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Enzymatic Activity and Plant Growth Promoting Potential of Endophytic Bacteria Isolated from *Ocimum sanctum* and *Aloe vera*

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ABSTRACT

Endophytic bacteria exhibit a complex web of interactions with host plants and have been extensively studied over the last several years as prolific sources of new bioactive natural products. The present study was conducted for isolation of endophytic bacterial population of *Ocimum sanctum* and *Aloe vera* and their evaluation for enzymatic activity and plant growth promotion. Morphological characteristics of the bacterial endophytes confirmed that all the bacterial isolates were either gram positive or gram negative rods and cocci. The biochemical study of the bacterial isolates confirmed their abilities to show extracellular enzymatic activity for urease, pectinase, cellulose, catalase, lipase, caseinase, gelatinase and chitinase enzymes. Bacterial isolates were also able to show plant growth promoting traits like phosphate solubilization, IAA, siderophore and ammonia production. On the basis of plant growth promoting traits four potential bacterial endophytes were screened and further evaluated for their plant growth promotory (PGP) potential through seed vigour assay with green gram var. Pant Moong- 4. The results of the seedling vigour assay confirmed that bacterium AVJR7II showed highest influence on seed germination and subsequent seedling growth followed by TNR15, TKR2II and AVJL6II bacterial treatment. Therefore, these bacterial strains could be developed as potential PGP candidates for sustainable agriculture and might be utilized as bioinoculant for organic farming after further evaluation.

Keywords

Endophytes, Plant growth promotion, Extra cellular enzyme, *Ocimum sanctum*, *Aloe vera*

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Introduction

Endophytes are microbes that reside within living plant tissue for all or part of their life cycle without causing substantive harm to their host. Endophytes enter the plant tissues primarily from the rhizosphere through the root zone and become localized at the point of entry or are able to spread throughout the

plant. One of the major contributions of these microorganisms towards plant growth is the production of auxin-like molecules (Spaepen *et al.*, 2007) which is shown to be produced by many root associated bacteria including *Enterobacter* sp., *Pseudomonas* sp. and *Azospirillum* sp. (El-Khawas and Adachi, 1999). In addition to the IAA production, plant growth promoting bacteria (PGPB) are

also shown to exhibit other properties like ACC deaminase, phosphate solubilization, siderophore production, etc. Plant associated bacteria can also have the capability to solubilize non-available phosphate to available form and there by enhance plant growth and yield (De Freitas *et al.*, 1997; Singh *et al.*, 2013). Besides these mechanisms, plant-associated microorganisms improve nutrient acquisition by supplying minerals and other micro/macro nutrients from the soil (Singh *et al.*, 2017; Singh and Singh, 2017). Therefore, isolation and characterization of endophytic bacteria with various properties from unexplored hosts will have many applications to improve plant growth promotion (Patten and Glick, 2002).

Microbial enzymes are always part of great importance in agriculture, industry and human health. Entrance of endophytic bacteria to plants through natural openings or wounds, is also seen to appear by utilizing hydrolytic enzymes like cellulases and pectinases for actively penetrating plant tissues. Since these enzymes are also produced by pathogens, as a result, more knowledge is required for their regulation and expression to distinguish endophytic bacteria from plant pathogens. Very few studies are conducted on isolation of endophytic bacteria and their enzymes production potential from indigenous plants. Jalgaonwala *et al.*, (2011) recorded highest proteolytic activity in bacterium *Lactobacillus fermentum* recovered from leaves of *Vinca rosea*, which is considered superior to non endophytic proteases. However, Yadav *et al.*, (2015) examined the enzymatic activity of endophytic fungi isolated from *Ocimum sanctum* and *Aloe vera* in India.

The medicinal plant, *Ocimum sanctum* ("Tulsi") belongs to the family *Lamiaceae* and on the other side *Aloe vera* ("Aloe") is an important and traditional medicinal plant belonging to the family *Liliaceae*. These

medicinal plants have high therapeutic values. Different parts of the plant (root, stem and leaves) have been recommended for the treatment of various diseases. The leaves of these two plant have been used as a prophylactic to control vomiting, malarial infection, diabetes mellitus, fungal infection and skin diseases (Prakash and Gupta, 2005). In the present study, *Ocimum sanctum* and *Aloe vera* were selected on the basis of their wide medicinal importance and therapeutic values. The aim of the present study was to find extracellular enzymatic activity and plant growth promoting (PGP) potential of the selected endophytic bacteria from *Ocimum sanctum* and *Aloe vera*.

Materials and Methods

Plant samples

Healthy plants of Tulsi (*Ocimum sanctum*) and Aloe (*Aloe vera*) were collected from two different places of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India) i.e. Medicinal plant research and development centre (MRDC) and Garden section. All the samples were kept in sterile zip lock polythene bags and used as source material for the isolation of endophytic bacteria.

Isolation of bacterial endophytes

Endophytic bacteria were isolated from roots, stems and leaves of healthy plants of Tulsi and Aloe vera. The root, stem and leaves were cut into small pieces by using sterile blade. Sections of root, stem and leaves of the plant were surface sterilized using the five step procedure described as; 3 min wash in 5% NaClO₃, followed by a 10 min wash in 2.5 % Na₂S₂O₃, a brief wash in 75 % ethanol, then a 10 min wash in 10 % NaHCO₃ and finally rinse in sterile distilled water for 5-8 times (Tiwari *et al.*, 2010). After final wash the

plant sections were transferred on nutrient agar plates to check the sterility of plant tissues and incubated at $28 \pm 2^\circ\text{C}$ for 24 h. After sterility check, each sample was macerated in a sterile pestle and mortar with sterile distilled water. Macerated tissues were serially diluted up to 10^{-6} dilutions. Three dilutions (10^{-3} , 10^{-4} , 10^{-6}) of macerated tissues were plated on three different medium i.e. Nutrient agar, Jensen's media and King's B media (Tejera *et al.*, 2006). The plates were incubated for 48 h at $28 \pm 2^\circ\text{C}$. Morphologically distinct and isolated colonies were transferred to the respective media and purified thereafter following standard protocols (Holt *et al.*, 1994). Pure culture of the bacterial isolates were maintained on nutrient agar slants at 4°C for regular use and in 20% glycerol stocks for long-time preservation at -20°C .

Morphological characterization

The cellular characterization of screened bacterial cultures was based upon cell shape and Gram's staining (Olympus microscope). The colony characteristics of bacterial cultures were carried under stereoscope microscope (Olympus, SZH 10) to get shape, edge, elevation, surface and chromogenicity.

Determination of hydrolytic enzymes

All the bacterial cultures were characterized for hydrolytic enzyme production such as protease, lipase, chitinase, cellulose, amylase and pectinase were detected on respective agar plates with variable substrates (Hankin and Anagnostakis, 1975; Cappuccino and Sherman, 2002; Kasana *et al.*, 2008). The bacterial isolates were inoculated on qualified agar plates and incubated at $28 \pm 2^\circ\text{C}$ for 3-4 days. Development of halo zone around the bacterial colonies indicated enzyme production for protease, lipase and chitinase enzymes. However, cellulose production was

confirmed through stained with 0.1% congo red solution and destained with 1M NaCl for 15 min. While, amylase and pectinase agar plates were flooded with 1% iodine in 2% potassium iodide to confirm.

The laccase production was confirmed by the conversion of colourless medium into blue due to oxidation of 1-naphthol by laccase. However, the urease production was confirmed through inoculation of bacterial strain in Christensen's urea broth (Cappuccino and Sherman, 2002). The conversion of medium colour from yellow to red indicates urease production.

Plant growth promotory traits

IAA production

The Indole-3-Acetic Acid (IAA) productions by the bacterial strains were qualitatively determined by the method of Patten and Glick (2002). The endophytic bacterial isolates were grown in Luria broth supplemented with L-tryptophan ($1\mu\text{g}/\text{ml}$) and incubated at $28 \pm 2^\circ\text{C}$ for 72 h. Afterwards, bacterial cultures were centrifuged at $10,000\text{g}$ for 10 min and the supernatant was collected. One ml of this culture filtrate was allowed to react with 2ml of Salkowski reagent (1ml of 0.5 M FeCl_3 in 50 ml of 35% HClO_4) and incubated at $28 \pm 2^\circ\text{C}$ for 30 min. At the end of the incubation, development of pink colour indicates the production of IAA.

Siderophore production

Qualitative productions of siderophore by the bacterial cultures were detected on the Chrome-azurol S medium (CAS-medium) as described (Schwyn and Neilands, 1987). Each endophytic bacterial isolate was inoculated on the surface of CAS agar plates and incubated at $28 \pm 2^\circ\text{C}$ for 72 h. The plates were observed for colour change i.e. orange to yellow halo zone around the bacterial colonies.

Phosphate solubilization

Ability of bacterial endophytes to solubilize the major plant nutrients i.e. phosphate (P) was investigated on Pikovskaya agar containing tricalcium phosphate as insoluble phosphate source (Pikovskaya, 1948). Each bacterial endophyte was spot inoculated in the centre of Pikovskaya agar plate and incubated at $28 \pm 2^\circ\text{C}$ for 72 h. The plates were observed for the appearance of halo zone around the bacterial colonies.

Ammonia production

Bacterial endophytes were tested for the production of ammonia in peptone water as described by Cappuccino and Sherman (1992). Freshly grown cultures were inoculated in 10 ml peptone water separately and incubated for 48-72 hrs at $28 \pm 2^\circ\text{C}$. After completion of incubation, Nessler's reagent (0.5 ml) was added in each test tube. Development of brown to yellow colour was a positive indication for ammonia production.

Plant growth promotion assessment

The plant growth promotion potential of the selected endophytic bacterial isolates were carried out through seed vigour assay. This assay was carried out according to In between paper (BP) method. To compile this assay the seeds of green gram (var. Pant Moong-4) were selected, which were free from obvious damage and surface sterilized by soaking in ethanol (95% v:v) for 30 sec and then a sodium hypochlorite solution (1.2% w:v) for 5 min, followed by 10 rinses with sterile tap water (Carrillo *et al.*, 2002). Afterwards, bacterial cultures were inoculated into flask containing 75ml nutrient broth and incubated overnight at $28 \pm 2^\circ\text{C}$. After end of incubation, surface sterilized seeds were transferred into bacterial culture flask. Flasks were then shaken for 6 hours to allow bacteria to adhere

on seed coat. Hundred bacterial treated seeds were sown aseptically in between paper (B.P) then kept at 25°C in germinator. Seeds were daily analysed for germination up to 8 days of incubation. This experiment had designed with 4 replicates per treatment. One un-inoculated, surface sterilised disinfected seed treatment was used as a control.

Observation recorded

(i) First count: Four replications of hundred seeds were taken for each treatment. Seeds were sown in between paper (BP) then samples were kept at 25°C in germinator. Only normal seedlings were counted on the 5th day of test.

(ii) Standard germination: Hundred bacterial treated seeds of green gram (variety Pant Moong-4) were placed with four replications in between paper (BP) and incubated at 25°C in germinator. The normal seedlings were counted on 8th day as final count.

(iii) Seedling root length: Ten normal seedlings were randomly selected on 8th day of the start of germination test from each replication. The length of radicle (in cm) was measured with the help of measuring scale and the mean root length was calculated.

(iv) Seedling shoot length: The shoot length (in cm) was measured with the help of a scale on 10 randomly selected seedlings from each replication. The value was obtained by calculating mean of 10 seedlings for each replication.

(v) Seedling length: The total length of seedlings (in cm) was obtained by adding shoot and root length as recorded earlier.

(vi) Seedling fresh weight: Seedling fresh weight was recorded at the end of seed germination test on 8th day. The 10 normal seedlings were randomly taken from each replication and were weighted and the seed

fresh weight was measured on an electronic balance in milligram.

(vii) Seedling dry weight: At the end of seed germination test on 8th day, the 10 normal seedlings were randomly taken from each replication. Seedlings were dried in the oven at 80°C for 24 hrs. The dried seedlings were weighed on an electronic balance and expressed in milligram.

(viii) Seedling vigour index: The seedling vigour index was calculated by two different methods.

(a) Seedling vigour index-I: Calculated as-

$$\text{Seed Vigour Index I} = \text{Standard germination (\%)} \times \text{Seedling Length (cm)}$$

(b) Seedling vigour index-II: Calculated as-
Seed Vigour Index II = Standard germination (%) × Seedling dry weight (mg)

(ix) Speed of germination: In this test, four replications of 100-seeds were taken from each treatment and placed in between paper (BP) and then kept at 25°C in germinator. After the seed began to germinate, they were checked daily at approximately the same time each day. Normal seedlings were removed from the test, when they reached a predetermined size. This procedure was continued until all seed that were capable of producing a normal seedling had germinated. An index was computed for each treatment by dividing the number of normal seedling removed each day by the corresponding day of counting.

(x) Relative growth index (RGI): Relative growth index was calculated according to the evaluation of Brown and Mayer (1986) as under-

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

(xi) Germination index (GI) - Germination index was calculated as described in the association of official seed Analysts (2002) using the following formula-

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

(xii) Mean germination time (MGT): Mean germination time (MGT) was calculated by the equation of Ellis and Roberts (1981) as

$$\text{MGT} = \frac{\sum Dn}{\sum n}$$

Where, n is the number of seeds which were germinated on day D and D is the number of days counted from beginning of germination.

(xiii) Time to 50% germination (T50): The time to 50% germination (T50) was calculated according to the following formula of Coolbear *et al.*, (1984) modified by Farooq *et al.*, (2005) as under

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

Where, N is the final number of germination and ni, nj be the cumulative number of seed germinated by adjacent counts at time ti and tj, when ni < N/2 < nj.

(xiv) Germination value: Calculated as = Peak value × Mean daily germination

(xv) Mean daily germination: Mean daily germination was calculated according to following formula:

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

(xvi) Peak value: Peak value was calculated according to following formula:

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

Statistical analysis

Statistical analysis was done by completely randomized design (CRD) using STPR3 programme. All experiments were performed in four replications. The critical difference at 5 per cent level of significance was calculated to compare the mean of different treatments.

Results and Discussion

Morphological characteristics

A total 10 bacterial cultures were isolated from roots, stem and leaves of *O. sanctum* and *Aloe vera*, those are presented well in table 1. Morphologically these all bacterial isolates were distinct to each other and confirmed through gram's staining and cultural characteristics. During gram's staining 8 bacterial isolates were either gram positive rods or cocci and remaining 2 were gram negative short rods (Table 1).

Extracellular enzyme activity

During biochemical studies most of the bacterial isolates showed extracellular enzymatic activity and exhibited amylase, caseinase, cellulase, chitinase, pectinase and urease activity by producing clear halo zone on respective agar plates. Extracellular enzymatic activities of most of the bacterial culture were positive for amylase, cellulose and chitinase activity. However, enzymatic activity of caseinase, pectinase and urease were limited for some of the bacterial cultures and mentioned in table 2. During investigation none of the bacterial isolates were positive for laccase, lipase and gelatinase activity (Table 2). These results are agreement with the finding of Yadav *et al.*, (2015) who described the extracellular enzymatic activity of the endophytic fungi isolated from *O. sanctum* and *Aloe vera*. In other hand Vijayalakshmi *et al.*, (2016) reported the enzymatic activity of

bacterial endophytes from medicinal plants. They found that most of the strains exhibited reasonable enzyme activity for amylase, cellulase and protease on LB agar plates amended with 1% substrate, however no lipase activity was observed in any of the six bacterial endophytes. Singh *et al.*, (2012) and Sharma *et al.*, (2013) reported the extracellular enzymatic activity of the bacteria recovered from mushroom compost and described the functional status of these enzymes through solid state fermentation.

Plant growth promotory traits

All the bacterial isolates were further studied for plant growth promoting traits like siderophore, IAA, ammonia production and phosphate solubilization (Table 3). The results of the study confirmed that out of 10 bacterial isolates only 3 isolates i.e. TNR15, TKR 1 II, and AVJR7 II having the ability to produce siderophore on Chrome-azurol S medium (CAS-medium) by producing orange to yellow halo zone around the bacterial colonies under iron limiting conditions. Ramanuj and Shelat (2018) characterize the siderophore producing plant growth promoting potential of bacterial endophytes from medicinal plants. Siderophore production from bacterial strains are considered one of the direct mechanisms of plant growth promotion. This characteristic one of the known and important characteristics of root associated PGPB/R (plant growth promotory bacteria/rhizobacteria) under iron deficient conditions. Indole-3-Acetic Acid (IAA) production is one of another plant growth promoting mechanisms of plant growth promotory bacteria. IAA is a plant growth regulatory hormone, known as auxin and involved in various physiological processes in plant development like cell division and elongation, tissue differentiation and root initiation (Gravel *et al.*, 2007). The results of the present study confirmed that 3 bacterial isolates i.e. TNR15, TKR 2 II and

AVJL6 II were able to produced indole-3-acetic acid (IAA) (Table 3). This finding also agreed with the findings of Ullah *et al.*, (2018) who confirmed IAA production potential of bacterial endophytes from two medicinal plants of Pakistan.

It is well known fact that phosphorous (P) is an important plant macronutrient, making up about 0.2 percent of plant dry weight and plant cannot survive without a reliable supply of this nutrient (Singh and Prasad, 2014; Singh *et al.*, 2018). Among all the bacterial isolates

only 2 isolates i.e. TNR 15 and AVKL 2 II were able to solubilize phosphate efficiently, that was evident by a clear halo zone around the bacterial colony on Pikovskaya agar plates (Table 3). Phosphate deficiency in soil can severely limit plant growth and productivity (Singh, *et al.*, 2010, 2010a, 2010b, 2011, 2013; Singh and Goel, 2015). Microbial phosphate solubilization is a complex phenomenon that depends on many factors like nutritional, physiological and growth conditions of the bacterial cultures (Reyes *et al.*, 2002).

Table.1 Morphological characteristics of bacterial isolates

S. No.	Bacterial isolates	Cellular characteristics		Colonial characteristics			
		Gram Reaction	Cell shape	Edge	Elevation	Surface	Chromogenicity
1.	TNS7	+ve	Cocci	Entire	Convex	Smooth	Creamy white
2.	TNR15	+ve	Cocci	Entire	Raised	Glistening	Yellowish
3.	TKR10	+ve	Cocci	Entire	Convex	Smooth	White
4.	TJR9	+ve	Long rods	Filamentous	Flat	Wrinkled	Orange
5.	TKR1II	+ve	Cocci	Entire	Raised	Smooth	White
6.	TKR2II	+ve	Long rods	Entire	Convex	Smooth	Dark yellow
7.	TKL5II	+ve	Long rods	Entire	Convex	Glistening	Yellowish
8.	AVKL2II	+ve	Cocci	Entire	Raised	Glistening	Light yellow
9.	AVJL6II	-ve	Short rods	Entire	Flat	Glistening	Orange
10.	AVJR7II	-ve	Short rods	Entire	Flat	Dry	Off-white

Table.2 Extracellular enzymatic activity of endophytic bacteria isolates

Sr No.	Bacterial Isolates	Catalase test	Amylase Test	Gelatinase Test	Caseinase Test	Cellulase Test	Chitinase Test	Pectinase Test	Laccase Test	Lipase Test	Urease Test
1.	TNS7	-	+	-	-	-	+	+	-	-	-
2.	TNR15	+	-	-	-	+	-	-	-	-	-
3.	TKR10	+	+	-	-	-	+	+	-	-	+
4.	TJR9	+	+	-	-	-	+	+	-	-	-
5.	TKR1II	+	-	-	+	-	-	+	-	-	-
6.	TKR2II	+	-	-	-	-	+	-	-	-	-
7.	TKL5II	-	+	-	+	-	+	+	-	-	-
8.	AVKL2II	+	+	-	+	-	+	-	-	-	-
9.	AVJL6II	+	+	-	-	-	-	-	-	-	-
10.	AVJR7II	+	+	-	+	+	+	-	-	-	-

Table.3 Plant growth promotory traits of endophytic bacterial isolates

Sr. No.	BACTERIAL ISOLATES	Siderophore	Phosphate	Ammonia	IAA
1.	TNS17	-	-	+	-
2.	TNR15	+	+	+	+
3.	TKR10	-	-	+	-
4.	TJR9	-	-	+	-
5.	TKR1II	+	-	+	-
6.	TKR2II	-	-	+	+
7.	TKL5II	-	-	+	-
8.	AVKL2II	-	+	+	-
9.	AVJL6II	-	-	+	+
10.	AVJR7II	+	-	+	-

Table.4 Effect of bacterial treatment on plant growth parameters of green gram

Bacterial 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 [*]
TNR15	82.25	89.5	5.85	11.36	17.16	1450.0	210.0	1534.7	18.8
TKR2II	76.25	85.5	5.86	21.9	27.8	1855.0	182.5	2381.9	15.6
AVJL6II	75.25	82.25	5.69	21.41	27.07	1642.5	187.5	2227.3	15.6
AVJR7II	90.5	95.25	4.40	20.57	24.97	1397.5	195.0	2378.8	18.6
CONTROL	67.25	75.25	2.19	6.56	8.72	962.5	155.0	657.0	13.18
SEm±	1.06	0.71	0.209	0.27	0.36	18.94	5.7	38.3	0.21

*Each value is the mean of four replicates.

Data were analysed statistically at the 5% (p<0.05) level of significance

Table.5 Effect of bacterial treatment on plant growth parameters of green gram

Bacterial Treatments	Speed of germination [*]	Relative growth index (RGI) [*]	Germination index [*]	Mean germination time (days) [*]	Time for 50% germination T ₅₀ (days) [*]	Germination value [*]	Mean daily germination [*]	Peak value [*]
TNR15	26.3	91.8	27.4	3.6	36.2	332.2	11.15	29.8
TKR2II	24.3	89.1	25.7	3.7	31.3	303.2	10.6	28.4
AVJL6II	24.10	91.4	25.1	3.6	30.0	279.8	10.2	27.3
AVJR7II	29.05	94.9	29.9	3.5	32.6	375.4	11.8	31.7
CONTROL	20.91	87.3	22.09	3.8	29.1	219.2	9.3	24.7
SEm ±	0.32	1.2	0.18	0.49	1.6	6.0	0.96	0.19

*Each value is the mean of four replicates.

Data were analysed statistically at the 5% (p<0.05) level of significance

The results of the present study confirmed that all the bacterial cultures were good ammonia producers (Table 3). Potential of ammonia production assumed that exploitation of such bacteria may be able to enhance the plant growth as a result of their ability to fix nitrogen (N_2) to ammonia (NH_3) making, which is an available nutrient for plant growth and development (Hayat *et al.*, 2010). These results of ammonia production are in agreement with the study of Sansanwal *et al.*, (2018) who described the maximum number of endophytic bacteria isolated from green gram were able to excrete ammonia.

Plant growth promotion assessment

Seed germination assay

In the present study the plant growth promotion potential of the selected endophytic bacterial isolates were carried out through seed vigour assay on green gram seeds by using In between paper (BP) method. On the basis of plant growth promoting traits 4 best potential bacterial isolates i.e. TNR15, TKR2II, AVJL6II and AVJR7II were selected for the seed vigour assay. After 8 days of incubation period for green gram the number of germinated seeds were determined. The results of the study confirmed that all these selected bacterial endophytes were able to improve seed vigour significantly throughout all the observations over un-inoculated control (Table 4). The first count, standard germination and shoot length, of the seedling were maximally improved by AVJR7II bacterial treatment i.e. 90.5%, 95.25% and 20.57cm, respectively, while the value for un-inoculated control were 67.25%, 75.25% and 6.56cm, respectively (Table 4). Maximum enhancement in root length, seedling length and fresh weight of the seedling were achieved by TKR2II bacterium treatment, while fresh weight was increased maximally by TNR15 bacterium treatment.

The numbers of normal seedlings recorded in the first count express the population of fast germinating seeds and thus function as a vigour measurement. The seed lot showing the higher standard germination is considered to be more vigorous (Abdul-Baki and Anderson 1973). These results are in agreement with the study of Yadav *et al.*, (2016) who reported the effect of endophytic fungi on seed vigour and germination on green gram seeds. On the day of final count (i.e. 8th day), ten normal seedlings were taken out carefully at random from each treatment and measured from the tip of primary root to the tip of apical shoot. The average length of ten seedlings was calculated and expressed as mean seedling length in centimetres. The root length, shoot length, seedling length and fresh weight of seedling were highly influenced by TKR2II bacterium over un-inoculated control (Table 4). A vigour index reveals a seed lot's ability to withstand a variety of different stress factors. The data depicted in Table 4 showed the influence of bacterial isolates on seed vigour index of green gram and it is evident that the treatment TKR2II was significantly enhanced vigour index-I with a value of 2381.9 followed by AVJR7II, AVJL6II and TNR15, while the un-inoculated control achieved the least value of 657.0 (Table 4). However, maximum value for seedling vigour index II was 18.8, which was achieved by TNR15 bacterium treatment followed by AVJR7II (18.6), AVJL6II (15.6) and TKR2II (15.6) over un-inoculated control (13.18) (Table 4). Seed germination and their establishment as a normal seedling are determining features for the propagation of plant species, and both are economic and ecologically important. Due to its high vulnerability to injury, disease, and biotic/abiotic stress, germination is considered to be the most critical phase in the completion of plant life cycle (Rajjou *et al.*, 2012). Germination is a complex phenomenon during which the imbibed mature seed must quickly

shift from a maturation to a germination driven program of development and prepare for seedling growth (Nonogaki *et al.*, 2010; Prasad *et al.*, 2016). The bacterium AVJR7II was more effective in rest parameters of seed vigour assay except mean germination time parameter. Speed of germination, relative growth index (RGI), germination index, germination value, mean daily germination and peak value were highly influenced by AVJR7II and achieve value 29.05, 94.9, 29.9, 375.4, 11.8 and 31.7, respectively, while the least value were recorded with un-inoculated control i.e 20.91, 87.3, 22.09, 219.2, 9.3 and 24.7, respectively (Table 5). However, value of mean germination time and time for 50% germination (T_{50}) of seedling highly influenced by TKR2II and TNR15 bacterial treatments, respectively (Table 5). Germination parameters were best way to know the extent of completeness of germination, rapidity and peak value of germination, which reflects the seeds and seedling quality. On the basis of seed vigour assay all the selected screened bacterial endophytes of *Ocimum sanctum* and *Aloe vera* were able to enhanced seedling vigour of green gram seeds up to certain levels. However, the values for all the parameters were varied with the bacterium treatments. Shah *et al.*, (2018) described the potential and prospect of plant growth promoting rhizobacteria in lentil. On the other hand Singh *et al.*, (2010b) studied the effect of plant growth promoting rhizobacteria (PGPR) on improving seed germination and seedling vigour of lentil.

In conclusion, collective summarized analysis of all the experiments done in the present study highlighted the effect of the isolated endophytic bacterial strains on enzymatic activity and plant growth promotion assessment which could be a valuable resource for applied agriculture and agricultural industries. All the selected

bacterial endophytes with PGP properties viz. P solubilization, production of hydrolytic enzymes such as cellulase, protease, chitinase and lipase responsible for endophytic colonization, production of IAA and siderophore which may be helpful directly or indirectly to enhance seed vigour, which reflects the seeds and seedling quality. The information acquired from these strains could open the door and exploit the valuable data for the benefit of mankind. Finally, the study concluded that the isolated bacteria have numerous plant growth promoting and enzymatic activities which directly or indirectly promote plant growth. These isolates can be developed as new inoculants to utilize in agro industrial sector after further evaluation.

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References

- Abdul-Baki, A. A. and Anderson, J. D. 1973. Vigour determination in soybean seeds by multiple criteria. *Crop Sci.* 13: 630-633.
- Brown, R. F. and Mayer, D. G. 1986. A critical analysis of maguirress germination rate index. *J. Seed. Tech.* 19: 101-110.
- Cappuccino, J. G. and Sherman, N. 2002. *Microbiology: A laboratory manual* (7th ed), Benjamin Cummings, Science - 491 pages.
- Cappuccino, J.C. and Sherman, N. 1992. *Microbiology: A Laboratory Manual*,

- Third ed. Benjamin/Cummings Publishing Company New York.* 179pp.
- Carrillo-Castaneda, G., Munos, J. J., Peralta-Videa, J. R., Gomez, E., Tiemannb, Duarte-Gardea. and Gardea-Torresdey, J.L. 2002. Alfalfa growth promotion by bacteria grown under iron limiting conditions. *Adv. Environ. Res.* 6: 391-399.
- Coolbear, P., Francis, A. and Grierson, D. 1984. The effect of low temperature pre-sowing treatment under thegermination performance and membrane integrity of artificially aged tomato seeds. *J. Exper. Bot.* 35: 1609-1617.
- De Freitas, J.R., Banerjee, M.R. and Germida, J.J. 1997. Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus*). *Biol. Fertil. Soils.* 24: 358-364.
- El-Khawas, H. and Adachi, K. 1999. Identification and quantification of auxins in culture media of *Azospirillum* and *Klebsiella* and their effect on rice roots. *Biol. Fertil. Soils.* 28: 377-381.
- Ellis, R. A. and Roberts, E. H. 1981. The quantification of ageing and survival in orthodox seeds. *Seed Sci.Technol.*, 9, 373-409.
- Farooq, M. S., Basra, M., Hafeez A. K. and Ahmad, N. 2005. Thermal hardening: A new seed vigor enhancement tool in rice. *Acta. Botan. Sin.* 47: 187-193.
- Gravel, V., Antoun, H. and Tweddell, R. J. 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). *Soil Biol. Biochem.* 39: 1968-1977.
- Hankin, L. and Anagnostakis, S.L. 1975. The use of solid media for detection of enzyme production by fungi. *Mycologia.* 67: 597-607.
- Hayat, Q., Hayat, S., Irfan, M. and Ahmad, A. 2010. Effect of exogenous salicylic acid under changing environment: A review. *Environ. Experim. Bot.* 68: 14-25.
- Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. 1994. Bergeys manual of determinative bacteriology, 9th edn. Williams and Wilkins, USA.
- Kasana, R.C., Salwan, R., Dhar, H., Dutt, S. and Gulati, A. 2008. A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. *Curr. Microbiol.* 57(5): 503-507.
- Nonogaki, H., Bassel, G.W. and Bewley, J. D. 2010. Germination—still a mystery. *Plant Sci.* 179:574-81.
- Patten, C. L. and Glick, B. R. 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Appl. Environ. Microbiol.* 68:3795-801.
- Pikovskaya, R.I. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya.* 17:362-370.
- Prakash, P. and Gupta, N. 2005. Therapeutic uses of *Ocimum sanctum* Linn (tulsi) with a note on eugenol and its pharmacological actions: a short review. *Indian J. Physiol. Pharmacol.* 49(2): 125-131.
- Prasad, B., Kumar, A., Singh, A. V. and Kumar, A. 2016. Plant growth and seed yield attributes as influenced by bacterial isolates under glass house. *Progressive Research*, 11 (IV): 2573-2576.
- Rajjou, L., Duval, M., Gallardo, K., Catusse, J. Bally, J., Job, C. and Job, D. 2012. Seed germination and vigor. *Annu. Rev. Plant Biol.* 63:507-533.
- Ramanuj, K. B. and Shelat, H. N. 2018. Plant growth promoting potential of bacterial

- endophytes from medicinal plants. *Advances in Research*. 13(6): 1-15.
- Reyes, I., Bernier, L. and Antoun, H. 2002. Rock phosphate solubilization and colonization of maize rhizosphere by wild and genetically modified strains of *Penicillium rugulosum*. *Microbiol. Ecol.*, 44: 39-48.
- Sansanwal, R., Ahlawat, U., Batra, P. and Wati, L. 2018. Isolation and evaluation of multi-trait novel bacterial endophytes from root nodules of mungbean (*Vigna radiata*). *Int.J.Curr.Microbiol.App.Sci*. 7(03): 2424-2430.doi: <https://doi.org/10.20546/ijcmas.2018.703.282>
- Schwyn, B. and Neilands, J.B. 1987. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* 160(1): 47-56.
- Shah, S., Ramanan, V. V., Singh, A. V. and Singh, A. K. 2018. Potential and prospect of plant growth promoting rhizobacteria in lentil. In: Scientific lentil production (Ed. Singh *et al.*, 2016). Satish Serial Publishing House, Delhi, India.
- Sharma, A., Singh, A. V. and Johri, B. N. 2013. Functional and genetic characterization of culturable bacteria associated with late phase of mushroom composting assessed by amplified rDNA restriction analysis. *Int.J.Curr.Microbiol.App.Sci*. 2(6): 162-175.
- Singh, A. V. and Goel, R. 2015. Plant growth promoting efficiency of *Chryseobacterium* sp. PSR10 on finger millet (*Eleusine coracana*). *Journal of Global Biosciences*. 4(6): 2569-2575.
- Singh, A. V. and Prasad, B. 2014. Enhancement of plant growth, nodulation and seed yield through Plant Growth Promoting Rhizobacteria in Lentil (*Lens culinaris* Medik cv. VL125). *Int. J. Curr. Microbiol. Appl. Sci.* 3(6): 614-622.
- Singh, A. V., Agarwal, A. and Goel, R. 2010a. Comparative phosphate solubilization efficiency of two bacterial isolates and their effect on *Cicer arietinum* seeds in indigenous and alternative soil system. *Environ. Ecol.* 28: 1979-1983.
- Singh, A. V., Chandra, R. and Reeta, G. 2013. Phosphate solubilization by *Chryseobacterium* sp. and their combined effect with N and P fertilizers on plant growth promotion. *Archives of Agronomy and Soil Science*. 59(5): 641-651.
- Singh, A. V., Prasad, B. and Goel, R. 2018. Plant Growth Promoting Efficiency of Phosphate Solubilizing *Chryseobacterium* sp. PSR 10 with Different Doses of N and P Fertilizers on Lentil (*Lens culinaris* var. PL-5) Growth and Yield. *Int. J. Curr. Microbiol. App. Sci.* 7(05): 2280- 2289. doi: <https://doi.org/10.20546/ijcmas.2018.705.265>.
- Singh, A. V., Prasad, B. and Shah, S. 2010b. Screening Plant growth promotory rhizobacteria for improving seed germination and seedling vigor of lentil (*Lens culinaris* Medik). *Environ. Ecol.* 28: 2055-2058.
- Singh, A. V., Prasad, B. and Shah, S. 2011. Influence of phosphate solubilizing bacteria for enhancement of plant growth and seed yield in lentil. *J. Crop Weed*. 7(1): 1-4.
- Singh, A. V., Shah, S. and Prasad, B. 2010. Effect of phosphate solubilizing bacteria on plant growth promotion and nodulation in soybean (*Glycine max* (L.) Merr). *J. Hill Agri.* 1(1): 35-39.
- Singh, A. V., Sharma, A. and Johri, B. N. 2012. Phylogenetic profiling of culturable bacteria associated with early phase of mushroom composting

- assessed by amplified rDNA restriction analysis. *Ann. Microbiol.* 62:675–682
- Singh, J. and Singh, A. V. 2017. Microbial Strategies for Enhanced Phytoremediation of Heavy Metals Contaminated Soils. In: Environmental Pollutants and their Bioremediation Approaches (Ed. Bharagava, R. N.). *Taylor & Francis*. CRC Press London, New York: 249-264.
- Singh, J., Singh, A. V., Prasad, B. and Shah, S. 2017. Sustainable agriculture strategies of wheat biofortification through microorganisms. In: Wheat a premier food crop (Ed, Anil Kumar, Amarjeet Kumar and Birendra Prasad). Kalyani Publishers, New Delhi, India.
- Spaepen, S., Vanderleyden, J. and Remans, R. 2007. Indole-3-acetic acid in microbial and microorganism-plant Signaling. *FEMS Microbiol. Rev.* 31: 1–24
- Tejera, N., Ortega, E., Rodes, R. and Lluch, C. 2006. Nitrogen compounds in the apoplastic sap of sugarcane stem: Some implications in the association with endophytes. *J. Plant Physiol.* 163: 80–85.
- Tiwari, A., Mahajan, C.S., Mishra, D.K., Shukla, K. and Porwal, P. 2010. Comparative *in vitro* antioxidant activity of *Pongamia pinnata* linn. leaves extracts and isolated compound. *Inter. J. Pharm. Biol. Arch.* 1: 69–75.
- Ullah, A., Mushtaq, H., Ali U., Hakim, Ali, E. and Mubeen, S. 2018. Screening, isolation, biochemical and plant growth promoting characterization of endophytic bacteria. *Microbiol. Curr. Res.* 2(2):24-30.
- Vijayalakshmi, R., Kairunnisa1, K., Sivvaswamy, N., S., Dharan, S. S. and Natarajan, S. 2016. Enzyme production and antimicrobial activity of endophytic bacteria isolated from medicinal plants. *Indian J. Sci. Tech.* 9(14): 1-8. DOI: 10.17485/ijst/2016/v9i14/83143
- Yadav, R., Singh, A. V., Joshi, S. and Kumar, M. 2015. Antifungal and enzyme activity of endophytic fungi isolated from *Ocimum sanctum* and *Aloe vera*. *African Journal of Microbiology Research.* 9(29): 1783-1788.
- Yadav, R., Singh, A.V., Kumar, M. and Yadav. S. 2016. Phytochemical analysis and plant growth promoting properties of endophytic fungi isolated from tulsi and aloe vera. *Int. J. Agricult. Stat. Sci.*, 12(1): 239-248.

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