

ISOLATION AND CHARACTERIZATION OF PLANT GROWTH PROMOTING AND BIO-CONTROL BACTERIA FROM VERMICOMPOST

Submitted in the partial fulfillment of the requirements for the award of Bachelor of
Science

Degree in

BIOTECHNOLOGY

By

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BONAFIDECERTIFICATE

This is to certify that this Project Report is the bonafide work of **Miss. R SUG ANTHI EVANGELINE** (Reg No: 40770051) who carried out the project entitled **"ISOLATION AND CHARACTERIZATION OF PLANT GROWTH PROMOTING AND BIO-CONTROL BACTERIA FROM VERMICOMPOST"** under our supervision from December 2022 to April 2023.

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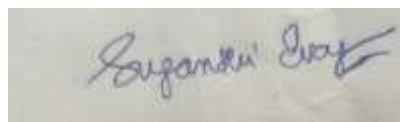
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DECLARATION

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A rectangular box containing a handwritten signature in blue ink. The signature appears to read 'Suganthi Evang' followed by a stylized flourish.

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ABSTRACT

Vermicompost is a nutritive organic fertilizer that is enhanced with plant-available forms of macronutrients (nitrogen, phosphorus, and potassium) and micronutrients (iron, copper, zinc, etc.) as well as helpful soil bacteria. Along with their function in promoting development, they also prevent plant illnesses by creating compounds that are toxic to pathogens and stimulating the plants' defense mechanisms. The main aim of the current research is to isolate the plant growth promoting and antagonistic bacteria from vermicompost and characterized for their *in-vitro* and pot culture plant growth promotion (PGP) and disease control ability as well as production and identification of volatile metabolite using headspace gas chromatography and mass spectroscopy (GC -MS). Totally, seven bacterial strains were isolated from the vermicompost sample. Out of which 4 strains showed siderophore positive in CAS agar medium. Similarly, the extracellular hydrolytic enzymes such as cellulase (1 strain), amylase (3 strains) and protease (2 strains) were also produced by vermicompost bacteria. Further, the bacterial strain VC2 showed maximum antagonistic activity against *Colletotrichum gloeosporioides* (60% of inhibition) and *Ralstonia solanacearum* (12mm zone of inhibition). Based on the *in-vitro* PGP and antagonistic activity, the bacterial strain VC2 was selected as a potential bacteria and characterized for *in-planta* PGP and antagonistic activity in tomato. It showed maximum germination percentage (100%) as well as higher shoot (7.3cm) and root (5cm) length in *in-planta* paper towel method. Similarly, in pot culture also it showed maximum shoot and root length when it compares with control plants. Finally, the production and identification of volatile compound by potential strain VC2 in headspace GCMS showed some interesting volatile metabolites such as oxalic acid, phenylephrine, acetone, DL-alanine ethyl ester, n-hexyl methylamine, cationone, may be the reason for their potential PGP and antagonistic activity. The potential vermicompost bacterial strain VC2 was identified as a genus-level *Lysinibacillus* by studying the microscopic, cultural, morphological, and molecular characterization. In conclusion, vermicompost has been shown to have several favorable effects on soil and plant growth. Because of its antagonistic ability against soil-borne illnesses, which improves plant health, vermicompost bacteria is seen as a potential replacement for hazardous chemical fertilizers and pesticides in agricultural production.

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LIST OF ABBREVIATIONS

VC	Vermicompost
PGP	Plant Growth Promotion
PGPR	Plant Growth Promoting Rhizobacteria
PGPB	Plant Growth Promoting Bacteria
PGR	Plant Growth Regulators
IAA	Indole Acetic Acid
GC-MS	Gas Chromatography - Mass Spectrometry

CHAPTER 1

INTRODUCTION

Vermicompost is a consequence of the joint action of earthworms and associated microorganisms in the non-thermophilic biodegradation of organic materials. It is a peat-like material that has been finely divided and has a high porosity, aeration, drainage, and microbial activity. Earthworms act as mechanical blenders, modifying the organic substrate's physical and chemical properties to increase the surface area that is favourable for microbial breakdown (Dominguez, *et.al.*, 2004). Earthworms generate vermicast, a nutritious organic fertilizer in the form of microscopic pellets after consuming soil and organic materials. Vermicast is rich in humus, macronutrients (nitrogen, phosphorus, and potassium), micronutrients, beneficial soil microflora, actinomycetes, and plant growth regulators (Adhikary *et.al.*, 2012). The digestion of both organic and inorganic elements depends heavily on the earthworm's digestive system. Cellulase, protease, and chitinase activity in the stomach, as well as endosymbiotic microbial activity (Zhang *et al.*, 2000). Vermicompost, also spelt vermi-compost, is the product of the decomposition process in which various worm species, primarily red wigglers, white worms, and other earthworms, are used to produce a mixture of bedding materials, decomposing food or vegetable waste, and vermicast. Raising worms for this activity, referred to as vermicomposting, is known as vermiculture (Thompson *et.al.*, 1998).

Vermicomposting is the practice of employing earthworms to decompose organic wastes. During the vermicomposting process, earthworms and bacteria collaborate to break down the material. However, they also fragment and condition the substrate, which significantly alters its biological activity. Microbes carry out the biochemical degradation of organic materials, which is driven by earthworms (Ludibeth *et al.*, 2012). Vermicompost is formed of organic waste that has been digested by diverse earthworm species into a homogenous material with good nutritional value and a wealth of possible uses in agriculture. *Eisenia foetida* and *Lumbricus rubellis* are the two kinds of earthworms that are grown in worm farms most frequently. These worms are widely used to produce vermicompost. Both have been referred to by a variety of common names, such as red

worms, red wigglers, tiger worms, brandling worms, and dung worms (Dawsonville Ga. *et.al.*, 2013).

Plant Growth Promoting Rhizobacteria's are regarded as promising alternatives due to their distinctive characteristics. Microbes have been recognized as being capable of solving complex problems in the life sciences and other fields because of their unusual abilities to demonstrate a variety of spontaneous biosynthetic activities in response to specific environmental and growth conditions (Zandonadi *et.al.*, 2007). There is evidence that some soil microbes, particularly those in the rhizosphere, secrete substances that are beneficial to plants and increase crop production. These substances include antibiotics, siderophores, growth promoters, and help plants absorb nutrients like phosphorus, potassium, and zinc, among others. Interaction between plants and microbes the foundation for soil fertility, plant growth, and production all lie within the rhizosphere (Baldotto *et.al.*, 2009).

Both the extracellular and intracellular PGPRs are parts of the PGPR. *Arthrobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, and *Pseudomonas* are a few examples of extracellular PGPR, as well as the rhizosphere itself, root surfaces, and even the cellular spaces of root tissues. Examples of intracellular PGPR include *Frankia* species and the *endophytes*, which include *rhizospheric* bacteria from the *Rhizobiaceae* family (Gray and Smith *et.al.*, 2005).

Vermicompost promotes the communities of plant-friendly bacteria by enhancing their populations, which in turn suppresses soil-borne illnesses, which both directly and indirectly speed up plant growth (Canellas *et al.*, 2002). By processes related to the stomach, earthworms increase the microbial diversity and enzymatic activity of ingested bacteria (Drake and Horn, 2007). Because of this, vermicompost contained a larger variety of bacteria, fungus, and actinomycetes that are beneficial to soil. Vermicompost improves soil's physio-chemical qualities, which promotes better crop growth and development in addition to introducing beneficial bacteria and nutrients. Vermicompost improves soil nutrient content, boosts soil microbial activity, increases oxygen availability, maintains normal soil temperature, promotes soil porosity and water infiltration, and encourages plant growth, yield, and quality (Arora *et al.*, 2011).

Many minerals, such as calcium, nitrates, phosphorus, and soluble potassium, which are crucial for plant growth, are abundant in vermicompost. Vermicompost, which originates from microorganisms, contains a variety of plant growth hormones, including auxins, cytokinins, and gibberellins (Pankaj Sood, 2021). Vermicompost has a significant impact on soil fortification in terms of growth traits like length, weight, root, shoot branch, number of leaves, and host plant metabolism against nematode infection. Plants that had been enriched with vermicompost produced more sugar, protein, and lipids than untreated controls. The production of more of these metabolites encourages excessive plant growth while assisting treated plants in metabolically overcoming the infection. Because vermicompost contains significant quantities of nutrients, humic acids, and humates, several research hypothesized that the growth responses of plants from vermicompost resembled "hormone induced activity" instead (Ferrerias *et.al.*, 2006).

Vermicompost (VC) is used to improve soil because it is a rich source of macro- and micronutrients. It is mostly regarded as an effective addition of organic matter to low-organic-content soils and horticultural container media. Lately, VC has also been thoroughly investigated for its many additional beneficial actions. Especially in the use of a variety of techniques to combat plant diseases (Rivera and wright *et.al.*, 2009). Because of its increased potential to alter soil qualities. The growth and multiplication of saprophytic soil microorganisms, including the biocontrol agents, is also encouraged by VC-mediated soil physicochemical characteristics. This aids in improving the effectiveness of most biocontrol agents against a variety of phytopathogens. Also, it has been determined that VC made using various techniques and resources has a variety of roles in fostering plant development and attaining biological control of phytopathogens (Reddy *et.al.*, 2012). Composts are widely acknowledged to reduce the occurrence of plant diseases, increase the availability of nutrients in the soil, and promote plant growth via both biotic and abiotic causes. By inducing systemic resistance against foliar pathogens and suppressing microbial populations found in composted organic matter, VC aids in the eradication of illnesses that are transmitted through the soil. Bacterial phytopathogens arise a number of problems worldwide in the cultivation of economically important agricultural crops especially in the tropical and subtropical regions (Ersahin *et.al.*, 2009) Chemicals are extensively used in current agriculture. However extensive

use of chemicals in agriculture has led to deteriorating human health, environmental pollution, and development of pathogen resistance to both bacterial and fungal pathogens. Micro-organisms as bio-control agents have high potential to control plant pathogens and have no effect on the environment and other non-target organisms. Over the last 25-30 years, improvement in alternative control methods, including use of micro-organisms as potential bio-control agents such as replacements or supplements for agrochemicals has been undertaken (Yang *et al.*, 2009).

CHAPTER 2

LITERATURE SURVEY

2.1 INTRODUCTION TO VERMICOMPOST

The product obtained by the process of decomposing various species of worms or earthworms is vermicompost. This process is known as vermicomposting. Vermicompost is an organic fertilizer with rich nutrients and soil conditioner. Vermicompost is used in organic farming, sustainable and gardening. Composting with vermicompost has become increasingly popular in both industrial and domestic settings because, compared to conventional composting, it provides a quicker way to treat organic wastes. as vermicompost contains water soluble nutrients and is an organic fertilizer it is relatively easy for the plants to absorb. Castings are often used as organic fertilizer. Since earthworms grind and mix minerals in simple forms, plants have little difficulty in obtaining them. Their digestive systems create environments that enable certain microbes to thrive. Except certain species all worms make compost. Vermicompost can be added straight to the soil or combined with water to create worm tea, a liquid fertilizer to be confused with worm tea is the light brown waste liquid, or leachate, that collects at the bottom of some vermicomposting systems. It is an uncomposed result of the breakdown of foods that are high in water and could be contaminated with infections and poisons. When additional moisture is required for processing, it is advisable to discard it or add it back to the bin. Depending on the inputs provided to worms, these fertilizer's, pH, nutritional, and microbial contents change. Improved soil aeration and microorganism enrichment are two benefits of vermicompost (adding enzymes such as phosphatase and cellulase). In comparison to soil and organic materials that worms consume, worm castings have 10 to 20 times more microbial activity. Draws in existing soil-based, deep-burrowing earthworms. Increases the capability of storing water. Vermicompost increases crop output, plant growth, and germination (Dilbaghi, *et.al.*,1997).

It promotes plant and root growth. Enhances soil microbial life (adding plant hormones such as gibberellin acid and auxin). It closes the "metabolic gap" by recycling trash locally. Manufacturing lowers the emissions of greenhouse gases including nitric

oxide and methane (produced in landfills or incinerators when not composted). Depending on the climate, vermicomposting techniques may vary. Since the raw materials or feedstocks utilized can compost, heating up the worm bins as they decompose and killing the worms, it is important to keep an eye on the temperatures of large-scale bin systems (which can have high heat-retentive qualities). Worms are so good at processing organic waste that they can consume over half of their own body weight in trash each day. Vermicomposting is a straightforward composting method that makes use of what earthworms naturally do but limits them to bins so that farmers can more easily feed them and collect the nutrient-rich compost.

2.2 NUTRITIONAL QUALITY OF VERMICOMPOST

Global social and environmental dangers have increased as a result of the extensive use of chemical fertilizers and pesticides. Also, it has decreased plant tolerance to insect-pests and other prevalent diseases as well as the fertility and soil bacteria in the area. The usage of these synthetic fertilizers has had a negative impact on agricultural output as well as human health. In order to avoid the risks associated with chemical fertilizers, ecologically acceptable, economically feasible, safe, and sustainable methods of soil fertilizing have been adopted. An eco-friendly method of bio-oxidation and stabilization of organic wastes is vermicomposting. Vermicompost, which is utilized as an alternative to agrochemicals, is rich in humus, macronutrients, micronutrients, beneficial soil microorganisms, *actinomycetes*, and plant growth regulators. Sometimes, earthworms are referred to be soil managers, nature's ploughmen, and farmers' friend. They eat organic matter and encourage soil aeration, mineral particle mixing, and fragmentation. Many organic wastes, such as animal manure, green manure, industrial wastes, sewage sludge, and agricultural residues, can be consumed by some species of earthworms. The benefits of vermicomposting over composting material include increased bulk density, water holding capacity, pH, electrical conductivity, nitrogen, phosphorus, and potassium content. Vermicompost also reduces the quantity of heavy metals incorporated into soil as compared to compost. According to another claim, vermicompost may include more substances than compost that are employed as plant hormones to promote plant growth and development (AlemayhuDiriba *et al.*,2020). Vermicompost contains nutrients like

NO₃, PO₄, Ca, K, Mg, S, and micronutrients that have similar effects on plant growth and production to inorganic fertilizers applied to soil. Vermicompost is predominantly composed of C, H, and O. (Singh et al., 2008). Vermicompost, has a high concentration of humic substances (humic acids, fulvic acids, and humin) that offer a variety of sites for chemical reactions, as well as microbial elements that promote plant growth and disease suppression through the actions of bacteria (*Bacillus*), yeasts (*Sporobolomyces* and *Cryptococcus*), fungi (*Trichoderma*), and bacteria (*Sporobolomyces*), as well as chemical antagonists such as phenol and amino acids (Nagavalemma et al., 2004).

Vermicompost has nutrients like NO₃, exchangeable P, K, Ca, and Mg that are readily absorbed by plants (Edwards and Burrows, 1988). Vermicomposts have been shown to include auxins, cytokinins, and other humic compounds, as well as other plant growth regulators and influencing components, created by bacteria. Many antibiotics and actinomycetes found in vermicompost contribute to the improvement of crop plants' "power of biological resistance" to pests and illnesses (Garg et al., 2009). For a variety of biological processes, micronutrients like iron, zinc, copper, and magnesium are crucial. Enhanced drought tolerance is another benefit, as is better resilience to disease and insect pests. It was discovered that zinc, which is included in vermicompost, is crucial for the conversion of carbohydrates and controls the absorption of sugars. It's possible that the presence of copper-containing oxidizing enzymes in vermicompost accounts for its higher copper content. Strengthening stalks, stems, and branches using copper results in healthy, vigorous growth. The earthworm activity's mineralization of manganese is the cause of the increase in its content in vermicompost (Rippu Daman. et al., 2016).

All macronutrients except sulphur were found to be higher in vermicomposting than in pit-composting. This may be related to the vermicompost's worm content and the composting process' duration. It has been discovered that vermicomposting increases total nitrogen, total phosphorus, total potassium, total calcium, and total magnesium (Bhat et al., 2017). Nitrogen is determined to be necessary for crop output (Nadeem et al., 2014), while phosphorus and potassium were discovered to be essential nutrients for plant growth and development (Huang et al., 2011; Zlatev and Lidon, 2012). Plants were discovered to be protected by the Ca against biotic and abiotic stressors (Yang et al.,

2013). Magnesium helps in plant photosynthesis and reduces the effects of heavy metal stress (Chen and Ma, 2013).

2.3 DIFFERENT SOURCES OF VERMICOMPOST

Brief descriptions of earthworms, the major players in the process of vermicomposting, highlight how these creatures can be significant decomposers and converters of organic waste. We review the life cycles of these species and the typical requirements of ideal vermicomposting earthworm species in order to better understand the requirements that the various earthworm species that are suitable for vermicomposting have for their optimal development, growth, and productivity in organic wastes. The biological and ecological process of vermicomposting is intricate. Despite the fact that earthworms are essential to the process, vermicomposting involves intricate interactions that cause the organic matter, bacteria, earthworms, and other soil invertebrates to fragment, bio-oxidize, and stabilize. In addition to sustaining intricate food webs, the vermicomposting system converts various nutrient elements' chemical forms into inorganic molecules that are easily accessible to plants and are crucial for nutrient dynamics. It is reasonable to assume that epigeic species are appropriate for vermiculture and vermicomposting. The ability to colonize organic wastes naturally; high rates of organic matter consumption, digestion, and assimilation; the ability to tolerate a variety of environmental factors; high reproductive rates, by producing large numbers of cocoons that should not have a long hatching time; and the ability to tolerate a wide range of environmental factors are additional requirements for a species to be considered suitable for use in vermicomposting. The majority of earthworm species lack some or all of these traits. *Eisenia andrei* (Bouche, *et.al.*, 1972), and *Eisenia fetida* (Savigny, *et.al.*, 1826), They are omnipresent and peregrine with a global distribution, and they naturally colonise numerous organic wastes. They have good temperature tolerance and can survive in organic wastes with a variety of moisture levels (Graff *et.al.*, 1974). This species typically grows in damp soils, especially those that have had animal manures or sewage solids added to them.

2.4 BACTERIA DIVERSITY IN VERMICOMPOST

The soul of all life, soil supports a wide variety of microorganisms. *Bacillus*, *Pseudomonas*, *Streptomyces*, and other soil bacteria are prolific producers of secondary compounds that fight off a variety of coexisting phytopathogenic fungi and human pathogenic bacteria. (Pathma et.al., 2011b). It has been discovered that earthworm activity increases the good microflora and suppresses the bad pathogenic microorganisms. A substantial source of micro- and macronutrients as well as microbial enzymes is found in soil wormcasts. (Lavelle Martin et.al., 1992).

The action of the earthworm gut microbiota is probably what allows them to boost plant nutrient availability. By comminuting the litter and influencing the activity of the soil microflora, earthworms have an indirect impact on the dynamics of soil chemical processes (Petersen and Luxton et.al., 1982). Along with rhizospheric soil, earthworms consume rhizospheric bacteria such as *Pseudomonas*, *Rhizobium*, *Bacillus*, *Azospirillum*, and *Azotobacter*, which may become activated or expanded due to the favourable microenvironment in the stomach. Therefore, earthworm activity increases the quantity of plant growth-promoting rhizobacteria (PGPR) (Sinha et al., 2010). According to reports, earthworms make up the drilosphere and are associated with these free-living soil bacteria. (Ismail et.al.,1995). The organic matter is mineralized by earthworm microbes, who also make it easier for metal ions to be chelated (Canellas et.al., 2002).

According to (Edwards and Lofty et al.,1977), earthworms increased the number of microorganisms in soil by up to five times. Earthworms improve soil aeration by their burrowing activities, which stimulates and accelerates microbial activities by increasing the population of soil microorganisms and their biomass. The initial microbial community of the garbage was altered in a variety of ways by vermicomposting (Binet et al.,1998).

Vermicompost is rich in *Actinobacteria* and *Gamma proteobacteria*, while traditional compost was higher in *Alpha proteobacteria* and *Bacteroidetes*, the bacterial phylogenetic groupings typical of uncured compost (Vivas et.al., 2009). Vermicompost contains nitrobacter, azotobacter, rhizobium, phosphate solubilizers, and actinomycetes (Suhane et.al., 2007).

Actinobacteria, *Planctomycetes*, *Firmicutes*, and *Bacteroidetes* were all found in the bacterial population of vermicompost according to molecular and culture-dependent investigations. In the worm-treated compost, several observations revealed a large rise in the overall viable counts of actinomycetes and bacteria (Yasir *et.al.*, 2009). The rise in microbial population may be caused by the favorable conditions of microbial development within an earthworm's digestive system and by the ingestion of nutrient-rich organic wastes that serve as a substrate for the growth of microorganisms as well as a source of energy (Tiwari *et.al.*, 1989).

Infected biomedical waste containing pathogens like *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas pyocyaneae*, and *Escherichia coli* are transformed into harmless waste by the process of vermicomposting, which also contains commensals like *Citrobacter freundii* and aerobic spore-bearing microorganisms that are typically found in soil and earthworms' (Umesh *et.al.*, 2006).

2.5 BACTERIA DIVERSITY IN VC AS PLANT GROWTH PROMOTER AND ANTAGONIST

Utilizing earthworms as adaptable natural bioreactors for the breakdown of organic materials, vermicomposting is an effective method for recycling nutrients. Vermicompost's increase soil fertility, promote plant growth, and reduce the population of plant diseases and pests due to their richness in nutrient availability and microbial activity. Earthworms are crucial to the cycling of carbon, the production of soil, the breakdown of cellulose, and the buildup of humus. The physics, chemistry, and biology of soil are all significantly impacted by earthworm activities. Earthworms consume a lot of organic material. Vermicompost's micro and macronutrients, vitamins, enzymes, and hormones can have a major impact on the growth and production of plants. (Makulecet.*et.al.*,2002). Vermicompost's have a large specific surface area that offers many microsites for microbial activity and for the strong retention of nutrients. They also include nutrients such as nitrates, exchangeable phosphorus, soluble potassium, calcium, and magnesium in forms that are available to plants (Sreenivas *et.al.*, 2000).

By introducing helpful bacteria to the soil, vermicompost's also increase biological fertility. Through the earthworm's digestive canal, mucus encourages conflict and

competition among various microbial populations, which in turn produces some antibiotics and biochemicals that resemble hormones and promote plant development (Edwards and Arancon *et.al.*, 2004). By incorporating vermicasts into the soil, crop output is increased while soil structure, fertility, and plant growth are all improved (Chaoui *et.al.*, 2002). Vermicompost provides helpful buffering properties that reduce the harm from excess nutrient intake that may otherwise result in phytotoxicity. As a result, vermicompost serves as a slow-release fertilizer and soil conditioner (Atiyeh *et al.*, 2000).

Vermicompost's are increasingly being used as biofertilizers because of their exceptional nutritional quality and increased microbial and antagonistic activity. Application of vermicompost increased plant growth significantly and consistently in both field and greenhouse settings, providing strong evidence that biological growth-promoting elements are important for seed germination and plant growth. Microorganisms produce auxins, gibberellins, cytokinins, ethylene, and abscisic acid, among other plant hormones and plant-growth regulating chemicals (PGRs) (Edwards *et.al.*, 2004). Gibberellins produced by microorganisms have an impact on plant development and growth, whereas auxins produced by *Azospirillum brasilense* have an impact on the growth of paoceae plants. Numerous studies on the biological functions of humic compounds revealed that they also have the ability to stimulate plant development (Barbieri *et.al.*,1988). Vermicompost's are abundant in microbial diversity and activity, and they also contain a variety of bacteria that are antagonistic to one another. As a result, they are excellent sources of biocontrol agents that help to prevent diseases brought on by soil-borne phytopathogenic fungi (Singh *et.al.*, 2008).

2.6 PLANT GROWTH PROMOTING BACTERIA

Plant growth-promoting bacteria (PGPB) use several different ways to both promote plant growth and shield plants from various biotic and abiotic stresses. Therefore, the use of PGPB in agriculture is practical since it provides environmentally acceptable, sustainable methods for preserving soil health and boosting crop output. Bacteria that stimulate plant development by colonizing plant roots are known as rhizobacteria (PGPR). The little area of soil that the root system specifically affects is known as the rhizosphere. Due to the buildup of various plant exudates, such as amino

acids and sugars, which serve as a rich source of energy and nutrients for bacteria, this zone is richer in nutrients than the bulk soil. Rhizobacteria are the bacteria that colonize the rhizosphere, which is home to a wide variety of microorganisms (Gray and Smith, *et.al.*, 2005). Rhizobacteria are typically used to describe beneficial free-living soil bacteria that aid in plant growth. Commercial exploitation of PGPR and its effects on plants offers significant potential for developing sustainable agriculture (Podile and Kishore, *et.al.*, 2006).

By solubilizing inaccessible forms of nutrients and by producing siderophores, which facilitate nutrient transport, PGPRs boost the availability of nutrients. The nutrient phosphorus, which is necessary for plant growth, can be found in large quantities in soil but is typically only present in insoluble forms. The transformation of phosphorus from insoluble forms to plant-available forms is aided by organic acids and phosphatases produced by rhizobacteria present in plant rhizospheres. By several mechanisms, including the fixation of atmospheric nitrogen transferred to the plant, the production of siderophores that chelate iron and make it available to the plant root, the solubilization of minerals like phosphorus, and the synthesis of phytohormones, PGPRs have been shown to directly enhance plant growth. Increases ion fluxes at the root surface brought on by the presence of PGPRs directly promote mineral absorption (Muhammad Zulqernain *et. al.*, 2021).

A complex ferric-siderophore is formed when siderophores are produced to solubilize iron from their surrounding surroundings. This complex siderophore can migrate through diffusion and return to the cell surface. The synthesis of siderophores by various microorganisms in soil has a major impact on their capacity to promote plant development. When plants can recognize the bacterial ferric-siderophore complex, microbial siderophores help plants absorb iron more effectively (Masalha *et.al.*, 2000). Siderophore synthesis gives PGPR a competitive edge, allowing it to colonize roots and keep other bacteria out of this ecological niche. The outcome of the competition for various carbon sources that are accessible as a result of root exudation or rhizodeposition may depend on the ability to obtain iron via siderophores (Crowley, *et al.*, 2006).

It is well known that when iron bioavailability is poor, microbial siderophores give plants nutrients to support growth. It has been proposed that siderophores could replace harmful pesticides as an environmentally acceptable option. Since it has been known for more than 30 years that certain *Pseudomonas* species can enhance plant development by creating siderophores, this bacterial group has been referred to as plant growth-promoting bacteria (Gamalero Glick, *et.al.*, 2011).

After nitrogen, phosphorus is the second most crucial nutrient for plants. It can be found in soil in the form of mineral salts or combined with organic molecules. Despite the fact that agricultural soils contain large amounts of these phosphorus compounds, the majority of them exist in an insoluble form. Therefore, it has been acknowledged that phosphorus deficiency in many soils is a significant growth constraint in horticultural and agricultural systems (Daniels *et.al.*, 2009). This implies the use of fertilizers containing phosphates, which are soluble forms of phosphorus that have limitations of their own since they too quickly get immobilized (fixed) to insoluble forms in the soil as a result of their interaction with the minerals of aluminium and iron. Rhizosphere-colonizing bacteria have the capacity to solubilize insoluble inorganic phosphate compounds such tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate or to liberate organic phosphates. In exchange for providing the plants with the soluble phosphates, these bacteria obtain root-borne carbon molecules, primarily sugars and organic acids, which are essential for bacterial growth (Khan *et.al.*, 2010).

By enhancing plant Phosphate uptake in rhizosphere soils, phosphate-solubilizing bacteria (PSB) play a crucial role in Phosphate cycling and fostering plant growth. The majority of PSB create indole-3-acetic acid (IAA), which promotes RNA/protein synthesis and plant development. Additionally, P solubilization in soils depends on the microbial metabolites and low molecular weight organic acids generated by PSB's metabolic processes (Alori ET *et.al.*, 2017).

2.7 HYDROLYTIC ENZYMES PRODUCING VERMICOMPOST BACTERIA

Vermicompost is a nutrient-dense material that includes calcium, magnesium, zinc, phosphorus, and nitrogen. In addition to the nutrients, it also has a large number of microorganisms that produce a variety of enzymes with remediation potential. Organic

waste is processed into nutrient-rich compost by the earthworms and their intestinal bacteria. The amylase, lipase, cellulase, and chitinase enzymes are few that are included in vermicompost which helps to break down the organic matter in the soil to release nutrients and make it accessible to plant roots even after they have been expelled. Protozoa, fungus, bacteria, and nematodes are all found in the soil that earthworms eat. They ingest the meal by using their powerful lips to do so. Food digestion is aided by enzymes and microorganisms. The waste, often referred to as castings, is discharged into the soil, where it enriches it with advantageous bacteria and enzymes while the helpful nutrients are absorbed by the bloodstream (Yasir *et.al.*, 2009).

The earthworms that consume plants, moulds, bacteria, and yeast all have the amylase enzyme. Due to its capacity to break down starch, polysaccharides, and glycogen, amylase plays a crucial role in the digestive process. Saliva contains amylase, which the earthworm's mouth uses to start the digestive process. The amylase enzyme is responsible for destroying the starch, xylan, pectin, galactine, and other compounds found in plants.

The digestion of proteins is aided by proteolytic enzymes. The earthworms' alimentary canals are the specific location of proteolytic enzymes. By breaking down proteins, they serve a crucial part in the digestive process of earthworms, enabling the animals to adequately absorb nutrients. Earthworms in compost piles consume the leftover plant material in the pile as a source of protein. Because of the protease, earthworms can organically decompose materials including eggshells, potato peels, fruits, vegetables, and more.

Endo-1, 4 glucanase, cellobiohydrolase, and cellobiase⁷ are the three main enzymes that make up the multienzyme complex known as cellulase. Together, these enzymes hydrolyze cellulose's crystalline structure. Plant cell walls' cellulose is broken down into simple sugars by cellulase enzymes so that bacteria can transform (ferment) them. An example of a carbohydrate is cellulose, which is composed of simple sugars like glucose that are bound together in long chains known as polysaccharides. Unravelling these polymers is essential for efficient ethanol fermentation because they are what provide plant cell walls their structural integrity (M. Himmel *et al.*, 2000).

2.8 CONCLUSION

Numerous beneficial effects of vermicompost on soil, plant growth, and health have been demonstrated. Additionally, it is seen as a possible replacement for dangerous chemical pesticides and fertilizers used in crop cultivation. It is gaining acceptance as a key element of organic agriculture, producing healthier meals and offering better options for the treatment of organic solid wastes. Vermicompost use in organic farming systems has a bright future as a result to research on earthworm-microbe interactions, exploration of potential earthworm species, soil-friendly microbes, use of various high-nutrient organic substances, efficient vermiculture systems, dose-specific use of vermicompost, and integration of vermicompost with other inorganic fertilizers (Janardan Lamichhane *et.al.*, 2017).

Since vermicompost enhances both soil quality and plant development and production, it can be used as an alternative to organic fertilizers in organic farming. Soil physio-chemical and biological qualities are reported to be improved. However, the raw materials used in its manufacturing have been proposed to mix some potentially toxic wastes, such as sewage, with some earthworm-friendly wastes to increase the efficacy of the plant growth stimulator on soil quality. Vermicompost use has been demonstrated to improve soil quality, which is reflected in plant production and growth (ShristiPiya *et.al.*, 2018). Vermicompost's are applied in the field to improve soil quality by boosting microbial activity and microbial biomass, which are essential elements in nutrient cycling, the generation of plant growth regulators, and the protection of plants against soilborne disease and arthropod pest attacks. To improve crop output and quality, usage of Vermicompost can be preferable (Geremew Taye Negeri *et.al.*, 2020).

CHAPTER 3

AIM AND OBJECTIVES

1. AIM

To isolate and characterize the plant growth promoting and antagonistic potential bacteria from vermicompost.

2. OBJECTIVE

- ❖ Isolation of plant growth promoting and bio control bacteria from vermicompost.
- ❖ Screening of plant growth promoting characters and antagonistic activity of vermicompost bacteria against plant pathogens.
- ❖ *In-planta* and pot culture study of potential vermicompost bacterial strains
- ❖ Volatile compounds isolation and identification from potential vermicompost bacteria.
- ❖ Morphological, physiological and molecular identification of potential vermicompost bacteria.

CHAPTER 4

METHODS AND MATERIALS

1. ISOLATION OF PLANT GROWTH PROMOTING AND BIO CONTROL BACTERIA FROM VERMICOMPOST.

1. *Soil sample collection*

The vermicompost sample was obtained from the neighbourhood nursery in Chennai's. The soils were gathered and placed in sterile plastic bags before being exposed to the isolation process in the storage room. Then about 9 grams of the pre-treated sample was diluted with 90ml sterile distilled water and kept in shaker for 1 hour.

2. *Media requirement*

- Actinomycete Isolation Agar (AIA)
- Nutrient Agar Media
- Chrome Azurol S (CAS)
- Carboxy Methyl Cellulose (CMC)
- Skim Milk Agar
- Potato Dextrose Agar (PDA)
- Casamino acid - Peptone Glucose (CPG)
- Mueller Hinton Agar (MHA)

4.1.3 *Composition of Media*

Table 4.1: Composition of media

S.No	Media	Components	g
1	Actinomycete Isolation Agar	AIA	2.17g
		Agar	2g
2	Nutrient Agar	Nutrient Broth	1.3g
		Agar	3g

3	Chrome Azurol S	CAS	0.6g
		FeCl ₃	0.0027g
		HDTMA	0.073g
4	CMC Agar	Potassium hydrate	0.5g
		Magnesium sulphate	0.25g
		Carboxy methylcellulose	15g
		Agar	20g
5	Skim Milk Agar	Skim milk powder	28g
		Casein enzymic hydrolysate	5g
		Yeast extract	2.5g
		Dextrose	1g
		Agar	15g
6	Potato Dextrose Agar	PDA	3.9g
		Agar	1g
7	CPG	Casein Hydrosylate	0.2g
		Peptone	2g
		Glucose	1g
		Agar	5g
8	Mueller Hinton Agar	MHA	2.1g
		Agar	2g

4. Isolation and Preservation

The serial dilution approach was used to perform the isolation. 1ml of the sample was diluted in 9ml of sterilized distilled water, which was then serially diluted to final concentrations of 10^6 , 10^7 , and 10^8 , respectively. Pour 100 μ l of the diluted material into the AIA and NA medium before starting a culture. Colony forming units on petri plates were identified and recorded after being cultured for seven to ten days at 28°C.

5. Bacteria Recovery and Storage

After the serial dilution process the bacteria obtained from vermicompost is recovered and stored by subculturing the bacteria by the streak plate method. The Bacteria was streaked in the AIA and NA medium which are then incubated at 28°C for

24 - 48 hours. The Bacteria is thus recovered and stored. Further research was done using preserved cultured bacteria.

2.SCREENING OF PLANT GROWTH PROMOTING CHARACTERS FROM VERMICOMPOST BACTERIA

1. ***Siderophore production in Qualitative analysis***

The strains were tested for siderophore synthesis using the Alexander and Zuberer (1991) technique on Chrome Azurol S agar. Vermicompost bacterial strains were spot inoculated onto CAS agar plates, which were then incubated at 28 °C for 7 days. The siderophore production of the colonies that produced yellow was deemed positive (Imran Sajid, *et.al.*, 2016).

2. ***Enzyme activity assay***

Qualitative screening for cellulose, protease, and amylase activity can be used to determine enzyme activity.

1. ***Cellulase Activity:***

CMC agar was used for the qualitative screening of the cellulase activity. On the isolation medium, bacterial isolates from vermicompost were obtained and qualitatively tested. The plates were mixed with 1% Congo red solution and left at room temperature for 20 minutes following the 72-hour incubation period. After that, the plates were stained using a 1M sodium chloride solution. Growing vermicompost bacteria colonies created a clear zone around them, and cultures that tested positive for cellulase were taken to mean that cellulase activity was occurring.

2. ***Amylase Activity:***

The starch hydrolysis test was used to screen bacteria strains that produce amylase. On plates of Nutrient agar medium supplemented with 1% starch, all of the bacterial isolates were spot inoculated. Next, the plates underwent a 7-day incubation period at 28 °C. The plates received an Iodine solution flood following the incubation time. The iodine solution was then removed from the plates after they had been left undisturbed

at room temperature for 10 to 15 minutes. It can be concluded that the bacterial isolates have generated amylase since a definite halo has formed around the bacterial colonies.

4.2.2.3 Protease Activity

Protease synthesis by bacterial isolates was measured using a skim milk agar medium. All the bacterial isolates were spot inoculated onto Nutrient agar medium that had been modified with 1% skim milk powder. The plates were incubated for 4 days at 30°C. A halo zone forming around the bacterial colonies was once considered a sign that protease was being generated.

3.SCREENING OF ANTAGONISTIC ACTIVITY OF VERMICOMPOST BACTERIA AGAINST PLANT PATHOGENS:

1.Antagonist activity by agar plug method against *Colletotrichum gloeosporioides*

In a Potato dextrose agar media, *Colletotrichum gloeosporioides* is cultivated. The agar plug method was used to perform anti-fungal activity. Choose the bacterial isolates from vermicompost like VC1, VC2, VC3, and VC4, then cultivate them in a different PDA medium. Cut agar plugs from PDA containing the *C. gloeosporioides* pathogen and placed them in the middle of the PDA medium containing the bacterial isolates. A definite zone of inhibition can be seen around the colonies after two to three days, and the zone must be measured. The vermicompost bacterial strain with the highest level of activity was chosen as a potential strain for additional research.

2.Antagonist activity by agar well diffusion method against *Ralstonia solanacearum*

In a CPG medium, *Ralstonia solanacearum* is cultivated. Take the bacterial isolates from vermicompost, such as VC1, VC2, VC3, and VC4, then inoculate one millilitre of distilled water into each separate centrifuge tube. *R. solanacearum* pathogen was used to stain the MHA plates after the MHA agar had solidified. Four wells were then drilled using a cork borer. The vermicompost bacterial isolates (50µl each) were added to

the wells. The plates were then incubated for 24hrs. After incubation, the zone of inhibition was calculated as millimeter in diameter.

4. *IN-PLANTA* EVALUATION OF VERMICOMPOST BACTERIA FOR PLANT GROWTH PROMOTION

1. *In-vitro studies for plant growth promotion*

Sort the potential strain into a nutrient broth, inoculate it there, centrifuge the result, collect the pellet, and put it away. Tomato seeds and other solanaceous seeds were removed and put in a beaker to wash for the night. The seeds were treated with pellets containing the potential vermicompost bacterial strain after being surface sterilized with sodium hypochlorite. Plant development is then accomplished through seed germination on conventional agar media.

5. PLANT GROWTH PROMOTION IN POT CULTURE

The surface of the tomato seeds has been sterilized after being cleaned with tap water. The VC2 pellets are used to treat the seeds with the prospective strain. Put the seeds in the pot; it needs daily watering. For the duration of the process (two weeks), keep the pot in a flexible environment. In pot culture, the plant's growth was monitored, and it demonstrated maximal shoot and root length in comparison to the control.

6. VOLATILE COMPOUNDS IDENTIFICATION FROM POTENTIAL VC BACTERIA

To screen the vermicompost bacteria by GC-MS with headspace, weigh the 1 gm of the homogenized sample (VC2) to the nearest 0.01 g, vial with PTFE-faced septa, and seal. Analyze using the headspace technique with column - VF 624 ms 60m 0.25mm, 1.8 m by placing the vial into the HS auto sampler. The carrier gas was helium (99.999%), flowing at a constant rate of 1.0 ml/min. The injection port was run in linear velocity mode with a steady flow of 1.0 mL/min. The oven's temperature was ramped up to 240 °C (with an increase of 6 °C/min) after remaining at 50 °C for one minute. The tuning file was 70 e, and the ion source was 280 °C in temperature.

7. MORPHOLOGICAL CHARACTERIZATION

Gram staining and KOH Test was done for the morphological characterization of the vermicompost bacterial strain VC2.

1. ***Gram Staining:***

A loopful of water was placed in the centre of a glass slide after it had been cleaned with ethanol. The water on the slide and the bacterial colony on the plate were combined using a loop until a thin, even layer of microorganisms covered the slide. The slide was left to dry naturally. After that, it was heatedly fixed before cooling. After a minute of crystal violet flooding, the slide was cleansed with water. The slide was flooded with iodine solution for a minute, and then immediately washed with water. For three seconds, flood the slide with Gram Stain Decolorizer to decolorize it. After that, immediately rinse with water. After being flooded with Safranin counterstain, the slide was given a 30-second waiting period before being thoroughly rinsed with water. After the slide had air dried, the organism was examined with the oil immersion objective of the microscope.

2. ***KOH Test:***

3g of KOH and 100ml of distilled water were combined to create a 3% KOH solution. The bacterial colony was mixed with a loop and added to a loopful of KOH solution in the middle of a glass slide until a thin layer of microbes coated the plate. Immerse the loop slowly into the mixture to raise it 1-2 cm.

8. MOLECULAR CHARACTERIZATION

1. **Extraction of chromosomal DNA**

Using a solute ready genomic DNA kit, the chromosomal DNA of the prospective vermicompost strain VC2 was extracted. The YEME broth was inoculated with the spores from the culture VC2 and incubated at 28 °C for 5 days at 95 rpm on a rotating shaker. One milliliter of the overnight culture was extracted and centrifuged for ten minutes at 6000 RPM in a 1.5 milliliter Eppendorf tube. The pellet was mixed with 320 l of solution A (Tris, EDTA, Guanidine thiocyanate) and 80 l of solution B (10% SOS) before being vortexed thoroughly to create a homogeneous suspension. This was incubated for 15

minutes at 65 °C in a water bath. once that, 130 l of solution C (potassium acetate) was added once it had cooled to room temperature. The tube was gently inverted, and incubated at room temperature for five minutes. At 10,000 RPM, it was centrifuged for ten minutes. Denatured protein and precipitated cell debris are produced as pellets as a result. A new tube containing DNA and RNA was used to transfer the supernatant into. After being introduced in equal volume, the PT buffer (isopropanol mix) was gently inverted five times and incubated for 10 minutes at room temperature. DNA and RNA precipitate as a result. To make the precipitated DNA and RNA form a pellet, it was once more centrifuged at 10,000 RPM for 5 minutes. The extra fluid was discarded. Without disturbing the pellet, 500 l of washed buffer (70 percent ethanol) was added and allowed to sit for one minute. The pellet's co-precipitate salts are eliminated by this solution. For one minute, it was centrifuged at 10,000 RPM and the excess material was thrown away. By turning the pellet over in a paper towel for ten minutes, the pellet was air-dried. The pellet was totally disintegrated after being suspended in 100 l of TE buffer. A 1% agarose gel was used to test the extracted DNA's purity, and 2 l of the isolated DNA was used for PCR amplification.

4.8.2 PCR Amplification of 16s RNA

Using a thermal cycler and two primers, 27F 5'-AGAGGATCMTGGCTCAG-3' (forward) and 1492R 5'-TACGGTACCTTGTTACGACTT-3' (reverse), the 16s ribosomal RNA gene of strain VC2 was amplified using PCR (Gothwal et al., 2007). 50.0 mg of template DNA (1.0 l), 10 pmol (each 2 l) of primer (forward and reverse separately), 10 l of PCR master mix (2 l Taq DNA polymerase 10X Taq reaction buffer, 2 mM MgCl₂, 1 l of 10 mM dNTPs and PCR additives), and 5.0 l of double-distilled water made up the final volume of the reaction mixture, which was 20 l. Thermo-cycler amplification was carried out using the following profile: 94°C for 3 minutes of initial denaturation (cycle 1), then (cycle 2) A four-step procedure was used to complete 30 amplification cycles, including (step 1) denaturation at 94°C for 1 minute, (step 2) annealing the primer for 1 minute at 58°C, (step 3) chain extension for 1 minute and 30 seconds at 72°C, and (step 4) final extension for 5 minutes at 72°C. 2g of agarose was dissolved in 100ml of 1X TAE buffer to create a 2% agarose gel. Each PCR vial was filled with 500 ml of the PCR sample and

around 8 l of 6X gel loading dye. This was put through electrophoresis at 50V until the dye was three-fourths of the way down, and the bands were then seen under a UV-transilluminator.

3. Agarose gel electrophoresis

The micro-oven was used to prepare and melt 2% agarose gel. Ethidium bromide was added in 5 l at a temperature of around 60°C to the agarose gel. In order to allow the agarose to solidify, the heated agarose solution was now slowly poured onto the gel platform. In the tank for the submersible gel, 1X TAE buffer was added. The buffer level was kept at 0.5 cm above the gel and the gel platform was carefully lowered into the tank. The PCR samples were loaded after being combined with 10 l of 100 bp DNA ladder and the gel loading dye. This was subjected to electrophoresis at 50V until the dye was three-fourths of the way down, at which point the band pattern was seen under a UV transilluminator.

4. Sequencing of 16S rRNA

DNA sequences were automatically sequenced using the Sanger dideoxy chain terminator method. Four reaction mixers were set up for this fluorescence-based DNA sequencing technique. Each contained a single ddNTP and was tagged with four distinct fluorescent dyes, requiring only one reaction mix. dATP, dGTP, dCTP, and dTTP were also added, along with other polymerase enzymes. Labelled ddNTP was put through one capillary tube to make the sequence detection. In one electrophoretic gel, DNA fragments of various colors were divided according to their sizes. A laser beam was used to spotlight the negatively charged 16S rRNA fragments as they moved through the gel's pores towards the positive end when current was introduced to it. A fluorescent After dyes were excited, a specialized recorder captured the light of particular wavelengths that was emitted from various bases. By figuring out the order of the colours that various peaks emanate as they pass the detector, the sequences were read. This data was directly entered into a computer, which chose the order. The computer produced a tracing electrogram of the four dyes' emitted light. Each dye's colour corresponds to a different nucleotide. The data of the light's emission was transformed by the computer into the nucleotide sequence.

5. BLAST analysis

Using the NCBI BLAST (www.ncbi.nlm.nih.gov) website, the closely related 16S rRNA gene sequence of the strain VC2 homologous sequence was identified and evaluated for similarity level with the reference species of *Lysinibacillus* stored in genomic database banks. Following BLAST analysis, a dataset of possible orthologs was created by taking into account database sequences that shared > 99% sequence identity with the strain VC2 query sequence.

6. Phylogenetic characterization

With the use of software from the MEGA version 11 (Takahasi and Omura, 2003) package, phylogenetic and molecular evolutionary analyses of prospective strain VC2 were carried out. The strain's 16S rRNA sequence was compared to nucleotide sequences of *Bacillus* species from GenBank in order to be aligned. In order to infer a phylogenetic tree, evolutionary distance matrices were created.

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 ISOLATION AND CHARACTERIZATION OF VERMICOMPOST BACTERIA

Totally 7 morphologically different bacterial strains were isolated from vermicompost sample. All isolates are recovered and stored AIA and NA medium and stored in. All the cultures showed good growth on AIA and NA and the colony consistency include smooth, leathery. The Aerial mass colour includes orange, yellow, white, mild yellow and mild orange. Similarly, Mirkashim H. *et.al.*, 2020 has also observed, various types of vermicompost bacterial colonies in the agar culture plates. Colonies of various sizes, shapes, textures, elevations, edges, and colours have been discovered. In the same way the Nutrient Agar plates with vermicompost bacterial strains showed cream-coloured colonies after 24 hours of incubation, indicating the presence of a microbial community Plabita Bora, *et.al.*, 2018.

TABLE 5.1 Morphological characteristics of Vermicompost Bacterial strains

S.NO	STRAIN NAMES	GROWTH	CONSISTENCY	AMC Aerial mass colour
1	VC1	Good	Smooth	Orange
2	VC2	Good	Leathery	Yellow
3	VC3	Good	Leathery	White
4	VC4	Good	Leathery	Yellow
5	VC5	Medium	Leathery	Mild Yellow
6	VC6	Poor	Leathery	Pale White
7	VC7	Poor	Leathery	Mild Orange



Fig 5.1 Vermicompost Bacterial Strains

2. PLANT GROWTH PROMOTING (PGPR) ACTIVITIES

Plant growth promoting activities is done for the Screening of Extracellular hydrolytic enzyme activity and Siderophore production.

1. **Enzymatic Activity**

The Hydrolytic enzyme activity such as The Cellulase, Amylase and Protease activity is done.

2. **Cellulase Activity**

A clear zone formed around the growing colonies the cellulase positive cultures are taken as the indication of cellulase activity. Out of seven vermicompost bacterial strains VC3 showed results as indication of cellulase activity. According to Mariyam *et.al.*, 2022 the enzyme activity was seen in 24 hours, and it was purified and used in the LDPE degradation process. The cellulase enzyme's use in the breakdown of cellulose is indicated was screening results. Similarly, Nongthombam *et.al.*, 2018 stated that cellulose and MBW substrate, CDM9 consistently shown high cellulase activity, and its co-inoculation with earthworm accelerated the degradation of the MBW mixture.

Table5.2: Cellulase Activity

STRAIN NAME	CELLULASE ACTIVITY
VC1	—
VC2	—
VC3	+
VC4	—
VC5	—
VC6	—
VC7	—



Fig 5.2: Zone of clearance for cellulose activity

5.2.3 Amylase activity

After the inhibition period, for the clear screening 1% iodine solution were mixed and kept for 15 min. Bluish-black complex is formed showing a clear zone around the colonies. VC1, VC2, VC3 showed positive results for amylase activity out of the 7 bacterial strains. In the same way T. Sivakumar, *et.al.*, 2012 isolated the bacteria from vermicompost soil on starch agar media and were tested for amylase production. Seven bacterial strains were identified from the soil samples. However, subsequent screening revealed that only 3 strains had amylase activity. Also, Soltan, *et.al.*, 2022 have stated that all vermicompost-derived bacterial isolates have shown amylase activity, as shown by the creation of clear zones on starch agar plates.

Table 5.3 Amylase Activity

STRAIN NAME	AMYLASE ACTIVITY
VC1	+
VC2	+
VC3	—
VC4	+
VC5	—
VC6	—
VC7	—



Fig 5.3: Zone of clearance for amylase activity

5.2.4 Protease Activity

The vermicompost bacterial strains were used for the qualitative screening of the protease activity. The clear zones were detected around the growing colonies. The result is positive in VC2, VC3 whereas the negative or lowest activity is shown in VC1 and VC4. According to Vijitra Luang-In, *et.al.*, 2010, 19 protease-positive isolates from soil displayed clear zones on skimmed milk agar with various halo: colony ratios. Colonies with clear zones were identified as having protein-degrading bacteria. Comparably Jayakumar, *et.al.*, 2013 used the protease synthesis to evaluate the functional characteristics of the bacteria isolated from vermicompost based on straw and goat dung. Protease was generated by 86 strains in total (44%).

Table 5.4: Protease Activity

STRAIN NAME	PROTEASE ACTIVITY
VC1	—
VC2	+
VC3	+
VC4	—
VC5	—
VC6	—
VC7	—

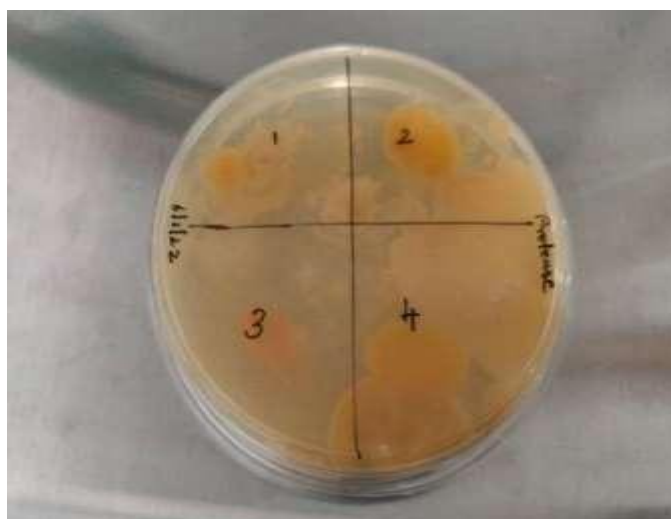


Fig 5.4: Zone of clearance for protease

5.2.5 Siderophore production

The vermicompost bacterial strains are used for the qualitative screening of siderophore activity. From the 7 isolates four isolates (VC1, VC2, VC3, VC4) produced siderophore in the CAS agar isolation medium. Similarly, Bhardwaj, *et.al.*, 2023 performed a quantitative estimation of siderophores using the CAS-shuttle test. Municipal waste compost bacteria were isolated from were grown in LB broth for 72 hours. The lack of blue color indicates the development of siderophores. Using a spectrophotometer (630 nm), the yellowish to orange colour change of the supernatant was measured. The

prospective Isolates demonstrated quantitative estimation of siderophore production. In the same way Bhattacharyya, *et.al.*, 2020 stated that tea rhizobacterial isolates were discovered to manufacture siderophore very well. 52 to 99% of the siderophore units produced by the isolates were determined to be siderophores. The synthesis of siderophores with more than 90% siderophore units was identified in almost 70% (21 isolates) of rhizobacterial isolates.

Table 5.5 Siderophore Production

STRAIN NAME	SIDEROPHORE PRODUCTION
VC1	+
VC2	+
VC3	+
VC4	+
VC5	—
VC6	—
VC7	—

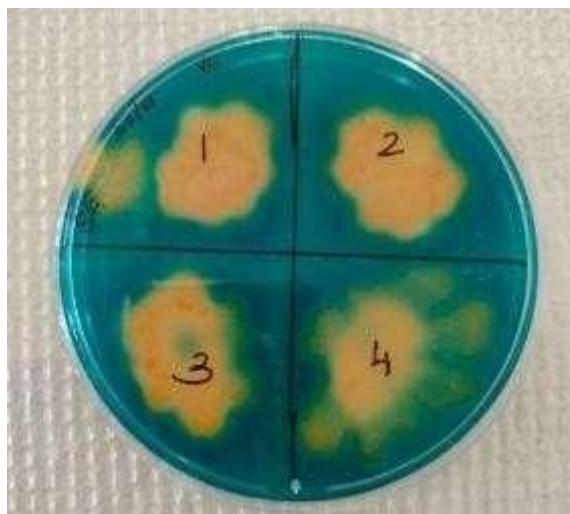


Fig 5.5: Siderophore Production

3.SCREENING OF ANTAGONISTIC ACTIVITY OF VERMICOMPOST BACTERIA AGAINST PLANT PATHOGENS

1.Screening of Antagonistic activity of vermicompost bacteria against *Colletotrichum gloeosporioides*

The vermicompost strains were screened for antifungal activity against the fungi *C. gloeosporioides*. The bacterial strain VC2 showed maximum antagonistic activity against the fungi with 60% of inhibition. According to Svetlana *et.al.*, 2010, the dual culture assay results demonstrated that all antagonistic bacteria, to varied degrees, reduced the mycelial growth of *C. acutatum* and *C. gloeosporioides*. The majority of *Colletotrichum* isolates were moderately inhibited by two fungi that belonged to growth inhibition categories. The pathogens' radial growth was decreased by more than 38% and 43%, respectively, by *T. harzianum* and *G. roseum*. In the same way Winston *et.al.*, 2023 have stated that The approach of poisoned food was used to test whether the mycelial growth of *C. gloeosporioides* and *C. lindemuthianum* was inhibited. The proportion of radial growth that was inhibited relative to the negative control was used to determine growth inhibition. The results are displayed as mean colony diameter values. Thymol at 352 and 704 M was found to strongly suppress *C. lindemuthianum* and *C. gloeosporioides* growth by roughly 90-100% over the course of the evaluation period.

TABLE 5.6: Antifungal activity against *Colletotrichum gloeosporioides*

STRAIN NAME	ANTAGONISTIC ACTIVITY	MEASUREMENT OF ZONE OF INHIBITION (%)
VC1	—	-
VC2	+	60%
VC3	—	-
VC4	—	-
VC5	—	-
VC6	—	-
VC7	—	-



Fig 5.6: Antifungal activity of the vermicompost bacterial strain

5.3.2 Screening of Antagonistic activity of vermicompost bacteria against *Ralstonia solanaecerum*

The vermicompost strains were screened for antimicrobial activity against the pathogen *R. solanaecerum*. The bacterial strain VC2 showed maximum antagonistic activity against the pathogen with 12mm of zone of inhibition. Accordingly Maja Ravnika, *et.al.*, 2015 reported that the minimum inhibitory concentration approach was modified for the initial screening of the activities of the mushroom protein extracts against *R. solanacearum* NIB Z 30 (NCPBP 4156). *R. solanacearum* was suppressed in vitro by 15 samples (14 mushroom extracts and 1 *C. geotropa* mycelium extract). Different levels of inhibition against *R. solanacearum* NIB Z 30 were displayed by these extracts, including full inhibition.

TABLE 5.7: Antimicrobial activity against *Ralstonia Solanaecorum*

STRAIN NAME	ANTAGONISTIC ACTIVITY	MEASUREMENT OF ZONE OF INHIBITION (mm)
VC1	—	-
VC2	+	12mm
VC3	—	-
VC4	—	-
VC5	—	-
VC6	—	-
VC7	—	-

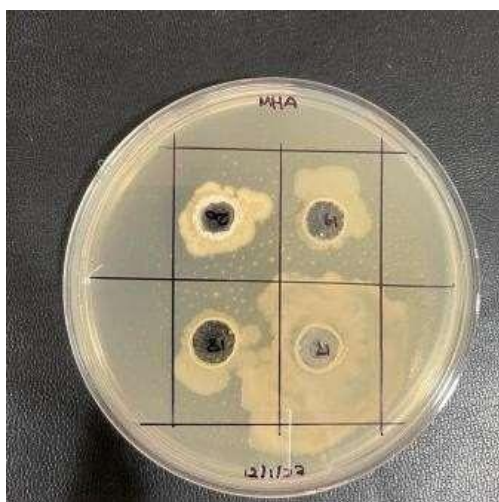


Fig 5.7: Antimicrobial activity of the vermicompost bacterial strain

4.IN-PLANTA EVALUATION OF POTENTIAL BACTERIA FOR PLANT GROWTH PROMOTION

1. *Invitro* Plant Growth Promotion

Based on the *in-vitro* PGP and antagonistic activity, the bacterial strain VC2 was selected as the potential bacteria and characterized for *in-planta* PGP and antagonistic activity in tomato. It showed maximum germination percentage (100%) as well as higher shoot (7.3cm) and root (5cm) length in *in-planta* paper towel method. In the similar way M. Motaher Hossain *et.al.*, 2022 has done the seed treatments with PGPR strains and

had better seed germination and seedling vigour than the control. According to the in vitro studies. *S. maltophilia* PPB3 and *B. subtilis* PPB9 treatments increased the percentage of tomato seeds that germinated by 15.11% and 20.27%, respectively, over the value obtained with the control.

Table 5.8 In- vitro plant growth promotion for *Solanum lycopersicum*

Strain Name	Root Length (cm)	Shoot Length (cm)	Seed Germination %
Control	4cm	6.8cm	80%
VC2	5cm	7.3cm	100%



Fig 5.8: In – vitro plant growth promotion for *Solanum lycopersicum*

5.5 PLANT GROWTH PROMOTION IN POT CULTURE

The growth of the tomato plant observed in pot culture which was treated with the VC2 strain, showed maximum shoot (12cm) and root (4cm) length compared to the control. Similarly, Hossain *et.al.*,2022 reported that rhizobacteria-treated seeds germinated more quickly and effectively than untreated seeds. They also documented that 5-day germination of tomato seeds in control pots was followed by a 4-day germination period for 1-2% of the PPB3 and PPB9-treated seeds. In the control treatment, on average, 63% of tomato seeds germinated 12 days after sowing. Compared

to the control, germination was increased by 25% and 38%, respectively, by inoculation with strains PPB3 and PPB9.

Table 5.9: Plant Growth promotion in pot culture

Strain Name	Root Length (cm)	Shoot Length (cm)
Control	2.5	8
VC2	4	12



Fig 5.9 Plant growth promotion in pot culture

5.6 VOLATILE COMPOUNDS IDENTIFICATION FROM POTENTIAL VC BACTERIA

The production and identification of volatile compound by potential strain VC2 in headspace GCMS showed some interesting volatile metabolites such as oxalic acid, phenylephrine, acetone, DL-alanine ethyl ester, n-hexyl methylamine, cathionone, may be the reason for their potential PGP and antagonistic activity.

In the same way (Yuxi He, Wenyu Guo, *et.al.*,2022) volatile substances produced by the strain of *Streptomyces* TOR3209. 48 compounds were found in the GC-MS study, of which eight (representing 15.22% of the total VOCs) were reported as PGP effective VOCs and fourteen (representing 37.36% of the total VOCs) had the potential to boost the plant's resistance to phytopathogens. The principal components, which were identified as seven chemicals, 1,4,5,6,7,8,9,9a-octahydro-1,1,7-trimethyl-[3aR-(3a.alpha.,7.alpha.,9a.beta)] trans-1,10-dimethyl-trans-9-decalol (9.43%)- 2,4-bis(1,1-dimethylethyl)-phenol (13.61%), 3,7-methano-3,4H-cyclopentacyclooctene (6.29%), and growth-promoting substances such n-hexadecanoic acid (6.53%).

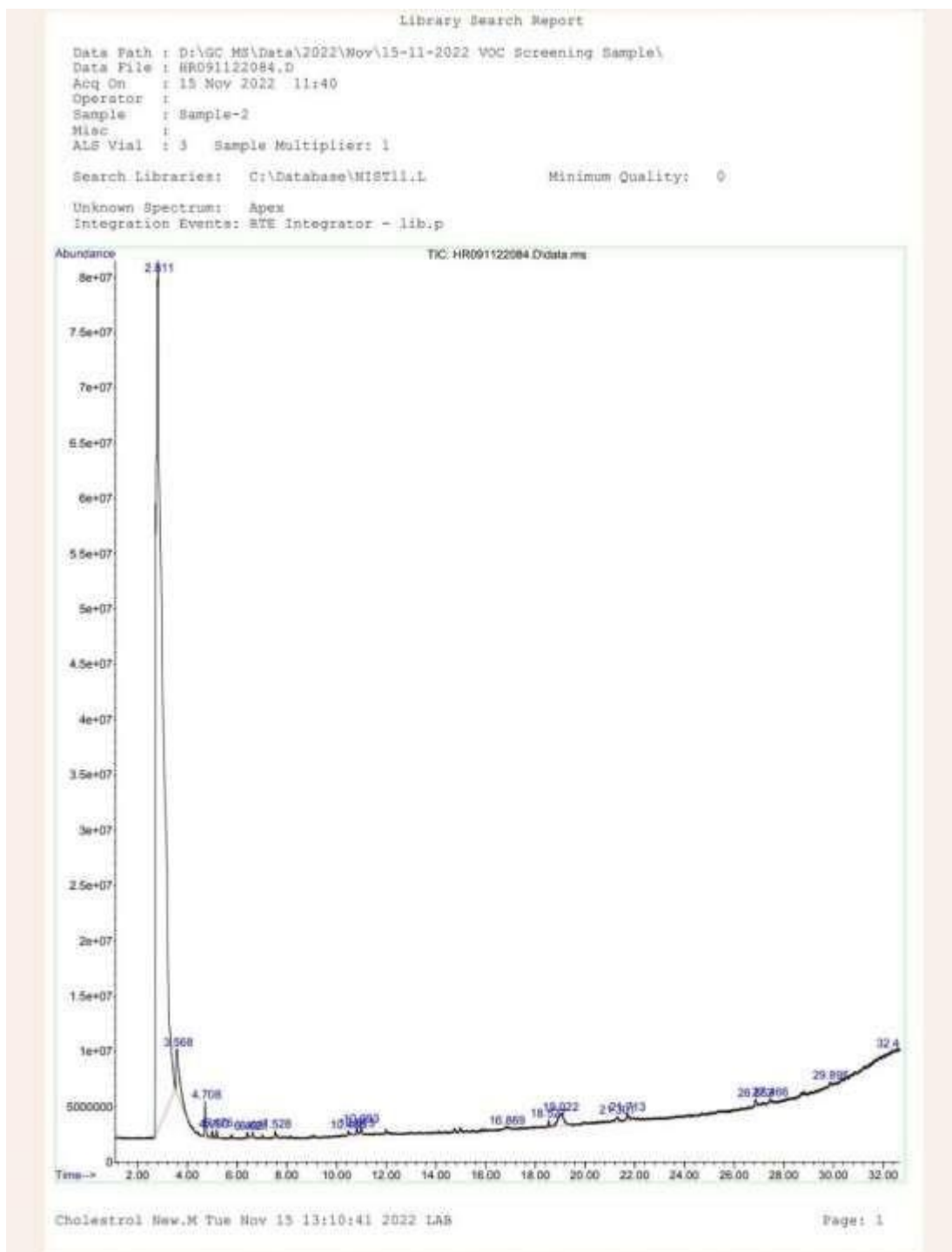


Fig 5.11 Headspace GC - MS

7. MORPHOLOGICAL CHARACTERIZATION

Gram staining and KOH Test is done for the morphological characterization of the vermicompost bacterial strain VC2.

1. *Gram Staining*

The gram staining involves four major steps, staining with crystal violet, gram's iodine, applying decolorizer and counter staining. After the slide had air dried, the organism was examined with the oil immersion objective of the microscope. Gram positive bacteria will stain blue/purple and gram-negative bacteria will stain pink/red. The result of the examined organism is Gram negative. Most likely Mirkashim H. Saha, et.al.,2020 reported that through examination of colony morphology, microscopy analysis, and biochemical characterization, the isolated bacterial strain was identified. Under a compound microscope, the bacterial strains that were isolated from the vermicompost sample were viewed, and the results are identified as BIFV H Gram-negative bacterial strain with a rod shape, BIFV G a Gram-negative bacterial strain with a coccus shape. Thus, the outcome of the microscopic investigation reveals the existence of bacterial strains that are Gram negative.

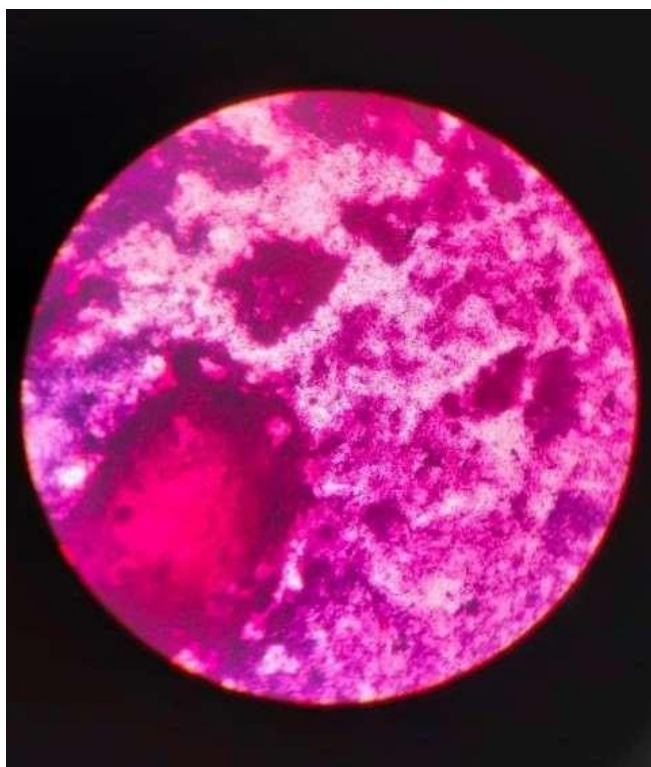


Fig 5.11 Gram Staining

5.7.2 KOH Test

A loopful of KOH solution was placed in the center of a glass slide and the bacterial colony were combined using a loop until a thin layer of microorganisms covered the slide. Slowly draw the mixture up 1-2 cm by immersing the loop in the mixture. A string of the mixture followed the loop when it was raised. Gram positive bacteria generally display no reaction when raised. Hence the bacteria is Gram Negative.



Fig 5.12 KOH Test

8. MOLECULAR CHARACTERIZATION

1. *Contig formation of potential strain sequence*

>>*Lysinibacillus* sp. VC2

TTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTACCTTATAGTTTGG
TTTCGGCTGTCGCTATAGGATGGGCCCCGCGGCGCATTAGCTAGTTGGTGAGGTAA
CGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACT
GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCA
CAATGGGCGAAAGCCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGATTTCGGTT
CGTAAAACTCTGTTGTAAGGGAAGAACAAGTACAGTAGTAACTGGCTGTACCTTGA
CGGTACCTTATTAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACG
TAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTC
TTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAACTGGG
AGACTTGAGTGCAGAAGAGGATAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTA
GAGATTTGGAGGAACACCAGTGGCGAAGGCGACTATCTGGTCTGTAAGTACGCT
GAGGAGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC
GTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCCTTAGTGCTGCAGCTAA
CGCATTAAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAAT
TGACGGGGGGCCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGA
AGAACCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTT
CGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATG
TTGGGTAAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTAGTT
GGGCACTCTAAGGTGACTGCCGGTGACAAACGGAGGAAGGTGGGGATGACGTC
AAATCATCATGCCCCCTTATGACCTTGGCTACAC

5.8.2 BLAST Result of Potential strain VC2

Sequences producing significant alignments

Download

Select columns

Show 100

☒ select all 100 sequences selected

GenBank

Graphics

Distance tree of results

MSA Viewer

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒	Uncultured bacterium clone AFEL3_aap16f...	uncultur...	1814	1891	100%	0.0	98.63%	1413	EU771565.1
☒	Uncultured bacterium clone AFEL3_aap14f...	uncultur...	1803	1880	100%	0.0	98.44%	1413	EU465948.1
☒	Lysinibacillus fusiformis strain BF13_16S ri...	Lysinibac...	1796	1889	100%	0.0	98.34%	1498	KJ524511.1
☒	Bacillus subtilis subsp. spizizenii strain 3E...	Bacillus...	1794	1882	99%	0.0	98.34%	1262	EU304958.1
☒	Bacillus subtilis subsp. spizizenii strain 3E...	Bacillus...	1794	1882	99%	0.0	98.34%	1262	EU304957.1
☒	Lysinibacillus fusiformis strain P-R2A48_16...	Lysinibac...	1784	1878	100%	0.0	98.14%	1396	MT533924.1
☒	Lysinibacillus fusiformis strain Uyi_38_16S r...	Lysinibac...	1784	1878	100%	0.0	98.14%	1427	MT507231.1
☒	Lysinibacillus fusiformis strain NBRC 1571...	Lysinibac...	1784	1878	100%	0.0	98.14%	1418	MN543857.1
☒	Lysinibacillus fusiformis strain NBRC 1571...	Lysinibac...	1784	1878	100%	0.0	98.14%	1439	MN543848.1
☒	Lysinibacillus mangiferihumi strain MGX18...	Lysinibac...	1784	1878	100%	0.0	98.14%	1438	MN543846.1
☒	Lysinibacillus fusiformis strain NBRC 1571...	Lysinibac...	1784	1878	100%	0.0	98.14%	1380	MN543814.1
☒	Lysinibacillus fusiformis strain NBRC 1571...	Lysinibac...	1784	1878	100%	0.0	98.14%	1425	MN543804.1
☒	Bacillus sp. (in: Bacteria) strain LP6_16S rib...	Bacillus...	1784	1872	100%	0.0	98.14%	1359	MN784658.1
☒	Lysinibacillus sp. strain LP5_16S ribosomal...	Lysinibac...	1784	1872	100%	0.0	98.14%	1394	MN784657.1
☒	Lysinibacillus sphaericus strain DH-B01_chr...	Lysinibac...	1784	22520	100%	0.0	98.14%	4602887	CP045583.1
☒	Lysinibacillus fusiformis strain RPW14_16S...	Lysinibac...	1784	1878	100%	0.0	98.14%	1426	MN559719.1
☒	Lysinibacillus fusiformis strain NBRC 1571...	Lysinibac...	1784	1878	100%	0.0	98.14%	1382	QQ618954.1
☒	Lysinibacillus fusiformis strain CD_16S ribo...	Lysinibac...	1784	1878	100%	0.0	98.14%	1516	QQ568519.1
☒	Lysinibacillus fusiformis strain G5.M11b.pl...	Lysinibac...	1784	1878	100%	0.0	98.14%	171838	CP118715.1
☒	Lysinibacillus fusiformis strain G5.M11b.ch...	Lysinibac...	1784	22535	100%	0.0	98.14%	4603763	CP118714.1
☒	Lysinibacillus sp. strain W11_16S ribosomal...	Lysinibac...	1784	1878	100%	0.0	98.14%	1403	QQ418029.1
☒	Lysinibacillus fusiformis strain Y61_16S rib...	Lysinibac...	1784	1878	100%	0.0	98.14%	1435	QQ406226.1
☒	Lysinibacillus fusiformis strain Y44_16S rib...	Lysinibac...	1784	1878	100%	0.0	98.14%	1451	QQ406209.1



Fig 5.13 BLAST sequence of VC2

5.8.3 Phylogenetic characterization of potential strain VC2

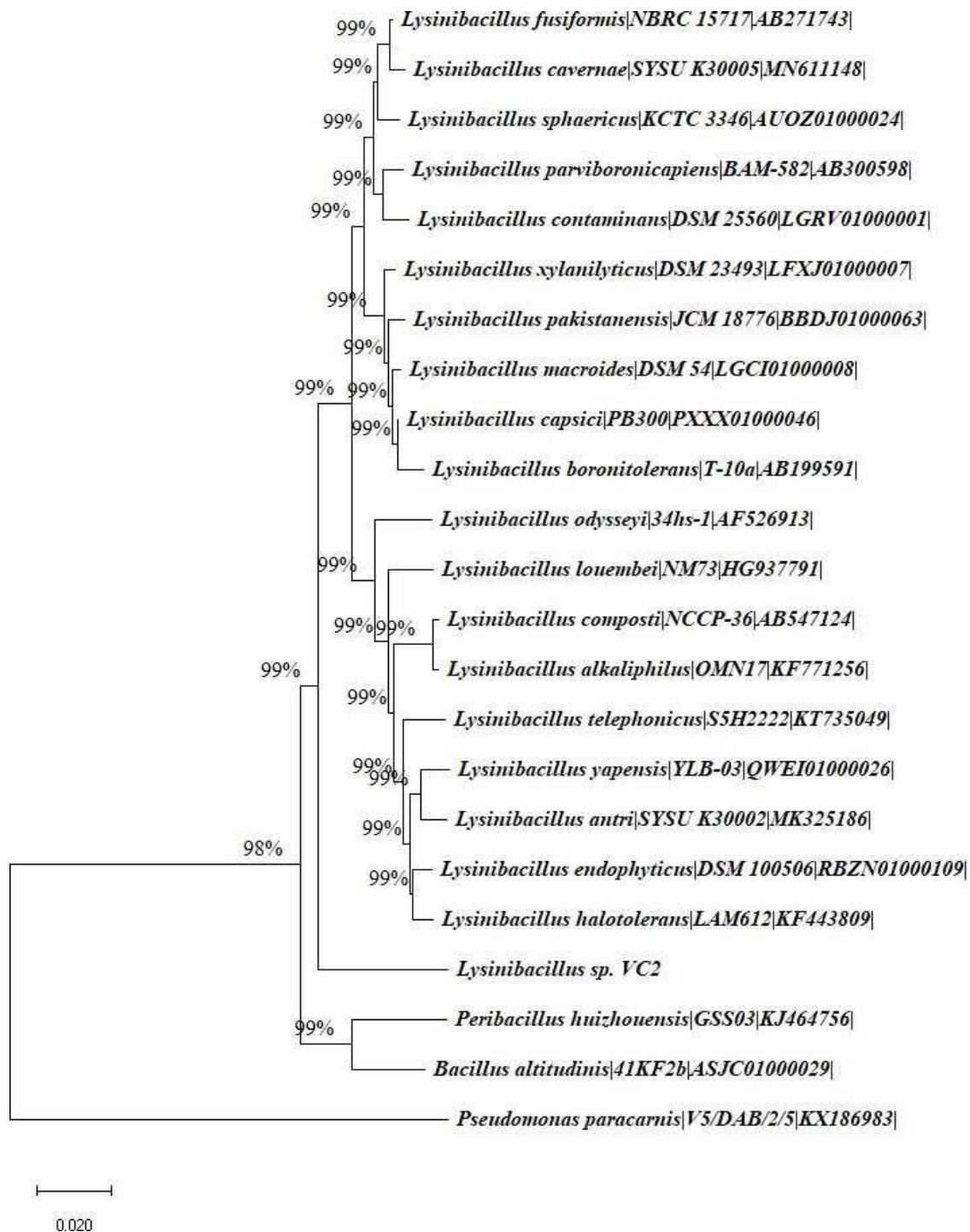


Fig 5.14 Phylogenetic Tree

The vermicompost bacterial strain VC2's 16S r RNA gene produced a sequence of 1070 base pairs. According to the BLAST analysis, *Lysinibacillus* is 99% similar (Fig. 5.14).

CHAPTER 6

SUMMARY AND CONCLUSION

The interactions between detritivorous earthworms and microorganisms during vermicomposting change the organic waste's biochemical and physical properties and hasten its stabilization. In order to decompose organic waste and turn it into useful manure, earthworms and microorganisms collaborate on several levels. Depending on the microbial inoculum utilized, the manure generated has diverse qualities and has advantageous impacts on plant growth. Vermicompost increases soil biodiversity by encouraging beneficial microbes, which in turn promotes plant growth directly by producing hormones and enzymes that regulate plant growth, as well as indirectly by preventing the spread of plant pathogens, nematodes, and other pests. This improves plant health. The isolates of Vermicompost bacteria were collected from vermicompost sample which was obtained horticulture nursery in Chennai, Tamil Nadu.

Out of 7 isolates 4 isolates produced siderophore production in CAS agar plate after a week of incubation. The vermicompost bacterial strains obtained produced an effective extra cellular hydrolytic enzymes; VC1 showed positive result for Cellulase activity. VC1, VC2 and VC4 showed positive results for Amylase activity and VC2 and VC3 showed positive results for Protease activity.

The Vermicompost bacterial isolate VC2 was chosen as the possible bacteria as it exhibited antagonistic action towards the pathogen *Ralstonia solanacearum* and the fungus *Colletotrichum gloeosporioides*. The isolate VC2 was identified as *gram negative* by morphological characterization and it was identified as *Lysinibacillus*.

Vermicompost's are fantastic organic amendments that act as an improvement of soil fertility, plant growth, and control of pathogens and pests as well as for sustainable agriculture. They have excellent physio-chemical properties and buffering ability, enriched with all nutrients in plant available forms, and contain antagonistic and plant growth-promoting bacteria. Vermicompost's natural biological, biochemical, and physiochemical characteristics make it suitable for use in promoting sustainable agriculture and in the safe management of agriculture.

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