Isolation and Characterization of Plant Growth-promoting Rhizobacteria from Bamboo Rhizosphere and Their Role in Plant Growth Promotion

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ISSN : 2382-5359(Online), 1994-1412(Print)

DOI:
https://doi.org/10.3126/njst.v21i1.49908

Date of Submission: 26/07/2021

Date of Acceptance: 21/07/2022

ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are a group of root-associated bacteria that intimately interact with the plant roots and consequently enhance growth by extemporising nutrient retrieval or phytohormone production. We isolated and screened indigenous phosphate solubilising and auxin-producing PGPR from bamboo rhizosphere. 66 soil samples from bamboo (*Bambusa nutans* subsp. *cupulata, B. balcooa* and *B. tulda*) rhizospheres were collected from Dhanusha, Mahottari and Sarlahi districts, Nepal. 120 isolates of PGPR were obtained by serial dilution method in (PVK) agar and Luria Bertani agar. 92 out of 120 isolates of PGPR with the ability to solubilise phosphate were selected based on the halo colony ratio in PVK agar medium and auxin production in Luria Bertani agar. Among them, six isolates having high phosphate solubilising index and high production capacity of indole-3-acetic acid were further screened. Biochemical analysis revealed that these isolates belonged to the genus *Pseudomonas*. Phosphate solubilising index and indole-3-acetic acid production by six isolates ranged from 4.19±0.8 to 7.65±1.3, and IAA production ranged from 72.93±0.2 to 82.48±0.9µg/ml respectively. These isolates significantly increased shoot length (13.26±0.56cm), shoot fresh weight (16.26±1.02mg), shoot dry weight (10.56±0.09mg), root length (4.9±0.5cm), root fresh weight (7.56±1.05mg), root dry weight (3.21±0.01mg), and chlorophyll 'a' and chlrophyll'b' and carotenoid (2.16±0.01mg/g, 1.19±0.06mg/g and 0.92±0.01mg/g respectively) of *B. nutans* subsp. *cupulata* seedlings. This study suggests that PGPR isolated from bamboo rhizosphere demonstrated outstanding contribution to the growth promotion of seedlings of *B. nutans* subsp. *cupulata* as compared to negative control.

Keywords: Bamboo, Biofertilizer, Rhizosphere, Siwalik region, Nepal

1. INTRODUCTION

The complex interactions among plants, soil and microbes about micronutrient dynamics represent a unique opportunity for improving soil fertility (Ramakrishna *et al.* 2019). Plant-associated bacteria are part of the natural microflora of healthy plants and the potential uses of plant-associated bacteria as agents for stimulating plant growth and managing soil and plant health (Compant *et al.* 2005). The rooting pattern and distribution of nutrients to the plant body is influenced by microbial activities. It further modifies the quality and quantity of the exudates from roots. The microorganisms metabolize a certain portion of the exudates as the source of carbon and nitrogen, and some of the molecules will then be reutilized by the plants for their growth and development (Kang *et al.* 2010). Plant growth-promoting rhizobacteria (PGPR) are beneficial to plants as they colonize the roots, accelerate plant growth, and alleviate the stress resulting by biotic and abiotic factors through various mechanisms. This PGPR are recognized as bio fertilizer, biocontrol agents and microbial inoculants (Andy *et al.* 2020). PGPR are the symbiotic, free living, soil microorganisms inhabiting the rhizosphere of several species of plants providing diverse beneficial effects to the host plants through nitrogen fixation, nodulation and production of phytohormones (Raza *et al.* 2016), solubilising insoluble phosphate available phosphorus especially in soils with limited phosphorus (Tripura *et al.* 2005). They are categorised as a group of neutral soil microbial flora capable of colonising in the rhizosphere and the surface of plant root, which positively impacts the overall growth promotion of plants (Goswami *et al.* 2016). They are accountable for defending plant health in an eco-friendly manner (Akhtar *et al.* 2012) and are said to have been functioning as a coevolution between plants and microbes through antagonistic and synergistic interactions with microorganisms and soil. Such an interaction as a direct mechanism includes production of plant growth regulators or facilitation of nutrient uptake (Long *et al.* 2008).

Microbes that hormonally promote plant growth are phytostimulators (Khare & Arora 2010). Indole-3-acetic acid (IAA), a phytohormone, secreted by rhizobacteria influences several plant developmental processes (Glick 2012). On the other hand, IAA also serves as a reciprocal signalling molecule since it affects gene expression in microorganisms. Therefore, it significantly contributes to rhizobacteria-plant interactions (Spaepen & Vanderleyden 2011).

Phosphorous (P), the second crucial plant growth-limiting nutrient, is found in soils in inorganic and organic forms (Khan *et al.* 2009). However, the amount of available .P forms is generally low as most of the soil P is found in insoluble forms. The plant roots acquire P in the form of monobasic (H_2PO_4-) and dibasic (H_pO_4--) ions (Bhattacharya & Jha 2012). The insoluble P is present as an inorganic mineral such as apatite or as one of several organic forms, including inositol phosphate (soil phytate), phosphomonesters, and phosphotriesters (Glick 2012).

To overcome the P deficiency in soils, there are frequent applications of phosphatic fertilisers in agricultural fields. Plants absorb little amount of applied phosphatic fertilisers and the rest is rapidly converted into insoluble complexes in the soil (Mckenzie & Roberts 1990). Regular application of phosphate fertilizers is very costly and undesirable in environmental point of view. It has compelled scientists to find an economically feasible and ecologically safe approaches for improving crop yield in the low P soils. Several rhizobacteria can solubilise plant-available phosphate from organic or inorganic bound phosphate (Lugtenberg *et al.* 2002). In this context, organisms coupled with phosphate solubilizing activity, often termed as phosphate solubilising microorganisms (PSMs), may provide the available forms of P to the plants and hence a viable substitute to chemical phosphatic fertilisers (Khan *et al.* 2006). Of the various PSMs inhabiting the rhizosphere, phosphate-solubilising bacteria (PSB) are considered as promising biofertilisers as they can provide plants with the required P (Zaidi *et al.* 2009).

As an alternative to timber, bamboo can positively impact many areas with multiple applications, including producing paper, scaffolding, diesel, artifacts, aphrodisiacs, musical instruments, medicine and food (Bansal & Zoologud 2002).

They have a higher raw material output than trees and are highly profitable renewable resources (Hunter & Wu 2002). Demand-supply gap of bamboo owing to the destruction of natural bamboo resources has become a pressing issue to explore appropriate methods for the mass propagation of bamboo (Ray & Ali 2017). Beneficial effects of PGPR have been reported in many economic-important crops like *Brassica juncea* (Asghar *et al.* 2002), *Vigna radiata* (Patten & Glick 2002), *Arachis hypogea* (Day *et al.* 2004), *Triticum aestivum* (Ali *et al.* 2008; Hussain & Hasnain 2011; Emami *et al.* 2019), *Zea mays* (Ruangsanka 2014), *Cucumis sativus* (Islam *et al.* 2015) *Lycopersicon esculentum* (Pathak *et al.* 2017; Qessaoui *et al.* 2019) Coffea arabica (Kunwar *et al.* 2018). To date, scanty work has been done regarding the effects of PGPR on the growth and development of bamboo seedlings. (K.C. *et al.*, 2020 & 2021). In our laboratory, we have isolated several PGPR from the rhizosphere of bamboo growing in the agroecosystem of the Siwalik region of Nepal. This PGPR exhibited plant growth-promoting traits like phosphate solubilization, indole-3-acetic acid (IAA) production and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. Therefore, we hypothesized that inoculation of PGPR could promote the seed germination and growth parameters of bamboo seedlings. As per the hypothesis, we have isolated the PGPR from the bamboo rhizosphere, and the interaction of PGPR in bamboo seedlings was investigated in an attempt to improve the major problems of poor seed set with seed-based propagation of bamboo.

2. MATERIALS AND METHODS

2.1 Soil Sampling Sites

The soil sampling sites were Sasapur, located at an altitude of 138 m (N 27°05'03.9''and E 085°36'59.9") towards the northern side of Lakhendehi river of Sarlahi District, Kantibazaar situated at an altitude of 105 m towards the Eastern side of Banke river of Mahottari district (N 26°57'50.1''and E 085°43'48.3") and Auribaba community forest located at an altitude of 198 m towards the northern side of Aauri river, Dhanusha district (N 26°57'33.9''and E 085°5832.3"). Three species of bamboo (*Bambusa tulda, B. balcooa* & *B. nutans subsp. cupulata*) distributed in the study area were selected from agroecosystem for sampling of rhizosphere soil (Fig.1). Three soil samples were taken from the rhizosphere of each species of bamboo from different depth (5, 10, 15cm) according to Barillot *et al.* (2013) during May 2016. The soil samples were kept in sterilized falcon tubes in ice bag, transported to Organic Farming and Natural Product Research Center (ONRC) laboratory, Kathmandu University, Dhulikhel, Nepal and stored at 4ºC until the time of isolation.

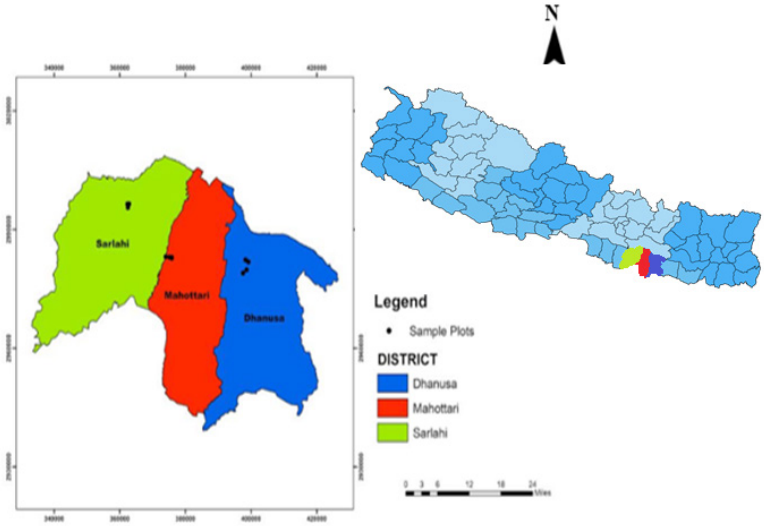

Fig.1. Soil sampling sites in Nepal

2.2 Isolation and Purification of Rhizobacterial Isolates

One gram rhizosphere soil sample was suspended in 9ml of sterilized distilled water. The soil suspension was subjected to serial dilution under aseptic condition. 0.1 ml of diluted samples (10-2 to 10-6) were spread on the sterile nutrient agar (NA) medium (Hi-media) followed by incubation for 24 hours at 30°C. Morphologically distinct colonies were selected from agar plates to avoid repetition of same strain. Altogether, 42 rhizobacterial isolates were obtained. Selected colonies were streaked repeatedly on NA agar for purity. Pure colonies were stored in nutrient broth (NB) containing 80% glycerol at -20ºC for further studies. These isolates were maintained by transferring them to NB after every 30-60 days.

2.3 Morphological and Biochemical Characterization

Efficient PSB and APB were identified on the basis of morphological and biochemical characteristics. For identification of PSB and APB isolates based on colony morphology isolates were grown in nutrient agar plates for 24 hours at 30°C. Gram reaction was done using the Gram Staining kit (Hi -Media) according to the manufacturer's instructions. Oxidase, KOH and citrate tests were performed according to Garrity *et al.* (2004).

2.4 Qualitative Estimation of Phosphate Solubilizing Index (PSI)

The selected bacterial isolates were streaked on PVK agar medium for their tri-calcium phosphate (TCP) solubilizing activity. The plates were incubated at 30°C for 192 hours and phosphate solubilization formed around the colonies were recorded. The PSB were peaked and subcultured in same medium until a pure culture was obtained. Pure cultures of PSB were spot inoculated at the center of PVK agar medium with Ca3(PO4)2 as the phosphate source and they were incubated at 30°C for 192 hours. The PSI was calculated by measuring the clear halo zone around the colony growth as solubilization index. The experiments were performed in triplicates and repeated three times for each isolates. Six isolates were finally obtained for further evaluation.

2.5 Spectrophotometric Quantification Indole-3-Acetic Acid (IAA)

Isolated pure colonies were inoculated into LB broth containing 1 mg/l L-trptophan (L-trp) and incubated at 30ºC with continuous shaking at 140rpm for 24 hours (Rahman *et al.* 2010) with slight modification. For each isolate, the experiment was repeated three times with three replicates. After 24 hours, 2 ml of cultured solution with triplicates were centrifuged at 10000 rpm for two minutes at 4ºC and amount of IAA per ml of culture was estimated by adding 2 ml of culture supernatant to 4 ml of Salkowski's reagent (2 ml of 0.5 M FeCl3 in 98 ml of 35% HClO4 (Asghar *et al.* 2002) and incubated for 45 minutes in darkness at room temperature for color development. Intensity of color was measured by spectrophotometer at 535nm (Hussain & Husnain 2011). IAA concentration was estimated using a standard curve prepared with known amount (10-100µg ml-1) of filtered IAA (Hi-media).

2.6 Effect of Bacterial Isolates on the Growth Performance of Bamboo Seedlings

To evaluate the growth performance of bamboo seedlings in in-vitro PGPR strains treatments, seeds of *B. tulda* were surface sterilized with 1% sodium hypochlorite for few minutes and washed three times with autoclaved distilled water and soaked in bacterial suspension (OD550 = 1.0) for 24 hours. The bacterial suspension was drained, and the seeds were dried overnight in a laminar hood. Seeds immersed in NB medium (HiMedia) only were used as a negative control treatment. There were 15 seeds per treatment. Petri plates with different treatments were arranged based on a completely randomized design and placed in a growth chamber at 28±10°C adjusted to 16 hours of light and eight hours of dark for 24 days. Treatments demonstrating promising results compared to control transferred to the greenhouse. For the greenhouse experiment, soil was sieved through 2 mm mesh and sterilized by autoclaving twice at 24 hour intervals. 1.125 kg of sterilized soil was placed in a plastic pot (32cm×15cm). Four seedlings of each treatment were sown in each pot. Each treatment was repeated and replicated three times, making

a total of 63 pots randomly distributed in the greenhouse. The floor was covered with plastic sheets to prevent microbial contamination into the pots. Water was applied to the experiment in the morning and evening to avoid water stress and no additional fertilizer was added. The plants were allowed to grow in greenhouse for four months followed by recording growth parameters including root length, shoot length, biomass (fresh weight and dry weight), and chlorophyll' a' and chlorophyll 'b' were quantified according to Hiscox & Israelstam (1979) and carotenoid according to Lichtenthaler & Buschmannan (2001) for all treatments.

2.7 Statistical Analysis

Statistical analyses were performed using IBM Statistical Package for the Social Science (SPSS) version 25. Treatments were compared by analysis of variance (ANOVA) using Duncan's Multiple Range Test (DMRT) at 5% ($P \leq 0.05$) probability level.

3. RESULTS

3.1 Isolation and Biochemical Characterization of Rhizobacterial Isolates

The rhizobacteria isolated from bamboo rhizosphere varied from 1×106CFU/g to 3×1017 CFU/g of soil samples. The highest number of bacteria per gram of soil sample were counted in 10 cm depth of each *bamboo* rhizosphere (Table1). Altogether 92 rhizobacterial isolates were obtained from the bamboo rhizosphere during pure culture. Morphologically distinct 6 isolates were identified to the genus *Pseudomonas* by morphological and biochemical characterization. These isolates demonstrated round to irregular shaped colonies, rod shaped, gram negative, catalase positive, oxidase negative, citrate test positive and KOH negative (Table 2). The colonies produced were circular, translucent, opaque, smooth or irregular margin. These isolates were represented by BUX39, BUX46, BUX71, BUX74, BUX82 and BUX89.

Table.1. Colony forming units of rhizobacteria of bamboo

Soil depth (cm)	CFUs/gm of dry soil	F- value	Significance
5	2 × 106	1.039	0.41
10	3 × 108		
15	1 × 106		

Table. 2. Biochemical characterization of rhizobacterial isolates

Isolates	Colony characteristics			Biochemical tests				
	Colony size	Colony shape	Texture	Morphology	KOH	Gram reaction	Citrate	Catalase	Oxidase
BUX_{39}	Small medium	Round	Creamy	Regular	-	- ve	+ve	+ve	-ve
BUX_{46}	Small	Erose filament	Creamy	Regular	-	- ve	+ve	+ve	-ve
BUX_{71}	Small medium	Lobate	Creamy	Irregular	-	- ve	+ve	+ve	-ve
BUX_{74}	Medium	Round erose	Yellowish	Irregular	-	- ve	+ve	+ve	-ve
BUX_{82}	Small	Round erose	Creamy	Regular	-	- ve	+ve	+ve	-ve
BUX_{89}	Small	Lobate	Creamy	Regular	-	- ve	+ve	+ve	-ve

3.2 Qualitative Estimation of Phosphate Solubilizing Index

All isolates released inorganic phosphate from TCP indicating the potential of these strains to release inorganic soluble phosphate from fixed phosphate sources for plant uptake. Highest PSI was demonstrated by BUX89 (7.65±1.32) followed by *Pseudomonas* sp (6.98±1.13) as control, BUX82 (5.34±0.07), BUX74 (5.01±0.93), BUX71 (4.92±0.69), BUX46 (4.78±1.12) and BUX39 (4.19±0.81) (Table.3).

Table. 3. Phosphate solubilization index (PSI) of rhizobacterial isolates isolated from bamboo rhizosphere

Isolates	Phosphate solubilization index PSI
BUX_{39}	4.19±0.81[a]
BUX_{46}	4.78±1.12[b]
BUX_{71}	4.92±0.69[c]
BUX_{74}	5.01±0.93[c]
BUX_{82}	5.34±0.07[d]
BUX_{89}	7.65±1.32[e]
Pseudomonas sp.	6.98±1.13[d]

Values are means±SE. Levels not connected by same letter in the same column are significantly different ($P < 0.05$) according to DMRT.

3.3 Spectrophotometric Quantification of Indole-3-Acetic acid (IAA)

All six rhizobacterial isolates and control (*Pseudomonas* sp.) showed the ability to synthesis IAA and varied in their potential of IAA production. IAA concentration determined with Salkowski reagent ranges from 72.93±0.2µg/ml to 82.48±0.9µg/ml. The more active producers of IAA were BUX89 and control (*Pseudomonas* sp.) (Fig. 2)

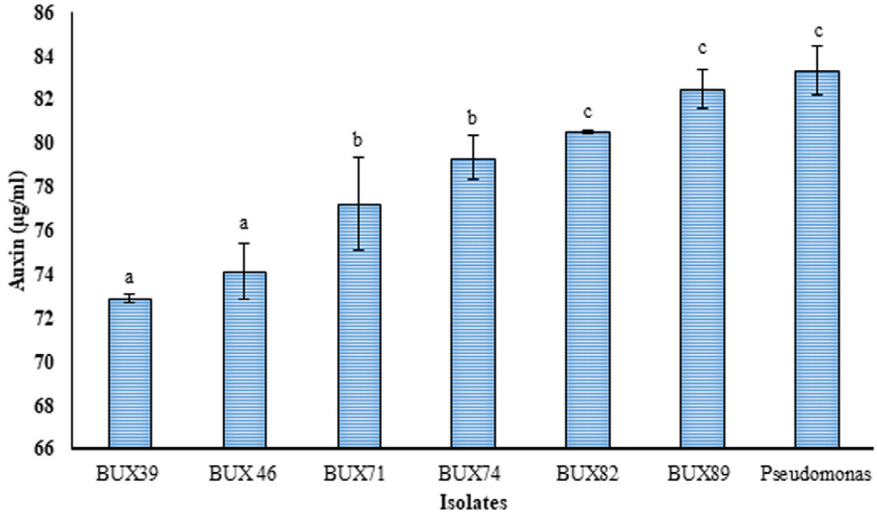

Fig. 2 Quantitative estimation of IAA produced by rhizobacterial isolates (Spectrophotometric Method). Bars show means±SE. Levels not connected by same letter are significantly different ($P < 0.05$) according to DMRT.

3.4 Effect of Rhizobacterial Isolates on Growth Parameters of Bamboo Seedlings

PSB-inoculate seedlings after greenhouse experiment for four months showed significant growth of shoot length, fresh shoot biomass, fresh root biomass and chlorophyll and carotenoid content as compared to the uninoculated bamboo seedlings. Isolate BUX39 showed the best effect on shoot length (13.26±0.56 cm), shoot fresh weight (16.26±1.01g) and root fresh weight (7.51±1.05g) on the bamboo seedlings in greenhouse experiment. Isolate BUX46 and BUX71 showed second and third best effect on shoot length and shoot fresh weight of bamboo seedlings respectively. Chlorophyll and carotenoid content were enhanced in the bamboo seedlings treated with rhizobacterial inoculum. BUX89 showed significant increase in chlorophyll content (2.16±0.0mg/g) while BUX39 significantly increased the carotenoid content (0.92±0.01 mg/g) in bamboo leaves as compared to negative control.

Table. 4. Effect of rhizobacterial isolates on growth parameters of bamboo seedlings

Isolates	Shoot parameters			Root parameters			Photosynthetic pigments ((µg/g))		
	SL (cm)	SFW (g)	SDW (g)	RL(cm)	RFW (g)	RDW (g)	Chl 'a'	Chl'b'	Carotenoid
BUX_{39}	13.26± 0.56a	16.26± 1.01a	10.56± 1.5a	4.92± 0.5a	7.51± 1.05	3.21± 0.01a	2.16± 0.0	1.19± 0.05	0.92± 0.01a
BUX_{46}	13.09± 0.32a	14.32± 09b	8.11± 09b	3.98± 1.2b	5.21± 1.8b	1.17± 0.5b	2.05± 0.3	1.03± 0.9	0.91± 0.03a
BUX_{71}	11.06± 09b	14.11± 1.1b	3.11± 1.5c	3.97± 1.5b	3.48± 0.9c	0.99± 0.5b	2.08± 0.2	1.01± 0.4	0.86± 0.05a
BUX_{74}	11.04± 07b	12.23± 1.5c	3.02± 09c	4.56± 0.9a	3.05± 0.5c	0.78± 1.2b	2.01± 0.1	0.96± 0.5	0.78± 0.04b
BUX_{82}	11.1± 1.1b	11.24± 09c	2.03± 01c	3.99± 1.7b	2.98± 0.1c	0.77± 0.9b	1.98± 0.3	0.94± 0.1	0.69± 0.01b
BUX_{89}	10.12± 08b	11.19± 1.2c	1.34± 08d	3.12± 0.7c	1.78± 0.9d	0.69± 0.7b	0.97± 0.3	0.76± 0.2	0.58± 0.3c
L.*B.* broth (-ve control)	9.15± 1.3c	8.56± 02d	1.02± 04d	1.34± 0.9d	0.99± 0.3e	0.62± 0.4b	0.88± 0.7	0.68± 0.92	0.52± 0.2c
Water (-vecontrol)	8.05± 07c	8.11± 01d	0.97± 03d	1.37± 0.4d	0.78± 0.2e	0.45± 02a	1.99± 0.8	1.02± 0.9	0.49± 0.9d
Pseudomonas (+ve control)	12.06± 1.21a	15.24± 07a	3.38 ± 1.4c	3.97± 1.5b	3.07± 0.9c	1.67± 0.4c	2.19± 0.01	1.88± 1.10	0.97± 0.03a

*SL: Shoot length; SFW: Shoot fresh weight; SDW: Shoot dry weight; RL: Root length; RFW: Root fresh weight and RDW: Root dry weight. Values are means±SE. Levels not connected by same letter in same column are significantly different ($P < 0.05$) according to DMRT.

4. DISCUSSION

Beneficial rhizospheric PGPR contribute through synthesizing particular compounds for plants or facilitating the uptake of particular nutrient from the soil or by preventing and protecting the plants from pathogens (Hayat *et al.* 2010). In our research, biochemical analysis revealed that all rhizobacterial isolates were gram negative and belonged to the genus *Pseudomonas*. Many species under *Pseudomonas* perform beneficial effects on plants. Among them P. putida and P. fluorescens are fluorescent *Pseudomonas* species that represent significant part of these members (Haas and Defago 2005). Baon *et al.* (2012) reported that the gram negative bacteria dominated the coffee rhizosphere. *Pseudomonas*

species are free living and most abundant in soil and can be cultured with ease in vitro and thus are more frequently encountered. Our study showed that the bamboo rhizosphere was found to be a rich source of the predominant genus *Pseudomonas*. Dominance of this genus in the root zones of various crops was also been reported by Agrawal et al (2015).

The ability of PGPR strains to solubilize insoluble P and convert it to plant-available form is an important characteristic under conditions where P is a limiting factor for crop production. In

general, out of the 96 isolates tested, only six were able to show P solubilization. This very low percentage of isolates capable of P solubilization ability in this study is comparable with Hameeda et al (2006) and Islam et al (2010) who reported 5% isolates capable of P solubilization in pearl millet and 23% in paddy field. The PSB rhizobacteria solubilize inorganic phosphates by several mechanisms like the production of organic acids (gluconic acid, ketogluconic acid, oxalic acid and succinic acid) (Vazquez *et al.* 2000), polysaccharides (Goenadi *et al.* 2000) and phosphatase enzymes mainly acid phosphatase (Rodriguez *et al.* 2000). PGPR convert the insoluble phosphorus into a soluble form which can be utilized by plants. PSB such as Azospirillum, Bacillus, Burkholderia, Erwinia, *Pseudomonas*, Rhizobium and Serratia convert insoluble phosphates into soluble form through the process of acidification, chelation, exchange reactions and production of gluconic acid (Pérez-Montaño *et al.* 2014). The P solubilizing microbial population from soil can be utilized as biofertililzer for enhancing crop productivity in view of sustainable agriculture. The presence of P solubilizing microbial population in soil may be considered a positive indicator of utilizing the microbes as biofertilizers for crop production and beneficial for sustainable agriculture.

One of the most commonly reported mechanisms of PGPR is the production of phytohormones such as IAA (Patten & Glick 2002). All the selected six isolates in our study produced IAA. Similar studies have shown that IAA production is very common among PGPR (Zahid *et al.* 2015). The amount of IAA produced by our rhizobacterial isolates was substantially lower than that reported earlier from other regions (Ali *et al.* 2008) but higher to that of reported by Zahid *et al.* (2015). However, it has been reported that the amount of indole compounds produced in vitro depends on the particular bacterial species, strain, or the conditions of the culture such as oxygenation and pH (Radwan *et al.* 2002). The variation among PGPR to produce IAA as found in the present study had also been reported earlier (K.C. *et al.* 2020 & 2021). This variation is attributed to the various biosynthetic pathways, location of the genes involved, regulatory sequences, and the presence of enzymes to convert active free IAA into conjugated forms (Patten & Galick 1996).

Treatment of bamboo seeds with rhizobacterial isolates significantly improved seedling emergence, and growth and development of bamboo seedlings. PGPR might indirectly enhance seed germination by reducing the incidence of seed mycoflora which can be detrimental to plant growth (Begum *et al.* 2003). Chin-A-Woeng *et al.* (2000) reported that the ability of *Pseudomonas* isolates to suppress disease relies mainly on their ability to colonise roots. The positive impact of PGPR is to foster the crop growth and yield (Karnwal 2019). Fatima *et al.* (2009) also proved that seed germination rate, growth of roots and wheat shoot were increased by inoculating bacterial IAA and PGPR. PGPR increases chlorophyll biosynthesis in plant leaves, enhancing the rate of photosynthesis (Nadeem *et al.* 2009). Our rhizobacterial isolates significantly increased the content of total chlorophyll' a', chlorophyll 'b' and carotenoid in comparison to negative control. Ahmed and Husnain (2010) conducted a similar kind of experiment in which they documented that Solanum tuberosum treated with *B.* flexus (Amb7) exhibited 26% accretion in chlorophyll' a', 82% in chlorophyll 'b' and no effect in carotenoid concentration. Sandeep *et al.* (2011) reported higher chlorophyll content (1.70 mg/g) from Lactuca sativa treated with *B.* megateriium.

5. CONCLUSION

In this study some PSB and APB rhizobacteria were isolated from bamboo rhizosphere and their potential in growth of bamboo seedlings was assessed. During the seed germination and greenhouse pot assay experiment, rhizobacterial

isolates and positive control (*Pseudomonas*) demonstrated notable higher growth performance of bamboo seedlings than those treated in negative controls. The present findings establish the potential for using efficient indigenous PSB and APB as reliable alternative source for production of biofertilisers. There is a need for identifying potential new PGPR from different bamboo rhizosphere in various geographical locations as this microbiota has the potential benefits in higher crop productivity, pathogen control and soil remediation. Rhizospheric microbial sources offer better prospects in terms of commercial production of biofertilizers.

ACKNOWLEDGEMENTS

Our sincere thanks go to the President Chure Tarai Madhesh Conservation and Development Board (KU/CHURE/PROJECT/01) for financial support and Organic Farming and Natural Product Research Centre (ONRC), Kathmandu University for laboratory support.

REFERENCES

Agrawal, P.K., R. Kundan, and S. Agrawal, 2015. Characterisation of *Pseudomonas* sp from rhizosphere of tomato plants (*Lycopersicon esculentum*) and its efficacy on plant growth promotion. *Journal of Biological and Scientific Opinion* 3: 114–121.

Ahmed, A., and S. Hasnain, 2010. Auxin-producing Bacillus sp.: Auxin quantification and effect on the growth of *Solanum tuberosum*. Pure and Applied Chemistry 82: 313-319.

Akhtar, M., S.*B.* Chali, and T. Azam, 2013. Bioremediation of arsenic and lead by plants and microbes from contaminated soil. *Research in Plant Sciences* 1: 68-73.

Ali, *B.*A.N. Sabri, K., Ljung, and S. Hasnain, 2009. Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of *Triticumaestivum* L. Letters in appliedmicrobiology 48: 542-547.

Andy, A.K., S.A. Masih, and V.S. Gour, 2020. Isolation, screening and characterisation of plant growth promoting rhizobacteria from rhizospheric soils of selected pulses. *Biocatalysis and Agricultural Biotechnology* 27, 101685.

Asghar, H., Z. Zahir, M. Arshad, and A. Khaliq, 2002. Relationship between in vitro production of auxins by rhizobacteria and their growth-promoting activities in *Brassica juncea*L. Biology and Fertility of Soils 4 : 231-237.

Baon, J. B., S. Wedhastri, and A. Kurniawan, 2012. The ability of phosphate solubilising bacteria isolated from coffee plant rhizosphere and their effects on Robusta coffee seedlings. Journal of Agricultural Science and Technology1064.

Barillot, C. D., C. O. Sarde, V. Bert, E. Tarnaud, and N. Cochet, 2013. A standardised method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. Annals of microbiology 63: 471-476.

Begum, M., V. R. RAI and S. Lokesh, 2003. Effect of plant growth promoting rhizobacteria on seed borne fungal pathogens in okra. Indian phytopathology.56:156-158

Bhattacharya, P. N., and D. K. Jha, 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology* 28: 1327-1350.

Chin-A-Woeng, T.F., G. V. Bloemberg, I.H. Mulders, L.C. Dekkers, and *B.*J. Lugtenberg, 2000. Root colonisation by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato foot and root rot. Molecular plant-microbe interactions 13: 1340-1345.

Compant, S. B., Duffy, J. Nowak, C. Clément, and E.A. Barka, 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Applied and environmental microbiology 71: 4951-4959.

Emami, S. H. A. Alikhani, A. A. Pourbabaei, H. Etesami, F. Sarmadian and B. Motessharezadeh, 2019. Assessment of the

potential of indole-3-acetic acid producing bacteria to manage chemical fertilisers application. International Journal of Environmental Research 13: 603-611.

Fatima, Z., M. Saleemi, M. Zia, T. Sultan, M. Aslam, R. Rehman, and M. F. Chaudhary, 2009. Antifungal activity of plant growth-promoting rhizobacteria isolates against Rhizoctonia solani in wheat. African *Journal of Biotechnology* 8: 219-225.

Garrity, G. M., J. A. Bell, and T. G. Lilburn, T. G. 2004. Taxonomic outline of the prokaryotes. Bergey's Manual of Systematic Bacteriology New York.

Glick, B. R., 2012. Plant growth –promoting bacteria mechanisms and application. Scientifica

Goenadi, D. H. and Y. Sugiarto, 2000. Bioactivation of poorly soluble phosphate rocks with a phosphorus-solubilising fungus. *Soil science society of America journal* 64: 927-932.

Goswami, D., J. N. Thakker, P. C. Dhandukia, and M. Tejada Moral, 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. *Cogent Food Agriculture* 1:19

Haas, D. and G. Défago, 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nature reviews microbiology 3: 307-319.

Hameeda, B., O. P. Rupela, G. Reddy, and K. Satyavani, 2006. Application of plant growth-promoting bacteria associated with composts and macrofauna for growth promotion of Pearl millet (*Pennisetumglaucum* L.). *Biology and Fertility of Soils* 43: 221-227.

Hayat, R., S. Ali, U. Amara,R. Khalid, and I. Ahmed, 2010. Soil beneficial bacteria and

their role in plant growth promotion: A review. Annals of Microbiology60: 579–598.

Hiscox, J. D., and G. F. Israelstam, 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* 57: 1332-1334.

Hussain, A. and S. Hasnain, 2011. Interactions of bacterial cytokinins and IAA in the rhizosphere may alter phytostimulatory efficiency of rhizobacteria. *World Journal of Microbiology and Biotechnology* 27: 2645-2654.

Islam M. R., M. Madhaiyan, H. P. D. Boruah, W. Yim, G. Lee, V. S. Saravanan, Q. Fu, H. Hu and T. Sa, 2010. Characterisation of plant growth-promoting traits of free-living diazotrophic bacteria and their inoculation effects on growth and nitrogen uptake of crop plants. *Journal of Microbiology and Biotechnology* 19:1213–1222.

Islam, S., A. M Akanda., A. Prova., M. T. Islam, and M. M. Hossain, 2016. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Frontiers in microbiology 6: 1-12.

K.C., B. M., D. P Gauchan, S. N. Khanal, S. Chimouriya and J. Lamichhane, 2020. Extraction of Indole-3-acetic Acid from Plant Growth Promoting Rhizobacteria of Bamboo Rhizosphere and Its Effect on Biosynthesis of Chlorophyll in Bamboo Seedlings. *Indian Journal of Agricultural Research* 54:781-786

K. C., B. M., D. P. Gauchan, S. N. Khanal, S. Chimouriya and J. Lamichhane, 2021. Amelioration of growth attributes of *Bambusa nutans* subsp. *cupulata* Stapleton by indole-3-acetic acid extracted from newly isolated *Bacillus mesonae* MN511751 from rhizosphere of *Bambusatulda* Roxburgh. Biocatalysis and Agricultural Biotechnology 13: 101920.

Kang, B. G.,W. T. Kim, H. S. Yun and S. C. Chang, 2010. Use of plant growth-promoting rhizobacteria to control stress responses of plant roots. Plant Biotechnology Reports 4: 179-183.

Karnwal, A., 2019. Production of indole acetic acid by Kocuriarosea VB1 and Arthrobacterluteolus VB2 under the influence of L-tryptophan and maise root exudates. BioTechnologia 100: 29-35.

Khan, M. S., A. Zaidi, P. A. Wani and M. Oves, 2009. Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environmental Chemistry Letters 7: 1-19.

Khan, M. S., A. Zaidi and P. A. Wani 2007. Role of phosphate-solubilising microorganisms in sustainable agriculture—a review. Agronomy for Sustainable Development 27: 29-43.

Khare, E. and N. K. Arora, 2010. Effect of indole-3-acetic acid (IAA) produced by *Pseudomonas aeruginosa* in suppression of charcoal rot disease of chickpea. Current Microbiology 61: 64-68.

Kunwar, V. S., S. Chimouriya, J. Lamichhane, and D. P. Gauchan, 2018. Isolation and Characterisation of Phosphate Solubilizing Bacteria from Rhizosphere of Coffee Plant and Evaluating Their Effects on Growth and Development of Coffee Seedlings. BioTechnology: *An Indian Journal* 14: 176-180

Lichtenthaler, H. K. and C. Buschmann, 2001. Chlorophylls and carotenoids: Measurement and characterisation by UV-VIS spectroscopy. Current protocols in food analytical chemistry 1:, F4-3.

Long, H. H., D. D. Schmidt and I. T. Baldwin, 2008. Native bacterial endophytes promote host growth in a species-specific manner; phytohormone manipulations do not result in common growth responses. PLoS One 3: 1-10

Lugtenberg, B., N. Malfanova, F. Kamilova, and G. Berg, 2013. Microbial control of plant diseases.In: Bruijn (ed) Molecular Microbial Ecology of the Rhizosphere, Wiley-Blackwell Publishing 2:575-586

McKenzie, R. H., and T. L. Roberts, 1990. Soil and fertilisers phosphorus update. In Proceedings of Alberta Soil Science Workshop Proceedings,pp20-22\

Nadeem, S. M., Z. A. Zahir, M. Naveed and M. Arshad, 2009. Rhizobacteria containing ACC-deaminase confer salt tolerance in maise grown on salt-affected fields. *Canadian journal of microbiology* 55: 1302-1309.

Pathak, R., V. Paudel, A. Shrestha, J. Lamichhane, D. P. Gauchan, 2017. Isolation of phosphate solubilising bacteria and their use for plant growth promotion in tomato seedling and plant. *Kathmandu University Journal of Science, Engineering and Technology* 13: 61-70.

Patten, C. L., and B. R. Glick, 2002. Regulation of indoleacetic acid production in *Pseudomonasputida* GR12-2 by tryptophan and the stationary-phase sigma factor RpoS. *Canadian journal of Microbiology* 48: 635-642.

Patten, C. L., and B. R. Glick, B. R. 1996. Bacterial accumulation of indole-3-acetic acid. Canadian JournalofMicrobiology, 42: 207-220.

Pérez-Montaño, F., C.Alías-Villegas, R. A. Bellogín, P. Del Cerro, M. R. Espuny, I. Jiménez-Guerrero, F. J. Ollero and T. Cubo, 2014. Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiological Research, 169: 325-336.

Qessaoui, R., R. Bouharroud, J. N. Furze, M. El Aalaoui, H. Akroud, A. Amarraque, and B. Chebli, 2019. Applications of new rhizobacteria *Pseudomonas* isolates in agroecology via fundamental processes complementing plant growth. Scientific Reports 9: 1-10.

Radwan, T. E. S. E. D., Z. K. Mohamed and V. Reis, 2002. Production of indole-3-acetic acid by different strains of *Azospirillum* and *Herbaspirillum* spp. Symbiosis.

Ramakrishna, W., R. Yadav and K. Li, 2019. Plant growth promoting bacteria in agriculture: two sides of a coin. Applied Soil Ecology 138: 10-18.

Raza, W., N. Ling, L. Yang, Q. Huang, and Q. Shen, 2016. Response of tomato wilt pathogen Ralstoniasolanacearum to the

volatile organic compounds produced by a biocontrol strain *Bacillus amyloliquefaciens* SQR-9. Scientific Reports 6: 1-13

Ruangsanka, S., 2014. Identification of phosphate-solubilising bacteria from the bamboo rhizosphere. Science Asia 40: 204-211.ss

Sandeep, C., M. S. Tejus, P. Sanjeev, G. Tejaswini, R. V. Raman, M. Radhika, C. K. Suresh, and S. R. Mulla, 2011. Growth response of Ayapana on inoculation with *Bacillus megaterium* isolated from different soil types of various agroclimatic zones of Karnataka. *Journal of Phytology* 3: 13-18.

Spaepen, S. and J. Vanderleyden, 2011. Auxin and plant-microbe interactions. Cold Spring Harborperspectives in Biology 3:a001438.

Tripura, C. B., B. Sashidhar, and A. R. Podile, 2005. Transgenic mineral phosphate solubilising bacteria for improved agricultural productivity. In: T.Satyanarayan and B. N. Johri (eds.) Microbial Diversity Current Perspectives and Potential Applications, New Delhi India. I.K. International Pvt.Ltd.pp. 375-392.

Vazquez, P., G. Holguin, M. E. Puente, A. Lopez-Cortes and Y. Bashan, 2000. Phosphate-solubilising microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biology and Fertility of Soils 30: 460-468.

Zahid, M., M. K. Abbasi, S. Hameed, and N. Rahim. 2015. Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maise (*Zea mays* L.). Frontier Microbiology 6:207

Zaidi, A., M. Khan, M. Ahemad and M. Oves, 2009. Plant growth promotion by phosphate solubilising bacteria. Acta Microbiologica Etimmunologica Hungarica, 56:263-284.