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MICROBIOLOGY

Enhancement of Growth and Yield Parameters of Wheat Variety AAI-W6 by an Organic Farm Isolate of Plant Growth Promoting *Erwinia* Species (KP226572)

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ABSTRACT

Plant growth promoting bacteria (PGPB) play an important role in agricultural production and soil fertility. Wheat is a highly consumed cereal food crop of the world population and sustainable wheat productivity achieved by the application of bacterium in combination with NPK is promising. In the present study bacterial isolate (PR6) from soil of organic farm was included. The isolate (PR6) was screened for its morphological, biochemical and plant growth promoting characteristics, sequenced by 16S rDNA method and submitted to NCBI for the confirmation of strain identification. Further, the inoculation effect of the bacterial isolate in combination with NPK on growth and yield parameters of wheat var. AAI-W6 were analysed. The isolate (PR6) was identified as *Erwinia* sp. with NCBI Accession No. KP226572. The organism possessed multiple plant growth promoting (MPGP) traits such as production of ammonia, siderophore, indole-3-acetic acid (IAA), ACC Deaminase (ACCD) and showed phosphate solubilization activity. The organism was found tolerant to 10% salt, wide range of pH 5- 9, higher levels of trace elements and heavy metals and possessed resistant to multiple antibiotics. Inoculation of wheat variety AAI-W6 with the *Erwinia* species showed significant increase in seed germination and enhancement in elongation of root and shoot compared to untreated control. The combined application of PGPB (*Erwinia* sp.) along with NPK treatments showed similar significant results in all growth and yield parameters of wheat. This study is the first report on the beneficial effects of organic farm isolated *Erwinia*-NPK treatment combinations on sustainable wheat productivity.

Highlights

- The organism was found tolerant to 10% salt, wide range of pH 5- 9, higher levels of trace elements and heavy metals and resistant to multiple antibiotics.
- The combined application of PGPB (*Erwinia* sp.) along with NPK treatments showed similar significant results in all growth and yield parameters of wheat.

Keywords: *Erwinia* sp. MPGP traits, stress tolerance, wheat, NPK, growth enhancement

Now-a-days agricultural practice based on organic farming is more important for nutritionally better crop produce as well as for the improvement of soil fertility. Organic farming helps to improve food quality and safety (Giles 2004). The organic farming utilizes organic manures including farm yard manure, compost, green manuring etc. These organic manures are easily degradable and ecofriendly and their application enhance soil fertility. The application of green manure on crops

can further improve the humus, organic carbon, nitrogen, soil microbial growth and activity with subsequent mineralization of plant nutrients (Randhawa *et al.* 2005; Eriksen 2005; Dubey *et al.* 2015).

A large number of bacterial population plays an important role in soil enrichment, these rapidly multiplying organisms are widely available and provide sufficient nutrients, carbon and nitrogen source. Colonization and multiplication of bacteria along the surface of inoculated plants can protect and promote plant growth (Mishra *et al.* 2008). These beneficial bacteria are known as plant growth promoting bacteria (PGPB). They posses plant growth promoting (PGP) traits that stimulate growth and yield of crops. Their application in assimilated agriculture handling can also play a vital role in crop protection, growth promotion and in maintaining soil fertility for susceptibility of agroecosystem (Dilantha *et al.* 2006; Rana *et al.* 2011). It has been established that PGPR isolates from soil can improve growth and yield in a wide range of cereal crops including wheat (Mehnaz *et al.* 2010; Zhang *et al.* 2012). The co-inoculation of salt tolerant PGPR species with wheat seeds would be useful for amelioration of saline stress and such practice can significantly increase the growth and yield of wheat crops in saline soil (Upadhyay *et al.* 2012; Upadhyay and Singh 2015). The results reported by Kumar *et al.* (2014) suggested that the combined application of indigenous PGPR, *Bacillus megaterium*, *Arthobacter chlorophenolicus* and *Enterobacter* is an efficient microbial consortium for wheat production. In the present study, a potential bacterial strain isolated from soil samples collected from SHUATS model organic farm (SMOF) was identified by morphological, biochemical tests and molecular characterization. The plant growth promoting activity of the identified strain along with its effectiveness in improving plant growth was evaluated *in situ* on a recently released promising wheat variety AAI-W6 under field conditions.

MATERIALS AND METHODS

Bacterial culture

Bacterial culture obtained from culture collection of Department of Biological Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Allahabad, India was included in the study. The organism was isolated from Model Organic Farm [SMOF] of SHUATS Allahabad.

Identification of bacterial culture

The organism was identified by its morphological and biochemical characteristics including Gram staining, oxidase test, indole test, methyl red test, Vogus Proskaur test, citrate utilization, glucose fermentation, acid from lactose, H_2S production, sugar fermentations, acetoin production, catalase activity and Gelatin liquefaction (Aneja 2001).

DNA isolation, PCR, 16S rDNA sequencing and identification of organism

The organism was cultured in 50 ml of nutrient broth, incubated overnight at 37 °C. Bacterial cells were harvested by centrifugation and genomic DNA was isolated (Kumar *et al.* 2013). The 16S rDNA gene of isolated genomic DNA was amplified using universal primers F-5′-TGGCTCAGATTGAACGCTGGCGG-3′ and R- 5′-GATCCAGCCGCAGGTTCCCCTAC-3′. The amplification reaction was performed in a Thermal cycler (Bio-Rad) programmed for an initial cycle of 95 °C for 5 min, followed by 40 cycles of 95 °C for 30s, 52.5 °C for 30s, 72 °C for 2 min, followed by a final extension at 72 °C for 7 min. The PCR products were separated by electrophoresis (at 75 V cm^{-1} for 50 min) on 1.5 % (w/v) agarose gels with 1X TAE buffer. The gels were then stained with ethidium bromide to visualize amplified products under UV light using a gel documentation system (Bio-Rad, Philadelphia, PA, USA). Isolates were genetically confirmed by 16S rDNA sequencing using Sanger method of sequencing. Further, the 16S rDNA sequences obtained from the isolates were compared with the reference strain sequences available in the GenBank database using the NCBI ((http://blast.ncbi.nlm.nih.gov) Blast program. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura *et al.* 2011).

Plant growth promoting traits

The PGP traits of the organisms studied were as follows:

Ammonia production (NH_3): NH_3 production was analyzed by inoculating the freshly grown organism

in 10 ml of peptone water and incubating for 48-72 hours followed by the addition of 0.5 ml of Nessler's reagent. Development of brown to yellow colour was positive for ammonia production (Cappucino and Sherman 1992).

Hydrogen cyanide (HCN) production: HCN production was detected using the method of Bakker and Schippers, 1987. King's medium was amended with 4.4 g glycine l^{-1} and the organism was streaked on agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate and 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated at 37°C for 4 days. Development of yellow to red colour on the filter paper indicated HCN production.

Siderophore (SD) production: SD production was observed by spot inoculation of **o**vernight grown organism on CAS agar plate and was incubated at 37˚C for 7 days. Appearance of orange halos around the colonies on the blue coloured agar indicated the production of SD (Schwyn and Neilands 1987).

Production of IAA: Production of IAA was detected as described by Brick *et al.* (1991)**.** The organism was grown in peptone water at 37°C for 72 hour. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with 2 drops of orthophosphoric acid and 4 ml of Salkowski reagent. Development of pink colour indicated IAA production.

ACC Deaminase activity: ACC Deaminase activity was performed as described by Safronova *et al.* (2006). The organism was grown in test tube containing 100 ml of liquid medium containing KH_2PO_4 (2g), K_2HPO_4 (0.5g), $MgSO_4$ (0.2g), Glucose (0.2g). The medium was supplemented with 0.3g ACC or 0.19g $(NH_4)_2SO_4$ as N source and incubated at 37°C for 24 -72hrs. The appearance of bacterial growth indicated ACC deaminase activity of the organism.

Phosphate solubilization (PS): For analysing PS, the organism was grown in Pikovskaya's agar medium and incubated at 28°C for 4-5 days. The appearance of transparent halo zone around the organism indicated the PS activity (Nautiyal 1999).

Chitinase production: Chitinase production was assessed by growing the organism on chitin plates with M9 agar medium amended with 1% (w/v) colloidal chitin and incubated at 37˚C for 24–72 hours. Zone of clearance around organism indicated chitinase production (Das *et al.* 2010).

Tolerance to salt and pH: The stress tolerance characteristics of the organism viz., salinity tolerance and pH tolerance were studied by inoculating the isolate in nutrient broth with 10% salt or different pH ranging from 5 to 9 and incubated at 37˚C for 48-72 hours. The organism's growth in respective salt medium or pH medium was considered as to salt tolerance or pH tolerance of the organism (Damodaran *et al.* 2013).

Tolerance to Heavy metals and Trace elements: The organism was tested for their tolerance to heavy metals by agar diffusion method (Cervantes *et al.* 1986). Freshly prepared agar plates were amended with various soluble heavy metals (Cr, Pb, As, Ag, Au, Hg) and trace elements (Al, Zn, Mo, Mn, Cu, Ni) at various concentration ranging from 0.6 to 3200µg/ml and inoculated with the organism. The incubated plates were kept at 37˚C for 48 hours and the effect of heavy metals and trace elements on growth of the organism was determined.

Susceptibility to Antibiotics: The organism was tested for its resistance to different antibiotics (viz., Cephalexin, Ampicillin, Cephataxime, Nalidixic, Neomycin, Streptomycin, Vancomycin, Kanamycin, Rifampicin, chloramphenicol, Trimethoprim, Tetracycline, Gentamycin) by agar diffusion method (Bauer *et al.* 1966). The organism was inoculated in freshly prepared agar plates amended with specific antibiotic, incubated the plates at 37˚C for 48 hours and determined the antibiotic resistance by observing growth of the organism.

Plasmid curing: The organism from 24 hours old culture was transferred to nutrient broth containing 200µg/ml of acridine orange solution (mutagenic agent), incubated at 37˚C for 24 hours. The organism grown in acridine orange solution was inoculated to petriplates containing individual antibiotic compound or heavy metals, kept for 24 hours of incubation at 37˚C. After the incubation period, the sensitivity of bacterial growth to a particular metal or antibiotic was observed and plasmid curing was determined on the basis of non-occurrence of colonies grown on the plate (Ramteke *et al.* 2012).

Wheat variety AAI-W6: Wheat variety AAI-W6 was collected from Department of Genetics and Plant Breeding, SHUATS, Allahabad. This semi-dwarf,

late sown, high terminal temperature tolerant wheat variety, AAI-W6 was developed and released from SHUATS during 2013 with low input requirement on irrigation (only 2-3 times), fertiliser application (i.e., 60-80 N, 40 P, 30 K per ha) is well suited for cultivation by marginal and small farmers (Ram, 2014).

Seed germination, root and shoot elongation: The seeds of wheat var. AAI-W6 were surface disinfected by immersion in 70% ethanol for 1 minute followed by washing with sterile distilled water for three times. The surface sterilized seeds were transferred to bacterial solution, kept for 30 minutes, then placed on germination paper and maintained for 9 days at 25 ˚C (Nandakumar *et al.* 2001). The percent germination was calculated by the following formula, Percent seed germination = Number of germinated seeds / Number of total seeds x 100. Control seeds without organism were used for comparison (Pandey *et al.* 2007). The effect of seed bacterization on root and shoot elongation of wheat seedlings was studied under *in vitro* condition.

Field experiments

Pot experimentation was carried out to record the data regarding growth, yield and yield component of wheat variety AAI-W6 under the influence of combined treatments of the organism and different ratios of NPK(120:60:40kg/ha)viz. 100,75, 50, 25%. Five plants were randomly tagged in each treatment of different sets for recording the data. The plant height (cm) was recorded from the ground level to the growing tip of the main shoot. Measurement was taken at 90 DAS (expand DAS) and the average height was calculated and expressed in centimetre. The data on number of tiller per plant was recorded at 100 days for all the tagged plants. The average spike length (cm) of five plants on the main culm from the base of the spike to the top of the last spikelet excluding awns was recorded in centimetre. The average spike weight (g) of five plants was taken in gram. The number of grains per spike was recorded by counting the number of grains in one spike of respective wheat plant. Grain yield per plant (g) was recorded from yield of grain per plant obtained after harvesting and threshing. The flag leaf width was measured in centimetre in the middle part of the leaf from one margin in each of the randomly selected flag leaf. The length flag leaf was measured in centimetre from the collar junction of the blade and leaf sheath to the tape of blade. The Test weight (g) was recorded by weighing 1000 grains in each entry. The Fresh weight of a plant was taken after harvesting a crop in each treatment of different sets and the average was worked out. Dry weight of the plant (g) was determined by drying the plant in an oven at 60^{o}C for 24 hours. The Harvest index **(%)** was calculated by applying the following formula, HI = Seed yield per plant (g)/ Total dry weight per plant (g) × 100. Total chlorophyll content was determined according to the method of Arnon (1949). Fresh leaves were collected and sample (200 mg) was taken from fully mature leaf. The sample was mixed with 2 ml (80%) acetone and ground well. These mixtures were centrifuged at 10,000 rpm for 5 min. After centrifugation, the supernatant was removed and transferred into a fresh test tube. Acetone was added to test tube containing the sample and volume was made up to 6 ml. Absorbance of the samples was read at 645nm and 663nm using spectrophotometer. The total chlorophyll content was calculated by using following formula, Total chlorophyll (µg/ml) =20.2 (A_{645}) +8.02 (A_{663}). The relative water content, RWC (%) was estimated by the method of Barrs and Weatherly (1962). Five leaf discs were collected and weighed. This was considered as the fresh weight. The weighed leaf discs were allowed to float on distilled water in a petridish and allowed to absorb water for four hours. After four hours, the leaf discs were taken out and their surface was blotted gently and weighed. This was referred to as turgid weight. After drying in hot air oven at 72 °C for 48 hours, the dry weight was recorded and RWC was calculated by applying the following formula, RWC (%) = Fresh weight – Dry weight/ Turgid weight – Dry weight × 100. Protein estimation was performed by adopting the method given by Lowry *et al.* (1951). The protein content was determined by the standard curve prepared using Bovine serum albumin protein and absorbance was measured at 660 nm. The total carbohydrate content (g) in plants was determined according to the methods of Hedge and Hofreiter (1962). Fully mature leaves were collected from plants and dried. Sample (200 mg) was taken and 10 ml water was added into the test tube and boiled for 1 h in a water bath. One ml of extract was taken from the test tubes and 3ml of 3% Anthrone reagent

(Sigma Aldrich) was added to the extract and the mixtures were kept in the water bath for 30 min at 100 °C. Samples (2 ml) were taken in cuvettes and absorbance was read at 630 nm using glucose as a standard. The amount of carbohydrate present in the sample tube was calculated.

Statistical analysis

The experiment was conducted in Completely Randomized Design (CRD) with 3 replications. Statistical analysis was performed for various comparisons using GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Characterization of the bacterial isolate

The data recorded for different morphological characteristics, biochemical tests and PGP traits of bacterial isolate obtained from SMOF are presented in Table 2. The isolate was gram negative rod, capsulated, motile with peritrichous flagella, non-spore forming and non-acid fast bacteria. The bacterial isolate showed positive reaction to voges-proskauer, citrate utilization, glucose formation, produced acid from lactose, trehalose, maltose, cellobiose, producted H_2S, acetion and possessed catalase activity, while it showed negative results for oxidase, indole, methyl red and gelatin hydrolysis tests. Based on these characteristics the isolate PR6 was presumptively identified as *Erwinia*.

Molecular identification and phylogenetic tress

The sequence generated for presumptively identified *Erwinia* strain through molecular characterization employing 16S ribosomal DNA was submitted to NCBI, which confirmed the identification as *Erwinia* sp. with Accession No.KP226572 (Table 1). The nearly complete 16S rRNA gene sequence of *Erwinia* sp. KP226572 was obtained and subjected to comparative analysis. On the basis of 16S rRNA gene sequence similarities of *Erwinia* sp., to another different nonpathogenic species of *Erwinia* taken from NCBI were used to construct the phylogenetic tree shown in Fig. 1. *Erwinia* sp. Showed the highest 16s rRNA gene sequence similarity to *E. herbicola* strain Eh252 (99%), followed by *E. teleogrylli* strain SCU-B244 (98%), *E. iniecta* strain B120 (98%), *E. billingiae* (97%) and *E. tasmaniensis* (97%).The 16S rDNA gene sequence based comparison combined with phenotypic tests indicated that it is the best PGPB for different crops Fig. 1.

Table 1: Morphological and Biochemical of *Erwinia* sp. (KP226572)

Characteristics	Characteristics	Positive /Negative
Morphological	Isolation from	Organic farm
	Organism	*Erwinia* sp.
	NCBI Accession no	KP226572
	Temperature	37°C - 42°C
	Gram staining	-
	Shape	Rods
	Motility	+
	Capsule	+
	Spore formation	-
	Flagella	Peritrichous
	Acid fast staining	-
Biochemical	Oxidase	-
	Indole production	-
	Methyl Red	-
	Voges-proskauer	+
	Citrate utilization	+
	Glucose fermentation	+
	Acid from Lactose	+
	Trehalose	+
	Maltose	+
	Cellobiose	+
	H_2S production	+
	Acetoin production	+
	Catalase	+
	Gelatin liquefaction	-

+ = presence, - = absence

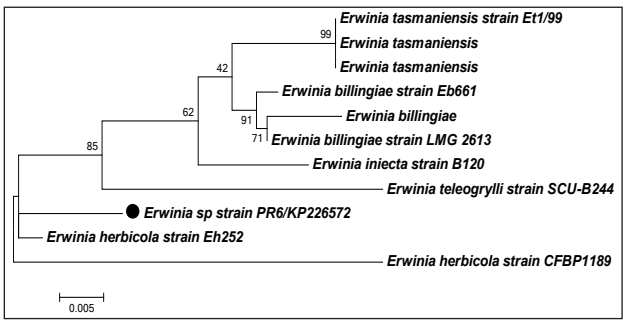

Fig. 1: Neighbor joining tree (Kimura two-parameter distance) of 16S ribosomal sequences of *Erwinia* isolates from soil of organic farms. The numbers given over branches indicate bootstrap (1000 replications) coefficient.

PGP traits of *Erwinia* sp. (KP226572)

The identified isolate of *Erwinia* sp. was further found positive for different plant growth promoting traits such as production of ammonia, siderophore, IAA, ACCD and phosphate solubilization activity. The isolate also showed tolerance to 10% salt stress and was able to grow on a wide range of pH from 5 to 9 (Table 2).

Table 2: PGP traits of *Erwinia* sp. (KP226572)

PGP traits	Positive /Negative
NH_3	+
HCN	-
SD	+
IAA(µg/ml)	5.5
ACCD	+
PS	+
Chitinase	-
Tolerance to salt %	10
Tolerance to pH	5-9

+ = presence, - = absence, production of ammonia= NH_3, hydrogen cyanide= HCN, Siderophore production =SD, indole acetic acid = IAA, 1-aminocyclopropane- 1-carboxylate deaminase =ACCD and phosphorus solubilization activity = PS

Tolerance to trace and heavy metal of *Erwinia* sp. (KP226572)

In the isolated strain of *Erwinia* species, response to trace and heavy metal tolerance was detected for 12 elements, the highest incidence of metal stress tolerance was recorded for Al (3200 µg/ml) and the lowest response was observed with Hg (0.6µg/ml). In case of other heavy metals viz., Mo, Zn (800 µg/ml), Mn (400 µg/ml), Cr, Pb (200 µg/ml), Ar, Cu, Ni (100 µg/ml), Ag (50 µg/ml) Au (25 µg/ml) the isolate of *Erwinia* sp. showed significant tolerance properties to trace and heavy metal stress (Table 3).

Table 3: Tolerance to trace and heavy metals of *Erwinia* sp. (KP226572)

Elements	Heavy metals	Tolerance to µg/ml
Trace elements	Aluminum	3200
	Zinc	800
	Molybdenum	800
	Manganese	400
	Copper	100
	Nickel	100
Heavy metals	Chromium	200
	Lead	200
	Silver	50
	Arsenic	100
	Gold	25
	Mercury	0.6

Susceptibility of antibiotic of *Erwinia* sp. (KP226572)

The antibiotic susceptibility spectrum showed resistance to 11 antibiotics viz., ampicillin, cephalexin, cephataximine, chloramphenicol, gentamycin, kanamycin, nalidixic, streptomycin, tetracycline, trimethoprim, vancomycin and susceptibility to 2 antibiotics, neomycin and rifampicin (Table 4).

Table 4: Antibiotic susceptibility of *Erwinia* sp. (KP226572)

Antibiotics	Resistance /sensitive
Cephalexin (Cp)	R
Ampicillin(A)	R
Cephataxime (Cf)	R
Nalidixic (NA)	R
Neomycin (Ne)	S
Streptomycin (S)	R
Vancomycin (V)	R
Kanamycin (K)	R
Rifampicin(Rf)	S
chloramphenicol(C)	R
Trimethoprim(TM)	R
Tetracycline(T)	R
Gentamycin(G)	R

Curing of plasmid of *Erwinia* sp. (KP226572)

In case of heavy metals viz., lead, silver and the antibiotics viz., kanamycin, streptomycin 100% curing followed by 75-80% curing in tetracycline, gentamycin was recorded (Fig. 2).

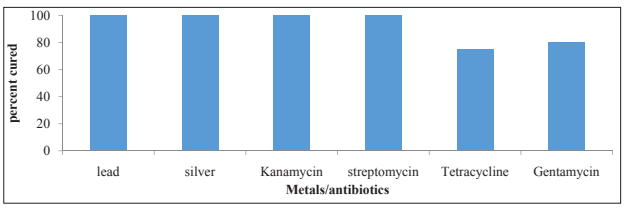

Fig. 2: Curing of heavy metals and antibiotics in *Erwinia* sp. (KP226572)

Inoculation effect of *Erwinia* sp. (KP226572) on wheat

The inoculation of PGP strain *Erwinia* sp. with AAI-W6 variety of wheat showed positive effects on percent seed germination, rate of root and shoot elongation during seedling growth (Table 5). Inoculation of the strain with wheat seeds resulted to 90 percent germination over 70 percent germination of untreated control seeds. Similar increaments were recorded in root and shoot elongation rates of the strain inoculated seeds (6.5, 10.7 cm) in comparison to the root and shoot length of control seeds (3.97, 8.1 cm) (Table 5).

Table 5: Effect of *Erwinia* sp. (KP226572) on seed germination and growth parameter of wheat variety AAI-W6 under lab condition

Treatments	Percent germination	Elongation in cm	
		Root	Shoot
Control	70	3.97	8.10
Erwinia sp. (KP226572)	90	6.50**	10.70

*$p<0.05$; **$P<0.01$; ***$p<0.001$

The effect of bacterial culture was further studied on different growth, yield parameters of wheat under field conditions and significant results were obtained due to the combined application of the bacterial culture along with NPK treatments (Table 6).

The production of ammonia is an important trait of PGPB that influences plant growth indirectly by fixing atmospheric nitrogen (N_2) and a reduction reaction in the presence of nitrogenase and provides ammonia to plants by symbiotic or non-symbiotic interactions. Many of the members of Entrobacteriaceae including species of *Erwinia* have the ability to fix atmospheric nitrogen. Neilson and Sparell (1976) reported high levels of nitrogen fixing activity in *E. herbicola* strain collected from paper mill process water samples. In a later communication, Neilson (1979) reported the identification of two specific biotypes of *E. herbicola* having the capacity to fix nitrogen and its close resemblance to *E.coli*, which ferments cellobios and utilizes malonate from paper mill process of water and compost samples. Papen and Werner (1979) reported the nitrogen fixation in four strains of *E. herbicola* grown under anaerobic conditions.

Secretion of siderophores is common in bacterial species. These high affinity ion chelating compounds bind to naturally occurring insoluble form of iron resources in soil, produce Fe^{3+}-siderophore complex that gets reduced to Fe^{2+} and release iron into a bioavailable form for its enhanced uptake by the plants. Leong and Neilands (1982) reported 3 types of siderophores viz., hydoxymate cateholate, carboxylate and *Erwina* produce carboxylate (complexones) siderophores.

The PGPR species associated with plant rhizosphere are known to produce IAA by four different metabolic routes of biosynthetic pathways (indole-3-acetamide- IAM, indole-3-pyruvic acid- IPyA, indole-3-acetonitrile, tryptamine) and demonstrates positive effect on seed germination, plant growth and development. The IAM pathway is reported to be involved in gall formation by phytopathogenic species of *Erwinia*, whereas the IPyA pathway enhances bacterial epiphytic fitness and is associated with non-pathogenic *Erwinia* sp. including *E. herbicola* (Brandl *et al.* 1996; Pattern and Glick, 1996; Manulis *et al.* 1998; Lambrecht *et al.* 2000).

Production of ACCD by PGPB is reported to be beneficial in their host plants by lowering plant ethylene concentration (Hall *et al.* 1996; Glick *et al.* 1998), and by reducing plant stress due to flood condition, water stress (Grincko and Glick 2001; Mayak *et al.* 2004a) and salt stress (Mayak *et al.* 2004b). The primary mechanism ascribed to ACCD production by PGPB is to diminish ethylene accumulation by cleavage of the immediate precursor of ethylene viz., ACC into ammonia and α-ketobutyrate thereby to reestablish a healthy root system in plants (Bhattacharyyaand Jha 2012).

PGPR enhances mineralization of insoluble form of inorganic phosphorus reserves of the soil and ensures phosphorus availability from the rhizosphere. In species of *Erwinia*, the phosphate solubilization mechanism has been related to the production of gluconic acid. Goldstein (1996) proposed direct glucose oxidation to gluconic acid as a major mechanism for mineral phosphate solubilization in gram negative bacteria including *E. herbicola*. The gene involved in mineral phosphate solubilization was cloned from *E. herbicola* and the expression of this gene allowed production of gluconic acid in *E.coli* HB101 (Goldstein and Liu, 1987). Later, Rodriguez *et al.* (2001) reported the

Table 6: Inoculation effect of *Erwinia* sp. (KP226572) on morphological, biochemical and yield parameters of wheat war. AAI-W6 under pot experiments

Treatments	Parameters															
	Morphological								Biochemical				Yield			
	Plant height (cm) 90 DAS	No. of tiller/ plant 80DAS	Length of spike (cm)	Weight of spike (g)	Flag leaf width (cm)	Flag leaf length (cm)	Fresh weight of plant (g)	Dry weight of plant (g)	Chlo-rophyll content (mg/g)	Relative water content (%)	Protein (%) in seed	Total car-bohydrate (g)	Grain yield/ plant (g)	No. of grain/ spike	Test weight (g)	Harvest index (%)
Control	23.9	1.1	5.4	1.5	1.1	11.5	10.1	2.1	2.0	41.7	4.9	32.3	11.3	20.5	21.5	20.7
NPK (100%)	37.1	2.0	8.9	2.9	1.2	18.2	14.9	3.5	4.0	50.2	8.4	60.2	15.3	26.1	28.8	33.1
NPK (75%)	34.6	1.7	8.7	2.4	1.1	17.4	13.6	3.2	3.4	48.6	8.3	59.9	14.5	25.8	28.4	32.6
NPK (50%)	30.6	1.6	7.6	2.4	1.2	16.2	12.8	3.0	3.3	48.4	8.2	59.5	14.4	25.5	28.3	32.1
NPK (25%)	25.9	1.3	6.4	2.1	1.2	14.8	12.3	2.9	3.0	48.1	8.1	59.2	14.2	25.3	27.4	31.2
Bacterial culture	40.2	1.8	8.8	2.2	1.1	15.5	14.4	3.5	3.1	54.4	8.3	64.3	16.6	25.4	26.9	31.3
Bacterial culture+NPK (100%)	53.2***	3.1**	9.9***	3.0***	1.7	19.4*	16.7*	5.3**	4.1**	63.9	10.6**	67.4***	19.5*	33.8*	36.4*	34.5*
Bacterial culture+ NPK (75%)	49.3***	2.9*	9.5***	2.8	1.6	18.5*	16.0*	4.4**	3.9**	63.1	10.3**	66.6**	18.3*	32.7*	34.5*	33.7
Bacterial culture+ NPK (50%)	48.2***	2.7*	8.9***	2.7	1.4	16.0	14.9	3.7	3.8	61.0	9.3	66.2**	18.1*	32.2*	32.5*	33.3
Bacterial culture + NPK (25%)	46.2***	2.0	8.3***	2.5	1.2	12.6	14.2	3.5	3.5	56.5	8.6	64.9**	17.4	31.6*	31.4*	32.3

*$p<0.05$; **$P<0.01$; ***$p<0.001$

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heterologous expression of phosphate solubilization gene from *E. herbicola*.

More evidences of catalase activity of *Erwinia* sp. were previously reported by few authors. The association of non pathogenic species of *Erwinia*, *E herbicola* CFBP1189, *E. teleogrylli* sp. nov. strain SCU-B244T (Liu *et al.* 2016), *E. billingiae* sp. nov. strain LMG 26133 (Mergaert *et al.* 1999), and the pathogenic species including E*. amylovora* CFBP 3049, CFBP 1430, 295/93, *E. amylovora* (Rhim *et al.* 1999), *E. carotovora* subsp. *Carotovora* (strains I1 to I6 and I8), *E. chrysanthemi* (strain I9) (Snehalatharani and Khan 2010) were enhanced by the catalase activity in their host plants. Calcutt *et al.* (1998) characterised ~150 bp fragment of rpoS gene in *E.carotovora* that regulates catalase synthesis and proposed the occurrence of rpoS sequences in other species of *Erwinia* including *E. herbicola*. The catalase activity of isolated *Erwina* strain can significantly enhance oxidative stress tolerance of the host plant. Further the ability of this PGPB strain to grow under 10% salt stress and withstand a wide range of pH from 5 to 9 could be utilized for better crop productivity by incubating the isolate to the rhizosphere of acidic / salt tolerant host plants to be grown under similar stressful field conditions.

Similar instances of antibiotic resistance in non-pathogenic spices of *Erwinia* were reported earlier. *E. herbicola* B247 was resistant to streptomycin and rifampicin (Kempf and wolf 1989). Watanabe and Sato (2002) reported resistance of *E. herbicola* to five antibiotics viz., streptomycin, kanamycin, ampicillin, tetracycline and chloramphenicol due to the transfer of pMUL1 and resistance to kanamycin in *E. herbicola* strain Eh252 (Vanneste *et al.* 1992). Among the pathogenic spices of *Erwinia*, *E. caratovora* was resistant to ampicilin, rifampicin, colistin (Laniewska-Trokenheim *et al.* 2006) and *E. amylovora* was resistant to cefotaxime (Islam *et al.* 2014). These findings further suggest that the non-pathogenic species of *Erwinia* having resistance to antibiotics could be better utilized by inoculating these bacterial species to the rhizosphere of crops growing on contaminated soil with antibiotics.

It is interesting to record heavy metal and antibiotic resistance from the non-pathogenic species of *Erwinia* strain isolated from an organic farm. The possible sources of antibiotic exposure of organic farm isolated *Erwinia* sp. could be the application of FYM ingredients including animal faeces (cow dung, cow urine, excreta from fowl, pet dogs, pigs etc.) from antibiotic administered domestic animals, leaves or plants sprayed with antibiotics, irrigation water contaminated with antibiotic compounds. Furthermore, several incidences related to the linkage of resistance characteristics between heavy metals and antibiotics of bacterial plasmids are reported (Ramteke 1997; Ghosh *et al.* 2000; Verma *et al.* 200l, 2002; Tewari *et al.* 2003; Siddiqui *et al.* 2005; Ramteke *et al.* 2012). The present result of 100% curing of kanamycin and streptomycin indicate that these antibiotic resistance factors are purely plasmid borne and the acridine orange treatment completely eliminate the plasmids bearing the resistance factors. The 70-80% curing recorded for tetracycline, gentamycin antibiotics due to acridine orange treatment suggests the probable intercalation of curing agent with plasmid DNA or the possibility of the formation of A+T region specific antibiotic resistance factor-acridine orange adducts in the plasmid DNA (Geoffrey *et al.* 1978). Such instances of plasmid curing by acridine orange and consequent selective interferences in the replication of bacterial plasmids have already been reported earlier (Hohn and Korn 1969; Yamagata and Uchida 1969; Salisbury *et al.* 1972). Chatterjee and Starr (1972) demonstrated the presence of plasmid borne, transferable antibiotic resistance factors viz., factor R100 *drd*-56 responsible for tetracycline resistance from *Escherichia coli* B/r strain and *Shigella flexneri* 1a strain responsible for transfer and induction of multiple antibiotic resistance to several species of *Erwinia* including non-pathogenic *E. herbicola* strains.

Most of the species of the genus *Erwinia* are phytopathogens causing fire blight to Rosaceae plants, bacterial blight to cucurbits and other dicotyledonous plants. At the same time, it is challenging to study the beneficial aspects of plant growth promotion in wheat crop with a nonpathogenic bacterial species of *Erwinia*. Reports are available on the non-pathogenic strains of *Erwina*, including *E. hebicola* pv. gypsophilae devoid of $pPATH_{Ehg}$ and $pPATH_{Ehb}$ (Nizan-koren *et al.* 2003), *E. herbicola* Eh252 (Vanneste *et al.* 1992), *E. billingiae* (Margaert *et al.* 1999) *E. tasmaniensis* sp. nov. (Geider *et al.* 2006), *E. tasmaniensi*s strain Et1/99 (Kube *et al.* 2008, 2010), *E. billingiase* strain

Eb661 (Kube *et al.* 2010), *E. billingiae* (Bielsa *et al.* 2012), *E. tasmaniensis* (Bielsa *et al.* 2012), *Erwinia iniecta* sp. Nov., strain B120^T (Campillo *et al.* 2015) and their positive association with plants. These non-pathogenic strains of *Erwinia* can be introduced to the rhizosphere of crops including wheat for higher productivity. The results on germination and seedling growth obtained in the present study are in agreement with the earlier finding of Robinsons *et al.* (2016) where they reported similar germination response (95-100%) and healthy seedling growth in wheat (*T. aestivum* cv. Hereward) seeds dominated with endophytic species of *Erwinia*. However, scanty references are available on the combined effect of treatments with NPK and PGPB (*Erwinia* sp.) on vegetative and yield parameters of wheat. The effect of NPK on growth and yield parameters of wheat are reported by few authors and are comparable with the present findings. The application of NPK and PGPB in wheat significantly resulted in enhanced growth and yield parameters when compared to control (Rana *et al.* 2012). Malik*et al.* (2012) also reported that the combined application of PGPR+ NPK significantly increased grain yield of 5150 kg/ha^{-1} of wheat.

CONCLUSION

A non-pathogenic strain of *Erwinia* sp. (KP226572) tolerant to 10% salt and a wide range of pH (5-9), bearing multiple plant growth promoting traits was isolated from the soil of an organic farm. Inoculation of this strain in the seeds of wheat var. AAI-W6 significantly induced germination, seedling growth including elongation of roots and shoot. In field conditions, the combined application of *Erwinia* sp. along with NPK treatments showed similar enhancement in the growth and yield parameters of wheat. This strain did not produce any symptoms of pathogenicity in wheat at any stage of its germination or vegetative growth. The potential of this non-pathogenic *Erwinia* strain could be further explored for the enhancement of wheat production in soils affected with acidic or alkaline pH stresses.

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