SCREENING, CHARACTERIZATION AND IDENTIFICATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA AS POTENTIAL BIOFERTILIZERS FOR OLERICULTURE

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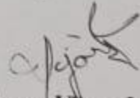
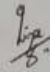
CERTIFICATE

This is to certify that the dissertation titled, " **SCREENING, CHARACTERIZATION AND IDENTIFICATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA AS POTENTIAL BIOFERTILIZERS FOR OLERICULTURE**", is an authentic record of work carried out by **ARATHI .S.R** under the supervision and guidance of **Dr. ALPHONSA VIJAYA JOSEPH**, Associate Professor, Department of Botany & Centre for Research, St Teresa's College (Autonomous), Ernakulam in partial fulfilment of the requirement for the Master's Degree of Science in Botany.





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INTRODUCTION

Bacteria that colonize the roots of plants (rhizosphere) and promote plant development are known as "plant growth promoting bacteria (Ahmad et al., 2008). The rhizosphere is the area of soil where the roots grow. The population of microbes found in rhizosphere differs from that of its surrounds because of the presence of root exudates, that serve as a source of nutrient for the microbial growth (Burdman et al., 2000). Weller and Thomashow (1994) proved that the narrow rhizosphere zone is rich in nutrients for microbes when compared to the bulk soil; this is demonstrated by the number of bacteria present around the roots of the plants, which is often 10 to 100 times higher than in the bulk soil. The use of PGPR as a multifunctional bio-fertilizer or bio-stimulant is an alternative method of preventing soil pollution and preserving agriculture for a long-term economic future. Microbes are ubiquitous in the biosphere, and their presence always has an impact on the environment in which they thrive. Microbe's effects on their environment can be positive, detrimental, or undetectable by human measurement or observation. Microbes have the most significant impact on the planet because of their ability to reproduce. To recycle the fundamental elements that all living systems are made up of, namely carbon, oxygen, and nitrogen (N). Primary production involves photosynthetic organisms which take up CO2 from the atmosphere and convert it to organic (cellular) material. The process is also called CO2 fixation, and it accounts for a very large portion of organic carbon available for synthesis of cell material. Decomposition or bio-degradation results in the breakdown of complex organic materials to other forms of carbon that can be used by other organisms. Naturally occurring organic compound can be broken down by bacteria. Although certain synthetic chemicals, such as Teflon, plastics, insecticides, and pesticides, are broken down very slowly or not at all by the microbes. Through the microbial metabolic processes of fermentation and respiration, organic molecules are eventually broken down to CO2 which is returned to the atmosphere for continuous process of primary production. Biological nitrogen fixing is a process that occurs only in bacteria and involves the removal of N2 from the atmosphere turns it to ammonia (NH3), which plants and animals can utilize. Nitrogen fixation also replenishes soil nitrogen that has been lost by agricultural activities. Microbes, in addition to all of these advantages, play an important role in environmental sustainability (Gupta et al., 2017).

Bacteria, fungi, actinomycetes, protozoa, and algae are among the microbes that colonize the rhizosphere (Kaymak et al., 2010). Bacteria, on the other hand, are the most prevalent microorganism in the rhizosphere. The utilization of these microbial communities to improve

plant growth is well-known and verified (Sharan et al., 2011; Battacharyya et al., 2012). The name "plant growth promoting rhizobacteria (PGPR)" was coined to describe these beneficial microbes. Kloepper and Schroth (1978) have paved the door for more PGPR findings. PGPR are not only related with the root and have beneficial effects on plant development, but they also have beneficial impacts on phytopathogenic microbe management (kloepper et al., 1980; Son et al., 2014). As a result, one of the active elements in bio-fertilizer formulation is PGPR.

PGPR can be divided into symbiotic bacteria, which live inside plants and exchange metabolites directly with them, and free-living rhizobacteria, which dwell outside plant cells (Gray et al., 2005). PGPR working mechanisms can also be divided into two categories. There are direct and indirect ones. The direct mechanisms are bio-fertilization, stimulation of root growth, rhizoremediation, and plant stress control. On the other hand, the mechanism of biological control by which rhizobacteria are involved as plant growth promotion indirectly is by reducing the impact of diseases, which include antibiosis, induction of systemic resistance, and competition for nutrients and niches (Egamberdieva et al., 2014). Symbiotic bacteria mostly reside in the inter-cellular spaces of the host plant, but there are certain bacteria that are able to form mutualistic interactions with their hosts and penetrate plant cells. In addition to that, a few are capable of integrating their physiology with the plant, causing the formation of specialized structures. Rhizobia, the famous mutualistic symbiotic bacteria, could establish symbiotic associations with leguminous crop plants, fixing atmospheric nitrogen for the plant in specific root structures known as nodules.

Role of Plant Growth Promoting Rhizobacteria for Plant Growth Enhancement is through a number of processes, PGPR plays a vital role in boosting plant development. PGPR promotes plant growth through (i) Abiotic stress tolerance in plants; Abiotic stressors are thought to be the primary cause of agricultural production loss. However, the severity of abiotic stress varies depending on soil type (hormonal and nutritional imbalances), as well as plant characteristics (physiological abnormalities such as disease susceptibility, abscission, and so on) (Nadeem et al., 2010). The mechanisms of PGPR in plants in response to abiotic stress have previously been thoroughly investigated. According to Pishchik (2002) the detrimental effect of cadmium pollution on barley plants could be mitigated by bacteria's ability to remove cadmium ions from the soil via binding processes, reducing cadmium availability in the soil. Furthermore, (Nautiyal et al., 2008) found that the *Bacillus lentimorbus* strain improved the antioxidant capacity of spinach, carrots, and lettuce edible sections while also enhancing growth. The findings are critical, especially in terms of improving the nutrient content of these crops. The

improvement of leaf water status, notably under salinity and drought stress, is another prominent effect of PGPR on plants under abiotic stress conditions (Ahmad et al., 2013; Naveed et al., 2014). According to Sarma and Saikia (2014) *Pseudomonas aeruginosa* strains boosted the growth of *Vigna radiata* (mung bean) plants under dry circumstances. Plants' ability to use water for growth is determined by their stomatal openings. The stomata on a plant's leaf balance the amount of water in the leaf and the amount of water taken up by the roots. (ii) Nutrient fixing for easy uptake by plants: By fixing nutrients, PGPR can increase the availability of nutrient concentration in the rhizosphere (Choudhary et al., 2011), preventing them from draining out. Nitrogen, for example, is the most limiting nutrient for plants since it is required for the synthesis of amino acids and proteins. Prokaryotes are the only organisms that can convert atmospheric nitrogen into organic forms that plants may use (Lioret et al., 2005). *Azospirillum* is a rare free-living nitrogen-fixing fungus that is commonly linked with cereals in temperate zones and has also been observed to boost rice crop yields. Some PGPR have the potential to solubilize phosphate (Wani et al., 2007), resulting in enhanced phosphate ion availability in the soil that can be easily absorbed by plants. Phosphate solubilizer, IAA producer, and siderophore producer were identified in *Kocuria turfanensis* strain 2M4 isolated from rhizospheric soil (Goswami et al., 2014). The effect of PGPR on rice nutrient uptake was investigated by (Lavakush et al., 2014). *Pseudomonas fluorescens*, *Pseudomonas putida*, were employed as PGPR strains. (iii) Plant growth regulators: Synthetic plant growth regulators, often known as plant external hormones, are identical to natural plant hormones. They are significant measurements for increasing agricultural productivity since they are used to regulate plant growth. Phytostimulator, or plant growth regulator, is one word for one of PGPR's main modes of action for growth promotion. Microorganisms that may create or modify the concentration of growth regulators like IAA, GA, cytokinin’s, and ethylene are classified as this. The synthesis of phytohormones (plant hormones) such auxins, cytokinin’s, and GA is thought to be the process. Phytohormones are organic compounds found in trace levels in plants that influence biochemical, physiological, and morphological processes; their synthesis is well-controlled. Plant growth regulators are phytohormones that are generated exogenously by natural and synthetic means rather than by the plants themselves. (iv) The creation of volatile organic compounds: Plant growth promoting rhizobacteria (PGPR) produce volatile organic compounds (VOCs) that aid plant growth and induce systemic resistance (ISR) to diseases. *Bacillus, Pseudomonas, Serratia, Acetobacter (PGBR)* and *Stenotrophomonas* are just a few of the bacteria that produce volatile organic compounds that affect the plant growth. The most well-known of these chemicals are acetoin and 2,3-butanediol, which are produced by Bacillus

and are responsible for major gains in plant development (Ryu et al., 2003). Other PGPR strains produce volatile stable compounds that can boost plant biomass, disease resistance, and abiotic stress tolerance directly or indirectly. Although the identity and quantity of volatile chemicals emitted vary among species, volatile stable compounds emission is a common trait of a wide range of soil microorganisms. and (v) The production of siderophores: Iron is one of the major minerals found on the earth's surface, however it is unavailable to plants in the soil. To deal with the fact that iron is typically found in nature in the form of Fe3+, which is very insoluble, PGPR secrete siderophores. Siderophores are low-molecular-weight iron-binding protein molecules that help chelate ferric iron Fe from the environment. Microbial siderophores deliver Fe to plants when Fe is scarce, allowing them to develop faster. Flores-Felix (Flores-Felix et al., 2015) demonstrated that a siderophore-producing *Phyllo bacterium* strain boosts strawberry growth and quality. Plants use siderophores released by the described PGPR to sequester iron. (vi) Production of enzymes: PGPR enhance plant growth by controlling phytopathogenic pathogens, primarily for the generation of metabolites that contribute to antibiosis and antifungal qualities utilized as defensive systems. Hydrolytic enzymes, such as chitinase and glucanase, would be produced as part of the procedure. Because chitin and beta-glucan are important components of fungal cell walls, bacteria that produce chitinases and beta-glucanases would stop fungal growth. *Sinorhizobium fredii* KCC5 and *Pseudomonas fluorescens* LPK2 generate chitinase and beta-glucanases, respectively, which are responsible for *Fusariumudum's fusarium* wilt (Kumar at al., 2010) Apart from producing chitinase and beta-glucanases, *Pseudomonas* spp. also suppresses *Rhizoctonia solani* and *Phytophthora capsic*i, two of the world's most damaging crop diseases (Arora et al., 2008). (vii) Production of hormones: Plant hormones are chemical messengers that regulate how a plant responds to its surroundings. These are organic substances that are produced in specific sections of the plant and transferred to another location. Plant hormones, often known as phytohormones, have a small effect on physiological processes. Growth, differentiation, and development are among the processes that are altered; other processes, such as stomatal movement, may also be affected (Davies et al., 2013). It's also worth noting that every plant reaction is frequently the consequence of the interaction of two or more hormones. Hormones are also known as plant growth regulators because they stimulate or hinder plant development and are produced by PGPR (Porcel et al., 2014). Auxins, ethylene, gibberellins, abscisic acid (ABA), and cytokinin's are a few well-known plant hormones that may well influence plant growth and development. However, depending on the type of host plant (Dey et al., 2004), the manner of action of different PGPR varies. The soil environment influences plant growth in a variety of

ways, which is a fundamental constraint for long-term agricultural productivity. These stressors are divided into two categories: biotic and abiotic. Plant diseases and pests such as viruses, fungi, and insects cause biotic stress. Abiotic stressors are caused by heavy metal content in soils, and include bacteria, nematodes, insects, and others. Drought, nutrient deprivation, salinity, temperature, and other factors can all affect the environment.

Beneficial and harmful aspects of plant promoting Rhizobacteria: The importance of rhizobacteria in preserving soil fertility and improving plant growth and development cannot be overstated. Several pathways are involved in improving growth, but some studies (Saharan et al., 2011) show the opposite. On the other side, it has the potential to harm plant growth (Bakker et al., 1987)). According to Vacheron (2013), auxin production by PGPR can have both beneficial and negative impacts on plant growth. It's vital to remember that auxin's effectiveness is determined by its concentration. For example, it promotes plant development at low doses while inhibiting root growth at high quantities. Certain *Pseudomona*s species, for example, are known to produce cyanide. The bacteria's synthesis of cyanide is regarded as both a growth promoter and a growth inhibitor. In addition, cyanide is a biocontrol agent for some plant diseases. Furthermore, rhizobitoxine produced by *Bradyrhizobium elkani*i also has a dual effect. Since it is an inhibitor of ethylene synthesis, it can alleviate the negative effect of stress-induced ethylene production on nodulation (Vijayan et al., 2013). On the other hand, rhizobitoxine is also considered a plant toxin because it induces foliar chlorosis in soybeans (Xiong et al., 1996). PGPR are particularly successful at promoting plant growth and development, although a few bacterial species can restrict growth. However, this detrimental influence may only occur in limited circumstances and due to certain characteristics. As a result, choose the right strain is critical for getting the best results in terms of plant growth and development.

The world's population is now estimated to be at 7 billion people, with this number expected to rise to around 8 billion by 2020. When one thinks about both the predicted growth in global population and an increase in environmental damage as a result of with ever-increasing levels of industrialization, it is apparent that the future feeding the entire world's population in the next ten to twenty years will be a significant challenge. It only gets better with time. There is no time to waste; in order to feed the world's rising population, the globe must begin to dramatically boost agricultural production. It should be done in a way that is both sustainable and environmentally friendly. It is vital to re-examine our food supply in order to feed a rising globe Many of the current agricultural practices, which include chemical fertilizers, herbicides,

fungicides, and other pesticides, insecticides. Sustainable agriculture, on the other hand, is more likely to succeed. Substantially by more use of both transgenic plants and transgenic animals and PGPB (plant growth promoting bacteria) (Lucy et al., 2004)

Chemical fertilizers were introduced in the last century. Chemical fertilizers are widely employed in modern intensive agriculture. These fertilizers are robust and potent since they are manufactured artificially from soil-essential macro-nutrients like nitrogen, phosphorous, and potassium. Depending on their structure and the crops and soils for which they are intended, they may contain ammonium sulphate, urea, potash, and ammonia, among other compounds. These fertilizes can be applied and disseminated in a number of ways, including mechanically and manually. This first made farmers happy because they were able to boost agricultural yields. Chemical fertilizers adaptability is undeniable: they have features that me

Chemical fertilizers are supplemented with bio-fertilizers. Bio-fertilizers are inexpensive and can help to reduce the use of chemical fertilizers. Bio-fertilizers use microbes to deliver nitrogen from the atmosphere directly to plants. They help to solubilize and mineralize other plant nutrients, such as phosphates. Hormones, vitamins, auxins, and other growth-promoting chemicals are better synthesized and available, which increases plant growth. On average, their use increases crop yield by 10–20 percent. They aid the growth and survival of beneficial microorganisms in the root zone (rhizospheric bacteria). They keep pathogenic soil microorganisms in check and suppress them. They improve soil texture and fertility by increasing humus content. They are Pollution-free and eco-friendly in nature.

Bio-fertilizers are essential for a variety of reasons: Bio-fertilizers improve the texture of the soil and increase plant output. They prevent infections from flourishing. They are both environmentally friendly and cost-effective. Because bio-fertilizers are natural fertilizers, they preserve the environment from contaminants. Many hazardous chemicals in the soil that can cause plant illnesses are destroyed by them.

Role of plant growth promoting rhizobacteria as a bio-fertilizer: Bio-fertilizer is rapidly becoming a critical component of organic farming and a key economic and agricultural production participant on a worldwide scale. Bio-fertilizers are products that contain living microorganisms that colonize seeds, plant surfaces, and soil when applied. It increases the delivery or availability of nutrients to the plant's rhizosphere or interior, and encourage plant

development to the host plant in the form of primary nutrients. Bio-fertilizer, according to Mishra (2013) , is a mixture of live or latent cells that encourage nitrogen fixing, phosphate solubilizing, or cellulolytic microorganisms and is applied to soil, seed, roots, or composting areas with the goal of increasing the quantity of nitrogen fixed, phosphate solubilizing, or cellulolytic microorganisms of those mutually beneficial microbes and speeding up the microbial processes that increase the availability of nutrients so that they can be easily ingested and absorbed by plants. A bio-fertilizer, according to Malusa and Vassilev (2014), is a designed product that contains one or more microorganisms that improve the plant's nutrient status (growth and yield) via either replenishing soil nutrients, boosting plant availability of nutrients, and/or increasing soil fertility nutrients available to plants. Plant growth-promoting bacteria are commonly used in bio-fertilizers (PGPM). Arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria (PGPR), and nitrogen-fixing bacteria (NFB) are the three major types of PGPM fixing rhizobia, which are thought to be favourable to plant nutrition and growth. It has, nonetheless, it has been claimed that PGPR have been employed as bio-fertilizers all over the world, resulting in higher crop yields. Yields and soil fertility are two factors to consider. As a result of the PGPR potential contribution, this leads to long-term stability. Previous research has shown that a biofertilizer made by mixing PGPR with composts can improve plant growth and biocontrol. Two PGPR have been reported to be efficient bio-control agents: *Bacillu*s spp. and *Pseudomonas* spp. Among *Bacillus subtilis*, *Bacillus amyloliquefacients*, and *Bacillus cereus* are the most common bacterium species. Through diverse ways, effective species at managing plant diseases. The capacity to create PGPR, particularly *Bacillu*s spp. and *Pseudomonas* spp., may survive in a wide range of environments thanks to endospores. Sufficient densities of PGPR in bio-fertilizer provide a beneficial role in creating a proper rhizosphere for plant growth and converting nutritionally important elements through biological process, for example increasing the availability of N, P, K, as well as inhibiting pathogen growth. The high availability of N, P, and K could enhance soil fertility, improve antagonistic isolates' bio-control effects, and extend microorganisms' survival rates in soil. When PGPR acts as a plant feeding and enrichment source that replenishes or reconstructs the nutrient cycle between the soil, plant roots, and microorganisms present, it is classed as a bio-fertilizer.

One of the branches of Horticulture that works with vegetables is Olericulture. Olericulture comes from the Latin word "oleris," which means "pot herb," and the English word "culture,"

which means "cultivation". However, it is now commonly used to refer to the cultivation of vegetables. vegetable farming is a significant economic activity as well as a vital source of vitamins, minerals, and money. Vegetable production, on the other hand, is far less developed than food grain production in the country. Innovative solutions are still needed to tackle biotic and abiotic problems in vegetable cultivation. Excessive use of chemical fertilizers to meet rising food demand has devastating consequences for soil microorganisms, causing soil fertility to deteriorate and increased pollution in the atmosphere. A variety of studies are still being conducted to better understand the diversity and value of plant growth promoting rhizobacteria (PGPR) and their role in improving vegetable output. Plant growth is aided by PGPR, which either aids in the acquisition of nutrients (nitrogen, phosphorus, and other necessary elements) or regulates hormone levels. In the form of bio-control agents, PGPR reduces the inhibitory effects of numerous diseases on vegetable growth and development. *Pseudomonas, Bacillus, Azotobacter, Enterobacter,* and *Azospirillum* are some of the most well-known PGPR capable of supporting the growth of vegetables such as potato, tomato, pepper, and onion. As a result, using PGPR in vegetable growing is advised for optimizing vegetable yield while reducing mineral fertilizer and pesticide inputs.

OBJECTIVES OF THE STUDY

- Isolation of Bacteria from the Rhizosphere of *Lycopersicum esculentum.*

- Screening of Isolates for Specific Enzyme Production.

- Screening of Isolates for Plant Growth Promoting Characters.

- Morphological and Biochemical Characterization of Screened Isolates.

- Molecular Identification of Screened Isolates using PCR.

REVIEW OF LITERATURE

Plant growth promoting rhizobacteria (PGPR) inhabit the rhizosphere region and often found to be beneficial for the development and growth of plants. A number of studies had conducted on the analysis of multifunctional activities of bacteria.

Ibiene and co-workers (2012) found that for the growth of tomato seeds, *Nitrobacter* species and *Nitrosomonas* species has the ability to solubilize phosphate while, *Azotobacter* species produce Indole acetic acid (IAA) and siderophore. The consortium of the three isolates gives the best performance in terms of growth parameters. The use of PGPR isolates *Azotobacter* species, *Nitrobacter* species and *Nitrosomonas* species as inoculants to biofertilizers might be beneficial for *Lycopersicum esculentus* cultivation. Thus, it is an efficient approach to replace chemical fertilizers and pesticides for sustainable cultivation of *Lycopersicum esculentus*.

The use of PGPR as a multifunctional bio-fertilizer or bio-stimulant is an alternative solution to prevent soil pollution and preserve agriculture for sustainable economy was demonstrated by Oo kayThi and Colleagues (2020). On this study 15 PGPR isolates from 102 bacterial strains isolated from have multifunctional activities for plant growth promotion and production of extracellular enzymes. From the study of seed germination, it is found that strains especially *Pseudomonas plecoglossicida* B3 strain and *Acromobacter insolitus* S3 strain could be useful for biofertilizer and as an alternate to chemical fertilizers.

Isolation of plant growth promoting rhizobacteria from rhizosphere soils of green gram, biochemical characterization and screening for antifungal activity against pathogenic fungi was done by Geetha and colleagues (2014). Out of 180 bacterial strains that were isolated from the rhizosphere soils of green gram fields, only 20 were antagonistic to the pathogens. Among antagonistic isolates, OUG21, OUG26, OUG38, OUG51, OUG57, OUG61, OUG62 exhibited significant antifungal activity. Isolate OUG38 shows high level of inhibition against *Colletotrichum capscici*, *Rhizoctonia solani*. Isolate OUG61 shows high level of inhibition against *Fusarium oxysporum*, *Macrophomia phaseolena*.

According to the findings by Glick (2012), PGPB aids in nitrogen fixation, phosphate solubilization, sequestering iron and also it can produce cytokinin's, gibberellins, indole acetic acid and ethylene. Many bacteria produce antibiotic and lytic enzymes to act against pathogens, produces siderophores as a biocontrol that have greater affinity for iron than the fungal pathogens so that the fungal pathogens are unable to proliferate in the rhizosphere of the roots of the host plant because of a lack of iron.

A study on the microbes and environment, there various functions and beneficent of microbes was conducted by (Gupta et al., 2017). Most significant of all those is the ability of microbes to recycle the primary elements like nitrogen, carbon and oxygen. Decomposition results in breakdown of complex organic materials to other forms of carbon that can be used by other organisms. Microbes also function in the process of fermentation, biological nitrogen fixation.

The advantages of using PGPR as an alternative to chemical fertilizers in agricultural production globally was pointed out by (Vejan et al., 2016) and Encouragement should be given in the implementation of PGPR in terms of biofertilization, biocontrol and bio remediation in agriculture all of which exert a positive influence in crop production and agriculture.

Hafeez and Colleagues (2006), Isolated seventeen rhizobacteria from Brazil, Indonesia, Mongolia and Pakistan and studied to develop inoculants for wheat, maize and rice. These isolates produced indole-3-acetic acid irrespective of the region. Fifteen isolates fixed Nitrogen. Isolate 8N-4 from Mongolia produced the highest amount of indole-3-acetic acid produced siderophores and was the only isolate that solubilized phosphate. Inoculation of the wheat variety Orkhon with 8N-4 isolate resulted in the maximum increase in plant biomass, root length, and total nitrogen and Phosphate contents in plants. It was concluded that *Bacillus pumilus* 8N-4 can be used as a bio-inoculant for bio-fertilizer production to increase the crop yield of wheat variety Orkhon in Mongolia. Thus, the characterization and screening of PGPB has helped in the selection of 8N-4 as a potent strain in stimulating growth promotion and yield increase in wheat, as well as having the potential to be used as a biocontrol agent.

The benefit of using emerging Biofertilizers as a suitable alternative to counteract the adverse environmental impact which is exerted by the synthetic agrochemical. Biofertilizers facilitate the overall growth and also the yield in an eco-friendly manner. The main candidate in biofertilizers is the PGPR, which is an eco-friendly alternative to chemical fertilizers. There are many benefits of using PGPR as biofertilizers. This study mainly focuses on the different types of PGPR -based bioferlizers, the challenges faced in the widespread adoption of biofertilizers, and deliberate the prospects of using biofertilizers to promote sustainable agriculture (Basu et al., 2021).

The benefits of Plant promoting rhizobacteria (PGPR) as an eco-friendly biopesticides is boon for the biocontrol of different plant pathogen was depicted by Bajracharya (2019). PGPR strains produces different growth plant promoting substance, which increases plant

productivity and immunity. It also elicits so called ‘induced systematic tolerance to salt and drought. PGPR can take up nutrients thus reducing the need for fertilizers and also preventing the accumulation of nitrates and phosphates in the soil. This study focuses on the adaptation of PGPR, there effects on plant physiology and growth, there induced systematic resistance, PGPR as a biocontrol of plant pathogens biofertilizers and potential green alternative for plant productivity. And potential green alternative for plant productivity, viability of co inoculating, plant microorganism interactions and mechanisms of root colonization.

Jha and co-workers (2015), depicts the role of PGPR which influence the direct promotion of plants by solubilizing insoluble phosphates, fixing atmospheric nitrogen, Secreting hormones such as GAs, IAA, kinetics besides ACC (1-Aminocyclopropane-1-carboxylic acid), deaminase production which will helps in regulation of ethylene. Some of the mechanisms that indirectly assist plant growth include induced systemic resistance (ISR), antibiosis, competition for resources, parasitism, and the generation of compounds (hydrogen cyanide, siderophores) that decrease harmful rhizobacteria. The creation of a plant growth promoting consortium (PGPC) has been recommended as a viable technique for increasing the activity and viability of plant growth promoting rhizobacteria (PGPR). When these strains are combined to form an inoculum consortium, each of the consortium's constituent strains not only out competes the others for rhizospheric establishment, but also works together to promote plant growth. Plant growth and nodulation were improved when certain Rhizobacteria were co-inoculated.

In the study conducted by Backer and Colleagues (2018), provides an update on the function of PGPR in agriculture, from their discovery to their commercialization as low-cost agricultural inputs. Inoculating plants with plant-growth promoting rhizobacteria (PGPR) or treating plants with microbe-to-plant signal chemicals has been shown to be an effective technique for boosting crop growth in studies. Furthermore, these tactics can help crops withstand abiotic stresses like drought, heat, and salinity, which are projected to become more common as climate change conditions worsen. This breakthrough has led to the development of multifunctional PGPR-based formulations for commercial agriculture, reducing the need for synthetic fertilizers and agrochemicals.

Riaz and Colleagues (2021) point outs the use of plant promoting rhizobacteria (PGPR) as potential biofertilizers and biopesticides. The Plant growth-promoting rhizobacteria (PGPR) increase plant development by many processes such as biological nitrogen fixation, phosphate solubilization, siderophore generation, and phytohormone synthesis. Because of its

environmentally favourable and efficient characteristics, PGPR can be used more in sustainable farming. It's being utilised as a substitute for synthetic fertilizers and insecticides, which are becoming more prevalent. Through the production of phytohormones such as indole acetic acid (IAA), cytokinin, gibberellins, and ethylene, PGPR have the ability to act as Phytostimulator. Biopesticides are bacteria and fungi that have the ability to boost plant growth by inhibiting the growth of plant diseases. Different strategies for the PGPR to function against plant diseases include cyanide biosynthesis, siderophore generation, and stimulation of systemic resistance genes in plants. PGPR can also act as a biocontrol agent, protecting plants while simultaneously promoting plant development by synthesizing antibiotics. Based on the many forms of microbial pesticides, the usage of bio-pesticides is slowly expanding at an annual rate of 8%.

Ahmad and Associates (2008), recognized that the plant growth promoting rhizobacteria (PGPR) have a variety of direct and indirect effects on plant growth. They isolated total of 72 bacterial strains belonging to *Azotobacter*, *fluorescent Pseudomonas*, *Mesorhizobium*, and *Bacillus* from diverse rhizospheric soil and plant root nodules in the region of Aligarh in the quest for efficient PGPR strains with multiple activities. Biochemical analysis was performed on these test isolates. These isolates were tested in vitro for plant growth-promoting properties such as IAA generation, NH (3) production, HCN production, siderophore production, phosphate solubilization, and antifungal activity. IAA was produced by more than 80% of *Azotobacter*, *fluorescent Pseudomonas*, and *Mesorhizobium ciceri* isolates, however only 20% of Bacillus isolates were IAA producers. Bacillus isolates had the highest rate of phosphate solubilization (80%), followed by *Azotobacter* (74.47%), *Pseudomonas* (55.56%), and *Mesorhizobium* (55.56%). All of the test isolates were able to generate ammonia, but none of them were able to hydrolyse chitin. With the exception of Mesorhizobium, (10-12.77%) of these isolates produced siderophores and had antifungal activity. Pseudomonas (88.89%) and Bacillus (88.89%) had the most HCN production (50 percent). Eleven bacterial isolates (seven *Azotobacter,* three *Pseudomonas, and* one *Bacillus*) were tested for quantitative IAA synthesis and broad-spectrum antifungal activity (active against three test fungi) based on numerous plant growth boosting activities. Pseudomonas isolates produced the most IAA at all concentrations of On Muller-Hinton medium, Azotobacter isolates (AZT (3), AZT (13), AZT (23), *Pseudomonas* Ps (5), and Bacillus (B (1)) shown broad-spectrum antifungal activity against *Aspergillus*, one or more *Fusarium* species, and *Rhizoctonia bataticola.*

The significance of root exudates in interactions between plant roots and other plants, microorganisms, and nematodes present in the rhizosphere was discussed by Bais and

colleagues (2006). It provides additional evidence that root exudates may be involved in the signalling events that trigger the execution of these interactions. From the molecular to the ecological scale, several positive and negative plant-plant and plant-microbe interactions are highlighted and described. In addition, techniques for addressing these interactions in the lab are provided.

Bhattacharyya and Jha (2012), pointed out the potentiality of plant promoting Rhizobacteria (PGPR) which can be used to replace the chemical fertilizers, pesticides and other supplements. The study focused on the role of plant growth promoting bacteria in a variety of applications related to the agriculture improvements along with their mechanism of action with regards to plant growth-promoting traits.

M. Lucy and Co-workers (2004), demonstrated the application of free-living plant growth-promoting rhizobacteria. In a variety of applications, PGPR offer an alternative to the use of pesticides for plant growth augmentation. It is shown that PGPR have the potential to improve crop yield in agriculture and horticulture. Free-living rhizobacteria that promote plant growth. When plant growth augmentation is necessary, PGPR can be employed in a variety of methods. Agriculture and horticulture have been the most extensively explored applications of PGPR. Several PGPR formulations are already available for agricultural use as commercial goods. Forest regeneration and phytoremediation of polluted soils are two newer applications of PGPR. Although research in these areas is limited, these species are also useful in forestry and environmental rehabilitation. When properly matched to the right plant and the suitable environmental situation, PGPR has been found to have very real and good benefits.

In the study conducted by Vessey (2003), focuses on the known, the putative, and the speculative modes-of-action of PGPR. Fixing N2, improving nutrient availability in the rhizosphere, positively influencing root growth and morphology, and promoting other beneficial plant -microbe symbioses are the some of the actions played by PGPR. The combinations of these modes of actions in PGBR and the challenges faced by the widespread utilization of PGPR as biofertilizers are also depicted in this study.

The biofertilizers an important role in the crop functional traits such as plant growth and productivity, nutrient profile, plant defence and protection, with a focus on their ability to trigger various growth and defence-related genes in the signalling network of cellular pathways, resulting in cellular response and crop improvement and the beneficial use of PGPR as an alternative to chemical pesticides (Bhardwaj et al., 2014).

The potential benefits of plant promoting bacteria in sustainable agriculture not only ensure that vital nutrients are available to plants, but they also improve nutrient use efficiency. PGPRs could be useful instruments for increasing agricultural yield and soil fertility in an environmentally sustainable way. According to the study conducted by Parewa and co-workers (2018), PGPR can be used for the sustainable agriculture as a biofertilizer. Through a variety of processes, PGPR improved plant growth and development as well as soil health, allowing for significant reductions in pesticide and chemical fertilizer use. It also enhances the growth and development of plants and soil health directly and indirectly through several mechanisms.

Singh and co-workers (2019) demonstrated the importance and utilization of plant beneficial rhizobacteria in agriculture. Which focuses on the role of Plant Promoting Rhizobacteria as an eco-friendly, sustainable and cost-effective approach for the agriculture practice without arising the environmental issues. PGPR has the capacity to provide directly or indirectly nutrients availability to the plants, restores the soil fertility, capacity to protect plant against soil-borne pathogens therefore promote the plant growth. Furthermore, using plant growth-promoting rhizobacteria decreases the use of chemical fertilizers, pesticides, and other artificial growth regulators, all of which have serious health and environmental consequences, including soil infertility, water pollution, and biodiversity loss. In this respect, the long-term usage of rhizobacteria has been proposed as an environmentally acceptable and cost-effective method for increasing agricultural yields and protecting plants from soil-borne diseases.

MATERIALS AND METHODS

The present study was conducted for Screening, characterization and identification of plant growth promoting rhizobacteria as potential biofertilizers for olericulture.

Isolation of Bacteria

1. Source of Sample

The soil samples were collected from rhizosphere of *Lycopersicum esculentum* from Manjummel village in Ernakulam district, Kerala.

2. Collection of Samples

The soil sample was collected from rhizosphere of *Lycopersicum esculentum* with a Trowel and transferred it into a clean polythene bag and stored in a refrigerator.

3. Isolation of the Bacterial Strains

Isolation of the bacterial strains was done following the protocol of (Benedetto et al., 2019)

The bacterial strains were isolated from the soil by the serial dilution and spread plate method. 10g of soil sample was weighed and mixed in 90 ml of distilled water in a 250 ml conical flask that was autoclaved. It was mixed well and then kept undisturbed for the sediments to settle down.

4 test tubes were taken and 9ml of distilled water was added to each test tube. It was covered with cotton plug and then autoclaved. 1ml of the sample was pipetted out from the conical flask and added to the first test tube with 9ml of autoclaved distilled water to get 10^{-3} dilution. It was thoroughly mixed with the pipette itself.

1 ml from the 10^{-3} sample was taken and added to the second test tube to get 10^{-4} dilution. It was mixed thoroughly. Again, 1ml was taken from the previously diluted sample, transferred to the third test tube and mixed thoroughly to get 10^{-5} dilution. Finally, 1ml was taken from this and transferred to the last test tube to get 10^{-6} dilution.

Now the sample has been diluted to 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions respectively.

Agar agar bacteriological (for general bacteriological purpose) that contains-

Moisture	12%
Ash Content	3%
Gel Strength	600 gm/cm^2

was taken along with Nutrient Broth that has the constitution-

Peptone	5.00 gm/L
NaCl	5.00 gm/L
HM peptone B (equivalent to beef extract)	1.50 gm/L
Yeast extract	1.50 gm/L
Final pH	7.4±0.2

Nutrient agar medium was made by adding nutrient broth and agar in the composition

Nutrient broth	3.9 gm
Agar agar	6 gm
Distilled water	300 ml

It was made in a 500 ml conical flask plugged with a cotton plug, covered and autoclaved. Cotton swabs were also made and autoclaved. Serially diluted samples- 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were spread on 4 agar plates each using the cotton swabs. These agar plates were kept for incubation for 24hr at 30-37° C in the incubator.

After 24hrs the plates were checked for individual colonies and the colonies that had distinct character like colour, texture and size were selected from the 10^{-3}, $10^{-4,}$ $10^{-5,}$ 10^{-6} plates. Those were streaked on to new agar plates by quadrant streaking method again kept for incubation in the same temperature requirement for 24hrs. Pure colonies were obtained.

Nutrient agar slants were prepared for temporary storage of the colonies. For that nutrient broth and agar was added in the following constitution-

Nutrient broth	2 gm
Agar agar	3 gm
Distilled water	150 ml

It was heated until dissolved and poured into test tubes, 10 ml each. The test tubes were covered with cotton plug and autoclaved. After sterilization, test tubes were placed in the Laminar air flow in a slanting position and waited until they get solidified. The colonies from the plates were transferred to each slant and those slants were kept for incubation at 30-37° C for 24hrs. After incubation period the slants were wrapped with cling film and stored in the refrigerator for further analysis.

Enzymatic Screening of Bacterial Strains

The isolates were screened for the production of amylase, gelatinase, and protease. It was conducted according to the protocol of (Geetha et al., 2014).

1. Test for Amylase

Starch agar medium plates were prepared by using ready-made starch agar medium along with agar.

The starch agar medium contains the following contents per litre,

Beef extract	3.0 gm
Peptone	5.0 gm
Soluble starch	2.0 gm
Agar	15.0 gm

200 ml medium was prepared. The following composition was followed for medium preparation.

Starch agar medium	5 gm
Agar	1 gm
Distilled water	200 ml

The medium was prepared in a conical flask, covered with a cotton plug and autoclaved. Petri plates were also autoclaved. After this process, the Petri plates were placed in the Laminar air flow and the medium was poured into each plate, waited for an hour to cool down. After solidification of the medium, each bacterial strain was spot inoculated on to the starch agar plates. These plates were then kept in the incubator at 30°C for 48 hours. After the incubation period the plates were flooded with iodine solution, held for a minute, and then drained off. Iodine forms a blue colour complex when it combines with starch. The colour blue fades quickly. The colourless zone around colonies suggests amylase production. The diameter of the zone of clearance formed around the colonies was measured using a scale.

2. Test for Gelatinase Activity

The presence of gelatinase production on plates were determined using gelatin agar plates containing 0.1 percentage of gelatin. For that the following constitution was made-

Nutrient media	2.6 gm
Agar	4 gm
Gelatin	0.2 gm
Distilled water	200 ml

The media was prepared and autoclaved. Petri plates were autoclaved separately. The medium was poured into each plate and kept to solidify. After solidification, the presence of gelatinase production on plates were determined by spot inoculating the bacterial isolates on gelatin agar plates containing 0.1 % of gelatin. After inoculation they were kept for incubation at 30°C for 48 hours. Gelatinase production was indicated by a zone of clearance surrounding the colonies. The diameter of the zone of clearance formed around the colonies was measured using a scale.

3. Test for Protease Activity

The qualitative assay for protease production was performed on sterile skim milk (1%) agar plates. The media was prepared by adding nutrient medium, agar and skimmed milk powder. The contents were taken in the following composition.

- Nutrient medium 2.6 gm
- Agar 4 gm
- Skim milk. 2 gm
- Distilled water 200 ml

The media was autoclaved only for 15 min. Extended exposure to heat damages the skimmed milk powder. Petri plates were autoclaved separately. The media was poured into the sterile plates. After solidification, isolates were spot inoculated and followed by incubation at 30 ° C for 48 – 72 hrs. After the incubation period, the plates were examined for zone of clearance around the colony. This zone of inhibition indicates the enzymatic degradation of protein by the protease enzyme. The diameter of the zone of clearance formed around the colonies was measured using a scale.

<u>Screening of Plant Growth Promoting Characters</u>

The isolates were screened for the production of IAA, phosphate solubilizing activity and ammonium production. It was conducted according to the protocol followed by (Benedetto et al., 2019)

1. Production of the IAA

Luria Bertani (LB) broth -(Himedia) medium modified with tryptophan is used as the test medium. LB broth contained the following constituents.

Tryptone	10 gm/L
Yeast extract	5 gm/L
Sodium chloride	10 gm/L
Final pH	7.4±0.2

The following constitution of the contents were made to prepare the medium.

LB broth	1.3 gm
Tryptophan	1 gm

The media was prepared and 5ml of the medium is taken in each test tubes, plugged with cotton plug and autoclaved for 20 min. After sterilization the test tubes containing medium was kept to cool down to room temperature. After cooling down, the bacterial isolates were transferred to these test tubes using an inoculation loop. The inoculated tubes were incubated for 24 hours at 28°C.

After the incubation period 2 to 3 drops of Orthophosphoric acid were added to it and 4 ml of Salkowski reagent was also added and incubated at room temperature for 25 minutes. Salkowski reagent was prepared by mixing

0.5M $FeCl_3$	1mL
70% perchloric acid	50 ml

Positive results showed formation of pink colour. This indicates IAA Production by the bacterial strains. Yellow coloration or no colour change showed a negative result.

2. Phosphate Solubilizing Activity

The plates were prepared with Pikovaskya's medium. The medium has the following composition in 500 mL

Glucose	5 gm
$MgCl_2 . 6 H_2O$	2.5 gm
$MgSO_4 . 7 H_2O$	0.125 gm
KCl	0.1 gm
$(NH_4)_2 SO_4$.	0.05 gm
Tricalcium phosphate	5gm
Agar	7.5 gm

The media was prepared and autoclaved along with the Petri plates. Then the medium was poured into each Petri plate and kept for solidifying. After solidification, the plates were inoculated with the bacterial strains by spot inoculation. It was incubated in an incubator at 28 - 30°C for 3-7 days. The plates were then examined and data were recorded. A clear zone around the colony indicated a positive result.

3. Production of Ammonia

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube separately. Peptone water was prepared according to the Himedia composition that contains-

Peptone	1 gm
Sodium chloride	0.5 gm
Distilled water	100 ml

The media was prepared and transferred to the test tubes 10 ml each. This was autoclaved with cotton plugs plugged in. After sterilization process, the test tubes containing the media was kept for cooling down the temperature to room temperature. Then the media was inoculated with the bacterial strains using an inoculation loop. It was then incubated for 48-72 h at 28 ± 2°C. After the incubation period, the isolates were tested for presence of ammonia by adding Nessler's reagent (0.5 ml) to each tube. Nessler's reagent consists of the following composition-

Mercuric chloride	10 gm
Potassium iodide	7 gm
Sodium hydroxide	16 gm
Distilled water	100 ml
pH	13.2±0.05

Development of brown to yellow colour was a positive test for ammonia production.

Morphological and Biochemical Characterization of Screened Isolates

1. Morphological Characterization

Gram staining was done by following the protocol of (Coico. R. 2006)

For Gram's staining the slide containing bacterial smear was dried in the vicinity of the flame and allowed to cool and then stained with crystal violet solution for 1 minute followed by rinsing with water and the slide was flooded with gram's iodine solution followed by 95% absolute ethyl alcohol for 1 minute. Then the slide was again washed with distilled water, drained and counter stained with safranin for 30 seconds. Finally, the slide was washed with distilled water, drained and air dried and observed under the microscope by using oil immersion objective.

2. Biochemical Characterization

2.1 Catalase Test

Catalyse test was according to the protocol by (Geetha et al., 2014).

Luria Bertani (LB) broth -(Himedia) medium modified with tryptophan is used as the test medium. LB broth contained the following constituents.

Tryptone	10 gm/L
Yeast extract	5 gm/L
Sodium chloride	10 gm/L
Final pH	7.4±0.2

The media was prepared and 5ml of the medium is taken in each test tubes, plugged with cotton plug and autoclaved for 20 min. After sterilization the test tubes containing medium was kept to cool down to room temperature. After cooling down, the bacterial isolates were transferred to these test tubes using an inoculation loop. The inoculated tubes were incubated for 24 hours at 28°C.

Under aseptic condition a drop of H2O2 is placed on a clean sterile glass slide with the help of a pipette. With a sterile inoculating loop place a small amount of culture on this and look for immediate bubbling. The bubbles resulting from production of oxygen gas clearly indicate a catalyse positive result.

The indole test [I], the Methyl test[M], Voges Proskauer test and the citrate test are the four tests of the IMViC series, which tests for evidence of an enteric bacterium. It was conducted according to the protocol followed by (Shoaib et al.,2020).

2.2 Indole Production Test

Tryptone broth medium was used as the test medium. Tryptone broth medium contained the following constituents.

Tryptone	1gm/L
Sodium chloride	0.5gm/L
Distilled water	100ml

Dispense 5mL of the medium into each test tubes, autoclave at 15 lbs for 15 minutes. The test tube of tryptone water was inoculated with the loopful of bacterial strains. Incubate for 48 hours

–96 hours at 37 ^{0}C. After incubation, about 0.5mL of Kovac's reagent was added to each test tube and made it stay for 1-5 minutes. Check the appearance of dark red colour on the surface layer which indicates the test is positive.

2.3 Methyl Red Test

Glucose-phosphate peptone water medium was used as the test medium. Glucose-phosphate peptone water medium contains the following composition-

Glucose	2.5 gm
Potassium phosphate	2.5 gm
Peptone	3.5 gm
Distilled water	500 ml

Dispense 5ml glucose-phosphate peptone water medium in small culture tubes, plug and autoclave at 15 lbs (112 ^{0}C) for 10 minutes. The test tubes were inoculated with the bacterial strains. Incubate for 24-96 hours at 28-37 ^{0}C or until good growth is obtained. After incubation, add approximately 5-10 drops of methyl red indicator were slowly added along the sides of each test tube to form a layer on the surface of the tube. Observe the ring of colour formed. Red colour indicates positive and yellow indicates negative

2.4 Voges-Proskauer Test

Glucose-phosphate peptone water medium was used as the test medium. Glucose-phosphate peptone water medium contains the following composition-

Glucose	2.5 gm
Potassium phosphate	2.5 gm
Peptone	3.5gm
Distilled water	500ml

Dispense 5ml glucose-phosphate peptone water medium in small culture tubes, plug and autoclave at 15 lbs (112 ^{0}C) for 10 minutes. The test tubes were inoculated with the bacterial strains. After inoculation, equal amount of Barritts reagent A and Barritts reagent B were added to each test tube. Agitate thoroughly and maintained for 1-2 hours. Development of pink or red coloration indicates positive test and no change or faint brown colour indicates negative.

2.5 Citrate Test

Isolates were streaked onto Simmon citrate agar slants. It has the following composition-

Magnesium sulphate	0.1gm/l
Ammonium dihydrogen phosphate	0.5gm/l

Dipotassium hydrogen phosphate	0.5gm/l
Sodium citrate	1gm/l
Sodium chloride	2.5gm/l
Bromothymol blue	0.04gm/l
Agar	7.5 gm/l
Distilled water	500ml

Medium was made in a conical flask and 5ml of the medium were taken in each test tube which was covered with a cotton plug and autoclave at 15 lbs (112 ^{0}C) for 10 minutes. The bacterial strains were streaked on to each simmon citrate agar slant and incubated at 28±2oC for 24hrs. Change in colour from green to Prussian blue indicated positive reaction for citrate utilization.

2.6 MacConkey Agar Test

The MacConKey agar test was conducted according to the protocol followed by (Devitt et al., 2009). The MacConkey agar media was used as the test medium. MacConkey agar medium contained the following constituents.

Pectic digest of animal tissue	8.5gm/l
Proteose peptone	1.5gm/l
Lactose	5 gm/l
Bile salts	0.75gm/l
Sodium chloride	2.5gm/l
Distilled water	500ml

The medium was prepared in a conical flask and covered with a cotton plug and autoclaved. It was then transferred to the autoclaved Petri plates. It was allowed to cool. Then the bacterial strains were streaked on the Petri plates. The plates were incubated for 18 to 24 hours at 35 ^{o} - 37 ^{o}C. If the strains are lactose fermenting strains, then they grow as red or pink colour and may be surrounded by a zone of acid precipitated bile.

Molecular Identification of Screened Isolates using PCR

Molecular Characterization and 16S rRNA Gene Sequence Analysis for Identification of

Species.

The screened isolates were subjected to molecular characterization using 16S rRNA partial gene sequencing and the sequences were analysed using bioinformatic tools for species level identification.

1. Genomic DNA Isolation

Genomic DNA isolation was done following the protocol of Esteban *et al.*, (1993).

- A single colony of the isolate was inoculated into Luria Broth (Hi media, India) and incubated at 37ºC overnight with constant shaking
- 1.5 mL culture was taken in a microfuge tube and centrifuged at 8000 rpm (Sigma, Germany) for 5 min.
- The pellet was resuspended in 567µL Tris-EDTA (TE) buffer (pH 8)
- To the suspension, 30µL of 10% sodium dodecyl sulphate (SDS) and 3µL Proteinase K (20mg/mL) were added and mixed well
- The culture was then incubated for 1h at 37ºC in a water bath.
- 100µL of 5M NaCl and 80µL Hexadecyltrimethylammonium bromide (CTAB) (10mg/mL) were added and incubated for 10min at 65ºC in a water bath.
- The tubes were allowed to cool to room temperature and an equal volume of chloroform-isoamyl alcohol (24:1) was added.
- The contents were mixed gently and centrifuged at 10000 rpm for 10min.
- The aqueous layer at the top, containing the DNA, was carefully transferred to a fresh microfuge tube using a blunt end sterile tip.
- An equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added to the aqueous layer and centrifuged again at 10000 rpm for 10 min.
- The aqueous layer was collected in a fresh tube and 0.6 volume of isopropanol was added to it.
- The contents were mixed gently and centrifuged at 12000 rpm for 10min.
- The supernatant was discarded and the pellet was washed with 70% ethanol and centrifuged.
- The supernatant was discarded and the pellet was dried at room temperature.
- The purified DNA was then dissolved in 100µL TE buffer (pH 8)

The concentration of genomic DNA thus obtained was estimated by the spectrophotometric method (Shimadzu UV-VIS Spectrophotometer, Japan). The purity of the

DNA was assayed spectrophotometrically using the automatic calculation of the ratio of optical densities at 260 and 280 nm. The quantification of DNA was done using DNA/Protein pack® software of spectrophotometer. DNA was stored at -20^{0}C until further use. An appropriate dilution (~80-100ng) of genomic DNA was used as template for PCR reactions.

2. 16S rDNA Sequence Analysis

A PCR based method using a primer pair for 16S rDNA was used for species identification of the *Vibrio* strains (Shivaji et al., 2000; Reddy et al., 2000, 2002a; b). A portion of the 16S rRNA gene (1.5kb) was amplified from the genomic DNA. Products after PCR amplification was purified by gene clean kit (Genei, India) and subsequently sequenced, followed by homology analysis.

Sequence	Primer	Reference
16SF	5' AGTTTGATCCTGGCTCA 3'	Shivaji et. al.,2000
16SR	5' ACGGCTACCTTGTTACGACT' 3'	Reddy et. al., 2000,2002 a; b

3. Polymerase Chain Reaction (PCR)

PCR was performed using the genomic DNA (~100ng) as template and16S rDNA specific primers as detailed above.

PCR Mix composition:

PCR buffer (10X)	2.0µL
dNTPs (2.5mM)	2.0µL
Forward primer (10µM)	1.0µL
Reverse primer (10µM)	1.0µL
Taq DNA polymerase (1U)	0.2 µL
Template DNA	0.5 µL
$MgCl_2$ (1.5mM)	1.2µL
Sterile distilled water to make the final volume to	20µL

PCR conditions:

Annealing	- 56ºC	-30 sec.
Extension	- 72ºC	-2 min.

PCR assays were performed in MJ Mini Thermal cycler (Bio-Rad, USA).

Agarose Gel Electrophoresis using PCR product

The agarose gel electrophoresis was carried out for the visualization of PCR products. Agarose gels of appropriate strength (1 - 2%) depending on the size of the PCR product were prepared in Tris-Acetate-EDTA (TAE) buffer. Ethidium bromide (EtBr) solution was added at a concentration of 10mg/mL. Definite volume of PCR product was mixed with gel loading dye and loaded into the wells. DNA markers were run along with the products for confirmation of amplicon size. Electrophoresis was performed at a constant volt (5V/cm) (Genei, India) and the gel pictures were captured with Bio-Rad Gel documentation system.

4. *n-Silico* Analysis of the 16S rDNA Sequences

DNA sequencing and analysis

Products after PCR amplification were purified by gene clean kit (Genei, India). The products were sequenced by Sanger's Dideoxy method using ABI 3730 Excel at Rajiv Gandhi Centre for Biotechnology. Thiruvananthapuram. The sequenced PCR products were analysed online using BLAST software (http://www.ncbi.nlm.nih.gov/blast) and the identity of the sequences were established.

RESULTS

Isolation and Characterization of Bacterial Isolates

A total of 24 bacteria strains were isolated from the rhizosphere soils which is collected from Manjummel village Ernakulam (Table 1). These isolates were taken from the colonies that had distinct character like colour texture and size.

Table 1 Showing the Names of the Bacterial Strains

Sl.no	BACTERIAL STRAINS
1	AR-01
2	AR-02
3	AR-03

4	AR-04
5	AR-05
6	AR-06
7	AR-07
8	AR-08
9	AR-09
10	AR-10
11	AR-11
12	AR-12
13	AR-13
14	AR-14
15	AR-15
16	AR-16
17	AR-17
18	AR-18
19	AR-19

20	AR-20
21	AR-21
22	AR-22
23	AR-23
24	AR-24

Screening for Extracellular Enzymatic Production

1. Amylase activity

The appearance of clear zone surrounding the colony indicates positive for starch hydrolysis test. 17 strains of bacteria showed Amylase activity (Table 2). The bacterial strain AR-10 showed the maximum clear zone of 4 cm (plate 1)

Plate 1 Strains Showing Positive Results for Amylase Activity:

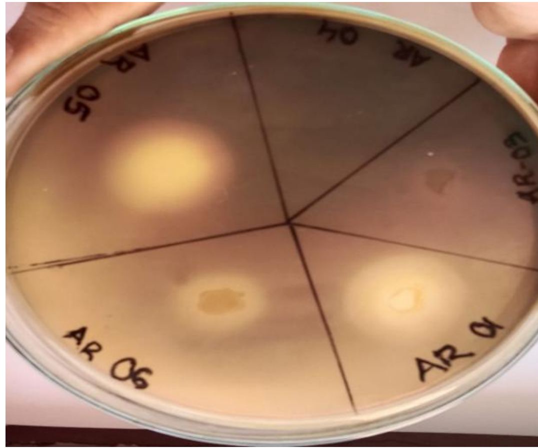


Plate: AR-01(+), AR-03(+), AR-04(-), AR-05(+), AR-06(+)

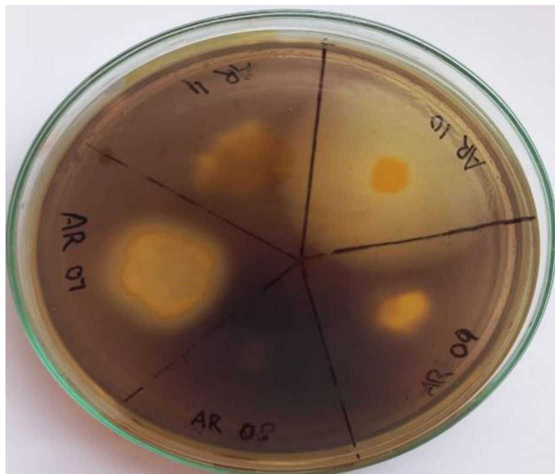


Plate: AR-07(+), AR-08(-), AR-09(+), AR-10(+), AR-11(+)

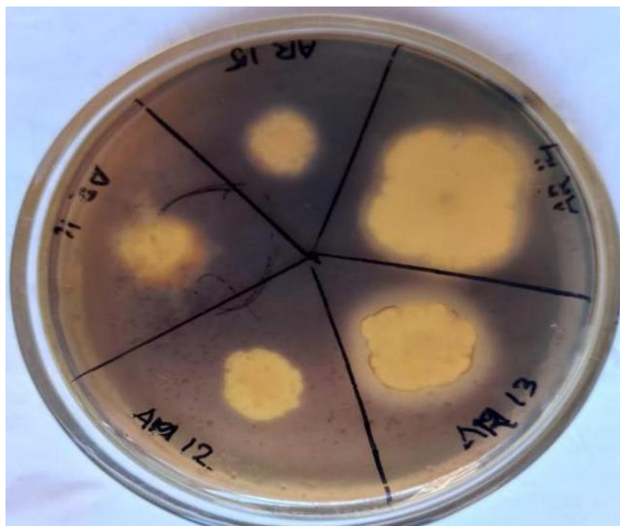


Plate: AR-12(+), AR-13(+), AR-14(+), AR-15(+), AR-16(+)

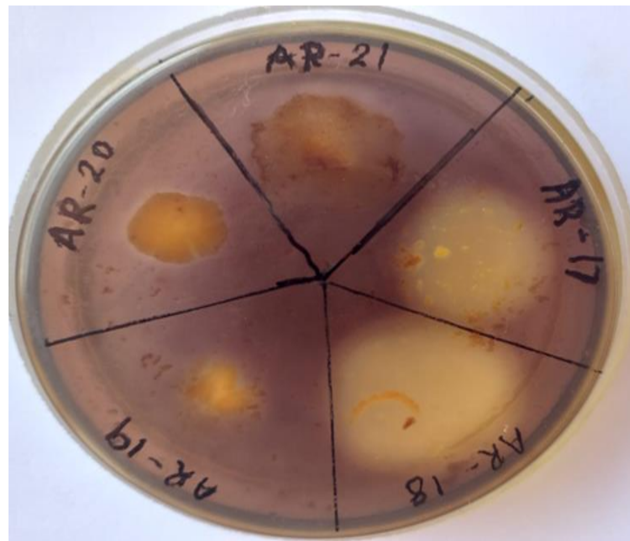


Plate: AR-17(+), AR-18(+), AR-19(+), AR-20(+), AR-21(-)

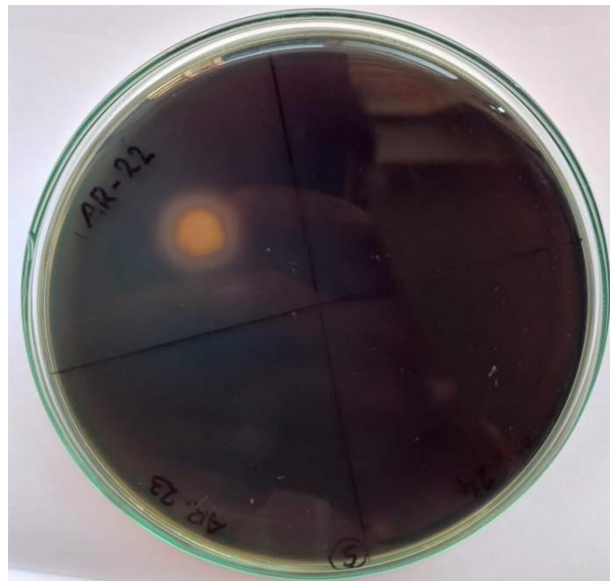


Plate: AR-22(+), AR-23(-), AR-24(-)

2. Gelatinase Activity

None of the strains exhibited clear zone for gelatinase activity (Table 2).

3. Proteases Activity

Proteolytic enzyme production was detected as formation of a clear zone around the colony on skim milk agar medium. 16 strains showed proteolytic activity (Plate 2). The bacterial strain AR-05 showed the maximum protein hydrolysis about 3.5cm (Table 2). This indicates that AR-05 produces the maximum protease enzyme which resulted in the hydrolysis of protein.

Plate 2 Strains Showing Positive Results for Protease Activity

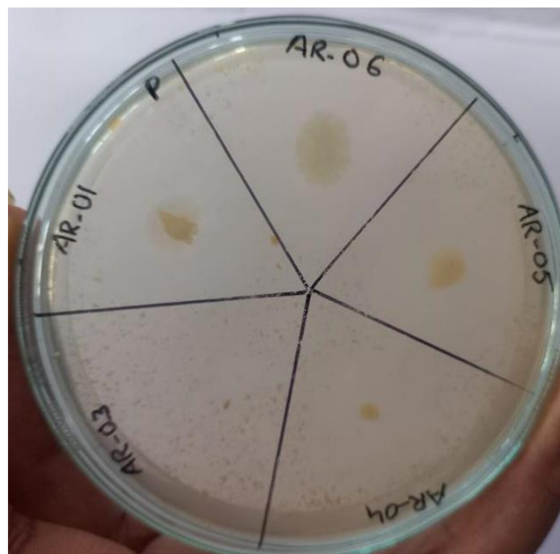


Plate: AR-01(+), AR-03(+), AR-04(-), AR-05(+), AR-06(+)

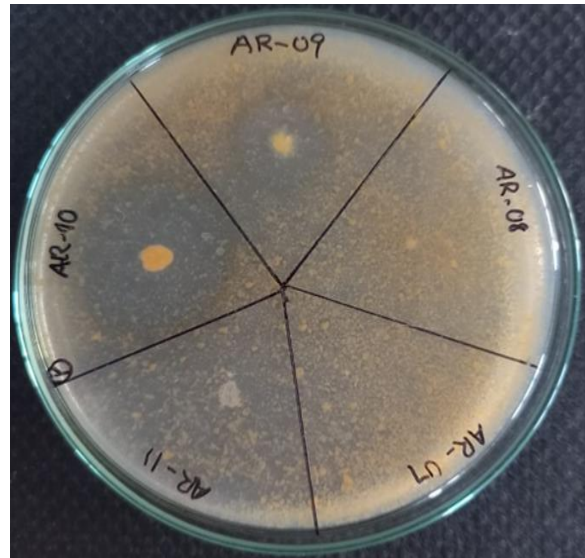


Plate: AR-07(-), AR-08(-), AR-09(+), AR-10(+), AR-11(-)

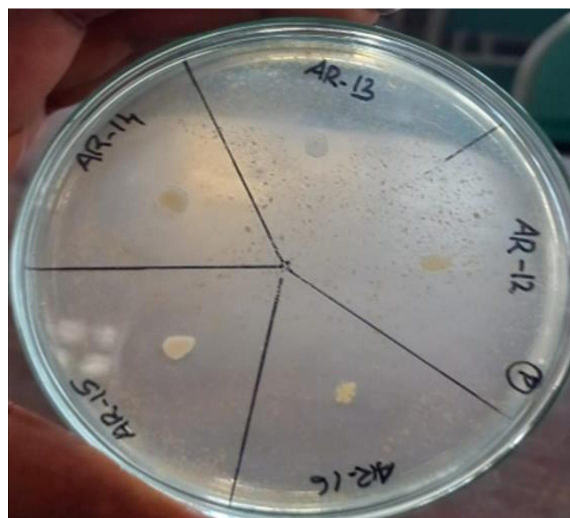


Plate: AR-12(-), AR-13(+), AR-14(+), AR-15(+), AR-16(+)

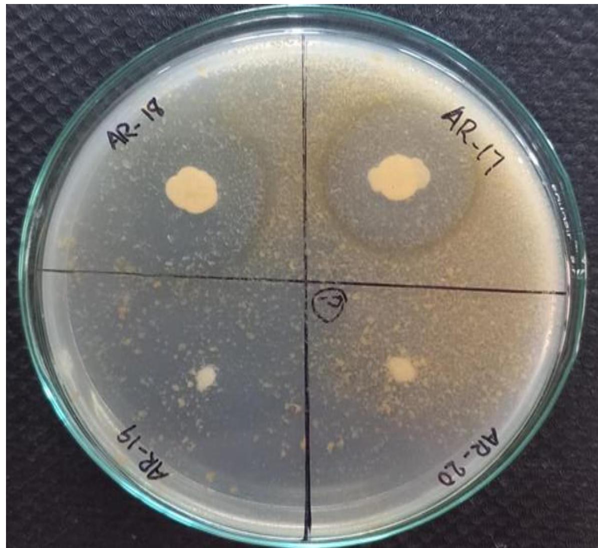


Plate: AR-17(+), AR-18(+), AR-19(-), AR-20(-), AR-21(-)

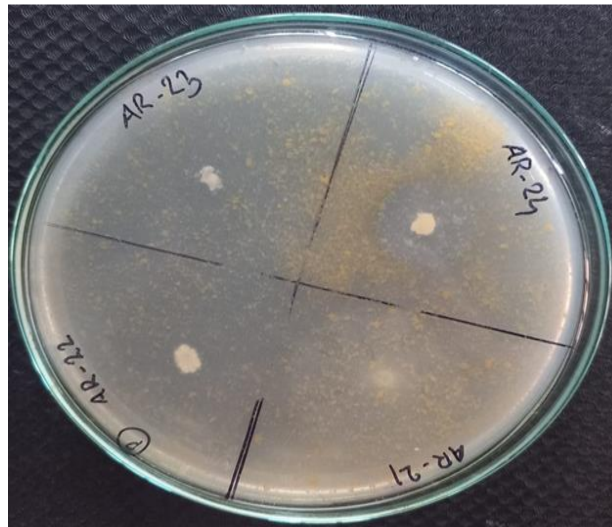


Plate: AR-22(+), AR-23(+), AR-24(+)

Table 2 Showing Enzyme Activity of Different Bacterial Strains.

The diameter of the zone of clearance formed around the colonies was measured using a scale (in cm).

BACTERIAL STRAINS	AMYLASE ACTIVITY (cm)	GELATINASE ACTIVITY (cm)	PROTEASE ACTIVITY (cm)
AR-01	2.1	-	3.2
AR-03	0.9	-	1
AR-04	-	-	2.5
AR-05	2	-	3.5
AR-06	1.9	-	2.5
AR-07	2.4	-	-
AR-08	-	-	-
AR-09	1.4	-	1.5
AR-10	4	-	3
AR-11	1	-	-
AR-12	1	-	-
AR-13	1.9	-	0.9

AR-14	2.9	-	2.6
AR-15	1.9	-	1.8
AR-16	1.4	-	1.6
AR-17	2.1	-	2.6
AR-18	2.5	-	3
AR-19	1.2	-	-
AR-20	1.1	-	-
AR-21	0.5	-	-
AR-22	-	-	0.7
AR-23	-	-	1
AR-24	-	-	1.7

Plant Growth Promoting Characters

1. Indole Acetic Acid (IAA) Production

After the incubation of the bacterial strains in Luria Bertani (LB) broth -(Himedia) medium modified with tryptophan. 2 to 3 drops of Orthophosphoric acid and 4 ml of Salkowski reagent is added to it.. The formation of pink colour was observed (Plate 3). 6 strains of bacteria showed positive activity (Table 3).

Plate 3 Strains Showing Positive Results for IAA Production

AR-10

AR-15

AR-01

AR-06

AR-16

AR-05

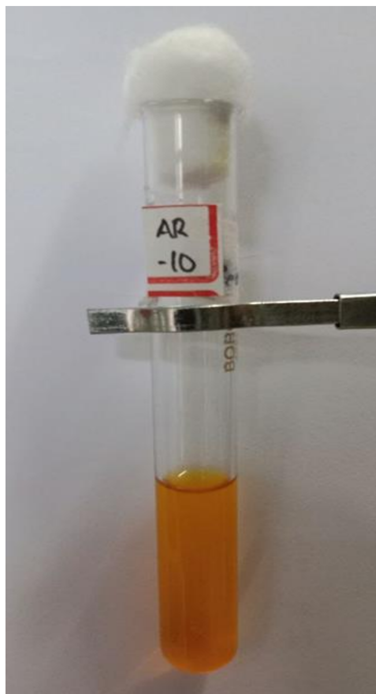
2. Phosphate Solubilizing Activity

None of the strains exhibited clear zone for Phosphate solubilizing Activity (Table 3).

3. Ammonia Production

After the addition of Nessler's reagent to the Peptone medium, formation of brown to yellow colour indicates the production of ammonia. (Plate 4) and light. It was observed in the eight isolates (Table 3).

Plate 4 Strains Showing Positive Results for Ammonium Production



AR-10

AR-09

AR-06

AR-17

AR-15

AR-18

AR-01

AR-16

AR-14

AR-05

Table 3 Showing Plant Growth Promoting Characters

BACTERIAL STRAINS	IAA PRODUCTION	PHOSPHATE PRODUCTION	AMMONIUM PRODUCTION
AR-01	+	-	+
AR-05	+	-	+
AR-06	+	-	+
AR-09	-	-	+
AR-10	+	-	+
AR-14	+	-	+
AR-15	+	-	+
AR-16	+	-	+
AR-17	-	-	+
AR-18	-	-	+

Morphological and Biochemical Characterization of Screened Isolates

1. Morphological Characterization of Screened Isolates.

The four strains, when subjected to gram staining revealed that, three were gram positive and one gram negative (Table 4).

Table 4 Shows the Results of Gram Staining

GRAM POSITIVE	GRAM NEGATIVE
AR-01	AR-10
AR-06	
AR-15	

2. Biochemical Characterization

Catalase test, Indole test, Methyl red test, Voges-Proskauer test, Citrate test, and MacConkey agar test was conducted for biochemical characterization of bacterial strains (Table 5).

2.1 Catalase Test

A drop of H2O2 was placed on a clean sterile glass slide to which a small amount of culture was placed with the help of a pipette. Immediate bubbling was not observed. This indicates that none of the strains shown positive result for catalase test (Table 5).

2.2 Indole Test

After the addition of Kovac's reagent to the Tryptone broth medium. The appearance of dark red colour on the surface layer of the medium was not observed. This indicates that none of the bacterial strains shown positive result for indole test (Table 5).

2.3 Methyl Red Test

After the addition of methyl red indicator into the glucose-phosphate peptone water medium, no red coloured ring was observed. This indicates that none of the bacterial strains shown positive result for methyl red test (Table 5).

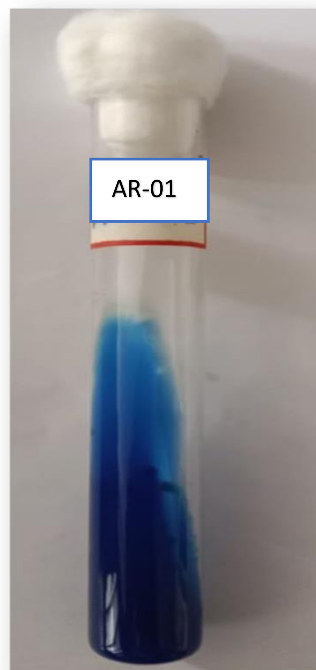
2.4 Voges-Proskauer Test

Equal amount of Baritts reagent A and Barittis reagent B were added to glucose-phosphate peptone water medium. Development of pink or red coloration was not observed. This indicates that none of the bacterial strains shown bacterial strains (Table 5).

2.4 Citrate Test

Among the 4 bacterial strains, AR-01 was tested as Positive for citrate test (Plate 5). A Change in color from green to Prussian blue was observed on the simmon citrate agar slant.

Plate 5 Strain Showing Positive Result for Citrate Test.



:

AR-01

Table 5 Showing the Results of Biochemical Test

BACTERIAL STRAIN	CATALASE TEST	INDOLE TEST	METHYL RED TEST	PROSKAUER TEST	CITRATE TEST	MacConkey AGAR TEST
AR-01	-	-	-	-	+	-
AR-06	-	-	-	-	-	-
AR-10	-	-	-	-	-	-
AR-15	-	-	-	-	-	-

Molecular Identification of Screened Isolates using PCR

1. Molecular characterization and 16 rRNA Gene Sequence Analysis for Identification of Species was done using PCR.

The 4 high potential bacterial strains, that is AR-01, AR-06, AR-10, AR-15 were identified using 16 rRNA gene sequence analysing. AR-01 was identified as the *Bacillus megaterium*. AR- 06 was identified as *Bacillus* acidiceler*,* AR- 10 is identified as *Cheyseobacterium rhizoplanae.* AR- 15 is identified as *Bacillus firmus.* The identity of the bacterial strains is listed in (table 6) based on the sequences (Fig1; Fig 2; Fig 3; Fig 4)

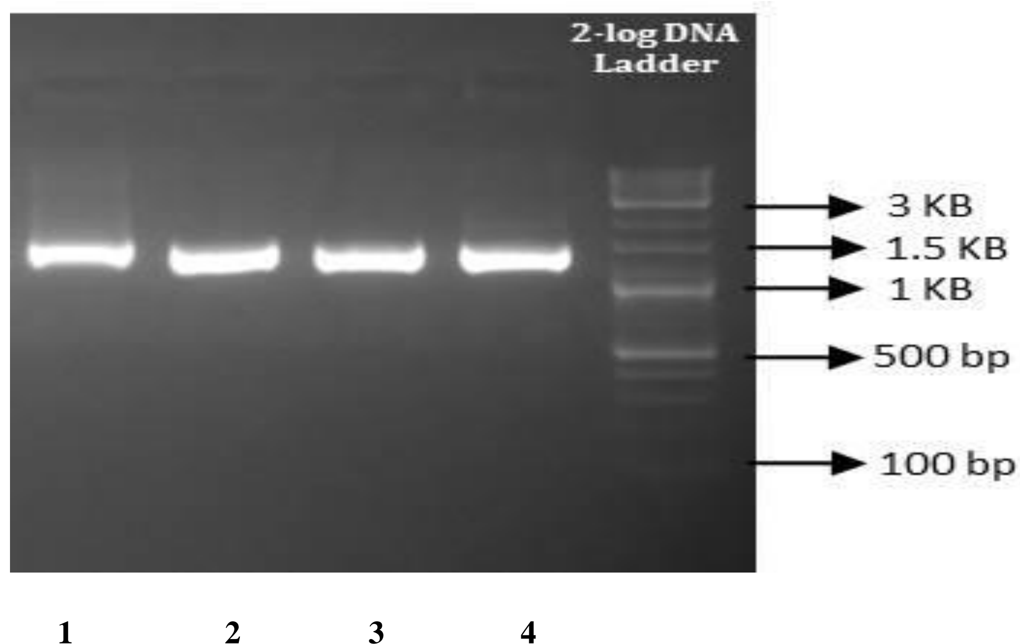
Table 6 Identity of the Bacterial Strains

Strain	Identification	% Of identity
AR- 01	*Bacillus megaterium*	99.19%

AR-06	*Bacillus* acidiceler	98.57%
AR-10	*Chryseobacterium rhizoplanae*	99.25%
AR-15	*Bacillus firmus*	99.57%

2. Gel Electrophoresis was done on these Bacterial Strains using PCR Product (plate 6).

Plate 6 shows the results of gel electrophoresis using PCR products.



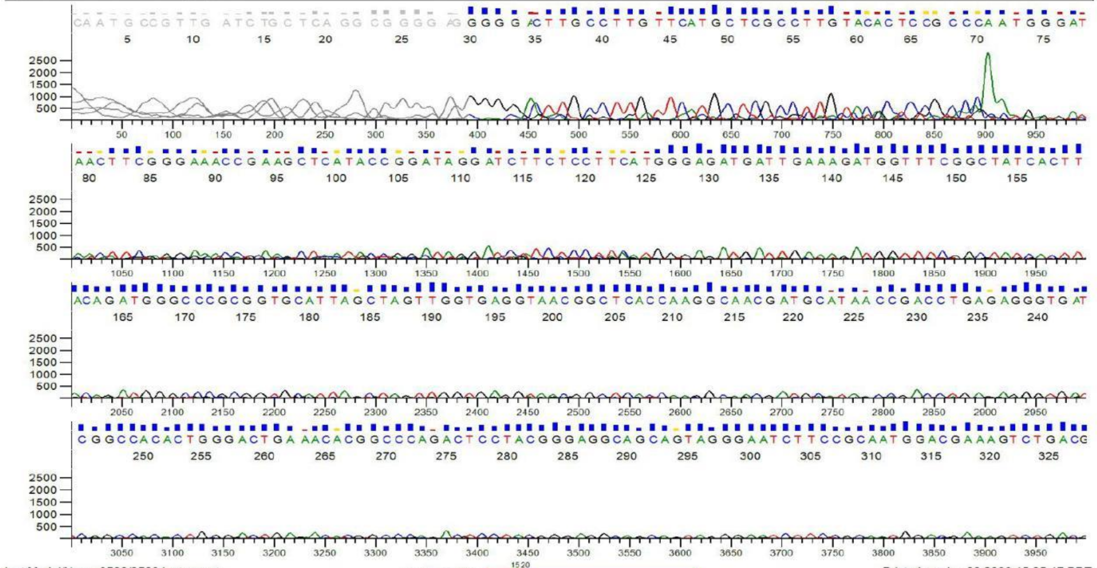
1 2 3 4

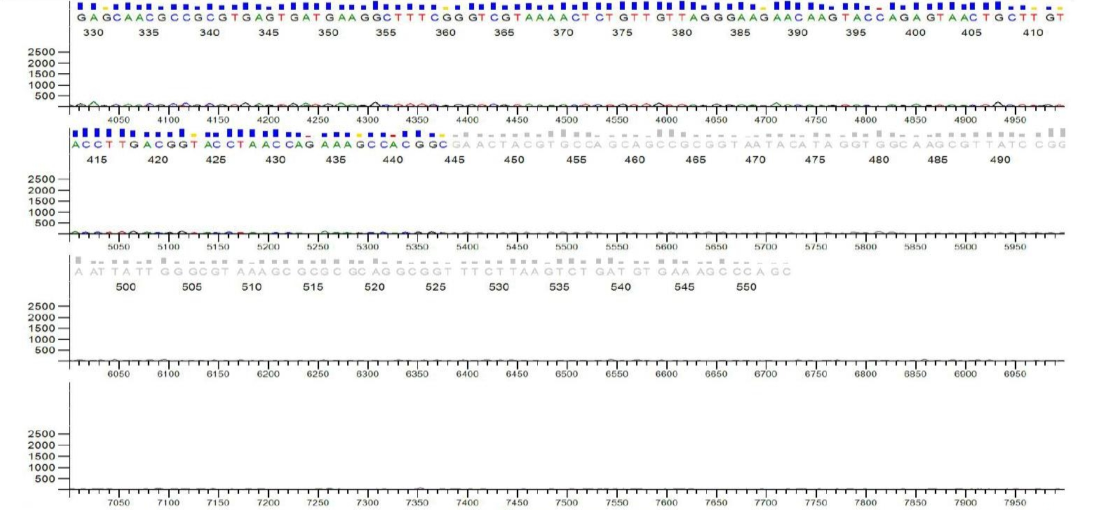
Lane 1: AR-01; Lane 2: AR-06; Lane 3: AR-10; Lane 3: AR-15

3. Electropherogram of the Identified Bacterial Strains

3.1 Electropherogram of Bacterial Strain AR-01 (figure 1)

Figure 1 Shows the Electropherogram of AR-01





Applied Biosystems

SR2842-AR01-RSF1_F01.ab1

SR2842-AR01-RSF1

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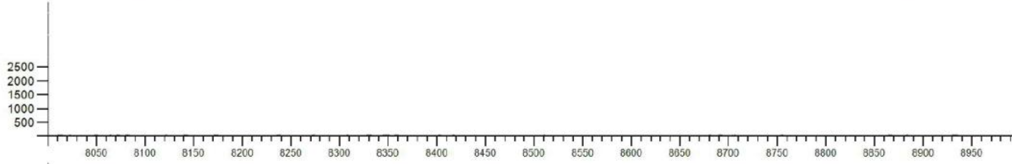

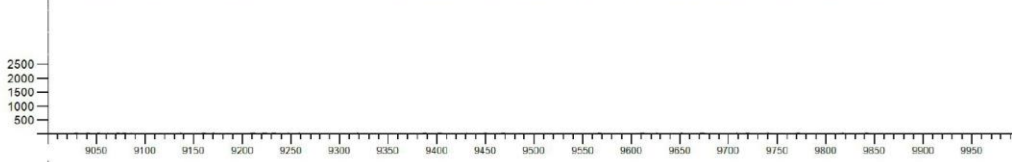

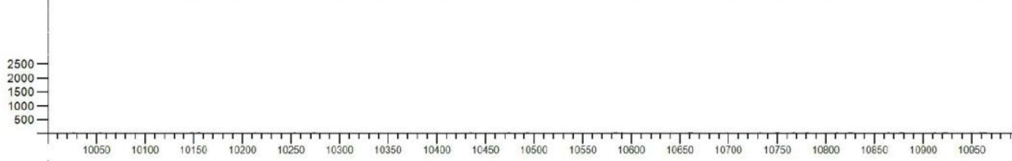

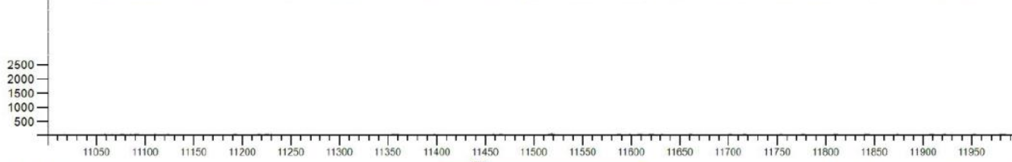

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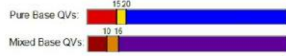

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SR2842-AR01-RSF1

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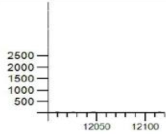

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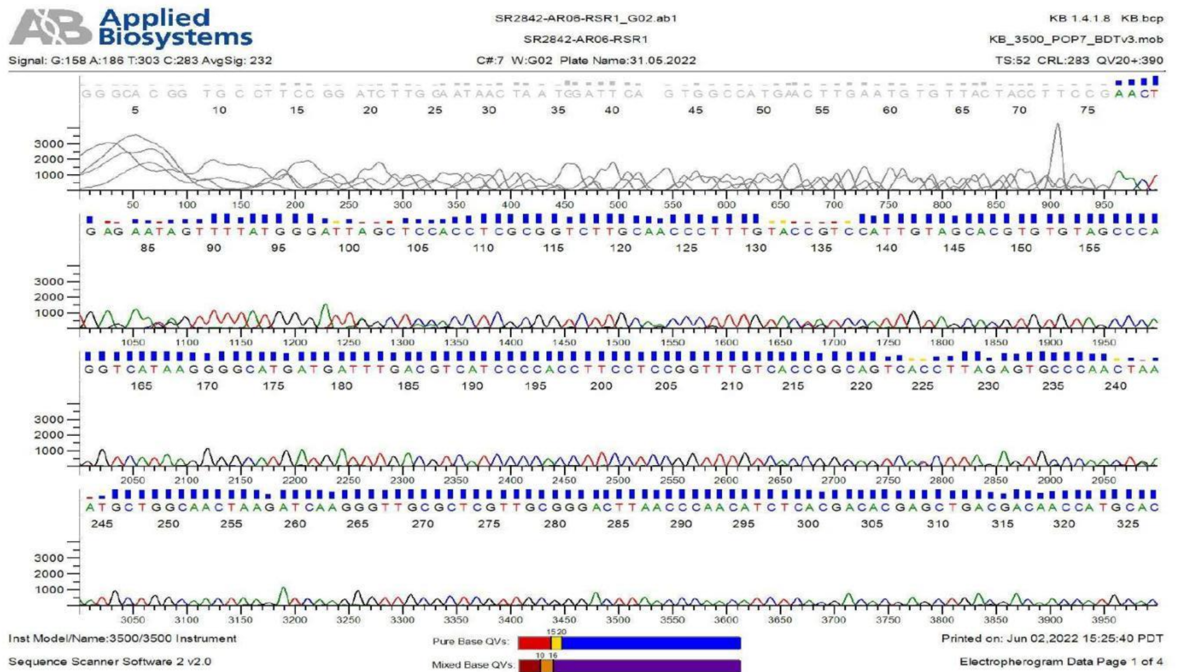
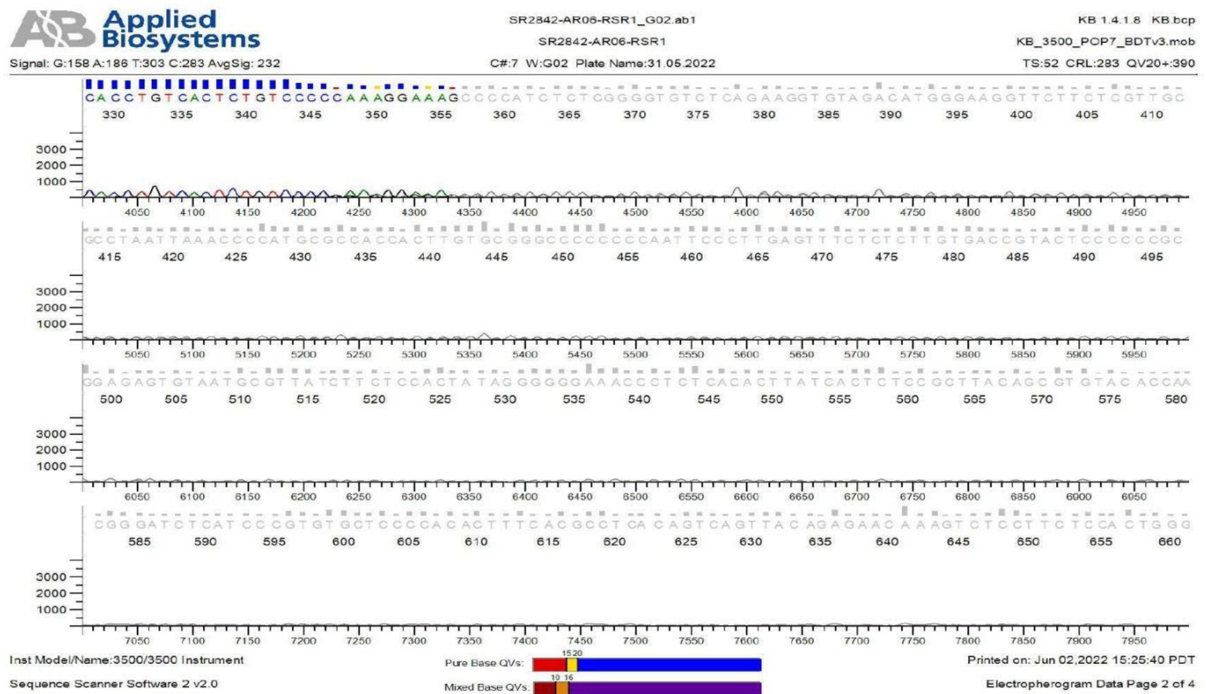
3.2 Electropherogram of Bacterial Strain AR-06 (figure 2)

Figure 2 Shows the Electropherogram of AR-06





Applied Biosystems

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SR2842-AR06-RSR1

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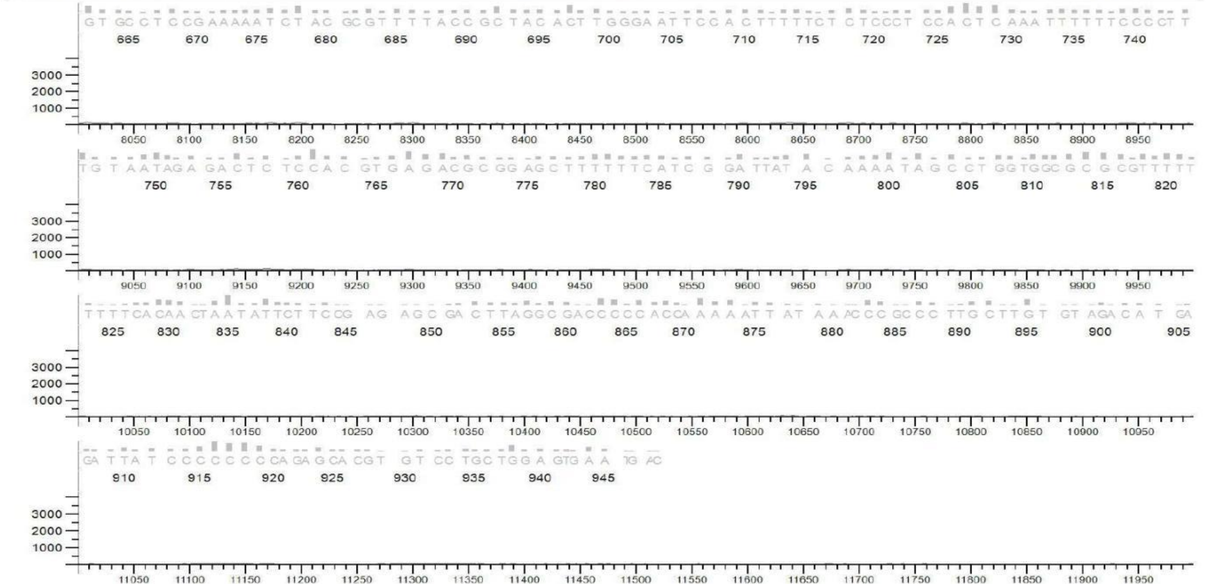

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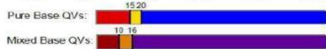

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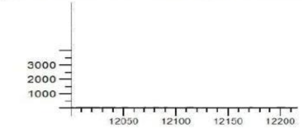

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Mixed Base QVs:

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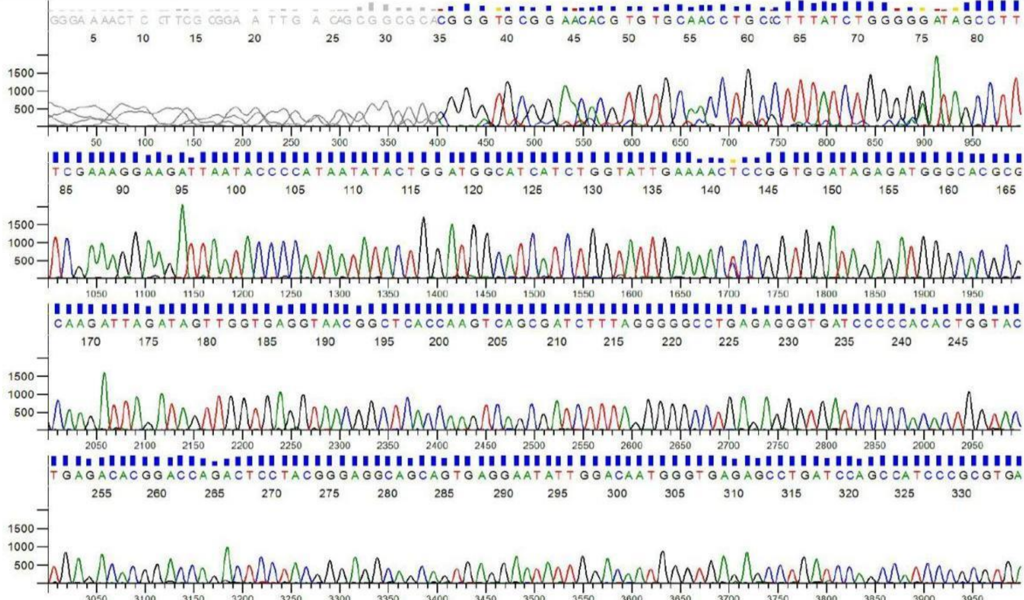
3.3 Electropherogram of Bacterial Strain AR-10 (figure 3)

Figure 3 Shows the Electropherogram of AR-10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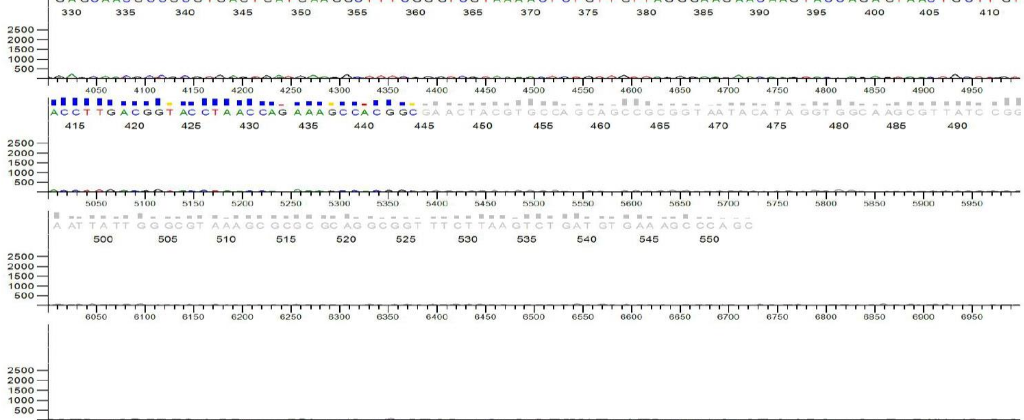
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KB_3500_POP7_BDTv3.mob
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TS:36 CRL:341 QV20+:416
Inst Model/Name:3500/3500 Instrument
Sequence Scanner Software 2 v2.0
Pure Base QVs:
Mixed Base QVs:
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SR2842-AR01-RSF1

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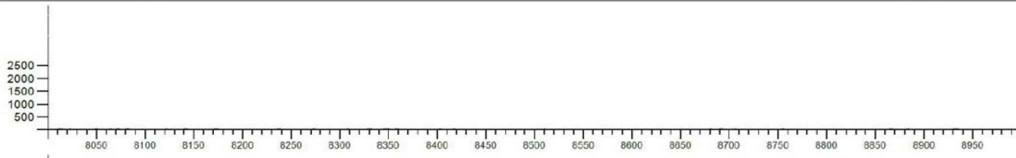

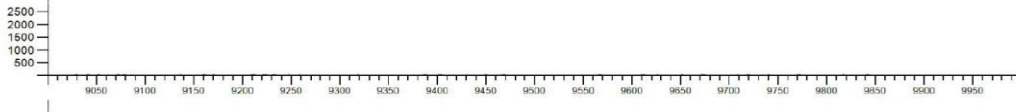

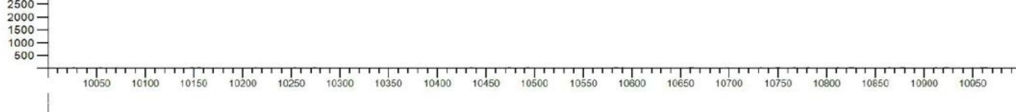

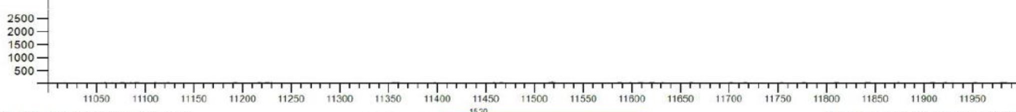

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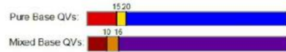

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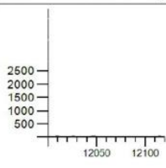

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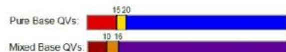

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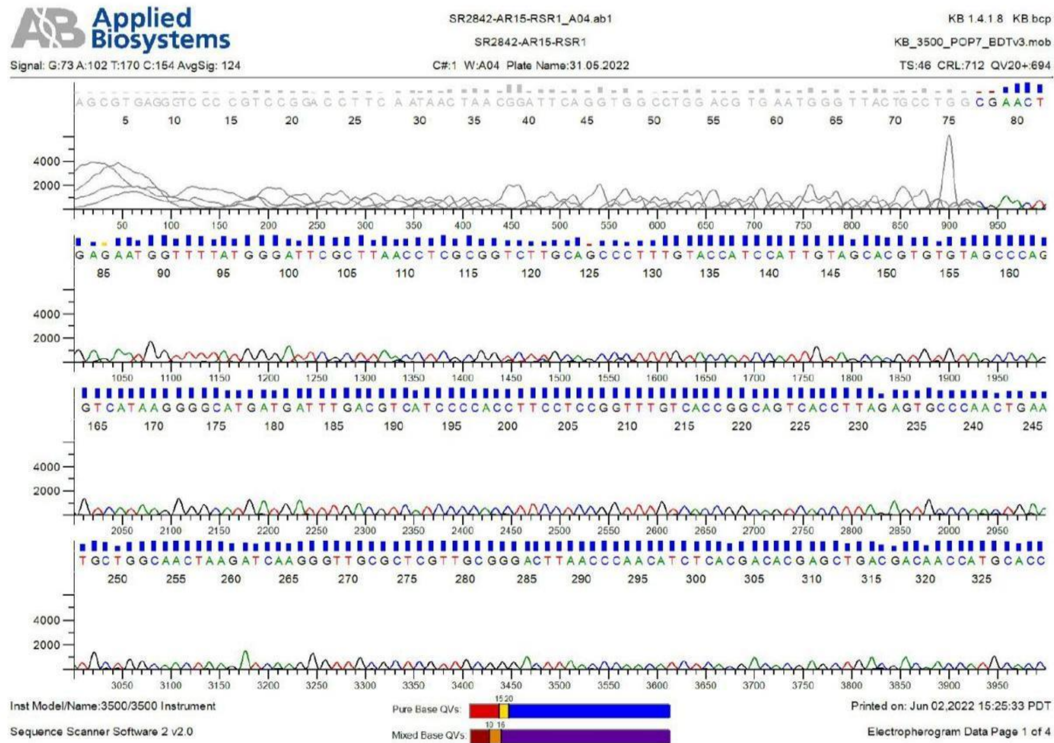
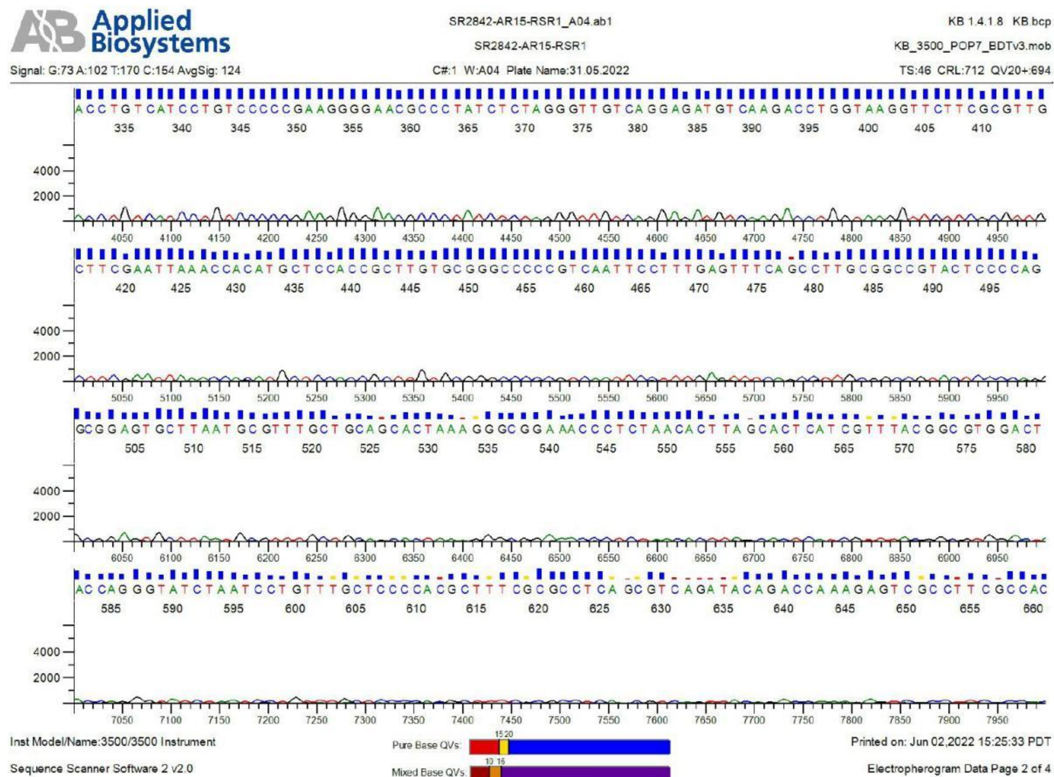
3.4 Electropherogram of Bacterial Strain AR-15 (figure 4)

Figure 4 Shows the Electropherogram of AR-15





Applied Biosystems

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SR2842-AR15-RSR1

KB 1.4.1.8 KB.bcp

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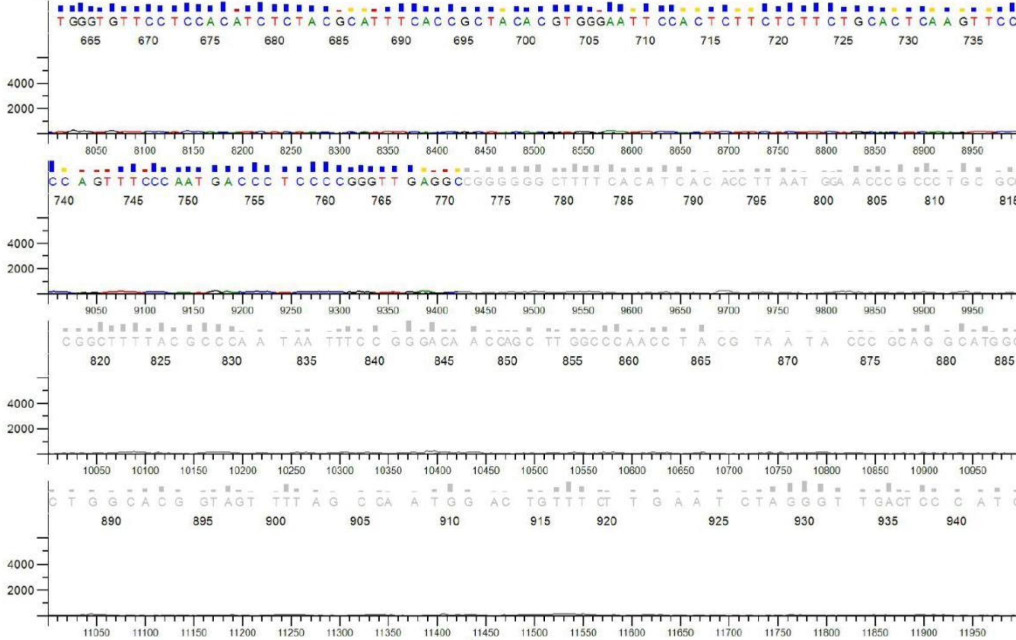

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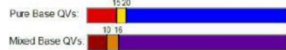

Mixed Base QVs:

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Applied Biosystems

SR2842-AR15-RSR1_A04.ab1

SR2842-AR15-RSR1

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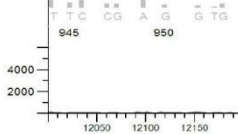

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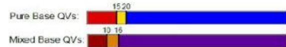

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DISCUSSION

This study revealed that the bacterial strains that were isolated from the rhizosphere of *Lycopersicum esculentum* exhibited positive results in both plant growth promoting characters and enzymatic test. Plant growth-promoting rhizobacteria (PGPR) are rhizospheric bacteria that can boost plant growth through a variety of mechanisms, including phosphate solubilization, siderophore production, biological nitrogen fixation, rhizosphere engineering, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), quorum sensing (QS) signal interference and phytohormone production. PGPR agricultural potential is continuously growing, since it provides an appealing alternative to conventional fertilizers, pesticides, and other additives.

Preliminary extracellular enzymatic screening was done on the isolated bacterial strains. Among 23 bacterial strains, amalyse activity was showed by 17 strains and 6 strains showed negative results. The positive strains indicate that these bacterial strains are capable of producing α amylase which result in the hydrolysis of the starch. The bacterial strain AR-10 showed the maximum clear zone of 4 cm. This indicates that AR -10 produces the maximum α amylase enzyme which resulted in the hydrolysis of starch. The α amylase is considered to be one of the primary enzymes which is responsible for the starch degradation in cereals and particularly in wheat. α Amylase initiates the conversion of starch into simple sugars during grain germination to fuel embryo and coleoptile growth in the first few days. In plants α Amylase was found in the aleurone layer. The amylase works to hydrolyse the endosperm starch into usable sugars. These sugars provide the necessary energy for root growth and act as reserve food storage (Esitken, 2009).

Gelatin is very useful for the plants, it has high nitrogen content, perfect for enriching the soil. Nitrogen is essential for plants to produce lush and green foliage. It is also much safer and inexpensive as gelatin releases nitrogen slowly, it enriches plants longer. It also saves the nitrogen leaching out of the soil or evaporating quickly. But none of the bacterial strains showed gelatinase activity. It indicates that these bacterial strains does not have potential to produce the gelatinase enzyme (a type of protease enzyme) which hydrolyse the gelatine (Goswami et al., 2016).

In Protease test among 23 bacterial strains, 16 strains showed proteolytic activity and rest of them were tested negative. It indicates that the positive bacterial strains are capable of producing the protease enzyme which hydrolyse the protein. Proteases play key roles in plants development by maintaining strict protein quality control and degrading specific sets of proteins in response to diverse environmental and developmental stimuli (Saharan & Nehra, 2011).

In the plant growth promoting characters, the bacterial strains showed positive results for both IAA and Ammonia production.

Indole acetic acid (IAA) test was conducted on 10 bacterial strains. And 7 bacterial stains showed positive result for IAA production. It indicates that these positive bacterial strain has the potential to produces IAA (indole-3-acetic acid) which is a plant growth regulator or hormone. It is a main auxin in plants. Which regulate the growth and developmental processes such as cell division and elongation, tissue differentiation, apical dominance and response to light, gravity and pathogens. In plants IAA plays a key role in both shoot and root development. The hormone moves from one part of the plant to another by a designated importer (AUX1) and efflux pumps (PIN1-7). Auxin concentration varies depending on the part of the plant and the various concentrations are strictly regulated by the plant. The regulation of the auxin concentrations creates a pattern-like system which helps determine plant growth and development. IAA is often used in horticulture to promote adventitious root growth and are used commercially to create root stem cuttings and to promote uniform fruit and flowering growth (Goswami et al., 2016).

Phosphorus is one of the essential elements that are necessary for plant development and growth. It makes up about 0.2 % of a plant dry weight. It is a vital component of ATP, the "energy unit "of plants. ATP forms during photosynthesis, has phosphorus in its structure, and processes from the beginning of seedling growth through to the formation of grain and maturity. It is important in cell division and development of new tissue. Phosphorus is also associated with complex energy transformations in the plant. Adding phosphorus to soil low in available phosphorus promotes root growth and winter hardiness, stimulates tillering, and often hastens maturity. Thus, phosphorus is essential for the general health and vigour of all plants. But none of the bacterial strains shown positive result for the phosphate solubilizing. It indicates that this bacterial strain does not have the potential to hydrolyse the organic and inorganic insoluble phosphorus compounds to soluble phosphorus that can be easily be

assimilated by the plants. Phosphorus solubilizing bacteria may aid the growth of plants by stimulating the efficiency of biological nitrogen fixation, synthesizing phytohormones and enhancing the availability of some trace elements such as zinc and iron (Goswami et al., 2016).

The production of ammonia was observed in the eight isolates. The ammonia is useful for plant as directly or indirectly. Ammonia production by the plant growth promoting bacteria helps influence plant growth indirectly. Maximum ammonium production was shown by AR-10. Plants use ammonium as a significant inorganic nitrogen source. Ammonium supports plant growth when external resources are low, but it causes toxicity when external supplies are abundant. Ammonium causes rapid changes in cytosolic pH, gene expression, and protein post-translational modifications, resulting in apo plastic acidification, coordinated ammonium absorption, increased ammonium assimilation, oxidative and phytohormonal status, and modified root system architecture. Some of these reactions are reliant on AMT-type ammonium transporters and are unrelated to a nutritional effect, implying that ammonium is seen as a signalling chemical by plant cells. Mineral fertilizer application is responsible for around half of the world's food output. Fertilizer consumption is steadily increasing as the world's population grows and people's diets shift to include more meat. Ammonia is one of the essential players. Ammonia binds nitrogen in the air, allowing the most important crop nutrient, nitrogen, to be used in nitrogen fertilizer manufacture. Ammonia is a key component of fertilizer, and it literally helps put food on the table. Approximately 80% of the ammonia produced annually is now utilized to make fertilizer. Fertilization boosts plant nutrition, encourages plant development, improves crop quality, and, in the end, preserves and even improves soil fertility (Goswami et al., 2016).

By conducting morphological characterization of bacterial strains. Out of 4 bacterial strains, AR-10 was identified as gram negative and AR-01, AR-16, AR-15 was identified as gram positive.

Catalase test, Methyl red test, Voges-Proskauer test, Indole test, Citrate test, and MacConkey agar test was conducted for biochemical characterization of bacterial strains.
None of the bacterial strains shown positive result for catalyst test. It indicates that this bacterial strain does not have the ability to produce the enzyme, catalyse. Catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide, was detected in this test (H_2O_2). It's used to distinguish between bacteria that make the catalase enzyme, like staphylococci, and bacteria that don't, like streptococci. For regular culture, 3 percent H_2O_2 is utilized, while 15 percent H_2O_2 is used to detect catalase in anaerobes. This test was done to differentiate bacterial strains of morphological similar *Enterococcus*or *Streptococcus* (catalase negative) and *Staphylococcus* (catalase positive), Also valuable in differentiating aerobic and obligate anaerobic bacteria. Semiquantitative catalase test is used

for the identification of Mycobacterium tuberculosis. It is used to differentiate aerotolerant strains of *Clostridium*, which are catalase negative, from Bacillus species, which are positive. It can be also used as an aid to the identification of Enterobacteriaceae (Geetha et al., 2014).

The indole test [I], the Methyl test[M],Voges Proskauer test and the citrate test are the four tests of the IMViC series, which tests for evidence of an enteric bacterium.

The indole test was conducted on bacterial strains and none of them shown positive results. It indicates that this bacterial strain does not have the ability to produce the enzyme tryptophanase. Which breaks the tryptone to release indole. When indole is combined with Kovac's Reagent (which contains hydrochloric acid and p-dimethylaminobenzaldehyde in amyl alcohol) the solution turns from yellow to cherry red. Since these bacterial strains does not show positive result, the solution does not turn from yellow to red colour. It is used to demonstrate the ability of certain bacteria to decompose the amino acid tryptophane to indole, which accumulates in the medium. Indole has recently been implicated as an important small molecule signal utilized by many bacteria to coordinate various forms of behavior. Indole plays a role in numerous bacterial processes, including: Biofilm formation and maintenance, virulence factor production, antibiotic resistance and persister cell formation. It is used to demonstrate the ability of certain bacteria to decompose the amino acid tryptophane to indole, which accumulates in the medium. Indole has recently been implicated as an important small molecule signal utilized by many bacteria to coordinate various forms of behavior. Indole plays a role in numerous bacterial processes, including: biofilm formation and maintenance, virulence factor production, antibiotic resistance and persister cell formation (Kloepper et al., 1980).

None of the bacterial strains shown positive result for Methyl red test. It shows that this bacterial strains does not have the ability to utilize glucose with production of a stable acid. The methyl red (MR) test is done to detects the production of sufficient acid during the fermentation of glucose and the maintenance of conditions such that the pH of an old culture is sustained below a value of about 4.5, as shown by a change in the colour of the methyl red indicator which is added at the end of the period of incubation. Some bacteria can use glucose as a source of energy and convert it to lactic acid, acetic acid, or formic acid as a by-product. The bacteria which will shows methyl test positive will be having ability convert glucose to pyruvic acid, which is then metabolized via the "mixed acid pathway" to create the stable acid.

The type of acid produced varies from species to species and is determined by the bacteria's particular enzymatic pathways. The resulting acid lowers the pH to 4.5 or below, as shown by a shift in the colour of methyl red from yellow to red (Saharan & Nehra, 2011).

None of the bacterial strains shown positive results for Voges-Proskauer test It shows that this bacterial strain does not have the ability to convert the glucose into a neutral end product 2,3 butanediol which follows 2,3 butanediol formation pathway give positive Voges-Proskauer test and negative Methyl red test. 2,3 butanediol is a neutral end product of glucose fermentation pathway which does not change the pH of media so colour of methyl red indicator does not change and remain yellow. The VP test is given by the intermediate product i.e., Acetoin formed just before butanediol in the pathway. Acetoin reacts with VP reagent (α-naphthol + KOH) to give pinkish red complex, which indicates positive test (Saharan & Nehra, 2011).
Citrate test was conducted on the bacterial strains and none of the bacterial strains shown positive results, except AR-01. It shows that AR-01 bacterial strains have the ability to use sodium citrate as the only source of carbon and inorganic hydrogen phosphate as a source of nitrogen. It is only possible to those bacteria which are capable of fermenting citrate. Bacteria that grow well on this medium produce citrate-permease, an enzyme that converts citrate to pyruvate. Pyruvate can then be used to generate energy in the organism's metabolic cycle. Citrate, an intermediate molecule in the Krebs cycle, is used in the growth of bacteria (Saharan & Nehra, 2011). The ammonium salts are converted down to ammonia by bacteria as they metabolize citrate, increasing alkalinity. The bromothymol blue indicator in the medium changes colour from green to blue as the pH rises above 7.6.
MacConkey agar test was conducted on the bacterial strains and all the 4 bacterial strains showed negative result. MacConkey agar test is used to isolate gram-negative enteric bacteria and distinguish lactose fermenting gram-negative bacteria from lactose non-fermenting gram-negative bacteria. The basic minerals, vitamins, and nitrogenous elements required for microorganism growth are provided by pancreatic digest of gelatin and peptones (meat and casein). Lactose monohydrate is a carbohydrate that can be fermented. Crystal violet and bile salts, which are inhibitory to most gram-positive bacteria, are responsible for this medium's selective activity. The osmotic balance of the medium is maintained by sodium chloride. Neutral red is a pH indicator that becomes red when the pH falls below 6.8 and is colourless when the pH rises above 6.8. The solidifying agent is agar (Devitt et al., 2009).

Molecular characterization and 16 rRNA Gene Sequence Analysis for identification of species was done. Molecular identification of screened isolates was done using PCR. The polymerase chain reaction (PCR) is a laboratory technique for rapidly making (amplifying) millions to billions of copies of a single DNA region, which may subsequently be analysed in greater detail. PCR includes selecting a portion of the genome to be amplified with short synthetic DNA fragments called primers, and then amplifying that segment with several rounds of DNA synthesis. It is the universal method which allows the detection of any bacterial rDNA leading to the identification of the bacterium. The method should allow prompt and accurate identification of bacteria. The principle of the method is simple; when a pure PCR product of the 16 s gene is obtained, sequenced, and aligned against bacterial DNA data base, then the bacterium can be identified.

Using PCR technique, 4 high potential bacterial strains, that is AR-01, AR-06, AR-10, AR-15 was identified. AR-01 was identified as the *Bacillus megaterium*. AR- 06 was identified as *Bacillus acidiceler.* AR- 10 is identified as *Chryseobacterium rhizoplanae.* AR- 15 is identified as *Bacillus firmus.*

AR-01 was identified as *Bacillus megaterium.* It is a rod-like, Gram-positive, mainly aerobic spore forming bacterium found in widely diverse habitats. With a cell length of up to 4 µm and a diameter of 1.5 µm. It is amongst the biggest known bacteria. It has been an important industrial organism for decades. It produces penicillin amidase used to make synthetic penicillin and several enzymes, like amylases used in the baking industry and glucose dehydrogenase used in glucose blood tests. It has the potential to produce plant growth promoting substances and enzymes which will induce growth and development of plant. Thus, it is regarded as the plant growth promoting rhizobacteria. According to (Ortiz-Castro et al., 2008), growth of *A. thaliana* and *P. vulgaris* seedlings were promoted by *Bacillus megaterium* strain. Inoculation with Bacillus megaterium affected the root system in Arabidopsis thaliana plants in a way suggested that the effects mediated by phytohormones, including an inhibition in primary root growth followed by an increase in lateral root number, lateral root growth and root hair length.

AR- 06 was identified as *Bacillus acidiceler*. It is a spore- forming, mesophilic, Gram-positive bacterium of the family Bacillaceae. It has the ability to produce enzymes and Plant growth promoting bacteria such as IAA, ammonium etc. So that it can the used as the potential plant growth promoting bacteria.

AR-10 was identified as *Chryseobacterium rhizoplanae.* It is a rod-shaped gram-negative bacterium. It belongs to the family Flavobacteriaceae. It is slightly yellow pigmented strain. It very significant in production of enzymes and certain plant growth promoting compounds. There for it can be used as plant growth promoting bacterium.

AR- 15 was identified as *Bacillus firmus.* It is a rod-shaped gram-positive bacterium. It belongs to the family bacillaceae. They are semi-transparent, flat colonies. It enhances the plant available forms of nutrients in rhizospheres, control disease- causing pathogenic microbial growth and induce pest defence system. It is very significant in production of enzymes and certain plant growth promoting compounds. Therefore, it can be used as plant growth promoting bacterium. It colonizes the plant roots and induces systematic resistance to some plants.

A biofertilizer contains living microorganisms that colonize the rhizosphere or the interior of the plant when applied to seeds, plant surfaces, or soil, and encourage growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers provide nutrients by enhancing plant development through the natural processes of nitrogen fixation, phosphorus solubilization, and the creation of growth-promoting chemicals. Plant growth-promoting rhizobacteria (PGPR) are a diverse group of soil bacteria that affect plant growth directly or indirectly by producing and secreting a variety of regulatory compounds in the rhizosphere, around/on the root surface. PGPR affect plant growth and development by releasing phytohormones or other biologically active substances, altering endogenous levels of plant growth regulators (PGR), improving nutrient availability and uptake through fixation and mobilization, reducing pathogenic microorganisms' harmful effects on plants, and/or using multiple mechanisms of action. PGPR has been used as a bio-fertilizer in many countries, resulting in increased crop yields. Two aspects to examine are yields and soil fertility. This leads to long-term stability as a result of the PGPR potential contribution. A biofertilizer created by mixing PGPR with composts has been found to promote plant growth and biocontrol in previous studies. *Bacillus* spp. and *Pseudomonas* spp. have both been reported to be effective biocontrol agents. The most prevalent bacterium species are *Bacillus subtilis, Bacillus amyloliquefacients,* and *Bacillus cereus.* Sufficient densities of PGPR in bio-fertilizer play a crucial role in establishing a healthy rhizosphere for plant growth and converting nutritionally significant elements through biological processes, such as boosting N, P, and K availability and preventing pathogen growth. The increased availability of N, P, and K in soil could improve soil fertility, improve antagonistic isolate bio-control actions, and increase

microorganism survival rates. It is classified as a bio-fertilizer when PGPR functions as a plant feeding and enrichment source that replenishes or reconstructs the nutrient cycle between the soil, plant roots, and microorganisms present. The soil environment influences plant growth in a variety of ways, which is a fundamental constraint for long-term agricultural productivity. These stressors are divided into two categories: biotic and abiotic. Abiotic stresses are caused by heavy metal concentration in soils, drought, nutrient deficiencies, salinity, temperature, and other factors, whereas biotic stresses are caused by plant pathogens and pests such as viruses, fungus, bacteria, nematodes, insects, and so on.

Not all the bacteria can be PGPR, A rhizobacterial strain is considered to be a putative PGPR if it possesses specific plant growth promoting traits and can enhance plant growth upon inoculation. An ideal PGPR strain should fulfill the following criteria such as high rhizosphere-competent and eco-friendly. The strains should colonize the plant roots in significant numbers upon inoculation. It should be able to promote promote plant growth and exhibit a broad spectrum of action. The strains should be compatible with other bacteria in the rhizosphere, tolerant of physicochemical factors like heat, desiccation, radiations, and oxidants and should demonstrate better competitive skills over the existing rhizobacterial communities (Vejan, P et al., 2016).

The use of PGPR as a biofertilizer for agroecosystem sustainability has gotten increased attention. Numerous studies have revealed that PGPR could be utilized as a useful addition to chemical fertilizers in an integrated nutrient management (INM) system for boosting crop yields and soil health on a long-term basis. In terms of a healthy and sustainable agriculture, the PGPR strategy has proven to be one of the finest options.

The 4 bacterial strains which shows positive results for both plant growth promoting characters and enzymatic test can be used as an inoculant to biofertilizer as a single strain or as a consortium. Thus, making it more eco-friendly way to the sustainable agriculture. In future, these bacterial strains can be used to conduct growth pattern studies in plants. The plants grown in the presence of these bacterial strains are expected to exhibit high rate of growth than the control.

As long as the human population grows, the world will have to cope with rising food consumption. The Green Revolution improved agricultural productivity worldwide seven decades ago, saving about one billion people from poverty and malnutrition; it also sparked the creation of chemical fertilizers and other innovations. We humans have been involved in different behaviours that have directly or indirectly impacted our ecology, whether for good or for harm, since the birth of civilization. Demand is soon driven by greed to enhance crop yield, resulting in soil ecosystem over exploitation. This must be laid to rest; the traditional crop method can no longer be used because anthropogenic activities such as intensive agriculture, crop monocultures, and the use of agrochemicals are serious environmental concerns

Given the benefits of PGPR in terms of biofertilization, biocontrol, and bioremediation, all of which have a favourable impact on crop productivity and ecosystem functioning, its adoption in agriculture should be encouraged. With the advancement of technology in the establishment of effective research and development, PGPR utilization will undoubtedly become a reality and will be helpful in critical processes that assure the stability and productivity of ago-ecosystems, leading us to a perfect agricultural system.

The study was conducted for Screening, characterization and identification of plant growth promoting rhizobacteria as potential biofertilizers for olericulture.

After preliminary extracellular enzymatic screening, Amylase test was conducted on the isolates. Among the 23 bacterial strains,17 strains showed positive result and 6 strains showed negative results. Gelatinase test was conducted on the 23 bacterial strains and no bacterial strains show gelatinase activity. Protease test was conducted on the isolated bacterial strains. Among 23 bacterial strains, 16 strains showed proteolytic activity and rest of them were tested negative.

In the plant growth promoting characters, the bacterial strains showed positive results for both IAA and Ammonia production. Indole acetic acid (IAA) Test was conducted on 10 bacterial strains. And out of 10 strains of bacteria ,7 bacterial stains showed positive result. None of the bacterial strains shown positive result for the phosphate production. Ammonium test was conducted on 10 bacterial strains. The production of ammonia was observed in the eight isolates.

By conducting morphological characterization of bacterial strains. Out of 4 bacterial strains, AR-10 was identified as gram negative and AR-06, AR-10, AR-15 was identified as gram positive.

Catalase test, Methyl red test, Voges-Proskauer test, Indole test, Citrate test, and MacConkey agar test was conducted for biochemical characterization of bacterial strains. Catalyse test was conducted on the bacterial strains and none of the bacterial strains shown positive result. The Indole test[I], the Methyl red test[M], Voges Proskauer test and the citrate test are the four tests of the IMViC series, which tests for evidence of an enteric bacterium. Indole test, Methyl red, Voges-Proskauer test was conducted on the four bacterial strains and none of the isolates shown positive results. Citrate test was conducted on the bacterial strains and none of the bacterial strains shown positive results, except AR-01. MacConkey agar test was conducted on the bacterial strains and none of the bacterial strains shown positive result.

Molecular characterization and 16 rRNA Gene Sequence Analysis for identification of species was done using PCR. 4 high potential bacterial strains, that is AR-01, AR-06, AR-10, AR-15 was identified. AR-01 was identified as the *Bacillus megaterium*. AR- 06 was identified as *Bacillus acidiceler*. AR- 10 is identified as *Chryseobacterium rhizoplanae*. AR- 15 is identified as *Bacillus firmus*.

Morphological and biochemical characterization of the bacterial strains were used for identification of bacterial strains. The molecular identification of screened isolates using PCR also aid in the identification and it further increased the authenticity.

The Results suggest that the isolated 4 strains of PGPR have high potential of amylase and protease activity and they are capable to enhance the production of IAA and ammonium, thereby improving growth of the plants. The use of PGPR as an inoculant to biofertilizers is an efficient approach to replace chemical fertilizers and pesticides for plant development and growth. PGPR as biofertilizers are well recognized as efficient soil microbes for sustainable agriculture and hold great promise in the improvement of agriculture yields.

These bacterial strains can be made commercially available by encapsulating it with a biofilm. For this n anotechnology can be used for improving the efficacy of PGPR. **N**ano-encapsulation technology can be used in improving PGR. Nanoencapsulation technology has the potential to safeguard PGPR while also improving their service life and dispersion in fertilizer formulations and enabling for regulated PGPR release.

Multiple activity of these 4 bacterial strains is more effective to be used as an inoculant to biofertilizer as a single strain or as a consortium. Thus, making it more eco-friendly way to the sustainable agriculture. The plants grown in the presence of these bacterial strains are expected

to exhibit high rate of growth than the control. In future, these bacterial strains can be used to conduct growth pattern studies in plants.

REFERENCES

1. Ahmad, F., Ahmad, I., & Khan, M. S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological research*, *163*(2), 173181.
2. Ahmad, M., Zahir, Z. A., Khalid, M., Nazli, F., & Arshad, M. (2013). Efficacy of Rhizobium and Pseudomonas strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. Plant Physiology and Biochemistry, 63, 170-176.

3. Arora, N. K., Khare, E., Oh, J. H., Kang, S. C., & Maheshwari, D. K. (2008). Diverse mechanisms adopted by fluorescent Pseudomonas PGC2 during the inhibition of Rhizoctonia solani and Phytophthora capsici. World Journal of Microbiology and Biotechnology, 24(4), 581-585.
4. Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., ... & Smith, D. L. (2018). Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in plant science*, 1473.
5. Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.*, *57*, 233-266.
6. Bajracharya, A. M. (2019). Plant growth promoting rhizobacteria (PGPR): Biofertiliser and Biocontrol Agent-Review article. *Journal of Balkumari College*, *8*, 42-45.
7. Bakker, A. W., & Schippers, B. O. B. (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp-mediated plant growth-stimulation. Soil Biology and Biochemistry, 19(4), 451-457.
8. Basu, A., Prasad, P., Das, S. N., Kalam, S., Sayyed, R. Z., Reddy, M. S., & El Enshasy, H. (2021). Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. *Sustainability*, *13*(3), 1140.
9. Benedetto, N. A., Campaniello, D., Bevilacqua, A., Cataldi, M. P., Sinigaglia, M., Flagella, Z., & Corbo, M. R. (2019). Isolation, screening, and characterization of plant-growth-promoting bacteria from durum wheat rhizosphere to improve N and P nutrient use efficiency. *Microorganisms*, *7*(11), 541.
10. Bhardwaj, D., Ansari, M. W., Sahoo, R. K., & Tuteja, N. (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial cell factories*, *13*(1), 1-10.
11. Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*, *28*(4), 1327-1350.
12. Burdman, S. A. U. L., Jurkevitch, E. D. O. U. A. R. D., & Okon, Y. A. A. C. O. V. (2000). Recent advances in the use of plant growth promoting rhizobacteria (PGPR) in agriculture. Microbial interactions in agriculture and forestry (Volume II), 229-250.
13. Choudhary, D. K., Sharma, K. P., & Gaur, R. K. (2011). Biotechnological perspectives of microbes in agro-ecosystems. Biotechnology letters, 33(10), 1905-1910.
14. Coico, R. (2006). Gram staining. *Current protocols in microbiology*, (1), A-3C.
15. Davies, P. J. (Ed.). (2013). Plant hormones: physiology, biochemistry and molecular biology. Springer Science & Business Media.
16. Devitt, S. (2009). Methyl red and voges-proskauer test protocols. American Society for Microbiology, 8.
17. Dey, R. K. K. P., Pal, K. K., Bhatt, D. M., & Chauhan, S. M. (2004). Growth promotion and yield enhancement of peanut (Arachis hypogaea L.) by application of plant growth-promoting rhizobacteria. Microbiological research, 159(4), 371-394.
18. Egamberdieva, D., & Lugtenberg, B. (2014). Use of plant growth-promoting rhizobacteria to alleviate salinity stress in plants. In Use of Microbes for the Alleviation of Soil Stresses, Volume 1 (pp. 73-96). Springer, New York, NY.
19. Esitken, A., Pirlak, L., Ipek, M., Donmez, M. F., Cakmakci, R., & Sahin, F. (2009). Fruit bio-thinning by plant growth promoting bacteria (PGPB) in apple cvs. Golden Delicious and Braeburn. Biological Agriculture & Horticulture, 26(4), 379-390.

20. Esteban, R., Rodriguez-Cousiño, N., & Esteban, L. M. (1993). Genomic organization of T and W, a new family of double-stranded RNAs from Saccharomyces cerevisiae. *Progress in nucleic acid research and molecular biology*, *46*, 155-182.
21. Flores-Félix, J. D., Silva, L. R., Rivera, L. P., Marcos-García, M., García-Fraile, P., Martínez-Molina, E., ... & Rivas, R. (2015). Plants probiotics as a tool to produce highly functional fruits: the case of Phyllobacterium and vitamin C in strawberries. PLoS One, 10(4), e0122281.
22. Geetha, K., Venkatesham, E., Hindumathi, A., & Bhadraiah, B. (2014). Isolation, screening and characterization of plant growth promoting bacteria and their effect on Vigna Radita (L.) R. Wilczek. *Int J Curr Microbiol Appl Sci*, *3*(6), 799-803.
23. Glick, B. R. (2012). Plant growth-promoting bacteria: mechanisms andapplications. *Scientifica*, *2012*.
24. Goswami, D., Pithwa, S., Dhandhukia, P., & Thakker, J. N. (2014). Delineating Kocuria turfanensis 2M4 as a credible PGPR: a novel IAA-producing bacteria isolated from saline desert. Journal of Plant Interactions, 9(1), 566-576.
25. Gray, E. J., & Smith, D. L. (2005). Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil biology and biochemistry, 37(3), 395-412.
26. Gupta, A., Gupta, R., & Singh, R. L. (2017). Microbes and environment. In *Principles and applications of environmental biotechnology for a sustainable future* (pp. 43-84). Springer, Singapore.
27. Hafeez, F. Y., Yasmin, S., Ariani, D., Renseigné, N., Zafar, Y., & Malik, K. A. (2006). Plant growth-promoting bacteria as biofertilizer. *Agronomy for sustainable development*, *26*(2), 143-150.
28. Ibiene, A. A., Agogbua, J. U., Okonko, I. O., & Nwachi, G. N. (2012). Plant growth promoting rhizobacteria (PGPR) as biofertilizer: Effect on growth of Lycopersicum esculentus. *J Am Sci*, *8*(2), 318-324.
29. Jha, C. K., & Saraf, M. (2015). Plant growth promoting rhizobacteria (PGPR). *JOURNAL OF AGRICULTURE RESEARCH AND DEVELOPMENT*, *5*(2), 0108-0119.
30. Kaymak, H. C. (2010). Potential of PGPR in agricultural innovations. Plant growth and health promoting bacteria, 45-79.
31. Kloepper, J. W. (1978). Plant growth-promoting rhizobacteria on radishes. In Proc. of the 4th Internet. Conf. on Plant Pathogenic Bacter, Station de Pathologie Vegetale et Phytobacteriologie, INRA, Angers, France, 1978 (Vol. 2, pp. 879-882).
32. Kloepper, J. W., Leong, J., Teintze, M., & Schroth, M. N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature, 286(5776), 885-886.
33. Kumar, H., Bajpai, V. K., Dubey, R. C., Maheshwari, D. K., & Kang, S. C. (2010). Wilt disease management and enhancement of growth and yield of Cajanus cajan (L) var. Manak by bacterial combinations amended with chemical fertilizer. Crop protection, 29(6), 591-598.
34. Lavakush, Y.J.; Verma, J.P.; Jaiswal, D.K.; Kumar, A. Evaluation of PGPR and different concentration of phosphorous level on plant growth, yield and nutrient content of rice (Oryza sativa). Ecol. Eng. 2014, 62, 123–128.

35. Lloret, L., & Martínez-Romero, E. (2005). Evolution and phylogeny of rhizobia. Revista Latinoamericana de Microbiología, 47(1-2), 43-60.
36. Lucy, M., Reed, E., & Glick, B. R. (2004). Applications of free living plant growth-promoting rhizobacteria. Antonie van leeuwenhoek, 86(1), 1-25.
37. MacWilliams, M. P. (2012). Indole test protocol. American Society for Microbiology, Washington, DC.
38. McDevitt, S. (2009). Methyl red and voges-proskauer test protocols. American Society for Microbiology, 8.
39. Malusa, E., & Vassilev, N. (2014). A contribution to set a legal framework for biofertilisers. Applied microbiology and biotechnology, 98(15), 6599-6607.
40. Mishra, D., Rajvir, S., Mishra, U., & Kumar, S. S. (2013). Role of bio-fertilizer in organic agriculture: a review. Research Journal of Recent Sciences ISSN, 2277, 2502.
41. Nadeem, S. M., Zahir, Z. A., Naveed, M., & Ashraf, M. (2010). Microbial ACC-deaminase: prospects and applications for inducing salt tolerance in plants. Critical Reviews in Plant Sciences, 29(6), 360-393.
42. Nautiyal, C. S., Govindarajan, R., Lavania, M., & Pushpangadan, P. (2008). Novel mechanism of modulating natural antioxidants in functional foods: involvement of plant growth promoting rhizobacteria NRRL B-30488. Journal of agricultural and food chemistry, 56(12), 4474-4481.
43. Naveed, M., Hussain, M. B., Zahir, Z. A., Mitter, B., & Sessitsch, A. (2014). Drought stress amelioration in wheat through inoculation with Burkholderia phytofirmans strain PsJN. Plant Growth Regulation, 73(2), 121-131.
44. Oo, K. T., Win, T. T., Khai, A. A., & Fu, P. (2020). Isolation, screening and molecular characterization of multifunctional plant growth promoting rhizobacteria for a sustainable agriculture. *American Journal of Plant Sciences*, *11*(6), 773-792.
45. Ortíz-Castro, R., Valencia-Cantero, E., & López-Bucio, J. (2008). Plant growth promotion by Bacillus megaterium involves cytokinin signaling. *Plant signaling & behavior*, *3*(4), 263-265.
46. Parewa, H. P., Meena, V. S., Jain, L. K., & Choudhary, A. (2018). Sustainable crop production and soil health management through plant growth-promoting rhizobacteria. In *Role of rhizospheric microbes in soil* (pp. 299-329). Springer, Singapore.
47. Pishchik, V. N., Vorobyev, N. I., Chernyaeva, I. I., Timofeeva, S. V., Kozhemyakov, A. P., Alexeev, Y. V., & Lukin, S. M. (2002). Experimental and mathematical simulation of plant growth promoting rhizobacteria and plant interaction under cadmium stress. Plant and Soil, 243(2), 173-186.
48. Porcel, R., Zamarreño, Á. M., García-Mina, J. M., & Aroca, R. (2014). Involvement of plant endogenous ABA in Bacillus megaterium PGPR activity in tomato plants. BMC plant biology, 14(1), 1-12.
49. Prasad, R., Jain, V. K., & Varma, A. (2010). Role of nanomaterials in symbiotic fungus growth enhancement. Current Science, 99(9), 1189-1191.
50. Reddy, G. S., Aggarwal, R. K., Matsumoto, G. I., & Shivaji, S. (2000). Arthrobacter flavus sp. nov., a psychrophilic bacterium isolated from a pond in McMurdo Dry Valley, Antarctica. *International Journal of Systematic and Evolutionary Microbiology*, *50*(4), 1553-1561.
51. Reddy, G., Prakash, J., Vairamani, M., Prabhakar, S., Matsumoto, G., & Shivaji, S. (2002). *Planococcus antarcticus* and *Planococcus psychrophilus* spp. nov. isolated

from cyanobacterial mat samples collected from ponds in Antarctica. *Extremophiles*, *6*(3), 253-261.

52. Riaz, U., Murtaza, G., Anum, W., Samreen, T., Sarfraz, M., & Nazir, M. Z. (2021). Plant Growth-Promoting Rhizobacteria (PGPR) as biofertilizers and biopesticides. In *Microbiota and Biofertilizers* (pp. 181-196). Springer, Cham.
53. Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Wei, H. X., Paré, P. W., & Kloepper, J. W. (2003). Bacterial volatiles promote growth in Arabidopsis. Proceedings of the National Academy of Sciences, 100(8), 4927-4932
54. Saharan, B. S., & Nehra, V (2011). Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res, 21(1), 30.
55. Sarma, R. K., & Saikia, R. (2014). Alleviation of drought stress in mung bean by strain Pseudomonas aeruginosa GGRJ21. Plant and soil, 377(1), 111-126.
56. Shivaji, S., Vijaya Bhanu, N., & Aggarwal, R. K. (2000). Identification of Yersinia pestis as the causative organism of plague in India as determined by 16S rDNA sequencing and RAPD-based genomic fingerprinting. *FEMS microbiology letters*, *189*(2), 247-252.
57. Shoaib, M., Muzammil, I., Hammad, M., Bhutta, Z. A., & Yaseen, I. (2020). A mini-review on commonly used biochemical tests for identification of bacteria. *A Mini-Review on Commonly used Biochemical Tests for Identification of Bacteria*, *54*(1), 8-8.
58. Singh, B. N., Rajawat, M. V. S., Hidangmayum, A., Ansari, W. A., Singh, D., Zeyad, M. T., ... & Kumar, M. (2019). Importance and utilization of plant-beneficial rhizobacteria in agriculture. In *Microbial Interventions in Agriculture and Environment* (pp. 171-187). Springer, Singapore.
59. Son, J. S., Sumayo, M., Hwang, Y. J., Kim, B. S., & Ghim, S. Y. (2014). Screening of plant growth-promoting rhizobacteria as elicitor of systemic resistance against gray leaf spot disease in pepper. Applied soil ecology, 73, 1-8.
60. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic acids research*, *25*(24), 4876-4882.
61. Vacheron, J., Desbrosses, G., Bouffaud, M. L., Touraine, B., Moënne-Loccoz, Y., Muller, D., ... & Prigent-Combaret, C. (2013). Plant growth-promoting rhizobacteria and root system functioning. Frontiers in plant science, 4, 356.
62. Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., & Nasrulhaq Boyce, A. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. Molecules, 21(5), 573.
63. Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and soil*, *255*(2), 571-586.
64. Vijayan, R., Palaniappan, P., Tongmin, S. A., Elavarasi, P., & Manoharan, N. (2013). Rhizobitoxine enhances nodulation by inhibiting ethylene synthesis of Bradyrhizobium elkanii from Lespedeza species: validation by homology modelingand molecular docking study. World J Pharm Pharm Sci, 2, 4079-4094.
65. Wani, P. A., Khan, M. S., & Zaidi, A. (2007). Synergistic effects of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on the performance of field-grown chickpea. Journal of Plant Nutrition and Soil Science, 170(2), 283-287.

66. Weller, D. M., & Thomashow, L. S. (1994). Current challenges in introducing beneficial microorganisms into the rhizosphere. Molecular ecology of rhizosphere microorganisms: biotechnology and the release of GMOs, 1-18.
67. Xiong, K., & Fuhrmann, J. J. (1996). Comparison of rhizobitoxine-induced inhibition of β-cystathionase from different bradyrhizobia and soybean genotypes. Plant and soil, 186(1), 53-61.
68. Yadav, J., Verma, J. P., Jaiswal, D. K., & Kumar, A. (2014). Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (Oryza sativa). Ecological engineering, 62, 123-128.