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Development of Potential Microbial Consortia and their Assessment on Wheat (*Triticum aestivum*) Seed Germination

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ABSTRACT

Application of plant growth promoting microorganisms and their consortia as potential bioinoculants depicts inexpensive and alternative approach of chemical fertilizers with ultimate solution for assisting plants through enhancing their growth and yield. In this view, the present study was conducted to develop potential plant growth promoting microbial consortia and their evaluation for enzymatic activities and plant growth promoting properties. Twenty four bacterial isolates were retrieved and screened on the basis of selective plant growth promoting traits. All selected isolates were evaluated for their compatibility with each other and finally four consortia were developed. All bacterial consortia were found to possesss several plant growth promoting properties such as siderophore production, zinc solubilization, phosphate solubilization, indole acetic acid and exopolysaccharide production. Furthermore, all bacterial consortia were assessed through seed germination assay on wheat. The outcomes of seed germination assay confirmed the efficiency of bacterial consortia through enhanced seedling germination and other agronomical parameters. Among all, bacterial consortia 3 and 4 exhibited maximum influence on seedling vigour. Therefore, these consortia could be used as potential bioinoculant to enhance plant growth and productivity in a more eco-friendly manner to improve sustainable agriculture.

Keywords : Plant growth promoting rhizobacteria, Microbial consortia, Wheat, Seedling vigour, Zinc solubilization.

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INTRODUCTION

According to the United Nation's report, the human population of the world is continuously increasing at an alarming rate and it is predicted to reach up to 8.9 billion by the year 2050 (Wood 2001).

As per increase in population food demand will also increased up to 70 % more, while at a similar period a battle of food security, hunger and climate change will also going on, which will be collectively considered as the key challenges for world agriculture. Various natural and as well as anthropogenic activities lead to change in climate conditions and soil properties which negatively affecting plant growth in terms of productivity, yield, nutrient and ultimately the deterioration of natural resources such as water and soil which causing the harmful effect on human health in terms of malnutrition, disease occurrence and also increasing the mortality. Facts pertaining that there is an urgent need to develop agriculture practices in order to improve food grain production as well as productivity and quality to accomplish food demand for world population in future (Singh and Singh 2017). Therefore, various techniques are practicing such as agronomic practices, breeding for high yielding varieties, transgenic crops and supplementation of foods with vitamins and minerals in the form of chemical fertilizers to enhance crop productivity (Parveen et al. 2018). But such strategies pursue their own limitations and it also negatively affects soil fertility and the environment (Upadhayay et al. 2018). Alternatively, a sustainable approach gained attraction due to utilization of plant growth promoting microorganisms to enhance plant growth and productivity through ecofriendly manner.

Generally microorganisms reside around the plant and classified into three groups such as beneficial, deleterious and neutral. Beneficial bacteria such as plant growth promoting rhizobacteria (PGPR) those reside in the vicinity of root help in the plant growth promotion through numerous direct and indirect mechanisms (Khan et al. 2019). But in recent decade's co-inoculation of PGPR is practicing because co-inoculation showed a better capability to perform plantgrowth promotion in more efficient manner than single strain inoculation (Guetsky et al. 2002). Therefore, researchers are exploring numerous plant growth promoting rhizobacterial strain in order to develop potential microbial consortia, suitable for plant growth enhancement. Each strain in multistrain consortia can effectively function in a synergistic manner to enhance the nutrient availability and plant growth by means of siderophore production for iron chelation, zinc, potassium and phosphate solubilization for Zn^{+2}, K^{+} and PO_4^{2-} ion, nitrogen fixation, phytohormone and various oxidizing, reducing enzymes production (Singh et al. 2017). They also stimulate the functioning of each other through physical and biochemical activities which leads to improvement in beneficial aspects. It has also been reported by many researchers that consortia are able to enhance nutrients and plant growth in a broad arrays of plants under stress as well as non-stress conditions (Walpola and Yoon 2013). Due to these ecofriendly and sustainable mechanisms application of consortia is a suitable approach to maintain crop yield and pollution free environment. Therefore, the present study was focused on the development of bacterial consortia from plant growth promoting microorganisms particularly rhizobacteria and their assessment on seed germination and as well as subsequent seedling vigour of wheat to improve yield in a sustainable manner.

MATERIALS AND METHODS

Retrieval of bacterial isolates

In total, twenty-four bacterial isolates were obtained from departmental culture collection, Department of Microbiology, GB Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, which was originally isolated from the rhizospheric region of wheat and soybean. To retrieve the bacterial isolates, 100 µl aliquot were inoculated in nutrient broth (Himedia, India) and incubated at 28±2°C with shaking speed at 100 rpm for 24h. All retrieved bacterial isolates were maintained on nutrient agar slants at 4°C and glycerol stock at -20°C for further studies.

Morphological and biochemical characterization

All retrieved test bacterial isolates were subjected to Gram's staining for determining cell morphology and gram reaction, colony morphology and biochemical characterization for carbon source utilization as per the procedure of Bergey's manual of determinative bacteriology for preliminary identification (Bergey et al. 1994).

Plant growth promoting traits

Siderophore production

The spot inoculation of all test bacterial cultures were performed on Chrom Azurole S (CAS) agar plates (Schwyn and Neilands 1987) and the plates were incubated for 72 h at 28±2°C. The appearance of the orange halo zone around the colony of bacteria was determined as siderophore mediated iron chelation complex formation. Clearing zone demonstrated (%) iron solubilization efficiency, which was measured as per Ramesh et al. (2014). Furthermore, the iron chelation efficiency was confirmed through quantitative estimation by CAS shuttle assay and for this the test bacterial strains were inoculated in M9 minimal medium broth and incubated at 28 ±2°C for 24h at 120 rpm. After incubation, the suspension was processed for centrifugation at 3000 rpm for 10 min for obtaining cell-free supernatant. Then, the aliquot of 0.5 ml cell-free supernatant was added with 0.5 ml CAS dye solution and 10 µl of shuttling solution.

Afterward, the solution was kept for incubation for 20 minutes at room temperature and optical density was recorded at 630 nm. An un-inoculated minimal broth was considered as blank while minimal broth with CAS dye and shuttling solution were served as a reference solution (Schwyn and Neilands 1987). The siderophore unit was determined by using the following formula :

$$\% \text{ Siderophore units} = [(A_r - A_s)/A_r] \times 100$$

Where, Ar - Absorbance of reference; As – Absorbance of sample.

Zinc solubilization

Zinc solubilization efficacies of bacterial isolates were determined on basal agar medium amended with different insoluble zinc source viz. zinc oxide (ZnO) and zinc carbonate ($ZnCO_3$) . Overnight grown bacterial isolates were inoculated on basal agar medium containing 1% zinc oxide (ZnO) and zinc carbonate ($ZnCO_3$), separately and the plates were kept for proper incubation at 28± 2°C for 4 to 5 days. Around the bacterial colonies, the appearance of a clear halo zone indicated zinc solubilization and width of zone indicates zinc solubilization efficiency, which was measured as per Ramesh et al. (2014).

Phosphate solubilization

The insoluble phosphate solubilization efficacies of bacterial isolates were determined on Pikovaskaya's agar medium supplemented with tricalcium phosphate (Pikovaskaya 1948). After spot inoculation of test bacterial isolates on plates containing Pikovaskaya agar medium were incubated at 28±2°C for 3 to 4 days and the positive result was determined on basis of appearance of clear halo zone around the bacterial colonies.

HCN production

The bacterial isolates were determined for producing their ability for HCN according to the method of Miller and Higgins (1970). In brief, each bacterial isolate was streaked on nutrient agar containing 4.4 g/1 glycine as a nitrogen source. Sterile filter paper soaked in suspension containing 2% sodium carbonate and 0.5% picric acid was put in the lid of each petri plate and after sealing all plates with parafilm was kept for incubation at 28 ± 2°C for 3–4 days. The appearance of color change of filter paper from yellow to brown indicated HCN production.

Ammonia production

To determine the ammonia production overnight grown test bacterial strains were inoculated in peptone broth tubes (10 ml) and incubated for 48–72 h at 28 ± 2°C. Following the incubation, Nessler's reagent (0.5 ml) was dispensed in each test tube. Ammonia production was detected on the basis of changing in color change from brown to yellow (Cappuccino and Sherman 2002).

Indole acetic acid production

All bacterial strains were determined for indole-3-acetic acid (IAA) production as described by Patten and Glick (2002). Overnight grown bacterial cultures were inoculated in tubes of Luria broth amended with L-tryptophan (100µg/ml) and incubated at 28± 2°C for 3 days. After incubation, the bacterial suspension

was centrifuged at 10,000 rpm (10 minutes) and lml supernatant was collected following the addition of Salkowsky reagent and tubes were left as such for 30 minutes at room temperature. The appearance of pink color indicated the production of indole acetic acid (IAA).

Exopolysaccharide (EPS) production

The test bacterial isolates were inoculated in 50 ml basal medium and plates were incubated at 28 ± 2°C for 3 days, subsequently centrifuged at 10, 000 rpm for 10 min and pellets were collected. Afterwards, it was suspended in two volume ice cold isopropanol and eppendrof tubes were stored at 4°C for 24 h for precipitation. The whole suspension was again centrifuged (at 10,000 rpm) for 20 minutes to collect the pellets and dried at 65°C (Vijayabhaskar et al. 2011). Presence of precipitated pellet confirmed the exopolysaccharide (EPS) production.

Antibiosis assay

Antifungal activities of bacterial isolates were determined by following the method of dual culture against phytopathogenic fungi against *Rhizoctonia solani* and *Fusarium oxysporum* (Bai and Shaner 2004). A 5 mm disc of fungal strain was placed on petri plates containing PDA : NA (1 : 1 v/v) then actively grown bacterial isolates were spotted towards the periphery of the culture plate and incubated at 25 ± 2°C for 5–6 days. Petri plates with no bacterial inoculation served as control. Presence of zone of inhibition due to suppression of fungal mycelia growth considered as positive results.

Development of microbial consortia

On the basis of different plant growth promoting traits, potential bacterial isolates were selected for consortium development according to their biocompatibility with other bacterial isolates. Consortia were formed by adding an equal amount of each strain. *In vitro* plant growth promoting traits such as the production of siderophore, IAA, HCN, exopolysaccharide and ammonia, solubilization of zinc and phosphate and antibiosis efficiency of all consortia were determined.

Assessment of plant growth promotion

Developed potential bacterial consortia were assessed for their plant growth promotion on wheat through seed vigour assay under *in vitro* conditions. For the study, seeds were provided by the Department of Genetics and Plant Breeding, GBPUA and T, Pantnagar, Uttarakhand, India. To compile this assay the healthy seeds of wheat (*Triticum aestivum* var UP2785) were taken under consideration and surface sterilized as described by Singh et al. (2010, 2013). Seeds were bacterized by placing it with overnight grown consortium suspension for 4 h at 28 ±2°C at 100 rpm. To carry out seed germination assay hundred bacterized seeds had been placed in between towel paper and kept in an incubator at 20 ± 2°C in tilted position. All treatments were replicated thricely along with control, treatments were frequently moistened with autoclaved water and germination behavior was observed up to 8 days of incubation.

Observation recorded

First count **:** At 4th day of incubation, normal germinated seedlings were counted and considered as first count of germination.

Standard germination **:** Subsequently seed germination was evaluated and normal seedlings was counted on 8th day of incubation and considered as standard germination.

Seedling root length : From each replication the random selection of ten normal seedlings were done on 8th day from the start of germination test. The length of radicle (in cm) was measured and the mean of root length was calculated for each replication.

Seedling shoot length **:** On 8th day from the start of germination, randomly ten normal seedlings were selected from each replication and shoot length (in cm) was measured for each treatment and mean of shoot length was calculated.

Seedling length **:** Seedling length (in cm) was determined by adding shoot and root length.

Seedling fresh weight **:** Fresh weights of 10 random-

ly selcted seedlings were determined with using an electronic balance at the end of seed germination test i.e. on the 8th day and weight in milligram.

Seedling dry weight **:** The 10 randomly selected seedlings were placed in oven at 80°C for 24 h to remove the moisture from seedlings and weight of dried seedlings were recorded by an electronic balance and measured in milligram.

Seedling vigour index **:** Seedling vigour index was calculated as follows.

Seedling vigour index -I : SVI was calculated as per the following formula :

$$\text{Seed vigour index-I} = \text{Standard germination (\%)} \times \text{Seedling length (cm)}$$

Seedling vigour index -II : Calculated as :

$$\text{Seed vigour index -II} = \text{Standard germination (\%)} \times \text{Seedling dry weight (mg)}$$

Speed of germination **:** To determine the seeds germination ability approximately at the similar time all treatments along with all replications were checked on the daily basis for germination. When normal seedlings attained a predetermined size then they were removed from the germination test. The procedure followed until all seeds get germinated. For calculating the index of seed germination was carried out by dividing the number of removed seedlings every day by the corresponding day of counting for each treatment.

Relative growth index (RGI) **:** Relative growth index were calculated by the formula as described by Brown and Mayer (1986) :

$$RGI = \frac{\text{No. of seeds germinated at first count}}{\text{No. of seeds germinated at final count}} \times 100$$

Germination index (GI) : Germination index of germinated seeds corresponding to the day of germination was recorded by using the following formula :

$$\text{Germination index} = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \ldots\ldots\ldots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Mean germination time (MGT) **:** Mean germination time (MGT) of seeds was calculated by following equation (Eills and Rober 1981) :

$$MGT = \frac{\Sigma Dn}{\Sigma n}$$

Where, D = Number of days counted from the beginning of germination; n = Number of seeds germinated on the D day.

Time to 50% germination(T_{50}) **:** The time taken for 50% germination (T_{50}) was evaluated by the following formula of Coolbear et al. (1984) :

$$T_{50} = t_i + \frac{\left[\frac{N}{2}\right] \quad n_i \quad [t_j - t_i]}{n_j - n_i}$$

Where, N = Final number of germination; when ni $<N/2<n_j$, n_i, n_j = Cumulative number of seed germination by adjacent counts at time t_i and t_j.

Germination value **:** Germination value for germinated seedling was deliberated by :

$$\text{Germination value} = \text{Peak value} \times \text{Mean daily germination}$$

Mean daily germination **:** Mean value of seed germinated daily was deliberate by formula under :

$$\text{Mean daily germination} = \frac{\text{Final germination percentage}}{\text{Total No. of days}}$$

Peak value **:** Peak value of seed germination was caculated by formula :

$$\text{Peak value} = \frac{\text{Final germination percentage}}{\text{No. of days to reach maximum germination}}$$

Table 1. Morphological characteristics of the bacterial isolates.

Sl. No.	Bacterial isolates	Cell morphology characteristics			Colony morphology				
		Gram's reaction	Cell morphology	Arrangement	Shape	Edge	Elevation	Surface	Chromogenesis
1	SRA1	+ve	Coccus	Bunch	Circular	Entire	Convex	Smooth	Off-white
2	SRA4	+ve	Long rod	Chain	Irregular	Undulate	Flat	Dry	White
3	SRA5	+ve	Long rods	Chain	Circular	Undulate	Raised	Dry	Creamy white
4	SRK13	+ve	Short rod	Chain	Circular	Entire	Convex	Smooth	Light yellowish
5	SRPI17	+ve	Coccus	Bunch	Circular	Entire	Convex	Smooth	Yellow
6	WRPA26	–ve	Coccus	Scattered	Circular	Entire	Convex	Smooth	Off–white

Statistical analysis

To determine the variability in data statistical analyses were done by using STPR3 program with Completely Randomized Design (CRD). Means of different treatments were compared at 5% level of significance.

RESULTS AND DISCUSSION

Screening of bacterial isolates

All retrieved bacterial strains were spot inoculated on CAS agar medium and basal medium add-on with 1% of $ZnCO_3$ and ZnO to determine their siderophore producing potential and zinc (Zn) solubilizing efficiency, respectively. On account of siderophore production and zinc solubilizing potential six bacterial isolates i.e. SRA1, SRA4, SRA5, SRK13, SRPI17 and WRPA26 were selected for plant growth promotion study and consortium development.

Morphological and biochemical traits of bacterial isolates

All selected six potential bacterial isolates were characterized by determining their colony characteristics including shape, edge, elevation, surface and pigmentation and bacterial cell morphological features such as cell shape, cell arrangement and gram reaction (Table 1). Gram's staining results confirmed that five bacterial isolates were Gram-positive rods or cocci and remaining WRPA26 was Gram-negative cocci (Table 1). Furthermore, extracellular enzyme production profile of all six bacterial isolates was illustrated. All six isolates exhibited amylase producing ability. However, other isolates were varying their capability for different enzymes (Table 2). None of bacterial strain was able to produce cellulose and laccase activity (Table 2).

Functional characteristics of bacterial isolates

All six isolates were further characterized for various plant growth promoting characters i.e. siderophore production, zinc solubilization, phosphate solubilization and production of ammonia, indole acetic, HCN and exopolysaccharide. During investigation, all cultures were positive for siderophore production and zinc solubilization. On plate assay bacterial isolates

Table 2. Plant growth promoting and antibiosis properties of the bacterial isolates.

Sl. No.	Bacterial isolates	Siderophore production efficiency	% siderophore unit	Zinc solubilization efficiency %		Phosphorus solubilization	IAA production	Exopolysaccharide	Antibiosis assay	
				$ZnCo_3$	ZnO				*Fusarium oxysporum*	*Rhizoctonia solani*
1	SRA1	150.00	20.00	225	214	+	–	–		
2	SRA4	142.85	37.33	200	150	+	–	–	–	–
3	SRA5	200.00	60.00	250	200	+	+	+	–	++
4	SRK13	111.11	26.66	180	143	+	+	–	+	++
5	SRPI17	133.33	22.66	225	160	+	–	–	–	–
6	WRPA26	200.00	26.66	225	266	+	–	–	–	+

SRA5 and WRPA26 exhibited highest siderophore production efficiency i.e. 200% as they formed largest orange zone around the bacterial colony (Table 2). Subsequently, confirmed by quantitative assessment and bacterial isolate SRA5 was produced highest percent siderophore unit i.e. 60% (Table 2). These results agreed with the findings of Sayyed et al. (2005), in which they estimated 83% and 87% siderophore production unit by *Pseudomonas putida* and *Pseudomonas fluorescens*, respectively. On the other hand, Joshi et al. (2018), Yadav et al. (2016) confirmed the siderophore production ability of endophytic microorganisms from Tulsi and Aloe vera. Availability of solubilized zinc in soil is a major problem for agriculture. To solubilize the complex compounds bacteria produces different organic acids inside the soil. To determine the zinc solubilization potential of bacterial isolates, cultures were inoculated on basal medium supplemented with different insoluble source of zinc. Results confirmed that bacterial isolate SRA5 reflected highest zinc carbonate solubilization efficiency i.e. 250% whereas, bacterial isolate WRPA26 marked flagged largest zone on basal media supplemented with zinc oxide and showed 266% solubilization efficiency (Table 2). Saravanan et al. (2004) also reported zinc solubilized zone of 2.8 cm by ZSB-O-1 *Bacillus* sp. and clearing zone of 3.30 cm by ZSB-S-2 (*Pseudomonas* sp.). Phosphate solubilization is another direct mechanism of PGP (Plant growth promotion) occurs through phytases and organic acids production to lower down the pH (Singh and Prasad 2014, Singh and Goel 2015). During current investigation, phenomena of phosphate solubilization were observed for all bacterial isolates (Table 2). The results of P solubilization were agreed with the observations of Singh et al. (2010a, 2010b, 2018) who observed phosphate solubilization by rhizospheric bacterial isolates and confirmed their potential in plant growth promotion. Phytohormones are the vital part of plant metabolomic and responsible for plant growth. Bacterial isolates also produce some phytohormone such as indole acetic acid. In current investigation, bacterial isolates SRA5 and SRK13 showed extracellular secretion of indole acetic acid (Table 2). Exopolysaccharide is a slime layer of bacteria, protects bacteria from desiccation and retains water for a longer period of time. Only one bacterial isolates i.e. SRK13 earmarked as significant exopolysaccharide production (Table 2). These findings are in agreement with results of Parveen et al. (2018) who estimated bacterial EPS production and verified their beneficial upshot on germination and vigour of green gram seedlings. Natural environmental factors exert various impacts on plant survival. Plant exposure to various phytopathogenic fungi reduces plant growth and productivity. Bacterial isolates produce some lytic enzymes, volatiles and some antibiotics, which ultimately suppress the mycelial growth of pathogenic fungi. In current study, bacterial isolate SRA5 was proficiently suppressed mycelia growth of both wheat pathogenic fungi (*Fusarium oxysporum* and *Rhizoctonia solani*) whereas, bacterial isolates SRA4, SRPI17 and WRPA26 had suppressed the growth of *Rhizoctonia solani* (Table 2). Singh et al. (2012) also confirmed the similar study of antibiosis assay against mushroom disease infested by *Verticillium fungicola* and *Mycogone perniciosa* through dual culture assay.

Table 3. List of bacterial consortia and their respective bacteria.

Sl. No.	Microbial consortia	Bacterial isolates
1	Consortia 1	SRK13, SRA1 and SRA5
2	Consortia 2	SRPI17, SRA4 and SRA5
3	Consortia 3	WRPA26, SRA1 and SRA4
4	Consortia 4	SRA4 and SRPI17

Consortia development

It is reported that consortia of microorganisms is a better approach due to its efficient function in comparison with single bacterial strain. All bacterial isolates subjected to compatibility test by preparing an individual lawn along with other isolates and according to its compatibility with each other four consortia were developed (Table 3). Furthermore, the plant growth promoting abilities of consortia were also determined.

Plant growth promotion efficiency of consortia

Inside soil, siderophore play a major role for iron availability in soil. Therefore, secretion of siderophore was estimated qualitatively as well as quantitatively for consortia. Consortia 2(C2) and consortia 4 (C4) showed the highest siderophore production efficiency i.e. 216.66% as producing maximum or-

Table 4. Plant growth promoting properties of developed consortia.

Sl. No.	Microbial consortia	Sidero-phore production efficiency	% sidero-phores unit	Zinc solubilization efficiency % $ZnCO_3$	Zno	Phosphorus solubilization	IAA production	Exopoly-saccharide production
1	Consortia 1	114.28	70.66	325	320	–	+	–
2	Consortia 2	216.66	68.00	300	325	+	–	–
3	Consortia 3	171.42	66.66	300	300	+	–	+
4	Consortia 4	216.66	69.33	260	375	–	–	+

ange halo region around the bacterial colony (Table 4). However, results of siderophore quantification demonstrated that a consortium 1 (C1) was highest percent siderophore unit producer i.e. 70.66% (Table 4). Similarly, 80.2% siderophore unit was reported by Thijs et al. (2014). Among all consortia Cl demonstrated highest zinc carbonate solubilization efficiency i.e. 325%, whereas consortia C4 showed highest zinc oxide solubilization efficiency i.e. 375% (Table 4). The present study agreed with the findings of Yasmin et al. (2013), who reported increment of cotton yield upon inoculation of zinc solubilizing consortia developed by *Bacillus* and *Pseudomonas*. Phosphate is a macronutrient and takes part in energy metabolism. Therefore, its availability may be very crucial for plant growth. In the current investigation, consortia 2(C2) and consortia 3 (C3) confirmed phosphate mobilization from insoluble tricalcium phosphate (Table 4). Present findings agreed with the observations of Aarti and Meenu (2014), who observed the maximum phosphate solubilization upon inoculation with consortia of *Pseudomonas aeruginosa, Bacillus cereus* and *Bacillus amyloliquefacien.* Moreover, all microbial consortia were subjected to Luria broth supplemented with L-Tryptophan to determine the indole acetic acid production. Results confirmed that consortium C1 was found as prominent indole acitic producer (Table 4), whereas, other consortia were unable to produce IAA Sethia et al. (2015) also reported the IAA production by consortia consisting of *Fluorescent pseudomonas, Bacillus firmus* and *Cellulosimicrobium cellulans*. Furthermore, exopolysaccharide production was demined qualitatively and found that consortia C3 and C4 significantly formed exopolysaccharide (Table 4), which were responsible for soil aggregation and water retention (Miller and Wood 1996).

Seed germination assay for consortia assessment

In the current investigation, plant growth promotion potential of all four microbial consortia were determined by carried out through seed germination assay on wheat (*Triticum aestivum* var UP2785) seeds by using between paper (BP) method. Seed germination is an important trait of planting value of seed. It is a process in which radicle and plumule emerge out from the seed coat. The seed germination assay was carried out for 8 consecutive days. After completion of experiment seed germination was determined for each treatment, which represents the seed vigour potential with respect to first count and standard germination by inoculated different bacterial consortia. The present findings confirmed significant improvement in seed vigour upon inoculation with consortia when compared with un-inoculated control. The seed showing the higher standard germination was considered as more vigorous germination potential. In the current research, first count, standard germination was significantly improved by consortia C1 i.e. 45.00% and 92.66% respectively, while the value for un-inoculated control were 26.33%, and 83.00%, respectively (Table 5). Similarly, the study of Jha and Saraf (2012) confirmed the application of consortium of *Bacillus brevis, Bacillus licheniformis* and *Alcaigen calcoaceticus,* which showed maximum seed germination percent in *Jatropha* curcus. Subsequently seedling vigour in terms of root, shoot, seedling length and seedling vigour index-I were measured and all vigour parameter was found highest for seeds inoculated with consortia C4 i.e. 21.15 cm, 12.52 cm, 33.76 cm and 2926.14 respectively (Table 5). These findings were also close confirmation with the findings of Pandey and Maheshwari (2007) who

Table 5. Effect of microbial consortia on germination parameters of *Triticum aestivum*. Each value is the mean of three replicates. Data were analyzed statistically at the 5% ($p<0.05$) level of significance.

Treatments	First count (%)*	Standard germination (%)*	Root length (cm)*	Shoot length (cm)*	Seedling length (cm)*	Fresh weight (mg)*	Dry weight (mg)*	Seedling vigour index-I*	Seedling vigour index-II*
Control	26.33	83.00	17.66	10.86	28.53	1149.99	138.45	2368.15	11491.63
Consortia 1	45.00	92.66	18.81	12.12	30.93	2007.00	160.66	2866.74	14888.48
Consortia 2	42.00	89.00	20.36	12.50	32.86	2159.00	178.05	2925.20	15846.61
Consortia 3	41.66	92.66	19.71	11.86	31.57	2178.00	174.21	2924.84	16143.81
Consortia 4	44.33	86.66	21.24	12.52	33.76	2148.00	171.86	2926.14	14895.09
SEm ±	0.63	0.63	0.29	0.28	0.42	16.32	0.10	35.93	105.80

observed enhanced root length, shoot length and dry weight of plants upon inoculation with consortia containing *Azospirillum, Pseudomonas, Azotobacter* and *Bacillus* strain in *Catharanthus roseus* plant. However, the influence of all microbial consortia on the fresh weight and dry weight of seedling was almost at par and significantly enhanced as compared to control (Table 5). The seedling vigour index reflects the survival capability of seed under stress conditions. Fresh weight of seedling and seedling vigour index-II were recorded highest for consortium C3 i.e. 2178.00 mg and 16143.81, respectively (Table 5). Seed germination and their establishment as standard seedling is a major seed quality determining features of economic importance. Germinated seedlings observed in the first count demonstrate earlyness of seed germination. Seeds inoculated with consortium C1 were showed the highest speed of germination and peak value i.e. 20.90 and 23.16 (Table 6), which was much higher than uninoculated control. Seed germination and subsequent seedlings vigour is a complex phenomenon during which seed quickly shift from germination to establishment in the field (Prasad et al. 2016). Moreover, highest relative growth index and lowest time for mean germination and time for 50% germination was recorded for seeds bacterized with consortium C4 i. e. 51.16, 4.58 and 3.63 respectively (Table 6). Among all treatments, seed germination index, germination value, mean daily time and peak value were higher in seeds inoculated with consortia C3 i.e. 16.83, 268.31, 11.58 and 23.16 (Table 6). These results are agreed with the study of Manjunath et al. (2011), Chatterjee et al. (2012) who reported the positive effect of consortia application on seed germination rate of wheat seeds.

CONCLUSION

The present study summarized the plant growth promoting features of potential microbial consortia and their stimulatory effect on wheat (*Triticum aestivum* var UP2785) seed germination and seedling vigour. Results of the present findings demonstrated that all microbial consortia posses numerous plant growth

Table 6. Effect of microbial consortia on other germination parameters of *Triticum aestivum*. *Each value is the mean of three replicates. Data were analyzed statistically at the 5% ($p<0.05$) level of significance.

Treatments	Speed of germination*	Relative growth index*	Germination index*	Mean germination time (days)*	Time for 50% germination T_{50} (days)*	Germination value*	Mean daily germination*	Peak value*
Control	16.91	31.71	9.69	5.46	4.52	172.21	10.37	16.60
Consortia 1	20.90	48.56	15.79	4.82	4.04	265.97	11.48	23.16
Consortia 2	19.68	47.19	15.91	4.83	4.11	247.51	11.12	22.25
Consortia 3	19.94	44.97	16.83	4.99	4.21	268.31	11.58	23.16
Consortia 4	19.68	51.16	15.12	4.58	3.63	234.74	10.83	21.66
SEm ±	0.10	0.83	0.20	0.06	0.18	3.82	0.09	0.15

promoting properties viz siderophore production, zinc and phosphate solubilization, IAA and EPS production. Moreover, the application of microbial consortia significantly improved the growth in terms of wheat seed germination percentage and seedling vigour. All the consortia possess various plant growth promoting traits which reflected significant improvement on seed germination and subsequent seedling growth in wheat. Finally, the present finding concluded that selected consortia have enough potential which may encourage plant growth promotion. Therefore, these bacterial consortia can be developed as bioinoculants for enhanced crop productivity after further evaluation.

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