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Theme

Contribution to study the role of earthworms to enhancing plant rhizosphere with PGPR bacteria

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Dedication

Last but not least I wanna thank me for believing in me, I want to thank me for doing all this hard work. I wanna thank me for having no days off. I wanna thank me for never quitting. I wanna thank me for always being a giver and trying to give more than I receive. I wanna thank me for trying to do more right than wrong. I wanna thank me for being me at all times.

RR

Abstract

This study focused on isolating and identifying effective Plant Growth-Promoting Rhizobacteria (PGPR) strains from earthworm castings. Earthworm castings, rich in nutrients and beneficial microbes, were found to be a significant source of PGPR. The isolated PGPR strains demonstrated their ability to solubilize phosphate, calcium, and potassium, produce siderophores and auxin, which are essential for plant growth. The use of PGPR supports sustainable agriculture by reducing the need for chemical fertilizers and pesticides, promoting healthier crops, and minimizing environmental impact. The findings highlight the potential of earthworm castings in sustainable agricultural practices and the importance of leveraging natural interactions between earthworms, beneficial bacteria, and plants. Future research should focus on wider application and long-term field studies to validate the effectiveness of these PGPR strains in diverse agricultural settings. Advanced genomic and biotechnological tools can further optimize PGPR strains, contributing to resilient agricultural systems that meet growing food demands while preserving environmental health. This research represents a significant step towards sustainable and productive farming practices .

Key Words : Plant Growth-Promoting Rhizobacteria (PGPR) ,Earthworm castings, Nutrient-rich , Phosphate solubilization ,Calcium solubilization, Potassium solubilization ,Siderophores ,Auxin production.

Résumé

Cette étude s'est concentrée sur l'isolement et l'identification de souches efficaces de Rhizobactéries Promotrices de la Croissance des Plantes (PGPR) à partir de déjections de vers de terre. Les déjections de vers de terre, riches en nutriments et en microbes bénéfiques, se sont révélées être une source importante de PGPR. Les souches de PGPR isolées ont démontré leur capacité à solubiliser le phosphate, le calcium et le potassium, et à produire des siderophores et de l'auxine, essentiels à la croissance des plantes. L'utilisation de PGPR soutient l'agriculture durable en réduisant le besoin d'engrais chimiques et de pesticides, en favorisant des cultures plus saines et en minimisant l'impact environnemental. Les résultats mettent en lumière le potentiel des déjections de vers de terre dans les pratiques agricoles durables et l'importance de tirer parti des interactions naturelles entre les vers de terre, les bactéries bénéfiques et les plantes. Les recherches futures devraient se concentrer sur une application plus large et des études de terrain à long terme pour valider l'efficacité de ces souches de PGPR dans des contextes agricoles divers. Les outils avancés de génomique et de biotechnologie peuvent encore optimiser les souches de PGPR, contribuant à des systèmes agricoles résilients qui répondent aux besoins alimentaires croissants tout en préservant la santé environnementale. Cette recherche représente une étape significative vers des pratiques agricoles durables et productives.

Mot clé : Rhizobactéries promotrices de croissance des plantes (PGPR), Déjections de vers de terre Riche en nutriments, Solubilisation du phosphate, Solubilisation du calcium, Solubilisation du potassium, Sidérophores, Production d’auxines.

ملخص

ركزت هذه الدراسة على عزل وتحديد سلالات فعالة من البكتيريا المحفزة لنمو النباتات (PGPR) من فضلات ديدان الأرض. وقد تبين أن فضلات ديدان الأرض، الغنية بالعناصر الغذائية والميكروبات النافعة، تشكل مصدرًا هامًا لـ .PGPR. وأظهرت السلالات المعزولة من PGPRقدرتها على إذابة الفوسفات والكالسيوم والبوتاسيوم وإنتاج السايدروفورات والأوكسين، وهي عناصر أساسية لنمو النباتات. يدعم استخدام PGPR الزراعة المستدامة عن طريق تقليل الحاجة إلى الأسمدة الكيميائية والمبيدات الحشرية، وتعزيز صحة المحاصيل، وتقليل الأثر البيئي. تسلط النتائج الضوء على إمكانات فضلات ديدان الأرض في الممارسات الزراعية المستدامة وأهمية الاستفادة من التفاعلات الطبيعية بين ديدان الأرض والبكتيريا النافعة والنباتات. ينبغي أن تركز الأبحاث المستقبلية على تطبيق أوسع ودراسات ميدانية طويلة الأجل للتحقق من فعالية هذه السلالات من PGPR في البيئات الزراعية المتنوعة. يمكن للأدوات المتقدمة في علم الجينوم والتكنولوجيا الحيوية تحسين سلالات PGPR بشكل أكبر، مما يساهم في أنظمة زراعية مرنة تلبي الاحتياجات الغذائية المتزايدة مع الحفاظ على الصحة البيئية. تمثل هذه الدراسة خطوة هامة نحو ممارسات زراعية مستدامة ومنتجة.

الكلمات المفتاحية:
البكتيريا المحفزة لنمو النباتات (PGPR
مخلفات ديدان الأرض
غني بالعناصر الغذائية
إذابة الفوسفات
إذابة الكالسيوم
إذابة البوتاسيوم
إذابة الحديد

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List of abbreviations

°C : degree Celsius

P : phosphate

K : potassium

Ca : calcium

Fe : iron

IAA : Indole Acetic Acid

SI P: Phosphate Solubilization Index

SI K : Potassium Solubilization Index

SI Fe : Iron Solubilization Index

SI Ca : Calcium Solubilization Index

ml : milliliter

g : gram

NBRIP : National Botanical Research Institute's Phosphate growth medium

nm : nanometer

PGPR : Plant Growth-Promoting Rhizobacteria

pH : Hydrogen Potentiel

GN : Nutrient agar

Introduction

Introduction

The pursuit of sustainable agricultural productivity has led researchers to investigate biological alternatives to traditional chemical fertilizers and pesticides. Plant Growth-Promoting Rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth through mechanisms such as nitrogen fixation, production of phytohormones, and suppression of pathogens. This research initiative was sparked by the need to develop sustainable agricultural practices that minimize environmental harm while maintaining high crop yields. The primary objective of this study is to isolate and identify effective PGPR strains from earthworm castings, which can enrich the plant rhizosphere and promote healthier and more productive crops (Vessey, 2003) .

Several studies have explored PGPR from various sources, with an increasing interest in earthworm-associated bacteria. Earthworms, known as ecosystem engineers, significantly enhance soil properties by their activity. Their castings, rich in organic matter and microbial communities, provide an ideal environment for beneficial bacteria. Earthworm-associated PGPR have shown great potential in improving soil fertility and plant health, contributing to increased agricultural productivity (Lavelle & Spain, 2001) . Previous research has successfully isolated potent PGPR strains from earthworm castings, demonstrating their effectiveness in promoting plant growth and resilience (Aira et al., 2007) .

The rationale for isolating bacteria from earthworm castings lies in the natural symbiotic relationship between earthworms and soil microorganisms. Earthworms consume soil and organic matter, which are then digested and excreted as nutrient-rich castings. These castings harbor high microbial activity and diversity, including beneficial bacteria like PGPR, which can be harnessed for agricultural use.

Studies have shown that earthworm castings have significantly higher microbial biomass and activity compared to surrounding soil, making them an excellent source for isolating effective PGPR strains (Edwards & Bohlen, 1996) .

PGPR are crucial for enhancing plant growth through various mechanisms. They facilitate better nutrient uptake, produce growth-promoting substances, and protect plants from diseases by outcompeting harmful pathogens. Enriching the plant rhizosphere with PGPR aims to create an optimal environment for plant growth, leading to higher yields and improved crop quality. This biological approach not only boosts agricultural productivity but also supports sustainable farming by reducing the reliance on chemical fertilizers and pesticides (Glick, 2012) .

Improving plant growth and soil health through PGPR is directly linked to enhanced agricultural productivity, essential for meeting the food demands of an ever-growing global population. The use of PGPR supports the principles of organic farming and integrated pest management, promoting a more

sustainable and eco-friendly agricultural landscape. By developing resilient agricultural systems that can withstand environmental stresses, PGPR contribute to long-term agricultural sustainability (Tilman et al., 2002) .

Earthworms have been recognized for their role in enhancing soil properties and plant growth through the production of vermicompost. Vermicompost, produced from earthworm castings, is rich in nutrients and beneficial microbes, making it an excellent organic fertilizer (Edwards & Lofty, 1977) . Research has shown that vermicompost application can improve soil structure, increase microbial biomass, and enhance plant growth, further highlighting the importance of earthworms in sustainable agriculture (Arancon et al., 2004) .

The isolation of PGPR from earthworm castings is not only advantageous due to the high microbial diversity but also because of the specific interactions between earthworms and these microorganisms. Earthworms selectively ingest organic matter and microorganisms, creating a unique microbial environment within their gut. This process results in the excretion of castings that are enriched with beneficial microbes, including PGPR (Byzov et al., 2007) . The unique conditions within the earthworm gut can lead to the proliferation of specific PGPR strains that are highly effective in promoting plant growth (Wurst et al., 2011) .

Furthermore, the use of PGPR isolated from earthworm castings can help mitigate the environmental impacts of conventional agricultural practices. Chemical fertilizers and pesticides are known to cause soil degradation, water contamination, and loss of biodiversity. By utilizing PGPR and vermicompost, farmers can reduce their dependence on these chemicals, leading to more sustainable agricultural practices (Mäder et al., 2002) . This approach aligns with global efforts to promote sustainable agriculture and reduce the ecological footprint of farming activities (Pretty, 2008) .

The beneficial effects of PGPR on plant growth have been widely documented. For instance, PGPR can induce systemic resistance in plants, making them more resilient to biotic and abiotic stresses. This is achieved through the production of secondary metabolites, which enhance the plant's defense mechanisms (Pieterse et al., 2014) . Additionally, PGPR can improve nutrient availability by solubilizing phosphate, fixing atmospheric nitrogen, and producing siderophores that chelate iron, making it more accessible to plants (Richardson et al., 2009) .

The integration of PGPR into modern agricultural practices offers a promising avenue for enhancing crop yields and soil health. As the global population continues to grow, the demand for food will increase, necessitating more efficient and sustainable farming methods. PGPR, particularly those isolated from earthworm castings, provide a natural solution to enhance plant growth and productivity while maintaining soil health (Singh et al., 2011) .

By harnessing the benefits of these microorganisms, farmers can achieve higher yields, improve crop quality, and contribute to the sustainability of agricultural systems (Berg et al., 2014) .

Recent advancements in molecular biology and genomics have further facilitated the study of PGPR. Techniques such as metagenomics and high-throughput sequencing have enabled researchers to identify and characterize microbial communities in greater detail, providing insights into their functional roles and interactions within the soil-plant system (Mendes et al., 2011) . These technologies have also allowed for the identification of novel PGPR strains with unique properties that can be applied in agricultural practices (Schlaeppi & Bulgarelli, 2015) .

The potential for PGPR to enhance plant growth and soil health extends beyond traditional agriculture. In urban and peri-urban areas, where soil contamination and degradation are prevalent, the use of PGPR can help restore soil fertility and promote plant growth in gardens, green spaces, and urban farms (Saharan & Nehra, 2011) . This urban application of PGPR aligns with efforts to promote urban agriculture and green infrastructure, contributing to food security and environmental sustainability in cities (Zhang et al., 2013) .

Moreover, the use of PGPR is not limited to food crops. These beneficial bacteria can also enhance the growth of ornamental plants, turfgrass, and biofuel crops, offering a wide range of applications in different sectors of agriculture and horticulture (Lucy et al., 2004) . The versatility of PGPR in promoting plant growth across various plant species underscores their importance in diverse agricultural systems (Compant et al., 2005).

The future of sustainable agriculture lies in the integration of biological approaches, such as the use of PGPR, with modern farming practices. This holistic approach can lead to the development of agroecological systems that are productive, resilient, and environmentally sustainable. Continued research and innovation in the field of PGPR will be essential to fully realize their potential in enhancing agricultural productivity and sustainability (Kloepper et al., 2004) .

In this study, we evaluated the capacity of these PGPR bacterial strains isolated from the earthworm waste to promote plant growth through IAA production and solubilization mechanisms of phosphate, potassium, calcium and iron.

Chapter 1

Bibliographic review

Bibliographic review

1. Overview of Rhizospheres

1.1. Etymology

The word rhizosphere was introduced in 1904 by Lorenz Hiltner (Anton et al., 2008), bacteriologist specializing in soil microbiology and professor of agronomy at the Technical College of Munich (Lombi et al., 2001). “Rhizo” comes from the Greek rhiza meaning root. “Sphere” comes from the Latin sphaera (same meaning), a word itself coming from the ancient Greek sfaira (meaning ball, balloon, or globe). The sphere defines the field of influence of the root system. Because of the volume it occupies, compared to the volume of the plant, the rhizosphere is also called the “hidden half” (Bowen and Roriva, 1991)

1.2. Definition

The rhizosphere is the region of soil located under the roots of plants and subject to their direct influence. It is a site of intense exchanges between the plant and the mineral substrate. It can be affected by soil compaction, prolonged waterlogging, salinization, eutrophication, or pollution, as well as by phenomena of aridification. Additionally, it is the region of intense microbial activity (Anoua et al., 1997).

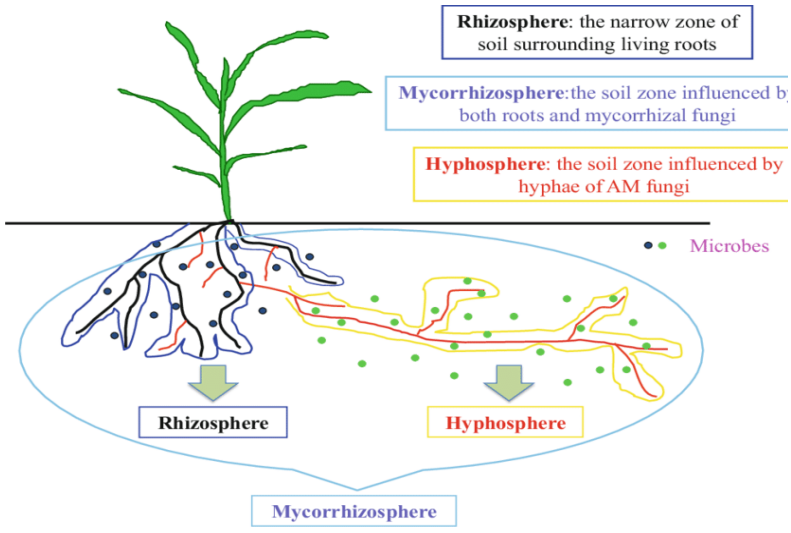

FIG .1. Conceptual diagram of rhizosphere, hyphosphere, and mycorrhizosphere. Modified from Wang (2014)

1.3. Rhizosphere activity

The plant releases root exudates which are made up of carbonaceous and nitrogenous organic substances: polysaccharides, organic acids and proteins (Mench, 1985). These exudates promote the development of pathogenic or non-pathogenic microflora. Thus, in response to the energy supply represented by root exudates, fungal propagules develop saprophytically to the root which they can infect and possibly parasitize (Schroth and Hildenbrand, 1964). Likewise, the density of bacteria is higher in the rhizosphere than in the soil distant from the roots: this is the "rhizosphere effect" (Foster and Rovira, 1978). The quantity and composition of root exudates also determine the nature of bacterial activities. These activities result from the synthesis of metabolites such as siderophores, antibiotics, growth substances, hydrocyanic acid, lipopolysaccharides (Lemanceau, 1992). A diverse bacterial flora, known as PGPR (plant growth promoting rhizobacteria), is beneficial to plant growth and health. There are two large groups: phytostimulatory PGPRs and phytoprotective PGPRs (Malek, 2015)

2. Rhizobacteria

The bacterial community of the rhizosphere is recruited from the reservoirs of microorganisms present in the soil (Bakker et al., 2013). Rhizobacteria are bacteria that have the ability to colonize roots intensively. Non-symbiotic bacteria meeting this definition belong to different genera and species, the most studied of which are: Agrobacterium sp, Azospirillum sp, Bacillus sp, Pseudomonas sp. The beneficial effects of rhizobacteria are linked to their strategic position at the soil-root interface. Indeed, the rhizoplane and the rhizosphere are the site of intense exchanges between the plant and the surrounding environment, these exchanges are reciprocal (Lemanceau, 1992)

2.1. Definition of Plant Growth-Promoting Bacteria (PGPR)

Several interactions, beneficial (symbioses) or not, even deleterious (pathogenesis) are observed between plants, bacteria and soil fungi which will flourish the biological activity of this soil. Among the interactions beneficial to plants, we can cite nitrogen-fixing symbioses, associations with growth-promoting bacteria (PGPR) or health, or interactions with mycorrhizogenic fungi. (Elaine, 2015). PGPR intervenes on the growth of plants through several mechanisms, directly or indirectly. These bacteria are capable of effectively colonizing root systems and beneficially influence the plant by stimulating its growth and/or protecting against infections by plant pathogens. These rhizosphere bacteria are then referred to

under the term PGPR (Plant Growth-Promoting Rhizobacteria). Most bacterial strains used as biopesticides belong to the genera Agrobacterium, Bacillus and Pseudomonas. (Haas and Defago, 2005).

3. Taxonomic Diversity of PGPR

Currently, many bacterial genera include PGPR, revealing very diverse taxa (Kloepper, 1992):

3.1. Alphaproteobacteria

The PGPR belonging to this class are the Rhizobia first classified by their ability to fix nitrogen and nodulate plants. These strains can behave like PGPR when they colonize the roots of non-leguminous plants in a non-specific relationship. Indeed, the genus Rhizobium also contains PGPR strains which were later considered as new genera: Bradyrhizobium, Sinorhizobium and Mesorhizobium (Sawada et al., 2003). The Gluconacetobacter genus of the Acetobacteraceae family, composed of obligate endophytic bacteria, colonizes the roots, stem and leaves of sugarcane (Tejera et al., 2003). Species of the genus Azospirillum described in the Rhodospirillaceae family are considered plant growth promoters. Strains belonging to this genus occur as free cells in the soil or associated with roots, stems, leaves and seeds mainly of cereals and forage grasses (Baldani et al., 2005).

3.2. *Betaproteobacteria*

In the family Burkholderiaceae, the genus Burkholderia forms a monophyletic group which contains various species with varied physiological and ecological properties, they are isolated from soils and plants. Some strains have the ability to symbiotically fix nitrogen. Ralstonia is a genus also assigned to the family Burkholderiaceae. It is, like the genus Burkholderia, omnipresent (Moulin et al., 2001).

3.3. Actinobacteria

The genus Frankia is a nitrogen-fixing symbiont. This capability is a characteristic of the genus. These bacteria are associated with actinorhizal plants that pioneer the colonization of poor or disturbed soils. Other Actinobacteria also promote plant growth but do not participate in symbiosis. They belong to the genera Arthrobacter, Micrococcus (Gray and Smith, 2005), Curtobacterium (Barriuso et al., 2005), and Streptomyces (Siddiqui and Mahmood, 1999).

3.4. Gammaproteobacteria

In the family Pseudomonadaceae, the genus Azotobacter is composed of bacteria that promote plant growth primarily due to their ability to fix nitrogen without nodulating plants (Sturz and Christie, 2003). Additionally, Pseudomonas is the most abundant genus in the rhizosphere among Gram-negative soil bacteria, and the PGPR activity of some of these strains has been known for many years, resulting from a broad understanding of the involved mechanisms. However, genera included in the family Enterobacteriaceae that function as PGPR include Citrobacter, Enterobacter, Erwinia, Klebsiella, Kluyvera, Pantoea, and Serratia (Garrity, 2005).

3.5. Firmicutes

Among the Gram-positive telluric bacteria, Bacillus are the most common and predominant types, they represent 95% of the isolated flora.

3.5.1. Bacillus

Les premières tentatives de classification des espèces de Bacillus étaient fondées sur deux caractéristiques principales : la croissance aérobie et la formation d'endospores. À partir des deux espèces formant des endospores, Bacillus anthracis et B. subtilis, le genre a progressé pour atteindre 146 espèces dans la quatorzième édition du Manuel de Bergey (Bergey et al., 1939). Cependant, ce nombre a été réduit à 22 espèces bien définies dans la huitième édition du Manuel de Bergey (Buchanan et Gibbons, 1974).

4. Effect and mechanisms of Action of PGPR

The beneficial effects of rhizobacteria on plant growth result from various mechanisms exerted by PGPR, whose modes of action can be direct or indirect, although the difference between the two is not always clear. Indirect mechanisms generally occur outside the plant, while direct mechanisms occur inside the plant and directly affect its metabolism. These mechanisms (see Fig. 3) can be active simultaneously or sequentially at different stages of plant growth:

1. Solubilization of phosphates, nitrogen fixation, and nutrient minerals, thereby making these elements available to the plant.
2. Production of phytohormones such as indole-3-acetic acid (IAA).
3. Suppression of soil pathogenic microorganisms, through the production of hydrogen cyanide, siderophores, antibiotics, and/or competition for nutrients (Gupta et al., 2000).

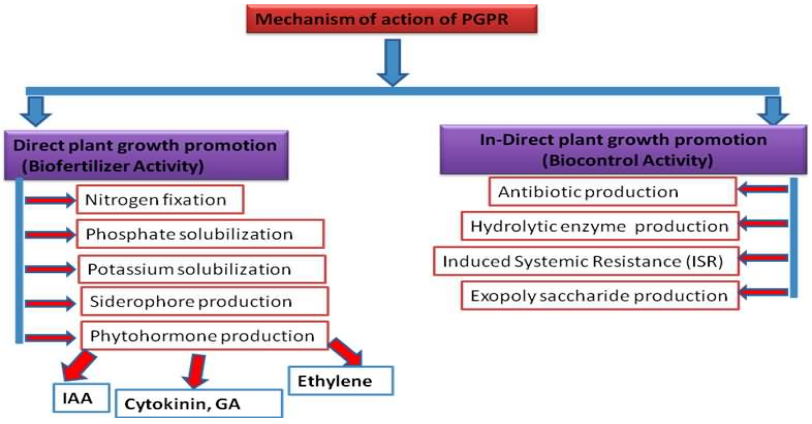

FIG .2. Mode of action of Plant Growth Promoting Rhizobacteria (PGPR) for enhancing plant/crop growth (Adapted from Gupta et al., 2015)

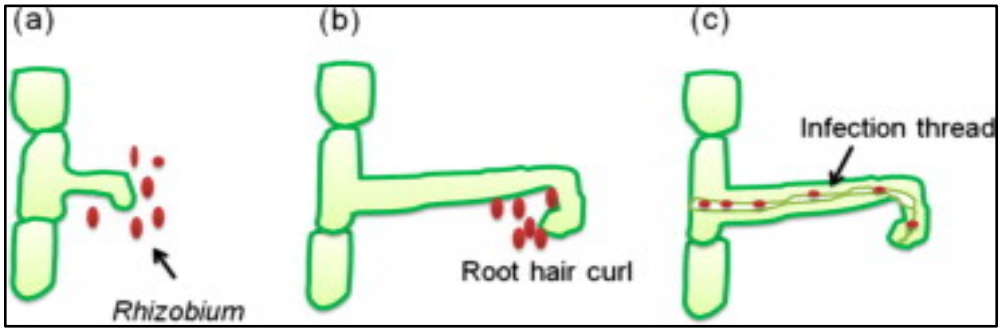

FIG .3. The nodulation process (a) Interaction of rhizobial rhicadhesin with host lectins and rhizobial attachment with root cells. (b) Excretion of nod factors by rhizobia causes root hair curling. (c) Rhizobia penetrate root hair and form an infection thread through which they penetrate the cortical cells and form bacteroid state thereby nodules are formed. (Ahemad, M. and Kibret, M., 2014)

4.1. Direct Effects of PGPR on Plants

PGPR bacteria facilitate plant growth directly by aiding in the acquisition of resources (nitrogen, phosphorus and essential minerals) or by modulating plant hormone levels (Munees and Mulugeta, 2014).

4.1.1. *Phosphate Solubilization*

Phosphorus is one of the main nutrients; second after nitrogen; in the requirement for plants. Plants are unable to utilize phosphate because 95-99% of phosphate present in the insoluble, immobilized and precipitated form. Plants absorb phosphate only in two soluble forms: monobasic (H2PO4) and basic (HPO42) ions (Govind et al., 2015).

Bacteria with phosphate solubilizing potential (BSP) could play an important role in providing phosphate to plants in a friendly and sustainable manner. These microorganisms can convert insoluble phosphate compounds into soluble forms and make them available to crop plants. Various bacterial species from the genera: Bacillus, Rhizobium and Pseudomonas were found to be the most powerful bacteria in phosphate solubilization (Banerjee et al, 2006).

The beneficial effects of BSP on plant growth vary significantly depending on environmental conditions, bacterial strains, plant and soil conditions (Şahin et al, 2004).

The solubilization capacity of phosphate also depends on the nature of the nitrogen source used in the medium, with greater solubilization in the presence of ammonium salts than when nitrate is used as a nitrogen source.

This has been attributed to the extrusion of protons to compensate for ammonium uptake, leading to a decrease in extracellular pH (Roos, 1984).

The lowering of soil pH by the microbial production of organic acids such as (lactate, citrate, etc.) as well as the extrusion of protons are the main mechanisms of mineralization of the organic form of phosphorus (Khan et al, 2009).

It plays a practically important role in all major metabolic processes in plants, including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration (Khan et al., 2010).

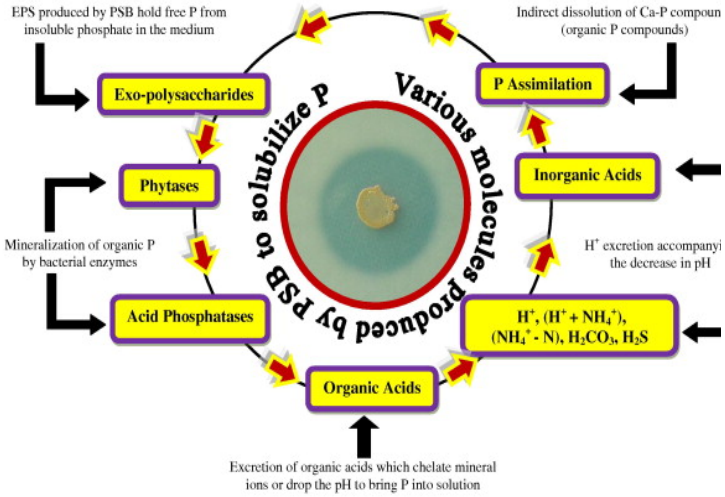

FIG .4. Various organic/inorganic substances produced by PSB responsible for phosphate solubilization in soils. (Ahemad, M. and Kibret, M., 2014)

4.1.2. Potassium Solubilization

It is the third major nutrient important to plants. Concentrations of soluble potassium in soil are generally very low and more than 90% of potassium in soil exists in the form of insoluble rocks and silicate minerals (Parmar and Sindhu, 2013).

Soil microorganisms play a key role in the natural K cycle and, therefore, potassium solubilizing microorganisms present in soil could provide an alternative technology to make potassium available for plant uptake (Rogers et al., 1998).

4.1.3. Siderophore Production

Iron is a vital nutrient for almost all forms of life. All microorganisms known hitherto, with the exception of certain lactobacilli, essentially require iron (Neilands, 1995). In the aerobic environment, iron occurs principally as Fe3+ and is likely to form insoluble hydroxides and oxyhydroxides, thus making it generally inaccessible to both plants and microorganisms (Rajkumar et al., 2010). Commonly, bacteria acquire iron by the secretion of low-molecular mass iron chelators referred to as siderophores which have high association constants for complexing iron. Most of the siderophores are water-soluble and can be divided into extracellular siderophores and intracellular siderophores. Generally, rhizobacteria differs regarding the siderophore cross-utilizing ability; some are proficient in using siderophores of the same genus (homologous siderophores) while others could utilize those produced by other rhizobacteria of different genera (heterologous siderophores) (Khan et al., 2009). In both Gram-negative and Gram-positive

rhizobacteria, iron (Fe3+) in Fe3+-siderophore complex on bacterial membrane is reduced to Fe2+ which is further released into the cell from the siderophore via a gating mechanism linking the inner and outer membranes. During this reduction process, the siderophore may be destroyed/recycled (Rajkumar et al., 2010, Neilands, 1995). Thus, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation (Indiragandhi et al., 2008). Not only iron, siderophores also form stable complexes with other heavy metals that are of environmental concern, such as Al, Cd, Cu, Ga, In, Pb and Zn, as well as with radionuclides including U and Np (Neubauer et al., 2000, Kiss and Farkas, 1998). Binding of the siderophore to a metal increases the soluble metal concentration (Rajkumar et al., 2010). Hence, bacterial siderophores help to alleviate the stresses imposed on plants by high soil levels of heavy metals.

Plants assimilate iron from bacterial siderophores by means of different mechanisms, for instance, chelate and release of iron, the direct uptake of siderophore-Fe complexes, or by a ligand exchange reaction (Schmidt, 1999). Numerous studies of the plant growth promotion vis-à-vis siderophore-mediated Fe-uptake as a result of siderophore producing rhizobacterial inoculations have been reported (Rajkumar et al., 2010). For example, Crowley and Kraemer (2007) revealed a siderophore mediated iron transport system in oat plants and inferred that siderophores produced by rhizosphere microorganisms deliver iron to oat, which has mechanisms for using Fe-siderophore complexes under iron-limited conditions. Similarly, the Fe-pyoverdine complex synthesized by Pseudomonas fluorescens C7 was taken up by Arabidopsis thaliana plants, leading to an increase of iron inside plant tissues and to improved plant growth (Vansuyt et al., 2007). Recently, Sharma et al. (2003) assessed the role of the siderophore-producing Pseudomonas strain GRP3 on iron nutrition of Vigna radiate. After 45 days, the plants showed a decline in chlorotic symptoms and iron, chlorophyll a and chlorophyll b content increased in strain GRP3 inoculated plants compared to control.

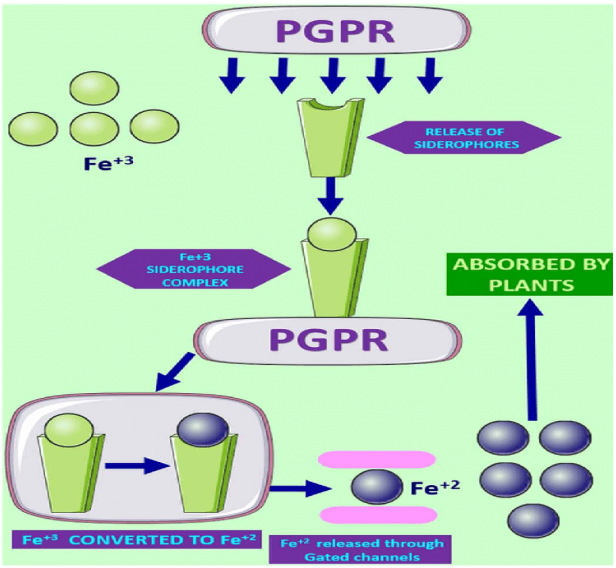

FIG .5. Schematic representation of Uptake of Iron (III) with the help of siderophore and conversion to Iron (II) by PGPR Sinha, D., Mukherjee, S., & Mahapatra, D. (2021)

4.1.4. Phytohormone Production

Phytohormones influence the physiological functions of plants at very low concentrations as chemical messengers. The phytohormones are key determinants of plant behavior and play a leading role in various physiological and developmental processes. Traditionally, plant hormones have been divided into five different classes: auxin, cytokinins, gibberellins, abscisic acid , and ethylene (Olenskaetal. , 2020) .

Besides, several phytohormones such as jasmonate, brassinosteroids, and salicylic acid also play significant roles in plant growth and development particularly under biotic and abiotic stress conditions (Wong et al., 2015; Kang et al., 2016). A wide range of rhizosphere inhabiting bacteria can produce phytohormone for facilitating plant growth and development. The phytohormones produced by plants and rhizobacteria are involved in all the communication in plant cells (Maheshwari et al., 2015).

4.1.2.1. Auxin

Auxin is the most imperative phytohormone which controls nearly all aspects of plant development. Indole-acetic acid (IAA) is the most common, well-characterized auxin produced by bacteria and plants. In plants, IAA plays an important role in apical dominance, division, and cell differentiation, seed germination, and the development of roots. It also contributes to processes like photosynthesis, biosynthesis of metabolites, and stress resistance (Maheshwari et al., 2015). Bacteria-produced IAA promotes the root length and surface area for enhanced uptake of nutrients and water. The majority of the microbes (>80%) inhabiting the rhizosphere are capable of synthesizing and releasing auxin (Olen´ska et al., 2020). Tryptophan has been identified as the main precursor of auxin biosynthesis. There are three main pathways involved in IAA synthesis by microbes: (1) Indole acetic acid synthesis via intermediates indole-3- pyruvic acid and indole-3-acetic aldehyde, is found in bacterial genera like *Erwinia, Agrobacterium, Pseudomonas, Azospirillum, Bradyrhizobium, Enterobacter, Klebsiella*, and *Rhizobium*. (2) IAA biosynthesis via tryptamine and indole-3-acetic aldehyde which has been reported in *Azospirillum* and *Pseudomonas*. (3) The IAA synthesis via indole-3-acetamide (IAM) formation, which operates in *Agrobacterium, Erwinia*, and *Pseudomonas* strains (Tahir and Sarwar, 2013). The indole-3-acetamide (IAM) pathway commonly found in bacteria, involves the conversion of tryptophan to indole-3-acetamide by the enzyme tryptophan-2- monooxygenase (IaaM), and then IAM is converted to IAA by the enzyme IAM hydrolase (iaaH). The two genes involved in the IAM pathway (iaaM and iaaH) have been identified in *Agrobacterium, Bradyrhizobium, Pantoea, Pseudomonas*, and *Rhizobium* strains. Genes involved in IAM pathway have been localized on the chromosome (*Pseudomonas* spp.) as well as on plasmids (e.g., *Pantoea agglomerans*). These auxin-producing PGPR modulate the plant response as reported through cucumber -*Bacillus amyloliquefaciens* strain SQR9 system, which showed that inoculation of *Bacillus amyloliquefaciens* leads to the high amount of tryptophan secretion through roots of cucumber, subsequently increasing the IAA synthesis by bacteria inhabiting the rhizosphere (Liu et al., 2016a). Several studies reported the improvement in root formation, growth, and yield of various crops through auxin-producing PGPR (Ali et al., 2014; Imran et al., 2015; Majeed et al., 2015). Moreover, bacteria- derived auxins might be involve in the mitigation of deleterious effects of various abiotic stresses, like salinity, drought, and soil pollution (Kudoyarova et al., 2019). The application of auxin producing *Bacillus thuringiensis, B. amyloliquefaciens, B. simplex, Enterobacter aerogenes, Moraxella pluranimalium*, and *Pseudomonas stutzeri* strains showed positive effect growth and yield parameters of wheat grown under drought condition and suggested to be used for rhizosphere engineering in drylands (Raheem et al., 2018).

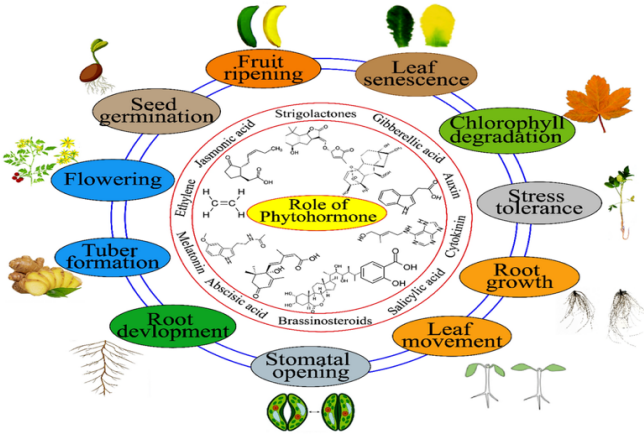

FIG .6. chemical structure and functions of phytohormones used in the growth and productivity of the horticultural crop Altaf, M. A. et al (2023)

4.1.2.2. Cytokinins and Gibberellins

Cytokinins are N6-substituted aminopurines that play a key role in a large number of physiological processes such as plant cell division, interruption of dormant bud quiescence, activation of seed germination, promotion of branching, root growth, chlorophyll accumulation, leaf expansion, and delayed senescence (Salisbury and Ross, 1992). In addition, cytokinins regulate the expression of the gene encoding expansin, a protein that induces the loosening of plant cell walls and facilitates the expansion of the plant cell and causes its turgidity, which has an impact on both the size and shape of the cells (Downes et al., 2001).

The gene encoding the enzyme responsible for cytokinin synthesis was initially characterized in Agrobacterium tumefaciens (Nester et al., 1984) and later in methylotrophic and methanotrophic bacteria (Ivanova et al., 2001). Since then, many PGRPs including Azotobacter, Azospirillum, Rhizobium, Bacillus and Pseudomonas spp. have produced this hormone (Nieto and Frankenberger 1989; Timmusk et al., 1999). Inoculation of seeds with cytokinin-producing bacteria usually leads to an increase in cytokinin content in plants, thus simultaneously influencing plant growth and development (Arkhipova et al., 2005). Various environmental stresses can also lead to the accumulation of elevated plant cytokinin levels (Arkhipova et al., 2007). A positive correlation has been observed in several legume species between the level of cytokinins in plants and the ability of Rhizobium to form nodules on the roots (Yahalom et al., 1990; Hirsch and Fang, 1994).

Gibberellins are synthesized by higher plants, fungi, and bacteria; they are diterpenoid acids made up of isoprenic residues. A significant number (136) of different gibberellins are identified and characterized (MacMillan, 2002). They affect cell division and elongation and are involved in several developmental processes such as seed germination, flowering, fruiting and delayed senescence in many organs of a wide range of plant species (MacMillan, 2002). Gibberellins are also involved in promoting root growth as they regulate the abundance of root hairs (Bottini et al., 2004). La capacité des bactéries à synthétiser des substances de gibbérellines a été initialement décrite chez A. brasilense (Tien et al., 1979) et Rhizobium (Williams and Sicardi de Mallorca, 1982) and then in various bacterial genera that populate the root system of the plant, including Azotobacter, Arthrobacter, Azospirillum, Pseudomonas, Bacillus, Acinetobacter, Flavobacterium, Micrococcus, Agrobacterium, Clostridium, Burkholderia, and Xanthomonas (Mitter et al., 2002; Tsakelova et al., 2006; Joo et al., 2009). The promotion of plant growth by gibberellin-producing PRMPs has been reported by several studies and this positive effect on plant biomass is often associated with increased gibberellin content in plant tissues (Atzhorn et al., 1988; Gutierrez-Manero et al., 2001; Joo et al., 2009).

4.2.2.3. Role of ethylene

Ethylene gas produced endogenously by plants has several effects on plant development and acts as a secondary signal molecule in the induction of plant defenses (Ecker, 1995). Ethylene is involved in many physiological processes, such as seed germination, tissue differentiation, root formation and elongation, lateral bud development, flowering, flower opening, organ senescence, fruit ripening, and leaf and fruit abscission (Frankenberger and Arshad 1995). At high concentrations, ethylene negatively affects many physiological steps of plants. An increase in the production of ethylene acting as a sensitive hormone stimulates fruit ripening and flower aging. These symptoms are associated with loss of leaf chlorophyll, degradation of proteins and RNAs, and loss of flower pigmentation (Oldroyd et al. 2001; VanLoon et al., 2006). In addition, ethylene at high concentrations inhibits the development of alfalfa (Medicago sativa) nodules (Glick et al., 2007) and peas (Pisum sativum) (Cheng et al., 2008) and weakens the plant's defence against pathogens (Wang et al., 2000).

4.2. Indirect Effects of PGPR on Plants

The main advantage of using PGPRs is the resistance conferred on plants against diseases caused by pathogens. Rhizobacteria play a major role in the fight against these agents, where a wide spectrum of bacterial, fungal and parasitic diseases is suppressed via the production of antibiotics, competition (for nutrients, oxygen and space), the 'Activation of induced systematic resistance (ISR) and the production of enzymes (chitinase, protease, lipase), this protection is named biocontrol. In addition, PGPRs can be used as an effective bi -fertilizer in improving crop yield by producing enzymes such as (cells, amylases, etc.)

4.2.1. Antibiotic Production

Antibiotic production is considered to be one of the most potent and studied biocontrol mechanisms in PGPRs. (Shilev, 2013) The production of antibiotics by PGPR is a crucial indirect effect that contributes to plant health and growth. These antibiotics can suppress the growth of phytopathogens in the rhizosphere, thereby reducing disease incidence and promoting plant vigor. For instance, strains of Pseudomonas fluorescens are known to produce a variety of antibiotics such as pyoluteorin, phenazines, and 2,4-diacetylphloroglucinol (DAPG), which exhibit broad-spectrum antimicrobial activity against soil-borne pathogens. This suppression of pathogen activity by PGPR antibiotics creates a favorable environment for plant growth and development. (Raaijmakers, J. M., & Mazzola, M. 2012).

4.2.2. Induction of Resistance System

PGPR can trigger a phenomenon in the plant known as systemic resistance induction which is phenotypically similar to acquired systemic resistance which occurs when the plant activates its defense mechanisms in response to infection by a pathogen (Abdesselam and Latache, 2017). Plants inoculated with PGPRs can also provide systemic resistance against a wide range of plant pathogens. Diseases of fungal, bacterial and viral origin and, in some cases, even damage caused by insects and nematodes can be reduced after the application of PGPR, it confers to the plant a certain degree of protection against subsequent attacks by a phytopathogen via stimulation of systemic defense mechanisms. This “immunity” is initiated following the perception by the plant of so-called “elicitor” molecules produced by microorganisms (Ara Naznin et al., 2012; Cherif, 2014).

4.2.3. Production of hydrolytic enzymes

One indirect mechanism employed by PGPR is the production of hydrolytic enzymes, which play a crucial role in enhancing nutrient availability for plants. These enzymes are capable of breaking down complex organic compounds in the soil into simpler forms that can be readily absorbed by plants. For instance, PGPR can produce enzymes like cellulases, proteases, and chitinases, which degrade cellulose, proteins, and chitin respectively, releasing nutrients such as carbon, nitrogen, and phosphorus into the soil solution for plant uptake. By facilitating the decomposition of organic matter and recycling nutrients, PGPR contribute to soil fertility and promote plant growth. (Bhattacharyya, P. N., & Jha, D. K. (2012)

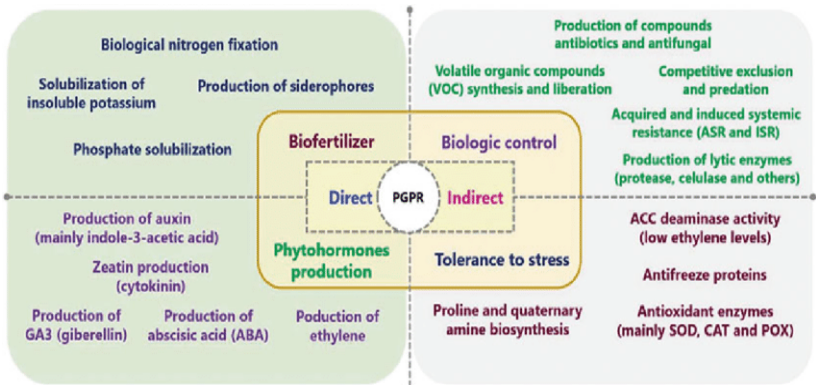

FIG .7. Direct and indirect mechanisms mediated by plant growth-promoting rhizobacteria (PGPR) with benefi cial effects on host plants (Chauhan et al. 2015 ; Pii et al. 2015)

Chapter 2

Materials and methods

Materials

1.Biological material (earthworms)

Earthworms represent a major component of soil macrofauna since, in most terrestrial ecosystems, they dominate in terms of biomass (pélosi.,2008).

Macrofauna is made up of animals between 4 and 80 mm. These are earthworms, insect larvae, woodlice, chilopod and diplopod myriapods, gastropod molluscs (slugs and snails), chelicerates (spiders and opilions) and various hexapods (Bachelier, 1978).

Macrofauna plays a key role in regulating the physical properties of soils and the biodiversity of smaller organisms (Lavelle and Spain, 2001).

Earthworms (*Annelids, Oligochaetes*) represent a major component of the soil macrofauna since, in most terrestrial ecosystems, they dominate in terms of biomass (Pelosi, 2008).

1.1. Aporrectodea caliginosa

Our choice fell on *Aporrectodea caliginosa* (Fig.9). The earthworms are taken from their natural soil where they live found in Tébessa and specifically in "the garden" then preserved in terrariums, in the laboratory (Provided by Dr. Bouazdia Karim " Master theme 2024")

1.2. Systematics of Aporrectodea Caliginosa (Savigny, 1826)

Kingdom:	**Animalia**
Junction:	Annelida
Class:	Clitellata
Order:	Crassiclitellata
Family:	Lumbricidae
Genus:	Aporrectodea
Species:	Aporrectodea Caliginosa

FIG .8. *Aporrectodea Caliginosa* (personal photo2024)

1.3. Eisenia fetida

Our choice fell on *Eisenia fetida* (Fig.10). The earthworms are taken from their natural soil where they live found in Tébessa and specifically in "Bouhaba" then preserved in terrariums, in the laboratory (Provided by Dr. Bouazdia Karim " Master theme 2024")

1.4. Systematics of Eisenia fetida (Savigny, 1826)

Kingdom:	**Animalia**
Junction:	Annelida
Class:	Clitellata
Order:	Haplotaxida
Family:	Lumbricidae
Genus:	Eisenia
Species:	Eisenia fetida

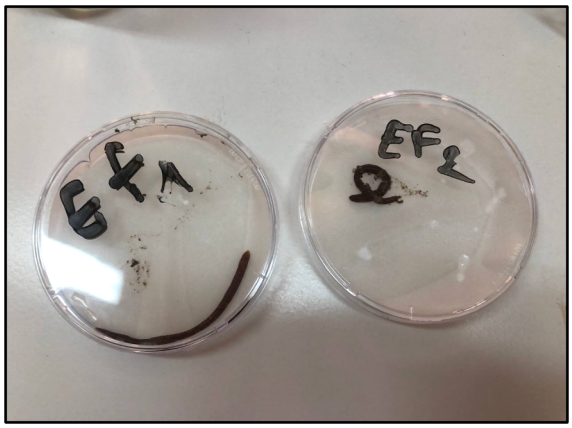

FIG .9. *Eisenia fetida* (personal photo 2024)

Methods

1. Isolation of PGPR from earthworms

Taking 2 earthworms from each species, rinse them with sterile distilled water. Then, place them in Petri dishes with filter paper and add a few drops of sterile distilled water.then we let them rest for 24h

After 24 hours, we will notice that the earthworms have left their waste. We will then collect the waste and place it in Eppendorf tubes.

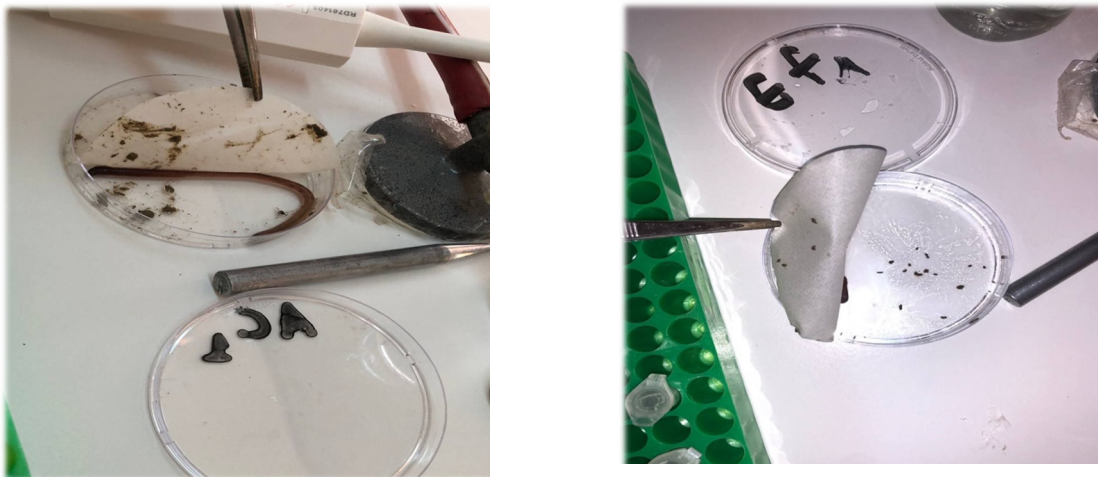

FIG .10. Earthworm waste (personal photo 2024)

Next, we will add 1ml of sterile distilled water using a micropipette, and then vortex them thoroughly.

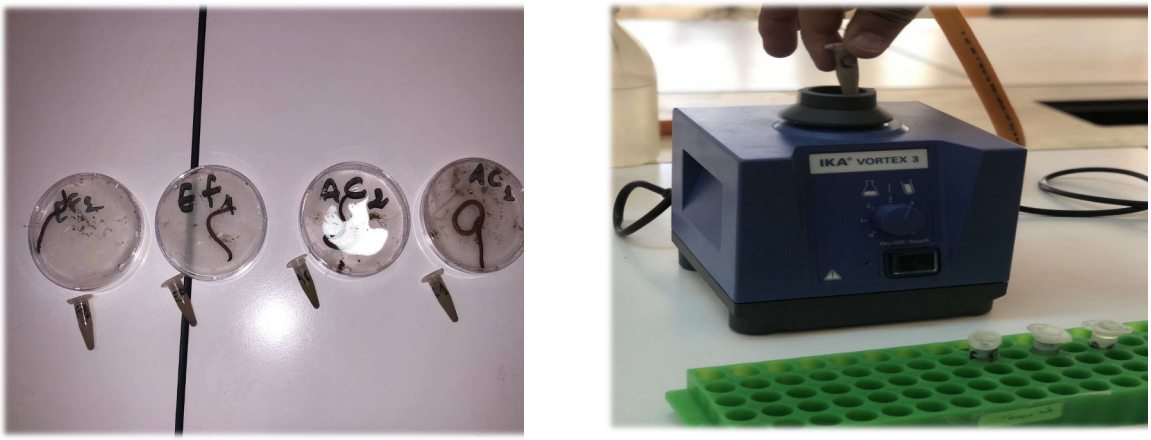

FIG .11. Vortexed waste (personal photo 2024)

We prepared the nutrient agar (NA) by dissolving it in one liter of distilled water, then autoclaved it. Afterward, we cooled 8 Petri dishes.

Next, from each tube, using a platinum loop, we take the vortexed waste and inoculate it onto 2 Petri dishes containing nutrient agar (NA) medium.

In the results, we obtained 2 Petri dishes from each species: AC11, AC12, AC21, AC22, EF11, EF12, EF21, and EF22.

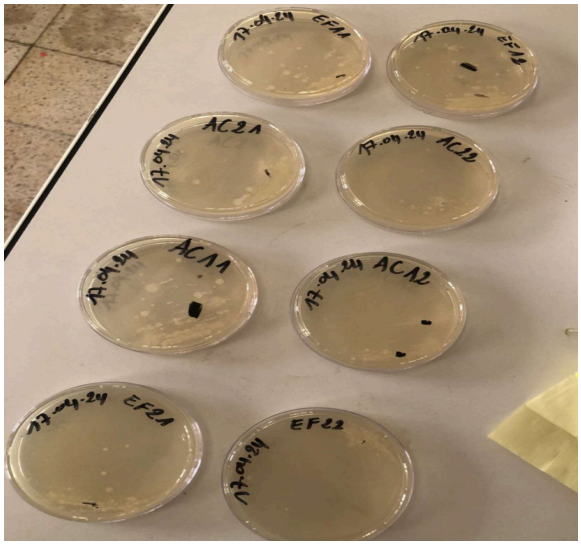

FIG .12. Bacterial strains (personal photo2024)

2. Preparation of strains

We prepared the nutrient broth in one liter of distilled water. Then, we added 10ml of the nutrient broth into 100 test tubes and autoclaved them.

Next, we collected the bacteria obtained from the previous 8 Petri dishes using a platinum loop and replicated them by placing each sample from each dish into 4 test tubes, resulting in 32 tubes. We then incubated them in a water bath at 32°C with gentle agitation for 24 hours.

FIG .13. Laboratary water bath (personal photo 2024)

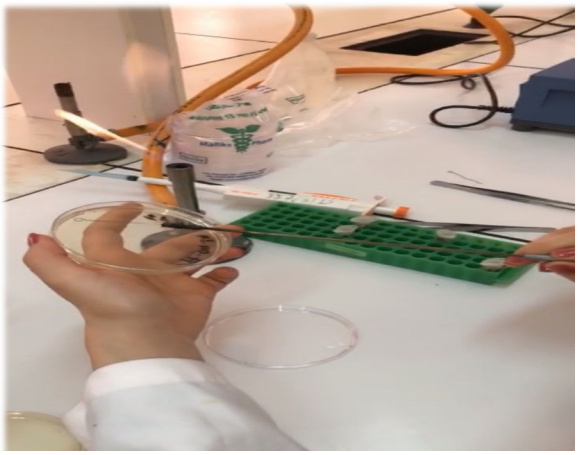

FIG .14. Bacteria inoculation (personal photo 2024)

After 24hours we added 5ml of glycerol to 16 tube using a micropipette which were then conserved while the remaining 16 tubes (AC111, AC112, AC121, AC122, AC211, AC212, AC221, AC222, EF111, EF112, EF121, EF122, EF211, EF212, EF221, and EF222) were used to repeat the bacterial inoculation onto 16 Petri dishes containing nutrient agar. Then, We preserved them in the incubator for 5 days.

Now, the bacterial strains are ready to be tested for their activity.

3. Media préparation and culture

To prepare the test media, we followed this method:

1) We agitated the medium with distilled water using an agitator until the components were dissolved.

2) In a 1-liter flask, we measured 1 liter of prepared medium by adding distilled water.

3) We divided each medium into 5 bottles, with 20ml of prepared medium in each bottle, and autoclaved them.

3.1. Phosphate solubilization test

The solubilizing capacity is determined by the presence of a transparent halo corresponding to the lysis zone around the bacterial colony, a solubilization index (P SI = Lysis Diameter/ Colony Diameter) has been estimated (Gonzalez et al., 2018; Abiala et al., 2015).

TAB .1. NBRIP media's protocol ingredients(Gonzalez et al., 2018 ; Abiala et al., 2015).

NBRIP media's protocol ingredients	g/l
- Glucose	10,0
- Tricalcium phosphate	5,0
- Magnesium chloride hexahydrate	5,0
- Magnesium sulphate heptahydrate	0,25
- Potassium chloride	0,2
- Ammonium sulfate	0,1
- Distilled water	1L
Ph 7	

3.2. Potassium solubilization test

The formation of the light area around the colony testifies to the solubilization of potassium, a solubilization index is calculated (K SI = Lysis Diameter/ Colony Diameter) (Meena et al. 2013).

TAB .2. Aleksandrov media's protocol ingredients(Meena et al. 2013).

Aleksandrov media's protocol ingredients	g/l
- Mg SO 4 7.H 2	0 0.005
- potassium (source of K)	2.0
- Ca HPO 4	2.0
- Ca CO 3	2.0
- Fe Cl 3	0.1
- Glucose	0.5
- Distilled water	1L
Ph. 7	

3.3. Siderophores production test

A change in color from blue to orange appears around the siderophore-producing colony. The color change is due to the transfer of ferric ions from the CAS to the siderophores. The calculation of the ratio (FE SI=diameter of the halo / the diameter of the bacterial colony) makes it possible to compare the differences in production between bacterial strains.

TAB .3. King's B media's protocol ingredients (Schwyn and Neilands (1987).

King's B media's protocol ingredients	g/l
- Casein	10
- Lactose	10
- FeSO4-7H2O	0.3
- Potassium Chloride	0.5
- Dibasic Potassium phosphate	3.0
- Distilled water	1L
Ph. 7	

3.3.1.Casein extraction

1. **Milk Collection:** collecting fresh, unpasteurized cow's milk
2. **Coagulation:** Add acetic acid to the milk to lower the pH and induce coagulation of milk proteins, including casein.
3. **Mixing:** Thoroughly mix the milk and acetic acid to ensure even distribution of the acid and homogeneous coagulation.

FIG .15. Centrifugation (5000/5min) (personal photo 2024)

4. **Centrifugation:** Place the milk-acetic acid mixture into a centrifuge and spin it at high speed. The centrifugal force separates the milk components based on density, with casein and fats forming a solid layer on the centrifuge walls
5. **Separation:** After centrifugation, stop the machine and gently remove the solid layer of casein and fats from the centrifuge walls. This layer mainly contains casein, although impurities may also be present.
6. **Washing and Drying:** Wash the recovered casein curds with cold water to remove any acid residues and impurities. Then, dry the curds to obtain casein powder.

3.4. Calcium solubilization test

The solubilizing capacity is determined by the presence of a transparent halo corresponding to the lysis zone around the bacterial colony, a solubilization index (Ca SI= Diametere désolbilisation/ Bacterial growth diameter) has been estimated.

TAB .4. Pikovskaya media's protocol ingredients (pikovskaya 1984)

MPVK media's protocol ingredients	**g/l**
- **MgSO4H2**	**0.005**
- **(NH4)2SO4**	**0.05**
- **NaCl**	**0.1**
- **KCl**	**0.1**
- **MnSO4.7H2O**	**0.125**
- **FeSO4.7H2O**	**0.1**
- **Agar**	**7.5**
- **Glucose**	**5.0**
- **Distilled water**	**1l**
Ph. 7	

3.5. IAA production test

The strains were inoculated in a nutrient broth containing 500 µg ml-1 tryptophan, the cultures were stirred continuously at 150 rpm for 90 hours at 30C, centrifuged them at 13200 rpm for 10 min and mixed the supernatant all is incubated in total darkness using Salkowski's reagent in a 2:1 ratio incubated at room temperature for 75 min and read at 535 nm into a spectrophotometer.

TAB .5. salkowski media's protocol

Nutritious Broth's Ingrédients	g/l	Salkowski's reagent's Ingredients	m/l
- Peptone **- Beef extract** **- distilled water** **- pH 7**	**5,0** **3,0** **1**	**–Fe Cl 3 (0,5 M)** **- H 2 SO 4** **- Sterile distilled water**	**5** **150** **250**

Next, for each test, we cooled 16 Petri dishes with the specific test medium. Thus, we obtained 16 Petri dishes with King's B for siderophore production, 16 Petri dishes with modified Pikovskaya medium for calcium production, 16 Petri dishes with Aleksandrov medium for potassium production, and 16 Petri dishes with NBRIP medium for phosphorus production.

Now, with the 16 Petri dishes containing the bacteria, we use a sterile toothpick to collect the active bacteria and place 4 replications in the form of a dot in each prepared test Petri dish and we let them incubate for 10 days before getting the results.

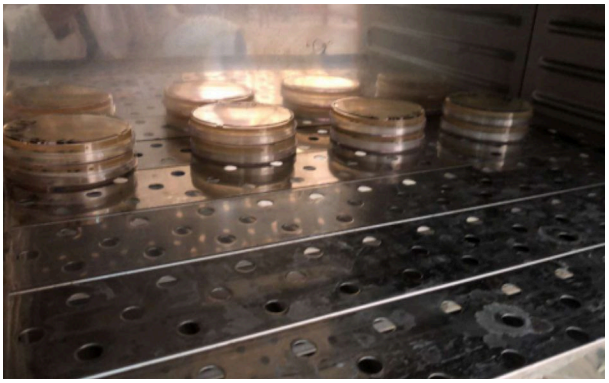

FIG .16. Incubator (30C°)

4. Statistical analysis

Obtained results are presented in histograms with standard deviation and the four replicate of each studied parameter is analysed by one way ANNOVA at $\alpha = 5\%$ significant level, significant treatment is followed by post-hoc Tukey analyses for multiple testing performed in XLSTAT 2014.

CHAPTER 3

RESULTS AND DISCUSSION

Results

1. Isolation of pure strains

The isolation of pure strains led to the isolation of sixteen isolates (Eight *A,Caliginosa* , Eight *E,Fetida*) based on their color, shaped and appearance . We observed a wide diversity of colonies in the used meduim (solid GN), known for distinguishing various bacterial forms (such as whitish viscous , reddish viscous, viscous with brownish color, viscous with veins).

2. Phosphate solubilization test

Figure 18 present the zone of lysis around the bacterial colonies, demonstrating the isolates ability to solubilize phosphate .

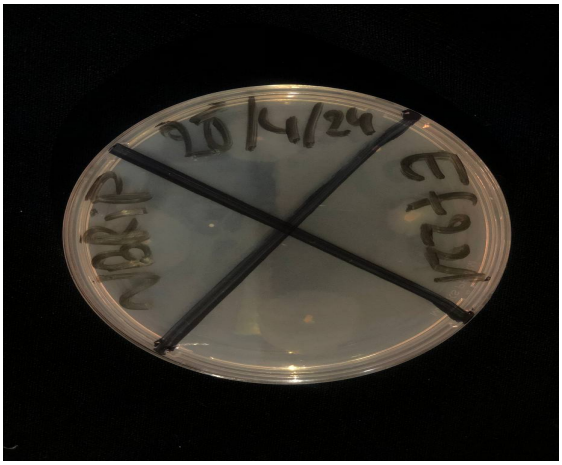

FIG .17. Phosphate solubilization by tested isolates
(personal photo 2024)

Histogram of phosphate solubilization indices by the isolates tested. Reveals a difference between the SI P indices recorded by the 6 isolates among the 16 tested, where it reaches as the highest value of isolate (10,5) and as the lowest value of isolate (2.5) .

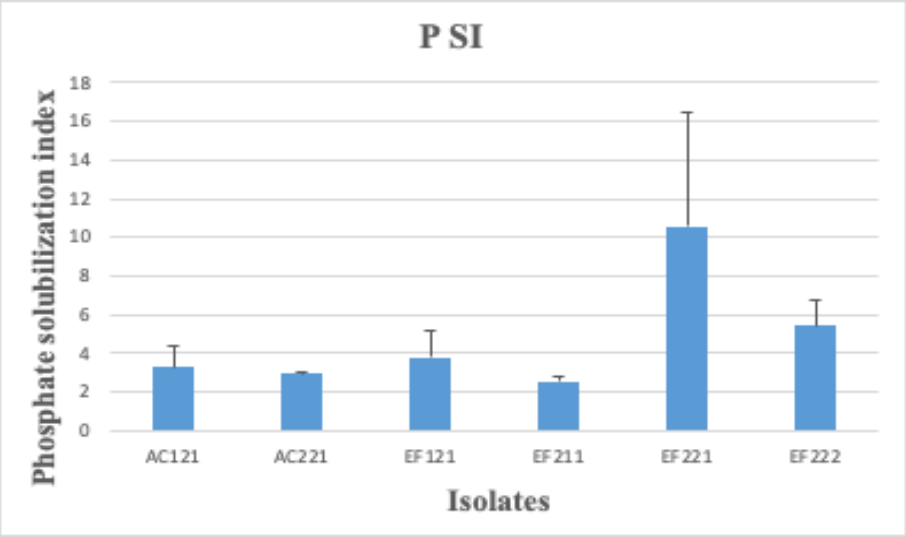

FIG .18. Histogram of the indices of phosphate solubilization by the isolate test

Statistical analysis revealed significant deference ($p<0.05$) between the SP indices recorded by the six isolates (tab), which showed solubilizing ability. Grouped into three different groups by the Tukey test.

TAB .6. Statistical analysis of SP

Analysis of variance:

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	5	182,032	36,406	4,117	0,011
Error	18	159,181	8,843		
Corrected Total	23	341,213			

Category	LS means	Groups		
EF221	10,563	A		
EF222	5,417	A		B
EF121	3,771			B
AC121	3,300			B
AC221	2,938			B
EF211	2,529			B

3. Potassuim solubilization test

The clear zone around the bacterial colonies indicates potassuim solubilization .

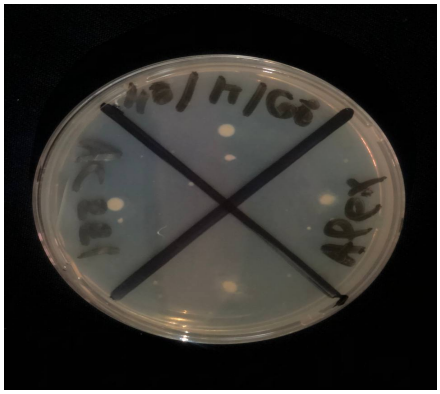

FIG .19. Potassuim solubilization by tested isolates

(personal photo 2024)

Histogram of potassuim solubilization indices by the isolate tested. Reveals a difference between the ISK indices recorded by the nine isolates among the 16 tested, where it reaches as the highest value of isolates (5,3) and as the lowest value of isolate (2,3) .

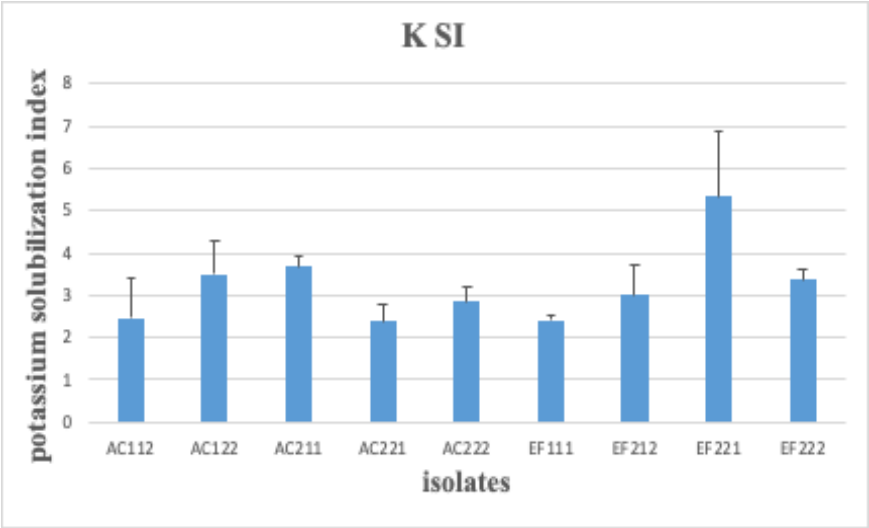

FIG .20. Histogram of the indices of potassuim solubilization by the isolate test

Statistical analysis revealed significant deference ($p< 0.05$) between the Sk indices recorded by the 9 isolates (tab), which showed solubilizing ability in both species *E.Fetida , A.Caliginosa* Grouped into 3 groups by the Tukey test.

TAB .7. Statistical analysis of SK

Analysis of variance:

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	8	27,315	3,414	4,892	0,001
Error	27	18,845	0,698		
Corrected Total	35	46,160			

Category	LS means	Groups	
EF212	5,325	A	
AC122	3,671	A	B
AC121	3,500	A	B
EF221	3,363	A	B
EF211	2,996		B
AC221	2,858		B
AC111	2,470		B
AC222	2,400		B
AC212	2,396		B

3. Calcuim solubilization test

The clear zone around the bacterial colonies indicates potassuim solubilization . It reveals a difference between the ISCa indices recorded by four isolates among the 16 tested .

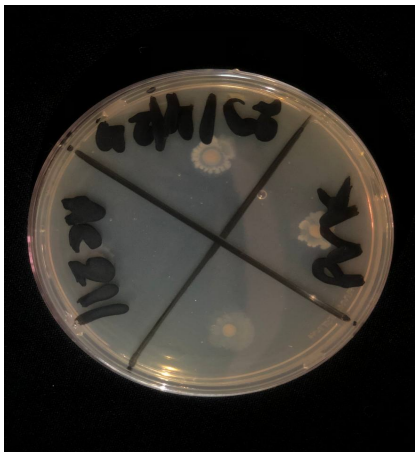

FIG .21. Calcuim solubilization by tested isolates

(personal photo 2024)

Histogram of calcuim solubilization indices by the isolate tested. Reveals a difference between the SI Ca indices recorded by the isolates among the 16 tested, where it reaches as the highest value of isolates (4,6) and as the lowest value of isolates (1.5).

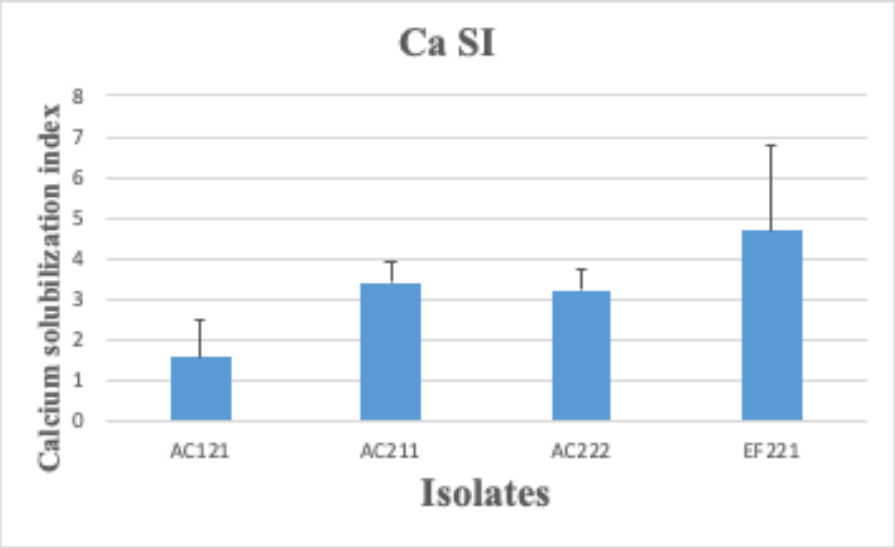

FIG .22. Histogram of the indices of calcuim solubilization by the isolate test

Statistical analysis revealed no significant difference (p>0.05) between the SICa indices recorded by the six isolates (tab), for calcium solubilizing .

TAB .8. Statistical analysis of SI Ca

Analysis of variance:

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	3	19,687	6,562	3,308	0,057
Error	12	23,808	1,984		
Corrected Total	15	43,495			

4. Siderophore solubilization test

The level of orange coloration is indicative of the fer solubilization the indice recorded by one isolate among the 16 tested .

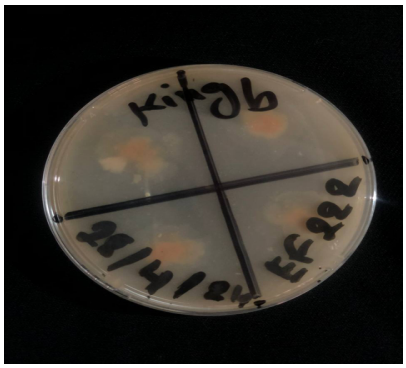

FIG .23. Siderophore solubilization by tested isolates

(personal photo 2024)

Histogram of siderophore solubilization indices by the isolate tested, recorded by one isolate among the 16 tested where it reaches as of the isolate (EF222).

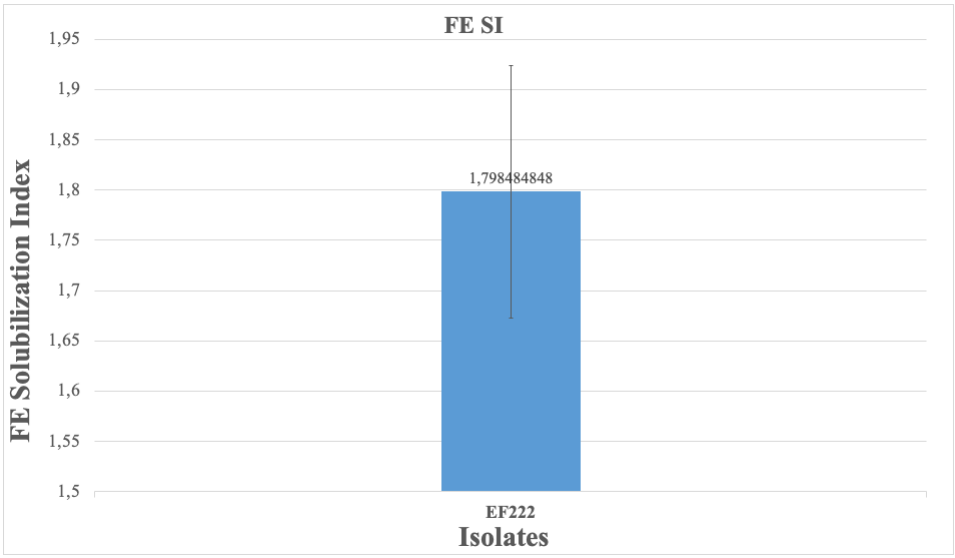

FIG .24. Histogram of the indices of siderophore solubilization by the isolate test

For siderophore solubilizing only one isolate (EF222) who showed a significant results

5. IAA production test

The qualitative test, marked by the color change of the culture medium from pinkish to brown after the addition of Salkowski reagent, indicates a clear production of indole-3-acetic acid (IAA) by all 64 isolates tested. This demonstrates that all isolates can transaminate tryptophan into IAA, with varying concentrations of IAA produced among the isolates.

FIG .25. Auxin Production by tested isolates
(personal photo 2024)

The histogram shows the concentration of IAA production by the tested isolates, with significantly high production noted for isolates AC111,EF111 and EF112.

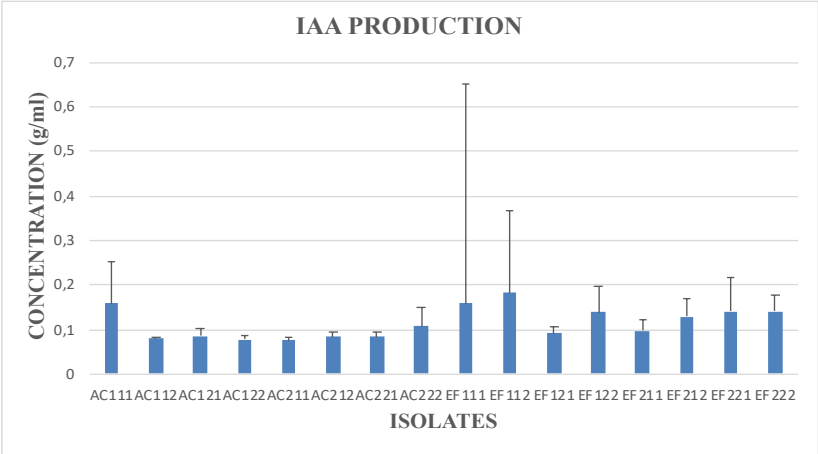

FIG .26. Histogram of concentration of IAA Production by the isolate test

The statistical analysis revealed no significant difference ($p>0.05$) among the isolates for IAA production, recorded between the 16 isolates .

TAB .9. Statistical analysis of IAA production

Analysis of variance:

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	15	0,342	0,023	0,947	0,522
Error	48	1,156	0,024		
Corrected Total	63	1,498			

DISCUSSION

Earthworms, particularly Aporrectodea caliginosa and Eisenia fetida, play a pivotal role in enriching the plant rhizosphere with Plant Growth-Promoting Rhizobacteria (PGPR). These earthworms contribute to soil health and fertility through their burrowing and feeding activities, which significantly impact microbial diversity and activity. (Blouin et al., 2013; Domínguez et al., 2019).

Aporrectodea caliginosa, a soil-dwelling earthworm, is known for its ability to ingest and mix large quantities of soil, enhancing soil structure and aeration (Blouin et al., 2013). This activity not only improves soil porosity and water infiltration but also creates a favorable environment for the proliferation of PGPR (Domínguez et al., 2019). The ingestion of organic matter and soil by A. caliginosa results in the production of nutrient-rich casts that are deposited in the rhizosphere (Aira et al., 2007). These casts are rich in microorganisms, including PGPR, which can promote plant growth by various mechanisms such as nitrogen fixation, phosphate solubilization, potassium solubilization, calcium solubilization, siderophore production, and the production of indole-3-acetic acid (IAA) (Bashan & Holguin, 1997; Richardson & Simpson, 2011).

In our study, the waste from A. caliginosa yielded four isolates identified based on other morphological characteristics. These isolates were found to possess significant PGPR traits. (Bashan & Holguin, 1997), specifically Bacillus spp. are well-documented for their phosphate-solubilizing capabilities and production of growth-promoting hormones (Richardson & Simpson, 2011).

Eisenia fetida, commonly known as the red wiggler, is typically used in vermiculture and vermicomposting due to its efficiency in breaking down organic waste (Edwards & Fletcher, 1988). E. fetida's digestion process significantly increases the microbial population in its casts, including beneficial bacteria such as PGPR (Atiyeh et al., 2000). The vermicompost produced by E. fetida is known to be rich in humic substances and microbial communities that enhance soil fertility and plant health (Suthar, 2010). Our study identified four isolates from the waste produced by E. fetida. These isolates displayed diverse PGPR activities, including phosphate solubilization, potassium solubilization, calcium solubilization, siderophore production, and auxin production. Notably, isolates similar to Pseudomonas and Rhizobium spp. were identified. Pseudomonas spp. are renowned for their phosphate-solubilizing abilities and production of siderophores, which enhance iron availability (Loper & Henkels, 1997). Rhizobium spp. are

symbiotic nitrogen fixers that form beneficial associations with leguminous plants (Lindström & Mousavi, 2019).

A study by Atiyeh et al. (2000) highlighted that vermicompost produced by E. fetida is particularly effective in promoting plant growth due to the presence of growth regulators and increased microbial activity. Edwards and Fletcher (1988) also noted that the microbial composition of E. fetida vermicompost includes a high concentration of phosphate-solubilizing bacteria, which are crucial for plant nutrient uptake. Moreover, Suthar (2010) reported that vermicomposting leads to a significant increase in beneficial microbial populations, including PGPR, which enhance soil nutrient availability and plant growth.

While both *A. caliginosa* and *E. fetida* contribute to the enrichment of the rhizosphere with PGPR, their modes of action and impacts on the soil microbial community differ (Blouin et al., 2013). A. caliginosa primarily enhances soil structure and aeration, creating a conducive environment for microbial proliferation (Domínguez et al., 2019). In contrast, *E. fetida* excels in organic matter decomposition, directly increasing the microbial population through its nutrient-rich casts (Edwards & Fletcher, 1988).

Research comparing the two species, such as the work by Brown et al. (2000), found that while *A. caliginosa* significantly enhances soil physical properties, E. fetida has a more pronounced effect on increasing microbial biomass and nutrient cycling due to its intensive decomposition activity. Furthermore, a study by Lavelle et al. (1997) indicated that earthworm activity leads to a greater diversity of PGPR in the rhizosphere, contributing to improved plant health and yield.

Our findings corroborate these studies, showing that the isolates from *E. fetida* exhibited more diverse PGPR activities compared to those from *A. caliginosa* (Blouin et al., 2013). This suggests that E. fetida may be more effective in introducing a wider range of beneficial bacteria into the soil, thereby enhancing overall soil fertility and plant growth (Domínguez et al., 2019).

To further substantiate the role of these earthworms in promoting PGPR, we conducted a series of solubilization tests for phosphate, potassium, and calcium, along with tests for siderophore production and auxin (IAA) production. These tests revealed the following:

Both *A. caliginosa* and *E. fetida* isolates showed significant phosphate solubilization, with E. fetida isolates demonstrating slightly higher solubilization indices. This aligns with previous studies highlighting the role of earthworm casts in enhancing phosphate availability (Nath & Singh, 2012; Sinha et al., 2010). Phosphate solubilization by PGPR is crucial as phosphorus is a vital nutrient for plant growth, often limited

in availability in soils (Rodríguez & Fraga, 1999). PGPR convert insoluble forms of phosphorus to forms accessible to plants, enhancing nutrient uptake (Vessey, 2003).

The isolates from both earthworm species showed clear zones of potassium solubilization, indicating their ability to mobilize potassium from insoluble sources. This is supported by findings from Sheng and He (2006), who reported on the role of potassium-solubilizing bacteria in soil fertility. Potassium is essential for various plant physiological processes, including enzyme activation, photosynthesis, and osmoregulation (Wang et al., 2013). Solubilizing bacteria release organic acids that can convert insoluble potassium into forms that plants can absorb (Meena et al., 2014).

The calcium solubilization tests revealed significant activity among the isolates, particularly those from *E. fetida*.
This can be attributed to the higher organic matter decomposition rates in *E. fetida* casts, which promote calcium availability (Pathma & Sakthivel, 2012). Calcium is a critical secondary nutrient for plants, influencing cell wall structure, signal transduction, and stress responses (Hepler, 2005). PGPR enhance calcium availability by producing organic acids that solubilize calcium from mineral sources (Gyaneshwar et al., 1999).

Siderophore production was evident in the isolates, with *E. fetida* isolates producing more siderophores. This is crucial for iron acquisition in plants, enhancing their growth and health (Kloepper et al., 1980). Iron is a vital micronutrient involved in chlorophyll synthesis and various enzymatic functions. Siderophores chelate iron from the soil, making it available to plants and suppressing pathogenic microbes by outcompeting them for iron (Ahmed & Holmström, 2014).

The isolates showed the ability to produce IAA, a key phytohormone that promotes root growth and plant development. This trait is particularly beneficial in enhancing root architecture and nutrient uptake (Patten & Glick, 1996). Auxins, such as IAA, regulate various aspects of plant growth, including cell elongation, division, and differentiation. PGPR that produce IAA can stimulate root development, increasing the root surface area for nutrient absorption (Spaepen et al., 2007).

These experimental results confirm the presence and activity of PGPR in the waste produced by A. caliginosa and E. fetida, highlighting their potential in sustainable agriculture.

CONCLUSION

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Conclusion

This study successfully isolated and identified Plant Growth-Promoting Rhizobacteria (PGPR) strains from earthworm castings, demonstrating their capability to solubilize phosphate, calcium, and potassium, as well as produce siderophores and auxin. These results highlight the potential of earthworm castings as a rich source of beneficial microbes that can significantly enhance plant growth, nutrient uptake, and resilience to environmental stresses. The use of PGPR presents a sustainable alternative to chemical fertilizers and pesticides, promoting healthier crops and reducing the environmental impact of traditional farming practices.

By leveraging the natural interactions between earthworms, beneficial bacteria, and plants, this study supports the development of eco-friendly agricultural systems that are both productive and sustainable. The findings provide a strong foundation for integrating PGPR into various agricultural practices, offering a viable solution for enhancing crop yields and soil health while minimizing ecological harm.

Moving forward, future research should focus on the wider application of these PGPR strains across different crops and soil types to validate their effectiveness in diverse agricultural settings. Developing efficient formulations and delivery methods for these bacteria will be crucial for practical field implementation. Long-term field studies are needed to assess the sustainability and consistent benefits of using PGPR from earthworm castings in real-world agricultural practices.

Moreover, leveraging advanced genomic and biotechnological tools can further optimize the selection and enhancement of PGPR strains with specific traits tailored to different agricultural needs. Combining PGPR with other sustainable agricultural practices, such as organic farming and integrated pest management, can maximize their benefits and contribute to a holistic approach to sustainable agriculture.

In conclusion, by continuing to explore and innovate in the field of PGPR and sustainable agriculture, we can develop resilient agricultural systems that meet growing food demands while preserving environmental health. This research represents a significant step towards achieving sustainable and productive farming practices.

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27. Ensure these citations are formatted according to the specific style guide required for your work (e.g., APA, MLA, Chicago, etc.).

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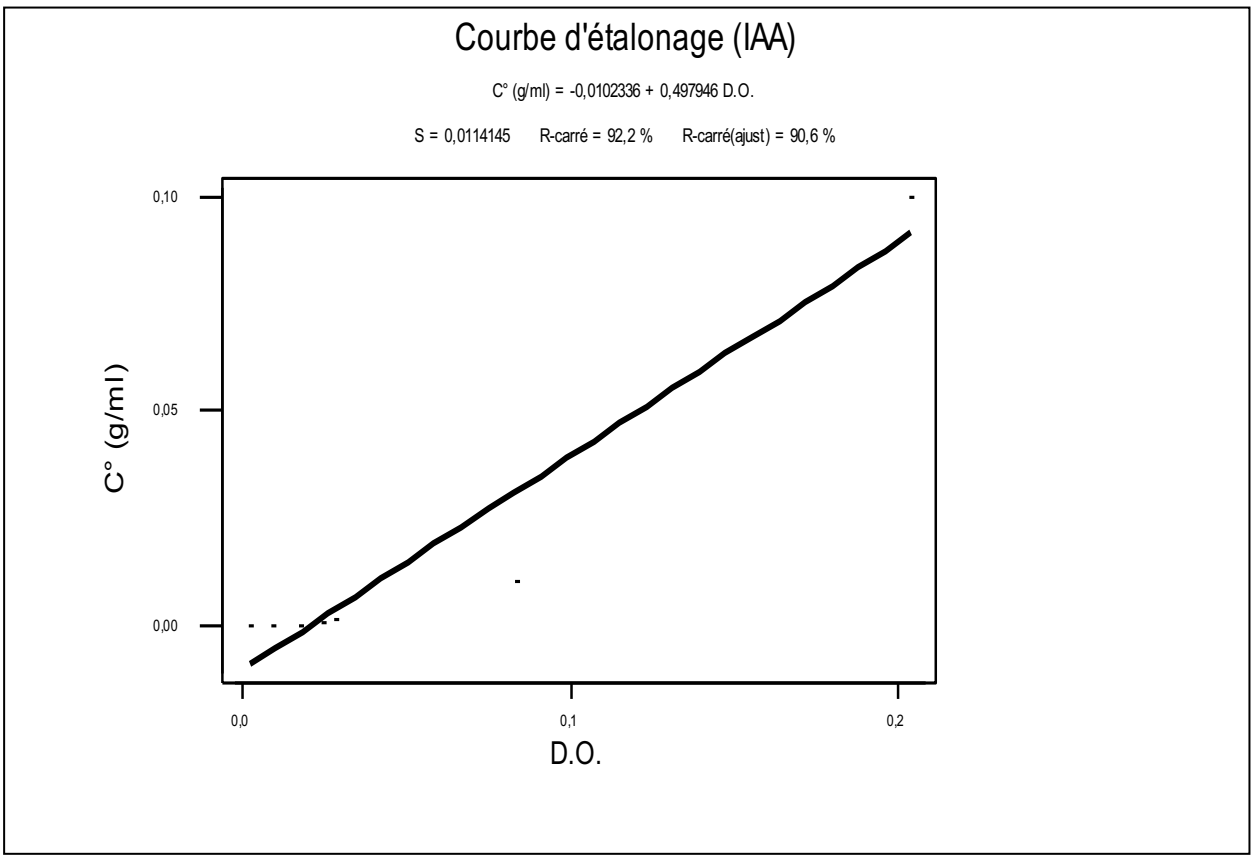
ANNEXS



IAA Calibration Curve