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ROLE OF BACTERIAL CONSORTIA IN PROMOTING PLANT GROWTH AND NUTRIENTS BIOAVAILABILITY

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In alkaline soils, most of the plant nutrients are either lost or become unavailable. Current study investigated the role of bacterial isolates for enhancing nutrients bioavailability in soil along with improvement in the plant growth characters under greenhouse conditions. Bacterial strains were characterized on the basis of morphological and biochemical features. Sequence analysis of 16S rRNA regions confirmed the bacterial identity as *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans* and *Desulfovibrio vulgaris,* and sequences were submitted to GenBank (Accessions: MK123808, MK123809, MK123680, MK123681, MK123861). Pot experiment was performed with 10 treatments including; T_1 (½ N fertilizer), T_2 (Full N fertilizer), T_3 (NPK fertilizers @ 100-50-30 mg kg^{-1} soil), T_4 (½ N + *T. thiooxidans*), T_5 (½ N + *T. ferrooxidans*), T_6 (½ N + *D. vulgaris*), T_7 (½ N + *T. thiooxidans* + *T. ferrooxidans*), T_8 (½ N + *T. thiooxidans* + *D. vulgaris*), T_9 (½ N + *T. ferrooxidans* + *D. vulgaris*), T_{10} (½ N + *T. thiooxidans* + *T. ferrooxidans* + *D. vulgaris*). Among all the treatments, T_7 enhanced the bioavailability of macro- and micro-nutrients in soil and maize plants; while T_{10} significantly improved the plant growth attributes. Study concludes that development and application of bacterial consortium based biofertilizers could help in improving the plant growth characters and nutrients bioavailability in plants and soil.

Keywords: Bacterial consortium, nutrients bioavailability, *Thiobacillus thiooxidans, T. ferrooxidans, Desulfovibrio vulgaris. Zea mays*.

INTRODUCTION

Maize (*Zea mays* L.) is one of the important cereal crops in terms of production and nutritive value (Harris *et al.*, 2007). Maize grains have high nutrient value and serve as a source of starch, protein, oil, fiber and sugar (Ignjatovic-Micic *et al.*, 2015). In Pakistan, 90% of soils are deficient in nitrogen (N) and phosphorus (P); while 50% soils have insufficient potash (K) and micronutrients (NFDC, 2003). A research study highlighted that N, P and B deficiency in our soils is 100, 90 and 55%, respectively (Leghari *et al.*, 2016). Most of the nutrients are available to the plants at a pH range from 6.5 to 7.5. As major part of soils in Pakistan is alkaline in nature having pH > 7.5, so most of the nutrients especially micro-nutrients remain unavailable due to higher pH (Jensen, 2010).

One of the critical issues in financially successful agribusiness is to supply the deficient nutrients (Fageria and Baligar, 2005). Since 1950s, 30-50% of increments in food production are credited to fertilizer utilization (Higgs *et al.*, 2000). In Pakistan, the recommended dose of fertilizer N-P-K for maize is 120-100-80 kg ha^{-1}. In modern agricultural systems, thousands of million tonnes of synthetic agrochemicals are used to achieve optimum crop yields. Synthetic chemicals are not entirely used by the plants, but these chemicals also persist in the soil in different forms. Chemical fertilizers leach down into the ground, and thus disturb the diversity and performance of rhizospheric microorganisms (Ai *et al.*, 2012), matrix of soil, and the human health via food chain (Ayala and Rao, 2002). In order to reduce the use of toxic chemicals, several safe management options such as resistant varieties, biocontrol agents and cultural practices have been suggested (Diby *et al.*, 2005). Use of synthetic fertilizers is not considered as a good practice because of high costs of the fertilizers and acute environmental hazards (López-Bellido *et al.*, 2013). Therefore, it is needed to find low cost and environment friendly alternative solutions (Adesemoye and Kloepper, 2009), and use biological sources to enhance plant growth (Kumar and Shah, 2006).

Plant growth promoting rhizobacteria (PGPR) directly stimulate the plant growth by phytohormone production (Kang *et al.*, 2019; Ahmad *et al.*, 2020), biological nitrogen fixation (Kumar *et al.*, 2020), siderophores

production (Ahmad *et al.*, 2008), phosphate solubilization (Paiter *et al.*, 2019) ACC (1-aminocyclopropane-1-carboxylate) deaminase activity and by decreasing ethylene concentrations in the plants (Duan *et al.*, 2009). As biocontrol agents, PGPRs suppress the plant pathogens (Bajracharya, 2019). Certain bacterial species play significant role in enhancing soil fertility and plant growth by making various unavailable nutrients available. Microorganisms secrete organic acids i.e. carboxylic acid (Deubel and Merbach, 2005) which reduces the pH of rhizosphere soil and consequently makes phosphate freely available to the plants.

Phosphate solubilizing bacteria (PSB) based biofertilizers are in use since 1950's (Krasilinikov, 1957) and have got more attention in recent years (Soumare *et al.*, 2019). Phosphorus is absorbed by the plants either in the form of $H_2P0_4^-$ (pH < 7) or $HP0_4^{2-}$ (pH > 7); while phosphate and micronutrients (Zn, Cu, Fe, Mn, B) availability is reduced at high pH (NFDC, 2003). In sulphur bacterial consortium, the sulfur oxidizing bacteria (SOB) and sulfate reducing bacteria (SRB) are most prominent. Elemental sulfur inoculated with *Thiobacillus* genera (*T. thiooxidans* and *T. ferrooxidans*) enhance the solubility of rock phosphate (apatite) and plant biomass (Yang *et al.*, 2010). In sulfur oxidation by *T. thiooxidans* and *T. ferrooxidans*, last product is H_2SO_4 due to which the pH of soil decreases thus, the availability of N, P, K, Fe, Cu, Zn, Mn and Co increases. Sulfate firstly reduced to H_2S by sulfate reducing bacteria (Mosley *et al.*, 2013) and then H_2S reacts with dissolved Fe (II) to form pyrite and this pyrite may lead to sulfuric material at pH < 4 (Mosley *et al.*, 2013). Diagrammatic presentation of sulphur oxidation and reduction is given (Fig. 1) and reaction channels are given below in the equation forms:

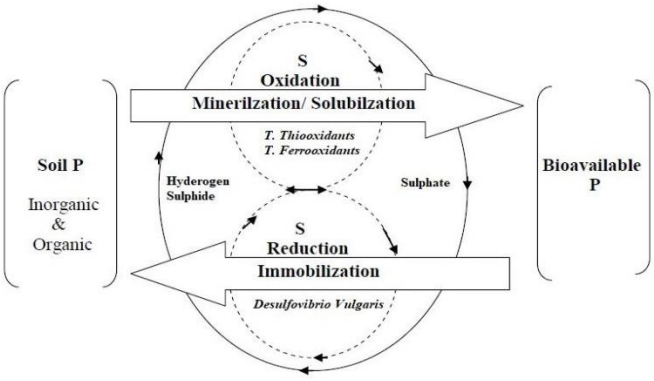

Figure 1. Diagrammatic explanation on role of bacterial isolates in the elevation of nutrient bioavailability.

(*Thiobacillus*)

1. $S^o \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow SO_4^{2-}$ (Ohba and Owa, 2005)
2. $S + H_2O + 1.5O_2 \rightarrow SO_4^{2-} + 2H^+$, Δ Go = -587.1kJ / reaction. (Hassan *et al.*, 2010)

Oxidation of sulphur by *T. thiooxidans* and *T. ferrooxidans* is given in the following chemical reactions:

1. $S° + 1.5\ O_2 + H_2O \rightarrow H_2SO_4$
2. $Fe_3S_4 + 7.5\ O_2 + H_2O \rightarrow 3FeSO_4 + H_2SO_4$

Insoluble calcium reacts with previously produced H_2SO_4 and gypsum is formed.

1. $CaCO_3 + H_2SO_4 + H_2O \rightarrow CaSO_4 \cdot 2H_2O + CO_2$
2. $Ca_5(PO_4)_3F + 5H_2SO_4 + 10H_2O \rightarrow 3H_3PO_4 + 5CaSO_4 \cdot 2H_2O + HF$
3. $Ca_5(PO_4)_3F + 7H_3PO_4 \rightarrow 5Ca(H_2PO_4)_2 + HF$ (Bhatti and Yawar, 2010)

T. ferrooxidans reduces the acetylene to ethylene and incorporates N_2 into its cell protein, thus considered a true nitrogen fixer (Mackintosh, 1978). Present research was aimed to investigate the effect of *Thiobacillus thiooxidans, T. ferrooxidans* and *Desulfovibrio vulgaris* on enhancing the nutrients bioavailability in soil and maize plants along with improvements in the plant growth characters under greenhouse conditions. Bacterial consortia with promising results could be further investigated to develop biofertilizer for enhancing the nutrient bioavailability and plant growth in cereal crops.

MATERIALS AND METHODS

***Soil and sewage water samples collection*:** During a survey in 2017, subsurface soil and sewage water samples (n = 30) from sewage water channels were collected from the industrial areas in Hattar (33.8521° N, 72.8501° E), Islamabad (33.6844° N, 73.0479° E) and Rawalpindi (33.5651° N, 73.0169° E) (Fig. 2). Soil samples were taken in zipped bags while the sewage water samples were collected in clean, sterilized plastic bottles and properly labeled. All the samples were brought to the Soil Science Institute's laboratory at PMAS Arid Agriculture University Rawalpindi, and stored at 4°C till further processing for bacterial isolation.

***Bacterial isolation*:** Sulfate reducing bacteria (SRB) were isolated from the collected samples by adopting the methodology of Pankhurst (1971) on agar medium (15 g L^{-1}) enriched with three solutions as reported by Lapage *et al.* (1970). All of these solutions were separately autoclaved and mixed together to form a homogenized solution. The mixture was aseptically poured into sterilized lid containing vessels.

For the isolation of *Thiobacillus thiooxidans* (SOB), Starkey's broth medium (Starkey, 1935) and thiosulphate broth medium (Parker, 1957) were used. For this, 10 g of elemental sulphur (S^o) was added in sterilized Starkey's broth medium and bromocresol purple (Difco, USA) was

Figure 2. Pin points of sampled areas in Rawalpindi (1); Islamabad (2) and Hattar (3), Pakistan.

used as an indicator. Serially diluted soil sample and 0.5 mL water samples were inoculated aseptically into 100 mL of broth in lid containing vials and incubated at 25 ± 2°C for 20 to 25 days. *Thiobacillus ferrooxidans* strains were isolated by inoculating serially diluted soil and 0.5 mL of sewage water samples separately onto iron-oxidizing medium (Atlas, 2010) and incubated at 25 ± 2°C for 20 to 25 days.

***Morphological and biochemical features of bacteria*:** Bacterial isolates were subjected to microscopic study to observe the morphological features, and biochemical assays were also performed. For morphological featuring, bacterial slides were aseptically prepared from 24 hours old bacterial cultures and were observed under microscope under various resolutions (10, 50 and 100X lens power). Integration of all characters (physiological and biochemical) was referred to the Bergey's Manual (Garrity *et al.*, 2005). In order to differentiate bacterial isolates, Gram staining was done according to the method reported by Vincent (1970). Methyl Red-Voges Proskauer (MR-VP) test was performed according to the procedure described by Atlas (1993). Ability of the bacterial strains to convert tryptophan into indole was determined by following the methodology published by Pandey and Chakraborty (2019). Urease production ability was determined according to the method reported by Brink (2010). H_2S production was tested on sterile triple sugar iron agar (TSIA) slants by following the procedure published by Bijitha and Bhai (2019). Bacterial motility test was carried out on semisolid agar medium contained in test tubes according to the method proposed by Swamynathan and Singh (1995).

***Molecular characterization of bacterial strains*:** Biochemically characterized bacterial strains were identified on molecular basis through 16S rRNA gene sequencing. Genomic DNA was extracted from bacterial strains by using the GeneJet Genomic DNA purification Kit (Thermo Scientific, Waltham USA). The 16S rRNA region of each bacteria was amplified by polymerase chain reaction (PCR) using primers 27F (5´-AGAGTTTGATCMTGGCTCAG-3´) and 1492R (5´-TACGGYTACCTTGTTACGACTT-3´). The reaction mixture (50 µL) contained 25–150 ng of DNA as template, 1X of Taq buffer, 200 µM of each dNTP, 1.25 mM of $MgCl_2$, 0.4 µM of each primer, and 0.5U of Taq DNA polymerase (Qiagen, Germany). PCR conditions were 95°C for 4 min followed by DNA denaturation at 95°C for 45 sec (30 cycles), annealing at 53°C for 45 sec and extension at 72°C for 2 min and a final extension step at 72°C for 5 min. Amplified DNA was visualized on 1% agarose gel and purified by using Gel and PCR Clean-Up System (Promega, USA) by following the manufacturer's instructions. DNA was quantified using NanoDrop and final DNA products were sent to Macrogen Inc. for sequencing. Sequences were deposited in GenBank and accession numbers MK123680, MK123681, MK123809, MK123861 and MK123808 were obtained. Both forward and reverse sequences were united together to get a final sequence. Sequences were checked in sequence alignment editor (BioEdit 7.1.9) and aligned by using MEGA 6 (Tamura *et al.*, 2013) by using the clustalW program. The edited sequences were aligned together with their best matches already deposited in GenBank nucleotide database (www.ncbi.nlm.nih.gov). A Maximum-Likelihood

tree was constructed by using Kimura two parameter model (K2) with Gamma distribution and with 1000 Bootstrap replicates.

***Greenhouse experiment*:** Effect of bacterial isolates on nutrient bioavailability in soil and plants and plant growth promotion traits in maize (variety Islamabad Gold) was tested in pots under greenhouse conditions. Detail of the treatments is as: T_1 (½ N fertilizer), T_2 (Full N fertilizer), T_3 (NPK fertilizer @ 100-50-30 mg kg^{-1} soil), T_4 (½ N + *Thiobacillus thiooxidans*), T_5 (½ N + *T. ferrooxidans*), T_6 (½ N + *Desulfovibrio vulgaris*), T_7 (½ N + *T. thiooxidans* + *T. ferrooxidans*), T_8 (½ N + *T. thiooxidans* + *D. vulgaris*), T_9 (½ N + *D. vulgaris* + *T. ferrooxidans*), T_{10} (½ N + *T. thiooxidans* + *T. ferrooxidans* + *D. vulgaris*). All the fertilizers were applied before sowing the seeds. Maize seeds were surface sterilized with 1% NaOCl for 5 min followed by three washings with sterile distilled water. Five seeds per pot were sown in 10 kg soil filled in plastic pots. Bacterial inocula (10^7 cfu mL^{-1}) were added in respective treatments @ 1% (v/v) in distilled water; while control treatments were given distilled water only. All the treatments were applied four times with fifteen days interval. Two independent experiments under the same conditions were carried out in plastic pots with ten treatments in three repeats (n = 30).

***Soil and plant chemical analysis*:** Before conducting the pot experiments, composite soil samples were analyzed for soil physical and chemical properties (Table 1).

Table 1. Physico-chemical characteristics of soil used in the pot experiments.

Characteristics	Units	Values
Soil texture	-	Silty clay loam
pH	-	7.80
Bulk density	g cm^{-3}	1.57
Electrical conductivity	dS m^{-1}	1.60
Phosphorous	mg kg^{-1}	7.00
Total nitrogen	mg kg^{-1}	8.00
Potassium	mg kg^{-1}	120.00
Iron	mg kg^{-1}	3.46
Manganese	mg kg^{-1}	0.17
Copper	mg kg^{-1}	0.69
Zinc	mg kg^{-1}	0.34

Soil texture was determined by using the hydrometric method as previously reported (Huluka and Miller, 2014). The pH and EC were measured in 1:5 suspension. AB-DTPA method was used for the determination of NO_3-N (Hussain *et al.,* 2019). Available-P was measured via method described by Recena *et al.* (2017). Extractable-K was tested by following the procedure reported by Affinnih *et al.,* (2014). Micronutrients were analyzed by DTPA method (Cancela *et al.,* 2002). For the plant analysis, Di-acid (Nitric acid, Perchloric acid) wet digestion was done for the determination of P, K and micronutrients (Zn, Cu, Fe, Mn). Total-N was measured by Kjeldahl method as reported by Stanley *et al.* (2019). Potassium was measured on flame photometer after running a series of K standards (Enders and Lehmann, 2012). Micronutrients were measured on atomic absorption spectrophotometer.

***Plant parameters measurement*:** Agronomic parameters such as seed germination percentage (GP), plant height (PH), leaves per plant (LPP), leaf area (LA), stem girth (SG), root volume (RV), root length (RL), root dry weight (RDW), root fresh weight (RFW), plant fresh weight (PFW), and plant dry weight (PDW) were measured after harvesting the maize crop. Effect of bacterial inocula on maize growth parameters, bioavailability of nutrients to plants and their inter-relationships were measured statistically by principal component analysis (PCA) through SPSS® version 16.0.

RESULTS

***Morphological and biochemical features of bacterial strains*:** Morphological and biochemical features of bacterial strains are given in Table 2. Bacterial isolates belonging to *T. thiooxidans* (Isolate: IRH, IRH, SH4), *Desulfovibrio vulgaris* (NFB, TNF) and *T. ferrooxidans* (DBN, HY2, TMF, DGI) were examined for biochemical responses in various assays. Results of the morphological and biochemical assays exhibited that all bacterial strains belonging to *T. thiooxidans* were short rods, motile and showed negative response towards urease and indole test, gelatin hydrolysis, H_2S production, methyl red test and Gram staining, while showed positive results towards Voges Proskauer test. Bacterial strains belonging to *D. vulgaris*

Table 2. Morphological and biochemical characterization of bacterial isolates.

Bacterial strain/Response	IRH	IRT	SH4	NFB	TNF	DBN	HY2	TMF	DGI
Morphology	SR	SR	SR	CR	CR	R	R	R	SR
Urease test	-	-	-	+/-	+	-	-	-	-
Indole test	-	-	-	+	-	-	-	-	-
Gelatin hydrolysis	-	-	-	+	+	-	-	-	NM
H_2S production	-	+/-	-	+	+	+	+	+	NM
Methyl red test	-	-	-	+	+	+	+	+	+/-
Voges proskauer	+	+	+	-	-	+	+	+	+
Gram staining	-	-	-	-	-	-	-	-	-
Motility	M	M	M	M	M	M	M	M	M

were Gram negative, curved rods (CR) and showed negative response towards Voges Proskauer test, while displayed positive results for all the other biochemical tests. *T. ferrooxidans* were rods (R) to short rods (SR), Gram negative, motile bacteria which showed negative results towards urease test, indole test and gelatin hydrolysis, while they were positive towards H_2S production, methyl red test and Voges Proskauer test.

***Molecular identification of bacterial strains*:** Morphologically characterized bacterial strains were further confirmed by molecular analysis and results of sequence homology are given in Table 3. The 16S rRNA gene sequence analysis showed that bacterial strains IRH (MK123680) and SH4 (MK123681) had 99% sequence homology to *Acidithiobacillus thiooxidans* while bacterial strains DBN (MK123809) and HY2 (MK123861) showed similarity with *Acidithiobacillus ferrooxidans*. Strain NBF (MK123808) corresponded 99% to the genus *Desulfovibrio*. Results of closest sequence matches are given in Fig. 3.

***Dynamics of soil and plant chemical parameter*:** Data on soil chemical analysis is presented in Table 4. Soil analysis after crop harvest revealed that the highest pH was 7.7 in T_1 where no bacterial inoculum was applied, followed by T_2 and T_6 which showed pH 7.5. The lowest pH 7.3 was observed in T_7 and T_{10}. The highest soil available-P was recorded in T_3 (17.5 mg kg^{-1}) followed by T_7 (14.7 mg kg^{-1}), and T_{10} (14.3 mg kg^{-1}), while the lowest P was found in T_1 (6.73 mg kg^{-1}). The highest contents of nitrate-N (19.7 mg kg^{-1}) were recorded in T_3 followed by T_2 and T_{10} with the contents of 19.0 and 18.0 mg kg^{-1}, respectively.

Figure 3. Maximum likelihood tree of the 16S rRNA of bacterial isolates belonging to the genus *Pseudchrobactrum*. Number above the nodes show bootstrap value >50 after 1000 pseudo replicates.

Table 3. Molecular characterization of bacterial strains by 16S rRNA sequencing.

Bacterial strain	GenBank accessions	Identified as	NCBI (match)	Identity
IRH	MK123680	*Acidithiobacillus thiooxidans*	MH017545	99%
SH4	MK123681	*Acidithiobacillus thiooxidans*	AB362190	99%
DBN	MK123809	*Acidithiobacillus ferrooxidans*	KX894698	99%
HY2	MK123861	*Acidithiobacillus ferrooxidans*	KX894691	99%
NBF	MK123808	*Desulfovibrio vulgaris*	NR112657	99%

Table 4. Effect of bacterial consortium applications on the nutrients bioavailability in soil

Trt.	Soil pH	Soil (P)	Soil (NO_3^-)	Soil (K)	Soil (Fe)	Soil (Zn)	Soil (Cu)	Soil (Mn)
T_1	7.7±0.06a*	6.73±0.15g	10.3±0.33f	124.3±2.33e	3.15±0.07d	1.00±0.00g	0.23±0.01f	0.54±0.03e
T_2	7.6±0.03ab	6.80±0.15g	19.0±0.09ab	125.3±2.40de	3.12±0.05d	1.15±0.05f	0.23±0.01f	0.64±0.01de
T_3	7.4±0.03b-e	17.5±0.29a	19.7±0.22a	141.0±2.08a	3.23±0.17d	1.32±0.01de	0.28±0.01e	0.70±0.03d
T_4	7.3±0.09de	13.5±0.29cd	13.3±0.06de	128.3±2.33cde	4.00±0.23c	1.71±0.09b	0.35±0.01c	0.96±0.03c
T_5	7.4±0.06b-e	12.0±0.58e	13.0±0.08de	127.7±2.60cde	4.33±0.07bc	1.55±0.01c	0.34±0.01c	0.96±0.01c
T_6	7.5±0.03abc	9.50±0.29f	11.7±0.23ef	126.0±2.65cde	3.37±0.05d	1.20±0.00ef	0.31±0.01d	0.87±0.01c
T_7	7.3±0.03e	14.7±0.15b	17.0±0.11bc	132.7±2.03bc	4.91±0.04a	1.85±0.01a	0.47±0.01a	1.18±0.06a
T_8	7.4±0.09cde	12.6±0.31de	15.0±0.08cd	132.0±2.08bcd	4.32±0.20bc	1.50±0.08c	0.36±0.01bc	1.00±0.10bc
T_9	7.5±0.09b-e	12.0±0.58e	14.3±0.11d	130.7±3.18cde	4.46±0.05b	1.43±0.03cd	0.38±0.01b	0.99±0.04c
T_{10}	7.3±0.06de	14.3±0.20bc	18.0±0.18ab	138.7±2.03ab	4.57±0.03ab	1.77±0.03ab	0.45±0.01a	1.13±0.05ab

* Mean values were separated via LSD test at $P<0.05$. Values with different alphabets are significantly different from each other. All values except pH were measured in mg kg^{-1}. Treatments include; T_1 (½ N fertilizer), T_2 (Full N fertilizer), T_3 (NPK fertilizer), T_4 (½ N + *T. thiooxidans*), T_5 (½ N + *T. ferrooxidans*), T_6 (½ N + *D. vulgaris*), T_7 (½ N + *T. thiooxidans* + *T. ferrooxidans*), T_8 (½ N + *T. thiooxidans* + *D. vulgaris*), T_9 (½ N + *T. ferrooxidans*+ *D. vulgaris*), T_{10} (½ N + *T. thiooxidans* + *T. ferrooxidans* + *D. vulgaris*).

The lowest nitrogen (N) contents were in T_1 (10.3 mg kg^{-1}) as shown in Table 4. The highest soil extractable K content (141 mg kg^{-1}) was observed in T_3 followed by T_{10} (138.6 mg kg^{-1}), while lowest was recorded in T_1 (124.3 mg kg^{-1}). Maximum concentration of iron 4.91 mg kg^{-1} was analyzed in T_7, followed by T_{10} (4.57 mg kg^{-1}), while the lowest concentration was observed in T_2 (3.12 mg kg^{-1}). The highest concentration of zinc (Zn) was recorded in T_7 (1.84 mg kg^{-1}) followed by T_{10} (1.76 mg kg^{-1}), while the minimum zinc concentration was recorded in T_1 (1.00 mg kg^{-1}). Maximum copper (Cu) concentration 0.465 mg kg^{-1} was observed in T_7 followed by T_{10} (0.446 mg kg^{-1}) and the minimum copper concentration was observed in T_1 (0.228 mg kg^{-1}). The highest content of manganese (Mn) was found in T_7 (1.184 mg kg^{-1}) followed by T_{10} (1.130 mg kg^{-1}), while the lowest Mn content was in T_1 (0.537 mg kg^{-1}).

Data on plants chemical analysis is presented in Table 5. The highest content of potassium (K) was recorded in T_7 (2.21%) followed by T_{10} (1.87%), while the lowest concentration was observed in T_1 (1.35 %). In case of N contents, maximum value (2.64%) was recorded in T_2 followed by T_3 (2.15%) and T_{10} (2.11%) while the lowest concentration 1.71% was recorded in T_9. Maximum phosphorus (P) concentration was observed in T_{10} (0.30%) followed by T_3 (0.29%), while the minimum concentration was recorded 0.15% in T_1. For iron contents, maximum concentration was recorded 140.00 mg kg^{-1} in T_7 followed by T_{10} (133.00 mg kg^{-1}), while the lowest (45.33 mg kg^{-1}) was observed in T_2. Maximum zinc (Zn) concentration (65.06 mg kg^{-1}) was observed in T_7 followed by T_{10} (62.93 mg kg^{-1}), while the lowest concentration was recorded 33.92 mg kg^{-1} in T_1. Maximum copper (Cu) concentration was 17.61 mg kg^{-1} in T_7 followed by T_{10} (15.57 mg kg^{-1}), while the minimum concentration was 5.95 mg kg^{-1} in T_2. The highest manganese (Mn) concentration was recorded in T_7 (65.22 mg kg^{-1}) followed by T_{10} (64.99 mg kg^{-1}) while the lowest concentration was recorded 37.92 mg kg^{-1} in T_1. The relationship between nutrients in soil and maize plant tissues indicated a positive

Table 5. The effect of bacterial consortium applications on the nutrients bioavailability in maize crop

Trt.	Plant (K)	Plant (N)	Plant (P)	Plant (Fe)	Plant (Zn)	Plant (Cu)	Plant (Mn)
T_1	1.36±0.05e	1.92±0.02c	0.16±0.00d	46.33±1.76e	33.92±2.26f	6.09±0.06g	37.92±1.42d
T_2	1.49±0.03d	2.64±0.05a	0.16±0.01d	45.33±2.73e	38.20±0.66e	5.95±0.18g	40.20±1.13d
T_3	1.73±0.05c	2.15±0.06b	0.29±0.02ab	48.33±1.76e	43.25±0.44d	6.50±0.08g	45.19±0.62c
T_4	1.84±0.05b	1.93±0.01c	0.28±0.01ab	89.00±5.13c	56.33±0.48b	11.50±0.37d	57.49±1.55b
T_5	1.76±0.04bc	1.92±0.01c	0.21±0.02c	98.67±4.63c	54.16±0.99b	9.66±0.34e	55.40±2.51b
T_6	1.72±0.03c	2.07±0.01b	0.26±0.01b	69.33±1.45d	48.10±1.97c	7.94±0.10f	53.76±1.69b
T_7	2.21±0.02a	1.81±0.01d	0.22±0.02c	140.00±2.89a	65.07±1.40a	17.62±0.41a	65.22±2.61a
T_8	1.82±0.02bc	2.07±0.03b	0.28±0.01ab	119.67±5.78b	54.31±0.35b	13.21±0.16c	55.31±2.00b
T_9	1.80±0.04bc	1.71±0.02e	0.27±0.01b	112.33±5.93b	55.91±0.75b	12.78±0.17c	55.91±0.75b
T_{10}	1.87±0.03b	2.11±0.02b	0.30±0.01a	133.00±2.08a	62.93±1.40a	15.57±0.30b	65.00±1.18a

* Mean values were separated according to LSD test at P<0.05. Values with different alphabets are significantly different from each other. Values for N, P and K are in %, while the rest are in mg kg^{-1}. Treatments include; T_1 (½ N fertilizer), T_2 (Full N fertilizer), T_3 (NPK fertilizer), T_4 (½ N + *T. thiooxidans*), T_5 (½ N + *T. ferrooxidans*), T_6 (½ N + *D. vulgaris*), T_7 (½ N + *T. thiooxidans* + *T. ferrooxidans*), T_8 (½ N + *T. thiooxidans* + *D. vulgaris*), T_9 (½ N + *T. ferrooxidans*+ *D. vulgaris*), T_{10} (½ N + *T. thiooxidans* + *T. ferrooxidans* + *D. vulgaris*).

Table 6. The effect of bacterial consortium applications on the plant growth enhancement in maize crop

Trt.	GP (%)	pH	LPP	LA (cm^2)	SG (cm)	RL (cm)	RV (cm^3)	RFW (g)	RDW (g)	PFW (g)	PDW (g)
T_1	66.1±2.11a	35.5±0.53f	7±0.59d	152.8±8.2e	8.1±0.11g	49.7±1.2f	21.7±0.88d	4.7±0.5e	2.1±0.08d	9.1±0.57f	4.3±0.33f
T_2	72.0±1.73b	39.5±0.73e	8±0.51cd	188.3±2.3d	9.3±0.09f	62.3±1.45e	25.7±0.67d	5.7±0.49e	2.6±0.43d	16.2±0.62e	5.0±0.58ef
T_3	84.3±0.88b	43.1±0.75d	9±0.48bc	217.2±9.2c	11.7±0.22b	72.0±1.73d	38.0±4.73c	9.4±0.32d	3.8±0.17c	20.2±1.92c	11.0±0.58ab
T_4	72.7±0.88b	47.6±0.97c	9±0.51bc	196.3±4.2c	9.7±0.06e	81.7±1.2c	35.7±0.67c	9.6±0.67c	3.8±0.06c	27.4±0.67d	7.7±0.33cd
T_5	72.3±0.88b	47.0±0.87c	9±0.68bc	204±6.9cd	9.9±0.08e	81.3±1.2c	36.0±1.53c	9.7±0.6c	3.8±0.09c	29.5±0.73d	7.3±0.88cd
T_6	72.3±2.03b	47.5±1.20c	10±0.33abc	210.1±4.7c	9.6±0.23ef	81.7±1.2c	35.0±0.58b	8.7±0.62c	3.7±0.19c	29.4±0.62d	6.7±1.20de
T_7	85.7±.76a	52.6±1.14b	10±0.51ab	245.7±3.3b	10.6±0.11d	94.0±2.08b	45.3±0.88b	11.8±0.49b	4.7±0.21ab	39.6±0.83b	10.7±0.9ab
T_8	77.0±2.65b	52.6±0.72b	10±0.58ab	244.8±6.5b	11.3±0.1bc	96.0±1.53b	45.0±0.58b	12.0±1.09b	4.5±0.14b	40.2±0.58b	10.3±0.8ab
T_9	75.3±1.45b	52.1±0.81b	9±0.88bc	245.3±2.8b	11.0±0.1cd	96.0±2.65b	45.3±1.45c	11.3±0.78c	4.2±0.25bc	40.4±1.3bc	9.0±0.58bc
T_{10}	83.3±1.76a	58.4±0.36a	11±0.19a	275.4±7.6a	12.9±0.18a	114±2.08a	52.3±1.45a	14.1±0.56a	5.2±0.16a	48.5±1.53a	12.0±0.58a

* Mean values were separated according to LSD test at P<0.05. Values with different alphabets are significantly different from each other. Treatments include; T_1 (½ N fertilizer), T_2 (Full N fertilizer), T_3 (NPK fertilizer), T_4 (½ N + *T. thiooxidans*), T_5 (½ N + *T. ferrooxidans*), T_6 (½ N + *D. vulgaris*), T_7 (½ N + *T. thiooxidans* + *T. ferrooxidans*), T_8 (½ N + *T. thiooxidans* + *D. vulgaris*), T_9 (½ N + *T. ferrooxidans*+ *D. vulgaris*), T_{10} (½ N + *T. thiooxidans* + *T. ferrooxidans* + *D. vulgaris*). Agronomic parameters of maize included; germination percentage (GP), plant height (PH), leaves per plant (LPP), leaf area (LA), stem girth (SG), root length (RL), root volume (RV), root fresh weight (RFW), root dry weight (RDW), plant fresh weight (PFW), and plant dry weight (PDW).

linear correlation for all the macro- as well as micro-nutrients (Fig. 4). The Pearson correlation coefficient "R^2" was 0.5 for P and K, while 0.9 for N, Fe, Zn, Cu and Mn.

***Maize crop agronomic parameters*:** Data on the impact of bacterial consortia applications on plant growth characters is displayed in Table 6. The highest germination percentage (GP) was recorded in T_7 (85.6%), followed by T_3 (84.3%) and T_{10} (84.3%), while the lowest seed germination percentage (66.1%) was recorded in T_1. Maximum maize plant height (58.39 cm) was observed in T_{10} followed by T_7 (52.63 cm) while minimum plant height was observed in T_1 (35.47 cm). Maximum leaves plant^{-1} (11.33) were observed in T_{10} followed by T_7 and T_6 (10 leaves plant^{-1}) while the minimum leaves plant^{-1} were recorded in T_1 (7.00 leave plant^{-1}). At the time of harvesting, maximum leaf area (275.40 cm^2) was recorded in T_{10} followed by T_7 and T_9 (245.7 cm^2), while the minimum leaf area (152.84 cm^2) was observed in T_1. Maximum stem girth 12.90 cm was observed in T_{10} followed by T_3 (11.70 cm), while the lowest stem girth 8.13 cm was recorded in T_1. The longest root was 114.00 cm in T_{10} followed by T_8 and T_9 (96.00 cm) while smallest root length was 49.66 cm in T_1. Maximum root

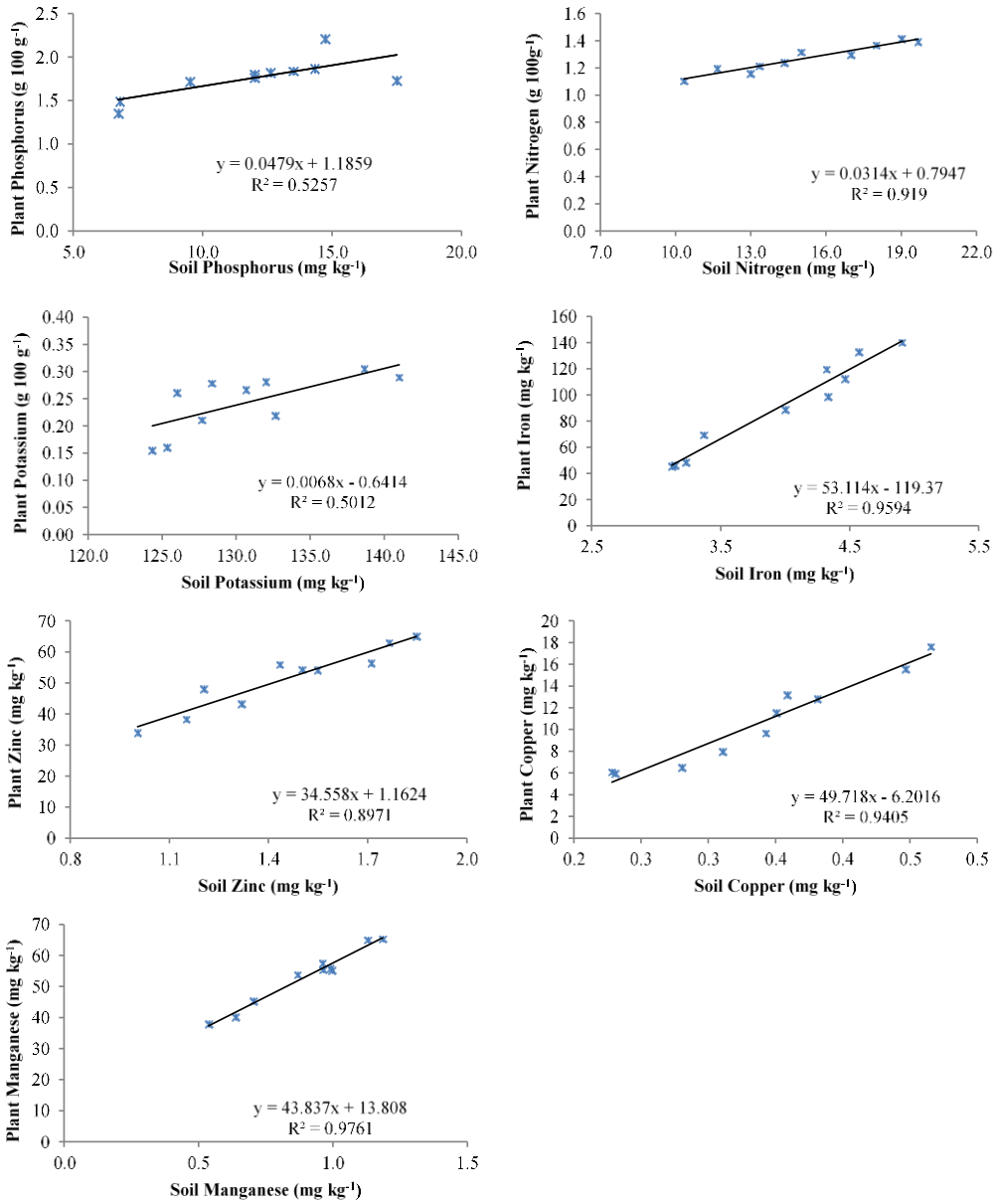

Figure 4. Soil and plant nutrients correlation of Phosphorus (P), Nitrogen (N), Potassium (K), Iron (Fe), Zinc (Zn), Copper (Cu) and Manganese (Mn).

volume 52.33 cm^3 was observed in T_{10} followed by T_7 and T_9 (45.33 cm^3), while the lowest root volume was noted in T_1 (21.66 cm^3). Fresh root weight (FRW) ranged between 4.8 to 15.8 g. Maximum FRW (15.8 g) was observed in T_{10} as compared to control. Maximum root dry weight was observed in T_{10} (5.1 g) followed by T_7 (4.7 g), while minimum root dry weight was observed in T_1 (2.0 g). Maximum fresh plant weight was recorded for T_{10} (48.5 g) followed by T_9 (40.4 g), while the minimum fresh weight was found in T_1 (9.11 g). Maximum plants' oven dry weight was recorded in T_{10} (12.00 g), followed by T_3 (11.00 g) and T_7 (10.70 g), whereas the minimum dry weight was observed in T_1 (4.33 g). Effect of bacterial inoculation on the plant growth promotion parameters, bio-availability of nutrients in maize plants and their inter-relationships were analyzed by principal component analysis (PCA) as presented in Fig. 5.

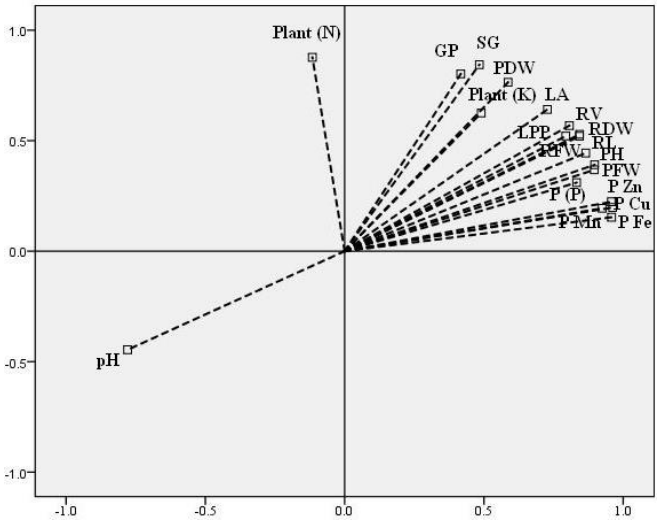

Figure 5. Principal component analysis of soil pH, plant nutrients and plant growth parameters. Abbreviations details can be found at the caption of Table 5 and 6. Data variations are mainly elucidated by first two components PC1 (60.6 %) and PC2 (29.4 %).

DISCUSSION

Plant growth promoting bacteria (PGPR) play significant role in crop yield enhancement and in the bioavailability of micro- and macro-nutrients. In this study, isolates of sulfur-oxidizing bacteria exhibited morphological, physiological and genotypic properties typical for the genus *Thiobacillus*. Some of the previous studies have characterized the bacterial strains belonging to genus *Thiobacillus* on the basis of morphological and biochemical features (Reddy *et al.*, 2018). Majority of the plant species display association with PGPR. Previous reports have highlighted the plant growth promoting (PGP) activities of the bacterial agents belonging to the genera: *Agrobacterium, Alcaligenes, Agromonas, Arthrobacter, Azospirillum, Azoarcus, Azotobacter, Acinetobacter, Beijerinckia, Bacillus, Caulobacter, Bradyrhizobium, Derxia, Chromobacterium, Erwinia, Enterobacter, Frankia, Flavobacterium, Herbaspirillum, Klebsiella, Hyphomycrobium, Pseudomonas, Micrococcous, Serratia, Rhizobium, Thiobacillus, Stenotrophomonas, Zoogloea* and *Xanthomonas* (Tripathi *et al.*, 2002; Hurek and Reinhold-Hurek, 2003; Gray and Smith, 2005; Choudhary and Johri, 2009).

In many countries across the world, PGPR are used as biofertilizers for sustainable agriculture (Habibi *et al.*, 2014). Previous studies have highlighted the role of PGPR in plant growth enhancement especially under stress conditions (Asghari *et al.*, 2020). The bacterial consortium promotes plant growth by synthesizing useful substances like nucleic acids, amino acids and sugars from the secretions of roots, organic matter by using the heat of soil and sunlight (Higa and Parr, 1994). The PGPR solubilize the phosphorus from soil and make it available to the plants (Paiter *et al.*, 2019). Phosphate solubilizing bacteria (PSB) increase the plants growth through their impact on numerous dynamic physiological processes of metabolism, which include the photosynthesis, utilization of starch, sugar and energy transfer. The PSB increase the availability of phosphorus to the plants by converting the organic compounds and by mineralizing the inorganic compounds into easily available forms (Tallapragada and Seshachala, 2012).

Sulfur oxidizing bacteria (SOB) help to control the environmental pollution by controlling H_2S pollution and help in sulfur cycle (Pokorna and Zabranska, 2015). A research study reported that *Thiobacillus* isolates can be incorporated to improve the sulfur oxidation in soil and to raise soil accessible sulfate (Vidyalakshmi and Sridar, 2007). When it comes to phosphorus solubility, apart from SOB, iron oxidizing bacteria (FeOB) have also been studied for their possible role in phosphorus solubilization. In the rhizosphere soil, PSB were more abundant than other microbes (Kucey, 1983). Sulfur reducing bacteria (SRB) are an ecological and physiological assemblage of morphologically very dissimilar types of anaerobic bacteria that have potential to reduce sulphate to hydrogen sulfide in the energy conserving reactions (le Gall and Postgate, 1973). The pH reduction by SOB and FeOB in different growth media under laboratory conditions has been reported (Nagendran *et al.*, 2008; Ullah *et al.*, 2014). The pH reduction by the *Thiobacilli* is due to biological sulfur oxidation (Stamford *et al.*, 2003). For SOB, the elemental sulfur (S^o) is an important substrate (Pokorna and Zabranska, 2015) and it is a biological process in which oxidation of S^o by SOB takes place. Acidity produced lowers the pH of soil significantly, and thus availability of plant nutrients including that of P increases (Hassan *et al.*, 2010; Yang *et al.*, 2010). Sulfur oxidation by *T. thiooxidans* and *T.*

ferrooxidans decreases the pH of soil (Hassan *et al.*, 2010) and increases the availability of phosphorus. These bacteria oxidize sulfur and enhance the plant growth (Khatibi, 2011).

Diverse microorganisms are involved in the alteration of potassium into soluble form (Setiawati and Mutmainnah, 2016). Optimum pH is 6 to 7 for the availability of phosphorus in the soil (Sarker *et al.*, 2014). Among the rhizospheric bacteria, *Bacillus* species also reduce the soil pH, produce plant hormones, fix nitrogen, and solubilize phosphate (Habibi *et al.*, 2014). Application of beneficial bacteria increases mobilization of insoluble nutrients and their uptake by the plants (Biari *et al.*, 2008), and produce antibiotics against soil-borne phytopathogens (Kenawy *et al.*, 2019), and stimulate the production of plant growth regulators (Goswami *et al.*, 2016). These PGPRs play role in symbiotic nitrogen fixation (Matse *et al.*, 2020) and nitrogenase activity (Kumari *et al.*, 2019). Kallar grass had nearly 70% nitrogen derived from atmosphere when inoculated with microbes (Malik *et al.*, 1997). Application of PGPR has been shown to enhance the plant growth of maize and many other crops (Ahmed *et al.*, 2020).

Micronutrients are required by plants in minute quantities, although these are very effective in regulating the plant growth as they form a part of the enzyme system and thus regulate plant life (Pathak *et al.*, 2011). Use of micronutrients increases crop yield. Zinc helps in protein formation and photosynthesis in plants (Cakmak, 2007). Application of beneficial microbes is reported to enhance the Zn availability (Gauri *et al.*, 2012). Plant height is attributed to the production of plant growth hormones (gibberellins and auxins) stimulated by phosphate solubilizing microbes (Siddiqui, 2005). Richardson *et al.* (2009) revealed that nitrogen availability enhances the plant vegetative growth. Elser and Bennett (2011) highlighted the role of phosphorus in promoting the plant growth parameters including leaves per plant. Soil microorganisms enhance the ability of soil and plants to uptake phosphorus by increasing roots surface area and displacement of sorption equilibrium which increases the net transfer of phosphate in soil (Paiter *et al.*, 2019). Beneficial microbial consortium enhances plant vegetative and reproductive growth by nutrients bioavailability (Habibi *et al.*, 2014). A research study has reported 20 to 40 % increase in nutrient bioavailability and nutrient use efficiency which promote the plant growth (Meena *et al.*, 2017). Nutrients bioavailability and plant growth parameters are interlinked and the relationship between plant growth parameters in Cagaita (*Eugenia dysenterica* DC.) and nutrient concentration has been reported (Bessa *et al.*, 2016). Another study has shown the relationship between growth in leaf area and biomass accumulation in *Arabidopsis thaliana*, and this relationship was found depended on carbon partitioning in different plant parts (Weraduwage *et al.*, 2015).

***Conclusion*:** Use of synthetic fertilizers is not only expensive but also poses ill effects on environment. It has become unavoidable to search some alternative biological sources, which could be inexpensive and ecofriendly in nature. Results of this study exhibited that the application of different microbial consortia lowered the soil pH, which enhanced the bioavailability of macronutrients (N, P, K) as well as micronutrients (Zn, Cu, Mn, Fe), and also improved the agronomic attributes of plants. This research concludes that by applying biological agents such as SOB, FeOB and SRB, dependency on chemical fertilizers could be reduced; so these bacterial agents need to be further explored to develop less expensive and efficient biofertilizers.

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