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Microbiology

Characterization of *Aneurinibacillus aneurinilyticus* Strain CKMV1 as a Plant Growth Promoting Rhizobacteria

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Abstract

A bacterial collection of approximately thirty native strains were isolated from rhizosphere soil associated with the seedlings of *Valeriana jatamansi* grown in moist temperate forest located in and around Chamba district of Himachal Pradesh. The strain CKMV1 showed PGP traits like, phosphate solubilization (257.0 mg l^{-1}), indole acetic acid (7.0 µ gml^{1}) and siderophore production (53.43%) at 35±2ºC. Besides, the strain also exhibited growth on nitrogen free medium, hydrogen cyanide production and antifungal activity against different fungal pathogens. Significant growth inhibition of fungal pathogens occurred in the order as *S. rolfsii* > *R. solani* > *D. necatrix* > *Alternaria spp*.> *F. oxysporum*. The results suggested that the rhizosphere of native *V. jatamansi* growing in their natural habitat of Chamba district of H.P. is a rich source of *Bacillus* sp., which have potential to be used in the future as PGP inoculants to improve crop productivity. Morphological, biochemical and molecular based characterization of a selected isolate CKMV1, based on sequence homology of a partial 1375-bp fragment of 16S rDNA amplicon with the ribosomal database sequence validated the strain as *Aneurinibacillus aneurinilyticus*. Therefore, these results suggested that out of 30 isolates, *Aneurinibacillus aneurinilyticus* strain CKMV1 possessed multiple PGP traits thus can be further explored for its efficacy as effective PGPR.

Highlights

- 30 phosphate (P)- solubilizing bacterial isolates were isolated from *Valariana jatamansi* and four were selected and examined for their multiple plant growth promoting traits.
- Strain CKMV1 showed maximum plant growth promoting traits in lab conditions and therefore it was characterized based on phenotypic and genotypic parameters.
- Strain CKMV1 was identified as *Aneurinibacillus aneurinilyticus*.
- It was for the first time that a P-solubilizing *Aneurinibacillus aneurinilyticus* strain having multiple PGP traits was isolated from the rhizosphere of *V. jatamansi*.

Keywords: *Valeriana jatamansi*, *Aneurinibacillus aneurinilyticus*, 16SrRNA, PGPR

Introduction

Himachal Pradesh has been a natural habitat for large variety of aromatic and medicinal plants, some of which are on the verge of extinction due to unsystematic and unscientific exploitation. Chauhan and Negi (1988) mentioned Indian Valerian to be one of the main medicinal plant collected and exploited for its immense medicinal importance. Farmers have started cultivation of *V. jatamansi* on large scale in agricultural fields which may result in the loss of naturally occurring plant growth promoting rhizobacteria as well as might also be exposed to various root rot causing plant fungal pathogens.

The use of beneficial microorganisms could be an environmental sound option in order to increase crop yields and reduction in disease incidence. Plant growth promoting rhizobacteria (PGPR) are known to influence plant growth through direct or indirect mechanisms (Glick, 1995). The direct promotion by PGPR entails either providing the plant with growth promoting substances that are synthesized by the bacteria or facilitating the uptake of certain plant nutrients such as phosphorus via phosphate solubilization, synthesizing stimulatory phytohormones like indole acetic acid (IAA) (Vessey, 2003). The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effect of one or more phytopathogenic microorganisms by excluding them from the roots by competition or by inducing systemic resistance (Compant *et al.,* 2005)

Strains of the genus *Bacllus* spp. are well know rhizosphere residents of many crops and are easily distinguishable from other bacteria owing to their ability to produce endospores which confers their high stability as biofertilizers and biofungicides (Schisler *et al.,* 2004). They usually show plant growth promoting (PGP) traits like phosphate solubilization that play a significant role in making phosphorus available to plants by bringing about favorable changes in soil micro environment leading to solubilization of inorganic phosphate sources. Microbial-mediated solubilization of insoluble phosphates through release of organic acid is often combined with production of other metabolites (siderophore, phytohormones and lytic enzymes), which take part in biological control against soil–borne pathogens (Vassilev *et al.,* 2006).

Exotic strains from commercial inoculants may not survive in local soils due to different edaphic or climatic conditions, or be out-competed by better adapted native bacteria during plant colonization resulting in the poor performance of PGPR (Chatli *et al.,* 2008). Therefore, isolation and screening of native stains is justified. *Bacillus circulans* have been previously isolated from apple rhizosphere (Mehta *et al.,* 2010) but nothing is much known about *Aneurinibacillus strains* associated with *V. jatamansi* growing in its natural habitat. Thus, there is a great possibility of finding an efficient genotype of PGPR which can perform better than the other isolates. The present work describes, a native soil isolate CKMV1 presumptively identified as *A. aneurinilyticus* which has been further characterized based on 16S rDNA homology. It was further investigated for its possible plant growth promoting traits and Phylogenetic affiliation.

Materials and Methods

Isolation and screening of plant growth promoting rhizoacteria

Twenty *V. jatamansi* plants along with soil and roots were randomly selected from moist temperate forest located around Chamba district of Himachal Pradesh for the isolation of rhizobacteria and endorhizobateria by replica plating technique. The technique involved the serial dilution and spreading on nutrient agar and incubated at 35ºC for 48h. A total of 200 different colonies were isolated on nutrient agar (Master plate) and were replica plated onto the selective media: nitrogen free medium (Jensen, 1942) for N_2-fixing ability, Pikovskaya medium (Pikovaskaya, 1948) for phosphate-solubilizing ability and chrome azurol S medium (Schwyn and Nellands, 1987) for siderophore production. Approximately 30 isolates were randomly selected for screening of phosphate solubilization (Sundra Rao and Sinha, 1963), IAA production (Gordon and Paleg, 1957), nitrogen fixing ability (growth on nitrogen free medium), HCN production (Baker and Schippers, 1987), siderophore production (Schwyn and Nellands, 1987) and antifungal activity (Vincent, 1947) against five fungi (*S. rolfsii, R. solani, D. necatrix, Alternaria spp., F. oxysporum*). All the experiments were conducted in triplicates along with equal number of appropriate controls. Data were statistically analyzed by analysis of variance technique (one way classification) using SPSS 16.0 software. Screened bacterial strains with multiple PGP traits were purified with repeated culturing and maintained in 20% glycerol at -80ºC.

Assays for plant Growth promoting traits

Bacterial isolates were screened for phosphate solubilization, IAA production, siderophore production, HCN production and antifungal activity by using the following assays.

Solubilization of organic phosphate

Bacterial isolates were first screened on Pikovskaya's agar plates for phosphate solubilization index as described by Gaur (1990). Quantitative analysis of solubilization of tricalcium phosphate in liquid medium was made as described by Bray and Kartz (1945). The absorbance of the developing blue color was read at 600 nm. The amount of soluble phosphorus was detected from standard curve of Potassium dihydrogen orthophosphate (KH_2PO_4).

Indole acetic acid (IAA) production

IAA production was detected by the modified method as descrbed by Bric *et al.,* (1991). Bacterial cultures were grown for 72h on their respective media at 35ºC. Fully grown cultures were centrifuged at 3000 rpm for 30 mins. The supernatant (2ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski agent, 35% perchloric acid, 1 ml of 0.5M Ferric chloride solution ($FeCl_3$ solution). Development of pink colour indicates IAA production. Optical density was observed at 530 nm and compared with standard curve of IAA.

Siderophore production

Bacterial isolates were assayed qualitatively for siderophore production on the chrome azurol S agar medium and quantitatively in liquid medium as described by Schwyn and Neilands, (1987). Development of yellow-orange halo around the growth was considered as positive for siderophore production. In case of quantitative estimation absorbance was measured at 630 nm.

HCN production

Bacterial isolates were screened for the production of hydrogen cyanide by adapting the method of (Baker and Schippers, 1987). Development of orange to brown color indicated HCN production.

Antifungal activity

Antifungal activity was observed by the formation of inhibition zone of mycelial growth, based on agar diffusion of extracellular bacterial metabolite. A small plug cut from a fresh agar culture of target fungus was seeded at the centre of Malt yeast extract (MEA) medium in a petri plate. Simultaneously, a loopful of 48h old bacterial isolate was streaked a little below the centre of prepared petriplates (MEA). For comparison, all fungal strains were also grown on MEA media to be used as control and growth inhibition was calculated as Vincent (1947).

Phenotypic characterization

The shape, size and arrangement of the cells were studied in native preparations and Hucker's Gram- and Schaeffer and Fulton's spore-stained smears (Barrow and Feltham, 2003). Physiological and biochemical tests performed with selected strain was as follows: catalase, indole test, oxidase activity, oxidation-fermentation test of D-glucose, methyl-red and Voges-Proskauer tests, nitrate reduction to nitrite or ammonia, digestion of casein and starch, hydrolysis of gelatine, Tween 80 (Barrow and Feltham, 2003). The influence of environmental factors (pH and salt) on growth was studied by varying the pH of the medium in between 7.0 to 9.0 and by varying the salt concentrations in the medium between 2.5-10% (w/v) NaCl for 72h at 35ºC. The carbon source utilization abilitiy of the strains was investigated Hi carbohydrate™ kit (Himedia, India) and Hi *Bacillus*™ Identification kit (Himedia, India).

Molecular taxonomic characterization

Molecular identification of strain CKMV1 was conducted by 16S rDNA sequence comparison. Genomic DNA was extracted and PCR- mediated amplification with Forward (5'GCAAGTCGAGCGGACAGATGGGAGC3') and Reverse (5'AACTCTCGTGGTGTGACGGGCGGTG 3') universal primer pair was carried out by the methods of Rainey *et al.,* 1996. PCR products were checked on 1% (w/v) agarose gel. The purified fragment was sequenced from commercial sequencing facility (Xcleris lab,Ahmedabad). The comparison of sequence was performed via the internet at National Center for Biotechnology Information (NCBI) database by employing BLAST algorithm. The Phylogenetic tree was constructed with the help of ClustalW from the website http://www2.ebi.ac.uk/clustalw/ (Higgins *et al.,* 1994) and phylip v3.6. Tree was viewed with the help of TreeView from the website http://taxonomy.zoology.gla.ac.uk/rod/treeview.html (Page, 1996).

Results

Isolation and *in vitro* screening of plant growth promoting rhizobacteria

In the present study, replica plating technique was used to initially screen the isolated bacteria for multiple plant growth promoting traits. Thirty bacterial isolates in total; twenty three from rhizosphere; and seven from endorhizosphere of *Valeriana jatamansi* showed variability in population of phosphate solubilizers and nitrogen fixers as obtained from different locations.

Assays for growth-promoting traits

All the thirty bacterial isolates showed variation in their ability for different plant growth promoting traits and thus, were ranked accordingly. Most of the isolates exhibited combination of two or more than two PGP traits. However, bacterial isolates CKMV1, CKMV2, CKMV3 and CKMV4 exhibited higher levels of multifarious plant growth promoting traits [evident from their high score, >60% data not shown] in comparison to other isolates and hence, were selected for quantitative assay of PGP traits. Of these four isolates, CKMV2* and CKMV3* were endophytic whereas, CKMV1 and CKMV4 were rhizospheric in origin.

The phosphate solubilizing activity of the selected bacterial isolates was compared on the basis of their phosphate solubilizing index in PVK agar medium and Psolubilization in PVK broth medium. The results (Table 1) revealed that in PVK agar medium, CKMV1 showed maximum phosphate solubilizing index (3.1) and minimum was recorded for CKMV2 (1.9). The PSI of isolates CKMV3 (2.5) and CKMV4 (2.1) was found statistically at par. In liquid PVK medium, significantly higher P- solubilization was recorded for isolate CKMV1 (257.0 mg /l) corresponding to the maximum viable count (29.08× 10^6 cfu/ml) and final pH decreased to 4.24 from initial pH 7.0 as compare to other other isolates. The isolate CKMV3 solubilized minimum TCP with the release of 89.0 mg /l phosphorus with the corresponding viable count 16.10× 10^6 cfu/ml) and final pH decreased to 5.64. TCP solubilization of all the isolates was significantly lower than TCP solubilization in CKMV1 (257.0 mg/l).

Quantitative estimation of siderophore using Chome-azurol-S (CAS) liquid assay revealed that CKMV1'produced maximum percent siderophore unit (53.43 %) which was significantly higher than the other isolates, whereas CKMV2, CKMV3 and CKMV4 were found to be statistically at par with each other.

Table 1 revealed that selected bacterial isolates showed large variation in their production of indole-3-acetic acid (IAA). The isolates CKMV1 showed statistically higher IAA production (7.0µg/ml) as compare to other isolates.

All the bacterial isolates exhibited variation in their inhibition towards different fungal pathogens used in the study. CKMV1 showed inhibition pattern in the order: *S. rolfsii* (93.58%) >*R. solani* (91.58%) > *D. necatrix* (75.73%) > *Alternaria* spp. (71.08%) > *F. oxysporum* (67.44%). In case of other isolates like CKMV3 maximum growth of inhibition (83.72%) was observed against *F. Oxysporum*, whereas CKMV2 and CKMV4 showed maximum growth inhibition (73.26%) against *S. rolfsii* which was found to be statistically at par to each other.

Table 1: Screening of selected P-solubilizing bacterial isolates of *Valeriana jatamansi* for multifarious plant growth promoting traits

Isolates	PSI %*	P-solubilization (mg/l)**	% siderophore unit**	IAA(µg/ml) ***	%growth inhibition against *F. oxysporum* ****	%growth inhibition against *R. solani* ****	%growth inhibition against *S. rolfsii* ****	%growth inhibition against *Alternaria spp.***	%growth inhibition against *D. necatrix* ****
CKMV1	3.1	257.00	53.43	6.5	67.44	91.58	93.58	71.08	75.73
CKMV2	1.9	120.00	40.21	3.21	76.16	74.42	73.26	70.00	60.00
CKMV3	2.5	89.00	37.08	2.1	83.72	77.91	70.93	68.04	45.00
CKMV4	2.1	119.00	38.75	3.98	72.19	69.77	73.26	72.06	53.00
LSD	0.38	3.82	1.70	0.87	3.15	2.71	2.31	1.28	3.15

*: $\frac{Clear\ zone\ diameter(colony+holozone)}{Growth\ diameter}$;

**T-C; Where, T= Inoculated PVK with TCP, C (uninoculated PVK with TCP)

*** % Siderophore unit= $\frac{Ar-As}{As}$C 100, ***A_r = Absorbance at 630nm of reference; A_s = Absorbance at 630 nm of test sample;

****I = $\frac{C-T}{C}$× 100

Where, I= Per cent growth inhibition, C= Growth of fungus in control, T= Growth of fungus in treatment

Phenotypic and metabolic characterization of CKMV1

Selected bacterial isolate CKMV1 was identified on the basis of morphological, physiological and biochemical characteristics (Table 2). The isolated colonies on nutrient agar medium after 48h of incubation were creamy grey, irregular, slightly crenate edges and glossy. Colonies become whitish and opaque as their component cells sporulate. The morphological characteristics of the isolate are as follows: the cells were Gram positive, Vegetative cells are 0.7 to 0.9µm by 3.0 to 5.0 µm, aerobic, rods, occurring in single, ellipsoidal spore lies subterminally. The biochemical characteristics of the isolate showed that the strain CKMV1 was positive for catalase, nitrate reduction, arginine hydrolysis, tyrosine utilization, and utilization of following carbon sources, D-trehalose, sucrose, Inulin, glycerol, D-ribose, salicin, inositol, sorbitol, fumarate, succinate, serine, lactose and D-alanine and adonitol and negative for all other biochemical tests mentioned in Table. 2

Molecular taxonomic characterization

Universal 16S rDNA primers were successfully used for the amplification of 16S rDNA from strain CKMV1. PCR product of expected size ~ 1375 bp was then cloned in pGEM-T easy vector system (Promega, USA) and transformed to competent *E. coli* (DH5α) cells. Positive

Table 2: Morphological, physiological and biochemical characteristics of *Aneurinibacillus aneurinilyticus* strain CKMV1

Morphological characteristics					
Colony morphology	Creamy greyish, irregular, slightly crenate edges and glossy, translucent				
Grams reaction/ cell shape	+ / rods /single				
Spore shape/ Position	Ellipsoidal/ sub terminal				
Physiological characteristics					
Growth in NaCl at concentration (2.5 to 4.5%)	+				
Growth at different temperature (25 to 45ºC)	+(optimum-37°C)				
Growth at different pH (5.5 to 9.0)	+(optimum-7.0)				
Aerobic	+				
Biochemical tests/ carbohydrates utilization					
Catalase	+	Fructose	+	Erythritol	-
Malonate	-	Dextrose	-	á-Methyl-D-glucoside	-
Voges Proskauer's	-	Galactose	-	Rhamnose	-
Citrate utilization	-	Raffinose	-	Cellobiose	-
ONPG	-	D-trehalose	+	D-melezitose	-
Nitrate Reduction	+	Melibose	-	á-Methyl-D-mannoside	-
Arginine dihydrolase	+	Sucrose	+	Xylitol	-
Hydrogen sulphide production	-	D-Mannose	-	D-Arabinose	-
Gelatin hydrolysis	-	Inulin	+	Sorbose	-
Starch hydrolysis	-	Sodium glucanate	-	Adonitol	-
Casein decomposition	-	Glycerol	+	Fumarate	+
Urea hydrolysis	-	Maltose	-	D-lactose	+
Tween 80	-	D-ribose	+	Histamine	-
Indole production	-	Salicin	+	D-sorbitol	-
Esculin hydrolysis	-	Dulcitol	-	Succinate	+
Tyrosine utilization	+	Inositol	+	Serine	+
Lactose	+	Sorbitol	+	Histamine	-
Xylose	-	L-arabitol	-	D-alanine	+
Adonitol	+	L-malate	+	L-serine	+

+, tested positive / utilized as substrate; -, tested negative / not utilized as substrate

clone containing insert was then identified using colony PCR. Plasmid was isolated from selected positive colony and was sequenced. 1215 bp sequence corresponding to 16S rDNA of CKMV1 was obtained and then analysed using BLASTn analysis (http://www.ncbi.nlm.nih.gov/blast). It was found to have 99 percent homology (Table 3) with *Aneurinibacillus aneurinilyticus* AB271755 (Japanese isolate) and X94194 (German isolate). The sequence was deposited in EMBL (Genbank) under accession number GQ980020 (*A. aneurinilyticus*). To trace out the evolutionary patterns a phylogenetic tree was constructed using Neighour-Joining (J) method of mathematical averages among 16S rDNA sequence of CKMV1 and corresponding sequence of 28 different *Bacillus* spp. Isolate CKMV1 was united with quite high statistical support by the bootstrap method estimates for 1,000 replications and values inferred greater than 50 per cent are only presented in Fig. 1. Phylogenetic tree (Fig. 1) also verified CKMV1 as *A. aneurinilyticus* as the isolate CKMV1 clustered closely with *A. aneurinilyticus* with high boot strap value (100%).

Table 3: Percent homology of 16S rDNA sequence of CKMV1 isolate with other nucleotide sequences present in the database using BLASTn analysis

IsolateName	Length(bp)	Closest match	Accession Number	Length(bp)	Percent Similarity
CKMV1	1215	*Bacillus vallimortis*	FJ009394B	1417	90.0
CKMV1	1215	*Bacillus subtilis*	AB383135	1540	90.0
CKMV1	1215	*Bacillus flexus*	EU884649	1482	90.0
CKMV1	1215	*Bacillus pumilus*	EU869282	1428	90.0
CKMV1	1215	*Bacillus megaterium*	EU879090	1515	89.0
CKMV1	1215	*Bacillus subtilis*	EU118756	1513	90.0
CKMV1	1215	*Bacillus alkalidiazotrophicus*	EU143680	1503	90.0
CKMV1	1215	*Bacillus firmus*	EU924075	1215	83.0
CKMV1	1215	*Bacillus pumilus*	EU869263	1453	90.0
CKMV1	1215	*Bacillus licheniformis*	EU869262	1454	89.0
CKMV1	1215	*Bacillus megaterium*	EU869261	1456	89.0
CKMV1	1215	*Bacillus clausii*	EU869250	1455	90.0
CKMV1	1215	*Bacillus circulans*	EU733231	1500	90.0
CKMV1	1215	*Bacillus circulans*	EU373401	1517	90.0
CKMV1	1215	*Bacillus amyloliquefaciens*	EU855195	1511	90.0
CKMV1	1215	*Bacillus amyloliquefaciens*	EU855192	1511	90.0
CKMV1	1215	*Bacillus aneurinilyticus*	X94194	1482	99.0
CKMV1	1215	*Aneurinibacillus aneurinilyticus*	AB271755	1464	99.0
CKMV1	1215	*Bacillus safensis*	JF411318	1410	90.0
CKMV1	1215	*Bacillus safensis*	JF798363	1442	90.0
CKMV1	1215	*Bacillus pumilus*	FN397514	1491	90.0
CKMV1	1215	*Bacillus pumilus*	HQ647270	1456	90.0
CKMV1	1215	*Bacillus methylotrophicus*	JQ023625B	1400	90.0
CKMV1	1215	*Bacillus methylotrophicus*	HQ325853	1451	90.0
CKMV1	1215	*Bacillus tequilensis*	JN205343	1416	90.0
CKMV1	1215	*Bacillus tequilensis*	HM770882	1462	90.0
CKMV1	1215	*Bacillus altitudinus*	JF343140	1447	90.0
CKMV1	1215	*Bacillus altitudinus*	HQ224625	1401	90.0

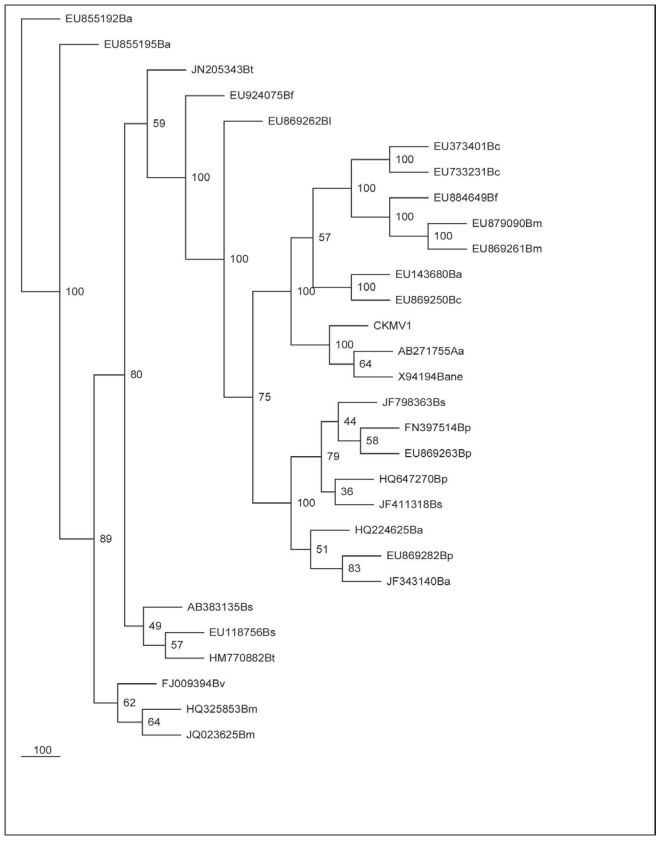

Fig. 1: Neighbour- joining tree based on 16S rDNA sequences showing the phylogenetic relationship of strain CKMV1. The numbers at the nodes indicate the levels of bootstrap support based on data for 1000 replicates; values inferred greater than 50 % are only presented. The scale bar indicates 100 substitutions per nucleotide position

Discussion

Many soil bacteria especially rhizospheric bacteria are known PGPR and *Bacillus* is an important member of such group. The present investigation evidenced a significantly high solubilization of TCP by *A. aneurinilyticus* strain CKMV1 which is considered as one of the most important traits associated with plant P-nutrition (Rodriguez *et al.*, 2006). Some plant-growth promoting bacteria solubilize phosphate from either organic or inorganic bound phosphates, thereby facilitating plant growth (Vassilev *et al.*, 2006). The phosphate solubilizing microorganisms (PSM) render insoluble phosphate into available forms by the process of acidification, chelation, and exchange reaction (Trivedi and Tongmin, 2008). CKMV1 also showed the production of secondary metabolites, viz., IAA, HCN, and siderophore, which have been assessed to elucidate the agronomic significance of the rhizobacterial isolate *A. aneurinilyticus* strain CKMV1. 115 isolates were isolated and characterized of having multiple PGP traits from the rhizosphere of Jaunpuri Gaint Raddish, representative PGP isolates were characterized using BIOLOG, ARDRA and were found they belongs to genus *Bacillus, Pseudomonas, Agarobacterium Leifsonia, Nesterenkonia* (Srivastava *et al.*, 2013). Our earlier studies demonstrated the PGP traits of *Bacillus circulans* strain (Mehta *et al.*, 2010). Earlier reports on PGPR with significantly higher levels of P-solubilization, siderophore (iron-chelating compound) production, HCN production and antagonism towards fungal pathogens, unequivocally underline the importance of these microbes in direct plant growth promotion (as bio-fertilizers and bio-stimulants) and indirect plant growth promotion (as bio-protectants) (Blumer and Hass, 2000; Selvakumar *et al.*, 2008).

A significant decline in the pH of medium to 4.24 from initial pH 7.0 was recorded during solubilization of TCP which suggested secretion of organic acids by the bacterial isolates. P-solubilization of inorganic phosphate has been attributed to the production and release of organic acids (Hwangbo *et al.*, 2003; Ivanova *et al.*, 2006). The strain CKMV1 by 16S rDNA phylogeny seemed to be a part of genus *Aneurinibacillus* and species *aneurinilyticus*, as it was closely related to *A. aneurinilyticus* AB271755 (Japanese isolate) and X94194 (German isolate) with high boot strap value (100%). The biochemical tests based on carbon source utilization described by Heyndrickx *et al.*, 1997 also strengthen the identity of CKMV1 as *A. aneurinilyticus.* It was also in confirmation with earlier results reported by Shida *et al.*, 1996 about *Aneurinibacillus* as a separate genus different from *Bacillus*. which is a well known PGPR (Nihorimbere *et al.*, 2010) *Aneurinibacillus aneurinilyticus* were earlier reported to have positive effects on plant growth promotion on different crop plants (Nihorimbere *et al.*, 2010).

The present study of isolation of *Aneurinibacillus spp* as phosphate solubilizer with multiple plant growth promoting traits from *Valeriana jatamansi* have not been earlier reported though, isolation of this particular strain from sludge of a pulp paper for degrading kraft lignin is known (Raj *et al.*, 2007). It is worth to note that strains belonging to *Aneurinibacillus* are considered safe for use in biotechnological applications.

In conclusion, *A.aneurinilyticus* strain CKMV1 isolated from *V. jatamansi* is the first known example of a phosphate solubilizing bacteria to have possible potential for the plant growth promotion under field condition due to its multifarious plant growth promoting traits. The

acquired knowledge can be used to improve the consistency and level of effectiveness of PGPR.

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