ISOLATION AND IDENTIFICATION OF PLANT GROWTH PROMOTING BACTERIA TO ALLEVIATE SALT STRESS TOLERANCE IN CROPS

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Isolation and Identification of Plant Growth-Promoting Bacteria to Alleviate Salt Stress Tolerance in Crops

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CONTENTS

CHAPTER 1

INTRODUCTION

Microorganisms associated with plants are broadly classified into beneficial, deleterious and neutral that does not have any effect on plant growth promotion (Beneduzi *et al*., 2012; Ngumbi and Kloepper, 2016; Illangumaran and Smith, 2017). Among them, the bacteria which showed a positive impact on the promotion of plant growth are called plant growth promoting bacteria (PGP) bacteria. They can colonize the rhizoplane (soil adhered within roots), rhizosphere (around rhizoplane) and within the root tissues (endophytes). The PGP bacteria affect plant growth through either a direct or indirect mechanism (Castro *et al*., 2009). The direct growth promotion includes the production of several secondary metabolites which directly affects the plant growth for example phytohormones or making the availability of certain nutrients to the plant from its environment like phosphorous and iron (Dinesh *et al*., 2015; Sharma *et al*., 2016). Whereas the indirect mechanism is mainly involved in the protection by the bacteria from phytopathogens by the production of antagonistic compounds or by inducing plant defense mechanisms against plant pathogens (Bhattacharyya and Jha, 2012). Some examples of PGPR strain showing plant growth promoting ability include *Pseudomonas*, *Flavobacterium*, *Burkholderia*, *Enterobacter*, *Bacillus*, *Rhizobium*, *Azotobacter*, *Mesorhizobium* (Singh *et al*. 2015; Wang *et al*., 2018).

Unavailability of phosphate was considered as the primary reason for limited plant growth being macronutrient that plays a vital role in the growth and development of the plants (Feng *et al*., 2004; Esitken *et al.,* 2010). Therefore, solubilizing inorganic phosphate by phosphate solubilizing bacteria was considered as one of the significant PGP traits to enhance plant growth

(Jeffries *et al*., 2003; Kaur *et al*., 2016). For example, *Pseudomonas fluorescens* can solubilize phosphate and improve plant growth (Ahmad *et al*., 2011). PGP bacteria also help in plant growth by triggering the plants to release various plant growth regulators like phytohormones such as auxin, cytokinin and gibberellin and other volatile organic compounds (Numan *et al.,* 2018). According to Pandey *et al*., 2005, *Burkholderia* sp. can produce IAA (Indole Acetic Acid) that regulates plant growth. Another important PGP trait is the production of siderophore to supply iron which is one the important micronutrient for plant growth (Radzki *et al*., 2013; Vejan *et al*., 2016). Other important beneficial effects of PGP bacteria which affect plant growth include the nutrient uptake and nitrogen fixation (Mirza *et al*., 2001; Souza *et al*., 2015; Goswami *et al*., 2016). Bacterial endophytes that live on the plants also can produce the enzyme known as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Shah *et al*., 1998) which helps the plant to tolerate certain stresses by decreasing the ethylene level. The function of this enzyme to the bacteria is unknown but it cleaves ACC which contributes to the plant growth promotion by acting as a precursor of ethylene in plants which in turn modulates the ethylene levels (Glick *et al*., 1998, Mayak *et al*., 2004a).

On the contrary, agricultural land worldwide is facing a serious threat due to several abiotic stresses such as salinity in the soil, contamination of soil by heavy metals, drought, extreme temperatures like heat and cold, and oxidative stress. This kind of stress leads to environment destruction (Annunziata *et al*., 2017). The average product of important crops is reduced by more than 50% due to salinity in the soil (Panta *et al*., 2014) and contributes to the major reason for crop destruction worldwide. Abiotic stresses change the physiological, morphological, biochemical and even molecular level which affect the plant growth and productivity. Therefore some strategies should be obtained by crop plants to cope with adverse external destruction

brought up by abiotic stresses (Meena *et al*., 2017). Among the abiotic stresses present in the soil, salinity is considered as one of the most serious problems that interrupt plant growth and development (Hu and Schmidhalter, 2005) and resulted in the major reductions in crop productivity as well as cultivated land area and quality (Shahbaz and Ashraf, 2013).

By definition, salinization is an increase of water-soluble salts present in the soil that may include the ions of carbonate (CO_3^{2-}), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), sulfate (SO_4^{2-}), chloride (Cl^-), bicarbonate (HCO_3^-) and sodium (Na^+). The water-soluble composition differs according to the salts that are dissolved depending on the soil. The solution extracted from the soil with electrical conductivity (EC), more than 20 mM (2 dS m^{-1}) can be considered as saline soil (Silva and Fay, 2012). Since salinization reduces the agricultural land which leads to low production of the crops and ultimately results in the destructive ecological and socio-economic outcomes. For instance, 70% of the agricultural land was destroyed in areas affected by the tsunami of Maldives due to the accumulation of salt. It damaged approximately 3, 70, 000 fruit bearing trees and around 15,000 farmers were affected (FAO. 2005). Salinity affects up to 20% of total cultivated land and 50% of the irrigated land in the world (Cheng *et al.,* 2012). Other than reducing the total cultivated land salinity also produces reactive oxygen species (ROS), like H_2O_2, O^{-2}, and OH^- that cause major damage to DNA, RNA, and proteins of the plant (Jaleel *et al.,* 2009; Mittler, 2002). These ROS compounds lead to chlorophyll destruction and damage the root meristem activity (Foreman *et al.,* 2003). To overcome all these problems caused by salinity stress chemical fertilizers and pesticides are used which in turn are not eco-friendly and cause serious problems to the environment. Therefore as mentioned before, an eco-friendly alternative for chemical fertilizers and pesticides are much needed. Different techniques to overcome salinity had been developed like the incorporation of conventional plant breeding

and molecular techniques are widely used to increase abiotic tolerance in crops (Ishitani *et al*., 2004; Breseghello and Coelho, 2013). Transgenic plants that adapt well to a high salt environment have been developed by overexpressing a varied range of genes. But the disadvantages of the transgenic plant are that it only grows well in laboratory and greenhouse conditions and cannot be adopted in agricultural fields (Roy *et al*., 2014).

Usually, these methods are not effective at high concentration of salt and are not successful in salinity tolerance or increase yield since it is time-consuming and requires immense effort (Coleman-Derr and Tringe, 2014). Therefore bioremediation is the best way to tackle salinity problems and PGP bacteria could be the best way to tackle the problem. This is well proven that PGP bacteria can reduce stress by reducing the ethylene level which is produced by the plant under stress condition by the production of ACC deaminase and helps in withstanding any type of abiotic stress. Not only this, PGP bacteria will also enhance the growth by producing siderophore, IAA, solubilizing insoluble phosphate and by having antagonistic activity against phytopathogens.

Mizoram which is a part of the Northeastern region of India is well known for its rich biodiversity and bioresources to have been identified as the important part of the Himalaya and Indo-Burma biodiversity hotspots (Myers *et al*., 2000). Mizoram is one of the 25 biodiversity hotspots of the world. There are many reports of indigenous medicinal plants with an ethnobotanical history in an area around Mizoram. A list of plant species of 159 ethnomedicinal plant species that belong to 134 genera and 56 families have been recorded from home gardens, tropical forests, roadsides, and Mizoram University campus have been recorded by Rai and Lalramnghinglova, 2010. It is well known that the diversity of microbial community especially Plant Growth-Promoting Bacteria in this region remains unexplored and uncharacterized.

Therefore it is assumed to have numerous potential plant growth promoting bacteria could be isolated and deep research on those bacteria is required. This type of study could help the local farmers in their agricultural fields for more crop production and income in a short period.

The present study aimed to isolate the plant growth promoting bacteria from rhizospheric soil and endophytes associated with medicinal plants and to check for their salinity tolerance to enhance plant growth. The main objectives set for the present work are:

- Isolation and screening of obtained bacterial isolates for their plant growth promoting (PGP) potential.
- *In vitro* and *in vivo* salinity stress tolerance of selected isolates.
- Identification of potential isolates using 16S rRNA gene amplification.

CHAPTER 2

Review of Literature

Plant Growth Promoting Rhizobacteria (PGPR) is a group of beneficial microorganism existed in rhizospheric soil or endosphere tissues of the plants that have an capability to inhabit the plant roots or any tissues of plants and plays significant role in plant growth and development (Kloepper and Schroth, 1978; Backer *et al*., 2018). Bacteria living around the region of the roots are called rhizospheric bacteria and bacteria living in any part of the plant tissues are called endophytic bacteria. There are some negative effects of rhizospheric bacteria to the plants like 40% of the plant product produced during photosynthesis from the roots is lost due to the existence of bacteria in the nutrient-rich rhizosphere (Lynch and Whipps, 1991). Whereas, endophytic bacteria have a mutualistic relationship with the host plant and do not create any harm to the host plant (Esitken *et al.,* 2010). Even though most of the rhizospheric bacteria are thought to have plant growth promoting abilities but some of the bacterial strains belonging to the same genus and species may have different metabolic abilities and interaction with plants. For instance, some strains of *Pseudomonas putida* can actively promote plant growth while others belonging to the same genus and species have no quantifiable effect on plants (Glick, 2014). Therefore screening and selection of bacteria for their PGP potential are essential to check whether a particular strain possesses efficient properties to promote plant growth to increase its productivity. Certain properties like germination percentage, total biomass of the plants, abiotic and biotic stress tolerance, root and shoot growth, seedling vigor, early flowering, seed weight and fruit yields etc., are taken into consideration while using any PGP isolate on the plant growth and development (Ramamoorthy *et al*., 2001; Mariani and Ferrante, 2017; Pandey *et al*., 2017). The effect of PGPR on the plant growth can be broadly classified into two mechanisms i.e. either

following direct mechanisms or by indirect mechanisms (Glick, 1995). Direct mechanism includes the production of phytohormones like cytokinins, auxins and gibberellin, increasing plant nutrition by solubilizing minerals like phosphorus, production of siderophores for chelating iron and extracellular enzymes, reducing ethylene levels by the production of ACC Deaminase (Bhattacharyya and Jha, 2012). The indirect mechanism includes the inhibition of the growth of plant pathogens by acting as a biocontrol agent and through the production of extracellular enzymes which helps in hydrolyzing the cell wall of fungus and decreasing pollutant toxicity (Zahir *et al*., 2003; Podile and Kishore, 2006; Bhattacharyya and Jha, 2012)

Among the PGP traits, siderophores production has been well explained to have direct benefits to enhance plant growth by providing iron directly and making it accessible to plants (Vansuyt *et al.,* 2007). Iron is a micronutrient that is essential to plants as it acts as a cofactor for various enzymes with redox activity and is also necessary for all the major physiological processes like respiration, photosynthetic pigment production, and N_2 fixation, etc. (Gouda *et al*., 2018). Siderophores are produced by many microorganisms and are usually less molecular weight molecules. There are different types of siderophores produced by microorganisms like hydroxamates, carboxylates, and phenol catecholate (Podile and Kishore, 2006).

In the past, many researchers have reported the direct plant growth-promoting effect by siderophore-producing microbes. For example, a siderophore-producing *Pseudomonas* strain GRP3 when inoculated in Mung bean plant under iron-limited condition has shown an increase in the growth and chlorophyll level (Sharma *et al.,* 2003). Bacterial siderophores from *Chryseobacterium* spp. C138 was delivered to the roots of iron-deprived tomato plants and were proved to be effective in supplying Fe. This suggests that the C138 bacterial strain can be used as

an effective organic biofertilizer (Radzki *et al*., 2013). It has also been reported that besides siderophore production some microbes like *Pseudomonas* sp. has the ability to utilize siderophores which are produced by various species of bacteria and fungi, it has also been reported that *Pseudomonas putida* can enhance the available iron level by utilizing the heterologous siderophores produced by rhizosphere microorganisms (Loper *et al*., 1999). Nadeem *et al*., 2016, has reported siderophore production in *Pseudomonas fluorescens*, *Bacillus megaterium*, and *Variovorax paradoxus.* There is also a report that states that the presence of heavy metal (abiotic stress) can induce siderophore production in *Chryseobacterium humi* and *Rhizobium radiobacter* (Moreira *et al*., 2016). There is also a report on different *Burkholderia* sp. like *Burkholderia phytofirmans* (Sun *et al*., 2009) to be able to produce siderophore (Pandey *et al*., 2005).

Another direct method to promote plant growth by the microorganisms is by the production of phytohormone, indole-3-acetic acid (IAA). Signal molecules that act as chemical messengers that play a significant role in the growth and development in the plants are known as phytohormones and IAA is included in the class of phytohormones. Phytohormones can affect the biochemical, morphological and physiological processes in plants in an extremely low concentration (Fuentes-Ramírez and Caballero-Mellado, 2006). Etesami *et al*., 2015 has reported that among the phytohormone produced by microbes IAA is the most important phytohormone that is produced most quantitatively and is responsible for enhancing plant growth. To cite a few examples, *Azospirillum* and fluorescent *Pseudomonas* which are a free-living PGPR can produce IAA which in turn increases the growth and development of the plant (Figueiredo *et al.,* 2010). *Azospirillum*, rhizobacteria that inhabit the internal tissues of plants helps in the enhancement of

plant growth and crop yield by the production of Indole3-acetic acid (IAA) (Perrig *et al.,* 2007). This phytohormone produced by *Azospirillum* species increases the growth by altering the morphology and metabolism of plant roots and helps in better absorption of mineral and water, producing healthier and bigger roots (Bashan and de Bahsan, 2010). There is a report by Moreira *et al*., 2016 that *Rhizobium radiobacter* can produce IAA for enhancing the growth of the plant and has also stated that the IAA production increases in the presence of heavy metal (Cadmium). Oves *et al*., 2013 has reported IAA production in *Pseudomonas aeruginosa* and increase the growth of Chickpea plant. *B. megaterium, Lactobacillus casei* and *B. subtilis* also can produce IAA which in turn increases the growth and the chlorophyll content in the wheat plant (Mohite, 2013). The IAA producing bacterial strains *Pseudomonas aureantiaca*, *Pseudomonas extremorientalis*, and *Pseudomonas extremorientalis* can significantly increase the growth of seedling root up to 52% at 100mM NaCl when compared with the control plants (Egamberdieva, 2009).

The next important PGP trait is the production of ACC deaminase. A study done by Glick (2014) has proven that many PGPR can produce 1- aminocyclopropane-1-carboxylate (ACC) deaminase. ACC deaminase is produced by PGPR under stress conditions. To reduce the stress faced by the plants the level of ethylene is lowered by hydrolyzing 1-aminocyclopropane-1-carboxylic acid (ACC) by the action of an enzyme ACC deaminase produced by PGP bacteria. ACC is the immediate precursor of ethylene hormone. ACC deaminase enzymes can degrade ACC to ammonia and α-ketobutyrate (Gamalero and Glick, 2015; Raghuwanshi and Prasad, 2018). Therefore, potential PGPR for producing ACC deaminase can withstand abiotic stress by lowering the ethylene production and in turn lowers the adverse effect on plants. Even though ethylene is required to break seed dormancy by normal plants for its normal course of their

growth, but if germination is followed by ethylene production it can be inhibitory for root elongation. There are several studies published that states that plants, when inoculated with PGPR containing ACC deaminase, make the plant more resistant to all kinds of abiotic stresses including salinity, flood, drought and against various pathogens (Pourbabaee *et al*., 2016; Ravanbakhsh *et al*., 2017; Saikia *et al*., 2018; Ghosh *et al*., 2018). So, for the bioremediation process, the more economical, environment-friendly and more practical in natural soil and plant system is using PGPR containing ACC deaminase activity. Microorganisms like *Burkholderia, Pseudomonas*, *Bacillus, Azospirillum*, *Enterobacter,* and *Kluyvera* have proved to have ACC deaminase activity (Saleem *et al.,* 2007). There are numerous reports of *Pseudomonas fluorescens* being able to produce ACC deaminase under salt stress conditions for the promotion of the growth of mung bean (Ahmad *et al*., 2011) and barley (Cardinale *et al*., 2015). There are several studies on *Burkholderia* sp. for the production of ACC deaminase to promote plant growth as well as tolerate salt stress. Sun *et al*., 2009 has reported that *Burkholderia phytofirmans*can produces ACC deaminase and helps the plant in growth promotion. Another study has stated that an ACC deaminase producing *Burkholderia cepacia* help *Capsicum annuum* to withstand both drought stress and salt stress and promote the plant growth (Maxton *et al*., 2017). Sziderics *et al*., 2007 has also reported ACC deaminase production by *Arthrobacter* sp. and *Bacillus* sp. and significantly increases the root biomass of *Capsicum annuum* when compared with the non-inoculated plant. Among the abiotic stresses, salinity can inhibit seed germination, growth of seedlings, and flowering due to the production of stress ethylene in more amounts. In such cases, the ACC deaminase producing PGPR can reduce the level of the stress ethylene produced and helps in salinity tolerance in the plants (Gontia-Mishra *et al.,* 2014). This is proven by Yoolong *et al*., 2019 where ACC deaminase producing *Streptomyces venezuelae*

has modulated the salt tolerance in *Oryza sativa* and can tolerate salt stress up to 3%. It does not only helps in salt tolerance it also promotes the growth of the plant.

Phosphate is a macronutrient for plants that are present in the soil. It is essential for plant growth but is present only in a limited amount in the soil; therefore it is considered to be one of the elements that limit plant growth (Feng *et al*., 2004). Generally, agricultural soils contain large amount of immobilized inorganic and organic phosphates that are unavailable to plants, but several PGPR strains like *Flavobacterium*, *Bacillus*, *Pseudomonas*, *Rhizobium,* and *Burkholderia* have been reported to have the potential to solubilize such inorganic phosphate and make it available to the plants. These phosphate solubilizing bacteria can increase the phosphate uptake by plants and therefore helps in the direct promotion of plant growth (Bashan and de Bahsan, 2010; Saharan and Nehra, 2011). Bhattacharyya and Jha, 2012 have also reported that certain bacterial genera like *Bacillus*, *Beijerinckia, Azotobacter*, *Burkholderia*, *Enterobacter, Flavobacterium*, *Pseudomonas*, *Erwinia*, *Microbacterium*, *Rhizobium* and *Serratia* are reported as the most significant phosphate solubilizing bacteria.

It was also reported that the growth of the cucumber plant has been enhanced by phosphate solubilizing *Pseudomonas fluorescens* (Nadeem *et al*., 2016). Another phosphate solubilizing *Serratia marcescens* along with other PGP traits like N_2 fixation and IAA production can enhance the growth of rice plants under saline conditions (Nakbanpote *et al*., 2014). An increase in fresh weight, dry weight and shoot length of *Capsicum annum* were observed in *Capsicum annum* plant inoculated with *Bacillus megaterium* (Wang *et al*., 2018). Besides providing Phosphate to the plants, the bacteria that solubilize phosphorus also help the plants by providing

other trace elements like iron and zinc for growth promotion (Ponmurugan and Gopi, 2006; Mittal *et al*., 2008). Certain PGPR also produces exopolysaccharides (EPS), for example *Pseudomonas* produce exopolysaccharides (EPS) which helps the bacteria to withstand different abiotic stress like water stress, and has the ability to stabilize and form soil aggregates, and also regulates plant nutrients as well as the water flow across plant roots by the formation of biofilm (Grover *et al.,* 2011). Bharti *et al*., 2012 have reported *Exiguobacterium oxidotolerans* having the ability to produce exopolysaccharides. There is also a report on *Pseudomonas fluorescens* and *Bacillus megaterium* by Nadeem *et al*., 2016 to be able to produce exopolysaccharide and help to promote the growth of cucumber plant.

Generally, salinity stress can cause an imbalance in the ion flux inside plants, but inoculation with PGPR results in decreasing Na+ and increased K+ concentration which in turn lower salt stress by binding the Na+ cation and therefore decreases the Na+ available for uptake (Kang *et al.,* 2014). It has been reported that various PGPR strains can produce osmolytes which helps the plants to enhance the osmotic potential within the cell and therefore reliefs the stress (Gururani *et al*., 2013). An ACC deaminase producing *B. cepacia* can tolerate salt stress up to 240mM concentration of salt and enhance the growth of *Capsicum annuum* (Maxton *et al*., 2017). A plant growth promoting bacteria, *B. megaterium* which can solubilize inorganic phosphate has increased the fresh weight, dry weight, shoot height and root length of *Capsicum annuum* under salt stress condition (Wang *et al*., 2018). Another study has reported that the growth of *B.monnieri* plants has increased with the inoculation of PGP *E. oxidotolerans* and *Bacillus pumilus* under salt stress conditions. Both the isolates have increased the herb yield when compared with the non-inoculated plants (Bharti *et al*., 2012). Another study was done by

Barnawal *et al*., 2017 reported that PGPR strains, *Arthrobacter protophormiae*, and *Dietzia natronolimnaea* to have a salt tolerating ability up to 100mM salt concentration. These reports have suggested that Plant Growth Promoting Bacteria are the potential microorganisms to alleviate salt stress in plants.

CHAPTER 3

MATERIALS AND METHODS

3.1. Isolation of bacteria from endosphere tissues of plant and rhizospheric soil samples

Endophytic bacteria were isolated from *Dillenia indica* and *Centella asiatica*. The tissues were rinsed for the 30s in 95% ethanol solution followed by a rinsed with sodium hypochlorite solution (2% available Cl^-) for 5 min. Finally, three washes were given with sterilized double distilled water and tissues were dried under laminar airflow. The dried tissues were kept in five different media (SCA, AIA, ISP7, ISP5 and King's Media). The plates were incubated at 28±2°C in BOD incubator and the growth of bacteria was observed once a day. Rhizospheric soil was taken from agricultural fields and bacterial isolation was done using the serial dilution technique (Manivannan *et al.,* 2012). The pure cultures were stored at 4°C in the refrigerator.

3.2. Determination of Plant Growth Promoting traits of the obtained bacterial isolates.

3.2.1. ACC Deaminase Activity Assay

To determine ACC activity, the isolates were grown in minimal salts agar medium which was supplemented with 3mM ACC instead of $(NH_4)_2SO_4$ as its nitrogen source. The ability of the isolates to grow on the prepared media and use ACC as its nitrogen source instead of $(NH_4)_2SO_4$ was tested to determine the presence of ACC deaminase (Shrivastava and Kumar, 2013).

3.2.2. Screening for phosphate solubilization of the isolates

Determination of insoluble phosphate was carried out by spotting the bacterial culture on Pikovskaya's agar plates (Pikovskaya, 1948) containing 2% tri-calcium phosphate and incubated at 28◦C for seven days and the appearance of solubilization was observed and measured and

phosphate solubilization index was calculated. For quantification of phosphate, the cultures were grown in Pikovskaya's broth and the supernatant was estimated using Barton's reagent (Singal *et al*., 1991)

3.2.3. Production of indole-3-acetic acid

Indole-3-acetic acid production was estimated using the method described by Sheng *et al.* (2008). The fifty isolates were grown in ISP1 (International *Streptomyces* Project 1) broth which contains 0.2% L-tryptophan and was incubated at 28°C with continuous shaking at 125 rpm for seven days. After the cultures are grown, it was centrifuged at 10,000 rpm for 20 minutes. Then 1 ml of the supernatant was mixed with 2 ml of Salkowski reagent. The mixture was incubated at a dark room for half an hour and the production of indole-3-acetic acid was observed by the development of pink color and the absorbance was measured at 530 nm using a spectrophotometer and the absorbance was compared with the standard curve of IAA and the concentration of IAA was expressed in µg/ml.

3.2.4. Cellulase and xylanase production

The screening was done by streaking the pure cultures on screening agar plates supplemented with 0.5% (w/v) CMC and 25% (v/v) oat spelt Xylan for cellulase and xylanase screening respectively at pH 7 for 3 to 5 days. After the cultures are grown all the isolates were screened for cellulose and xylanase production using Congo red assay (Teather and Wood, 1982). The plates were flooded with Congo red (0.5%) for 5 minutes which was followed by distaining with 1M NaCl for 15 minutes. The diameter of the clear zone which is the indication of the magnitude of cellulose and xylanase production (Teather and Wood, 1982) was observed.

3.2.5. Amylase production

Media composing of 1% starch and 2% agar was prepared for the screening of amylase production. The cultures were streaked and kept for incubation at 28°C until the cultures were grown. Then the cultures were flushed with a mixture of 1% iodine and 2% Potassium iodide (Kammoun *et al*., 2008).

3.2.6. Laccase production

Screening for laccase production was done by streaking the isolates at 2% agar supplemented with guaiacol. The plates were checked for the purple color zone after four days of incubation at 28°C (Muthukumarasamy *et al*., 2015).

3.2.7. Production of siderophore by the isolates

For the screening of siderophore production, the cultures were streaked in Chrome Azurol S agar plates (Ames-Gottfred *et al.,* 1989) which is blue. The appearance of an orange halo zone around the cultures was observed after 48 hours incubation at 28°C (Ali *et al.,* 2014).

3.2.8. Antagonistic potential of obtained bacterial isolates

All the selected bacterial isolates were screened for their antifungal activity using four fungal pathogens (*Fusarium lycopersicum*, *Fusarium graminearum* and *Fusarium oxysporum*) according to Khanna *et al*., 2008. Briefly, 0.5 cm mycelia disc were cut from the actively grown fungal pathogens that have been cultured on PDA plates incubated at 25°C for 7 days. The tested bacterial isolates will then be streaked on opposite sides of the same plate and incubated at 28°C for 7 days, and the percentage of inhibition was calculated.

3.3. Molecular characterization and phylogenetic analysis of the isolates

3.3.1. Genomic DNA extraction, amplification of 16S rRNA gene and sequencing

Bacterial universal primers- PA: 5′-AGA GTT TGATCC TGG CTC AG-3′as forward and PH: 5 ′- AAG GAG GTG ATC CAG CCG CA-3′ as reverse (Qin *et al*. 2009) was used for the amplification. The amplified products were quantified by agarose gel electrophoresis (1.5%) and analyzed using a Bio-rad Gel Doc XR+ system (Hercules, CA, USA). The PCR products were purified using Pure-link PCR Purification Kit (In-vitrogen), and was sequenced commercially at Chromegene Pvt. Ltd. India.

3.3.2. Phylogenetic analysis of the isolates

The sequences of the 16S rRNA gene were compared with the GenBank database using BlastN and the most similar match sequence was selected. The sequences were aligned with pair wise alignment using the program Clustal W packaged in the MEGA 5.05 software (Thompson *et al*., 1997). From this data, a phylogenetic tree was constructed using the neighbor-joining tree and (Saitou and Nei, 1987). Bootstrap analysis was performed with MEGA 5.05 using Kimura 2-parameter (K2) for gram-negative bacteria and Tamura-Nei (TN93) for gram-positive bacteria.

3.4. Screening for salinity stress tolerance of the identified isolates

Salinity stress tolerance was tested by evaluating the bacterial cultures in their respective growth media which was amended with 5%, 10%, 15% and 20% salt concentrations of salt (NaCl) (Amaresan *et al.,* 2016). The media with different concentrations of salt was prepared and the bacteria were streaked and incubated at 28°C and the growth was observed after 2-4 days.

3.5. Effect of selected isolates on seed germination under salt stress

Two best isolates were selected and were used to check the seed germination effect of the isolates. For seed germination, the seeds were surface sterilized with 5% sodium hypochlorite for 5 minutes followed by 95% ethanol wash for three minutes and a final wash with sterilized water for 2 minutes three times. The sterilized seeds were soaked in the respective 10^{-2} bacterial suspension for 8 hours and were transferred to 0.8% agar media supplemented with 5%, 10% and 15% of NaCl for its germination (Upadhyay and Singh, 2014).

3.6. Pot experiments of selected isolates using chickpea crop

The ability of two selected PGPB strains to promote plant growth was evaluated using the pot experiment (Upadhyay and Singh, 2014). The experiment was done using a sandy soil and in each pot 500grams of the soil were added. 15 seeds for each bacterial isolates were selected, the seeds were surface sterilized and soaked in 10^{-2} bacterial dilution for 8 hours. Control seeds were similarly soaked in water for the same amount of time. After the seed is germinated it was transferred into pots with different treatment of salt concentrations (5%, 10%, 15%). Treatment 0: Seeds are grown without salt treatment. Treatment 1: Seeds treated with 5% of salt. Treatment 2: Seeds treated with 10% of salt. Treatment 3: Seeds treated with 15% of salt. The seeds were given treatments by spraying with different salt concentrations for 30 days. Control plants i.e treatment 0 was treated with the same amount of water. Hoagland solution was given once per week to fulfill nutrient requirements. Each treatment will be performed in three replicates with five plants per pots.

3.7. Determination of plant growth parameters of the chickpea plants

On the 31st day, the grown plants were removed from the pots. The root and shoot length was measured and the number of branches was counted. The fresh weight of the plant was measured using a weighing balance.

3.8. Determination of chlorophyll content of treated and control plants

The method given by Arnon (1949) was followed to estimate the photosynthetic pigments. The plant with each treatment was ground in the acetone (80% v/v) and the supernatant was measured at 663 and 645 nm in a spectrophotometer. The concentration of Chlorophyll a, Chlorophyll b and the total chlorophyll content was calculated using the formula

Total Chlorophyll: 20.2(A645) + 8.02(A663)

Chlorophyll a: 12.7(A663) – 2.69(A645)

Chlorophyll b: 22.9(A645) – 4.68(A663)

3.9. Determination of catalase activity of treated and control plants

Catalase activity in the leaf was determined following the method of Aebi (1984) where the reaction mixture was made by 100mM phosphate buffer (pH 7.0), 6.6mM H_2O_2 and 50µl of plant extract. H_2O_2 was added at the end of the experiment and the reduction of H_2O_2 was monitored at 240 nm for 3 minutes and the activity was calculated using Fick and Qualset (1975) formula.

3.10. DPPH (1,1-Diphenyl-2-picryl-hydrazyl) assay

The methanolic extract prepared from the leaf was used for the determination of 1,1-Diphenyl-2-

picryl-hydrazyl assay.100-200µl of the sample was mixed with 3.8 ml of DPPH solution. The mixture was incubated in a dark room for 1 hour (Villano *et al*., 2007). Then the absorbance of the mixture was taken at 517 nm.

3.11. ABTS radical scavenging assay

For the determination of the ABTS radical scavenging assay, the method given by Re *et al*.,1999 was followed. The stock solution was made using a 7mmol/L ABTS solution and 2.4mmol/L potassium persulfate solution. Then the two solutions were mixed in a 1:1 ratio for working solution. The mixture was allowed to react for 12-16 hours in the dark at room temperature. The resulting solution was then diluted by mixing 1 mL of freshly prepared ABTS solution to obtain an absorbance of (0.706±0.001) units at 734 nm using the spectrophotometer. Plant extracts (1 mL) were allowed to react with 2.5 mL of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The percentage of inhibition was calculated as:

$$\text{ABTS radical scavenging activity (\%)} = \frac{(\text{A control} - \text{A test})}{\text{A control}} \times 100$$

Where A control is the absorbance of ABTS radical+methanol

A test is the absorbance of ABTS radical+sample extract/ standard

CHAPTER 4

RESULTS

4.1. Isolation of bacteria from endosphere tissues of plants and rhizospheric soil samples

In total, 50 bacterial isolates were isolated from rhizospheric soil and endosphere of selected plants. Out of which 30 were obtained from rhizospheric soil and 20 were obtained as endophytes from the selected plants (*Dillenia indica* and *Centella asiatica*). All the isolates were maintained on their respective nutritional media (**Figure 1**). Based on the media composition, 16 isolates were obtained from King's Media, 12 isolates from Starch Casein Agar (SCA), 10 isolates from Tyrosine Agar (ISP7) and 6 isolates each from Actinomycetes Isolation Agar (AIA) and International *Streptomyces* Project 5 (ISP5) (**Figure 2**).

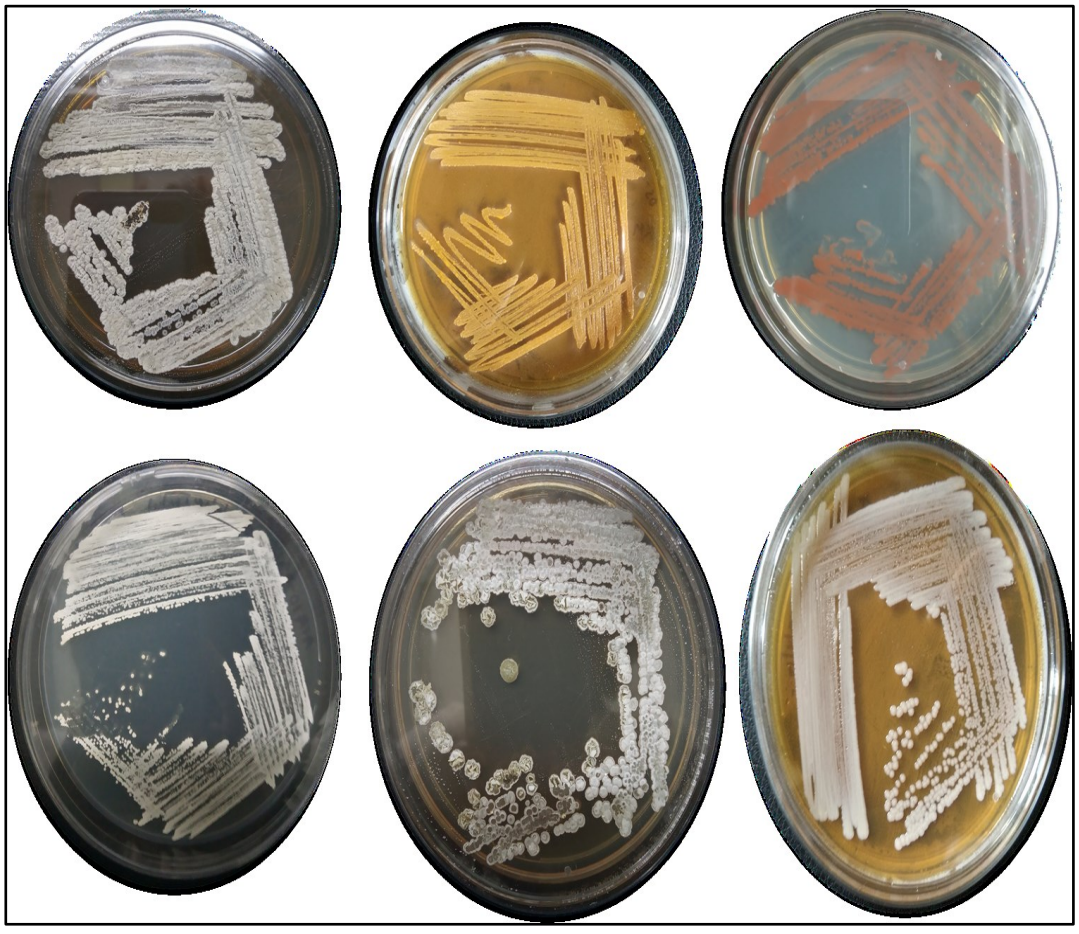

Figure 1: Pure cultures of different isolates showing morphological characters

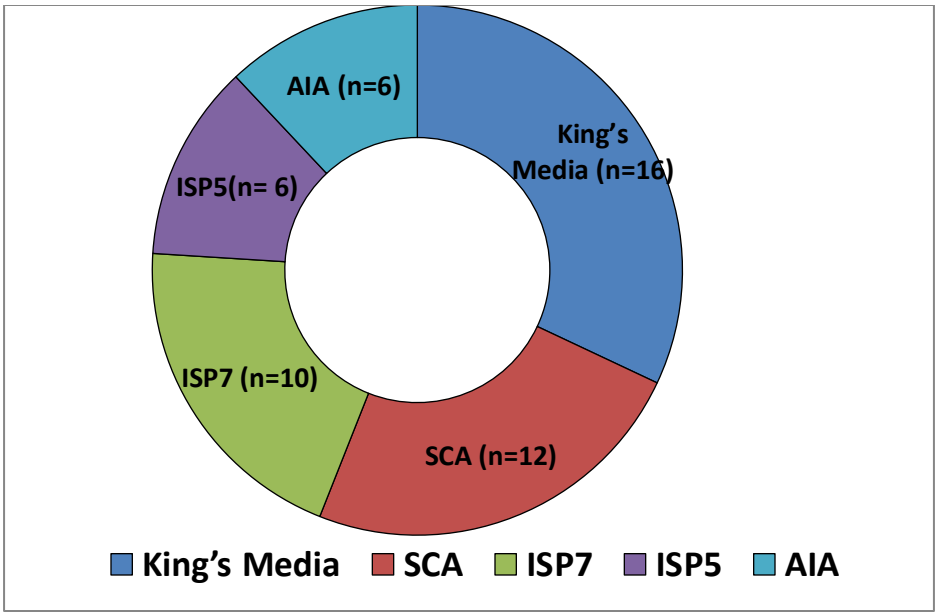

Figure 2. Pie chart showing the distribution of obtained bacterial isolates using different nutritional media.

4.2. Determination of Plant Growth Promoting (PGP) traits of obtained isolates.

4.2.1. ACC Deaminase production Assay

All the obtained isolates were screened for their ACC Deaminase production ability and 22 (44%) isolates showed positive which is indicated by the growth of respective bacteria on nutritional media supplemented with ACC (**Figure 3**).

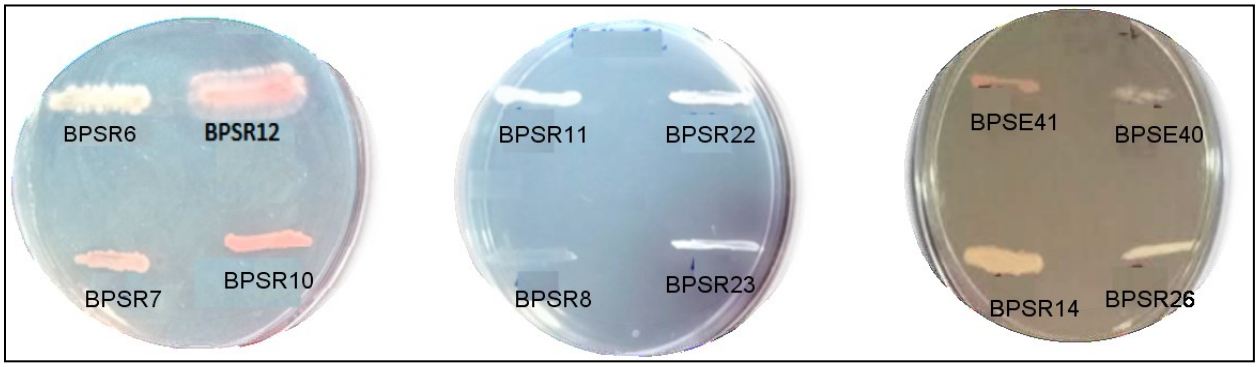

Figure 3: Screening of bacterial isolates using ACC supplemented nutritional media. Growth indicates positive and isolates which could not grow indicates negative.

4.2.2. Screening for phosphate solubilization potential

Among the 50 isolates, 29 (58%) isolates showed positive for phosphate-solubilizing ability by showing a halo zone nearby the colony on Pikovskaya's agar media. The Phosphate Solubilizing Index was calculated for all the 29 isolates and the maximum phosphate solubilization was observed in **BPSR28** followed by **BPSR19** with the Phosphate Solubilizing Index of **7.3±0.8** and **6.8±0.7** respectively **(Figure 4).** All the isolates which show the ability to solubilize phosphate were analyzed quantitatively to determine how much phosphate the bacterial isolate can solubilize. The maximum phosphate-solubilizing ability was shown by BPSR28 with a value of 152.61µg/ml.

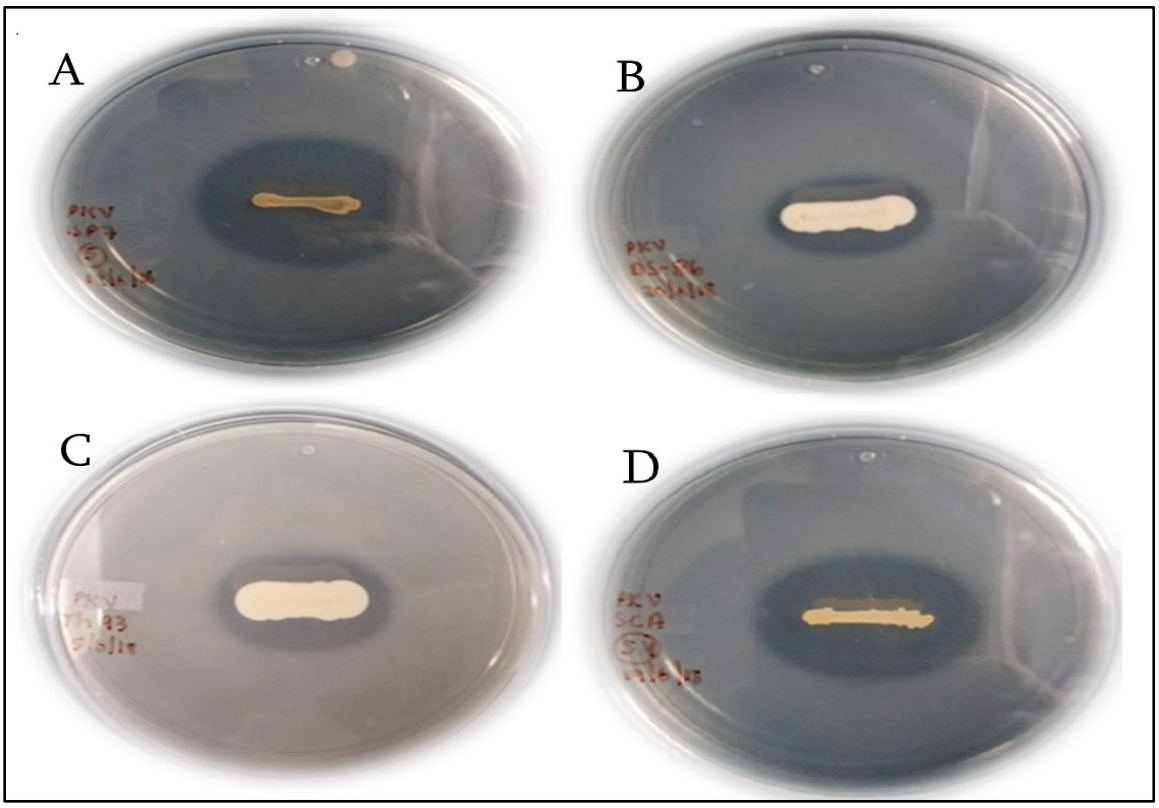

Figure 4: Phosphate solubilization ability of the bacterial isolates using Pikovskaya'sagar. Formation of the zone indicates the phosphate solubilization ability.

4.2.3. Production of indole-3-acetic acid

A total of 17 (34%) isolates were observed to produce Indole-3-acetic acid. The amount of IAA produced was known through quantitative analysis. The IAA production value ranges from 2.08µg/ml to 53.60µg/ml. The highest amount of IAA was produced by BPSR28 followed by BPSR14 with the value of 53.60µg/ml and 48.04µg/ml respectively.

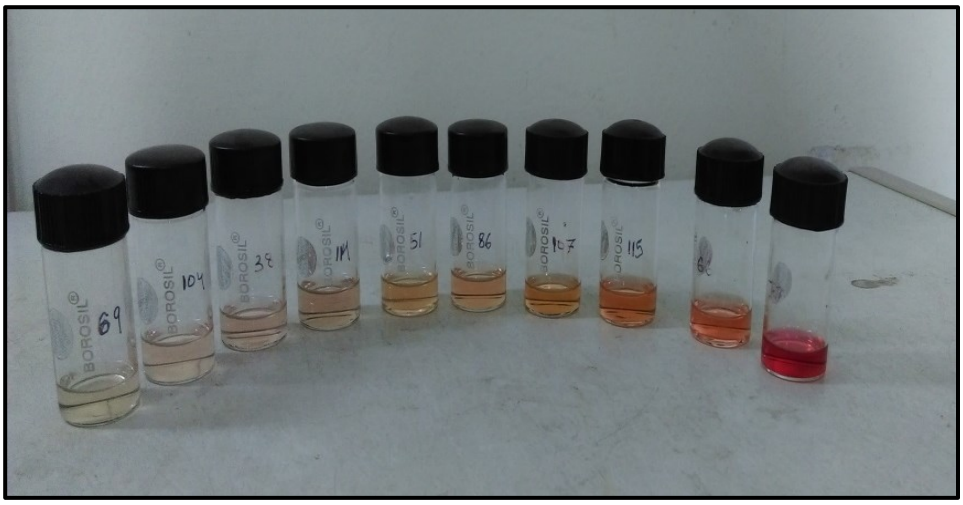

Figure 5: Color formation due to IAA production, the production of IAA increases from left to right side

4.2.4. Extracellular enzymes production of the isolates

Amylase, Laccase, Cellulase and Xylanase enzyme production was screened for all the 50 isolates (**Figure 6**). Among them, only 7 isolates were observed to be positive for xylanase production. One isolate showed positive for cellulase production. None of the isolates have shown positive for Amylase and Laccase.

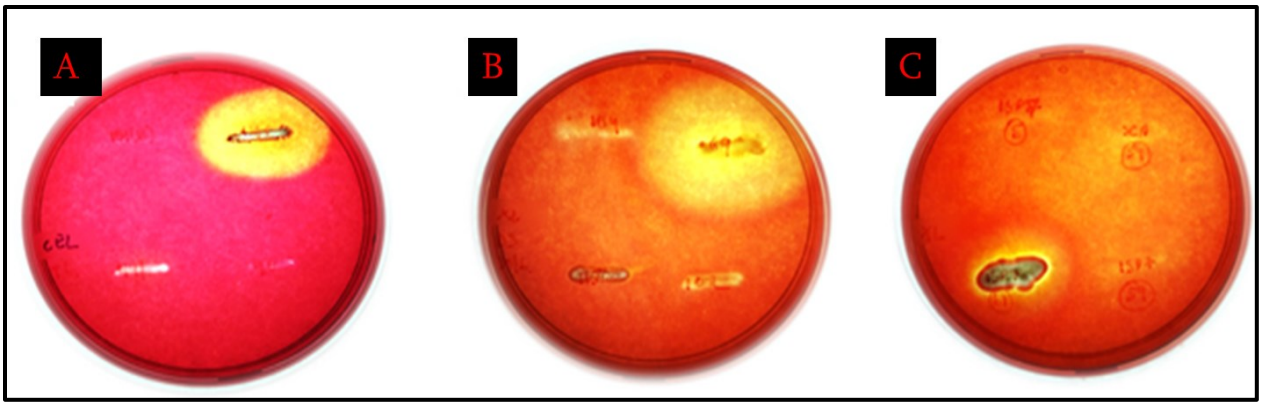

Figure 6: Cellulolytic zone (orange) formed by **BPSE36, B.** Xylanolytic zone (orange) formed by **BPSR13 and C.** Xylanolytic zone (orange) formed by **BPSR22**

4.2.5. Production of siderophore

All the 50 isolates were screened for the ability to produce siderophore. Out of 50 isolates, 66% (n=33) isolates were observed as positive for siderophore production by producing an orange halo zone in CAS media **(Figure 7).**

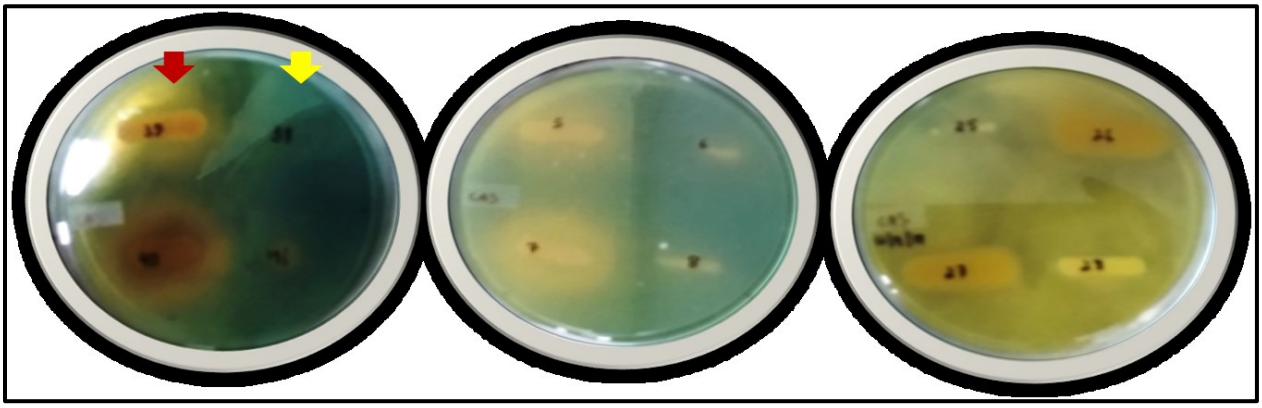

Figure 7: Siderophore production screening on CAS media. Siderophore production is indicated by an orange zone around the isolates. Red arrow indicates positive and yellow arrow indicates negative for siderophore production.

4.2.6. Screening for the antagonistic potential of the isolates

Three fungal pathogens namely *Fusarium oxysporum* (MTCC1893), *Fusarium lycopersicum* (ITCC1322), and *Fusarium graminearum* (ITCC3237), were used to test the antagonistic potential of all the isolates. From 50 isolates, 28.2% of the isolated bacteria showed inhibitory activity against at least one of the tested pathogens with the percentage of inhibition ranging from 23.3-67.6 % (Fig: 8). Among all the 50 isolates, 8 isolates (*Burkholderia* sp. BPSR41, *Achromobacter xylosoxidans* BPSR6, *Brevibacterium* sp. BPSR9, *Pseudomonas aeruginosa* BPSR17, *Pseudomonas aeruginosa* BPSR18, *Pseudomonas aeruginosa* BPSR19, *Leucobacter komagatae* BPSR22, *Ochrobactrum* sp. BPSE36) showed activity against all the tested three fungal pathogens.

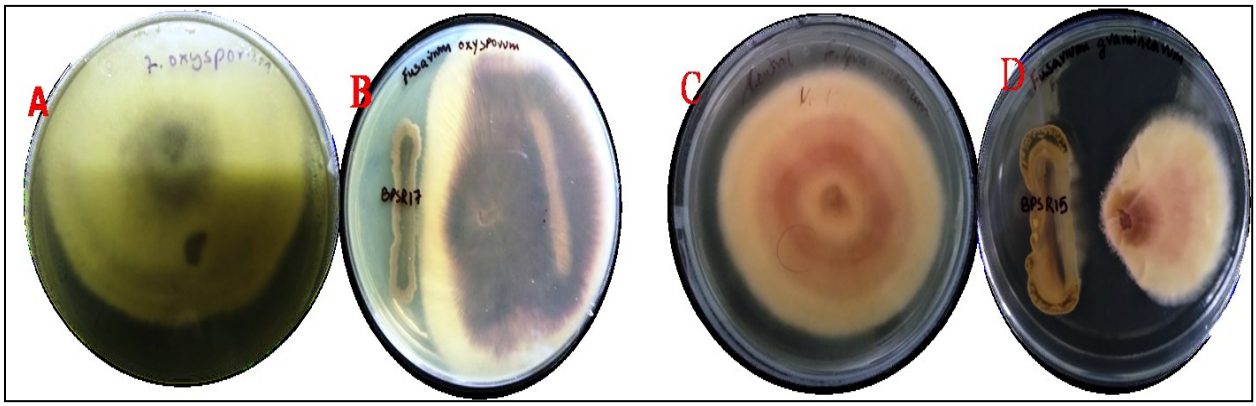

Figure 8: A. Control of *F. oxysporum*, B. Inhibition of *F. oxysporum* by BPSR17, C. Control of *F. graminearum*, D. Inhibition of *F. graminearum* by BPSR15

Table1: Overview of both direct and indirect plant growth promoting properties of all the isolates.

Sample no.	IAA (µg/ml)	Phosphate (µg/ml)	ANTIFUNGAL (%)			Sidero-Phore	ACC deaminase	Cellulase	Xylanase	Laccase	Amylase
			F.oxysporum(MTCC-1893)	*F.lycopersicum*(ITCC-1322)	*F.graminearum*(ITCC-3437)						
BPSR1	-	124.90±0.14	43.9±0.2	31.3±0.1	41.4±0.7	+	-	-	-	-	-
BPSR2	-	-	-	-	-	+	+	-	-	-	-
BPSR3	-	-	-	-	-	+	-	-	-	-	-
BPSR4	-	111.30±0.56	-	-	-	+	+	-	-	-	-
BPSR5	15.33±0.30	67.68±0.19	-	-	-	-	+	-	-	-	-
BPSR6	-	-	42.6±0.2	32.4±0.5	40.9±0.5	+	+	-	-	-	-
BPSR7	-	105.80±0.42	-	-	-	-	+	-	-	-	-
BPSR8	-	135.55±0.64	43.9±0.4	55.3±0.6	-	+	-	-	-	-	-
BPSR9	10.04±0.05	-	42.2±0.2	42.8±0.8	54.6±0.7	+	-	-	-	-	-
BPSR10	-	51.15±0.33	-	-	-	+	+	-	-	-	-
BPSR11	-	117.60±0.70	-	-	-	+	+	-	-	-	-
BPSR12	-	139.70±0.53	-	-	-	+	-	-	-	-	-

BPSR13	-	70.84±0.49	-	-	-	-	-	-	+	-	-
BPSR14	48.04±0.80	-	-	-	-	+	+	-	-	-	-
BPSR15	28.45±0.20	130.67±0.53	-	-	-	+	-	-	-	-	-
BPSR16	23.42±0.50	114.32±0.74	-	-	-	+	-	-	-	-	-
BPSR17	-	137.40±0.30	23.3±0.8	-	-	+	-	-	-	-	-
BPSR18	28.67±0.30	110.05±0.63	55.6±0.8	61±0.9	67.6±0.4	+	-	-	-	-	-
BPSR19	27.18±0.40	128.95±0.63	49.3±0.8	31.03±0.5	37.4±0.2	+	-	-	-	-	-
BPSR20	2.62±0.40	-	-	-	-	+	-	-	-	-	-
BPSR21	2.08±0.60	-	-	-	-	+	-	-	-	-	-
BPSR22	-	141.40±0.28	31.3±0.1	31.9±0.7	28.9±0.6	+	+	-	+	-	-
BPSR23	-	80.33±0.71	-	-	-	+	+	-	-	-	-
BPSR24	6.26±0.50	137.40±0.56	-	-	-	+	+	-	-	-	-
BPSR25	14.33±0.50	84.38±0.89	-	-	-	+	+	-	-	-	-
BPSR26	32.04±0.02	-	-	-	-	-	+	-	-	-	-
BPSR27	14.57±0.30	141.85±0.35	-	-	-	+	+	-	-	-	-
BPSE28	53.60±0.20	152.61±0.86	59.8±0.2	60.6±0.9	67.3±0.4	+	+	-	-	-	-
BPSE29	-	122.90±0.42	-	-	-	+	+	-	-	-	-
BPSE30	-	134.15±0.63	-	-	-	+	+	-	-	-	-

BPSE32	-	85.47±0.95	-	-	-	+	+	-	-	-	-
BPSE34	-	-	-	-	-	+	-	-	-	-	-
BPSE35	-	30.07±0.03	45.5±0.8	-	-	+	+	-	-	-	-
BPSE36	23.42±0.60	103.45±0.77	31.3±0.1	35.7±0.7	31.1±0.1	+	-	+	+	-	-
BPSE37	-	67.08±0.74	-	-	-	-	-	-	+	-	-
BPSE38	-	86.48±0.31	-	-	-	-	-	-	+	-	-
BPSE39	-	-	-	-	-	+	-	-	-	-	-
BPSE40	46.27±0.30	41.15±0.50	-	-	-	+	+	-	-	-	-
BPSE41	26.93±0.30	143.57±0.81	-	-	-	+	+	-	-	-	-

Data presented in Mean ± Standard deviation from triplicate sample, + indicates positive results and – indicates negative results

4.3. Genomic DNA extraction, amplification of 16S rRNA gene and sequencing

Genomic DNA was extracted using Pure-Link Genomic DNA extraction Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol **(Figure 9).**

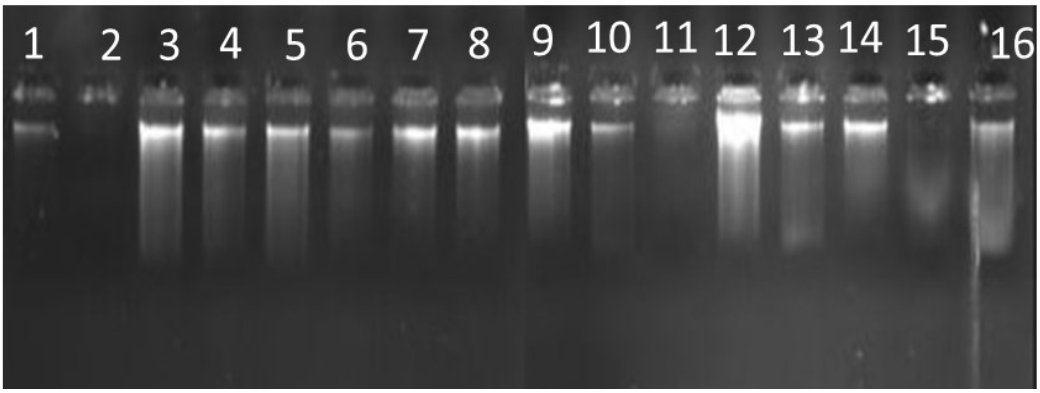

Figure 9: Isolated genomic DNA, Lane 1-16 are the representative bacterial isolates.

Amplification of 16S rRNA gene was done using Applied Biosystems thermal cycler PCR. The PCR product was run on 1.2% agarose gel with low range DNA ruler plus (100 bp to 3 kb) as molecular markers. For all the isolates a single amplicon of 1500 bp was amplified **(Figure 10).**

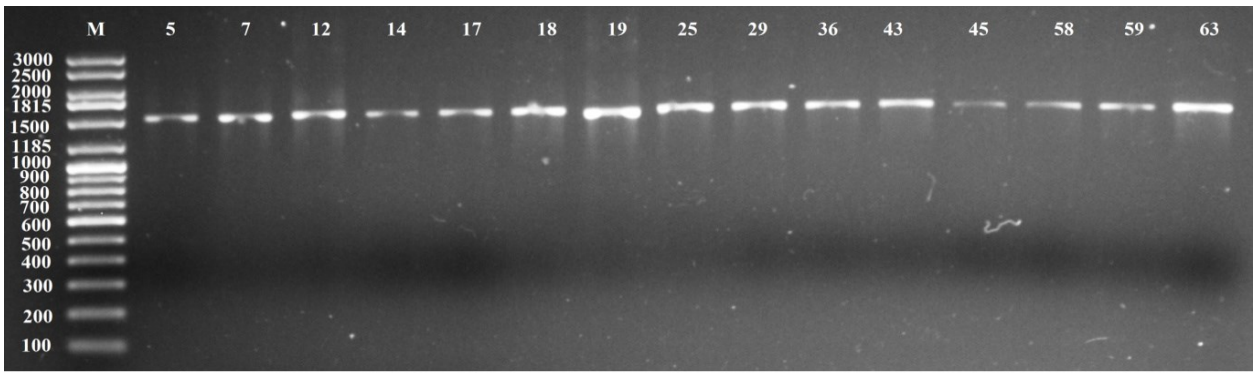

Figure 10: PCR amplification of 16S rRNA gene of the isolates. Numerical numbers represent the isolated DNA of some isolates and M represents 3kb DNA ladder.

The amplified PCR product was commercially sequenced and the sequences were analyzed using NCBI BLAST (Basic Local Alignment Search Tool) and the analyzed sequences were submitted to NCBI Gen Bank. The accession number was obtained for 39 isolates (MK786691 to MK786729) as shown in **Table 2.**

Table 2: Identification of the positive bacterial isolates based on 16S rRNA Gene sequences

Isolate no.	Gene Bank accession no.	Closest species with accession no.	Similarity	Identification
BPSR1	MK786691	*Burkholderia* sp.(KM362438)	97.21%	*Burkholderia* sp.
BPSR2	MK786692	*Stenotrophomonas maltophilia*(LC389419)	100%	*Stenotrophomonas maltophilia*
BPSR3	MK786693	*Lysinibacillus fusiformis*(MH921651)	100%	*Lysinibacillus fusiformis*
BPSR4	MK786694	*Alcaligenes* sp. (MH793376)	100%	*Alcaligenes* sp.
BPSR5	MK786695	*Burkholderia cenocepacia* (MK615919)	99.73%	*Burkholderia cenocepacia*
BPSR6	MK786696	*Achromobacter xylosoxidans* (MH793398)	99.75%	*Achromobacter xylosoxidans*
BPSR7	MK786697	*Burkholderia cenocepacia* (MK615919)	100%	*Burkholderia cenocepacia*
BPSR8	MK786698	*Pseudomonas aeruginosa*(MK713646)	99.72%	*Pseudomonas aeruginosa*

BPSR9	MK786699	*Brevibacterium* sp.(KU145657)	98.55%	*Brevibacterium* sp.
BPSR10	MK786700	*Burkholderia* sp. (MK691482)	100%	*Burkholderia* sp.
BPSR11	MK786701	*Burkholderia* sp. (MK691482)	99.85%	*Burkholderia* sp.
BPSR12	MK786702	*Alcaligenes faecalis*(MH793406)	100%	*Alcaligenes faecalis*
BPSR13	MK786703	*Bacillus licheniformis*(MK648261)	99.72%	*Bacillus licheniformis*
BPSR14	MK786704	*Brevibacterium* sp. (KP126811)	99.18%	*Brevibacterium* sp.
BPSR15	MK786705	*Pseudomonas aeruginosa* (MK713646)	100%	*Pseudomonas aeruginosa*
BPSR16	MK786706	*Pseudomonas aeruginosa* (AY548953)	99.51%	*Pseudomonas aeruginosa*
BPSR17	MK786707	*Pseudomonas Aeruginosa* (CP040127)	99.86%	*Pseudomonas aeruginosa*
BPSR18	MK786708	*Pseudomonas Aeruginosa* (MH368362)	99.59%	*Pseudomonas aeruginosa*
BPSR19	MK786709	*Pseudomonas aeruginosa* (MK713646)	99.86%	*Pseudomonas aeruginosa*
BPSR20	MK786710	*Achromobacter*	99.48%	*Achromobacter*

		xylosoxidans (MK089550)		*xylosoxidans*
BPSR21	MK786711	*Burkholderia cenocepacia* (MK615919)	99.74%	*Burkholderia cenocepacia*
BPSR22	MK786712	*Leucobacter Komagatae* (JF792093)	99.74%	*Leukobacter komagatae*
BPSR23	MK786713	*Pseudomonas Aeruginosa* (LR590474)	100%	*Pseudomonas aeruginosa*
BPSR24	MK786714	*Serratia rubidaea* (KC953862)	100%	*Serratia rubidaea*
BPSR25	MK786715	*Mesorhizobium* sp. (JX949237)	97.74%	*Mesorhizobium* sp.
BPSR26	MK786716	*Serratia* sp. (JX193587)	99.87%	*Serratia* sp.
BPSR27	MK786717	*Alcaligenes* sp. (MK312571)	99.74%	*Alcaligenes* sp.
BPSE28	MK786718	*Pseudomonas cedrina* (MG905307)	99.62%	*Pseudomonas cedrina*
BPSE29	MK786719	*Alcaligenes aquatilis* (MH106703)	100%	*Alcaligenes aquatilis*
BPSE30	MK786720	*Pseudomonas aeruginosa* (MK713646)	99.87%	*Psedomonas aeruginosa*
BPSE32	MK786721	*Chryseobacterium indologenes* (KX817277)	99.74%	*Chryseobacterium indologenes*
BPSE33	MK786722	*Enterobacter*	100%	*Enterobacter*

		cancerogenus (KF224913)		*cancerogenus*
BPSE34	MK786723	*Streptomyces malachitospinus* (KY767028)	99.59%	*Streptomyces malachitospinus*
BPSE35	MK786724	*Brachybacterium paraconglomeratum* (KJ094581)	99.47%	*Brachybacterium paraconglomeratum*
BPSE36	MK786725	*Ochrobactrum* sp. (MK063698)	99.88	*Orchrobactrum* sp.
BPSE37	MK786726	*Streptomyces* sp. (KT443827)	99.23	*Streptomyces* sp.
BPSE38	MK786727	*Streptomyces* sp .(MF960781)	97.87	*Streptomyces* sp.
BPSE39	MK786728	*Brevibacterium*sp .(KU145657)	100%	*Brevibacterium* sp.
BPSE41	MK786729	*Burkholderia* sp. (JN643561)	100%	*Burkholderia* sp.

4.4. Phylogenetic analysis of the isolates

To observe the relationship among the isolates, 16S rRNA gene sequences were aligned along with the type strains retrieved from EZTaxon database. A separate tree was constructed for Gram-positive (**Figure 11**) and Gram-negative bacteria (**Figure 12**). *Burkholderia* sp. was used as an out-group for Gram positive bacteria and 30% of the isolates belong to Brevibacteriaceae and another 30 % belongs to Streptomycetaceae followed by 20% Bacillaceae and 10%

Dermabacteraceae and Microbacteriaceae. For Gram negative bacteria *Clostridium septicum* (AF385694) was used as an out group. Out of which 31.04% belong to the family Pseudomonadaceae (31.04%) followed by 20.68% of the isolates belong to Burkholderiaceae (20.68%) family, 20.68 % of the isolates belong to Alcaligenaceae (20.68%), 10.35% of the isolates belong to Enterobacteriaceae (10.35%) family followed by Moraxellaceae (3.45%), Brucellaceae (3.45%), Phyllobacteriaceae (3.45%), Flavobacteriaceae (3.45%), Xanthomonadaceae (3.45%). The phylogenetic tree was constructed using Maximum likelihood method with Kimura 2 parameter for Gram negative bacteria and Tamura Nei parameter for Gram positive bacteria according to the lowest BIC values using Mega5.05 with the estimated Transition/Transversion bias (R) value is 1.82 and 1.35 respectively.

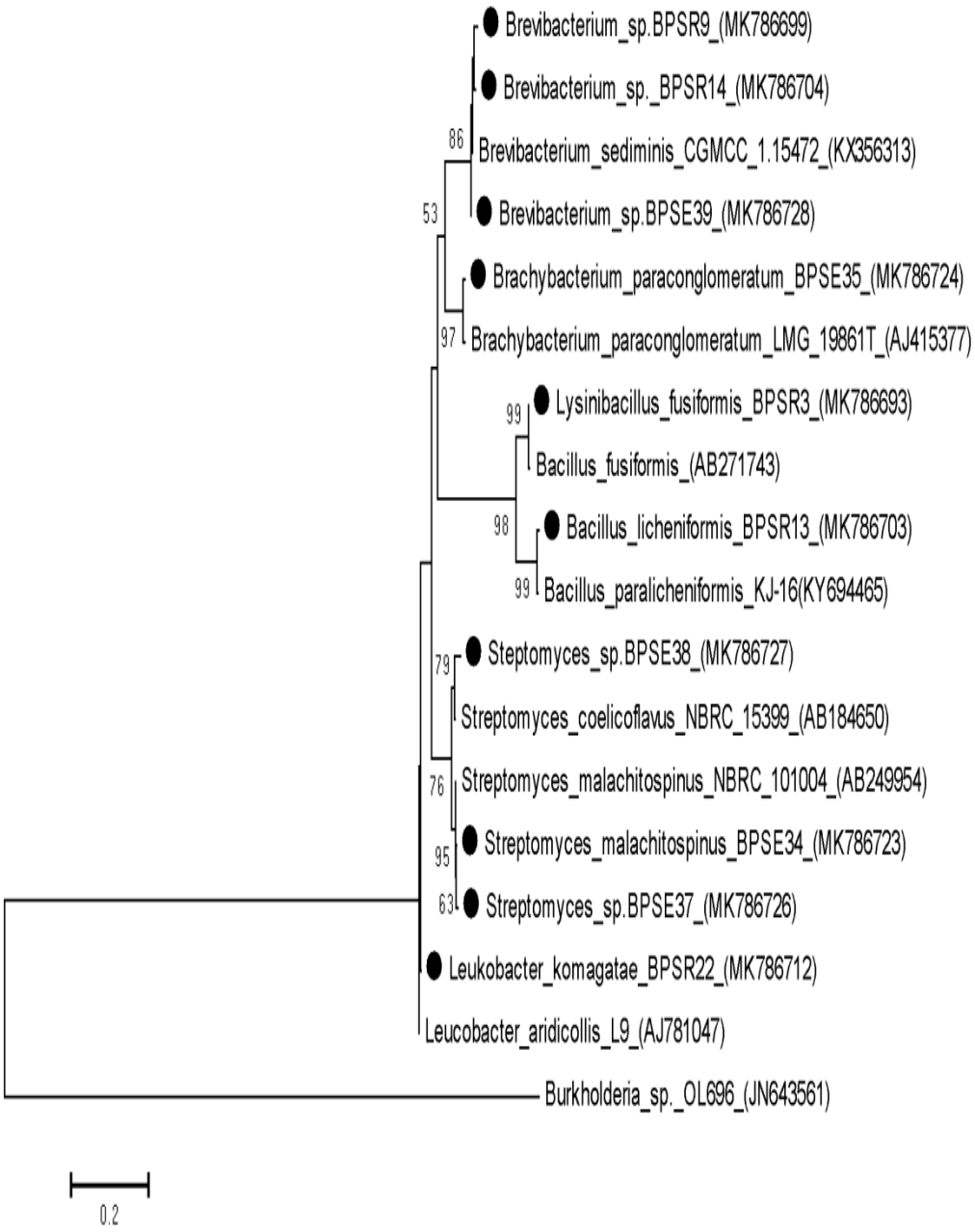

Figure 11: Maximum Likelihood Phylogenetic tree for Gram Positive Bacteria based on 16S rRNA gene

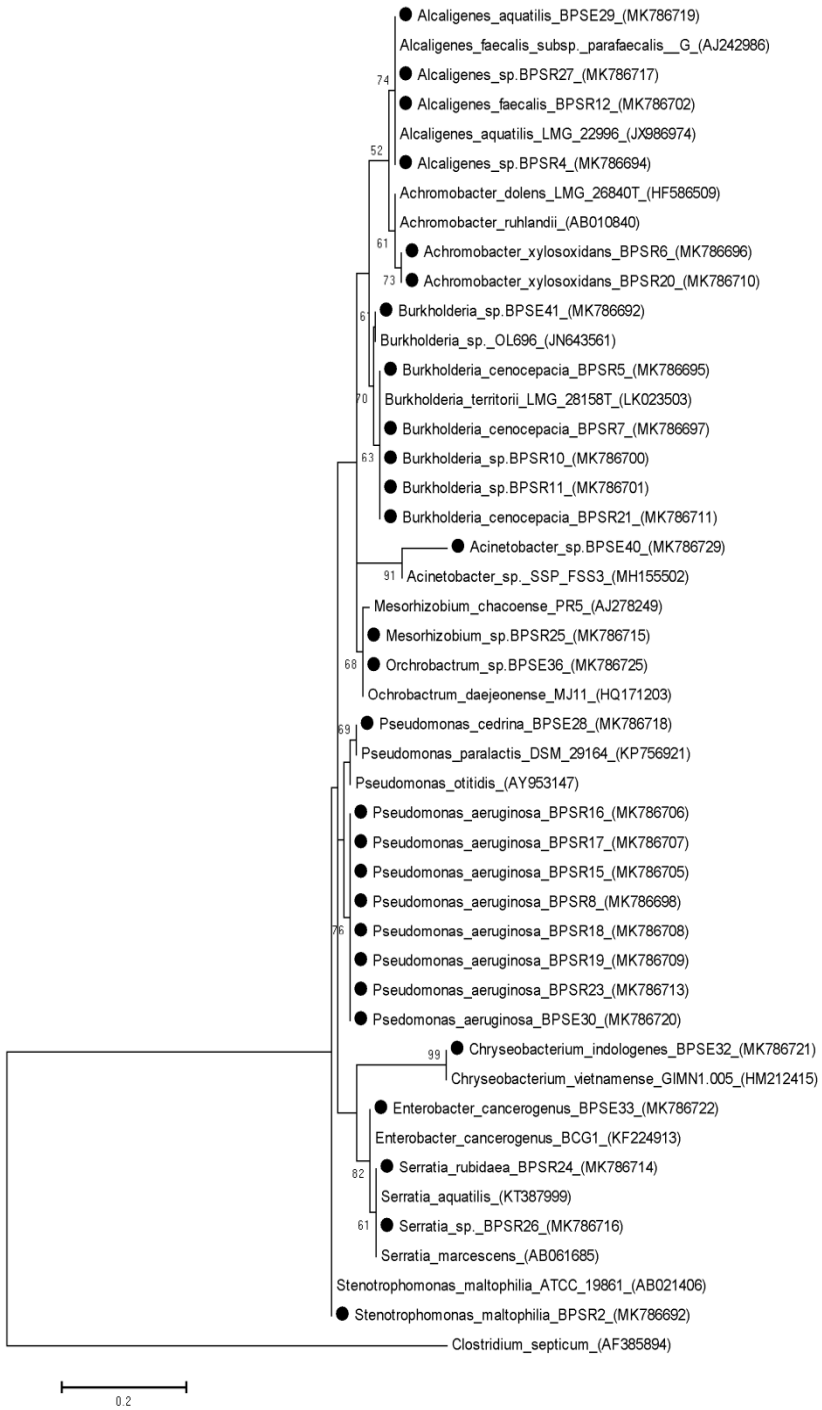

Figure 12: Maximum Likelihood phylogenetic tree for Gram Negative bacteria based on 16S rRNA gene

4.5. Screening for salinity stress tolerance of the isolates

The tolerance level of the isolates was observed by the growth of the isolates in the respective media supplemented with different concentrations of salt. The highest salt tolerance (25%) was observed in BPSR4, BPSR7, BPSR11, BPSE39, and BPSE41 (**Figure 13**).

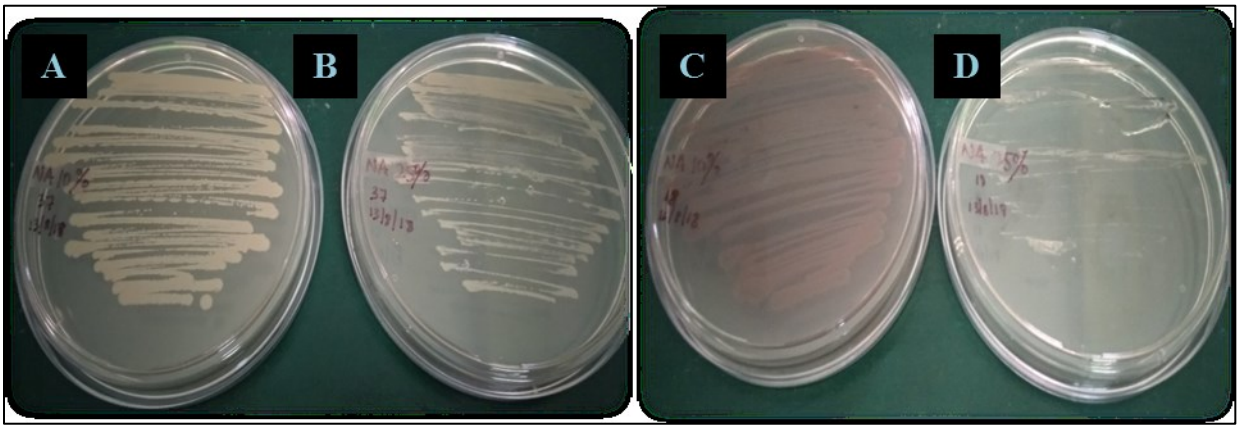

Figure 13: Growth on the salt-supplemented media indicates positive (A, B and C) and no growth on the media (D) indicates negative.

Table 3: **Ability to tolerate different concentration of salt by the isolates**

Sample No.	**NaCl concentration (%)**				
	5%	**10%**	**15%**	**20%**	**25%**
BPSR1	+	-	-	-	-
BPSR2	+	+	+	-	-
BPSR3	+	+	-	-	-
BPSR4	+	+	+	+	+
BPSR5	+	+	+	-	-
BPSR6	+	+	+	-	-
BPSR7	+	+	+	+	+
BPSR8	+	+	-	-	-

BPSR9	+	+	+	+	-
BPSR10	+	+	-	-	-
BPSR11	+	+	+	+	+
BPSR12	+	+	+	+	-
BPSR13	+	+	+	+	-
BPSR14	+	+	+	=	-
BPSR15	+	-	-	-	-
BPSR16	+	-	-	-	-
BPSR17	+	-	-	-	-
BPSR18	+	-	-	-	-
BPSR19	+	-	-	-	-
BPSR20	+	-	-	-	-
BPSR21	-	-	-	-	-
BPSR22	+	+	+	+	-
BPSR23	+	+	+	+	-
BPSR24	+	+	+	+	-
BPSR25	-	-	-	-	-
BPSR26	+	-	-	-	-
BPSR27	+	+	+	-	-
BPSE28	+	+	-	-	-
BPSE29	+	-	-	-	-
BPSE30	+	-	-	-	-
BPSE32	+	+	+	-	-
BPSE33	-	-	-	-	-
BPSE34	+	-	-	-	-

BPSE35	+	+	+	+	-
BPSE36	+	-	-	-	-
BPSE37	+	-	-	-	-
BPSE38	+	-	-	-	-
BPSE39	+	+	+	+	-
BPSE40	+	+	-	-	-
BPSE41	+	+	+	+	-

+ indicates positive activity and – indicates negative activity of slat tolerance

4.6. Synergistic effects among the selected isolates

Based upon the initial PGP activities and salt tolerance ability, two best isolates were selected and were used for further studies. BPSE41 (*Burkholderia* sp.) grows well at 25% of salt and BPSE28 (*Pseudomonas cedrina*) showed a maximum number of PGP traits. Selected two isolates were checked for their synergy of growing together and was observed that there was no inhibition on the growth of both the isolates as shown in **Figure 14.**

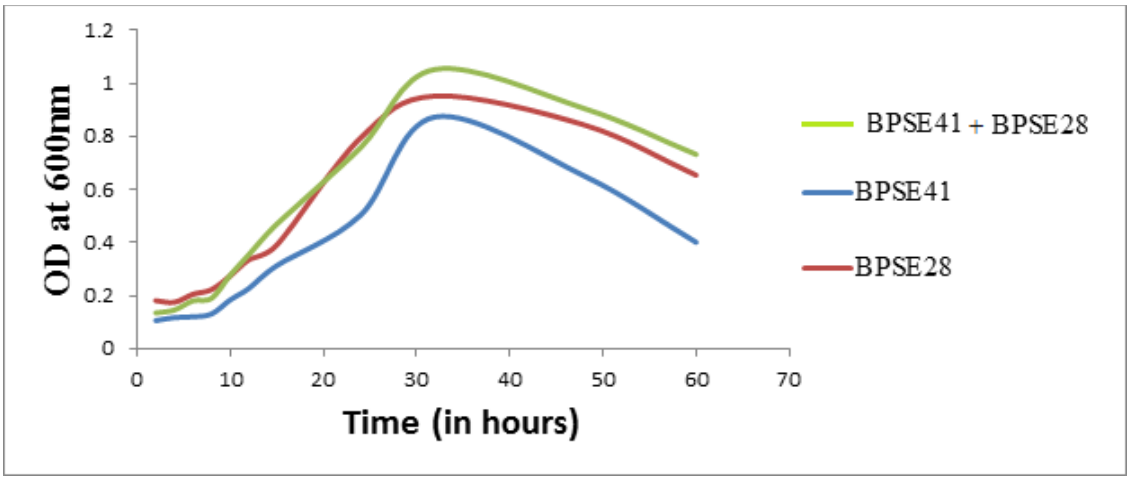

Figure 14: Growth curve of isolate no. BPSE41, BPSE28 and BPSE41+ BPSE28 to show the synergistic effect of BPSE41 and BPSE28

4.7. Effect of selected isolates on seed germination under salt stress

A chickpea plant seed was selected for the *in vivo* growth promotion assay. The seeds were surface sterilized and soaked in the bacterial suspension of selected groups (Group 1: BPSE41, Group 2: BPSE28 and group 3: Mixed). The seed germination percentage of Group 1, Group 2 and Group 3 was found to be 100% whereas the control group was only 80%. The shoot length of the germinated seeds was measured on the 8th day and the average was calculated and found that Group 1 and Group 2 was 3cm whereas in Group 3 it was 2 cm (**Figure 15**).

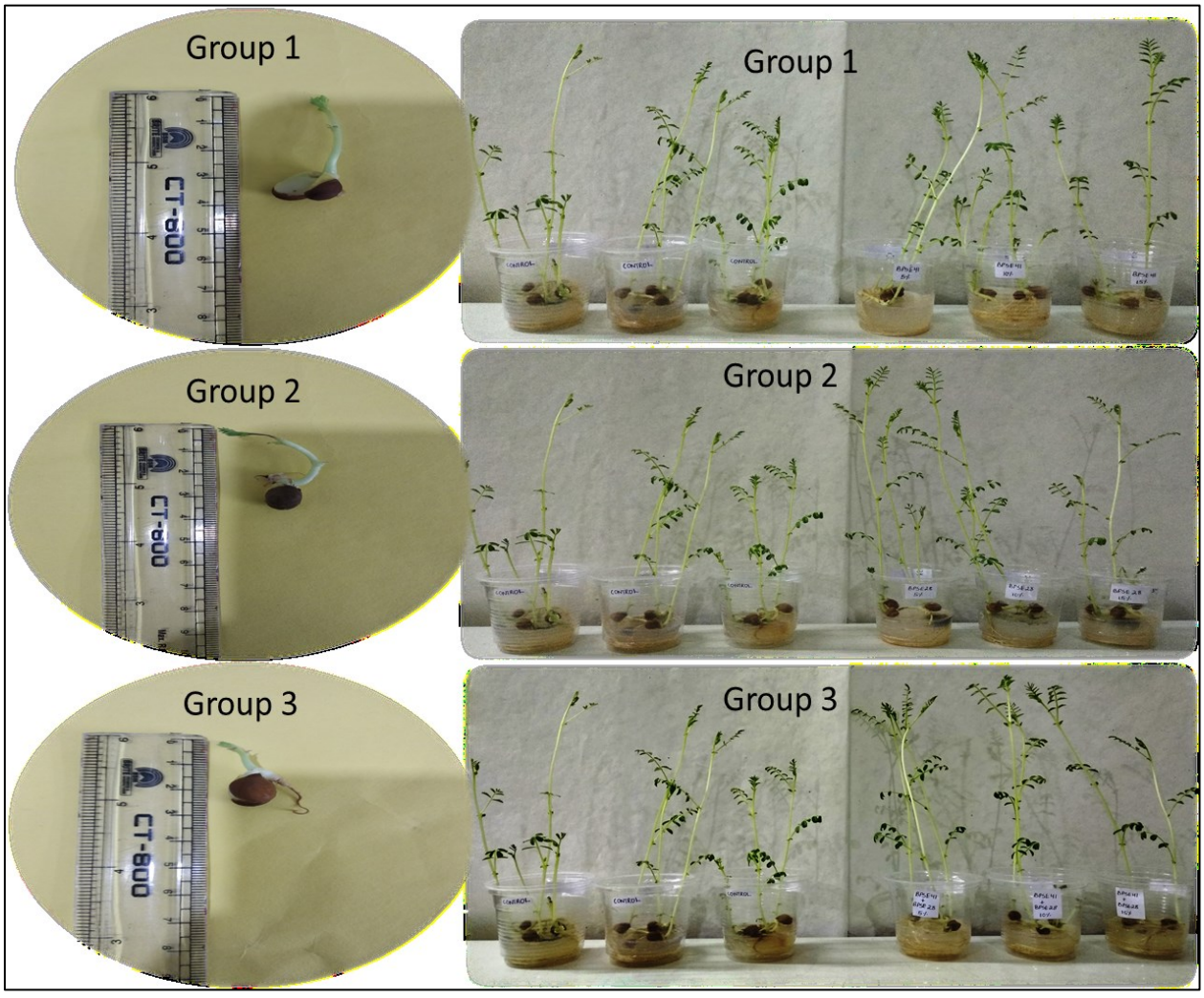

Figure 15: Seed Germination assay performed for Group 1 (BPSE41), Group 2 (BPSE28) and Group 3 (BPSE41+BPSE28). All the experiments were performed in triplicates.

4.8. Pot Experiments using selected three groups

Pot experiment was carried out for 30 days for all the three groups. On every alternative day the plant was given with Hoagland solution **(Figure 16)**. On the 31st day the plant physical parameters like the shoot length, root length, no. of branches and fresh weight was recorded and the plants were used for further experiments. As expected all the three groups have significantly enhanced the growth of Chick pea plant when compared with the un-inoculated control even in salt stress conditions. It was found that *Burkholderia* sp. has produced better and higher shoot length as compared to *Pseudomonas cedrina* under salt stress conditions. The benefit of *Burkholderia* sp. was most evident for plants grown at 10% salt as compared with other treatments.

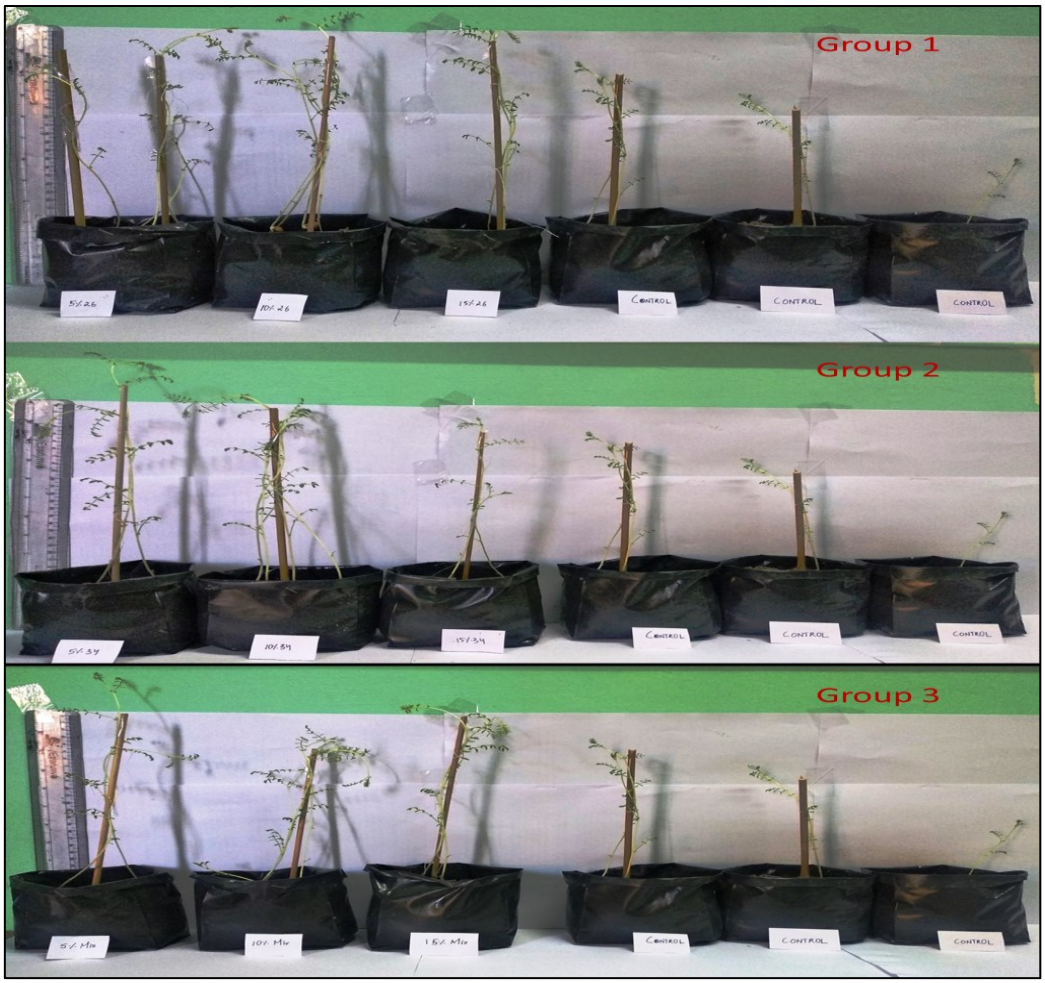

Figure 16: Plant grown on different concentrations of salt (5%, 10%, 15%) inoculated with Group1 (BPSE41), Group 2 (BPSE28) and Group 3 (BPSE41+BPSE28) along with Control.

4.9. Determination of Plant Growth Parameters

Root length, shoot length, the number of branches and the fresh weight of the plant was measured on the 31st day (**Table 4**). Group 1 showed maximum growth parameters at the 31st day as compared to other groups. The results showed that fresh weight, fresh weight and shoot and root length of the plant inoculated with Group 1 were not inhibited by salt stress (**Figure 17**). The results showed that the inoculation of BPSE41 may have eliminated the evident harmful effect of salt stress and have the same growth promotion effects.

Table 4: **Growth parameters of different groups with different concentrations of salt**

	Treatments	Root Length(cm)	Shoot Length(cm)	Branches
CONTROL	T0	14.0±3.1	14.3±2.6	6.3±0.4
BPSE28 (Group 2)	T1	17.6±2.1	20.0±3.6	8.0±0.8
	T2	16.6±2.4	19.6±2.0	7.6±0.4
	T3	15.8±3.2	15.8±4.6	8.0±0.8
BPSE41 (Group 1)	T1	17.0±1.8	17.0±2.9	8.0±0.0
	T2	**18.3±2.8**	**18.3±3.6**	**8.0±0.8**
	T3	17.3±1.8	17.3±2.8	8.3±0.9
BPSE28+	T1	16.5±3.7	16.5±1.2	7.6±0.4
BPSE41 (Group 3)	T2	16.6±4.6	16.6±2.8	7.3±0.4
	T3	16.6±3.8	16.16±3.6	7.6±0.4

T0: Soil without any salt ; T1- Soil amended with 5% salt, T2- Soil amended with 10-% salt; T3- Soil amended with 15% salt; data presented in Mean ± Standard deviation.

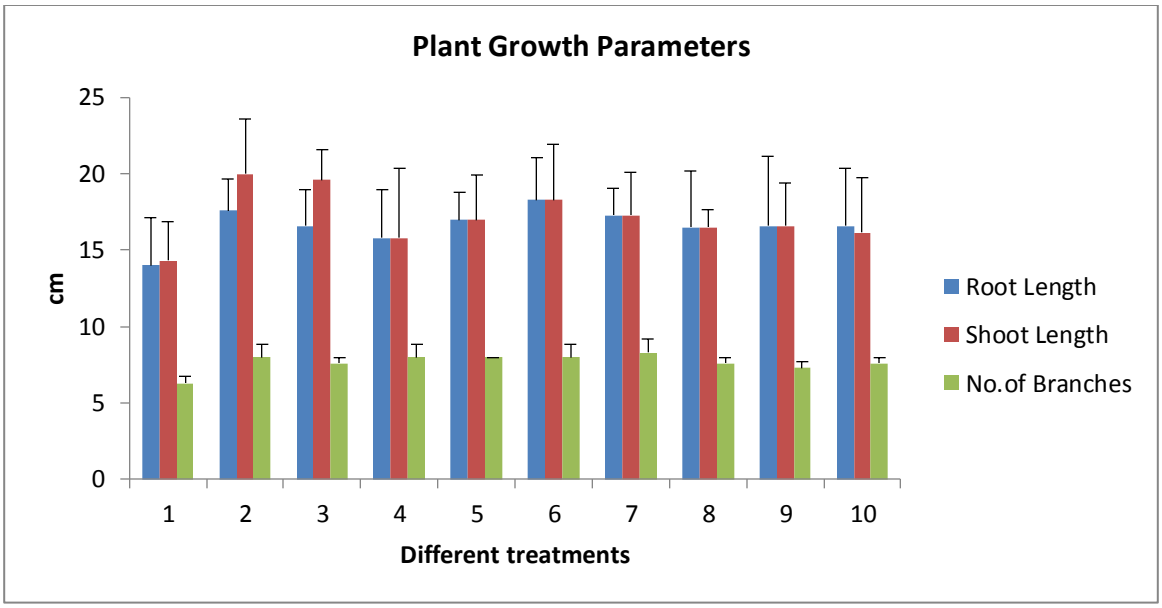

Figure 17: Root length, Shoot length and no. of branches of Chickpea plants. Bars represent means standard error

Figure 18: Chickpea plants with different salt concentrations inoculated with Group1 (BPSE28), Group2 (BPSE28) and Group3 (BPSE41+BPSE28) and Control.

4.10. Determination of Plant Physiological Parameters

4.10.1 Estimation of Chlorophyll content

On the 31st day when the fully grown plants were harvested, the chlorophyll content was estimated. Chlorophyll a content ranges from 1.28µg/ml to 2.98 µg/ml and Chlorophyll b ranges from 2.35µg/ml to 5.58µg/ml. The highest Total Chlorophyll (a+b) was found in Treatment 1 of Group 1 with the value of 8.27µg/ml as shown in **Table 5**.

Table 5: Chlorophyll content of Chickpea plant with different treatments

Samples	Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll (a+b)
CONTROL	T0	1.87	3.60	5.47
BPSE41	T1	2.90	5.37	8.27
	T2	2.00	3.74	5.74
	T3	1.28	2.35	3.63
BPSE28	T1	2.23	4.17	6.40
	T2	2.32	4.34	6.66
	T3	2.98	5.58	8.56
BPSE41+BPSE28	T1	2.29	4.27	6.56
	T2	1.67	4.60	6.27
	T3	1.39	2.58	3.97

T0: Soil without any salt ; T1- Soil amended with 5% salt, T2- Soil amended with 10-% salt; T3- Soil amended with 15% salt; data presented in Mean ± Standard deviation

4.10.2. Estimation of Catalase activity

The Methanolic extract was prepared for each plant inoculated with Group1, Group2 and Group3 grown in different concentrations of salt and the untreated control. All the extracts were tested

for catalase activity by adding H_2O_2 .All the ten extracts were found to have a catalase activity and among them, Treatment 2 of Group 3 showed maximum catalase activity with the value of 1.15U.

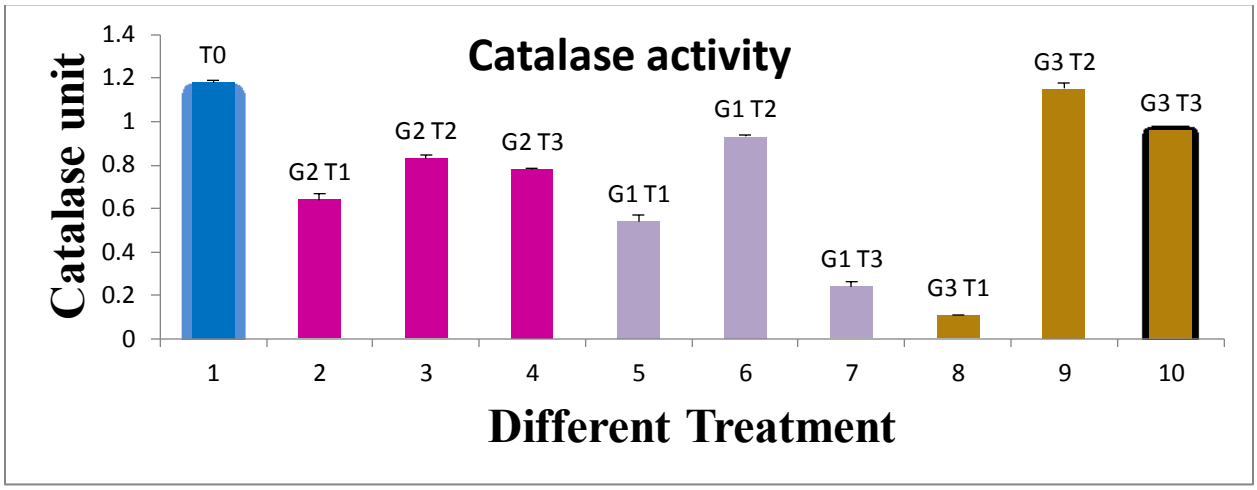

Figure 19: Catalase activity of Chickpea plant. G1: Group1, G2: Group 2 and G3: Group 3. T1: Treatment with 5% salt, T2:Treatment with 10% salt, T3: Treatment with 15% salt. T0: Treatment without salt

4.10.3. ABTS and DPPH Scavenging Assay

All the ten methanolic extracts were used for ABTS and DPPH assay. It was found that the DPPH activity increases as the concentration of the plant extracts increases. The methanolic plant extract of Group 1 T3 with IC50 values of 38.59µg/ml showed the highest DPPH scavenging assay (**Table 6**). Similarly, ABTS activity increases with the increase in concentration and the methanolic plant extract of Group 1 T3 with IC50 values of 0.007µg/ml shows the highest ABTS radical scavenging activity (**Table 7**).

Table 6: DPPH scavenging activity of different groups using different treatments

SAMPLE	TREATMENT	IC_{50} values
BPSE41(Group1)	T1	49.30±0.69
	T2	269.30±0.43
	T3	**38.59±0.47**
BPSE28 (Group2)	T1	1347.0±0.95
	T2	340.80±0.74
	T3	103.70±0.08
BPSE28+BPSE41(Group3)	T1	392.40±0.32
	T2	73.490±0.21
	T3	39.360±0.12
Control		969.80±0.30

Data presented in Mean ± Standard deviation from triplicate sample

Table 7: ABTS assay of different groups using different treatments

SAMPLE	TREATMENT	IC_{50} values
BPSE41(Group1)	T1	0.873±0.46
	T2	3.186±0.34
	T3	**0.007±0.93**
BPSE28 (Group2)	T1	10.68±0.65
	T2	8.602±0.09
	T3	2.062±0.87
BPSE28+BPSE41(Group3)	T1	9.717±0.76
	T2	0.419±0.89
	T3	0.044±0.87
CONTROL	T0	10.75±0.96

Data presented in Mean ± Standard deviation from triplicate sample

Chapter 5

DISCUSSION

Plant Growth Promoting Bacteria (PGPB) is the group of potential microorganisms that promote plant growth and development by regulating nutritional availability to the plants and maintaining hormonal balance, as well as solubilizing nutrients for the easy uptake of the plants (Patten and Glick, 2002). Inoculation of PGPR on the plants does not only enhanced the plant growth it also makes the plant to tolerate or withstand several stress conditions (Nadeem *et al*., 2014). Therefore this study was focused on the screening of potential bacteria from rhizospheric soil and endophytic bacteria from the endosphere of selected two traditional medicinal plants to promote plant growth as well as withstand salinity stress in the soil. Fifty bacterial isolates were isolated and were critically investigated for their Plant Growth Promoting (PGP) properties along with their salt tolerance potential.

Phosphate solubilization ability is one of the prominent PGP traits as Phosphorus is a macronutrient and is a basic component of a fertilizer (Pereira and Castro, 2014). But the availability of phosphorus in the soil is very limited since it is mostly present in the form of insoluble phosphate (Feng *et al*., 2004). Our results suggested that *Pseudomonas cedrina* (BPSE28) showed the highest phosphate solubilizing activity among the 50 isolates with the Phosphate Solubilizing Index (PSI) value of 7.3±0.80. The quantitative assay showed that the amount of phosphate solubilization was 61µg/ml. Similarly, Kaur and Sharma, 2013 have reported 13.45mg/100ml in *Pseudomonas* sp. whereas Yang *et al*., 2018 have reported 31.82 mg/L in *Pseudomonas fluorescens*. Liu *et al*., 2019 has also demonstrated the phosphate-solubilizing ability of *Pseudomonas* sp. P34 and was able to solubilize 101.6 µg/ml and in turn,

enhance the growth of Wheat plant. Kaundal *et al*., 2017 has also demonstrated the phosphate-solubilizing ability of *Pseudomonas* sp. which was able to solubilize 84 µg/ml insoluble phosphate. Majeed *et al*., 2018 has reported the Phosphate Solubilizing Index of *Pseudomonas* sp. as 3.03 and was able to solubilize 59.37 µg/ml of insoluble phosphate. Majeed *et al*., 2018 has demonstrated the ability of the phosphate solubilizing *Pseudomonas* sp. to enhance the growth of *Helianthus annuus* plant. There are also several reports on other strains of bacteria to be able to solubilize Phosphate like *Burkholderia* sp. with PSI value of 2.15 (Pandey *et al*., 2005), *Serratia marcescens* with PSI value of 4.3 (Nakbanpote *et al*., 2014). From all these findings we can conclude that *Pseudomonas cedrina* (BPSE28) is one of the best Phosphate Solubilizing Bacteria that solubilize insoluble phosphate to soluble form for easy uptake for the plants and therefore can continue to act as plant growth promoting agent.

1-Aminocyclopropane-1-carboxylate (ACC) deaminase is a PGP trait responsible for modulating stress which is produced by some beneficial bacteria (Yoolong *et al*., 2019). ACC is the immediate biosynthetic precursor of the hormone ethylene in plant tissues; the enzyme ACC deaminase hydrolyzes 1-aminocyclopropane-1- carboxylic acid (ACC) to ammonia and α-ketobutyrate. In this way, it reduces the amount of the inhibitory effect of ethylene on root elongation, and thus promoting plant growth (Laslo *et al*., 2012). From our results, we have found that among 50 bacterial isolates 22 (44%) isolates can produce ACC deaminase enzyme. Similarly, Bal *et al*., 2012 has reported that out of 66 isolates 7 (10.6%) isolates were positive for ACC deaminase production. At the same time reports on ACC deaminase production was given by Nadeem *et al*., 2010, where 37.2% i.e18 isolates out of 55 isolates that were isolated from the rhizospheric soil have been recorded to have the ability to produce ACC deaminase. Qin *et al*.,

2013 has isolated 126 isolates from plant tissues and out of that only 10.31% of the total isolates showed positive for ACC deaminase production which is also in agreement with our study.

Production of Indole-3-acetic acid (IAA) is a key regulator of most of the plant growth and development *(*Ahemad and Kibret, 2014). Among our reported 50 isolates, *Pseudomonas cedrina* can produce a maximum amount of IAA with the value of 53.60µg/ml. Production of IAA by *Pseudomonas fluorescens* was reported by Nadeem *et al*., 2016 where the IAA production reported is 5.24mg/L which is comparatively lesser than our reported IAA value. Cardinale *et al*., 2015 has also reported 12.82 µg/ml IAA productions by the same organism, *Pseudomonas fluorescens*. Oves *et al*., 2013 has reported a comparable decrease in IAA production by *Pseudomonas aeruginosa* with an IAA concentration of 32µg/ml. Similarly, *Pseudomonas putida* GR12-2 can produce 32.7µg/ml IAA (Patten and Glick, 2002). Another study was done by Majeed *et al*., 2018 has reported 23.9 µg/ml. IAA productions by *Pseudomonas* sp. and Tiwari and Singh, 2017 has reported 13.2µg/ml IAA production by *Pseudomonas aeruginosa.*

Bacteria not only inhibit the fungal growth by the production of siderophore but it also induced systematic resistance in plants by taking up iron from the soil and promote the plant growth indirectly (Ghyselinek *et al*., 2013). Several past studies have suggested a close relationship between siderophore productions with the antifungal activity of plant growth promoting rhizobacteria. According to our results, 33 (66%) isolates out of 50 have exhibited siderophore production. Similarly, Wang *et al*., 2018 has reported more or less the same percentage for siderophore production where it was reported that 8 out of 13 isolates can produce siderophore which means 61.5% of the total isolates can produce siderophore which is comparatively lower than our reported value.

Antagonistic activity against fungal pathogens increases the resistance of different plant pathogens and is one of the most important PGP traits. In the present study, the maximum inhibition percentage was shown by *Pseudomonas fluorescens* (BPSE28) with an inhibition percentage of 67.3% against *Fusarium graminearum.* Similarly, the growth of *Fusarium oxysporum* was also inhibited by *Pseudomonas* sp. with 51.11 percentage of inhibition (Verma *et al*., 2017) of Likewise, other bacterial strains have also been reported by several researchers, like the ability of *Burkholderia cepacia* to inhibit the growth of different fungal pathogens like *Fusarium oxysporum*, *Fusarium culmorum*, and *Fusarium sambucinum* has been reported by Recep *et al*., 2009. Extracellular enzymes produced by bacteria can degrade fungal cell wall and therefore the production of extracellular enzymes and anti-fungal properties are well connected (Wang *et al*., 2018) From our results, the ability to produce Xylanase occurs the most when compared with other extracellular enzymes. The production of laccase and amylase is hardly seen, and one isolate is positive for Cellulase enzyme production. PGPB which can produce extracellular enzymes has a better chance to withstand salinity as compared with other PGPB (Kumar *et al*., 2017)

Thirty-nine isolates were identified using 16S rRNA gene sequencing. The bacterial strains showed diversities at the genus-species to- strain level. All these 39 isolates were confirmed to have at least one PGP trait including phosphate solubilization, IAA and siderophore production, ACC deaminase production and having antagonistic activity against fungal pathogens.

Soil salinity destroyed the plant by changing the physiological and biochemical processes like membrane degradation etc. which results in the production of reactive oxygen species (ROS) such as hydroxyl radical, hydrogen peroxide (Geros *et al*., 2016). From the 39 selected PGP bacterial strains, four isolates were able to tolerate salt up to 25% which suggested that those

four isolates not only can promote plant growth, they also can withstand salt stress. According to our results, *Burkholderia* sp. can tolerate up to 25% salt. Where Pandey *et al*., 2005 has reported 2% salt tolerance of *Burkholderia* sp., which suggested that bacteria from these locations are more efficiency

Comparing all the 39 positive PGP strains, BPSE28 (*Pseudomonas fluorescens*) was the best among all the tested isolates and have the best PGP traits, and BPSE41 (*Burkholderia* sp.) was able to tolerate the highest concentration of salt i.e. 25%. The synergistic effect study suggested that *Pseudomonas fluorescens* (BPSE28) and *Burkholderia* sp. (BPSE41) showed that both organisms grow well together without inhibiting each other growth. This was seen when the growth curve of the mixed culture was compared with the growth curve of single isolates i.e. BPSE41 and BPSE28.

There was an increase in seed germination percentage when the seeds were inoculated with the selected bacterial strains (BPSE41 and BPSE28) when compared with the seeds without bacterial inoculation This suggested that *Pseudomonas fluorescens* and *Burkholderia* sp. are plant growth promoting bacteria that enables the seedling growth. In addition to that, *Pseudomonas fluorescens* and *Burkholderia* sp. has increase the fresh weight, root length and shoot length of the chickpea plant when grown in pots with salt amended with different concentration of salts (5%,10%, 15%) *Burkholderia* sp. has better growth promoting ability than *Pseudomonas fluorescens* in case of pot experiments which could be due the salt tolerance ability of *Burkholderia* sp. in other words, *Burkholderia* sp. has the ability to promote plant growth as well as the ability to tolerate high concentration of salt. Even though, *Pseudomonas fluorescens* can promote plant growth it cannot tolerate salt which explains the reason why *Pseudomonas fluorescens* exhibit lesser plant growth promoting ability. It is obvious from the result that

Burkholderia sp. BPSE41 may have the ability to eliminate the evident damaging effect of salt stress and exhibit the same plant growth promotion. Maxton *et al*., 2017 has reported *Burkholderia cepacia* to have the ability to tolerate salt up to 240mM in the pot as well as increase the plant growth. Previous studies have also reported that the inoculation of *Burkholderia phytofirmans* has significantly increased the fresh weight, dry weight, shoot height and shoot length of *Arabidopsis thaliana* under salt stress up to 250mM (Pinedo *et al*.,2015). Other than *Burkholderia* sp., *Bacillus megatarium* can significantly increase the fresh weight, dry weight, shoot height and shoot length of *Capsicum annum* under salt stress condition with a salt concentration of 300mM (Wang *et al*., 2018). Upadhayay and Singh *et al*., 2014 have reported the salt tolerance ability of *Arthrobacter* sp. and enabling the growth of the wheat plants. Habib *et al*.,2015 demonstrated a salt tolerance of *Enterobacter* sp. on Okra plant and was able to conclude that *Enterobacter* sp. can increase the shoot length, dry weight and fresh weight of Okra plant under 75mM salt concentration.

Chlorophyll-a and chlorophyll-b are the photosynthetic pigments that are responsible for the process of photosynthesis. In our results, Chickpea plant inoculated with *Burkholderia* sp. (BPSE41) showed a significant accumulation of total chlorophyll on the plants treated with 5% of salt. It has increased the chlorophyll content by 38.9% as compared with the non-inoculated control. However, there was a report in decreased chlorophyll content with plants grown under salt stress in the paddy plant (Kumar *et al*., 2017). There are several reports on the increasing production of chlorophyll in different strains of plant growth promoting bacteria like Wang *et al*., 2018 has reported that the inoculation of *Bacillus subtilis* on *Capsicum annuum* has significantly increased the chlorophyll content under saline condition.

The reactive oxygen species (ROS), like peroxides, superoxide, singlet oxygen, etc. causes oxidative damage to cells when the plant is under abiotic stress (Apel and Hirt, 2004). ROS scavenging enzyme like catalase can reduce the level of these molecules to a normal level (Ahmad *et al*., 2014). The higher levels of the catalase in the PGP inoculated plants can be connected with the enhanced tolerance towards salinity stress (Kohler *et al*., 2010). In the present study, the catalase activity increases in the plant inoculated with both *Burkholderia* sp. (BPSE41) and *Pseudomonas cedrina* (BPSE28), this result suggested that the inoculation of *Pseudomonas cedrina* which is a good Plant growth promoting bacteria (PGPB) and *Burkholderia* sp. which is a Salt tolerant Plant growth promoting bacteria (ST-PGPB) increases the antioxidant activity and therefore reduces the Reactive Oxygen Species. Other researchers have reported on *Klebsiella michiganensis* to be able to increase the antioxidant enzymes activity on the plant seedlings under stress (Mitra *et al*., 2018). There are several methods to measure the antioxidant capacity and among them, DPPH and ABTS assay is a simple and rapid method. In our present study, the plant treated with 15% of salt inoculated with *Burkholderia* sp. (BPSE41) has the best antioxidant activity against DPPH free radicals with IC50 value of 38.59µg/ml and the same treated plant inoculated with *Burkholderia* sp. (BPSE41) showed the best ABTS decolourization activity with IC50 value of 0.007µg/ml. This result suggested that BPSE41 is a potent antioxidant.

CONCLUSION

In the present study, a total of fifty isolates were isolated from rhizospheric soil and two selected plants (*Dillenia indica* and *Centella asiatica*). All the fifty isolates were investigated for their plant growth promoting potential. King's Media was the best suitable nutritional media for growing the isolated bacteria and yields the maximum number of isolates (n=16). From the fifty isolates 44% (n=22) of the isolates were positive for ACC deaminase production and 58% (n=29) were able to solubilize inorganic phosphate, the phosphate solubilization value ranges from 30.07µg/ml to 152.61µg/ml. The highest Phosphate solubilization ability was shown by *Pseudomonas cedrina* (BPSE28). Seventeen isolates (34%) of the total isolates can produce IAA phytohormones and the concentration of IAA produced by the isolates ranges from 2.08µg/ml to 53.60µg/ml. The highest IAA production was shown by *Pseudomonas cedrina* (BPSE28). Among the 50 isolates, 7 isolates can produce Xylanase whereas; Cellulase production ability was shown by only 1 isolate. Screening for siderophore production showed 66% of the total isolates were positive and were having the ability to produce siderophore. All isolates were screen for their *in vitro* antagonistic potential against three fungal pathogens, where 28.2% of the isolated bacteria showed antagonistic activity against at least one of the tested pathogens in which the percentage of inhibition ranges from 23.3 to 67.6%. Isolates that exhibit at least one Plant Growth Promoting (PGP) trait were further selected for molecular characterization and phylogenetic analysis. Out of 50 isolates, 39 isolates were identified based on 16S rRNA gene sequences. 39 identified isolates were screened for their salt tolerance ability and the highest salt tolerance (25%) was observed in BPSR4, BPSR7, BPSR11, BPSE39 and BPSE41. Two best isolates (BPSE41 and BPSE28) based on the salt tolerance ability and plant growth promoting ability was selected. The synergistic effect study from the selected two isolates showed that the

two isolates do not inhibit the growth of each other. We conclude that BPSE41 has the maximum growth promoting potential based on the *in vivo* seed germination assay and pot experiments which can be due to its salt tolerating ability. The highest shoot length and root length of the chickpea plant were shown by the plant treated with BPSE41 (*Burkholderia* sp.). Chlorophyll content was the maximum in plant inoculated with BPSE41 (*Burkholderia* sp.). Antioxidant assay like ABTS and DPPH activity was also the highest in plants inoculated with BPSE41 (*Burkholderia* sp.). Therefore, we conclude that the strain can be used to make bioformulation for the agricultural fields affected by salinity stress.

ABBREVIATIONS

PGP	:Plant Growth Promoting
ACC	:1-aminocyclopropane-1-carboxylic acid
DNA	:Deoxyribonucleic acid
RNA	:Ribonucleic acid
ROS	:Reactive Oxygen Species
mM	:Millimolar
SCA	:Starch Casein Agar
AIA	:Actinomycetes Isolation Agar
ISP7	:International Streptomyces Project 7
ISP5	:International Streptomyces Project 5
IAA	:Indole acetic acid
PSI	:Phosphate Solubilizing Index
PDA	:Potato Dextrose Agar
PCR	:Polymerase Chain reaction
µg	:Microgram
ml	:Millilitre
NCBI	:National Centre for Biotechnology Information
U	:Unit
ABTS	:2,2'-Azino-Bis-3-Ethylbenzothiazoline 6-Sulfonic Acid
DPPH	:2,2-diphenyl-1-picrylhydrazyl
ITCC	:Indian Type Culture Collection
MTCC	:Microbial Type Culture Collection
WHO	:World Health Organization

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NAME OF CANDIDATE : LALROKIMI

DEGREE : MASTER OF PHILOSOPHY

DEPARTMENT : BIOTECHNOLOGY

TITLE OF DISSERTATION : ISOLATION AND IDENTIFICATION OF PLANT GROWTH-PROMOTING BACTERIA TO ALLEVIATE SALT STRESS TOLERANCE IN CROPS

DATE OF PAYMENT OF ADMISSION : 25/08/2017
(Commencement of first semester)

COMMENCEMENT OF SECOND SEM/DISSERTATION : FEBRUARY 2018
(From conclusion of end semester exams)

APPROVAL OF RESEARCH PROPOSAL

1. BOS : 26/04/2018
2. SCHOOL BOARD : 03/05/2018

   REGISTRATION NO. & DATE : MZU/M.Phil./472 0f 03.08.2017

   DUE DATE OF SUBMISSION : 31 JANUARY 2019

   EXTENSION (IF ANY) : 28 JUNE 2019

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Educational Qualification:

SL. No	Qualification	Subject	Board/University	Percentage
1.	Master (M.Sc)	Zoology	University of Madaras	78%
2.	Bachelor (B.Sc)	Advanced Zoology and Biotechnology	University of Madaras	82%
3.	HS (12^{th})	Biology, Chemistry, Physics, Mizo, English	MBSE	72%
4.	HSLC (10^{th})	Science, Maths, English, Mizo, Social Studies, IT	MBSE	68%

- **Computer skills:** Course on Computer Concept (CCC) under National Institute of Electronics and Information technology (NEILIT)

Present Position:

- Doing Master of Philosophy (M.Phil) entitled "**Isolation and Identification of Plant growth promoting bacteria to alleviate salt stress tolerance in crops**" under the supervision of Dr. Bhim Pratap Singh in Department of Biotechnology, Mizoram University.

Conference/Seminar/Training attended:

- **Lalrokimi,** Ajit Kumar Passari, Zothanpuia and Bhim Pratap Singh*. Presented poster entitled "**Isolation of plant growth promoting bacteria and seed germination effects in *Cicer arietinum***". In "4th National Conference on plant growth promoting rhizobacteria (PGPR) for sustainability of agriculture and environment" to be held during May 11-12' 2018 organized by

Department of Biotechnology, Mizoram University and India Chapter of Asian PGPR Society for Sustainable Agriculture in association with Asian PGPR Society for Sustainable Agriculture.

- Training in State Biotech Hub, Department of Biotechnology, Mizoram University, to learn "**Basic techniques in Molecular Biology" under the guidance of Prof. N. Senthil Kumar** (01st Apr- 30th May, 2014).
- Training in BIF, Department of Biotechnology, Mizoram University, to learn "**Basic tools in Bioinformatics and Molecular Phylogenetic stduy" under the guidance of Prof. N. Senthil Kumar** (20th May- 20th June, 2016).

Publications : 02

1. Lalrokimi, Souvik Ghatak, Ravi Prakash Yadav, Lalhma Chhuani, Doris Lallawmzuali, Jeremy L. Pautu, N. Senthil Kumar (2016). Relevance of GSTM1, GSTT1 and GSTP1 gene polymorphism to breast cancer susceptibility in Mizoram population, NorthEast India. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences.
2. Tochhawng, L., Mishra, V. K., Passari, A. K., & Singh, B. P. (2019). *Endophytic Fungi: Role in Dye Decolorization. In B. P. Singh (ed.), Advances in Endophytic Fungal Research, Fungal Biology, https://doi.org/10.1007/978-3-030-03589-1_1 Springer.* **1–15**.doi:10.1007/978-3-030-03589-1_1.

Declaration

I hereby declare that all the information mentioned above is true to the best of my knowledge and belief. I will be solely responsible if any of the information is found wrong.

Place: Aizawl, Mizoram **LALROKIMI**

Date: 26-06-19