un-inoculated control.

Biochemical characterization

Native KMB isolates were characterized by tests like utilization of sugars and substrates and production of enzymes. Individual bacterial suspension (50 μ l of 107 CFU ml-1) was inoculated in kits available from HiMedia (HiPure Bacterial Identification Kit) and allowed to incubate for 24 h at 30±2°C followed by observations applying appropriate reagents.

Molecular characterization

To confirm results of morphological and biochemical studies, sequencing of the 16S rRNA gene was performed [38] using the set of primers (27 F-5'AGAGTTTGATCCTGGCTCAG3' and 1492 R-5'GGTTACCTTGTTACGACTT3'). Partial 16S rRNA gene sequencing was carried out for promising isolates using ABI Applied Biosystems 3500 genetic analyzer and assembled using MEGA 4 software, compared with other strains using NCBI BLAST analysis for identification purpose and comparison of homologies of isolated strains with previously characterized. A neighbor-joining phylogenetic tree was constructed using Phylogeny.fr software.

Results

Isolation of KMB

Total 116 bacterial isolates were obtained on GYC agar from 17 different rhizospheric soils of Anand, Middle Gujarat. Out of these, 42 isolates were able to solubilize insoluble K on solid media, but only 17 were able to give constant and promising results for K solubilization on repeated sub-culturing on Aleksandrov agar. The solubilisation capacity was in the range of 1.4 to 8.0 Solubilization Index (SI) and the highest by maize isolate M-2. The isolates were further studied up to 10 days for K release from Aleksandrov liquid media supplemented with mica wherein isolate M-2 gave the highest K release 21.41 μ g ml-1 at 4 DAI which declined to 20.16 μ g ml-1, 18.57 μ g ml-1 and 17.23 μ g ml-1 at 6, 8 and 10 DAI, respectively and considered as the best K solubilizer, followed by W-1 which showed 20.30 μ g ml-1 at 6 DAI, CS-1, B-1 and M-3 exhibited 20.17, 19.39 and 18.31 μ g ml-1 respectively. Five ilsolates M-2, M-3, W-1, B-1 and CS-1 were found to be the best K mobilizer [Table-1], [Fig-1].



Fig-1 Zone of potash solubilization by KMB on Aleksandrov agar supplemented with mica

Cultural and Morphological characterization of KMB isolates

All the 17 isolates were having different colony characteristics on GYCa plates

after 24 h. For morphological characterization, isolates were observed under microscope following Gram reaction and wet mount. They were gram negative, short rods either single, pair or short chains, non-spore former and motile in nature.

K solubilization Mechanism

Among 17 isolates, 9 isolates gave dark red color in MR test showing profuse acid whereas, 8 gave red-orange color indicating less acid production. Nine bacterial isolates were found to produce capsular polysaccharide while five isolates shown proficient acidic exopolysaccharide production. Five isolates also showed high CPS production whereas three isolates showed high fluorescence observed under UV-light.

PGPR traits of KMB isolates

Phosphorus and Zinc solubilization

Fifteen isolates showed halo zone indicating solubilization of tri-calcium phosphorus (TCP) in solid medium whereas eleven isolates showed clear zone on zinc oxide agar [Table-2].

IAA production

All the isolates shown pink color development confirming IAA production [Table-2]. Isolate M-2 showed high amount of IAA production 5.2, 7.6 and 12.2 µg ml-1 at 2, 4 and 6 DAI, respectively followed by isolate W-1.

ACC-deaminase activity

Sixteen bacterial isolates were found to grow luxuriously on MS media containing (NH4)2SO4 whereas, grew poorly on nitrogen free media. Moreover, five isolates showed luxurious growth on plates having ACC as sole source of nitrogen showing their ability to produce enzyme ACC deaminase [Table-2].

Siderophore

Fifteen isolates produced yellow- orange colour zone on CAS agar plate hence considered as siderophore producers wherein ten isolates produced big zone [Table-2].

Cell wall degrading enzymes

Selected seventeen native KMB isolates were grown on their specific medium wherein, 14 were capable of producing lipase while 11 for protease and 7 for cellulase responsible for cell wall degradation of plant pathogens [Table-2]. Out of all seventeen native KMB isolates, five isolates namely M-1, M-2, W-1, B-1 and CS-1 were selected on the basis of above PGP traits. These five isolates were checked for biocontrol potential, biochemical qualitative tests and Molecular characterization.

Biocontrol potential

Antifungal activity of selected isolates on plant pathogenic fungi is presented in [Table-3]. All the five isolates showed growth inhibition percentage against *F. oxysporum i.e.*, 26 -40%. KMB-1 showed the highest growth inhibition percentage against *F. solani* (19.12%) and *P. aphanidermatum* (20.00%) whereas KMB-3 against *A. niger* (36.25%) and *Al. alternata* (12.24%). KMB 2 showed the highest growth inhibition percentage against *M. phaseolina* (15.79%).

Test pathogenic fungi	% Growth inhibition						
	KMB 1	KMB 2	KMB 3	KMB 4	KMB 5		
Fusarium solani	19.12	-	10.29	13.24	16.18		
Pythium aphanidermatum	20.00	-	-	12.86	11.43		
Aspergillus niger	-	32.50	36.25	-	-		
Thanatephorus cucumeris	-	-	-	-	-		
Fusarium oxysporum	38.75	26.25	35.00	40.00	36.25		
Macrophomin aphaseolina	10.53	15.79	6.58	-	-		
Alternaria alternata	10.20	-	12.24	-	6.12		

Physiological characterization

Chosen bacterial isolates had wide pH tolerance range 5.0, 7.0 and 9.0. Maximum

			1 auie-4 p	n i, Sait e	inu rennp		JIEI AIILE	01 1501416	5	
	Isolates	Optical density (OD @ 600nm) at 24 h								
			pH range		NaCl c	oncentrati	ion (%)	Te	ıre	
		5* 7* 9*			2.5 %*	5.0 %	7.5 %	37ºC	40°C	50°C
	KMB 1	0.565	0.734	0.679	0.617	0.121	0.019	+++	++	-
	KMB 2	0.521	0.638	0.599	0.675	0.261	0.016	+++	++	-
	KMB 3	0.518	0.531	0.454	0.689	0.149	0.044	+++	++	-
	KMB 4	0.532	0.723	0.564	0.658	0.127	0.024	+++	++	-
	KMB 5	0.560	0.588	0.494	0.681	0.091	0.017	+++	++	-
ľ	Noto * 9	Samplas	dilutod 1	1 with D	11/ +++ //	wurious d	nrowth · ⊥.	+ acod a	rowth	No aro

Table-4 pH, Salt and Temperature tolerance of isolates

Note: * Samples diluted 1:1 with D/W, +++ luxurious growth; ++ good growth; - No growth

growth was obtained at pH 7.0 of all the isolates however; KMB-1 showed growth at all pH tested. Maximum growth of selected isolates was found in 2.5% NaCl concentration, while increasing the concentration of NaCl the growth decreased. Isolate KMB-3 showed higher growth (OD 0.689) at 2.5% NaCl concentration as compared to other isolates. KMB-3 was found tolerant at 7.5% NaCl concentration. This might be due to the proline which acts as predominant compatible solute independent of constant higher osmolality. Maximum growth of selected isolates was found at 37°C, while increasing the temperature the growth decreased. There was moderate growth observed for all the isolates at 40°C. At 50°C none of the isolates grew [Table-4].

Biochemical characterization

All five isolates were positive for Indole and MR tests, Utilization of citrate, malonate, lysine and ornithine; nitrate reduction, catalase, urease test and utilized various carbon sources *viz*. glucose, lactose, rhamnose, xylose, melibiose and cellobiose while isolate KMB-2, KMB-3 and KMB-4 were found positive for arabinose.

	 Acinetobacter calcoaceticus strain ATCC 23055 16S ribosomal RNA, partial
•	Acinetobacter pittii strain ATCC 19004 16S ribosomal RNA, partial sequence
	Acinetobacter pittii strain LMG 1035 16S ribosomal RNA gene, partial seque
0.001	Acinetobacter pittii strain KMB-1 16S ribosomal RNA gene, partial sequence
	 Acinetobacter pittii strain CIP 70.29 16S ribosomal RNA gene,
	 Acinetobacter oleivorans strain DR1 16S ribosomal RNA gene, partial
•	Acinetobacter oleivorans strain KMB-2 16S ribosomal RNA gene, part
	Acinetobacter oleivorans strain DR1 16S ribosomal RNA, partial sequ
0.001	Acinetobacter pittii strain ATCC 19004 16S ribosomal RNA, partial se
12:	Acinetobacter calcoaceticus strain NCCB 22016 16S ribosomal RNA g
	Acinetobacter junii strain ATCC 17908 16S ribosomal RNA, partial seque
	Acinetobacter baumannii strain DSM 30007 16S ribosomal RNA gene, part
	Acinetobacter baumannii strain ATCC 19606 16S ribosomal RNA, partial
0.003	Acinetobacter baumannii strain KMB-3 16S ribosomal RNA gene,
	Acinetobacter baumannii strain JCM 6841 16S ribosomal RNA gene, part
	 Acinetobacter calcoaceticus strain ATCC 23055 16S ribosomal RNA, partial seq
•	 Acinetobacter calcoaceticus strain KMB-4 16S ribosomal RNA gene, partial seq
	Acinetobacter calcoaceticus strain NCCB 22016 16S ribosomal RNA gene, part
0.001	Acinetobacter dijkshoorniae strain JVAP01 16S ribosomal RNA, par.
	Acinetobacter lactucae strain NRRL B-41902 16S ribosomal RNA,
	 Acinetobacter indicus strain A648 16S ribosomal RNA gene, partial sequence
•	Acinetobacter junii strain ATCC 17908 16S ribosomal RNA, partial sequence
	Acinetobacter junii strain DSM 6964 16S ribosomal RNA gene, partial sequence
0.001	Acinetobacter junii strain KMB-5 16S ribosomal RNA gene, part
	A cinetobacter junii strain Mannhaim 2723/50 165 ribasomal PNA gana partial

Fig-2 Phylogenetic trees derived from 16S rRNA gene sequences showing the relationships of native KMB isolates A) KMB-1, B) KMB-2, C) KMB-3, D) KMB-4 and E) KMB-5

Molecular characterization

16S rRNA partial gene sequence of ~ 1500 bp was carried out and the output data were stored in FASTA format. Isolate KMB 1 was identified as *A. pittii* (Accn. No: MF614918) with 100 % similarity and 100 % query coverage to *A. pittii* strain ATCC 19004. Similarly, isolate KMB 2 was identified as *A. oleivorans* (Accn. No: MF614919) with 99 % similarity and 100 % query coverage to *A. oleivorans* strain

DR1. Isolate KMB 3 was identified as *A. baumannii* (Accn. No: MF614920) with 99 % similarity and 100 % query coverage to *A. baumannii* strain DSM 30007. Isolate KMB 4 was identified as *A. calcoaceticus* (Accn. No: MF614921) with 100 % similarity and 100 % query coverage to *A. calcoaceticus* strain NCCB 22016. Isolate KMB 5 was identified as *A. junii* (Accn. No: MF614922) with 99 % similarity and 99 % query coverage to *A. junii* strain ATCC 17908. Additionally, the phylogenetic position of the isolates was also worked out within the available database of NCBI (presented as phylogenetic tree in [Fig-2].

Discussion

Verma et al. [35] isolated KSB using mica from the soil samples U.P and M.P on Aleksandrov agar. Out of fourteen, seven isolates showed SI ranging from 3.13 to 5.00. Similarly, Dhaked et al. [23] isolated four KSB from rice, maize, cotton and sorghum rhizosphere soil collected from Student and College farm Rajendranagar, PJTSAU, Hyderabad. The solubilization zone for potassium ranged from 85 to 160 mm. The isolate KSB-2 showed maximum solubilization. Archana et al. [24] reported K release from muscovite mica by bacterial isolates ranging from 2.41 to 44.49 µg ml-1 wherein, at 20 DAI KSB11 released maximum amount of K followed by KSB42 gave 37.07 µg ml-1. Similarly, Parmar and Sindhu [25] studied the solubilization of potassium by wheat rhizospheric bacteria on modified Aleksandrov medium supplemented with mica powder and found K release of 15 to 48 mg L-1. In similar study, Parmar [26] showed that some rhizobacterial isolates caused solubilization of potassium in mica by acid production only, whereas other isolates caused K solubilization by production of CPS and EPS. Wang et al. [27] reported Si and K concentrations were increased by 1.3 to 4.0 fold and 1.1 to 1.7 fold respectively, in the bacterium-inoculated cultures compared with the controls.

Kumar *et al.* [28] reported that Enterobacter (LCR1, LCR2 and LCR3) and Exiguobacterium sp. (LCR4, LCR5 and LCR6) isolated from paddy fields of eastern Uttar Pradesh showed phosphorus solubilization. Pseudomonas sp ZSB-S-2 showed more solubilizing ability in the zinc oxide, with 3.30 cm clearing zone and 20.43 cm2 area. Vasanthi *et al.* [29] reported that silicate solubilizing Bacillus sp. isolated from sugarcane field shown Zinc (Zn) solubilizing potential.

Archana *et al.* [30] reported IAA production by KSB ranging from 1.10 to 16.50 μ g/25 ml broth. Verma *et al.* [31] reported out of 45 isolates, 32 strains exhibited IAA production. Strain IARI-HHS1-3 showed the highest IAA production (70.8±1.5 μ g mg-1 day-1) followed by IARI-HHS1-8 (69.1±0.5 μ g mg-1 day-1). ACC deaminase is useful to plants to fight against several biotic and abiotic stresses such as attack by phytopathogens, salinity, drought and higher concentration of heavy metals. Verma *et al.* [31] reported out of 45 representatives, 26 strains exhibited ACC deaminase activity. Siderophore bind most of the available iron (Fe+3) in rhizosphere and thereby preventing proliferation of fungal pathogens in immediate vicinity due to lack of iron [32]. Verma *et al.* [33] reported out of 45 representatives, 35 strains exhibited siderophore production.

A positive role is played by rhizosphere antagonistic microorganisms, which protect the plants from pathogenic microbes and thus improve their healthiness [34]. Verma *et al.* reported fourteen strains showing antagonistic activity against *F. graminearum, R. solani and M. phaseolina.* Parmar and Sindhu reported that bacterial strain WPS73 caused maximum solubilization (49.0 mg L-1) at 25°C whereas bacterial strain NNY43 caused maximum solubilization at 30°C. K solubilization was found more when bacterial strains were grown in broth with pH 7.0. Bacterial strain UPS1C1 showed maximum solubilization (5.0 cm) at 25°C and decreased with increase in temperature.

Verma *et al.* [34] that bacterial strain UPS2C1 showed maximum solubilization (4.13 cm) at $28\pm2^{\circ}$ C, whereas other bacterial strains showed significant solubilization in the temperature range of 25° C to 35° C. K solubilization decreased at higher temperature of incubation i.e., 45° C with all the bacterial strains. Ahmad and Zargar [35] characterized KSB isolates from rhizospheric soils of apple in Kashmir shown positive results for catalase, urease, casein hydrolysis, acid production tests. Twenty-three isolates were gram positive rods belonging to Bacillus genera and four were gram negative rods belonging to Pseudomonas. Verma *et al.* studied different biochemical analysis wherein, seven bacterial strains were positive for starch hydrolysis, Simmon's citrate test, Triple sugar iron agar test and catalase. Rokhbakhsh-Zamin *et al.* [36] isolated 31 bacteria from the rhizosphere of Pennisetum glaucum and identified on the basis of 16S rRNA gene sequences. All the strains were designated as Acinetobacter sp. and submitted to Gene-Bank.

Conclusion

Overall results indicated that five efficient Acinetobacter KMB strains from various crops' rhizosphere exhibited PGPR activity like P and Zn solubilization, IAA, Siderophore, ACC deaminase production. Additionally, they were found to tolerate wide pH, temperature and salt ranges. Moreover, these isolates showed inhibitory effect on soil borne phytopathogenic fungi. The results endow with a basis for understanding the beneficial effects of KMB for deploying the strains in industrial production of biofertilizer cum bio-control agents.

Application of research: Results of the present study strongly reflected that the Potash mobilizing bacteria play important role as biofertilizer cum bio-control agents.

Research Category: Agricultural Microbiology

Abbreviations: KMB: Potash mobilizing bacterial, cm: centimetre, mm: millimeter, °C: Degree centigrade, %: percent, mg: Milligram, µg: Microgram, ml: Milliliter, L: Liter, CFU: Colony forming unit

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Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Anand Agricultural University

Cultivar / Variety / Breed name: Nil

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