Journal of Nepal Biotechnology Association, 4 (1), 2023: 17-26 ISSN: 1728-1628

Research Article

Growth Promoting Role of Phytase Producing Bacteria Isolated from *Bambusa tulda* Roxb. Rhizosphere in Maize Seedlings Under Pot Conditions

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ARTICLE INFO

Submission: 07/12/2022
Acceptance: 29/11/2022
Published: 05/03/2023

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Abstract

Phytase enzymes have focused on their role in agriculture to generate bioavailable phosphorus (P) requires for plant nutrition. Herein, the feasibility of phytase-producing microbes as biofertilizers was studied. Bacteria with greater potential for hydrolysing calcium phytate based on the halo-to-colony ratio from *Bambusa tulda* Roxb. rhizosphere was isolated. Phytase activity by incorporating wheat bran, phytase screening and Luria Bertani (LB) medium after acetone precipitation was measured. Bacterial genomes were screened for the presence of β-propeller phytase gene corresponding to the *Bacillus* spp. using polymerase chain reaction (PCR). Finally, the effect of the isolates on the growth of maize seedlings under pot conditions in P-deficient loamy soil was evaluated. Ten distinct bacterial isolates collected from *B. tulda* rhizosphere were capable of mineralizing phytate and the maximum effect was observed for designated SRBR-04. Most isolates solubilized $Ca_3(PO_4)_2$ as a sole P source in Pikovskaya's agar. Five isolates selected for the study synthesized auxin in the LB broth supplemented with 1 mg mL^{-1} L-Tryptophan (1.63 to 4.5 µg mL^{-1}). Phytase production was highest in wheat bran with isolate SRBR-04 producing a maximum of 0.34 U mg^{-1}. Two isolates (SRBR-01, SRBR-04) screened positive for the presence of *Bacillus* phytase gene. Pot assay in P-deficient soil showed significant ($p < 0.05$) biomass promotion for the isolate SRBR-07 in shoot height (57%), dry shoot weight (178%), dry root weight (104%) and leaf area (113%) over the untreated control. Amendment of P-deficient agricultural soils with phytase-producing bacteria would provide a sustainable approach for P nutrition management in *Zea mays*.

Keywords: Biofertilizer, Phytate, Pikovskaya's agar, Plant growth promotion, Pot assay

Introduction

Phosphorus (P) is one of the major limiting macronutrients for plant growth and development (Gyaneshwar et al., 2002). Although accumulation of total P present in the soil is sufficient (400-1200 mg kg^{-1}) to sustain crop yield for another hundred years, plants cannot use them because they tend to form insoluble complexes with Fe^{3+} and Al^{3+} in acidic soil and with Ca^{2+} in calciferous soil (Khan et

al., 2009; Sharma et al., 2013). In soil abundant in organic materials, the organic P comprises 20-80% of total soil P (Richardson, 1994). Phytic acid or phytate is a major storage form of P in plants and constitutes up to 80% of the total organic P in soil (Lim et al., 2007). Bacteria in the soil produce phytase enzymes that convert phytates into inorganic phosphates (Pi), which are then readily available for P nutrition in plants (Ariza et al., 2013). Phytases are distinctly classified into four major classes, among them, β-propeller phytases (BPP) are particularly important as they have a major role in phytate-P cycling in nature (Lim et al., 2007). Several studies have been conducted on BPP showing their importance in the animal feed industry because of their high thermostability. The role of phytases has been underexplored in agriculture, even though it provides a sustainable source of soluble P.

Biotransformation of insoluble Pi to a soluble form like HPO_4^{2-} or $H_2PO_4^{-1}$ by the various mechanism of solubilization and mineralization is one of the key characteristics of the plant growth promoting rhizobacteria (PGPR) (Rodríguez et al., 2006). Incorporation of phosphate solubilizing bacteria (PSB) in agricultural soils can mitigate deleterious environmental impacts such as eutrophication and depletion of soil fertility by providing a sustainable source of soluble P (Rodrıguez & Fraga, 1999). Exhaustive studies were carried out to show an increase in plant biomass and yield of cereals, legumes, root crops and vegetables due to the mineral phosphate solubilizing (MPS) ability of the PSB (Abbasi et al., 2015; García-López et al., 2016; Pereira & Castro, 2014; Singh et al., 2014). However, only a handful of research was carried out to show the growth-promoting effect of the phytase-producing microorganisms (Hanif et al., 2015; Jorquera et al., 2012; Kumar et al., 2013; Liu et al., 2018). Along with the release of bioavailable P, the presence of phytin in the soil was also shown to induce mycorrhiza formation (Krey et al., 2013a).

To our best knowledge, there is no report on the isolation of phytase-producing bacteria from the bamboo rhizosphere. Based on the reasoning that bamboo is the fastest-growing plant, it can harbour robust microorganisms required for P assimilation. Therefore, in this study, we isolated potential phytate mineralizing bacteria from *Bambusa tulda* Roxb. rhizosphere and investigated their effect on the growth promotion of maize seedlings under pot conditions. Maize is selected to study the effect of treatment on growth promotion because of its low inherent root-induced P-solubilization capacity (Liu et al., 2016).

Materials and Methods

In this study**,** we collected a total of ten soil samples (0-10 cm depth) from a Bamboo (*Bambusa tulda* Roxb. rhizosphere) community forest located in Siraha district, Nepal (26.69° N, 86.49° E). Serially diluted soil samples were spread in a phytase screening medium (PSM) developed by Jorquera et al., 2011, containing 0.4% (w/v) calcium phytate ($C_6H_{16}CaO_{24}P_6$) from rice (RM3255, HIMEDIA®, India) as a sole P source (pH 7.0). After 4 days of incubation at 28 ± 2° C, cultures were examined for the appearance of a clear halo zone around the vicinity of the colony. We sub-cultured isolates again in the PSM using the spot inoculation method to calculate the phytate solubilizing index (PSI). After 4 days, PSI was calculated based on the ratio of total diameter to colony diameter, where total diameter includes both colony and halo zone diameter (Kumar et al., 2013). We qualitatively tested the ability of the isolates to solubilize mineral Pi based on their ability to produce organic acids in Pikovskaya's agar (M520, HIMEDIA®) containing 5 g L^{-1} tri-Calcium Phosphate (TCP) as a sole P source (Pikovskaya, 1948). In addition, isolates were grown in modified Pikovskaya's medium containing bromophenol blue (Gupta et al., 1994). Using the spot inoculation method, we cultured isolates in Pikovskaya's agar plate and incubated them at 28 ± 2° C for 7 days (Pikovskaya, 1948). We calculated the phosphate solubilising index (PhSI) with the same formula used for calculating PSI. *Bacillus subtilis* ATCC6633 was used as a reference organism for the comparison of PhSI and PSI data.

To differentiate isolates, we performed a series of biochemical tests such as Gram staining, citrate utilization, starch hydrolysis, total sugar iron (TSI), catalase, methyl red (MR), Voges-Proskauer (VP), indole and growth in YEM (Yeast Extract Mannitol) medium. Ten distinct bacterial isolates capable of hydrolysing phytate were obtained. We selected five isolates for further studies based on their high phytate mineralizing ability. The ability of the five selected isolates to produce auxin was determined according to Pedraza et al. (2013). We inoculated isolates in Luria Bertani (LB) broth (M1245,

HIMEDIA®) consisting of (g L-1): casein enzymic hydrolysate (10), yeast extract (5) and NaCl (10), maintaining pH at 7.5. Further, we tested the ability of the isolates to augment auxin production in the presence of 1 mg mL^{-1} L-Tryptophan (Trp). LB broths were incubated at 30° C for 72 h. Spectroscopic determination was carried out at 540 nm, using indole-3-acetic acid (RM384, HIMEDIA®) as standard.

We accessed the enzyme production abilities of the bacterial isolate with the highest PSI in three different mediums: (i) PSM, (ii) LB broth supplemented with sodium phytate (4 g L^{-1}) and (iii) wheat bran medium (WB) in 100 mL volume. WB medium was prepared as described by Powar and Jagannathan 1982. Briefly, 100 g L^{-1} WB (purchased from a local mill) was sterilized at 121° C for 60 min, cooled and squeezed through a muslin cloth. 100 mL extract of WB was transferred to the Erlenmeyer flask and added with the following ingredients: $(NH_4)_2SO_4$, 0.04 g; $MgSO_4.7H_2O$, 0.02 g; casein, 1 g; KH_2PO_4, 0.05 g and K_2HPO_4, 0.04 g. WB medium was autoclaved (121° C for 20 min) and separately sterilized $CaCl_2$ solution was added to give a final concentration of 0.2% (w/v). In the best production medium, phytase activity was determined for all the isolates. Isolates were loop-inoculated and incubated for 96 h at 30° C on a rotary shaker (200 rpm). Broths were centrifuged at 10000 x g for 30 min at 4° C (NF 800R, Nüve). Clear supernatants were transferred into sterile falcon tubes, and $CaCl_2$ was added at 1 mM final concentration and stored at 4° C until assays (Kim et al., 1998a). Phytase activity was assayed using the ascorbic acid method (Kim et al., 1998b). Spectroscopic measurement of the released Pi was taken at 820 nm (Genesis 10, Thermo Scientific, USA). One unit (U) of enzyme activity was defined as the amount of enzyme required to liberate 1 µmol of Pi per minute under assay conditions. KH_2PO_4 was used as a standard to estimate the amount of Pi released. Lowry method was used to determine total protein concentration in the enzyme preparations (Waterborg & Matthews, 1994). Bovine serum albumin (MB083, HIMEDIA®) was used as a protein standard. Specific activity is reported in µmol min^{-1} mg^{-1} protein.

The screening of isolates genomes for the presence of BPP was done using PCR. Total genomic DNA was extracted using Quick-DNA™ bacterial/fungal miniprep kit (Zymo Research, USA). To amplify the phytase gene, primer set, phyF (5'-CTGTCTGATCCTTATCATTT-3') and phyR (5'-TCCGCTTCTGTCGGTCA-3') were chosen (Bawane et al., 2011) and procured from Takara Bio, USA. We procured a Taq PCR kit (E5000S) from New England Biolabs, UK. The thermal cycler (Biorad T100, USA) conditions were as follows: pre-denaturation at 95° C for 5 minutes followed by 35 cycles of denaturation at 95° C for 1 minute, annealing at 53° C for 1 minute and extension at 72° C for 1 minute, followed by a final extension at 72° C for 10 minutes. 100 base pairs (bp) DNA ladder (MBT049, HIMEDIA®) was used to estimate the tentative size of the PCR amplicons**.**

The effect of the treatments in the growth promotion of maize seedlings was carried out in pots using P-deficient loamy soil (Gholami et al., 2008). This involved surface sterilization of maize seeds followed by the transfer to medium-sized pots of 11 cm diameter and 12 cm height containing 800 g of double autoclaved loamy soil (soil pH: 6.96, available Olsen-P: 0.835 mg Kg^{-1}). The experiment was conducted at the Kathmandu University greenhouse facility (altitude: 1550 m, in August-September, 2018). The soil was not supplemented with any kind of chemical fertilizer. Seeds were allowed to grow for two days before bacterial treatments. Fresh bacterial broth (4 mL) containing a cell concentration of ~108 CFU mL^{-1} was used to treat maize plants in the form of soil drenching twice in a gap of a week. Tests were made on five replicated pots of each treatment. Negative control constitutes 4 mL LB broth without bacteria. Seedlings were irrigated every two days. The seedlings were harvested after 30 days of bacterial treatment. Growth response variables were analysed after drying at 60° C. Leaf area was calculated as K × length × width, where K = 0.75 (Gholami et al., 2008). Soluble Pi content in the soil was determined according to Olsen et al. 1954.

Data in the study were represented as mean ± standard deviation (SD). For the comparison of two-sample data, Student's T-test was performed. One-way analysis of variance (ANOVA) followed by a contrast procedure with Tukey's honestly significant difference (HSD) test was performed for multiple comparisons at an alpha level of 0.05. Pearson product-moment correlation was used for testing the significant correlation between various parameters. Differences in the mean value with $p < 0.05$ were considered statistically significant. The statistical

analyses were conducted in R-Studio version 1.2.5033 (R-Studio Team, 2016)

Results and Discussion

Organic and inorganic P solubilization by the bacterial isolates

Based on the biochemical reaction (Table 1) and PSI data (Figure 1), we isolated ten distinct bacterial isolates capable of hydrolysing calcium phytate from *Bambusa tulda* Roxb. rhizosphere. However, biochemical tests were insufficient to identify the genera of the isolates. Calcium phytate and TCP appear turbid in the medium, and the formation of clear solubilization zones around the vicinity of the colony indicates the hydrolysis of these P sources. Among the isolates, SRBR-04 had the highest PSI of 9.78 ± 1.95 and SRBR-07 had the highest PhSI of 2.36 ± 0.23 (Figure 1). It was interesting to find that two of the isolates, namely SRBR-02 and SRBR-08, mineralized phytate but did not solubilize TCP as growth was not observed in Pikovskaya's agar (Figure 1). A similar trend of utilizing organic P sources but not inorganic P sources by the bacteria was noticed previously (Jorquera et al., 2011). All isolates capable of solubilizing TCP were further inoculated on modified Pikovskaya's agar containing bromophenol blue. We observed the appearance of yellow-coloured halos due to the drop in pH in the medium. The release of a vast repertoire of organic acids from direct oxidation pathways is responsible for the hydrolysis of TCP by the PSB (Sharma et al., 2013). Isolates *viz*. SRBR-01, SRBR-02, SRBR-03, SRBR-04, SRBR-07 and SRBR-09 showed significantly higher ($p < 0.05$) PSI than *Bacillus subtilis* (Figure 1). In contrast, *Bacillus subtilis* had significantly higher ($p < 0.05$) PhSI among the isolates (Figure 1). Five isolates *viz*. SRBR-01, SRBR-02, SRBR-03, SRBR-04, and SRBR-07 having comparatively higher PSI in the range of 5.78 to 9.78 were selected for further study. Visualization of the halo zones for these isolates is shown in Figure 2.

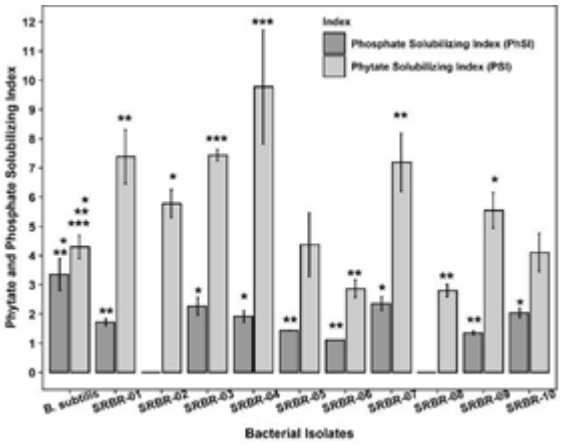

Figure 1: Phytase and phosphate solubilizing indices of the ten isolates. *Bacillus subtilis* strain ATCC6633 was used as a reference organism for comparison of PSI and PhSI. Each index of the isolates was compared with *B. subtilis* with two-sided student's T-test at $p < 0.05$.

Table 1: Biochemical reactions shown by the phytase producing bacterial isolates.

Biochemical test	Phytate mineralizing bacterial isolates									
	SRBR-01	SRBR-02	SRBR-03	SRBR-04	SRBR-05	SRBR-06	SRBR-07	SRBR-08	SRBR-09	SRBR-10
Gram staining	+	-	+	+	+	-	+	+	-	+
Citrate utilization	+	-	+	+	-	-	+	-	+	-
MR	+	+	+	+	+	-	+	-	+	+
VP	+	+	+	+	+	-	+	-	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Indole production	-	-	-	-	-	-	-	-	-	-
Growth in YEM	+	+	+	+	+	+	+	+	+	-
Starch hydrolysis	-	-	-	-	-	+	-	-	-	-
TSI test										
Slant color	r	r	r	r	o	o	r	o	r	x
Butt color	o	o	o	o	o	r	o	r	o	x
H_2S production	-	-	-	-	-	-	-	-	-	x
Air cavity	-	-	+	+	-	-	+	-	+	x

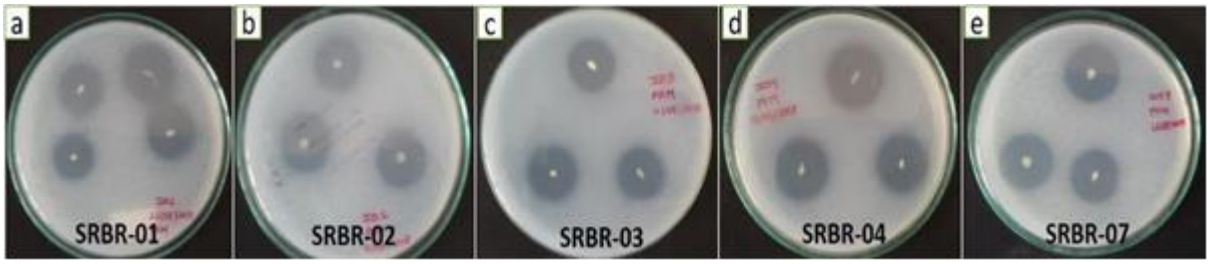

Figure 2: Visualization of halo zones due to calcium phytate mineralization by (a) SRBR-01, (b) SRBR-02, (c) SRBR-03, (d) SRBR-04 and (e) SRBR-07 in phytase screening medium after 4 days of incubation at 28±2 °C.

Auxin production ability

The production of auxin is an important attribute of rhizobacteria. All five selected isolates were able to produce auxin in the medium not fortified with Trp, suggesting that they were PGPR. A significant increase in the production of auxin was observed with 1 mg mL^{-1} Trp fortification (Figure 3). Trp acts as a precursor in the auxin synthesis pathway (Pedraza et al., 2004). An increase in the production of auxin (µg mL^{-1}) by 6-fold (2.82 ± 0.076, t2 = -36, $p < 0.001$), 5.8-fold (2.88 ± 0.15, t2 = -23.66, $p < 0.01$), 3.5-fold (4.5 ± 0.09, t2 = -72.95, $p < 0.001$ and 2.25-fold (3.29 ± 0.1, t2 = -29.85, $p < 0.01$) were observed for SRBR-01, SRBR-02, SRBR-03 and SRBR-04 respectively. SRBR-07 was unable to augment auxin in the presence of Trp (1.63 ± 0.076 µg mL^{-1}, t2 = -4, $p > 0.05$).

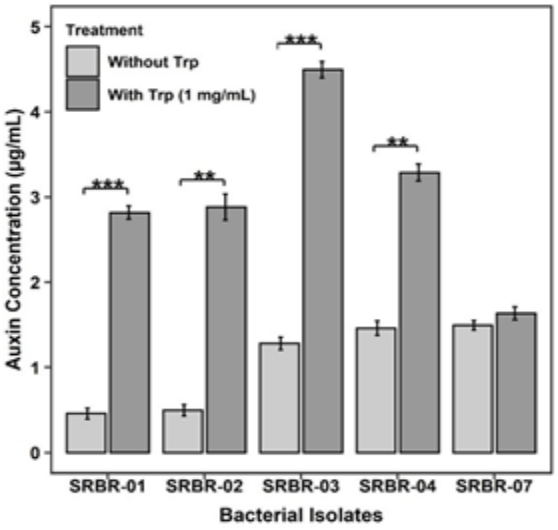

Figure 3: Comparisons of the auxin concentrations produced by the phytase producing isolates in the presence (1 mg ml^{-1}) and absence of L-Tryptophan (Trp) in LB medium after 3 days of incubation at 30°C. Significant differences in the augmentation of auxin due to the presence of L-Tryptophan was studied with paired Student's t-test at $p < 0.05$. Asterisk (*) was assigned to show the significant differences in the mean. **[$p < 0.01$], ***[$p < 0.001$]. Error bars denote SD (n = 3).

Optimum medium for enzyme production and enzyme activity

With isolate SRBR-04, an optimum medium for enzyme production was selected. Phytase activity (U mg^{-1}) among the production media differed significantly after 96 h of enzyme cultivation ($F_{2,6} = 1467.2$, $p < 0.001$). Enzyme-specific activity in WB (0.34 ± 0.009) was 4.35 times greater than LB + phytate medium (0.078 ± 0.008, $p < 0.001$, Figure 4a). Similarly, there was a 7.5-fold increase in enzyme production in WB than in PSM (0.045 ± 0.005, $p < 0.001$, Figure 4a). Assessment of the phytase activity in liquid cultures among all the selected isolates was conducted in WB owing to its high enzyme production ability. Statistically significant differences in phytase-specific activity among the isolates were obtained in WB after acetone precipitation ($F_{2,10} = 26.8$, $p < 0.001$, Figure 4b). Isolates SRBR-01, SRBR-02 and SRBR-03 had a comparable activity of 0.28 ± 0.017, 0.26 ± 0.01 and 0.26 ± 0.017 respectively and were statistically higher ($p < 0.05$) than SRBR-07 (0.22 ± 0.017). Specific activity obtained for SRBR-04 (0.34 ± 0.0087) was found to be statistically higher than the isolate SRBR-01 ($p < 0.01$), SRBR-02 ($p < 0.001$), SRBR-03 ($p < 0.001$) and SRBR-07 ($p < 0.001$). An increase in enzyme activity in WB showed a mild positive and significant correlation with the PSI data in PSM ($r = 0.63$, $p = 0.013$). The high enzyme production in the WB medium is attributed to the presence of a high amount of phytate in WB which acts as a stimulus for phytase production as suggested by Powar & Jagannathan (1982). WB was also supplemented with 1 g L^{-1} casein hydrolysate. According to Kim et al. (1998a), both WB and casein hydrolysate provide a good source of carbon and nitrogen for the optimum production of phytase enzyme. A small amount of Pi in the form of KH_2PO_4 and K_2PO_4 was added in the WB medium to impersonate phosphate starvation

conditions required for the induction of the phytase gene to Kim et al. (1998a). The addition of $CaCl_2$ in the culture supernatant promotes the binding of Ca_{2+} to high- affinity Ca-binding pockets in the case of BPP, maintaining its six-bladed propeller configuration (Ha et al., 2000). Previously, 0.59 U mg_{-1} phytase activity was reported after acetone precipitation produced from *Bacillus* sp. DS11 (Kim et al., 1998a).

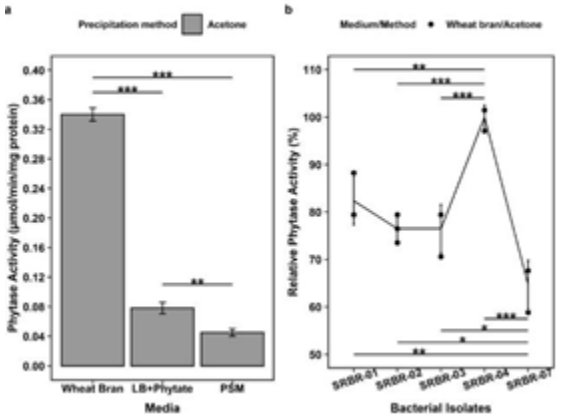

Figure 4: Activity of phytase enzyme in three different liquid media. Highest phytase activity was found in WB medium with acetone precipitation (a). Relative phytase activity of the five selected isolates in WB medium after acetone precipitation. The highest phytase activity for SRBR-04 was defined as 100% which corresponds to 0.34 µmol of Pi min^{-1} mg^{-1} protein (b). Data from three independent experiments (n = 3) were subjected to pairwise comparison with Tukey's HSD test at $p < 0.05$. Asterisk (*) was assigned to show the significant differences in the mean. *[$p < 0.5$], **[$p < 0.01$], ***[$p < 0.001$]. Error bars represent SD.

Phytase gene PCR

With *Bacillus* primer set (phyF and phyR), we were able to amplify the phytase gene for only two isolates, namely SRBR-01 and SRBR-04 (Figure 5). Detection of the BPP gene in these two isolates indicates their belonging to the *Bacillus* genus. With a 100 bp DNA ladder, the size of the PCR amplicon was found to be tentatively around 1059 bp. Among the four different classes of phytases, BPP is mostly present in the species of *Bacillus* and *Pseudomonas*, which in turn are PGPR (Rathinasabapathi et al. 2018). Multiple sequence alignment showed that the phytase gene-TS-phy from *Bacillus* sp. DS11 (Kim et al., 1998b) and phyC from *B. subtilis* VTTE-68013 (Kerovuo et al., 1998) perfectly aligned with >99% sequence similarity. Therefore, this template sequence was used for the selection of a degenerate primer set. Forward primer (phyF) representing the amino acid residues -LSDPYH which is located in the polypeptide at the end of the tentative 29 amino acid signal peptide and reverse primer (phyR) representing the amino acid residues-TDRSG were chosen (Kerovuo et al., 1998). A total of 353 amino acids polypeptides is flanked by these primers corresponding to the nucleotide length of 1059 bp. The nucleotide length reconciles in our experiment as we observed a PCR amplicon size of around 1059 bp for SRBR-01 and SRBR-04 (Figure 5). The size of the amplicons is in concordance with the amplicon size of the phy gene from two *B. subtilis* strains NCDC-070 and NCIM-2712 reported by Bawane et al. (2011). Positive amplification of the phytase gene for only two isolates may indicate the diverse form of BPP genes (TS-phy, phyC, phyL,168phyA) present among different *Bacillus* species (Mullaney & Ullah, 2003) or the isolation of the bacteria other than the *Bacillus* species.

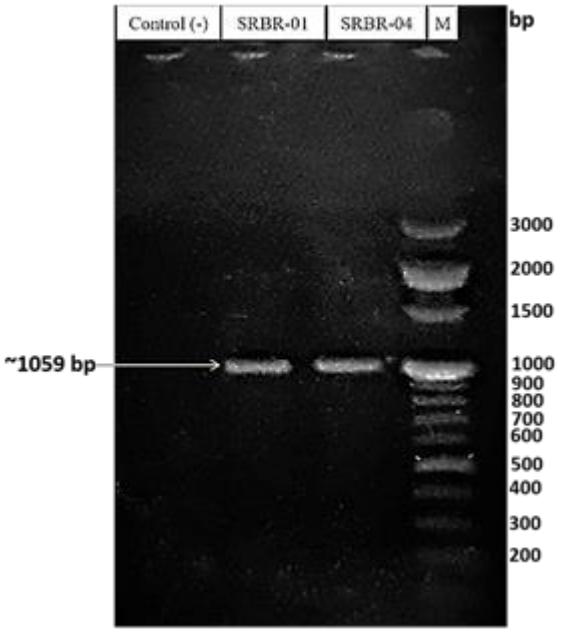

Figure 5: Gel electrophoresis image of the phytase gene amplicon amplified with phyF/phyR primer set. An amplicon of around 1059 bp was amplified for the isolate SRBR-01 and SRBR-04. Electrophoresis was carried out in 0.5 X Tris-Borate EDTA at 60 V for 30 min at 0.8% (w v-1) agarose concentration. 'M' denotes the lane for 100 bp DNA markers whose corresponding sizes are given in base pairs. Control (-) denotes the lane for negative control.

Plant growth promotion under pot conditions

Visualization of growth enhancement of maize seedlings statistically a significant among the treatment groups were established for all selected growth parameters: shoot length, dry shoot weight, dry root weight and leaf area (Table 2).

Table 2: Effect of phytase producing bacterial isolates on the growth promotion of maize seedlings after 30 days of inoculation under pot conditions.

Treatment	Plant growth parameter			
	Shoot length (cm)	Shoot weight (g)	Root weight (g)	Leaf area (cm^2)
Control (-)	12.2 ± 0.72^{a}	0.71 ± 0.18^{a}	1.67 ± 0.78^{abc}	165 ± 50.9^{a}
SRBR-01	15.6 ± 0.46^{acd}	1.03 ± 0.19^{ab}	1.24 ± 0.24^{ab}	204 ± 39^{a}
SRBR-02	$\mathbf{19 \pm 2.55}^{bd}$	$\mathbf{2.24 \pm 0.88}^{bc}$	2.7 ± 0.73^{cde}	$\mathbf{323 \pm 119}^{b}$
SRBR-03	$\mathbf{19.9 \pm 3.2}^{b}$	$\mathbf{2.13 \pm 0.9}^{b}$	1.84 ± 1.02^{ae}	$\mathbf{338 \pm 98}^{b}$
SRBR-04	14.6 ± 1.4^{ace}	0.94 ± 0.14^{ad}	1.31 ± 0.12^{a}	184 ± 45.5^{a}
SRBR-07	$\mathbf{19.2 \pm 1.28}^{bdf}$	$\mathbf{1.98 \pm 0.35}^{bd}$	$\mathbf{3.42 \pm 0.64}^{df}$	$\mathbf{353 \pm 47.7}^{b}$
R^{2*}	0.67	0.52	0.57	0.49
$F_{5,23}$	12.06	7.17	8.44	6.28
p-value	<0.001	<0.001	<0.001	<0.001

Data from five replicates (n = 5) were subjected to Tukey's HSD test at $P < 0.05$. Different superscript letter(s) along the same column indicate significant differences between treatment groups. Mean ± SD is given in bold for those groups showing significant differences with the negative control group. * R2 value present is 'adjusted'.

The treatment group SRBR-07 was able to significantly promote shoot height (57%, $p < 0.001$), dry shoot weight (178%, $p = 0.026$), dry root weight (104%, $p < 0.01$) and leaf area (113%, $p = 0.011$) over the negative control (Table 2). Treatment group SRBR-02 was able to significantly promote shoot height (55%, $p < 0.001$), dry shoot weight (215%, $p < 0.01$) and leaf area (95%, $p = 0.043$) (Table 2). Similarly, treatment group SRBR-03 was able to significantly promote shoot height (63%, $p < 0.001$), dry shoot weight (200%, $p = 0.01$) and leaf area (104%, $p = 0.022$) over the non-inoculated control group (Table 2). Between the treatment with PSB, an increase in shoot height showed a moderate positive correlation with dry shoot weight ($r = 0.77$, $p < 0.001$). We obtained a strong positive correlation between chlorophyll content in the leaves and shoot height ($r = 0.87$, $p < 0.001$).

Although, the P content in the seedlings' biomass compared to the negative control was not determined, given that the alkaline loamy soil (pH: 6.96) used in the pot experiment contains a very low amount of soluble P (Olsen-P: 0.835 mg kg-1), we conclude the growth-promoting effect of these isolates was due to their ability to increase the bioavailability of P. The release of soluble P might be due to the expression of phytase enzyme as the alkaline nature of the soil induces the expression of phytase enzyme in the previous report (Kumar et al., 2013). A significant promotion in the growth of maize was observed under pot conditions, but a correlation with high phytate mineralizing ability for plant growth promotion was not established. Isolate SRBR-04 was unable to promote any of the plant growth parameters. This may seem counterintuitive, however, the inoculation of PSB in soil could help by affecting a myriad of other factors. It involves the assimilation of macro-and micronutrients to the production of growth regulators, signalling molecule, biocontrol agents, biotic and abiotic stress regulators as well as involves the expression of other MPS traits like the release of exopolysaccharide, siderophores etc. (Sharma et al., 2013; García-López et al., 2016). In previous studies, the inoculation of phytase- producing bacteria on P-deficient soil was shown to enhance maize biomass production (Krey et al., 2013a; Krey et al., 2013b; Liu et al., 2018). The promotion of root growth is particularly important in low nutrient-rich soil because the increment in the volume of the root is proportional to the scavenging activity of the root for nutrients (Eltlbany et al., 2019). In this regard, SRBR-07 would act as a better biofertilizer in the nutrient-limiting soil than SRBR-02 and SRBR-03.

Conclusion

We isolated phytase-producing bacteria and the tested the hypothesis that these bacteria would provide a bioavailable P to the maize plants as a potential biofertilizer. We found *Bambusa tulda* Roxb. rhizosphere to harbour bacteria with greater phytate mineralizing ability. The ability of these bacterial isolates to solubilize mineral Pi and auxin production partly augments their plant growth-promoting capability. We detected the BPP gene in two isolates, among them, SRBR-04, had higher enzyme activity in the WB medium that could have implications in the animal feed industry. In pot assay, SRBR-02, SRBR-03 and SRBR-07 showed

significant growth enhancement of various plant growth parameters in maize seedlings indicating their huge potential in the sustainable management of the P in the nutrient-limiting soil.

Acknowledgements

We acknowledge the President Chure Terai-Madhesh Conservation Project (KU/CHURE/ PROJECT/01) for financial support. We are thankful to Mr. Siddhi Nath Paudel (Teaching Assistant) and Dr. Meena Maiya Suwal from the Department of Biotechnology and Dr. Uttam Budhathoki (Associate Professor) from the Department of Pharmacy, Kathmandu University for their assistance and guidance.

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