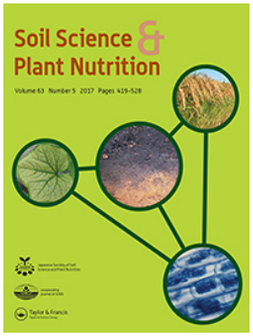

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ORIGINAL ARTICLE

Enhancement of maize plant growth with inoculation of phosphate-solubilizing bacteria and biochar amendment in soil

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ABSTRACT

Maize plant has an absolute requirement of nutrients (N, P, and K) for growth and development. The microbial application can facilitate in addressing limited access to chemical fertilizer concern. Moreover, biochar and phosphorus-solubilizing bacterial (PSB) community can contribute together in nutrient availability. Both have the P-supply potential to the soil, but their interaction has been tested less under semiarid climatic conditions. The purpose of the study was to evaluate the potential of biochemically tested promising PSB strains and biochar for maize plant growth and nutritional status in plant and soil. Therefore, two isolated PSB strains from maize rhizosphere were biochemically tested *in vitro* and identified by 16S rDNA gene analysis. The experiment was conducted in the greenhouse where the plant growth and nutrient availability to the plants were observed. In this regard, all the treatments such as PSB strain-inoculated plants, biochar-treated plants, and a combination of PSBs + biochar-treated plants were destructively sampled on day 45 (D_{45}) and day 65 (D_{65}) of sowing with four replications at each time. PSB inoculation, biochar incorporation, and their combinations have positive effects on maize plant height and nutrient concentration on D_{45} and D_{65}. In particular, plants treated with sawdust biochar + *Lysinibacillus fusiformis* strain 31MZR inoculation increased N (32.8%), P (72.5%), and K (42.1%) against control on D_{65}. Besides that, only *L. fusiformis* strain 31MZR inoculation enhanced N (23.1%) and P (61.5%) than control which shows the significant interaction of PSB and biochar in nutrient uptake. PSB and biochar have the potential to be used as a promising amendment in improving plant growth and nutrient absorption besides the conventional approaches.

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KEY WORDS
Bacillus subtilis; *Lysinibacillus fusiformis*; phosphorus; nitrogen; potassium

1. Introduction

In Pakistan, maize variety consumption is increasing on a regular basis. To properly exploit the potential of current maize plant varieties regarding profitability, the addition of chemical fertilizers and other growth-promoting inputs is inevitable. Considering the low phosphorus (P) status in Pakistani soils (80–90% soils are deficient) (Ahmad and Rashid 2004), it is of basic importance to make P application essential in the rhizosphere. Hence, application of chemical fertilizer-P may be used efficiently to overcome yield gaps. But the application of fertilizer-P demands high cost as most of the P makes a complex with calcium (Ca), aluminum (Al), and iron (Fe) which make it unavailable for plant uptake (Herrera *et al*. 2016). In undisturbed natural soil, a considerable amount of P (400–1200 mg kg^{-1}) is present (Rodríguez and Fraga 1999). Besides that, the addition of chemical fertilizer accumulates a large proportion of insoluble P in the form of a complex with Ca/Mg carbonates in alkaline soil, while for acidic pH soil, Al/Fe mineral complexes are formed. Organic forms of P may constitute 30–50% of the total phosphorus in most soils (Rodríguez and Fraga 1999). Currently, shifting the insoluble proportion of P into the soluble pool is a key objective in sustainable agriculture by adopting all possible soil management protocols and optimizing the P-availability for plant growth with minimum losses from soil. For mining of P-minerals, phosphorus-solubilizing bacteria (PSB) and phosphorus-solubilizing fungi constitute about 1–50% and 0.1–0.5% of soil biota, respectively (Sharma *et al*. 2013; Zaidi *et al*. 2009). Soil biota contribution got the attention of researchers for plant growth promotion (PGP) and yield enhancement due to P solubilization potential (Chen *et al*. 2006; Fasim *et al*. 2002). The indigenous PSB in combination with chemical fertilizer (superphosphate and rock phosphate) reduces dose requirement by 25–50% (Sundara *et al*. 2002). Some studies reported that inoculation of PSB can solubilize the Ca/Mg/Al/Fe-bound P which becomes available to the plant roots for growth enhancement and root proliferation (Liu *et al*. 2016). The release of enzymes and other chemicals by PSB (organic acids, phosphorus-solubilizing enzymes, phytase, and siderophore) breaks down the bond between P and the fixing element to make it available for the plant uptake (Hayes *et al*. 2000). Several bacteria such as *Azospirillum, Azotobacter, Bacillus, Burkholderia, Pseudomonas, Klebsiella*, etc., have been identified as phosphate solubilizers in soil and phytohormone producers in maize plant (Sharma *et al*. 2013).

Conventional farming and intensive use of the chemical fertilizers developed a one-way movement of the nutrients. They are being applied to the soil where most of them are fixed in the soil and become unavailable for plant uptake. An available form of the fertilizer is taken by plant and fixed in

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biomass. Whereas its recycling from agricultural residues is need of the time for enhancing soil quality and agricultural sustainability (Cordell *et al*. 2009; Metson *et al*. 2014). Maximum utilization of P under minimum loss can be achieved through biochar application to the soil.

Biochar is a valuable by-product of pyrolytic biomass during generation of biofuel. It is a potential source of P recycling from the agricultural wastes to enrich the soil quality. As a new area of research, biological and chemical effects on the P release from biochar still need further investigation (He *et al*. 2014). Biochar production and its application as soil amendment achieved promising results for crop production (Dickinson *et al*. 2015; Ortas 2016; Changxun *et al*. 2016), soil quality improvement (Fang *et al*. 2016), biochemical property enhancement to facilitate soil biota (Puga *et al*. 2015; Hairani *et al*. 2016), mitigation of climate change effects in a long run (Smith 2016), and disposal of large-scale waste biomass (Jeffery *et al*. 2015). Conventional scheme of using agricultural waste as a soil amendment in the recycling of fixed P is supposed to be less effective than biochar-routed P cycling (Dai *et al*. 2016).

However, currently, available information on the use of PSB and biochar together in association with maize plant for nutrient uptake is still limited. Here, the study aims to clarify the effects of plant residue-based biochar and PSB inoculation on P recycling for maize plant growth response. It was hypothesized that combined PSB and plant-based biochar could increase nutrient availability to plants.

2. Materials and methods

2.1. Biochemical characterization of bacteria

Rhizospheric bacteria were isolated from 1-g soil tightly adhering to the root by serial dilution plating on Luria-Bertani (LB) agar plates (Somasegaran and Hoben 1994). The soil was mixed by shaking for 20 min to separate microorganisms completely from the soil in autoclaved dispersion flasks. The plates were incubated at 28 ± 2°C till the appearance of bacterial colonies. Individual colonies were picked and streaked on LB plates for further purification. Isolated bacterial strains were biochemically characterized by respective methods described here. Catalase activity was determined by obtaining a bacterial culture from LB medium incubated for 24 h, and few drops of H_2O_2 (30%) were added to a glass slide. Oxygen bubble formation indicated the catalase activity (Schaad *et al*. 2001). To determine oxidase activity, Kovacs oxidase reagent was employed (one to two drops) in 24-h-old culture on a small filter paper. Change in color (dark purple) of filter paper in 60–90 min showed bacterial positivity for oxidase. Phosphate solubilization was examined on Pikovskaya's medium (Pikovskaya 1948) where the bacterial colony was spotted in the center of plates which had tricalcium phosphate [$Ca_3(PO_4)_2$] as the insoluble phosphate source. After 7 days incubation at 28 ± 2°C, halos formation confirmed the P-solubilization activity of bacteria. N-fixation quality of the bacteria was determined by incubation at 28 ± 2°C on nitrogen-free media for 3 to 4 days (Okon *et al*. 1977). Growth exhibition on media confirmed nitrogen fixation quality of bacteria. Nutrient gelatin stabs were inoculated and incubated at 25°C for 1 week; liquefication of gelatin shows the positivity of gelatinase in bacteria (Wood and Krieg 1994). In the determination of gelatin hydrolysis, milk agar was prepared and inoculated with bacteria. Clear zone formation indicated the hydrolysis of casein (Smith *et al*. 1952). Simmon's citrate broth was used for the citrate utilization test, whereas Christensen's agar was used with incubation for 4 days (Christensen 1946; Graham and Hodgkiss 1967). Indole acetic acid (IAA) was measured through a colorimetric method on a spectrophotometer using ferric chloride–perchloric acid reagent ($FeCl_3$–$HClO_4$) by drawing a standard curve (Gordon and Paleg 1957). Sulfide Indole Motility agar medium tubes were inoculated with both bacteria, incubated for 48 h at 37°C to determine the hydrogen sulfide production (Clarke 1953).

2.2. Bacterial strain characterization and genetic identification

Total genomic deoxyribonucleic acid (DNA) of bacterial strains was extracted using Bacterial Genomic DNA Purification Kit (GM Biolab Co, Ltd., Taichung, Taiwan) according to the supplier's instructions and used as DNA template in polymerase chain reaction (PCR) for amplification of the 16S rDNA gene as previously reported protocol (Araújo *et al*. 2002). The DNA purity was quantified at 260 and 280 nm using NanoDrop Spectrophotometer (ND 1000, Thermo Fisher Scientific Inc., Waltham, MA, USA), 1.6–2.2, to detect protein contamination in the DNA (Calvo *et al*. 2001). PCR amplification was performed in a reaction mixture containing 3 µl of 10× buffer, 2.4 µl of deoxyribonucleotide triphosphate (dNTP)s mixture (2.5 mM of each dNTP), 0.6 µl (20 pmol μl^{-1}) of each primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TTCAGCATTGTTCCATCGGCA-3′) (Weisburg *et al*. 1991), 0.18 µl of One Taq DNA Polymerase (Thermo Fisher Scientific), and 1.8 µl of template DNA. The PCR program (Bio-Rad DNA Engine, Hercules, CA, USA) started with an initial denaturation step for 5 min at 95°C followed by 40 amplification cycles of denaturation at 95°C for 30 s, annealing at 59°C for 30 s, and 1 min extension at 72°C. Before cooling to 4°C, final extension period of 5 min at 72°C was incorporated into the program (Branco *et al*. 2005). The suitability of DNA amplification was visualized by electrophoresis of PCR products with 6X loading dye (Thermo Scientific™) at 5:1 (PCR product:dye) ratio and a marker (1 kb DNA ladder, Fermentas GeneRuler™) in 1% (w/v) agarose gel in 1X Tris-acetate EDTA buffer for 1 h at 80 V. The agarose gel was stained with GelRed™ (Biotium Inc., Hayward, CA, USA) for 40 min and examined under UV light in a UV transilluminator (Bio-RadMolecular Imager Gel Doc™ XR+ System, Bio-Rad, Hercules, CA, USA). Gel image was captured with Image Lab software (Version 4.1, Bio-Rad Laboratories, Segrate, Italy). The sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) Sequence Similarity Search to identify the most closely related members in the National Center for Biotechnology Information (NCBI) GenBank DNA database (www.ncbi.nlm.nih.gov/geo). The partial 16S rDNA sequences of the phosphorus-solubilizing strains were submitted to the NCBI database under their respective accession number as follows: *Bacillus subtilis* strain 18MZR (KX710213) and *Lysinibacillus fusiformis* strain 31MZR (KX710214).

2.3. Phylogenetic analysis

The partial 16S rDNA sequences of both strains were aligned with the closely related bacterial sequences obtained from the NCBI database using Multiple Sequence Comparison by Log-Expectation (Edgar 2004). The neighbor-joining phylogenetic tree was constructed after calculation of a maximum composite likelihood method from distance matrix using the Molecular Evolutionary Genetic Analysis 4.0 software by the method of Kimura two-parameter model with a discrete gamma distribution (Tamura *et al*. 2007).

2.4. Biochar preparation and analysis

The woody sawdust was collected from a local sawmill in Rawalpindi, Pakistan. The wood chips were ground and sieved. Bagasse was collected from sugar mill, i.e., Frontier Sugar Mill Thaktbhai Mardan. The obtained material was passed through a 50-mesh screen to move large lumps. Particle size was reduced to 0.7–0.8 nm, and it was dried at 110°C for 24 h and stored in a container before initial characterization. The samples were pyrolyzed from room temperature to the 350°C temperature using a heating rate of 10°C min^{-1}. The process was carried out in a closed muffle furnace with an outlet for the gases release (Sánchez *et al*. 2009). Biochar samples were prepared at 350°C with a residence time of 1 h and obtained samples were properly labeled. The prepared biochar samples were characterized; pH and electrical conductivity (EC) of biochar were also measured according to Novak *et al*. (2009). To measure pH and EC, 2 g of biochar shaken with 40 ml DI water for 30 min. and the sample was allowed to settle for 15 min before recording pH and EC. The cation exchange capacity (CEC) of biochar was measured by ammonium acetate (NH_4OAC) extraction method (Song and Guo 2012; Melo *et al*. 2013) (Table 1).

2.5. Setting pot experiment and plant-soil analyses

The experimental soil (Nabipur soil series, Fine-loamy mixed hyperthermic Udic Ustochrept) was collected from 0 to 15 cm soil depth at the National Agricultural Research Centre located (33° 43′ 11.9784″ N, 73° 5′ 45.7764″ E) at an altitude of 518 m above sea level in Islamabad. The soil collected from the research field area was air-dried, sieved (2 mm mesh), and analyzed for its physicochemical properties. The soil had 15% clay and 45% silt, and texture was loamy with 3.1% $CaCO_3$, whereas pH 8.34 (1:1, soil:water ratio; MeterLab® PHM210, Radiometer Pacific Limited, Copenhagen, Denmark) (McLean 1982); nitrate-N (ammonium bicarbonate-diethylenetriaminepentaacetic acid [AB-DTPA] extractable) (Soltanpour 1985), 3 mg kg^{-1}; organic carbon (Walkley 1947), 4.9 g kg^{-1}; available P (Soltanpour 1985), 1.9 mg kg^{-1}; and exchangeable K (Soltanpour 1985), 120 mg kg^{-1}. The soil was autoclaved at 121°C for 20 min; exactly 3 kg of the soil was weighed and placed in each pot (21 cm, D × 18 cm, H). Uniform doses of urea (46% N), diammonium phosphate (18% N and 46% P_2O_5), and Muriate of Potash (60% K_2O) at the recommended rates of 160 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, and 60 kg K_2O ha^{-1} equivalents (NARC 2017) were applied to each pot, respectively. The experiment was conducted in a randomized complete block design, with two harvests at 45 (D_{45}) and 65 (D_{65}) days after planting with four replications at both stages (eight replications in total). The nine treatments were executed: (i) control (C) (uninoculated and untreated), (ii) 1% bagasse biochar (BC-1) addition (equals to 30 g for 3 kg soil), (iii) 1% sawdust biochar (BC-2) addition, (iv) *B. subtilis* strain 18MZR inoculation (B1), (v) *L. fusiformis* strain 31MZR inoculation (B2), (vi) *B. subtilis* strain 18MZR + 1% bagasse biochar (M1), (vii) *L. fusiformis* strain 31MZR + 1% bagasse biochar (M2), (viii) *B. subtilis* strain 18MZR + 1% sawdust biochar (M3), and (ix) *L. fusiformis* strain 31MZR + 1% sawdust biochar (M4). Biochar was thoroughly mixed with the soil before seed sowing for each treatment.

The PSB inoculum was grown in LB media for 24 h (200 rpm, 26 ± 2°C), and cell suspensions were adjusted to OD_{600} between 1.4 and 2.0, which corresponded to the total plate counts of 10^9 cfu ml^{-1}, as determined on the LB media agar. Five maize seeds of similar size and shape, var. Islamabad Gold, were added in each pot and thinned to two per pot at tenth day after sowing. Each pot with PSB inoculation treatment was injected with 15 ml of respective inoculum on D_0 to the seed and D_{10} in the rhizosphere after thinning. The pots were watered every second day to maintain at 80% of field capacity throughout the experiment to avoid any possible loss of applied fertilizer through denitrification in excessive moisture.

Leaves of the plant were harvested at D_{45} and D_{65}. They were dried and ground to determine N by Kjeldahl method (Van Schouwenburg and Walinga 1975) using a UDK 142 Automatic Distillation Unit (VELP Scientifica, Milan, Italy) and P by ammonium molybdate-vanadate solution by reading on spectrophotometer (Hitachi U-1500, San Jose, CA, USA) (Isaac and Johnson 1975), and wet digested filtrate was directly used to determine K by Flame Photometer (Jenway PFP7, Jenway, UK). Representative soil samples of 200 g from the rhizosphere were collected by removing all the soil + root from a pot into a tray and shake gently. The soil surrounding the roots was collected in a separate bag, air-dried, and sieved (2 mm mesh). Soil NO_3-N, P, and K by AB-DTPA extraction were analyzed, and pH was also determined.

Table 1. Properties of biochar used in study.

Elements	Sawdust biochar	Bagasse biochar
Yield (%)	56	49
N (%)	4.6	4.1
P (%)	1.6	1.1
K (%)	2.14	1.7
pH	8.1	7.9
EC (dS m^{-1})	0.8	1.2
CEC $cmol_c$ kg^{-1}	35	27
Ash (%)	2.9	3.2

2.6. Statistical analysis

Data were analyzed using one-way analysis of variance procedure followed by Duncan Multiple Range Test at $p < 0.05$ using Statistical Analysis System software (SAS version 9.0) (Robert *et al*. 1997). Pearson's correlation of coefficient test was performed to estimate the relationships between measured parameters of plant and soil at both stages of harvesting.

3. Results

3.1. PSB strain characterization

Phosphorus-solubilizing ability of two selected strains and biochemical characteristics are shown in Table 2. Bacterial strains gave positive reactions for phosphorus solubilization and nitrogen fixation. Both strains *B. subtilis* strain 18MZR and *L. fusiformis* strain 31MZR showed the clear halo zone formation around their bacterial colonies grown on the Pikovskaya media, indicating phosphate solubilization abilities.

3.2. Molecular characterization of PSB

Both PSB strains were identified by 16S rRNA gene sequence. Based on the sequences of strains strain 18MZR and strain 31MZR, BLAST search results showed that the strains are more closely related to the species of genus *Bacillus* and *Lysinibacillus* (Fig. 1) with 96% sequence similarity in both strains. The sequence analysis showed that both strains showed less similarity values (96%) with previously characterized validly published species. Strain 18MZR and strain 31MZR clustered together and belonged to the genus *Bacillus* and *Lysinibacillus*, respectively. The bacterial strains were indicated to belong to same phylum and two different genera of bacteria, and they could be (i) *B. subtilis* strain 18MZR and (ii) *L. fusiformis* strain 31MZR.

3.3. Plant height

Biochar addition to the soil increased plant growth, and a significant increase was observed in sawdust biochar (BC-2)-amended maize root (43.6 cm) and shoot (55 cm) at D_{45}, while at D_{65}, it was 49.6 and 62 cm, respectively. Inoculation with PSB significantly increased the growth of maize plants at both harvestings (D_{45} and D_{65}); particularly, *L. fusiformis* strain 31MZR-inoculated plant height was significantly increased at D_{65} for root (44.4 cm) and shoot (63.1 cm) (Figs. 2 and 3). The highest increase in root and shoot length (54.2 and 92.4 cm) was observed for sawdust-amended soil with *L. fusiformis* strain 31MZR inoculation (D_{65}). Bagasse biochar also increased root and shoot length on D_{45} (39.8 and 40.3 cm) and D_{65} (45.9 and 52.1 cm) in comparison to the control treatment. Similarly,

Table 2. Biochemical characteristics of bacteria used in study.

Characteristics	Properties: Bacillus subtilis 18MZR	Properties: Lysinibacillus fusiformis 31MZR
Gram staining	+	+
Cell shape	Rod	Rod
Catalase	+	+
Oxidase	+	+
Phosphate solubilization	+	+
Nitrogen fixation	+	+
Hydrolysis of gelatin	+	+
Hydrolysis of casein	+	+
Citrate utilization	+	+
Indole production	+	+
Hydrogen sulfide	–	–
Urease	–	+

(+) Positive results; (–) negative results.

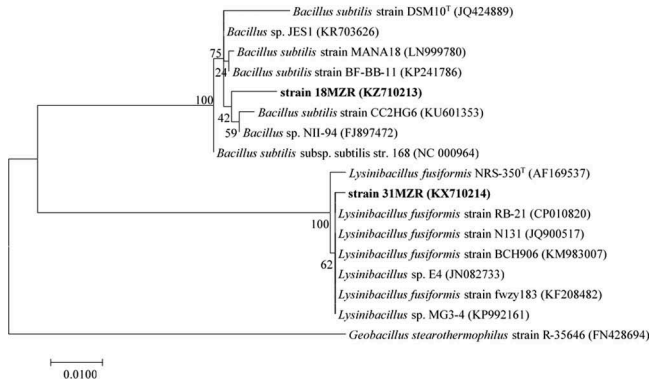

Figure 1. Phylogenetic tree showing interrelationship of strain 18MZR (KX710213) and 31MZR (KX710214) with closely related species of the genus *Bacillus subtilis* and *Lysinibacillus fusiformis*, respectively, inferred from aligned unambiguous sequences of 16S rRNA gene. Numbers at nodes indicate percentages of occurrence in 500 bootstrapped trees. The analysis involved 16 nucleotide sequences. There were a total of 1002 positions. Scale bar, 0.1 substitutions per nucleotide position. Tree was generated by maximum composite likelihood method and was rooted by *Geobacillus stearothermophilus* strain R-35646 (FN428694) as an out-group. Accession number of each type strain is shown in parentheses.

in sawdust biochar-treated soil, the root and shoot length increase was significant on D_{45} (43.6 and 55 cm) and D_{65} (49.6 and 62 cm). Comparatively, only PSB inoculation, such as *B. subtilis* 18MZR on D_{45} (32.5 and 48 cm) and D_{65} (36.7 and 54.1 cm), while *L. fusiformis* strain 31MZR on D_{45} (36.4 and 42.9 cm) and D_{65} (42.7 and 59.2 cm), increased plant growth which is less than the biochar-amended soils. The combination of *L. fusiformis* strain 31MZR with bagasse biochar significantly increased plant height on D_{45} (38.5 and 45 cm) and D_{65} (44.5 and 63.1 cm). When *L. fusiformis* strain 31MZR was inoculated with sawdust biochar, plant height increased on D_{45} (45.3 and 78.8 cm) and D_{65} (54.2 and 92.4 cm). Moreover, *B. subtilis* strain 18MZR inoculation with bagasse biochar increased plant height on D_{45} (37.7 and 39 cm) and D_{65} (41.4 and 52.8 cm), while inoculation with sawdust biochar increased plant height on D_{45} (40 and 60 cm) and D_{65} (42.6 and 68.1 cm).

3.4. Plant nutrient concentration

Biochar-amended soil significantly increased the N concentration in both harvestings on D_{45} and D_{65} (Table 3). Inoculation with *B. subtilis* strain 18MZR and *L. fusiformis* strain 31MZR also significantly increased N concentration on D_{45} (3.7% and 23.1%) and D_{65} (7.7% and 20.1%) in comparison to control. Meanwhile, inoculation with bacteria in biochar-amended soil increased N uptake by D_{45} and D_{65} as *L. fusiformis* strain 31MZR + sawdust biochar (35.4% and 32.8%), followed by *B. subtilis* strain 18MZR + sawdust biochar (25.2% and 23.3%), *L. fusiformis* strain 31MZR + bagasse biochar (21.8% and 19.8%), and *B. subtilis* strain 18MZR + bagasse biochar (19.9% and 17.5%).

Phosphorus concentration in the control was 0.23% (D_{45}) and 0.25% (D_{65}). In biochar-amended treatments, total P was significantly high against control, i.e., bagasse and sawdust biochar at D_{45} (37.8% and 59.6%) and D_{65} (59% and 58.3%), respectively. Application of PSB increases P concentration in the plant; it was significantly increased in *B. subtilis* strain

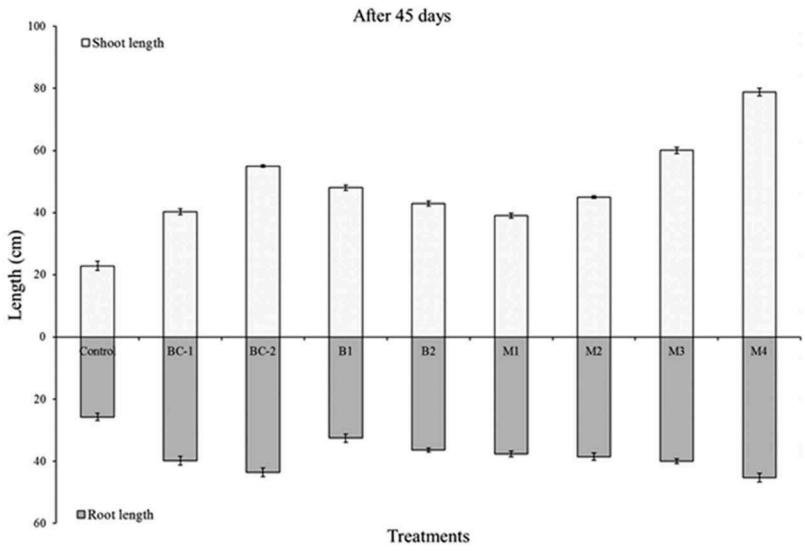

Figure 2. Root and shoot length of maize plant after 45 days harvesting for all treatments: control (uninoculated and untreated); BC-1: bagasse biochar; BC-2: sawdust biochar; B1: *B. subtilis* strain18MZR inoculation; B2: *L. fusiformis* strain 31MZR inoculation; M1: *B. subtilis* strain 18MZR + bagasse biochar; M2: *L. fusiformis* strain 31MZR + bagasse biochar; M3: *B. subtilis* strain 18MZR + sawdust biochar; M4: *L. fusiformis* strain 31MZR + sawdust biochar.

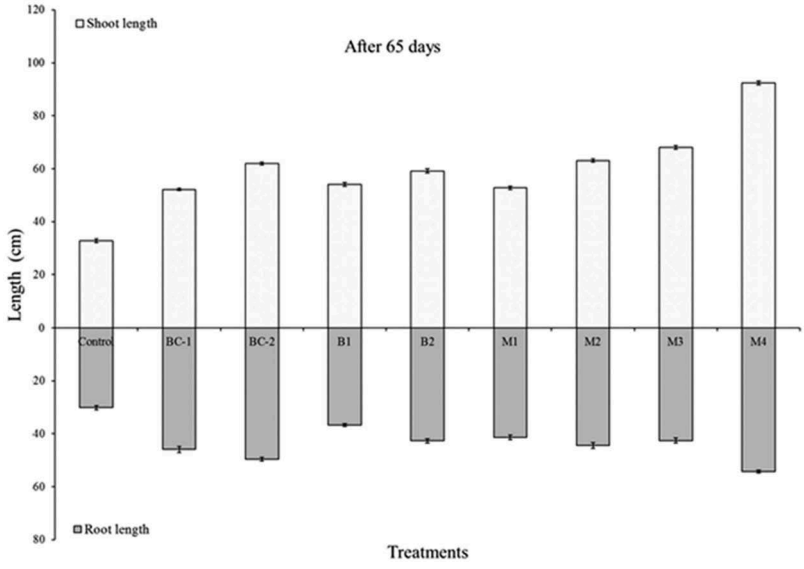

Figure 3. Root and shoot length of maize plant after 65 days harvesting for all treatments: control (uninoculated and untreated); BC-1: bagasse biochar; BC-2: sawdust biochar; B1: *B. subtilis* strain 18MZR inoculation; B2: *L. fusiformis* strain 31MZR inoculation; M1: *B. subtilis* strain 18MZR + bagasse biochar; M2: *L. fusiformis* strain 31MZR + bagasse biochar; M3: *B. subtilis* strain 18MZR + sawdust biochar; M4: *L. fusiformis* strain 31MZR + sawdust biochar.

18MZR (58.2%) on D_{45} and D_{65} (58.3%) and also in *L. fusiformis* strain 31MZR inoculation D_{45} (62.9%) and D_{65} (61.5%) than control plant. When PSB strains were inoculated with biochar, P concentration was highest in all combinations on D_{45} and D_{65} than control, as *B. subtilis* strain 18MZR + bagasse biochar (63.5% and 62.1%), *L. fusiformis* strain 31MZR + bagasse biochar (70.1% and 68.4%), *B. subtilis* strain 18MZR + sawdust biochar (72% and 70.6%), and *L. fusiformis* strain 31MZR + sawdust biochar (73.6% and 72.5%).

Biochar-amended soil significantly increased the K concentration in both harvestings of D_{45} and D_{65}. Inoculation with *B. subtilis* strain 18MZR and *L. fusiformis* strain 31MZR also significantly increased K concentration by D_{45} (1.3% and 17.3%) and D_{65} (1.3% and 17%) in comparison to control. Meanwhile, inoculation with bacteria in biochar-amended soil also increased K uptake by D_{45} and D_{65} as *L. fusiformis* strain 31MZR + sawdust biochar (42.2% and 42.1%), followed by *B. subtilis* strain 18MZR + sawdust biochar (36.8% and 36%), *L. fusiformis* strain 31MZR + bagasse biochar (28.8% and 30%), and *B. subtilis* strain 18MZR + bagasse biochar (23.3% and 23.4%).

3.5. Soil nutrient concentration

Soil N concentration in bagasse biochar-amended soil was 12% (D_{45}) and 8.5% (D_{65}) more than control, while N was observed high in sawdust biochar, amended the soil as

Table 3. Plant nutrient concentration at D_{45} and D_{65} harvesting.

	N (%)		P (%)		K (%)	
	D_{45}	D_{65}	D_{45}	D_{65}	D_{45}	D_{65}
Control	2.37 ± 0.35 e	2.50 ± 0.29 e	0.23 ± 0.03 e	0.25 ± 0.05 e	1.48 ± 0.04 e	1.51 ± 0.05 d
BC-1	2.62 ± 0.51 d-e	2.72 ± 0.55 de	0.37 ± 0.05 d	0.38 ± 0.05 d	1.78 ± 0.12 d	1.82 ± 0.11 c
BC-2	3.34 ± 0.30 ab	3.56 ± 0.19 ab	0.57 ± 0.04 c	0.61 ± 0.03 c	1.89 ± 0.12 d	1.92 ± 0.11 c
B1	2.46 ± 0.52 de	2.71 ± 0.36 de	0.55 ± 0.07 c	0.60 ± 0.06 c	1.50 ± 0.04 e	1.53 ± 0.05 d
B2	3.08 ± 0.08 bc	3.13 ± 0.10 b-d	0.62 ± 0.06 c	0.65 ± 0.05 c	1.79 ± 0.05 d	1.82 ± 0.06 c
M1	2.96 ± 0.18 b-d	3.03 ± 0.17 cd	0.63 ± 0.13 c	0.66 ± 0.11 c	1.93 ± 0.04 cd	1.97 ± 0.04 c
M2	3.03 ± 0.15 bc	3.09 ± 0.15 cd	0.77 ± 0.07 b	0.79 ± 0.07 b	2.08 ± 0.11 c	2.18 ± 0.16 b
M3	3.17 ± 0.13 ab	3.26 ± 0.07 bc	0.82 ± 0.05 ab	0.85 ± 0.06 ab	2.34 ± 0.14 b	2.36 ± 0.13 b
M4	3.67 ± 0.43 a	3.72 ± 0.40 a	0.87 ± 0.06 a	0.91 ± 0.07 a	2.56 ± 0.26 a	2.61 ± 0.28 a

[a–e]Means of different treatments for various parameters.
Treatments: control (uninoculated and untreated); BC-1: bagasse biochar; BC-2: sawdust biochar; B1: *B. subtilis* 18 inoculation; B2: *L. fusiformis* 31 inoculation; M1: *B. subtilis* 18 + bagasse biochar; M2: *L. fusiformis* 31 + bagasse biochar; M3: *B. subtilis* 18 + sawdust biochar; M4: *L. fusiformis* 31 + sawdust biochar.

29.9% (D_{45}) and 27.8% (D_{65}) more. The PSB strains inoculated soil increased N concentration by 9.4% (D_{45}) and 8.1% (D_{65}), whereas 18.6% (D_{45}) and 18% (D_{65}) N was increased in both treatments of *B. subtilis* strain 18MZR- and *L. fusiformis* strain 31MZR-inoculated soil. Highest N concentration was observed in *L. fusiformis* strain 31MZR + sawdust biochar-amended soil with 37.2% (D_{45}) and 36.2% (D_{65}) increased than control.

Similarly, soil available P concentration was increased by 15.2% (D_{45}) and 17.1% (D_{65}) in bagasse biochar-amended soil than control, while for sawdust amendment, it was 24.1% (D_{45}) and 26.6% (D_{65}). PSB inoculation alone solubilized more P by 13.3% (D_{45}) and 22.7% (D_{65}) via *B. subtilis* strain 18MZR and 16.7% (D_{45}) and 25.9% (D_{65}) via *L. fusiformis* strain 31MZR inoculation than control. The combination of *L. fusiformis* strain 31MZR + sawdust biochar-amended soil showed 58.3% more P than control on D_{65}, while in *L. fusiformis* strain 31MZR + bagasse biochar, it was 47.9% (Table 4).

Biochar-amended soil increased the K concentration in both harvestings of D_{45} and D_{65}. The addition of biochar enhanced K concentration is soil, as 29% increase was observed in bagasse-amended soil on D_{45} and D_{65}. Whereas for sawdust biochar-amended soil, it was 47% increase. Meanwhile, inoculation with *B. subtilis* strain 18MZR and *L. fusiformis* strain 31MZR increased K concentration on D_{45} (12.5% and 24%) and D_{65} (11.5% and 25%) in comparison to control. Inoculation with bacteria in biochar-amended soil increased K by D_{45} and D_{65} as *L. fusiformis* strain 31MZR + sawdust biochar (58.3% and 59.5%), followed by *B. subtilis* strain 18MZR + sawdust biochar (53.2% and 54%), *L. fusiformis* strain 31MZR + bagasse biochar (48% and 49%), and *B. subtilis* strain 18MZR + bagasse biochar (37% and 38%).

In the present study, the plant height and nutrient concentration were found positively correlated in all treatments of biochar amendment, bacterial inoculation, and their combination (Tables 5 and 6). Root and shoot height was significantly correlated with phosphorus uptake. In general, the nutrient uptake induces the increase in plant growth.

4. Discussion

In the present study, biochar from two feedstocks (bagasse and sawdust) and two PSB strains (*B. subtilis* strain 18MZR and *L. fusiformis* strain 31MZR) were used as inoculants for plant growth under greenhouse conditions with different combinations of biochar and PSB application. According to 16S rDNA sequence analysis, isolated PSB strains belonged to *Firmicutes: Bacillus* sp. and *Lysinibacillus* sp. (Fig. 1). Similarly, in previous studies, an association of maize plant with specific bacterial genera (*Bacillus* and *Lysinibacillus*) has been reported (Cavaglieri *et al*. 2005; Vigliotta *et al*. 2016). Further studies also showed that *B. subtilis* and *L. fusiformis* are present in soil ecosystem where they interact with plant roots and particularly in maize plant (Posada *et al*. 2016; Singh *et al*. 2013; Zhang *et al*. 2016). Bacterial strains such as *B. subtilis* and *L. fusiformis* have already been reported as effective maize plant growth-promoting rhizobacteria through P-solubilization, and this activity was confirmed by the biochemical test in the present study (Chauhan *et al*. 2016; Sgroy *et al*. 2009). Inoculation with *Bacillus* and *Lysinibacillus* for various crops significantly promoted plant growth resulted in plant height and biomass increase, as observed in the current study, where

Table 4. Soil nutrient concentration at D_{45} and D_{65} harvesting.

	N (mg kg^{-1} soil)		P (mg kg^{-1} soil)		K (mg kg^{-1} soil)	
	D_{45}	D_{65}	D_{45}	D_{65}	D_{45}	D_{65}
Control	7.51 ± 0.67 e	7.40 ± 0.68 e	13.14 ± 1.30 e	12.05 ± 0.63 e	86.63 ± 5.15 f	82.75 ± 2.06 f
BC-1	8.53 ± 1.16 de	8.09 ± 0.89 de	15.50 ± 0.49 de	14.54 ± 0.28 d	122.00 ± 5.77 e	116.400 ± 8.52 e
BC-2	10.71 ± 1.04 ab	10.25 ± 1.07 b	17.32 ± 0.76 c	16.41 ± 1.03 c	166.00 ± 14.35 c	157.50 ± 15.15 c
B1	8.29 ± 1.36 de	8.05 ± 1.37 de	15.16 ± 0.10 de	15.59 ± 0.83 cd	99.00 ± 2.58 f	93.55 ± 3.55 f
B2	9.23 ± 0.27 cd	9.03 ± 0.33 b-d	15.78 ± 0.66 d	16.27 ± 0.60 c	114.00 ± 10.2 e3	110.43 ± 10.37 e
M1	8.79 ± 0.40 de	8.59 ± 0.39 d-e	16.41 ± 0.65 cd	15.62 ± 0.60 cd	138.00 ± 6.48 d	133.90 ± 6.49 d
M2	10.24 ± 0.51 bc	9.78 ± 0.16 bc	17.19 ± 0.68 c	16.68 ± 0.43 c	166.25 ± 5.62 c	161.65 ± 5.55 c
M3	10.70 ± 0.70 ab	10.30 ± 0.71 b	18.73 ± 0.56 b	18.22 ± 0.72 b	185.00 ± 8.37 b	179.95 ± 7.58 b
M4	11.96 ± 1.01 a	11.60 ± 1.01 a	21.53 ± 1.68 a	22.13 ± 1.78 a	207.75 ± 10.78 a	204.54 ± 11.15 a

[a–e]Means of different treatments for various parameters.
Treatments: control (uninoculated and untreated); BC-1: bagasse biochar; BC-2: sawdust biochar; B1: *B. subtilis* 18 inoculation; B2: *L. fusiformis* 31 inoculation; M1: *B. subtilis* 18 + bagasse biochar; M2: *L. fusiformis* 31 + bagasse biochar; M3: *B. subtilis* 18 + sawdust biochar; M4: *L. fusiformis* 31 + sawdust biochar.

Table 5. Pearson's correlation coefficients among plant and soil parameters at D_{45}.

Parameters	Root	Shoot	Plant N	Plant P	Plant K	Soil N	Soil P	Soil K
Root	1.00							
Shoot	0.74**	1.00						
Plant N	0.59	0.63**	1.00					
Plant P	0.62**	0.77**	0.57	1.00				
Plant K	0.66**	0.77**	0.68**	0.76**	1.00			
Soil N	0.65**	0.75**	0.94**	0.66**	0.76**	1.00		
Soil P	0.72**	0.87**	0.59	0.81**	0.82**	0.70**	1.00	
Soil K	0.75**	0.82**	0.69**	0.76**	0.90**	0.81**	0.85**	1.00

Significant at $p < 0.01$**, $n = 36$.

Table 6. Pearson's correlation coefficients among plant and soil parameters at D_{65}.

Parameters	Root	Shoot	Plant N	Plant P	Plant K	Soil N	Soil P	Soil K
Root	1.00							
Shoot	0.82**	1.00						
Plant N	0.66**	0.72**	1.00					
Plant P	0.58	0.82**	0.58	1.00				
Plant K	0.70**	0.83**	0.63**	0.75**	1.00			
Soil N	0.70**	0.82**	0.91**	0.67**	0.76**	1.00		
Soil P	0.73**	0.94**	0.60**	0.87**	0.78**	0.70**	1.00	
Soil K	0.77**	0.86**	0.70**	0.77*	0.91	0.80*	0.80**	1.00

Significant at $p < 0.01$**, $p < 0.005$*, $n = 36$.

inoculation of PSB enhanced plant growth regarding root and shoot length (Chauhan *et al*. 2016; Sgroy *et al*. 2009).

Similarly, biochar addition to the soil may increase soil inorganic nitrogen which assists plant to increase its biomass regarding plant height. Moreover, it improves moisture content in the soil for enhanced nutrient availability, and the current study showed that application of bagasse biochar and sawdust biochar improved soil condition in comparison to the control (Nguyen *et al*. 2017; Chen *et al*. 2010). Biochar made from sawdust feedstock has been reported to assist plant growth by improving soil physicochemical properties such as enhancing nutrient retention up to 59%, and nutrient content of plant, which was also observed in the sawdust-amended soil of the current study which enhanced nutrient concentration in soil and plant than the control (Laghari *et al*. 2016). Biochar addition widely enhances the potential of soil to boost plant growth as observed in the bagasse- and sawdust biochar-amended soil where the plant length was notably increased than control by increasing soil porosity (De Tender *et al*. 2016; Mollinedo *et al*. 2016). According to an estimate, more than 80% of rhizospheric bacteria can produce growth-promoting chemicals and increase in plant height which is endorsed in the PSB-inoculated plants in the current study by enhancement of root and shoot length, particularly the *L. fusiformis*-inoculated maize plant than the control (Arruda *et al*. 2013). The combination of bacteria and biochar for plant growth has been reported as a promising approach against various crops. Similar results are monitored in the present study where the combination of PSB and biochar is more effective to enhance the plant growth than a single application of PSB or biochar. The addition of biochar recruits the microbiome which produces certain compounds in the rhizosphere to make nutrients available for plant growth and biomass production (De Tender *et al*. 2016; Shanta *et al*. 2016), and in this study, a combination of PSB and biochar increased in plant growth. Plant height increase can be attributed to nutrient availability such as N and P due to biochar amendment and PSB inoculation where they may produce various organic compounds in the rhizosphere such as IAA, gibberellin, and cytokinin.

The selected strains showed a positive reaction for P-solubilization on Pikovskaya's agar media, a differing media for the screening of P-solubilizing organisms by halo zone formation (Kaundal *et al*. 2016). This medium contains tricalcium phosphate which is broken down by the activity of PSB into various acids (malic acid, formic acid, citric acid, succinic acid, lactic acid, and tartaric acid), and their chelation capacity implicates major mechanism in the solubilization of inorganic phosphates by microorganisms (Park *et al*. 2009). During screening procedure, clear halo zone formation due to organic acid production was observed for both isolated bacterial strains. In addition, some PSBs such as *B. subtilis* are also reported in N-fixation confirmed by acetylene reduction activity, and it fixes N from the atmosphere (82.9 mg l^{-1}) which is converted into ammonium (Xie *et al*. 1998; Suksabye *et al*. 2016). The N derived from biological nitrogen fixation is fixed as ammonia with minimum losses to the environment. According to an estimate, about 50–70% of chemical N-fertilizer in soil losses through denitrification, leaching, and volatilization (Hodge *et al*. 2000). Besides that, both bacterial strains had PGP abilities such as IAA and chitinase production which make the plant stress tolerant. *L. fusiformis* had produced IAA (32.1 µg ml^{-1}) and chitinase (3.2 mU ml^{-1}) and solubilize P (198.2 µg ml^{-1}) (Trivedi *et al*. 2011). *B. subtilis* inoculation on *Artemisia annua* L. yielded plant height as 93.3 cm and assisted in N (1.52%), P (0.2%), and K (2.1%) uptake (Awasthi *et al*. 2011). Rhizospheric bacteria and plant roots release specific exudates in the rhizosphere which induce the activity of phosphatase and enhance P uptake (Geneva *et al*. 2006). Besides that, *Bacillus* sp. helped the plant to uptake K (Sheng and He 2006).

Application of biochar to the soil modify microbial community, and bacterial population affiliated with *Actinobacteria, Bacteroidetes, Firmicutes*, and *Proteobacteria* phyla is activated by making 92–95% of the community which benefits the plant growth (Kolton *et al*. 2011). In this study, both isolated bacteria belong to the *Firmicutes* phylum and results showed significant correlation with biochar and bacterial combination on plant growth and nutrient uptake. Moreover, biochar treated with *L. fusiformis* released P up to 54% under rigorous chemical conditions (He *et al*. 2014). However, positive growth effects from bacteria and biochar were less apparent at D_{45}. Biochar as a porous material enhances the soil porosity and allows water and air to infiltrate which facilitated extension of the root system. Some rhizospheric factors along with photosynthetically assimilated carbon compounds such as the root exudates (vitamins, amino acids, amides, and carbohydrates) influence the rhizospheric microbial community (Lugtenberg and Kamilova 2009). Moreover, the addition of low pyrogenic biochar in the soil can do positive priming of the carbon which facilitates microbial community to grow in the rhizosphere (Zimmerman *et al*. 2011). PSB inoculation (*B. subtilis* strain 18MZR and *L. fusiformis* strain 31MZR) significantly increased total N and P concentration in the maize plant on D_{45} and D_{65}. These increments were attributed to the inherent bacterial growth-promoting abilities.

5. Conclusion

This greenhouse study demonstrated that biochar (bagasse and sawdust) addition to the soil, inoculation with indigenously isolated PSB strains (*B. subtilis* strain 18MZR and *L. fusiformis* strain 31MZR), and their various combinations significantly increased plant growth by enhancing nutrients uptake (N, P, and K) in maize plant. The increments in plant growth are mainly attributed to the P-solubilization by bacterial strains in the soil, P from the biochar, and other PGP abilities such as IAA, cytokinin, and gerbilline production. Biochar and bacterial interaction may form soluble-P which resulted in 0.87% absorption to the plant leaves in *L. fusiformis* strain 31 MZR + sawdust biochar-amended maize on D_{65}. Plant height and available nutrient concentration are strongly correlated among all treatments. Maize plants inoculated with bacteria and biochar together exhibited maximum growth and nutrient concentration than biochar and bacterial treatments alone. Thus, this study shows that PSB and biochar have the potential to use as a promising approach in improving plant growth and nutrient absorption besides the conventional approaches under semiarid soil conditions. Further studies are necessary to evaluate (i) the suitability of *B. subtilis* strain 18MZR and *L. fusiformis* strain 31MZR on maize yield under field conditions and (ii) the suitability of PSB in combination with biochar for nutrient availability in the field and carbon sequestration potential.

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