



# The Open Microbiology Journal

Content list available at: <https://openmicrobiologyjournal.com>



## RESEARCH ARTICLE

### Plant Growth-promoting and Bio-control Activity of *Micrococcus luteus* Strain AKAD 3-5 Isolated from the Soybean (*Glycine max* (L.) Merr.) Rhizosphere

Anamika Dubey<sup>1</sup>, Ashwani Kumar<sup>1,\*</sup> , Mohammed Latif Khan<sup>2</sup> and Devendra Kumar Payasi<sup>3</sup>

<sup>1</sup>Department of Botany, Metagenomics and Secretomics Research Laboratory, Dr. Harisingh Gour University (A Central University), Sagar 470003, Madhya Pradesh, India

<sup>2</sup>Department of Botany, Dr. Harisingh Gour University (A Central University), Sagar 470003, Madhya Pradesh, India

<sup>3</sup>Jawaharlal Nehru Krishi Vishwa Vidyalaya, Regional Agriculture Research Station, Sagar, MP470002, India

#### Abstract:

#### Background:

Applications of bioinoculants for improving crop productivity may be an eco-friendly alternative to chemical fertilizers. Rhizosphere or soil-inhabiting beneficial microbes can enhance plant growth and productivity through direct and indirect mechanisms, *i.e.*, phosphate solubilization, nutrient acquisition, phytohormone production, *etc.*

#### Objective:

This study is based on the hypothesis that diseases resistant plants can act as a source of potential microbes that can have good plant growth-promoting traits and bio-control potential.

#### Methods:

In this study, we have isolated the rhizobacterial strains (AKAD 2-1, AKAD 2-10, AKAD 3-5, AKAD 3-9) from the rhizosphere of a disease-resistant variety of soybean (JS-20-34) (*Glycine max* (L.) Merr.). These bacterial strains were further screened for various plant growth-promoting traits (phosphate solubilization, indole acetic acid (IAA), ammonia, biofilm, HCN, Exopolysaccharide (EPS), and enzyme production activity (catalase, cellulase, and chitinase)).

#### Results:

Among four, only bacterial strain AKAD 3-5 has shown plant-growth-promoting and biocontrol (98%) activity against *Fusarium oxysporum*. Morphological, biochemical, and molecular characterization (16S rRNA) revealed that this rhizobacterial isolate AKAD 3-5 closely resembles *Micrococcus luteus* (Gene bank accession: MH304279).

#### Conclusion:

Here, we conclude that this strain can be utilized to promote soybean growth under varied soil stress conditions.

**Keywords:** Rhizobacteria, PGPR, Rhizosphere, Phosphate solubilization, Soybean, Microbes.

#### Article History

Received: March 22, 2021

Revised: May 31, 2021

Accepted: June 23, 2021

## 1. INTRODUCTION

Soybean has turned out to be a major oilseed crop in India, and a good source of protein supplement, wherein more than 40% population suffers from energy-protein malnutrition [1, 2]. By the year 2050, global food production needs to be

doubled to feed 9.1 billion people of the world, as reported by FAO [3]. To achieve this ambitious goal, cereal production must be increased, with a high emphasis on sustainability. Therefore, the development of eco-friendly strategies can help to ameliorate the effect of abiotic and biotic stress in plants, and there is an urgent need for such strategies in our agricultural systems that have to cope with the impact of climate change [4 - 7]. The beneficial role of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal

\* Address corresponding to this author at the Department of Botany, Metagenomics and Secretomics Research Laboratory, Dr. Harisingh Gour University (A Central University), Sagar (M.P.) India; Tel: 91-7697432012; E-mail: [ashwaniitd@hotmail.com](mailto:ashwaniitd@hotmail.com)

fungi (AMF) in improving plant growth under stressful conditions are well documented in the literature [1, 8 - 12]. Various reports are available demonstrating the role of PGPRs in mitigating abiotic stress in many crops, such as tomato, pepper, pea, maize, wheat, mung bean, and chickpea [13 - 19]. PGP microbes-induced plant growth potential is explained through numerous anticipated mechanisms [9, 20 - 22]. Several plant growth-promoting (PGP) microbes help to increase the availability of nutrients, like nitrogen (N), phosphorus (P), iron (Fe), as well as produce metabolites that can regulate growth and defense responses in plants [10, 23]. These microbes also produce plant hormones like auxin, cytokinin, gibberellic acid, abscisic acid, strigolactones, brassinosteroids, salicylic acid, jasmonic acid, and ethylene [24 - 26]. Groups of some plant growth-promoting (PGP) microbes produce some defensive molecules, like melatonin, proline, and carotenoids which play a defensive role under abiotic stress conditions. The study conducted by Zahedi and Abbasi [27, 28] investigated the effect of inoculation of three beneficial bacterial strains *Azotobacter chroococcum*, *Azospirillum brasilense*, and *Rhizobium japonicum*, and their combination on polyamines and phytohormones of soybean growing under different irrigation systems. The study conducted by Martins *et al.* [28] used three bacterial strains, *Bacillus cereus*, *B. subtilis*, and *B. thuringiensis*, for mitigating the effect of drought stress in soybean. Previous studies conducted on *Micrococcus luteus*

were majorly focused on exploring its plant growth-promoting attributes [29 - 32], but plants under stress conditions are susceptible to different kinds of disease. In nature, plants are concurrently exposed to a combination of abiotic and biotic stresses that limits crop production [33]; however, the study related to PGPRs combating both abiotic and biotic stress is often limited. In the present study, we have isolated, identified, and screened a potential isolate *Micrococcus luteus*, from a disease-resistant variety of soybean (JS-20-34), with their effective plant growth-promoting, desiccation-tolerant, antifungal, and antagonistic activity against *Fusarium oxysporum*. The experimental work performed is shown in Fig. (1).

## 2. MATERIALS AND METHODS

### 2.1. Soil Sampling

Rhizosphere soil samples from a disease-resistant variety of soybean (JS-20-34) were collected by grubbing up soybean plants and the roots with attached soils from Jawaharlal Lal Nehru Krishi Anusandhan Kendra (JNKV) Sagar M.P. The location of the sampling site is at 23°49'44 N latitude and 78°42'46 E longitude. These rhizosphere soil samples were placed in sterile polythene bags, labelled, and stored at 4°C in a refrigerator until use.

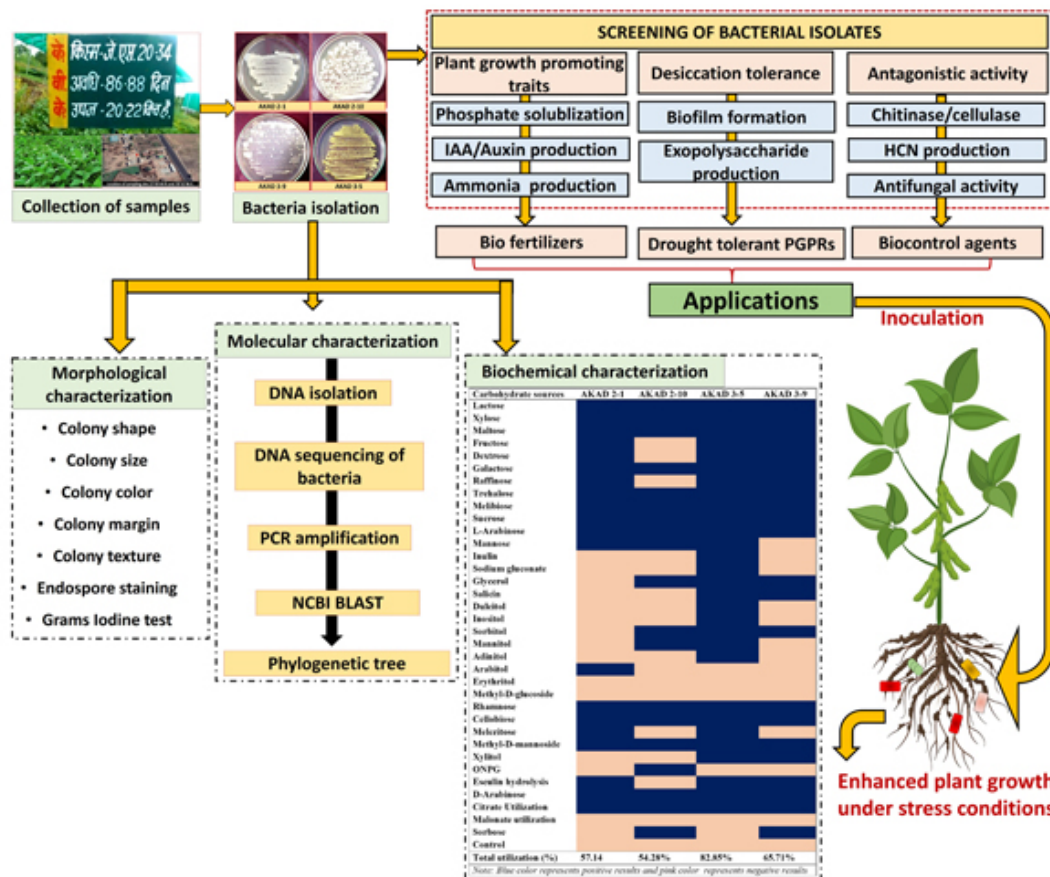


Fig. (1). Graphical representation of the work.

## 2.2. Isolation of Rhizobacteria:

Aliquots of 1 g rhizosphere soil were diluted with 9 mL of a sterile 0.9% NaCl solution. Serial dilutions were carried out and streaked onto Nutrient Agar (NA) plates (1 M NaOH, NaCl 5.0g/L, peptone 10g/L, 10ml/L, agar 15g/L, yeast extract 10g/L, and pH was maintained at 7) medium and the plates were incubated at 30 °C for 24 h and stored in glycerol at 4 °C. Rhizobacterial strains were cultured in the nutrient broth media and maintained in glycerol at -20°C.

## 2.3. Morphological and Biochemical Characterization

Bacterial isolates were morphologically identified based on the shape, size, color, and texture of colonies. The Grams staining was performed by following the protocol of Vincent and Humphrey [23]. Carbohydrate and the biochemical test were performed by using (KB009 HiCarbohydrate™ Test kit and KB002 HiAssorted™ Biochemical Test Kit). The kit was opened aseptically, and 50µl of pure bacterial cultures were inoculated to each well by using the surface inoculation method and incubated at 37°C for 1 day [11]. These biochemical tests are based on the principle of color change, change in pH, and substrate utilization by bacteria isolates.

## 2.4. Molecular Identification

Bacteria genomic DNA was extracted by using Bacterial g DNA Mini Kit according to the manufacturer's protocol. The quality and purity of isolated DNA were checked at 260/280 nm absorbance using a spectrophotometer. PCR amplification of the 16S rRNA gene was done by using universal primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGTACCTTGTTACGACTT) and amplified by PCR using an automated thermal cycler (Biorad, Hercules, USA) by the following steps: denaturation at 94°C for 5 minutes followed by 30 cycles of amplification for 30 seconds at 94°C, annealing for 60 seconds at 52°C, extension for 2 minutes at 72°C, and elongation for 10 minutes at 72°C. Amplified products were visualized using 1% agarose gel and were sent for sequencing (outsourced). Obtained 16S rRNA gene sequences were blast on the NCBI BLAST database (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) and were compared to other sequences already present in the Gene Bank. These bacterial sequences were deposited to NCBI GenBank for accession numbers. The phylogenetic analysis of sequences with the closely related sequence of NCBI blast results was performed by using the automated NCBI pipeline. A phylogenetic tree of AKAD 3-5 was constructed by using iTOL (<https://itol.embl.de/>) after establishing a relationship with already available bacterial sequences [34].

## 2.5. Screening for PGP Traits

### 2.5.1. Phosphate Solubilizing Activity

The qualitative phosphate solubilization potential of bacteria was estimated by observing the large clear/halo zones on Pikovskaya agar media [35]. Phosphate solubilization activity was performed by growing the bacterial culture on Pikovskaya's agar plate and then incubating the plate at 37°C for 3 days following protocols [36]. The development of the

clear halo zone around the bacterial colony specifies the positive result for phosphate solubilization. For quantitative estimation of phosphate solubilization, 1 ml of bacterial cell suspension ( $3 \times 10^7$  cells/ml) was inoculated in 50 ml of Pikovskaya's liquid medium (pH 7) in 150 ml Erlenmeyer flasks and incubated for a week in an orbital shaking incubator (Remi). It was followed by centrifugation of bacterial cell suspension for 10 min at 10,000 rpm. The phosphate content in the supernatant was estimated spectrophotometrically by the ascorbic acid method. The pH of the medium was recorded with a digital pH meter [37]. Soluble P ( $\mu\text{g/mL}$ ) was estimated by plotting standard curve of  $\text{KH}_2\text{PO}_4$ .

### 2.5.2. Ammonia Production

Bacteria were tested for the production of ammonia in the liquid media containing peptone water and NaCl. 200µl of fresh bacterial culture was inoculated in 20 ml peptone broth and incubated for about 2-3 days at 37°C. Change in color from yellow to orange after the addition of Nessler's reagent was considered as a positive test for the production of ammonia [38].

### 2.5.3. Production of Indole-3-Acetic Acid

Bacterial cultures (100 µl) were added to Dev tryptophan broth (HiMedia) and allowed to grow by incubating at 37°C for 5 days on a rotary shaker [39]. The bacterial culture was then centrifuged for 20 min at 1000 rpm. Then 2 ml of Salkowsky's reagent (containing 2 mL 0.5M  $\text{FeCl}_3$  and 48 mL 35%  $\text{HClO}_4$ ) was added to 1 ml of cell-free supernatant. Therefore, the development of the pink color was considered as a positive result for IAA/auxin production. IAA production was measured by recording the absorbance at 530 nm using a UV-Vis spectrophotometer. A standard curve of commercial Auxin/IAA was plotted for quantification of auxin in the solution, and an un-inoculated medium served as a control. The amount of IAA in the culture was expressed as  $\mu\text{g/ml}$ .

### 2.5.4. Biofilm Formation

Biofilm forming ability of bacterial strains was assayed using CV (crystal violet) by following the methods of Feoktistova *et al.* [33]. Bacterial strains were grown in nutrient broth for about 3-4 days and incubated at 37°C. After incubation, bacterial cell cultures were stained by using a crystal violet solution (1%) and extracted with ethanol. The biofilm formation by bacterial strains was quantified by recording the absorbance at 570 nm in a 96 well microliter plate reader.

### 2.5.5. Exopolysaccharide Production (EPS)

For exopolysaccharide production (EPS), *Micrococcus luteus* cells were grown in EPS media by Kimmel *et al.* [39]. Bacterial strain AKAD 3-5 was grown at 37 °C for 72 hrs with constant shaking at 150 rpm. Cell-free extracts were collected by centrifugation 25 °C at 16000 rpm for 15 min and filtered through a 0.2 µm filter. Exopolysaccharides were precipitated by using cold acetone and kept at -20°C for 2-3 hrs. The precipitate was separated by centrifugation at 16000 rpm for 15 min at 4°C and re-suspended in distilled water. This suspension

was lyophilized and stored at  $-20^{\circ}\text{C}$ . EPS concentration was determined by the phenol sulfuric acid method, described previously by Dubois *et al.* [40]. The concentration of EPS was determined with reference to a standard curve prepared with glucose.

### 2.5.6. Screening of Isolates for HCN

Hydrogen cyanide (HCN) production by bacterial strains was tested by using the method of Lorck [41]. Bacterial colonies were streaked on a nutrient agar plate supplemented with 4.4 g/l of glycine. Therefore, the production of cyanide was detected by placing Whatman filter paper No.1 saturated in a 0.5% picric acid solution. The change in yellow to red color of filter paper after 48 hrs of incubation specified positive results for HCN production.

### 2.5.7. Assessment of Bacterial Isolates for Enzymatic Activity

For catalase activity, 100 $\mu\text{l}$  of 3% hydrogen peroxide was added to 24 hrs old bacterial culture in Eppendorf tubes. The instant bubble formation is considered a positive test for catalase activity [42]. Cellulase production was determined by following the methods of Kasana *et al.* [43]. Bacterial strains were inoculated into the plates containing the media (CMC,  $\text{NaNO}_3$ , KCl,  $\text{K}_2\text{HPO}_4$ ,  $\text{MgSO}_4$ , peptone, yeast extract, and agar) and incubated for 3-5 days at  $35^{\circ}\text{C}$ . Then these CMC agar plates were flooded with grams iodine solution and allowed to stand for about 1 min at room temperature, and 1M NaCl solution was used for counterstaining. Clear halos zones were observed around bacterial colonies indicating hydrolysis of cellulose [43]. Chitinase activity was measured by following the protocol of Babashpour *et al.* [44]; therefore, the development of clear halos zones was observed around growing bacterial colonies, indicating hydrolysis of chitin (Table 1).

### 2.5.8. Bio-control Activity against *Fusarium oxysporum*

For testing the bio-control potential of different bacterial isolates against *F. oxysporum*, selected bacterial isolates were co-cultured by using a dual plate culture assay. A fungal disc (2

mm) was placed in the center, and bacterial isolates were inoculated at four opposing corners on the PDA plates. The cultures were incubated at  $28^{\circ}\text{C}$  for 4-5 days, after which the diameter of the fungal and bacterial colony was measured. Antifungal activity was expressed as a percentage (%) inhibition rate calculated by the formula  $\text{C-T}/\text{C}\times 100$ , where C and T are the fungal mycelial diameter on control and the test plate, respectively [45, 46].

## 3. RESULTS AND DISCUSSION

In the present study, four rhizobacterial isolates were successfully isolated from the rhizosphere of a disease-resistant variety of soybean (JS-20-34). These four bacterial isolates (AKAD 2-1, AKAD 2-10, AKAD 3-5, AKAD 3-9) were identified morphologically based on color, shape, size, margin, the texture of colonies, and Gram staining. Most of the bacterial strains were rod shape, Gram-negative, and forming a white color colony except AKAD 3-5 strain. Bacterial isolate AKAD 3-5 were found to be Gram-positive, non-motile, coccus shape, forming smooth yellow colonies (Table 2).

Bacterial isolates were tested for their carbohydrates utilization potential against 35 different carbohydrate sources. Bacterial isolate AKAD 3-5 had utilized maximum (82.85%) carbohydrate sources, followed by AKAD 3-9, which utilized 65.71% carbohydrate sources, as shown in Table 1. Bacterial strains AKAD 2-1 and AKAD 2-10 utilized only 57.14% and 54.28% of carbohydrate sources, respectively. AKAD 3-5 was further used for biochemical characterization, and it utilized almost all the (91.6%) biochemical sources like ornithine, urease, adonitol, phenylalanine deamination, lysine utilization, nitrate reductase, arabinose, glucose, lactose sorbitol citrate utilization, except  $\text{H}_2\text{S}$ . Strain AKAD 3-5 was identified as *Micrococcus luteus* (Gene bank Accession no MH304279), showing 98% similarity and 100% query cover with *Micrococcus luteus* strains. The phylogenetic tree was prepared by using the iTOL v3 (Interactive tree of life) (Fig. 2). This strain has the potential to promote plant growth, as shown by its various activities that can be used for promoting soybean and other plant growth under soil stress conditions.

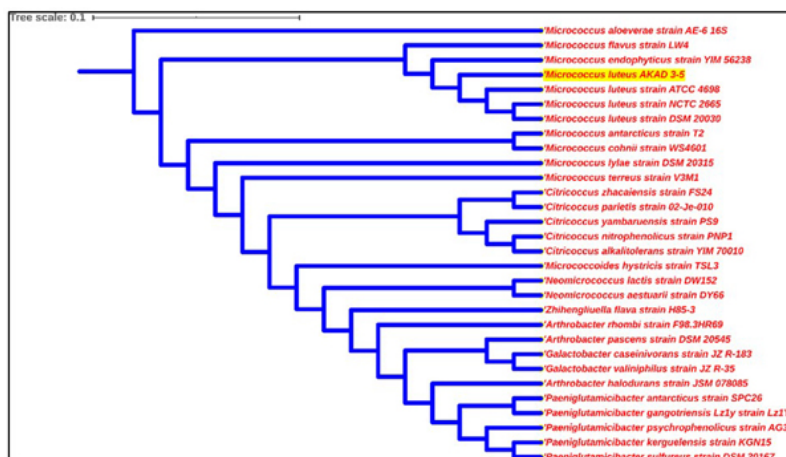


Fig. (2). Phylogenetic tree created by iTOL software to present the relationship between closely related species of *Micrococcus luteus* strain AKAD 3-5.

These rhizobacterial isolates were further screened for various plant growth-promoting traits (phosphate solubilization, indole acetic acid (IAA), ammonia production, biofilm production, HCN production, catalase, chitinase, and cellulase production). All the bacterial isolates were selected as phosphate solubilizers and produced a clear halo zone ranging from 10-13 mm. *Micrococcus luteus* AKAD 3-5 strain was found to be the best phosphate solubilizer by producing  $135 \mu\text{g mL}^{-1}$  of phosphate. Our study was supported by the study conducted by Raza and Faisal [32], who reported that phosphate solubilizing bacteria *Micrococcus luteus*-chp37 can tolerate desiccation and enhance plant growth in maize by producing plant growth-promoting compounds hydrogen cyanide (HCN) and cytokine (Fig. 2). Phosphate solubilizing bacteria (PSB) have been shown to solubilize and mineralize phosphorous from inorganic and organic sources of total soil P, thereby increasing the availability of the P to the plants, which may promote plant growth [47]. Three bacterial isolates (AKAD 2-1, AKAD 2-10, and AKAD 3-5) were forming biofilm, and bacterial strains AKAD 3-5 possesses a strong biofilm-forming capacity ( $2.05 \pm 0.1$ ). It was validated previously that biofilm-producing bacteria play an imperative role in improving soil fertility and bioremediation [48]. Our study reported that  $440 \mu\text{g mL}^{-1}$ . EPS production was recorded

in *Micrococcus luteus* when cells were grown in EPS medium (Table 3). Most of the functions attributed to EPSs are defensive. However, some studies describe some direct evidence illustrating the role of biofilm formation and exopolysaccharides production in protection against abiotic and biotic stresses, especially drought stress [47 - 49].

All the bacterial isolates showed a positive result for ammonia production (Fig. 3) in the range of  $2.45$ - $5.15 \mu\text{g mL}^{-1}$  and AKAD 3-5 produced  $34.4 \mu\text{g mL}^{-1}$  of IAA (Table 3). Therefore, it was well reported that IAA-producing microbes help in enhancing the surface area, root length, and also relax plant cell walls, which enables better nutrient uptake and creates strong plant-microbe communication by amassing more root exudates [50]. Production of HCN, hydrolytic, or cell wall degrading enzymes by bacterial isolate AKAD 3-5 enzymes may be attributed to being used as biocontrol agents against pathogen attack. The production of extracellular enzymes such as cellulase, chitinase, and protease can hydrolyze cell wall components consisting of proteins and chitin,  $\beta$ -1, 3-glucanase. They have been shown to mediate the bio-control of fungal pathogens [43]. Enzymes like cellulases produced by beneficial bacteria play a very important role in bacterial penetration inside the plant and help in plant colonization [51, 52] (Fig. 3).

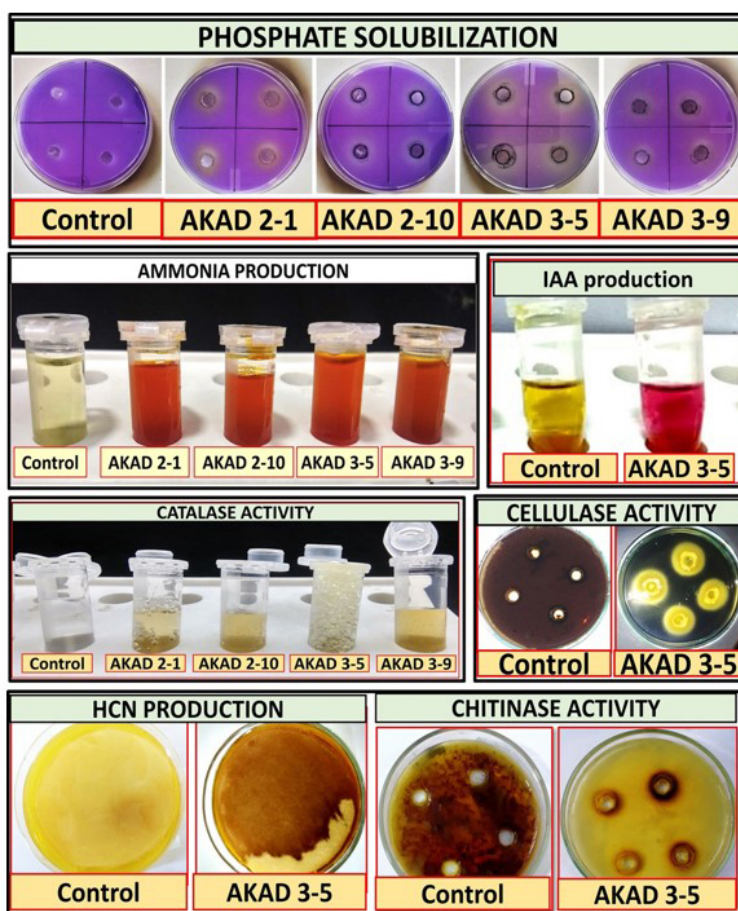


Fig. (3). Plant growth-promoting activities by bacterial strains isolated from the soybean.

Table 1. Heat map showing carbohydrates utilization of by bacterial isolates.

Carbohydrate sources	AKAD 2-1	AKAD 2-10	AKAD 3-5	AKAD 3-9
Lactose	Blue	Blue	Blue	Blue
Xylose	Blue	Blue	Blue	Blue
Maltose	Blue	Blue	Blue	Blue
Fructose	Blue	Pink	Blue	Blue
Dextrose	Blue	Pink	Blue	Blue
Galactose	Blue	Blue	Blue	Blue
Raffinose	Blue	Pink	Blue	Blue
Trehalose	Blue	Blue	Blue	Blue
Melibiose	Blue	Blue	Blue	Blue
Sucrose	Blue	Blue	Blue	Blue
L-Arabinose	Blue	Blue	Blue	Blue
Mannose	Blue	Blue	Blue	Pink
Inulin	Pink	Pink	Blue	Pink
Sodium gluconate	Pink	Blue	Blue	Pink
Glycerol	Pink	Blue	Blue	Blue
Salicin	Pink	Pink	Blue	Blue
Dulcitol	Pink	Blue	Blue	Pink
Inositol	Pink	Blue	Blue	Pink
Sorbitol	Pink	Blue	Blue	Blue
Mannitol	Pink	Blue	Blue	Pink
Adinitol	Pink	Pink	Blue	Blue
Arabitol	Blue	Pink	Pink	Pink
Erythritol	Pink	Pink	Pink	Pink
Methyl-D-glucoside	Pink	Pink	Pink	Pink
Rhamnase	Blue	Blue	Blue	Blue
Cellobiose	Blue	Blue	Blue	Blue
Melezitose	Blue	Pink	Blue	Pink
Methyl-D-mannoside	Blue	Blue	Blue	Blue
Xylitol	Pink	Pink	Blue	Blue
ONPG	Pink	Blue	Pink	Pink
Esculin hydrolysis	Blue	Pink	Blue	Blue
D-Arabinose	Blue	Blue	Blue	Blue
Citrate Utilization	Blue	Blue	Blue	Blue
Malonate utilization	Pink	Pink	Pink	Pink
Sorbose	Blue	Blue	Blue	Blue
Control	Pink	Pink	Pink	Pink
<b>Total utilization (%)</b>	<b>57.14</b>	<b>54.28%</b>	<b>82.85%</b>	<b>65.71%</b>
<i>Note: Blue color represents positive results and pink color represents negative results</i>				

After testing for various cell wall degrading enzymes (chitinase and cellulase) and HCN production, these bacterial strains were further tested for their inhibitory effect on *Fusarium oxysporum* (ITCC 2389). Only *Micrococcus luteus* strain AKAD 3-5 showed 98% inhibition against mycelia growth of *Fusarium oxysporum* (ITCC 2389) in dual plate culture assay (Fig. 4). Similar observations were made by Patel *et al.* [52], reported plant-growth-promoting and biocontrol activity of marine bacteria *M. luteus* and tested their effects on vegetative parameters. The results from this study suggested that *M. luteus* can protect chickpea from Fusarium wilt disease

as it displayed strong biocontrol ability against fungal pathogens and acted as a promising plant growth-promoting bacterial strain to enhance overall plant growth in chickpea [51]. Most of the studies conducted by different scientists have shown the positive plant-growth-promoting [30, 52 - 54], desiccation-tolerant [32], and biocontrol properties [53] of *M. luteus* individually, but the present study showed plant-growth-promoting together with abiotic and biotic stress tolerant potential of *M. luteus* AKAD 3-5 strain compared to other reported studies (Table 4).

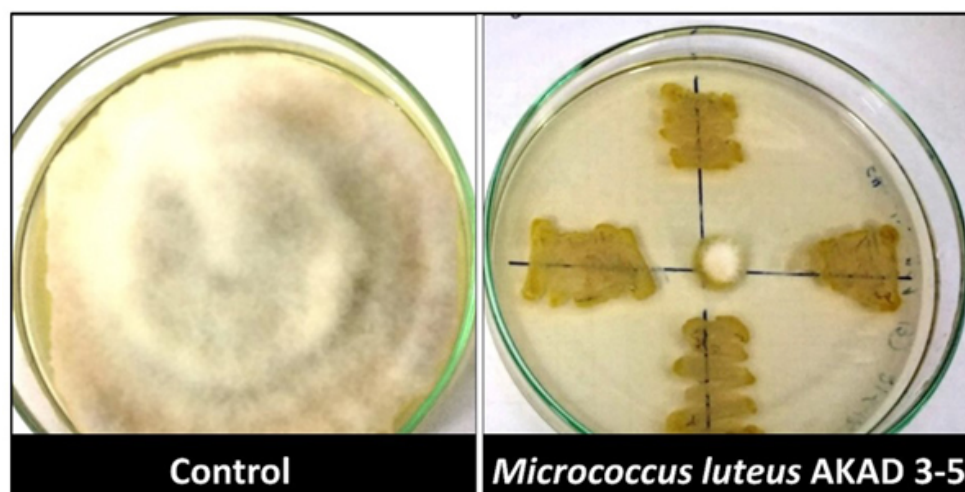


Fig. (4). Biocontrol activity of *Micrococcus luteus* AKAD 3-5 against *Fusarium oxysporum*.

Table 2. Morphological and biochemical characteristics of strain *Micrococcus luteus* AKAD 3-5 strain.

Morphological Characteristics	<i>Micrococcus luteus</i> AKAD 3-5 strain	Biochemical Utilization	<i>Micrococcus luteus</i> AKAD 3-5 Strain
Colony shape	Circular	Citrate Utilization	Negative
Colony color	Yellow	Lysine Utilization	Positive
Colony elevation/ margin	Convex /Entire	Ornithine Utilization	Positive
Cell shape	Cocci	Urease	Positive
Gram staining	Gram-positive	Phenylalanine Deamination	Positive
Capsules staining	Positive	Nitrate reduction	Positive
Spore staining	Positive	H <sub>2</sub> S Production	Negative
Motility	Non-motile	Glucose	Positive

Table 3. Quantitative screening activities by bacterial isolates.

Isolate	IAA ( $\mu\text{g mL}^{-1}$ )	Ammonia ( $\mu\text{g mL}^{-1}$ )	Phosphate Solubilization ( $\mu\text{g mL}^{-1}$ )	Biofilm (OD at 570 nm)	EPS Production ( $\mu\text{g mL}^{-1}$ )
AKAD 2-1	-	3.10±0.02	50.3 ± 1.34	-	-
AKAD 2-10	-	5.15±0.09	110.2 ± 31	-	-
AKAD 3-5	34.4 ± 0.23	4.6±0.03	135 ± 2.4	2.05 ± 0.1	440 ± 3.56
AKAD 3-9	-	2.45±0.021	90.45 ± 2.45	-	-

Note: Data presented is the mean of three replicate ± SD (n=3)

**Table 4. Comparison of plant-growth-promoting and biocontrol properties of *Micrococcus luteus* AKAD 3-5 strain with other *Micrococcus luteus* strains reported in different studies.**

Bacterial Strain	IAA	Ammonia	Phosphate Solubilization	Siderophore Production	Biofilm Activity	EPS Production	HCN Production	Chitinase	Cellulase	Antifungal Activity	References
<i>Micrococcus luteus</i> strain AKAD 3-5	Present	Present	Present	NT	Present	Present	Present	Present	Present	Present	Present study
Marine <i>Micrococcus luteus</i> strain	Present	Present	Present	Present	NT	NT	NT	Present	NT	Present	[53]
<i>Micrococcus luteus</i> -chp37	NT	NT	Absent	Absent	NT	Present	Present	NT	NT	NT	[32]
<i>Micrococcus sp</i> NII-0909	Present	NT	Present	Present	NT	NT	NT	NT	NT	NT	[54]
<i>Micrococcus luteus</i> RWL-3	Present	NT	NT	NT	NT	NT	NT	NT	NT	NT	[30]
<i>Micrococcus luteus</i> strain 4.43	Present	NT	NT	NT	NT	Present	NT	NT	NT	NT	[31]
NT: not tested											

## CONCLUSION

Different studies conducted on members belonging to *Micrococcus sp.* have proven their role as excellent biofertilizers due to their multifarious plant growth-promoting activity. However, the dual potential of these bacterial isolates is less explored. In the present study, we have isolated *Micrococcus luteus* from a disease-resistant variety of soybean (JS-20-34) with multifarious plant growth-promoting traits (Auxin/IAA, ammonia production, phosphate solubilization), desiccation-tolerant potential (biofilm and EPS production), colonization potential (cellulase activity), antifungal activity (chitinase, HCN production), and antagonistic activity against *Fusarium oxysporum*. Screening of these rhizobacteria from the rhizosphere of disease-resistant varieties of a plant may be a sustainable option to improve soil health and biomass production on marginal land. This study suggests that bacterial *Micrococcus luteus* strain AKAD 3-5 can be formulated into tailor-made bio-fertilizer and bio-control agent for promoting plant growth under abiotic and biotic stress conditions in soybean. The application of PGPR as an alternative to chemical fertilizers offers a sustainable, safe, and eco-friendly approach to maintain soil health and increase crop production.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data supporting findings of this study is present within the article.

## FUNDING

The authors are grateful to DST Inspire Ph.D. Fellowship (IF160797), DST Govt. New Delhi, India for funding this study.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

Anamika Dubey would like to acknowledge her thesis supervisor, Ashwani Kumar, for the supervision and successful conduction of the work, manuscript writing, editing, and critical comments.

## REFERENCES

- [1] Dubey A, Kumar A, Abd\_Allah EF, Hashem A, Khan ML. Growing more with less: breeding and developing drought resilient soybean to improve food security. *Ecol Indic* 2019; 105: 425-37. [http://dx.doi.org/10.1016/j.ecolind.2018.03.003]
- [2] Conley SP, Gaska JM, Pedersen P, Esker P. Soybean yield and *Heterodera glycines* response to rotation, tillage, and genetic resistance. *Agron J* 2011; 103(6): 1604-9. [http://dx.doi.org/10.2134/agronj2011.0043]
- [3] FAO. How to Feed the World in 2050. Insights from an Expert Meet FAO 2009.
- [4] Kumar A, Dames JF, Gupta A, Sharma S, Gilbert JA, Ahmad P. Current developments in arbuscular mycorrhizal fungi research and its role in salinity stress alleviation: a biotechnological perspective. *Crit Rev Biotechnol* 2015; 35(4): 461-74. [http://dx.doi.org/10.3109/07388551.2014.899964] [PMID: 24708070]
- [5] Kumar A, Sharma S, Mishra S. Evaluating effect of arbuscular mycorrhizal fungal consortia and *Azotobacter chroococcum* in improving biomass yield of *Jatropha curcas*. *Plant Biosystems- Intern J Deal Aspects Plant Bio* 2016; 150(5): 1056-64. [http://dx.doi.org/10.1080/11263504.2014.1001001]
- [6] Dubey A, Malla MA, Khan F, *et al.* Soil microbiome: A key player for conservation of soil health under changing climate. *Biodivers Conserv* 2019; 28(8): 2405-29. [http://dx.doi.org/10.1007/s10531-019-01760-5]
- [7] Kumar A, Dubey A. Rhizosphere microbiome: Engineering bacterial competitiveness for enhancing crop production. *J Adv Res* 2020; 24: 337-52.



- [8] Kumar A, Sharma S, Mishra S. Influence of arbuscular mycorrhizal (AM) fungi and salinity on seedling growth, solute accumulation, and mycorrhizal dependency of *Jatropha curcas* L. *J Plant Grow Regul* 2010; 29(3): 297-306.
- [9] Kumar A, Sharma S, Mishra S, Dames JF. Arbuscular mycorrhizal inoculation improves growth and antioxidative response of *Jatropha curcas* (L.) under Na<sub>2</sub>SO<sub>4</sub> salt stress. *Plant Biosystems- Intern J Deal Aspects of Plant Bio* 2015; 149(2): 260-9.
- [10] Dubey A, Kumar A, Khan ML. Role of biostimulants for enhancing abiotic stress tolerance in Fabaceae plants *Plant Family Fabaceae*. Singapore: Springer 2020; pp. 223-36.
- [11] Vyas P, Kumar D, Dubey A, Kumar A. Screening and characterization of *Achromobacter xylosoxidans* isolated from rhizosphere of *Jatropha curcas* L.(energy crop) for plant-growth-promoting traits. *J Adv Res Biotechnol* 2018; 3(1): 1-8. [http://dx.doi.org/10.15226/2475-4714/3/1/00134]
- [12] Bhattacharyya D, Duta S, Yu SM, Jeong SC, Lee YH. Taxonomic and functional changes of bacterial communities in the rhizosphere of Kimchi cabbage after seed bacterization with *Proteus vulgaris* JBL5202. *Plant Pathol J* 2018; 34(4): 286-96. [http://dx.doi.org/10.5423/PPJ.OA.03.2018.0047] [PMID: 30140182]
- [13] Mayak S, Tirosh T, Glick BR. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Sci* 2004; 166(2): 525-30. [http://dx.doi.org/10.1016/j.plantsci.2003.10.025]
- [14] Zahir ZA, Munir A, Asghar HN, Shaharoona B, Arshad M. Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. *J Microbiol Biotechnol* 2008; 18(5): 958-63. [PMID: 18633298]
- [15] Sandhya VS, Ali SZ, Grover M, Reddy G, Venkateswarlu B. Effect of plant growth promoting *Pseudomonas spp.* on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Regul* 2010; 62(1): 21-30. [http://dx.doi.org/10.1007/s10725-010-9479-4]
- [16] Kasim WA, Osman ME, Omar MN, Abd El-Daim IA, Bejai S, Meijer J. Control of drought stress in wheat using plant-growth-promoting bacteria. *J Plant Growth Regul* 2013; 32(1): 122-30. [http://dx.doi.org/10.1007/s00344-012-9283-7]
- [17] Kumari S, Vaishnav A, Jain S, Varma A, Choudhary DK. Induced drought tolerance through wild and mutant bacterial strain *Pseudomonas simiae* in mung bean (*Vigna radiata* L.). *World J Microbiol Biotechnol* 2016; 32(1): 4. [http://dx.doi.org/10.1007/s11274-015-1974-3] [PMID: 26712619]
- [18] Grover M, Nain L, Singh SB, Saxena AK. Molecular and biochemical approaches for characterization of antifungal trait of a potent biocontrol agent *Bacillus subtilis* RP24. *Curr Microbiol* 2010; 60(2): 99-106. [http://dx.doi.org/10.1007/s00284-009-9508-6] [PMID: 19777301]
- [19] Hashem A, Kumar A, Al-Dbass AM, et al. Arbuscular mycorrhizal fungi and biochar improves drought tolerance in chickpea. *Saudi J Biol Sci* 2019; 26(3): 614-24. [http://dx.doi.org/10.1016/j.sjbs.2018.11.005] [PMID: 30899180]
- [20] Kumar A, Dubey A, Malla MA, Dames J. Pyrosequencing and phenotypic microarray to decipher bacterial community variation in *Sorghum bicolor* (L.) Moench rhizosphere. *Curr Res Microb Scien* 2021; 2100025 [http://dx.doi.org/10.1016/j.crmicr.2021.100025]
- [21] Ahmad P, Alyemeni MN, Abass Ahanger M, et al. Upregulation of antioxidant and glyoxalase systems mitigates NaCl stress in *Brassica juncea* by supplementation of zinc and calcium. *J Plant Interact* 2018; 13(1): 151-62. [http://dx.doi.org/10.1080/17429145.2018.1441452]
- [22] Vyas P, Kumar D, Dubey A, Kumar A. Screening and characterization of *Achromobacter xylosoxidans* isolated from rhizosphere of *Jatropha curcas* L.(energy crop) for plant-growth-promoting traits. *J Adv Res Biotechnol* 2018; 3(1): 1-8. [http://dx.doi.org/10.15226/2475-4714/3/1/00134]
- [23] Dubey A, Malla MA, Kumar A, Dayanandan S, Khan ML. Plants endophytes: unveiling hidden agenda for bioprospecting toward sustainable agriculture. *Crit Rev Biotechnol* 2020; 40(8): 1210-31. [http://dx.doi.org/10.1080/07388551.2020.1808584] [PMID: 32862700]
- [24] Santoyo G, Moreno-Hagelsieb G, Orozco-Mosqueda MdelC, Glick BR. Plant growth-promoting bacterial endophytes. *Microbiol Res* 2016; 183: 92-9. [http://dx.doi.org/10.1016/j.micres.2015.11.008] [PMID: 26805622]
- [25] Wani SH, Kumar V, Shiriram V, Sah SK. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *Crop J* 2016; 4(3): 162-76. [http://dx.doi.org/10.1016/j.cj.2016.01.010]
- [26] Zahedi H, Abbasi S. Effect of plant growth promoting rhizobacteria (PGPR) and water stress on phytohormones and polyamines of soybean. *Int J Agric Res* 2015; 49(5): 427-31.
- [27] Hossain Z, Khatoon A, Komatsu S. Soybean proteomics for unraveling abiotic stress response mechanism. *J Proteome Res* 2013; 12(11): 4670-84. [http://dx.doi.org/10.1021/pr400604b] [PMID: 24016329]
- [28] Martins SJ, Rocha GA, de Melo HC, et al. Plant-associated bacteria mitigate drought stress in soybean. *Environ Sci Pollut Res Int* 2018; 25(14): 13676-86. [http://dx.doi.org/10.1007/s11356-018-1610-5] [PMID: 29502259]
- [29] Shahzad R, Waqas M, Khan AL, et al. Indoleacetic acid production and plant growth promoting potential of bacterial endophytes isolated from rice (*Oryza sativa* L.) seeds. *Acta Biol Hung* 2017; 68(2): 175-86. [http://dx.doi.org/10.1556/018.68.2017.2.5] [PMID: 28605980]
- [30] Namwongsa J, Jogloy S, Vorasoot N, Boonlue S, Riddech N, Mongkoltharuk W. Endophytic bacteria improve root traits, biomass and yield of *helianthus tuberosus* L. under Normal and Deficit Water Conditii [http://dx.doi.org/10.1016/j.jplph.2014.11.008] [PMID: 25546584]
- [31] Raza FA, Faisal M. Growth promotion of maize by desiccation tolerant ' *Micrococcus luteus* '-cp37-chp37 isolated from Cholistan desert, Pakistan. *Aust J Crop Sci* 2013; 7(11): 1693-8.
- [32] Ramegowda V, Senthil-Kumar M. The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. *J Plant Physiol* 2015; 176: 47-54.
- [33] Letunic I, Bork P. Interactive tree of life (iTOL) v3: An online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res* 2016; 44(W1)W242-5 [http://dx.doi.org/10.1093/nar/gkw290] [PMID: 27095192]
- [34] Pikovskaya RI. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya* 1948; 17: 362-70.
- [35] Mehta S, Nautiyal CS. An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Curr Microbiol* 2001; 43(1): 51-6. [http://dx.doi.org/10.1007/s002840010259] [PMID: 11375664]
- [36] Fogg DN, Wilkinson AN. The colorimetric determination of phosphorus. *Analyst (Lond)* 1958; 83(988): 406-14. [http://dx.doi.org/10.1039/an9588300406]
- [37] Cappuccino JC, Sherman N. *Microbiology: A laboratory manual*. 1992; 125-79.
- [38] Frankenberger WT Jr, Poth M. Biosynthesis of indole-3-acetic acid by the pine ectomycorrhizal fungus *Pisolithus tinctorius*. *Appl Environ Microbiol* 1987; 53(12): 2908-13. [http://dx.doi.org/10.1128/aem.53.12.2908-2913.1987] [PMID: 16347506]
- [39] Kimmel SA, Roberts RF, Ziegler GR. Optimization of exopolysaccharide production by *Lactobacillus delbrueckii* subsp. bulgaricus RR grown in a semidefined medium. *Appl Environ Microbiol* 1998; 64(2): 659-64. [http://dx.doi.org/10.1128/AEM.64.2.659-664.1998] [PMID: 9464404]
- [40] Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F. Colorimetric method for determination of sugars and related substances. *Anal Chem* 1956; 28(3): 350-6. [http://dx.doi.org/10.1021/ac60111a017]
- [41] Lorck H. Production of hydrocyanic acid by bacteria. *Physiol Plant* 1948; 1(2): 142-6. [http://dx.doi.org/10.1111/j.1399-3054.1948.tb07118.x]
- [42] Dacre JC, Sharpe ME. Catalase production by lactobacilli. *Nature* 1956; 178: 700.
- [43] Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A. A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine. *Curr Microbiol* 2008; 57(5): 503-7. [http://dx.doi.org/10.1007/s00284-008-9276-8] [PMID: 18810533]
- [44] Babashpour S, Aminzadeh S, Farrokhi N, Karkhane A, Haghbeen K. Characterization of a chitinase (Chit62) from *Serratia marcescens* B4A and its efficacy as a bioshield against plant fungal pathogens. *Biochem Genet* 2012; 50(9-10): 722-35. [http://dx.doi.org/10.1007/s10528-012-9515-3] [PMID: 22555558]
- [45] El-Sayed WS, Akhkha A, El-Naggar MY, Elbadry M. In vitro

- antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. *Front Microbiol* 2014; 5: 651. [http://dx.doi.org/10.3389/fmicb.2014.00651] [PMID: 25538687]
- [46] Kumar A, Kumar A, Devi S, Patil S, Payal C, Negi S. Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: An *in vitro* study. *Rec Res Sci Tech* 2012; 4(1): 1-5.
- [47] Auger S, Krin E, Aymerich S, Gohar M. Autoinducer 2 affects biofilm formation by *Bacillus cereus*. *Appl Environ Microbiol* 2006; 72(1): 937-41. [http://dx.doi.org/10.1128/AEM.72.1.937-941.2006] [PMID: 16391139]
- [48] Kasim WA, Gaafar RM, Abou-Ali RM, Omar MN, Hewait HM. Effect of biofilm forming plant growth promoting rhizobacteria on salinity tolerance in barley. *Ann Agric Sci* 2016; 61(2): 217-27. [http://dx.doi.org/10.1016/j.aogas.2016.07.003]
- [49] Chen Y, Yan F, Chai Y, *et al*. Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. *Environ Microbiol* 2013; 15(3): 848-64. [http://dx.doi.org/10.1111/j.1462-2920.2012.02860.x] [PMID: 22934631]
- [50] Wasai S, Minamisawa K. Plant-associated microbes: From rhizobia to plant microbiomes. *Microbes Environ* 2018; 33(1): 1-3. [http://dx.doi.org/10.1264/jsme2.ME3301rh] [PMID: 29593170]
- [51] Carro L, Menéndez E. Knock, knock-let the bacteria in: Enzymatic potential of plant associated bacteria *Mol Aspec Plant Bene Micro Agri*. Academic Press 2020; pp. 169-78.
- [52] Patel P, Patel K, Dhandhukia P, Thakker JN. Plant growth promoting traits of marine *Micrococcus sp.* with bio-control ability against *Fusarium* in chickpea plant. *Vegetos* 2021; 34(1): 94-101. [http://dx.doi.org/10.1007/s42535-021-00191-4]
- [53] Dastager SG, Deepa CK, Pandey A. Isolation and characterization of novel plant growth promoting *Micrococcus sp* NII-0909 and its interaction with cowpea. *Plant Physiol Biochem* 2010; 48(12): 987-92. [http://dx.doi.org/10.1016/j.plaphy.2010.09.006] [PMID: 20951599]
- [54] Matsuura K, Asano Y, Yamada A, Naruse K. Detection of *Micrococcus luteus* biofilm formation in microfluidic environments by pH measurement using an ion-sensitive field-effect transistor. *Sensors (Basel)* 2013; 13(2): 2484-93. [http://dx.doi.org/10.3390/s130202484] [PMID: 23429511]

© 2021 Dubey *et al.*

This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International Public License (CC-BY 4.0), a copy of which is available at: <https://creativecommons.org/licenses/by/4.0/legalcode>. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.