

Qualitative *in vitro* Evaluation of Plant Growth Promoting Activity of Electrogenic Bacteria from Biohydrogen Producing Microbial Electrolysis Cell towards Biofertilizer Application

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ABSTRACT

DBio-fertilizers confer a sustainable alternate to chemical fertilizers through their plant growth promoting activities and contribute significantly to enhance soil fertility and crop yields. The present study identifies five bacterial strains (K7, K8, K9, K11 and K18) isolated from a different microbiome (Microbial Electrolysis Cell; MEC) to have nitrogen fixing capability. Consequently, the isolates were identified by 16S ribosomal RNA gene sequencing analysis as *Bacillus safensis* strain K7, *B. nealsonii* strain K8, *B. nealsonii* strain K9, *Leifsonia shinshuensis* strain K11 and *Streptomyces griseoviridis* strain K18. Since the isolates exhibited nitrogen fixation property which is one of the important characteristics of plant growth promoting activity, these strains were further evaluated for other biofertilizer properties viz. phosphate solubilization, indole acetic acid (IAA) production, siderophores activity and anti-fungal activity. The results showed that siderophore activity was observed in both K7 and K18 strains while IAA production and antifungal activity was observed in K7 and K18 strains respectively. Phosphate solubilization was observed only in K9. The results illustrate that *Streptomyces griseoviridis* strain K18, *Bacillus safensis* strain K7 and *B. nealsonii* strain K9 hold promise to be used as a biofertilizer with potential plant growth promoting activity.

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1. Introduction

Chemical based fertilizers in agriculture have imposed a negative impact on the overall environment and soils. Thus, bio-fertilizer, which is ecofriendly and have no adverse effects on environment and living beings as well are in great demand (Khan et al., 2009). Biofertilizers accelerate certain microbial processes in the soil by increasing the extent of availability of nutrients in a form easily assimilated by plants and are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their by-products (Rajasulochana and Krishnamoorthy., 2014). They add nutrients to the soil through natural processes of atmospheric nitrogen fixation, phosphate solubilization and mobilization, translocate minor elements like zinc, and copper, secreting plant growth promoting substances like Indole acetic acid (IAA), siderophores, vitamins and amino acids etc., and also shun plant pathogenic fungi, thereby improving the soil health and crop production. Symbiotic integration of plant and microorganisms is synergistic in nature as plants supply root borne carbon compounds, that can be metabolized for bacterial growth and bacteria provide soluble nutrients in return (Perez et al., 2007). Use of biofertilizers is one of the important components of integrated nutrient management, as they provide eco-friendly organic agro-input and restore the soil's natural nutrient cycle and build soil organic matter for sustainable agriculture (Mohammadi and Sohrabi, 2012). Knowledge of using organic biofertilizers dates back to history with production and usage of compost. Bio-fertilizers do not contain any synthetic chemicals which are detrimental

to the living soil and does not add any pollution to nature like chemical fertilizers. Efficient strains of nitrogen fixing, phosphate solubilizing, siderophores activity, IAA productivity and antifungal activity in combination will provide good scope for production of biofertilizers having plant growth promoting properties (Shahab et al., 2009).

In the present study, an attempt was made to evaluate the biofertilization abilities of the isolates from Microbial Electrolysis Cell (MEC). Previous reports on microbial diversity in MEC have shown that the microbial populations are rich in electrogenic bacteria having biohydrogen production capacity (Venkata Mohan and Lenin Babu., 2011; Venkata Mohan et al., 2007; Kannaiah Goud et al., 2012). The microbial diversity studies have also demonstrated that the enriched electrogenic bacteria are mostly anaerobic or microaerophilic having the ability to form biofilm or colonize on biotic (plant roots) or abiotic (electrode) surfaces (Puenta et al., 2004). These electrogenic bacteria has exhibited the ability to colonize plant roots and utilize root exudates formed by plant rhizosphere as substrate for their metabolic activities and simultaneously release electrons, protons and microbial metabolites to the external environment enumerating the positive function of rhizosphere (Chiranjeevi et al., 2012). So, it was assumed that the isolated strains might also exhibit plant growth promoting activity. However, so far no reports are available on using electrogenic bacteria for evaluating plant growth promoting and biofertilizer activity. Thus in the present study, the bacterial isolates from MEC were initially screened for diazotrophy and later on for other biofertilizer capabilities.

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2. Experimental Methodology

2.1 Bioreactor

A single chambered MEC is used to produce Bio-H₂ by electro fermenting the volatile fatty acid (VFA) effluents (4000 mg/l and 8000 mg/l) generated from acidogenic fermentation with simultaneous waste treatment is used for isolation of potent organisms which can withstand the potential of 0.6 V. Maximum cumulative H₂ production (CHP) and hydrogen production rate (HPR) of 39.35 ml and 0.057 mmol/h was observed at 0.6 V respectively and maximum amount of VFA utilization as substrate is observed to be 68% at 0.6 V (Annie Modestra et al., 2015).

2.2 Bacterial strains Cultivation

Five bacterial strains viz., K7, K8, K9, K11 and K18 screened from MEC system were used in the study to evaluate the biofertilizer activity. Cultures were grown on nutrient agar plates by streak plate method for 48 hours at 37°C and the representative colonies, according to their morphologies and nitrogen fixing ability, were selected for further characterization. Colonies were re-streaked on nutrient agar plates until a pure culture was obtained. A single colony was picked from these plates and transferred to 50 ml of nutrient broth and incubated for growth at 37°C (150 rpm). Overnight grown cultures were used for various biofertilization assay procedures unless until mentioned.

2.3 Characterization and Identification of isolates

Following the selection of pure isolates, 5 isolates were identified based on grams staining and microscopy. Colony morphology of all the isolates grown on nutrient agar plates was also studied. Sequencing of 16S rRNA gene was done to identify the bacteria. Colony PCR was used to amplify the 16S rRNA gene using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGTCCARCC-3'). Small amount of pure isolated colony obtained after streaking was picked and subjected to lysis by heating at 95°C for 10 min. The cell lysate obtained cells was used as DNA template for amplification of 16S rRNA gene. Polymerase chain reaction was performed in a 50 µl reaction volume using the primers described above. PCR was performed using the thermal cycler (Eppendorf, Germany) and according to the protocol described previously (Kannaiah Goud and Venkata Mohan., 2013). PCR product was separated by electrophoresis on 1% agarose gel stained with ethidium bromide and photographed under UV illumination using Gel Doc (Molecular Imager G: BOX EF System; Syngene). The 1 kb ladder was used to check the amplification of the product obtained. PCR product was cleaned using QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions and purified PCR product was used for sequencing. 16S rRNA gene sequencing was done as described previously (Lane, 1991) using above described primers. For the resultant sequences obtained by sequencing, a BLAST algorithm search was performed to identify the nearest taxa. The 16S rRNA sequences of the related members were downloaded from the database (<http://www.ncbi.nlm.nih.gov>) and aligned using MEGA 6 (Tamura et al., 2007). Phylogenetic trees were constructed based on neighbour joining method using MEGA 6.

2.4 Screening for Nitrogen Fixation Activity

Nitrogen free media (1000 ml; Sucrose- 20 g, Dipotassium phosphate- 1 g, Magnesium sulphate- 0.5 g, Sodium chloride- 0.5 g, Ferrous sulphate- 0.1 g, Sodium molybdate- 0.005 g, Calcium carbonate- 2 g, Agar- 15 g) was dissolved in distilled water and transferred to test tubes for autoclaving (Jensen., 1942). After autoclaving, the cooled media was inoculated with the specific strains, incubated for 2 days and checked for growth by measuring the optical density (OD) at 600nm using UV-Visible Spectrophotometer

2.5 Screening for IAA production

Bacterial isolates were screened for their ability to produce IAA in tryptophan media (5 g of casein peptone, 0.5 g of D-tryptophan, 2.5 g of sodium chloride dissolved in 500 ml of distilled water and adjusted to pH 7.5). The media was transferred into test tubes and autoclaved for 15 min at 121°C. The autoclaved media was inoculated with specific strains in duplicate with standard inoculum 350 x10⁵ CFU/ml and incubated for 48 h (37°C). 1 ml of chloroform was added to the test tube followed by 2.1 ml of Kovac's reagent (25 ml of Conc. HCl mixed with 75 ml of amyl alcohol and 5 g of para dimethyl amino benzaldehyde; stored in closed vials at 4°C). The appearance of pink color in the test tube indicates the capability of that particular strain for producing IAA (Mohite., 2013).

2.6 Phosphate solubilization

The phosphate solubilisation activity of the isolated strains was evaluated using PVK medium (1000 ml; Glucose-10 g, tri-calcium phosphate-5 g, ammonium sulphate-0.5 g, sodium chloride-0.2 g, magnesium sulphate-0.1 g, potassium chloride-0.2 g, yeast extract-0.5 g, manganese sulphate-0.002 g, ferrous sulphate-0.002 g and agar powder-15 g) (Pikovskaya., 1948). The dissolved media was subjected to autoclaving for 15 min prior to preparation of petri plates. The petriplates filled with PVK medium was spread with serially diluted samples and incubated for 48-72 h. The halo and colony diameters were measured after 5 days of the incubation where halo size was calculated by deducting colony diameter from total halozone diameter (Nguyen et al., 1992).

2.7 Screening of bacterial isolates for Siderophore Activity

Simple succinate media (SSM; 1000 ml, ammonium sulphate- 1 g, dipotassium phosphate-6 g, magnesium sulphate-0.2 g, potassium dihydrogen phosphate-3 g, succinic acid- 4 g; pH 7) was used to detect siderophore activity colorimetrically. Autoclaved media after inoculation with test strains were subjected to incubation at room temperature for 48 h. Fully grown culture was centrifuged (9000 rpm; 12 min) and absorbance was recorded at wavelengths between 350 and 450 nm. The presence of peaks at these particular wavelengths signifies the presence of siderophores (Ines et al., 2012).

2.8 Antifungal Activity

The antifungal activity of the strains was assessed by performing germination test (Hernández-Albíter et al., 2007). Fine ground nut (*Arachis hypogaea*) seeds were used to determine the effectiveness of the isolated strains. Plastic trays were spread with wet sterilized cotton to provide moisture content for the seeds to germinate and the whole setup was autoclaved prior to experiment. In control, the seeds were treated with sodium hypochlorite (5% NaOCl) for 5 min to disinfect them from fungi present on the seed coat and then washed with distilled water to remove traces of sodium hypochlorite. Two day old culture is centrifuged (8000 rpm, 10 min) and the pellet obtained was washed twice in saline and coated on the seeds. The trays were incubated for 4 days and checked for seeds sprouting activity

3. Results and Discussion

3.1 Bacterial strains isolation and characteristics

In the present study, isolates from MEC reactor were used for screening the nitrogen fixing capabilities. Out of eleven, five bacterial isolates viz., K7, K8, K9, K11 and K18 were selected for further characterization according to their morphologies and nitrogen fixation ability (Table 1). Selected strains showed various colony morphologies when grown on nutrient agar plates aerobically at 37°C and were also observed to be positive for gram staining. They also showed rod shape morphology and were motile in nature. The gene sequences obtained were used as BLAST queries against the NCBI database with the results indicating that the bacteria isolates were *Bacillus safensis* NBRC 100820 (NR_113945), *Bacillus nealsonii* DSM 15077 (NR_044546), *Bacillus nealsonii* DSM 15077 (NR_044546), *Leifsonia shinsuensis* DB 102 (NR_043663) and *Streptomyces griseoviridis* NBRC 12874 (NR_112313). All the sequences showed 99% similarity in 16S rRNA coding gene sequence with their nearest neighbours (Fig. 1). However, the phylogenetic analysis revealed that the sequences were distantly clustered with their nearest neighbouring species. Further, it was observed that the strains K7, K8 and K9 belonged to phylum *Firmicutes* and strains K11 and K18 belonged to phylum *Actinobacteria*. Apart from this, pair wise comparison of 16S rRNA gene sequences of *Bacillus nealsonii* strains K8 and K9 was done to identify the similarity between them and results showed that only 96% similarity between each other. Difference in 16S rRNA gene sequence is also evidenced in their phenotypic characteristics such as colony morphology and in phosphate solubilization properties. The strains characterized from MEC may be regarded as a safe industrial microorganism as there was no study till date reporting their specific pathogenicity (Lateef et al., 2015; Chauhan et al., 2014; Monteiro-Vitorello et al., 2004; Sharma., 2014)

3.2 Nitrogen Fixation Activity

Nitrogen fixation is the second most important process after photosynthesis which has significant function in crop production. Nitrogen is an essential component of soil and plays an important role in plant growth by forming ammonia or nitrates from atmospheric nitrogen fixation (Kanimozhi and Panneerselvam., 2010). The strains isolated were checked for nitrogen fixing activity, as elemental nitrogen is one of the key element

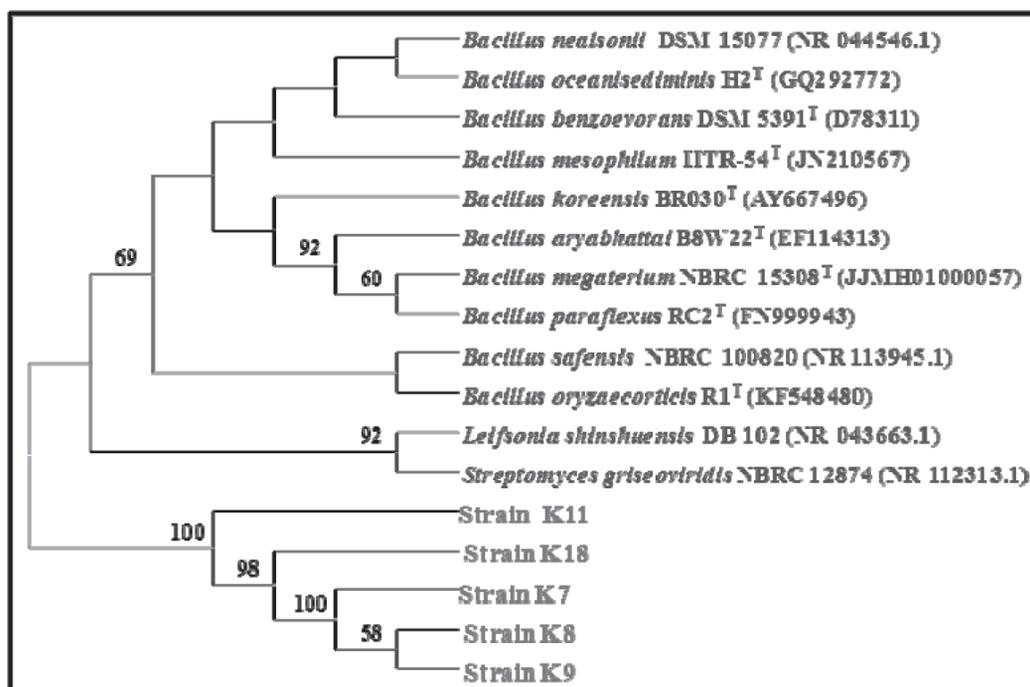


Figure 1: Dendrogram of isolates depicting nearest neighbouring species. strains K7, K8 and K9 belonged to phylum *Firmicutes* and strains K11 and K18 belonged to phylum *Actinobacteria*.

Table 1: Characteristics of the strains isolated from MEC

Isolate	Colony morphology	Gram's stain and Motility	16S rRNA sequence similarity (%)	Nearest neighbour	Taxonomy
K7	Flat irregular margin, opaque beige colored colony	+	99	<i>Bacillus safensis</i> NBRC 100820 (NR_113945)	<i>Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus</i>
K8	Big shiny, yellow colored slimy colony	+	99	<i>Bacillus nealsonii</i> strain DSM 15077(NR_044546)	<i>Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus</i>
K9	Small pale orange slimy circular colony	+	99	<i>Bacillus nealsonii</i> strain DSM 15077 (NR_044546)	<i>Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus</i>
K11	Small slimy, transparent, white colored colony	+	99	<i>Leifsonia shinshuensis</i> DB 102(NR_043663)	<i>Actinobacteria; Actinobacteridae; Actinomycetales; Micrococcineae; Microbacteriaceae; Leifsonia.</i>
K18	Small circular, slimy, orange colored colonies	+	97	<i>Streptomyces griseoviridis</i> NBRC 12874(NR_112313)	<i>Actinobacteria; Actinobacteridae; Actinomycetales; Streptomycineae; Streptomycetaceae; Streptomyces.</i>

of proteins, nucleic acids and other cellular constituents and is the important parameters for the isolate to act as biofertilizer. Despite its abundance in atmosphere, it cannot be directly used by plants because of the high bond energy of nitrogen molecule. In order for nitrogen to be used as nitrogen source, it must be fixed (combined) in the form of ammonium (NH_4) or nitrate (NO_3) ions. Many bacteria and fungi can degrade organic matter or symbiotically fix nitrogen releasing useable nitrogen for reuse by other organisms (Gopalakrishnan et al., 2012; Nagendranatha Reddy et al., 2014).

Defined medium lacking nitrogen source is inoculated with respective cultures and incubated for 48 hrs. Any growth observed is because of the utilization of atmospheric nitrogen as sole nitrogen source. Out of eleven isolates screened for diazotrophy only five isolates depicted growth in the presence of atmospheric nitrogen and showed turbidity in the test tubes which is comparable to the growth in control having NH_4Cl as nitrogen source. Activity and growth of isolates varied with time to other isolates depicting the capabilities of individual strain towards nitrogen fixation. The isolates K7, K18, K8, K11 and K9 followed the order of highest absorbance recorded and all the strains showed gradual increment in the absorbance measure till 48 h depicting the growth of microorganisms by utilizing N_2 from atmosphere. K8, K9 and K11 isolates showed nominal

variation in the absorbance recorded. K18 showed little increment in the OD from the start of operation but K7 showed highest by 48h. Till 24 h, the K18 isolate depicted maximum growth. Ding et al., (2005) stated that almost all the *Bacillus* spp. are able to fix nitrogen from atmosphere.

3.3 IAA Production

IAA, produced by plant for its internal regulation of growth, is one of the most important physiologically active and abundant auxins in plant (Davies, 1995; Shahab et al., 2009). Specific microbes have the capability of producing IAA as secondary metabolite due to rich supply of substrates and it is the common product of L-tryptophan metabolism followed by several microorganisms. All the electrogenic bacterial isolates were screened for their ability to produce plant growth regulator, IAA. Out of the five bacterial strains assayed for IAA production *B. safensis* strain K7 showed red color reaction with Kovac's reagent indicating their ability to produce IAA. It is also demonstrated previously that *B. safensis* has the inherent character of producing IAA which is the indication of beneficial effect by the bacterial isolate on the plant growth stimulation (Kothari et al., 2013) and therefore, termed as plant growth promoting bacteria. About 80% of the soil isolates possess the activity of IAA and vary greatly in their inherent ability to produce IAA. Initially, the tryptophan

is first converted to indole 3-acetamide (IAM) by the enzyme tryptophan-2-monooxygenase followed by conversion of IAM to IAA by an IAM hydrolase. The genes that encode the pathway are *iaaM* and *iaaH* genes respectively. Interactions between IAA-producing bacteria and plants lead to diverse outcomes on the plant side, varying from phytostimulation to productivity (Spaepen et al., 2007).

3.4 Phosphate Solubilisation Activity

One of the essential mineral macronutrients, which are required for maximum yield of agriculturally important crops, is phosphorus. Due to its non-availability in useable form, phosphorus deficiency is a major constraint to crop production. The Phosphate solubilizing bacteria (PSB) have the ability to convert insoluble phosphates into available forms for plant via the process of acidification, chelation, exchange reactions, and production of gluconic acid. PSBs render more phosphates into the soluble form than required for their growth and metabolism by secreting organic acids and/or enzymes, the surplus get the plants (Vessey, 2003). It is possible that bacteria capable of phosphate solubilization may improve plant productivity both by hormonal stimulation and by supplying phosphate.

Qualitative estimation of phosphate solubilization was carried out using different bacterial isolates grown on PVK agar media for determining phosphate solubilizers. All the 5 isolates found to grow on PVK media but could not form halo zone around them except in K9 isolate. The criterion for phosphate solubilizers isolation is based on the formation of a visible halo zone on agar plates. According to deFreitas et al. (1997), good phosphate-solubilizers have the capability of forming halo zones with diameters higher than 15 mm. *B. nealsonii* strain K9 exhibited a halo zone of 6 mm around the colony depicting the frail phosphate solubilisation property. Reports also state that the strain *B. nealsonii* has the capability to solubilize insoluble phosphate in the liquid inoculants (Velineni and Brahmaprakash., 2011).

3.5 Siderophores Activity

Apart from phosphate in available form, iron is also essential for plant growth and for almost all life processes such as respiration and DNA synthesis. Despite being one of the most abundant elements in the Earth's crust, the bioavailability of iron in many environments such as the soil or sea is limited by the very low solubility of the Fe^{3+} ion (Kraemer., 2005). The predominant state of iron in aqueous, non-acidic, oxygenated environments cannot be readily utilized by organisms. Microorganisms utilize various iron uptake systems to secure sufficient supplies from their surroundings. One such kind is siderophores, strongest soluble Fe^{3+} binding agents, which are small and high-affinity iron chelating compounds secreted by microorganisms. Microbes release siderophores to scavenge iron from these mineral phases by formation of soluble Fe^{3+} complexes that can be taken up by transport mechanisms. Production of IAA, siderophores, etc., by microbial isolates varies greatly among different species and strains and depends on the availability of substrate.

Spectrophotometric analysis of the five isolates of the present study showed an absorption area between 350 and 450 nm with a sharp peak at about 400 nm, indicating the production of PVD type siderophores (Fig. 2). The maximum absorbance obtained for the isolate K18 was at 413 nm. The other strain, K7, showed the maximum absorbance between

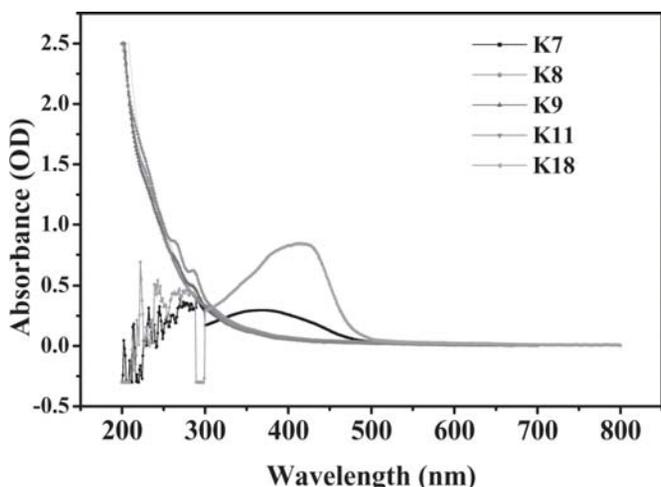


Figure 2: Siderophore activity depicted by K18 and K7 strains with the peaks observed at absorbance 350-450 nm

350 and 450 nm. Early reports supports the siderophore activity of *B. safensis* (Lateef et al., 2015; Kothari et al., 2013) and *S. griseoviridis* (Alam et al., 2012). Under iron deficiency, bacteria synthesize siderophore, which when excreted into extracellular environment, solubilizes and sequester the iron by binding and transport the iron in to the cell via., cell specific receptors recognition present on the outer membrane of the cell (Kraemer., 2005).

3.6 Antifungal Activity

Plants, apart from internal regulation of plant growth promoting substances, also face infections from Fungi thus reducing the yield of the plant and have adverse effects on the plant health. Evidence exist supporting the role of antibiotic activity of many bacterial strains prompting to screen these bacteria for antifungal property. To reduce the fungal infection to seed or to plant, strains that have inherent anti fungal activity have to be screened and applied as spray or as biofertilizer (Kaewchai et al., 2009). It has been reported that enhancement of plant growth characters, resistance to fungal diseases and yield components may be due to ability of soil microorganisms to provide plant by nutritional requirements, plant growth regulates.

Antifungal activity of bacterial isolates involves the usage of their genes, and/or products, such as metabolites, that reduce the negative effects of plant pathogens and promote positive responses by the plant (Couillerot et al., 2013). It has been evaluated by Germination test performed on ground nut seeds. The activity of all the bacterial isolates was evaluated in comparison to control (NaOCl treated). Fine groundnut seeds were soaked and coated with individual bacterial culture pellets and incubated (aseptic conditions) for 4 days. After 2 days of incubation, all the seeds except control were observed to have fungal contamination. But the seeds treated with K18 isolate showed comparatively lower (only one seed) fungal contamination as the strain inherently have antifungal properties. K18 isolate obtained from MEC bioreactor showed antifungal property whereas other bacterial isolates could not show any antifungal property. *Streptomyces griseoviridis* strain K18 exhibited broad-spectrum antifungal activity in vitro. *Streptomyces griseoviridis* is a naturally occurring soil bacteria which secretes various enzymes and metabolites that inhibit pathogen growth. It has been shown to promote the growth and yield of plants even in healthy crops. It is used for the control of damping off, root and stem rot, and wilt caused by *Fusarium* in greenhouse ornamentals and vegetables such as cucumbers, tomatoes and peppers (Jog et al., 2014). Seeds were also evaluated to check the impact of these bacterial isolates on the sprouting activity of seeds. Seeds coated with K18 strain sprouted immediately after 1 day whereas other strains depicted late sprouting activity. The seeds treated with K7, K8, K9 and K11 showed sprouting after 2 days. The antifungal activity of K18 strain inducing the sprouting activity indicates that it might be an ideal plant growth promoting as well as biocontrol agent, for its integrated use in disease and nutrient management strategies.

Overall *in vitro* plant growth promoting activities of all the electrogenically active bacterial isolates from MEC is depicted in Table 2. The data shows that isolates K18 (*S. griseoviridis*), K7 (*B. safensis*) and K9 (*B. nealsonii*) have multifaceted beneficial characteristics. K7 isolate isolated from MEC bioreactor has all the properties that biofertilizer has except the antifungal and phosphate solubilization activity. But, whereas K18 and K9 strains have antifungal and phosphate solubilization activity respectively. With the result obtained, three isolates namely K7, K9 and K18 isolates can be combined and evaluated for better and efficient biofertilizer activity. This gives the scope of developing biofertilizers with mixture of efficient bacteria for all the plant growth promoting towards production of biofertilizer. Due to its ability to protect plants, these isolates should be widely studied and commercially marketed as biopesticides, biofertilizers and soil amendments.

4. Conclusion

Biofertilizer production and application is a sustainable technology which helps to maintain natural soil resources, reduce negative impact on the environment and enhances crop productivity. Present study demonstrates the bio-prospecting of electrogenic bacteria for their ability to be used as biofertilizer and improve soil productivity. Besides nitrogen fixation, isolates exhibited different abilities of plant growth promotion. Hence, based on the demand for required biofertilizer activity, an integrated approach of co-culturing 3 different strains of the present study viz., *B. safensis* strain K7, *B. nealsonii* strain K9 and *S. griseoviridis* strain K18 may be employed to enhance crop yield to significant levels. This concept of co-culturing helps in providing the plant with all the essential growth promoting factors which are difficult to find in a single organism.

Table 2: Overall plant growth promoting activity of Bacterial isolates

Isolate	Phosphate Solubilization	IAA Production	Siderophores	Nitrogen Fixation	Antifungal
K7	-	+	+	+	-
K8	-	-	-	+	-
K9	+	-	-	+	-
K11	-	-	-	+	-
K18	-	-	+	+	+

From the result, it can be also be suggested that different microbiomes can also serve as a potential source for isolating bacteria with plant growth promoting activity apart from soil borne bacteria. This approach of biofertilizer development may reduce the dependence on chemical fertilizers and provides a step forward towards sustainable agriculture.

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