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Assessment of Potential Bacterial Isolates for Enhancing Plant Nutrient Uptake and Growth of Wheat

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ABSTRACT

Wheat is the major cereal crops in Pakistan and inoculation of beneficial microbes plays a major role in crop growth enhancement. This study was conducted to evaluate bacteria with potential to contribute to enhanced plant nutrient uptake and wheat growth. A total of 10 bacterial strains, isolated from the wheat crop, were characterised. The results showed that selected isolates were able to produce Indole-3-acetic acid (IAA) and biofilm, fix atmospheric nitrogen and solubilise inorganic phosphate. However, NIA-2 and NIA-5 were the most efficient among the isolated bacteria in regard to N_2-fixation, biofilm production, P-solubilisation (57.32 and 45.38 %) and IAA production (4.28 and 3.49 mg L^{-1}). A pot study was conducted on wheat crop to investigate the effect of NIA-2 and NIA-5 isolates with full NP fertilisers (120 kg N ha^{-1} in the form of urea, 90 kg P_2O_5 ha^{-1} in the form of DAP) and at half rates of NP fertilizers (60 kg N ha^{-1} in the form of urea, 45 kg P_2O_5 ha_{-1} in the form of DAP). The highest plant height (34.49 cm) and root length (10.38 cm) were observed in half NP fertiliser application inoculated with NIA-5 inoculated treatments. Similarly, the highest plant dry biomass (1.270 g $plant^{-1}$) was recorded in half fertiliser application inoculated with NIA-5. All bacterial inoculated treatments showed the existence of microbes in the soil after 45 days of sowing. Nevertheless, the highest bacterial population was recorded in half NP fertiliser with NIA-05 (5.560 log CFU g^{-1} soil). Significantly highest plant nitrogen (1.344 %), phosphorus (0.697 %) and potassium uptake (0.310 %) were observed in half NP fertiliser with NIA-05. Overall, the half rate of the NP fertiliser inoculated with bacterial isolate NIA-5 improved nutrient uptake and growth of the wheat crop. Thus, this study suggests that these bacterial isolates might be used as an inoculum for enhancing plant growth by supplying plant nutrient and phytohormones.

Keyword: Bacterial population, inoculation, plant uptake, potential, wheat growth inoculation.

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INTRODUCTION

Wheat (*Triticum-aestivum* L.) is grown in most parts of the world. China is the largest wheat grower (30 million hectares), followed by the Russian Federation, India, the USA, Australia, Canada, Turkey and Pakistan. Wheat is one of the important agricultural cereal crops in Pakistan, contributing about 10 % of value added to agriculture and 2.1 % to GDP of the country. Wheat is Pakistan's dietary staple food and its flour accounts for 72 % of Pakistan's daily caloric intake with a per capita wheat consumption of around 124 kg per year (PES 2017). Wheat is cultivated all over the world in various environments. It takes up 36 % of the total cultivated land, 30 % of the value added by major crops and 76 % of the total production of food grains. It offers livelihood to 43.5% of the rural population (PARC 2013). Wheat supplies high energy to the average diet and is the main food cereal crop which is better from the nutritional point of view than most cereals and other food staples (Abedi *et al.* 2010).

Wheat requires nutrients for growth improvement and yield. These nutrients exist in soil but are depleted during the cultivation of various crop plants. Hence, to attain better growth and higher yields of wheat and other agricultural crops, fertilisers are applied for nutrient restoration (Ramteke and Shirgave 2012). The mineral nutrients in soil are solubilised in water and taken up by plant roots. However, nutrient contents in soil are mostly irregular and not sufficient for plants growth. The major nutrients (NPK) which are taken up by plants in higher amounts by crops are frequently found in mixed fertilisers (Khan *et al.* 2009). Increased flux into plant stem could probably deliver supplementary micronutrients for seed biofortification through several mechanisms that enhance uptake of micronutrients by plant roots (Rana *et al*. 2011). NPK influences growth and development of the plant. Nitrogen performs a vital role in plant development as it makes a portion of amino acids, proteins, enzymes and chlorophyll molecules. Phosphorus plays a role in many life processes like photosynthesis, synthesis and breakdown of carbohydrates and the transference of energy in the plant (Obreza 2001). Potassium is crucial for primary physiological purposes e.g. development of sugars and starch, synthesis of proteins and cell division and growth (Abbas and Fares 2009).

Chemical fertilisers in excess have great adverse effects on the soil and environment. One possible way to optimise crop production and maintain a healthy environment is the usage of organic sources and soil microorganisms, mainly bacteria that perform several biological processes in plant growth and nutrient cycles. Biofertilisers comprise microorganisms which are helpful to plant growth and enhance crop yield. Several free living bacteria, useful for plant growth and for high yields, are known as plant growth promoting *rhizobacteria* (PGPR) (Kloepper 1994). The beneficial microbes contribute to plant growth through the production of Indole-3-acetic acid (IAA), gibberellic acid, indole-3 butyric acid, siderophore, ammonia, HCN and solubilisation of inorganic phosphate. Anand and Nikhilesh (2015) showed that application of isolated microbes was able to promote growth of wheat resulting in higher productivity. These beneficial

bacteria perform a vital role in improving growth and yield of crop plants (Ahemad and Khan 2009). Nitrogen fixating bacteria are important and perform a positive role in supplying nitrogen to the soil. The sensible utilisation of chemical fertilisers including that of N_2 fixing inoculants and rhizobium can be an alternate or supplementary source for crop production (Dobrei *et al*. 2001). In this case, soil beneficial microbes can be an alternative method for viable crop production (Panhwar *et al.* 2014). The use of several beneficial microbes such as *Azospirillum* sp. and *Azotobacter* sp. as bio-inoculants for plant growth has been confirmed by studies (Naher *et al.* 2016; Panhwar *et al.* 2014; Ribaudo *et al.* 2006). The use of biofertilisers in agriculture offers several benefits: (i) is an organic source ii) minimises environmental pollution; (iii) improves soil health; and (iv) reduces input cost of agriculture (Shamshuddin *et al.* 2016). In addition, the cumulative effect of humic substances and biofertilisers in soil enhances nutrient absorption by increasing the availability of nutrients to improve the physical structure of soil. Biofertilisers are safe substitutes to the use of chemical fertilisers as they are environmentally friendly, have less impact on animals and human beings and reduce pollution of the environment (Naher *et al*. 2016).

The application of biofertilisers increases the absorption accessibility of several minerals to the plant and allows the plant to develop resistance to; this results in a 25% reduction in nitrogen requirement to the plants (Kannaiyan 2002). In addition, the combined application of beneficial microbes results in a significant upsurge in spikes, number of tillers, grain weight, grain size, spikelet per plant, spike length etc. Thus the use of 75% mineral N and beneficial microbes as biofertiliser enhanced all the growth components in wheat (Chauhan *et al.* 2011). These microbes (*Azotobacter* etc.) enhance the yield of many agricultural crops by about 10-12 % (Jaga and Singh 2010). A study by Kaushik *et al.* (2012) found that inoculation of beneficial microbes improved nitrogen fixation and phosphate solubilisation as well as enhanced straw and grain yield of plants compared to non-inoculated treatments. Mehnaz *et al*. (2010) found that the use of soil microorganisms such as *Rhizobium* and *Azotobacter* can fix atmospheric N_2 and synthesise growth enhancing substances which provide higher amounts of humus in soils in an environment friendly soil ecosystem. Panhwar *et al*. (2011) found that the use of beneficial bacteria in soil rhizosphere improved plant growth and yield. There has been much research interest in the potential of beneficial microbes and there is now an increasing number of microbes being commercialised for various crops. Several reviews have discussed specific features of plant growth promotion by these microbes (Saharan and Nehra 2011). Hence, the study was conducted to screen out, characterise and assess the potential of selected bacterial isolates to improve wheat growth.

MATERIALS AND METHODS

The research was conducted in the Soil Microbiology Laboratory at the Nuclear Institute of Agriculture (NIA) Tandojam, Sindh, Pakistan during the 2016-17 wheat season. A total of 10 bacteria isolated from wheat crop were selected to

assess their potential. The preliminary study was conducted under laboratory conditions for characterisation of isolates while the second study was done in a net house.

Laboratory Experiment

Screening and characterisation of potential bacteria for improving wheat growth
The experiment was conducted in the Soil Microbiology Laboratory at Nuclear Institute of Agriculture (NIA) Tandojam in three replications. The following observations were recorded.

Determination of Indole acetic acid (IAA) production
The bacterial strains were cultured on nutrient media containing 2 mg mL^{-1} tryptophan and incubated at 28 ± 2°C for 48 h. The cultures were centrifuged at 7000 rpm for 7 m and one mL of the supernatant was added to 2 mL of Salkowsky's reagent (Gordon and Weber 1951). The IAA-concentration was determined using spectrophotometer at 535 nm.

Determination of phosphate solubilisation activity
The P-solubilising activity of the bacteria was determined by spotting 10 µl of 48 h cultures on NBRIP media plates. The media plates were incubated at 30°C for one week and observed for halo zone formation. The solubilising activity was calculated by following the formula of Nguyen *et al.* (1992).

$$P \quad Solubilization\ efficiency = \frac{solubilization\ diameter(halo\ zone)}{growth\ diameter\ of\ colony} \times 100$$

Determination of nitrogen fixation activity
The nitrogen fixing activity of the bacteria was determined by culturing one loop of fully-grown bacterial culture in Nfb semi-solid liquid medium (Gyaneshwar *et al.* 2001). Results were confirmed by pellicle formation.

Biofilm production
The inoculated broth cultures of the bacteria were incubated at 30°C on a Kotter man 4020-shaker at a medium speed of 80 cycle's min^{-1}. After 72 h, the culture was observed for biofilm production. Detection of biofilm production was done using the tissue culture plate method (TCPM). The development of film is described as biofilm productive bacteria (Sultan and Nabiel 2018).

Net House Study
The soil for the experiment was taken from the NIA Farm Tandojam and soil physico-chemical properties were determined before the experiment. Soil texture was assessed by Bouyoucos hydrometer method (Bouyoucos 1962); organic matter by Walkely and Black (1934) method; electrical conductivity was measured

by an electrical conductivity meter at a ratio of 1:5-soil water extract; soil pH was measured in soil:water (1:5) extract using PHM210 Standard pH meter at 30°C (Benton 2001); total nitrogen was determined by Kjeldahl digestion method (Bremner and Mulvaney 1982) and available P and K by AB-DTPA method (Soltanpur and Schwab1977).

Treatment and experimental design

The soil was air dried, ground and passed through a 2-mm sieve. A total of 5 kg of sieved soil was sterilised at 121°C for 60 m by autoclaving. The sterilised soil was transferred into plastic pots (17 cm diameter × 23 cm height) and the experiment was conducted in a net house at the Nuclear Institute of Agriculture (NIA) Tandojam during the 2016-2017 season. The experimental set up involved three replications in a complete randomised design (CRD) with factorial (bacteria and chemical fertiliser) arrangements (Table 1). Chemical fertilisers consisting of 120 kg of N ha^{-1} in the form of urea, 90 kg of P_2O_5 ha^{-1} in the form of DAP as a full recommended dose and 60 kg of N ha^{-1} in the form of urea, 45 kg of P_2O_5 ha-1 in the form of DAP was the half rate of recommended fertilizer were applied. However, 5 kg of Zinc in the form of zinc sulfate were applied in all treatments.

TABLE 1
Description of treatments in pots

Treatment	Description
T1	$N_F + P_F$ without Bacteria
T2	$N_H + P_F$ without Bacteria
T3	$N_F + P_H$ without Bacteria
T4	$N_H + P_H$ without Bacteria
T5	$N_F + P_F$ NIA-02
T6	$N_H + P_F$ NIA-02
T7	$N_F + P_H$ NIA-02
T8	$N_H + P_H$ NIA-02
T9	$N_F + P_F$ NIA-05
T10	$N_H + P_F$ NIA-05
T11	$N_F + P_H$ NIA-05
T12	$N_H + P_H$ NIA-05

N_H= nitrogen half; N_F = nitrogen full; P_H= phosphorus half; N_F = phosphorus full

Inoculum preparation and seed inoculation

The two best bacteria with potential were selected from the previous study to assess its effect on growth and nutrient uptake of wheat. A pure culture of bacterial strains was cultured in nutrient broth for 48 h. The bacterial cells were collected by centrifugation at 13500-rev min^{-1} for 10 min in Eppendorf tubes and washed with 0.85% sterilised phosphate buffer saline (PBS). The optical density (OD600) of washed cells was checked and adjusted consequently. The bacterial population was confirmed by using drop plate method on nutrient agar (NA). The wheat seeds were soaked in the bacterial solution at a population of 10^9 CFU mL^{-1} for 2 h prior to planting. The non-inoculated seeds were given the same amount of killed cells (autoclaved for 30 min at 121°C).

Determination of total bacterial population

After 45 days of sowing, rhizospheric bacterial population was determined. Approximately 10g of soil sample was added into a conical-flask containing 90 mL sterilised distilled water. The mixture was shaken vigorously on a rotary shaker for 10-m to suspend bacterial cells. A serial dilution was prepared and the total bacterial population was determined following spread plate count method on nutrient agar plate (Somasegaran and Hoben 1985).

Agronomic data collection

Plant height, root length and plant leaf numbers were observed fortnightly. After 45 days plant samples were harvested and cleaned and dried in an oven at 70°C for three days.

Nutrient uptake (NPK)

Total plant nitrogen was determined by Kjeldhahl method (Bremner and Mulvaney 1982) P was analysed by wet digestion method of Havlin and Soltanpur (1980) and exchangeable K was extracted using 1 mol L^{-1} NHOAc buffered at pH 7.0 (Benton 2001).

Statistical analysis

The collected data were analysed statistically using STATISTIX 8.1. Tukey's LSD test was employed for multiple comparisons.

RESULTS

Laboratory Experiment

Biochemical characterisation

A total of 10 bacteria strains with potential were screened, characterised and studied further based on their different colony morphological-characteristics (Table 2). Futher table 2 shows eleven of the strains had N_2 fixation ability while NIA-4, NIA-7, NIA-8 and NIA-10 did not; for phosphate solubilisation ability, only NIA-1 and NIA-3 did not possess this ability. All strains, except for NIA-4 and NIA-9, were able to produce biofilm (Table 2).

TABLE 2
Biochemical properties of potential bacteria isolated from the wheat crop

S. No.	Isolates	Colony morphology	Nitrogen fixing ability	Phosphate-solubilising ability	Biofilm production
1	NIA-1	Circular, translucent, dull	+ve	-ve	+ve
2	NIA-2	Transparent, oval	+ve	+ve	+ve
3	NIA-3	Translucent, gummy	+ve	-ve	+ve
4	NIA-4	Circular, translucent, off white, shiny	-ve	+ve	-ve
5	NIA-5	Irregular, off white	+ve	+ve	+ve
6	NIA-6	Circular, off white	+ve	+ve	+ve
7	NIA-7	Circular, translucent	-ve	+ve	+ve
8	NIA-8	Circular, light yellow, gummy	-ve	+ve	+ve
9	NIA-9	Brown, circular, sticky	+ve	+ve	-ve
10	NIA-10	Oval, light yellow	-ve	+ve	+ve

Note: +ve = positive, -ve = negative

Determination of IAA production by bacteria

All bacterial isolates were examined for IAA production and all strains were able to produce IAA in the nutrient broth culture in the range of 1.87−4.28 mg L^{-1}. The highest IAA production of 4.28 mg L^{-1} was observed in NIA-2 followed by NIA-5 with 3.49 mg L^{-1} (Figure 1).

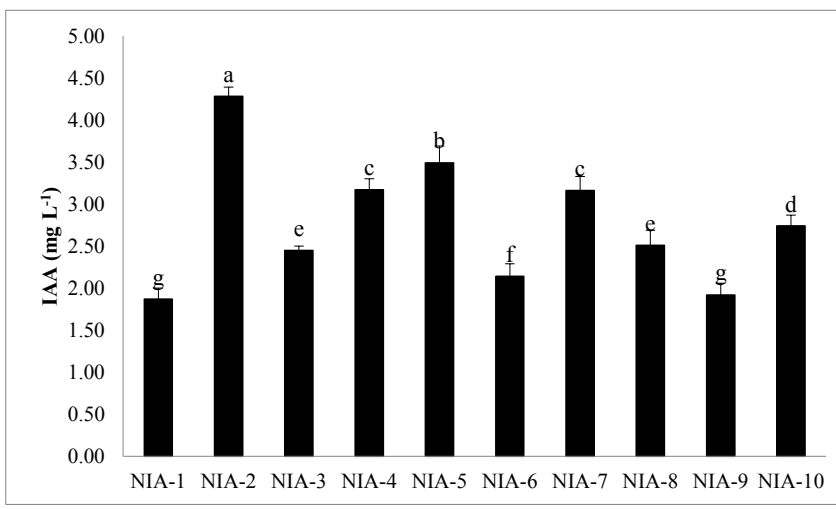

Figure 1. Production of IAA by potential bacteria isolated from wheat crop
Means within the same column followed by the same letters are not significantly different at P<0.05

Determination of phosphate solubilising activities by bacteria

The selected bacteria (10) were observed for phosphate solubilising ability on the NBRIP agar media plates. The P solubilisation proficiency of the selected isolates was different as they produced a different diameter of halo zones (Figure 2). The highest P solubilisation efficiency was found by NIA-2 (57.32 %) followed by NIA-5 (45.38 %). The lowest solubilisation efficiency was recorded by NIA-8 (16.42 %).

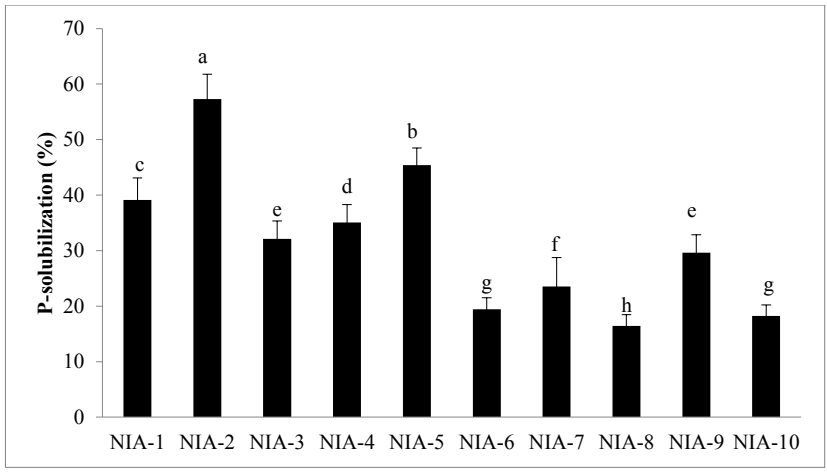

Figure 2.Phosphate solubilisation by potential bacteria isolated from wheat crop
Means within the same column followed by the same letters are not significantly different at P<0.05

Net house study

Soil physico-chemical properties

Table 3 shows the physic-chemical properties of the soil used for the pot experiment. The soil was non-saline and slightly alkaline in nature. However, the soil was low in organic matter (0.88 %), organic carbon (0.512%) and nitrogen percentage (0.03 %) but levels of phosphorus and potash were low to adequate.

Effect of beneficial bacteria on the agronomic parameters of wheat

There were significant differences observed among the various treatments (Table 4) after 15 days of sowing. The highest plant height (16 cm) was observed in N_H + P_H NIA 02, and N_F + P_H NIA-05 followed by N_F + P_H NIA 02 (15 cm). The longest root was found in N_H + P_H NIA-05 (4.73 cm) followed by N_H + P_H NIA 02(4.50 cm). However, there was no significant difference in leaf numbers (3 plant^{-1}) after 15 days of sowing among the treatments. The highest plant dry biomass (0.071 g plant^{-1}) was reported in N_H + P_H NIA 05 followed by N_H + P_H NIA-02 (0.060 g plant^{-1}).

TABLE 3
Soil physic-chemical properties of experimental soil

Parameters	*EC	pH	*OM	*OC	*N	*P	*K
	(dSm^{-1})	----------%----------				------(mg kg^{1})------	
	1.125	7.7	0.88	0.512	0.03	4.17	127

*OM = Organic matter, *OC = Organic carbon

After 30 days of sowing there were significant diverse observations between the various treatments (Table 4). Significantly (P<0.05), the highest plant height (28.84 cm) was observed in $N_H + P_H$ NIA 05, followed by $N_H + P_H$ NIA 02 (28.57 cm). The highest root length (7.30 cm) was found in $N_H + P_H$ NIA 05 followed by $N_H + P_H$ NIA 02 (7.29 cm). However, no differences were found in leaf numbers of plant (5 $plant^{-1}$) after 30 days of sowing. The highest plant dry biomass (0.093 g $plant^{-1}$) was reported in $N_H + P_H$ NIA 05 followed by $N_H + P_H$ NIA 02 (0.085 g $plant^{-1}$).

After 45 days of sowing, the highest plant height (34.50 cm) was observed in $N_H + P_H$ NIA 05 followed by $N_H + P_H$ NIA 02 treatment (33.47 cm) (Table 4) while the highest root length was found in $N_H + P_H$ NIA 05 (10.38 cm) followed by $N_F + P_H$ NIA 05 (10.30 cm). However, no difference was found in number of leaves (7 plant-1) after 45 days of sowing. The highest plant biomass (1.270 g plant-1) was reported in $N_H + P_H$ NIA 05 followed by $N_H + P_H$ NIA 02 (1.210 g $plant^{-1}$).

Total bacterial population trend during the wheat growth

Table 5 shows the bacterial population during the growth period after 15, 30 and 45 days of sowing wheat inoculated by the beneficial bacteria. There were significant varied observations among the treatments. The bacterial population initially showed an increasing trend up to 30 days of sowing but after 45 days, the bacterial population began to show a slightly decreasing trend among all treatments. However, the highest total bacteria population after 15 days (4.840 log CFU g^{-1} soil) was observed in $N_F + P_H$ NIA-05; in 30 days, (6.910 log-CFU g^{-1} soil) was observed in in $N_F + P_F$ NIA-02; and after 45 days (5.560 log CFU-g^{-1} soil) was observed in $N_F + P_F$ NIA-02 followed by $N_H + P_H$ NIA-05 treatment (5.340 log CFU-g^{-1} soil).

Effect of beneficial bacteria on the plant nutrient uptake of wheat

Table 6 shows that significantly (P<0.05) high nitrogen uptake (1.353 %) was noted in $N_H + P_H$ NIA-05 followed by $N_H + P_H$ NIA-02 (1.344%). However, the highest phosphorus uptake (0.697 %) was found in the $N_H + P_H$ NIA-05 treatment followed by $N_F + P_H$ NIA 02 treatment with 0.696 %. Significantly (P<0.05) high potassium (0.310 %) plant uptake was observed in $N_H + P_H$ NIA-05 followed by $N_F + P_H$ and $N_F + P_F$ inoculated with NIA-05 treatments. The non-inoculated treatments received less plant uptake among all treatments.

TABLE 4
Effect of beneficial bacteria on agronomic parameters of wheat

Treatments	Plant height (cm)			Root length (cm)			Number of leaves (plant^{-1})			Plant dry biomass (g plant^{-1})		
	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS
N_H + P_H without bacteria	13.50cd	26.663de	31.160e	3.58e	5.65e	8.59g	3	5	7	0.0357f	0.0556e	0.883d
N_H + P_F without bacteria	12.00e	24.000g	31.000e	3.40ef	3.21h	8.31g	3	5	7	0.0203j	0.0359i	0.470h
N_F + P_H without bacteria	14.00c	26.470e	31.503de	3.47ef	5.60e	8.48g	3	5	7	0.0328g	0.0504f	0.810e
N_F + P_F without bacteria	12.00e	23.833g	30.000f	2.50g	5.19g	8.30g	3	5	7	0.0161k	0.0331j	0.333i
$N_H + P_H$ NIA-02	16.00a	28.573a	33.473b	4.50b	7.29a	9.22bcd	3	5	7	0.0607b	0.0847b	1.210a
$N_H + P_F$ NIA-02	13.00d	26.427ef	30.993e	3.29f	5.33f	8.92dc	3	5	7	0.0239i	0.0403h	0.546g
$N_F + P_H$ NIA-02	15.00b	27.497c	32.667bc	3.90d	6.12c	9.11cd	3	5	7	0.0477d	0.0714c	1.083b
$N_F + P_F$ NIA-02	13.00d	26.440e	30.997e	3.45ef	5.50e	8.65ef	3	5	7	0.0338g	0.0504f	0.760e
$N_H + P_H$ NIA-05	16.00a	28.840a	34.490a	4.73a	7.30a	10.38a	3	5	7	0.0705a	0.0933a	1.270a
$N_H + P_F$ NIA-05	13.00d	26.160f	31.020e	3.35f	5.41f	9.46b	3	5	7	0.0290h	0.0433g	0.646f
$N_F + P_H$ NIA-05	16.00a	28.170b	32.660bc	4.14c	6.36b	10.30a	3	5	7	0.0519c	0.0732c	1.136b
$N_F + P_F$ NIA-05	13.66cd	26.827d	32.160cd	3.63d	5.85d	9.30bc	3	5	7	0.0410e	0.0605d	0.960c
LSD	1.66	0.274	0.846	0.228	0.171	0.326	1.685	1.685	2.148	0.0014	0.0028	0.621

Means within the same column followed by the same letters are not significantly different at $P<0.05$

TABLE 5
Total bacterial population trend during wheat growth

Treatment	15 days after sowing	30 days after sowing	45 days after sowing
	----------(log CFU g^{-1} soil) ----------		
N_H + P_H Without bacteria	0.000	0.000	0.000
N_H + P_F without bacteria	0.000	0.000	0.000
N_F + P_H without bacteria	0.00	0.000	0.000
N_F + P_F without bacteria	0.000	0.000	0.000
$N_H + P_H$ NIA-02	4.520d	6.690b	5.240d
$N_H + P_F$ NIA-02	4.360e	6.430f	5.080c
$N_F + P_H$ NIA-02	4.533d	6.603d	5.253d
$N_F + P_F$ NIA-02	4.767b	6.910a	5.560a
$N_H + P_H$ NIA-05	4.620c	6.690b	5.340b
$N_H + P_F$ NIA-05	4.580cd	6.650c	5.300c
$N_F + P_H$ NIA-05	4.840a	6.546e	5.196e
$N_F + P_F$ NIA-05	0.0389	0.0430	0.0854
LSD	4.520d	6.690b	5.240d

Means within the same column followed by the same letters are not significantly different at $P<0.05$

TABLE 6
Effect of beneficial bacteria on nutrients uptake by wheat plant

Treatment	N	P	K
	---------- (%) ----------		
$N_H + P_H$ without bacteria	1.288b	0.555e	0.160ef
$N_H + P_F$ without bacteria	1.190cd	0.617c	0.160ef
$N_F + P_H$ without bacteria	1.055e	0.645b	0.230c
$N_F + P_F$ without bacteria	1.050e	0.476f	0.230c
$N_H + P_H$ NIA-02	1.344a	0.685a	0.190d
$N_H + P_F$ NIA-02	1.022e	0.584d	0.150f
$N_F + P_H$ NIA-02	1.288b	0.696a	0.280b
$N_F + P_F$ NIA-02	1.218c	0.547dc	0.280b
$N_H + P_H$ NIA-05	1.353a	0.697a	0.310a
$N_H + P_F$ NIA-05	0.924f	0.629bc	0.170e
$N_F + P_H$ NIA-05	1.204cd	0.684a	0.300a
$N_F + P_F$ NIA-05	1.171d	0.574d	0.300a
LSD	0.046	0.026	0.0169

Means within the same column followed by the same letters are not significantly different at $P<0.05$

DISCUSSION

Beneficial bacteria are a cluster of microorganisms which vigorously colonise plant roots and improve plant growth and yield. The mechanism of these bacteria by which they stimulate plant growth is their capability to produce phytohormones, fix N_2, synthesise antibiotics and enzymes and solubilise phosphates and micronutrients. The bacteria mostly show adequate persistence and survival in the plant rhizosphere. The significant propagation in plant growth and increased

nitrogen level both in shoot and root on bacterial isolates application is vibrant, suggestive of the fact that the bacteriological isolates could be capable of delivering improved nutrient flux to the host plant resulting in an upsurge of plant biomass and increased N. The development in root length due to the inoculation of useful isolates which enhanced N uptake in plant shoot.

The ten selected bacteria with potential were screened from wheat crop and characterised for their beneficial traits. Most of the bacteria had nitrogen fixation abilities. The application of soil microbes such as *Rhizobium* and *Azotobacter* can fix-atmospheric N_2 and promote production of growth enhancing abilities by decomposition of plant-residues for better plant nutrient management (Mehnaz *et al*. 2010). Similarly, Panhwar *et al*. (2012) reported that several soil microbes have beneficial plant traits. Furthermore, PGPR are free-living soil microbes which vigorously inhabit the plant rhizosphere and stimulate the growth and yield of several plants after their application to seed or plants (Kumar *et al.* 2014). In the case of biofilm formation, all bacterial strains isolated in our study were able to form biofilm with the exception of NIA-4 and NIA-9. Earlier we had already reported that biofilm was formed by the most of the isolates (*Bacillus* followed by *Pseudomonas* and *Azotobacte*r) (Table 2). Further assessment of the *Bacillus* for their efficiency on survival in the rhizopshere showed better survival compared to control. Previous studies have also shown that in vitro biofilm formation has significantly positive correlation with plant root colonisation (Panhwar *et al.* 2011; 2015). Therefore, such beneficial microbes with their biofilm formation should play an effective role in preventing competing organisms, nutrient uptake, quick reactions, and adaptating to changing environmental conditions. An earlier study by Seneviratne *et al.* (2011) showed that plant associated biofilms has a significant capability to defend themselves from external stresses and other microbial competitors in the rhizosphere, and to create beneficial effects on plant growth.

Most of the bacteria isolated in our study had the capability to produce IAA, a major beneficial characteristic of beneficial microbes. Several mechanisms have been assumed to clarify how microbes affect positively the plant host. These comprise the capability to produce plant growth regulators as indole acetic acid (IAA), cytokinins and gibberellins (Marques *et al.* 2010). Furthermore, IAA production by beneficial bacteria screened from the rhizosphere of wheat, maize, peanut, and rice had been previously defined in a number of research studies (Ali *et al*. 2016; Naher *et al*. 2016; Panhwar *et al.* 2014).

The selected beneficial bacteria (10) were found to be positive for P solubilising activity on the Pikovskaya agar media plates, while their efficiency varied as they produced various hallo zones around their colonies. The highest P solubilisation efficiency was exhibited by NIA-2 (57.32 %) while the minimum was recorded in NIA-8 (16.42 %). Many bacteria with potential to contribute to plant growth isolated from various crops belong to *Pseudomonas*, *Bacillus, Enterobacter, Serretia, Pantoea, Azospirullum, Azotobacter, Rhizobium, Burkholderia* and *Flavobacterium* (Deepa *et al*. 2010). Among the 23 bacterial

isolates Singh *et al*. (2017) isolated, they found 17 isolates exhibited IAA production, three showed siderophore production capability and 13 showed NH_4 production ability and exhibited a clear halo zone around the colonies on tricalcium phosphate containing Pikovaskaya's agar plates. P solubilisation is generally production of bacteriological metabolites containing organic-acids that decreases the pH of the culture medium (Shahid *et al.* 2012).

Wheat plant-growth in the pot study benefited from the bacterial inoculation and inoculated treatments as it was reflected in better plant enhancement compared to the non-inoculated treatments. The maximum plant height, number of leaves, root length and plant dry biomass was observed in NIA-05 followed by NIA-02 at half rate (60 kg of N and 45 kg of P_2O_5 ha^{-1}) of fertilised treatments throughout the planting period. The inoculation of beneficial microbes to the plants showed significant correlation of weight of panicles and grains, plant biomass, N, P and iron (Fe) with acetylene reduction (ARA) activities, demonstrating the impact of N_2 fixation in whole crop productivity (Rana *et al.* 2012).

Plant growth promoting microorganisms vigorously inhabit plant rhizosphere and increase growth and yield of plant when smeared on seed or crops (Kumar *et al.* 2014). Additionally, use of chemical fertilisers and plant growth promoting bacteria enhance urease actions of the soil. The occurrence of beneficial bacteria in arrangement of various doses (25 and 50 % reduced) of chemical fertilisers improved the effect on soil enzymatic activities. Maximum plant growth and leaf protein, and maximum upsurge in root length was developed by *Azotobacter* and *Azospirillum* microbes with a mixture of chemical fertilisers (25 and 50 % reduced). Leaf area and chlorophyll content significantly increased through *Azotobacter* combined with half dosage (50 % reduced) of chemical fertilisers (Nosheen and Ban 2014). The beneficial bacterial strains of *P*. sp. NUU-1 and *P. fluorescens* NUU-2 considerably enhanced shoot and root length and dry weight of wheat. According to Egamberdieva (2010), the inoculation of the wheat plant with *Pseudomonas* strains is able to increase plant growth in calcareous soils but this growth also depends on the wheat cultivar used.

Our study showed that bacterial inoculums were capable of inhabiting the plant roots as well as inducing a significant optimistic effect on enhancing plant growth. However, there were significant variations among the various bacterial populations during the planting period. The bacterial population initially showed an increasing trend up to 30 days of sowing but after 45 days, the population showed a slightly decreasing trend among all the treatments.

The application of positive microbes to seeds is an effective mechanism for employment of bacterial inoculation into the soil where it will colonise roots of seedlings and defend the plant against several infections and pests (O'Callaghan 2016). Philippot *et al.* (2013) states that inoculation of beneficial microbial inoculants to the rhizosphere zones of soil surrounding the roots allow the plants to act directly with microbes resulting in beneficial effects on plant growth and development The plant rhizosphere is a region of strong bacterial activity that promotes plant growth. Furthermore, beneficial microbes rely on the reciprocal

facility of plant nutrients and a wide range of supplementary traits including plant growth regulators and antibiotics. Several microbes have significant agricultural significance in the rhizosphere as they have the capability to enhance plant-growth through a range of mechanisms (Babalola 2010).

The inoculation of bacterial isolates promotes plant growth by providing adequate nutrients. Comparatively, all beneficial bacterial isolates improved nutrient uptake compared to non-inoculated treatments. Significantly (P<0.05) the highest N (1.344 %), P (0.684 %) and K (0.310%) uptake was found in NIA-05 inoculated treatment with half dose (60 kg of N and 45 kg of P_2O_5 ha^{-1}) of the fertiliser. Comparable results were obtained by Turan *et al.* (2015) who found that bacterial inoculations along with fertiliser applications significantly enhanced wheat growth, total biomass yield and nutrients compared with control. Furthermore, inoculation of a bacterial consortium of OSU - 142 + M - 13 + *Azospirillum* sp. 245 significantly increased grain yields of crop at half rate (60 kg of N ha^{-1}) of nitrogenous fertiliser doses compared with a full dose of nitrogen. In addition, inoculation of a bacterial consortium has been found to significantly improve uptake of major nutrients such as N, P, K, S, Ca and Mg including micro-nutrients of Fe, Mn, Cu and Zn in the wheat plant as reflected in grains, leaves, and straw parts of the plants.

Bacterial strains have several useful effects on enhancing plant growth as observed by developments in seed germination, increase in roots, shoot & root weight, leaf area, yield, hydraulic activities, chlorophyll content, protein content and nutrient uptake. The use of helpful microorganisms in agricultural production systems has long been in use and there is strong evidence that helpful microorganisms can increase plant tolerance to adverse environmental stresses including salt stresses (Egamberdieva 2010). Several research studies (Rana *et al.* 2015; Pnahwar *et al.* 2014; Panhwar *et al*. 2011) have reported the beneficial effect of bacteria on plant growth when inoculated with rhizobacterial strains AW-1 *Bacillus* sp., AW-5 *Providencia*-sp. and AW-7 *Brevundimonas* sp. Inoculated along with 2/3-recommended dosage of N and full dose of P & K fertilizer applications. About 14 to 34 % in plant agronomic parameters and 28 to 60 % in micronutrient contents were enhanced in treatments inoculated with a mixture of rhizobacterial isolates with a full rate of fertiliser application. These treatments, including inoculation using rhizobacterial strains, were analysed for the maximum percent of P & N and found a two-fold improvement in P and 66.7 % enhancement in N with full P and K fertiliser application. Likewise a substantial association was reported in plant biomass, grain weight, panicle weight, N & P, and Fe with decreased activity in acetylene, showing the consequence of N_2 fixation for crop production (Rana *et al.* 2015).

CONCLUSION

Bacterial isolates with potential, selected from the wheat crop, exhibited various beneficial traits such as N_2 fixation, P-solubilisation, IAA production and biofilm formation. Inoculation of the bacterial isolates enhanced growth of the wheat

crop. The results showed that at half the NP fertilizer dose (60 kg of N and 45 kg of P_2O_5 ha^{-1}) wheat plants inoculated with NIA-05 exhibited the highest beneficial effects on plant growth parameters and nutrient uptake. Based on the results of our study, it is concluded that isolates (NIA-2 and NIA-5) which produced the highest amount of IAA offered maximum effect on enhancing growth of the wheat crop. This study suggests that the bacterial isolates isolated in our study have the potential for IAA production, besides having other beneficial traits such as; Nitrogen fixation, P-solubilsation which could be used for developing biofertiliser products for plant growth enhancement and as a supplementary source of plant nutrients.

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