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Title

Isolation and screening of IAA producing bacteria and their potential in promoting plant growth

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Dedication

With honor, I dedicate this humble work carried out with the help of GOD to:

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My dear brothers *BOUBKEUR, SAMIR, YAHIA for their support and encouragement.*

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To all those who know me, whether near or far, and whose names I have not been able to mention.

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Dedication

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To all those who know me, whether near or far, and whose names I have not been able to mention.

Melissa

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Abbreviations

ABA	Abscisic Acid
ANOVA	Analysis of Variance
CK	Cytokinins
DMSO	Dimethylsulfoxide
DO	Optical density
Fe	Iron
H_2O_2	Hydrogen peroxide
$H_2PO_4^-$	Ortho phosphorique acid
IAA	Indole-3-Acetic Acid
ISR	Induce systemic resistance
JA	Jasmonic Acid
LB	Luria-Bertani
$NO3^-$	Nitrate
P	Phosphor
PAL	Phenylalanine ammonia lyse
PBS	Phosphate Buffer solution
PCA	Plat Count Agar
PGR	Plant Growth Regulator
PGPR	Plant Growth Promoting Rhizobacteria
pH	Potential hydrogen
PSMs	Phosphate solubilizing microorganisms
Rpm	Rotation per minute
Cu	Copper
Zn	Zinc
µg	Micro gramme

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Introduction

Introduction

The challenge of agricultural production is to continually increase agricultural output, improve its quality, and enhance processing and storage capabilities. Chemical fertilizers are widely employed to enhance soil condition, increase fertility, and boost crop productivity (Uzakbaevna, 2022; de Andrade et *al.*, 2023). Despite their apparent advantages, this practice raises serious environmental concerns and has negative impacts on human health due to the accumulation of toxic metals and biocide residues in soils and food products (Kouassi, 2001). The extensive use of synthetic chemical fertilizers has therefore emerged as a significant and pressing issue in agricultural cultivation (Soni et *al.*, 2022; Barun and Colla, 2023).

In recent years, scientists have found more advantageous ways to reduce the use of chemical fertilizers (Davies, 1995; Badr et *al.*, 2022**)**, the use biofertilizers, which contain living micro-organisms (PGPR), helps to preserve the soil´s physical, chemical and biological structure over the long term, and to provide plants with sufficient nutrients (Misra el *al*., 2020).

PGPR are soil microorganisms that colonize the rhizosphere and play a crucial role in improving plant health, growth and development through a variety of mechanisms (Suliasih and Widawati, 2020; Rehman et *al.*, 2020; Mustami et *al.*, 2024). PGPR also affect plant growth through the synthesis of phytohormones such as indole 3-acetic acid and other plant growth regulators such as jasmonic acid, ethylene, abscisic acid (Wang et *al*., 2024; Bhat et *al*., 2024).

IAA (Acid Indole Acetic) is one of the most important phytohormones for plant growth, (Lebrazi et *al*., 2020), naturally present in plants, it influences various physiological processes and controls plant expansion and development (Roopa et *al*., 2023). Plays an important role in a number of plant activities such as leaf formation, embryo development, root initiation and development, abscission, fruit development, etc (Chandra et *al*., 2018).

In this study, our focus is on investigating the production of indole-3-acetic acid (IAA) by rhizospheric bacterial isolates obtained from Fava bean, potato, Onion and Turnip rhizosphere. Additionally, we aim to assess the potential of these isolates to enhance wheat growth.

The manuscript is divided into three parts:

- The first part is devoted to a literature review, with two brief chapters: the first gives an overview of PGPRs and their direct and indirect mechanisms, while the second discusses the various phytohormones and their roles.

- The second part describes the methodology used in this study, in particular the methods employed to carry out various tests to optimize IAA production, as well as the realization of an *in Vivo* test

- The third part is reserved to results and discussion.

Finally, a general conclusion summarizes the main findings of this study.

Bibliographic synthesis

1. Rhizosphere

The term “Rhizosphere” is derived from a Greek word rhizo or rhiza, which means "root", and “sphere” which denotes an area or field of influence (Sebihi, 2016). Hilnter (1904) described the rhizosphere zone as the area of soil that is directly affected by the living roots. This zone spans approximately 1 mm in width but lacks a distinct edge (Hilnter, 1904; Chaitanya and Meenu, 2015).

The rhizosphere of a plant hosts a vast and active microbial population (Rhizobacteria) that can have positive, neutral, or negative impacts on plants through direct or indirect processes (Kumar et al., 2012).

2. Rhizospheric microbium or rhizobacteria

The diverse bacteria inhabiting the rhizosphere can be classified based on their impact on plants and interactions with roots. These rhizobacteria play crucial roles in supporting plant health and ecosystem functions, they enhance plant nutrition and soil fertility, control plant disease, and contribute to nutrient cycling (Saharam and Nehra, 2011; Nabti and Slimane, 2020).

3. Plant growth promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) are a potential microbe of the rhizosphere that enhances plant growth, and improve soil health status. By using root exudates, these bacteria thrive in the closely adhering region around plant roots, where they can colonize the surface or interior of roots (Bent et *al*., 2001; Bensidhom and Nabti, 2020; Su et *al*., 2024).

PGPR can enhance plant development either directly or indirectly (Utami et *al*., 2024) through various mechanisms. These involve the production of various phytohormones that enhances plant nutrition, and tolerance to biotic and abiotic stress (Alhaithloul et *al*., 2020), as well as solubilization of phosphorus, production of siderophore, antimicrobial compounds and hydrolytic enzymes, and induction of plant resistance system (Kaymak, 2011; Igiehon et *al*., 2024).

4. The mechanisms involved in plant growth stimulation by PGPR

PGPR are classified into different categories based on their functions: biofertilizers, which enhance the availability of plant nutrients, phytostimulation, which promote plant growth typically through the production of plant hormones, and biocontrol agents, which mainly control plant diseases by producing antibiotics and antifungal metabolites (Somers et *al.*, 2004).

4.1. Biofertilization

Biofertilizers are biological preparations of efficient microorganisms that enhance nutrient availability and uptake by plants.

4.1.1. Nitrogen fixation

Nitrogen is the first essential macronutrient and one of the most crucial nutrients for plant growth (Bensidhom and Nabti, 2020; Hyder et al., 2023). It is found in the air in significant concentrations, but in a gaseous form that plants cannot directly assimilate. One of the methods to convert nitrogen into a form available to plants, such as nitrate (NO^{3-}), ammonium, amino acids, and ammonia, is through the biological process of nitrogen fixation by soil microorganisms (Chakraborty and Akhtar, 2021; Bhat et *al*., 2023).

4.1.2. Phosphorous solubilization

Phosphorus (P), the second important plant growth-limiting nutrient after nitrogen, is widely present in soils in organic and inorganic forms (Khan et *al*., 2009). Despite being largely distributed in the soil, the amount of readily accessible forms for plants is typically limited (Ahmed and Kibret, 2014). The limited accessibility of phosphorus to plants is due to the fact that most soil phosphorus exists in insoluble forms, whereas plants can only absorb it in two soluble forms: the monobasic ($H_2PO_4^-$) and the dibasic (HPO_4^{2-}) ions (Bhattacharyya and Jha, 2012). Several phosphate solubilizing microorganisms (PSMs) are now recorded to convert the insoluble form of phosphorus to soluble form (Sharma et *al*., 2011; Bhattacharyya and Jha, 2012; Chaitanya and Meenu, 2015).

The ability to solubilize phosphates has been studied in several mycorrhizal bacteria, including *Bacillus*, *Rhizobium*, *Penicillium*, *Aspergillus*, and *Staphylococcus* (Timofeeva et *al*., 2022; Wang et *al*., 2024).

4.1.3. Hydrolytic Enzymes

Soil enzymes are produced by plants, animals, and microorganisms which considered to be the main source (Chernysheva et *al.,* 2021; Kompała et *al.*, 2021). Soil enzymatic activities are considered "sensors" of soil degradation, as they reflect the physicochemical conditions of the soil and serve as indicators of its microbial status (Baum et *al.,* 2003). Enzymes catalyze all biochemical reaction and are an integral part of nutrient cycling in the soil. Their activity after fertilization can be used as early sensitive indicators of soil nutrient changes after fertilization application (Bandick and Dick, 1999; Yagüe et *al.*, 2023).

The interest in microorganisms producing hydrolytic enzymes, is based on their application as biofertilizers and biocontrol agents. Enzymes such as proteases, lipases, amylases and cellulases are of remarkable agricultural interest due to their involvement in soil fertilization through the degradation of organic polymers (Bensidhoum, 2016). Hydrolytic enzymes make nutrients available to plants and soil microorganisms (Dilly et *al.*, 2007; Joanisse et *al.*, 2008**).**

4.2. Phytostimulation

The production of phytohormones by bacteria is pivotal in the interaction between plants and microorganisms (Yaghoubi Khanghahi et *al*., 2024). Phytostimulation often occurs due to the production of plant growth regulators by microbe, which can enhance plant development by influencing elongation, differentiation, and cell division (Beattie, 2007; Benaissa, 2019). Five classes of phytohormones are established: auxins, gibberellins, cytokinins, ethylene, and abscisic acid (Zahir et *al*., 2004).

Several PGPRs are reported to produce IAA and other plant growth regulators in rhizospheric soil, thereby play a significant role in promoting plant growth.

4.3. Biocontrol

Microorganisms, principally rhizobacteria, can effectively colonize root systems and beneficially influence plant growth and health by controlling plant pathogens. Most bacterial strains used as biocontrol agent belong to the genera *Bacillus* and *Pseudomonas*. PGPR can control phytopathogenic agent by several mechanisms including: production of antimicrobial substances; the secretion of hydrolytic enzymes, induction of plant resistance and the competition for nutrients and space.

4.3.1. Antibiosis

The main mechanism of biocontrol by PGPR involves producing antimicrobial compounds (Bashan and Bashan, 2005; Kenawy et *al*., 2019; Hassan et *al*., 2023). These compounds are secondary metabolites with low molecular weights that are toxic and effective against pathogenic organisms. The antimicrobial substances produced by the antagonistic organism are of diverse nature, including: Antimicrobial peptides or proteins; Polyketides; Phenolic compounds; Biosurfactants; etc. (Fernando et al., 2005). *Bacillus* and *Pseudomonas* actively suppress plant pathogens by secreting inhibitory extracellular metabolites at very low concentrations (kumar et *al*., 2024).

4.3.2. Siderophore production

Siderophore is a protein molecule capable of solubilizing and sequestering iron from the soil, making it available to plant cells. It has a high affinity for Fe3+. PGPRs utilize siderophores as mechanisms for biofertilization and in the biocontrol of phytopathogens. By producing siderophores, PGPRs prevent phytopathogens from acquiring sufficient iron, thereby limiting their ability to multiply (Glick, 2012; Olanrewaju et *al*., 2017; Bensidhoum and Nabti, 2020). Several studies have confirmed that the siderophores produced by the PGPR influence significantly plant uptake of various metals, including Fe, Zn, and Cu (Egamberdieva, 2007).

4.3.3. Induced systemic resistance (ISR)

The interactions of PGPR with plants involved in the fight against pathogenic agents consist of stimulating plant defense mechanisms. This phenomenon has been named 'induced systemic resistance' or ISR (Induced Systemic Resistance) (Van Loon et *al*., 1998). Systemic resistance can be induced by various microorganisms, including Gram-positive bacteria like *Bacillus pumilus*, as well as Gram-negative bacteria belonging to the genus *Pseudomonas* (*P. fluorescens*, *P. putida*, *P. aeruginosa*) (Jourdan et *al*., 2008).

Effective colonization of roots by PGPR is a crucial condition for optimal expression of biocontrol activity through ISR (Bloemberg and Lugtenberg, 2001), where the bacterial population must reach a sufficient threshold level on roots to trigger the phenomenon. For instance, in *Pseudomonas*, there must be at least 10^5 cells per gram of roots (Raaijmakers et *al*., 1995).

Rhizobacteria such as *Pseudomonas*, *Bacillus*, *Rhizobium*, and *Azospirillum* offer protection against plant pathogens. These microorganisms can induce systemic resistance in plants by recognizing the microbial compounds that pathogens produce (Bloemberg and Lugtenberg, 2001; Ghiasian, 2020; Vincze, 2024).

4.3.4. Lytic enzymes production

PGPR produce lytic extracellular enzymes such as proteases, chitinases, hydrolases and glucanases (Vincze, 2024). These enzymes protect plants against fungal and other soil pathogens by the hydrolysis of polymeric components such as protein, cellulose, chitin, and hemicellulose. Some of these target compounds are in the plant cell wall which are prone to attacks (Kumar et *al*, 2024). It has been observed that the β-1,3-glucanase produced by Paenibacillus and Streptomyces spp. strains is capable of readily breaking down the fungal cell walls of pathogenic F. oxysporum (Dweipayan et *al*., 2016).

The enzyme chitinase hydrolyzes ß (1,4) N-acetylglucosamine's insoluble linear polymers (Bensidhom and Nabti, 2020). Since these components represent the majority of the fungal cell wall, microorganisms that generate this chitinase impede the growth of fungal growth (Hasan et *al*., 2023). It has been demonstrated that chitinase-producing bacteria associated with plants and soil belonging, specifically those from the genera Bacillus, Pseudomonas, and Streptomyces, are beneficial against phytopathogenic fungi (Matilla and Krell, 2018).

5. Nutrients and niche competition

Competition for nutrient acquisition and rhizosphere niche occupation is thought to be an indirect biocontrol mechanism by which PGPRs interact and protect plants from plant pathogens (Lugtenberg and Kamilova, 2009; Pathak *al*., 2017). Furthermore, the physical occupation of the site by PGPRs is enhanced by delaying tactics, preventing pathogen colonization until the available substrate is exhausted (Odoh, 2017). in Addition, root colonization by Pseudomonas has been shown to protect tomato plants against stem and root rot (Pathak et *al*., 2017; Panpatte et *al*., 2019).

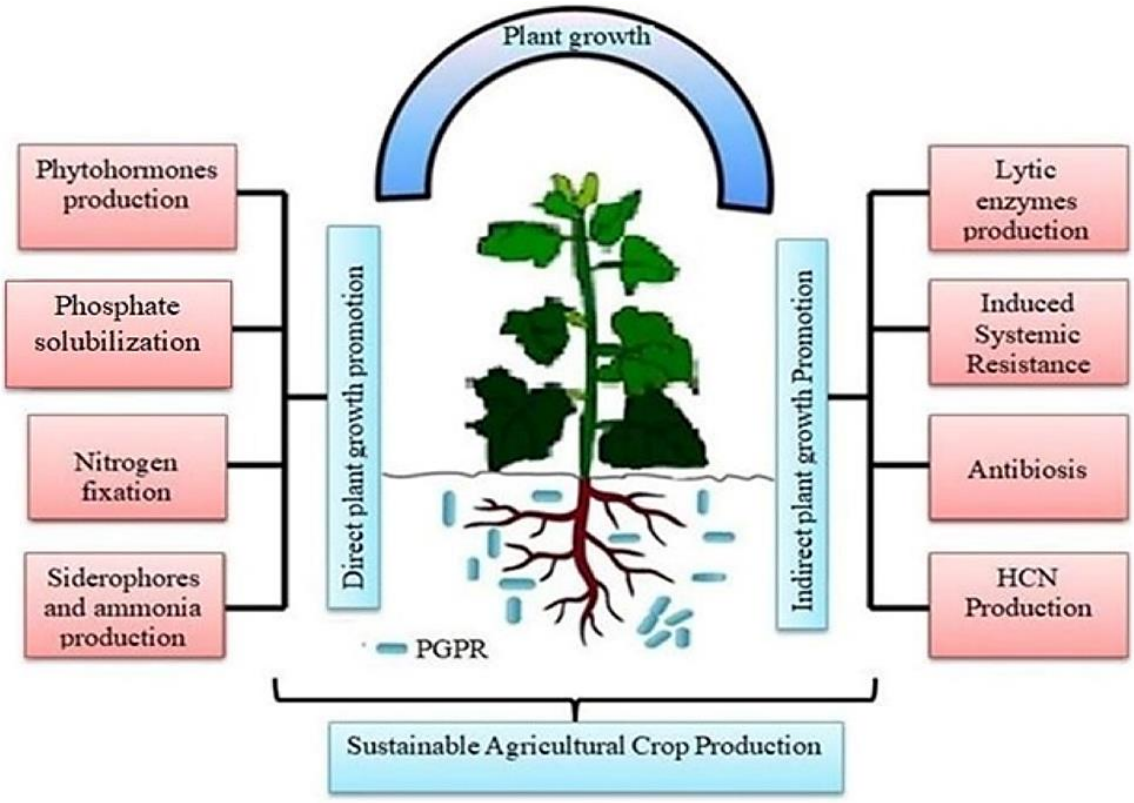


Figure 1: Mechanisms of plant growth promoting (Mekonnen and Kibret. 2021)

1. Introduction

1.1. Plant hormones

Plant hormones or phytohormones are a group of organic substances produced by higher plants and many of soil micro-organisms (PGPR). The production of phytohormones is one of the mechanisms by which rhizospheric bacteria can promote plant growth and development (Kukreja et *al*., 2004Amara et *al*., 2015; Pons, 2020). These hormones, derived from essential metabolic pathways, play crucial roles in regulating internal development processes and exert significant physiological influence at minimal concentrations (Santner et *al*., 2009; Davies, 2010; Nadeem et *al*., 2016).

Phytohormones play an active role in every phase of the plant's life cycle, they are mainly classified into three groups according to their physiological effects on plants: hormones controlling vegetative development (auxin, cytokinin, gibberellin); hormones controlling reproduction (ethylene, abscisic acid); and hormones responding to stress (salicylic acid, jasmonic acid) (Williams, 2011).

It is well established that there are two sources of plant hormones naturally available for the plants: endogenous production by plant tissues and exogenous by associated microorganisms (Baca and Elmerich, 2007), *Bacillus, Rhizobium, Sinorhizobium, Azospirillum, Bradyrhizobium, Pseudomonas* and *Paenibacillus* (Utami et *al*., 2024).

1.2. History of plant hormone

The first scientific investigations on phytohormones date back to the beginning of the 18th century (Sezgin and Kahya, 2018). Charles Darwin discovered in 1880 that a specific chemical transferred from coleoptile tips regulates plant phototropism (Baca and Elmerich, 2007; Williams, 2011). This discovery was published in a book entitled "The power of movement in plant" was published by Charles in 1898 (Baca and Elmerich, 2007; Sezgin and Kahya, 2018).

Ernist Starling used the term "hormone" for the first time in 1905 in reference to animals, and it has since been applied to plants as well (Bakshi et *al*., 2015). Fitting was the first person to use the term "hormone" in a botanical context in 1909, 1910. In 1919, Paàl adopted the name hormone in his research on phototropism (Weyers and Paterson, 2001).

Several years later, by 1926, the Dutch botanist Frits W. Went discovered auxin and described a bioassay for its quantitative detection. The first generally accepted report of the occurrence of IAA in a higher plant was published by Haagen-Smit et al. in 1946 (Moore, 1979). Since then, there have been an increasing number of reports on the discovery of new phytohormones or related compounds in plants.

2. The classes of plant hormones

2.1. Auxin

The term auxin is derived from the Greek word “auxein” meaning “to grow”. Indole-3-acetic acid (IAA), the predominant natural form of auxin, was the initial plant hormone isolated and was previously thought to be derived from tryptophan (Went and Thiman, 1937; Turnbull and loveys, 1999). Most of the total auxin in plants found in the conjugated form, which is one of the important regulatory mechanisms for the activation or inactivation of IAA (Bari and Jone, 2009). Auxin is synthetized by plant in the apical meristematic region of plants, including the buds and tips of shoots and roots. The production of this hormone in green leaves is influenced by light conditions (Went and Thiman, 1937; Mukherjee et *al*., 2022). Furthermore, IAA is synthesized from tryptophan or indole in young leaves and once synthesized, it is distributed throughout the plant via a cell-to-cell transport system (Santner et *al*., 2009).

2.2. Abscisic acid (ABA)

The term "Abscisin" was initially used to describe the substance that controlled the abscission of cotton bolls; it was also designated as "dormin" due to its role in bud dormancy (Davies, 2010). Later several studies have revealed that the Abscisin or Abscisic Acid (ABA) is involved in a number of plant growth and development processes, including stress responses, seed germination, embryo maturation, leaf senescence, seed and bud dormancy (Wasilewska et *al*., 2008; Santner et *al*., 2009; Mukherjee et *al.*, 2022). ABA is synthesized in different parts of plant, but is most concentrated in all cells with chloroplasts or amyloplastes and found in major organs and tissues (Taiz et *al*., 2015).

2.3. Gibberellins

Gibberellins are a plant hormone that form a large group of carboxylic acid. There are at least 125 forms of gibberellin, they are found in buds, roots, young leaves, flowers, fruits and cambium of plants (Sezgin and Kahya, 2018). These hormones have translocated from roots to the aerial parts of plants, their synthesis takes place mainly in developing leaves and stems, in developing seeds and during germination. (Went and Thiman, 1937; Turnbull and loveys, 1999; Goswami et *al*., 2016). Moreover, several PGPRs including Bacillus and Acinetobacter been documented as capable to produce multiple types of GA with substantial quantities (Singh et *al*., 2023).

2.4. Cytokinins

Cytokinins (CKs) are an essential plant hormone, mostly produced by inter-root bacteria (Raza et *al*., 2023; Wang et *al*., 2024). CKs are involved in a number of plant growth and development processes including stem-cell control, vascular differentiation, chloroplast biogenesis, seed development, growth of shoots and roots, leaf senescence, nutrient balance and stress tolerance (Muller and Sheen, 2007). Moreover, CKs are synthetized in roots, where they regulate senescence processes and development, and via the xylem, they move to shoots to regulate its development (Javid et *al*., 2011).

2.5. Ethylene

Ethylene is a gaseous plant hormone, it is a powerful regulator of plant growth and development despite having a simple two-carbon structure (Wang et *al*., 2002; Lin et *al*., 2009). Ethylene is produced in all cells at different rates during plant development, it is bioactive in minute amounts and in addition to its countless activities on plant it has significant commercial implications (Lin et *al*., 2009).

Moreover, Ethylene plays multiple roles in plant development and environmental responses it affects principally the root architecture by regulating bending lateral root initiation (Vandenbussche et *al*., 2012; Anfang and Shani, 2021; Azhar et *al*., 2023).

3. The role of phytohormones

3.1. The role of Indole-3-acetic acid

Indole-3-acetic acid (IAA) is one of the most abundant endogenous auxins in plants (Checker et *al*., 2018). It plays an important role in regulating plant growth by controlling cell elongation, tissue development and developing a response to biotic and abiotic stresses (Javid et *al*., 2011; Checker et *al*., 2018). The IAA released by PGPRs as a secondary metabolite mainly affects the root system by increasing roots size and weight, branching numbers and surface area in contact with the soil. It allows plants to maximize their nutrient acquisition abilities and contributes to their growth (Dweipayan et *al*., 2016; Goswami et *al*., 2016; Mukherjee et *al*., 2022).

Furthermore, IAA can act as a reciprocal signaling molecule in plant-bacteria interaction (Lebrazi et al., 2020). However, the effect of auxin on plants depends on its concentration; low amounts of IAA allow primary roots to proliferate and at optimal concentrations it increase root surface area and length, thus contributing to enhance nutrient absorption. However, high levels of IAA have a negative effect on plant growth and have been shown to inhibit root elongation (Raj et *al*., 2020; Mukherjee et *al*., 2022).

3.2. The role of Abscisic acid

Abscisic acid (ABA) plays a significant role in regulation of many plant physiological processes. The concentration of ABA in plants fluctuates depending on environmental conditions, leading to variable effects on physiological processes (Sezgin and Kahya, 2018). ABA promote the maturation of somatic embryos and the synthesis of storage reserves, it acts as a controlling factor of germination and dormancy in somatic embryos (Rai et *al*., 2011). It plays role in the initiation of adaptive responses to various environmental conditions, like adaptation to drought, to low temperature and to salinity, as well as the regulation of water status and stomatal functioning in various environmental stress (Much-Mani and Much, 2005; Rahman et *al*., 2023).

Additionally, ABA is essential for photoperiodic flowering induction, which promotes plant growth and development, and increases the plant's sensitivity or resistance to various diseases. Another function of ABA under biotic stresses conditions is the activation of stomatal closure; that acts as a barrier against bacterial infection and contribute to the plant defense (Gomez-Cadenas et *al*., 2015; Vincze et *al*., 2024).

3.3. The role of Ethylene

Ethylene is known as a maturing hormone and even at very low concentration, it has a physiological effect on the plant. Its production enables the regulation of a wide range of crucial plant processes, such as abscission, fruit ripening, leaf and fruit shedding, and enhances stress tolerance as well (Gutierrez et *al*., 2009; Sezgin and Kahya, 2018; Bhat et *al*., 2023).

Ethylene plays also an important role in the regulation of the molecular and cellular metabolism of plants which promote cell division and elongation, leaf growth, flower and root development (Khan et *al*., 2024).

3.4. The role of Cytokinin

Cytokinin is an essential hormone that acts as a stimulator, it plays a key role in a wide range of physiological processes in plants, such as cell division, production of chloroplast, seed germination and response to biotic and abiotic stress (Gaspar et *al*., 1996; Gamalero and Glick, 2011; Kieber and Schaller, 2018; Hyder et *al*., 2023). It can also affect the activities of meristem cells, and nodule formation during the establishment of nitrogen-fixing symbiosis and other interactions between plants and microbes (Miransari and Smith, 2014). Cytokinins are thought to delay senescence by preventing protein degradation through the inhibition of nuclease and protease formation in foliage.

Although the auxines promote root formation, cytokinins promote shoot formation. They contribute to organ formation and development in tissue culture media (Sezgin and Kahya, 2018).

3.5. The role of Gibberellin

Gibberellins are a type of plant growth regulator, they influence seed germination and plant development (Castro- Camba et *al*., 2022; Warisman et *al*., 2024). They regulate lateral branches growth, flowering, stem elongation, and leaf expansion (Javid et *al*., 2011; Egamberdieva et *al*., 2017).

When plants are exposed to abiotic stress, gibberellic acid are rapidly accumulated, it provides a mechanism to regulate the metabolic process based on sugar signaling and anti-oxidant enzymes (Fahad et *al*., 2015). It contributes also to the regulation of heavy metals toxicities by activating several defense mechanisms (Rahman et *al*., 2023).

Material and Methods

1. Soil Sampling

Four soil samples were collected in March 2024, from different agricultural fields located in Bejaia: Ait Idris Taskriout (36°33'43.3"N 5°17'17.9"E); Guendouza, Akbou (36°27'11.7"N 4°32'13.4"E); Tichy (36°39'24.5"N 5°09'55.3"E) and Adkar (36°44'49.1"N 4°42'44.9"E). Sampling was conducted in the root zones (rhizospheres) of the following crops, respectively: fava bean, onion, potato, and turnip. The soil collected was placed in sterile vials and transported to the laboratory (figure 2).

Figure 2: Sampling location

2. Isolation and isolates purification

One gram of each soil sample was suspended in 10 ml PBS (Phosphate-buffered Saline) (Annex I) and 1 ml of each soil solution was serially diluted from 10^{-1} to 10^{-7} g/ml in the same broth medium. 1 ml of each dilution was spread on Plat Count Agar (PCA) (Annex II) in duplicate, using the "flooding technique", and then incubated for 24h at 30°C. Finally, successive subcultures of all colonies are performed until getting pure colonies (figure 3).

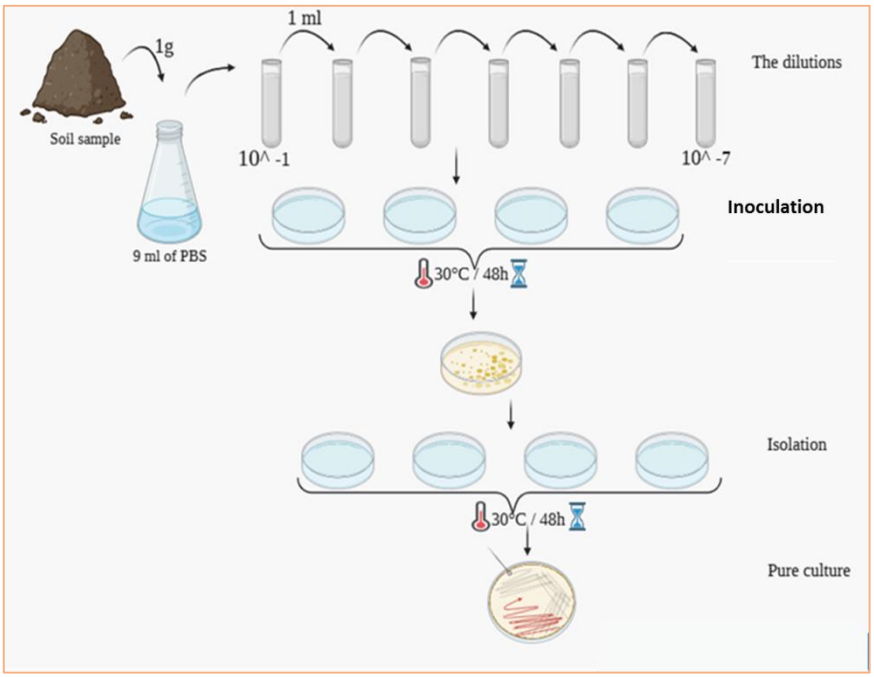


Figure 3: Isolation and purification steps

3. Indole 3-AceticAcid (IAA) detection and quantification

The production of IAA by bacterial isolates was determined according to the method of Bric et al. (1991) with slight modification. 24h grown cultures of bacterial isolates were inoculated into LB medium (Annex III) supplemented with 0.5% glucose and 0.5 mg/ml tryptophan, then incubated on a rotary shaker at 120rpm for 4 days at 30°C.

After incubation, the cultures were centrifuged at 5000 rpm for 15min, then 800µl of supernatant were collected and mixed with an equal volume of Salkowsky´s reagent (Annex IV), the mixture was kept in the dark at room temperature for 20min. Development of pink color indicate the production of IAA. Measurement of the solution absorbance was performed at 530 nm. Then, the concentration of IAA was determined based on a calibration curve (Annex V) prepared with standard IAA (BIOCHEM Chemopharma) (figure 4). All experiments were done in triplicate.

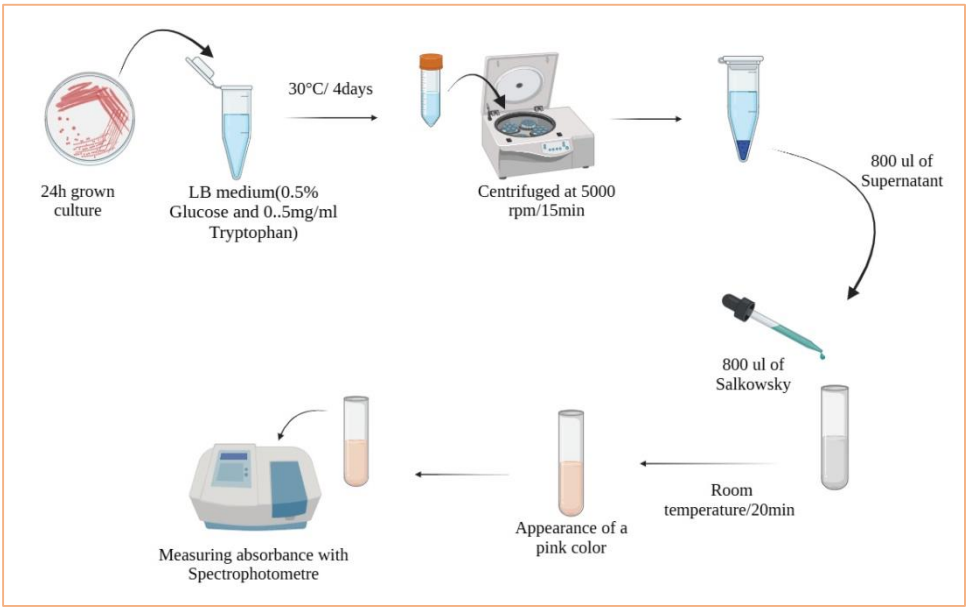


Figure 4: IAA production test

After IAA quantification, four isolates from each sample crop: F4 (Fava bean), P17 (potato), O3(Onion) and N14(Turnip), producing variable amounts of IAA, were selected for further tests

4. Optimization of IAA production

For IAA production, the culture medium was inoculated with 24h grown cultures (OD 0.08-01) of selected isolates. Three different parameters, namely temperature, pH and L-tryptophan concentration, were considered for the study to optimize IAA yield. LB medium was used as the basic medium to test IAA production.

4.1. Effect of incubation temperature

Temperature is known as an important parameter for IAA production since the growth of bacteria is affected by low or high temperatures. Thus, IAA production was quantified at 20, 25, 30, 35, 40 and 45 °C/4 days.

4.2. Effect of pH

One of the crucial physicochemical factors influencing the overall production of IAA is pH. To ascertain the optimal pH for IAA production by the selected isolates, IAA investigation was conducted using LB medium (supplemented with 0.5 mg/ml L-tryptophan

and 0.5% glucose) adjusted to different pH values: 5, 6, 7, 8, and 9. The media were inoculated and incubated at 30°C for 4 days.

4.3. Effect of *L*-tryptophan concentration

L-tryptophan is the precursor of IAA and significantly influences its production by bacteria. Its effect was investigated using LB medium supplemented with 0.5% glucose and varying final concentrations of L-tryptophan: 0, 200, 350, 500, 650, and 800µg/ml. The cultures were then incubated at 30°C for 4 days.

5. Biochemical identification

The selected isolates were identified through a few biochemical tests only, specifically Gram staining, catalase test, and oxidase test. This represents a partial identification rather than a comprehensive one.

5.1. Gram staining

Gram staining was performed for the selected isolates using the following standard method:

The technique begins with heat-fixing a bacterial smear on a glass slide, followed by staining with crystal violet for one minute, then fixed with a solution of iodine (Lugol's solution) for another minute. Decolorization is then achieved by briefly washing with alcohol for 30 seconds, and then rinsed with water. Counterstaining with fuchsin was applied for one minute, followed by a final rinse. The slides were dried and a drop of immersion oil was applied. The slides were then observed under a light microscope with a 100X objective.

5.2. Catalase test

A small amount of bacterial colony from each isolates was mixed with a drop of hydrogen peroxide on a glass slide. Immediate effervescence indicates a positive catalase reaction, confirming the presence of catalase enzyme

5.3. Oxidase test

Add a disc of oxidase to a tube containing the bacterial suspension. A development of purple coloration indicates a positive oxidase reaction.

6. Plant growth stimulation tests

6.1. Surface disinfection of wheat seeds

Wheat seeds were surface-disinfected as described by Götz et al. (2006). First, wheat seeds were treated with ethanol (70%) for 1 min and then with 12% acid hypochlorite for 15 min. Six consecutive rinses with sterile tap water were performed to remove chlorine (figure 5).

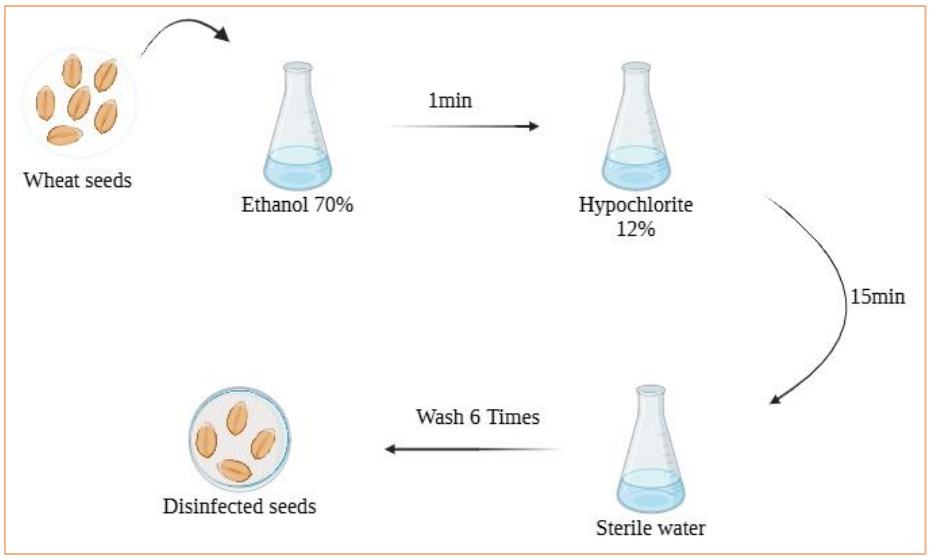


Figure 5: Surface disinfection of wheat seeds

6.2. Preparation of bacterial suspension

The cultures were grown on LB medium (annex II) overnight at 30°C. The bacterial culture was centrifuged at 5000 rpm/7min. The supernatant was discarded, and the cell pellet was washed twice with 20 ml of phosphate buffered saline (PBS, pH 7.2), and suspended in PBS. The optical density of bacterial suspension was 0.1 at 600 nm, corresponding to a cell density of 10^8 cells/ml (figure 6).

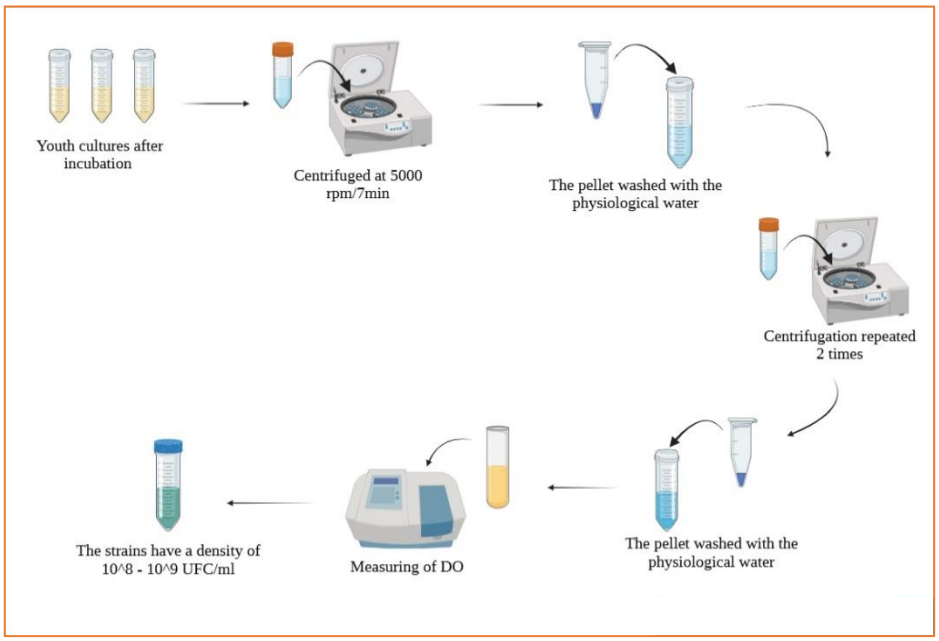


Figure 6: Preparation of bacterial suspension

6.3. Germination test

Surface sterilized wheat seeds were incubated with bacterial suspension for 2h at room temperature. Control seeds were incubated in sterile distilled water under the same conditions. The seeds were then placed in Petri dishes (16 seeds per dish) containing moistened sterile paper and kept in the dark at room temperature. Germinated seeds were counted each daily (figure 7).

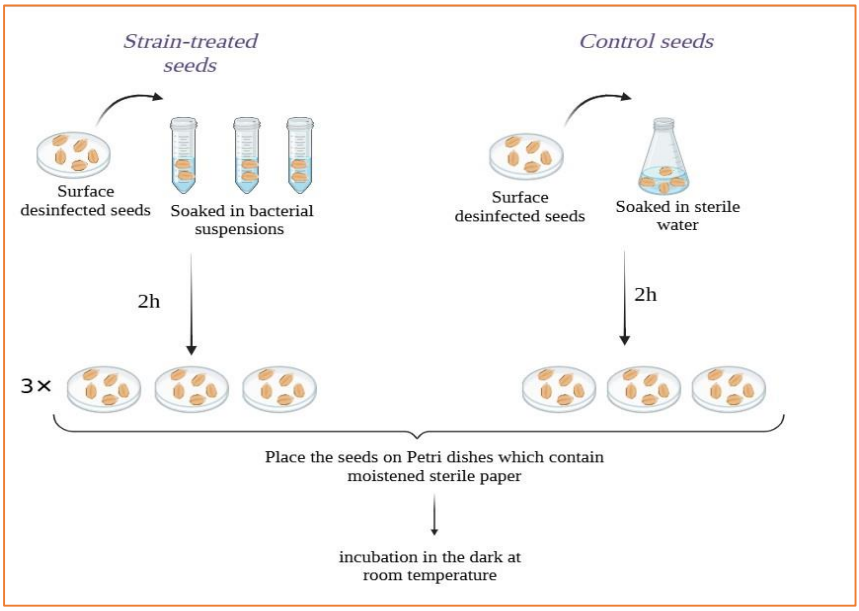


Figure 7: Germination test

6.4. Wheat growth promotion test

This test is conducted using germination trays arranged in seven batches, with each batch consisting of seven pots. Each pot is filled with a mixture of ¾ soil and ¼ peat, and watered accordingly. The wheat seeds used in this test are identical to those used in the aforementioned germination test. They are planted at a rate of one seed per pot, buried to a depth of 1 cm.

1ml of the bacterial suspension of each isolate was added to each treated batch, and 1ml of physiological water was added to the control batch. The experiment was carried out under natural conditions at 25-35°C for 15 days (figure 8). Growth parameters were measured after 15 days, including shoots and roots length, fresh and dry weight of shoots and roots.

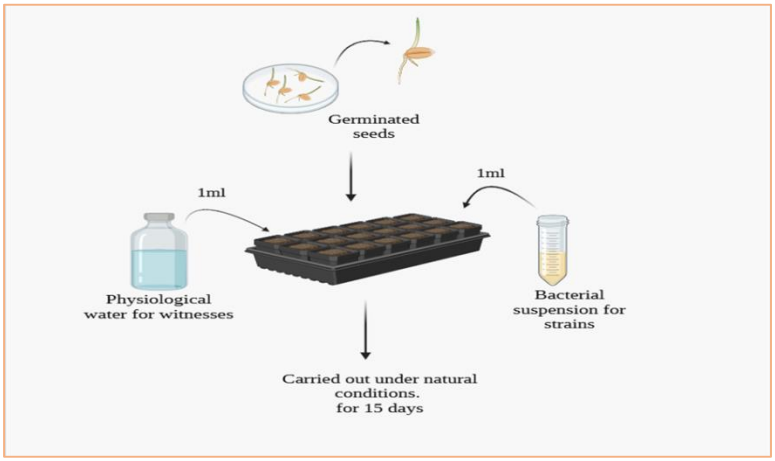


Figure 8: Realization of growth test

6.5. Chlorophyll assay

Photosynthetic pigment content was determined using the Hiscox and Tsraelstam (1979) method. 50 mg of fresh material were cut and placed in a flask containing 7 ml of DMSO (Dimethyl sulfoxide). The mixture was incubated at 65°C/30 min. After incubation, the volume was adjusted to 10 ml with DMSO. Absorbance readings were immediately taken at 645 nm and 663 nm. Chlorophyll a, chlorophyll b, and total were calculated using the equations established by Arnon (1949).

$$\text{Chla } (g\ l^{-1}) = 0,0127 \times A663 - 0,00269 \times A645$$
$$\text{Chlb } (g\ l^{-1}) = 0,0229 \times A645 - 0,00468 \times A663$$
$$\text{Chl total } (g\ l^{-1}) = 0,0202 \times A645 + 0,00802 \times A663$$

7. Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) by the least significant difference (LSD) test at $p \leq 0.05$ with statistical software GraphPad Prism

Results and Discussion

1. Isolation and isolates purification

Macroscopic observation of the morphological appearance of colonies on PCA medium revealed a wide diversity in size, shape, color and surface characteristics. Therefore, 61 different colonies were isolated from each sample crop: 9 (Fava bean), 21 (potato), 13 (Onion) and 18 (Turnip).

Agricultural soils, particularly rhizospheric soils harbor an exceptionally high microbial biomass and species diversity. Indeed, just 1 gram of rhizosphere soil contain between 10^8 and 10^{11} cultivable cells. Due to its significant genetic, ecological, functional, and taxonomic diversity (Saleem et al., 2015; Fierer, 2017), the soil microbiome serves as a crucial reservoir of microbial traits that could benefit plant growth and health (Saleem et al.,2019).

2. Indole 3-AceticAcid (IAA) detection and quantification

IAA production was carried out on LB medium supplemented with 0.5 mg/ml of tryptophan. The appearance of a pink color indicates IAA production by the isolate. The IAA values produced by each bacterium are shown in the graphs below.

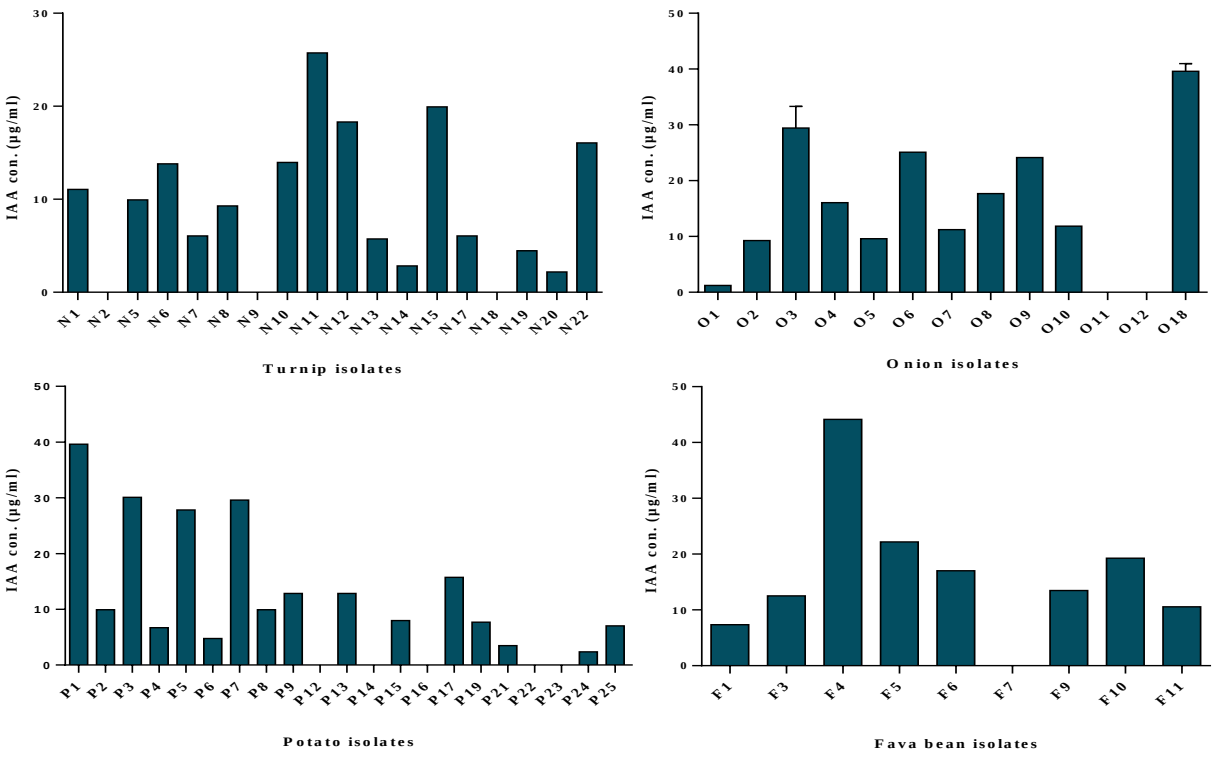


Figure 9: The concentration of IAA produced by all isolates

The obtained data show that the majority of tested isolates have the capacity to produce IAA, and the IAA-production ranged from 1.22 to 44.12 µg/ml. The isolates N11, O3, P1 and F4, represent the most proficient isolates producing IAA from each sampling site.

It is well established that the most important mechanism in the direct plant growth stimulation by rhizobacteria involves the production of growth-regulating substances (Baca and Elmerich, 2007). According to Zakharova et al. (1999), approximately 80% of rhizospheric bacteria are capable of producing IAA. IAA-producing bacteria stimulate seed germination, cell and tissue division and enlargement, leaf expansion, and also play a significant role in root elongation (Maleki et al., 2010; Martínez-Viveros et al., 2010).

The analyze of the obtained results, illustrated in figure above, showed that most of the isolates had IAA production activity in the range of 5 to 45 µg/ml, which is in the range of other rhizospheric bacteria. According to Beneduzi and Passaglia (2011), a low amount of IAA stimulates plant growth, while a high concentration can inhibit root development. Egamberdieva et al. (2010) revealed that two strains, *Pseudomonas trivialis* 3Re27 and *Pseudomonas extremorientalis* TSAU20, produce 12 µg/ml and 10.1 µg/ml of IAA, respectively.

Egamberdieva (2009) observed a 52% improvement in root growth after wheat inoculation with three IAA-producing *Pseudomonas* strains (producing 5, 5.7, and 7.4 µg/ml of IAA). These rates are similar to those produced by the majority of our isolates, affirming their potential use as seed inoculants to enhance plant growth and yield.

Three isolates from each sample crop; P17 (potato); O3(Onion) and F4 (Fava bean), producing 15.74; 29.45 and 44.13 respectively, were selected for further tests.

3. Optimization of IAA production

3.1. Effect of incubation temperature

The effect of different temperatures (20, 25, 30, 35, 40 and 45) on the production of IAA is shown in the graph below (figure 10).

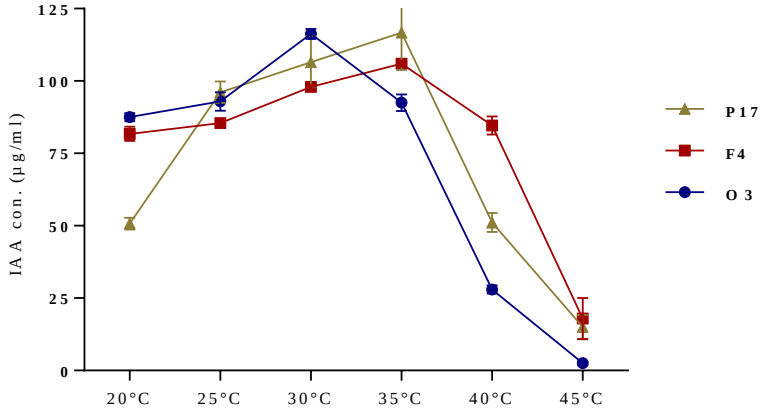


Figure 10: The effect of temperature incubation on the IAA production

Temperature plays a crucial role in IAA production as it impacts bacterial growth; both low and high temperatures can affect the synthesis of IAA, which is closely related to the optimal growth conditions of the microorganisms (Chandra et al., 2018).

After analyzing the IAA values obtained at temperatures ranging from 20°C to 40°C, it was determined that the optimal temperature for IAA biosynthesis was 30°C for isolate O3, and 35°C for isolates P17 and F4. The IAA concentrations are as follows: 116.25 µg/ml; 116.71 µg/ml and 106 µg/ml respectively. Furthermore, there was a reduction in IAA production observed at temperatures exceeding 35°C.

30°C has been reported as the optimal temperature for IAA production by *Streptomyces* sp. (Khamna et al., 2010), and by *Acetobacter diazotrophicus* L1 isolated from sugarcane (Patil et al., 2011), Van Giang et al. (2024) have also reported that 30°C was the optimal temperature.

The results obtained with the isolates P17 and F4, are in accordance with Widawati et al. (2020), who reported that the maximum production of IAA by *Bacillus siamensis* was recorded at 35^{o}C (8.42 µg/ml)**.** Randive et al. (2024) found that their isolates produce the maximum amount of IAA at 30°C, with production decreasing as temperature increases. In other studies, it was shown that 37°C was the best temperature for IAA production in the culture medium (Panigrahi et al., 2020).

3.2. Effect of pH

The effect of different pH (5, 6,7,8 and 9) on the production of IAA is shown in the graph below (figure 11).

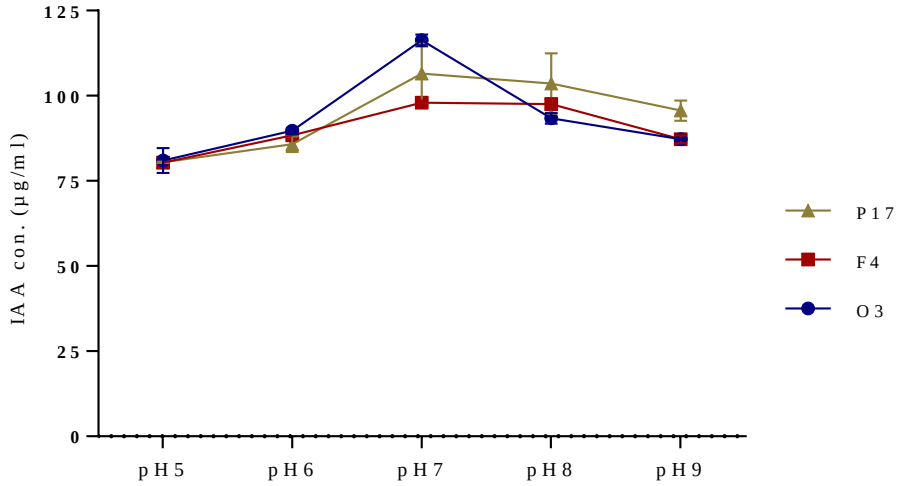


Figure 11: The effect of pH on the IAA production

The pH affects the function of enzyme systems also the solubility of many substances that are important for bacterial growth (Parvin et al. 2020).

In our investigation, we found that the pH 7 and 8 are the optimal pH for IAA production. The IAA concentrations are as follows: 120µg/ml and 105µg/ml for O3 and P17 respectively at pH 7 and 95µg/ml for F4 at pH 7 and 8.

Similar results were obtained by Ait Bessai et al (2022), who found that the optimal pH was ranging between 7 and 9. Harikrishnan et al. (2014) reported that the maximum IAA production by *Streptomyces* sp VSMGT1014, was recorded at 30 °C and pH 8 for the production of 4.76 µg/ml and 26.63 µg/ml respectively. In our research the pH below 6 (acidic) was found to be unfavorable for IAA production by all tested isolates. This results align with those reported by Yousef (2018) who showed that high IAA concentration was observed at pH 8. Moreover, Minakshi et al (2020) reported that their isolate showed maximum IAA production at pH 8 and indicate that neither acidic nor alkaline conditions promotes IAA production. Lebrazi et al (2020) found that the maximum of IAA production by their strain has been detected at pH 9.

3.3. Effect of L- Tryptophan concentration

The effect of different concentration of Tryptophane (0, 200, 350, 500, 650 and 800 µg/ml) on the production of IAA is shown in the graph below (figure 12).

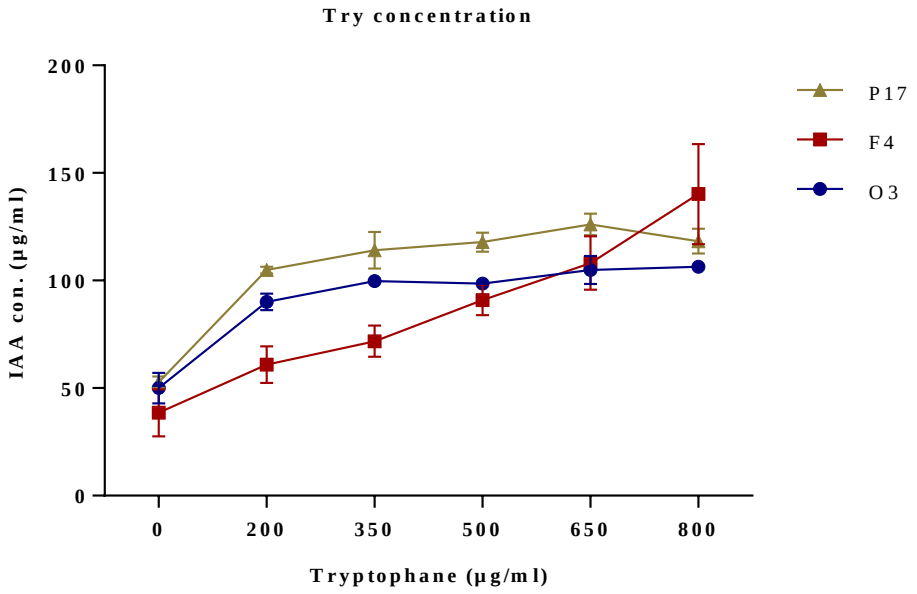


Figure 12: The effect of L- tryptophane concentration on the IAA production

Tryptophan serves as the precursor for IAA, and its inclusion in the growth medium enhances bacterial biosynthesis of IAA (Mohite, 2013; Rahayu et al., 2024). Our research results demonstrated that IAA production increases with higher concentrations of L-Tryptophan, similar to findings reported by Patil et al (2011) and Sandur and Onkarappa (2023). Karnwal (2009) tested Pseudomonas isolates for their ability to produce IAA in the absence and presence of tryptophan, and found that IAA production increased with higher concentrations of tryptophan. We found that the maximal concentration for the production of IAA are as follows: 800µg/ml for F4 (130µg/ml), 650µg/ml for P17 and O3 (120µg/ml and 100µg/ml) respectively. Our results are not consistent with those reported by Parvin et al. (2018), where the maximum IAA production by Bacillus cepacia UPMB3 was recorded at a concentration of 4 $\mu g.ml^{-1}$ of tryptophan, and with other reports where 0.2 $mg.ml^{-1}$ of tryptophan was the optimal concentration for maximum IAA production (Khalid et al., 2004; Bharucha et al., 2013).

4. Biochemical identification

The results of the chemical tests for the selected isolates are shown in the following table and figures.

Table I: Results of Gram staining

Strains	Gram	Shape
O3	-	Bacille
F4	-	Bacille
P17	-	cocci

Table II: Biochemical identification test

Chemical test / Strain	O3	F4	P17
Catalase test	+	+	+
Oxidase test	+	+	+

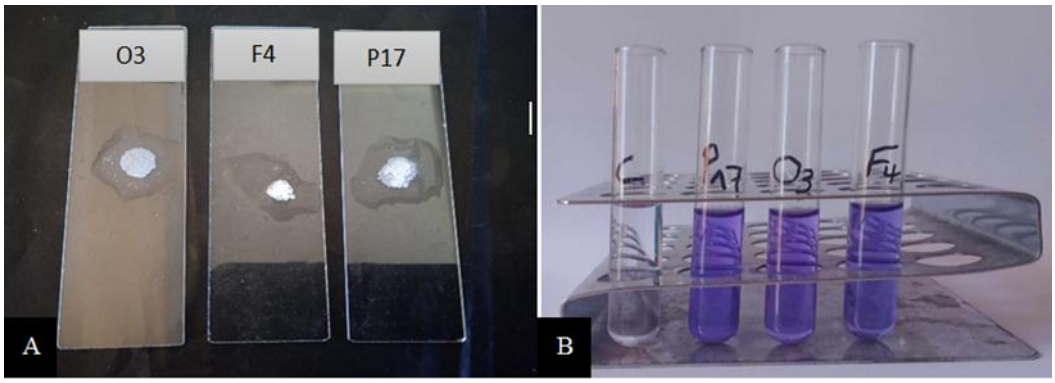


Figure 13: Biochemical test: A) Catalase test; B) oxidase test

All isolates were Gram-negative. Similar results were obtained by Grobelak and al (2015) who showed that the rhizosphere is colonized mainly by a Gram-negative microbial potato community.

A bacterium that is Gram-negative, bacillus-shaped, oxidase-positive, and catalase-positive (O3 and F4) could belong to the genus *Pseudomonas*. *Pseudomonas* bacteria are typically Gram-negative, rod-shaped, and often test positive for oxidase and catalase. These characteristics align with numerous species within this genus, such as *Pseudomonas protegens*, *Pseudomonas fluerescens*, and other *Pseudomonas* species commonly found in soil

and other natural environments. P17 is a cocci gram negative bacteria, catalase and oxidase positive, could belong to the genus *Acinetobacter* or *Moraxella*.

Pseudomonas sp. are known as plant growth-promoting rhizobacteria (PGPR), they are widely used in agriculture as natural biocontrol agents (Kumar et al., 2014). The genetically best-characterized biocontrol agents belong to the genus *Pseudomonas* (Bloemberg and Lugtenberg, 2001). The complex of *Pseudomonas fluorescens* includes several species described as plant growth-promoting rhizobacteria (PGPR) potentially active in biocontrol and biofertilization (Garrido-Sanz et al., 2016).

Moraxella osloensis has shown significant potential due to its production of LPS, which acts as an active endotoxin against the grey garde slug *Deroceras reticulatum*, representing a novel biological toxin effective against mollusks.

Khalil et al. (2021) have reported that *Acinetobacter calcoaceticus* displays IAA and siderophore production that promotes wheat plant growth, with antagonistic activity to different phytopathogens such as *Fusarium oxysporum*, *Aspergillus flavus* and *A. niger*. Kwon and Song (2014) have studied Interactions between Indole-3-acetic Acid producing *Acinetobacter* sp. SW5 and Growth of Tomato Plant. This strain produced 4.06 μM of IAA from root exudates of 8 tomato seedlings. Given its ability to grow in tomato root exudates, the IAA secreted by this bacterium could potentially enhance the growth of tomato plants.

5. Plant growth stimulation tests

5.1. Germination test

To study the effect of the selected isolates on germination, we tested them on wheat seeds, and the germination percentage is represented in the following figure.

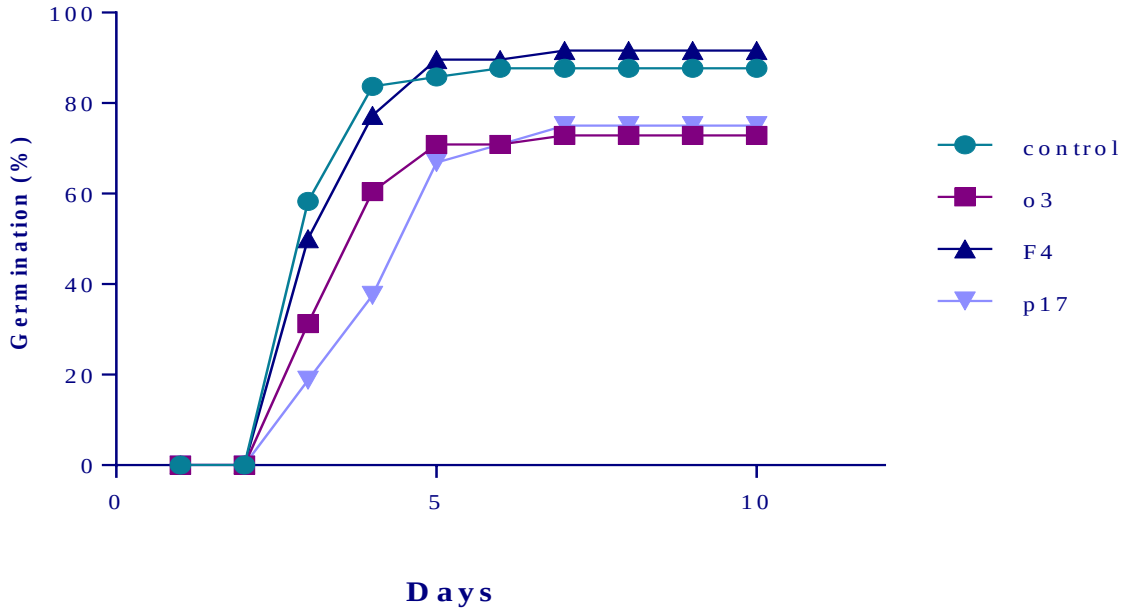


Figure 14: Germination percentage of wheat seeds

F4 isolate shows a 5% difference in germinated seeds compared to the uninoculated control (95%; 90% respectively), whereas isolates P17 and O3 reduced the seed germination rate (75% of germinated seeds). According to Shen et al (2013), all the inoculated bacterial strains showed a positive effect on the growth of wheat seedling. Arzoo and al (2024) found that the percentage of germination in plants treated with PGPRs was reported to be enhanced by the production of plant growth regulators such as auxins, gibberellins, cytokinin and ethylene.

The stimulation of seed germination may also be due to the production of IAA. According to Wahyudi et al. (2011), the ability of *Pseudomonas* spp. to stimulate seed germination is closely linked to auxin production.

5.2. Wheat growth promotion test

The results of the different growth parameters (shoots and roots length, dry weight and fresh weight) of wheat treated with the isolates are presented in the following graphs.

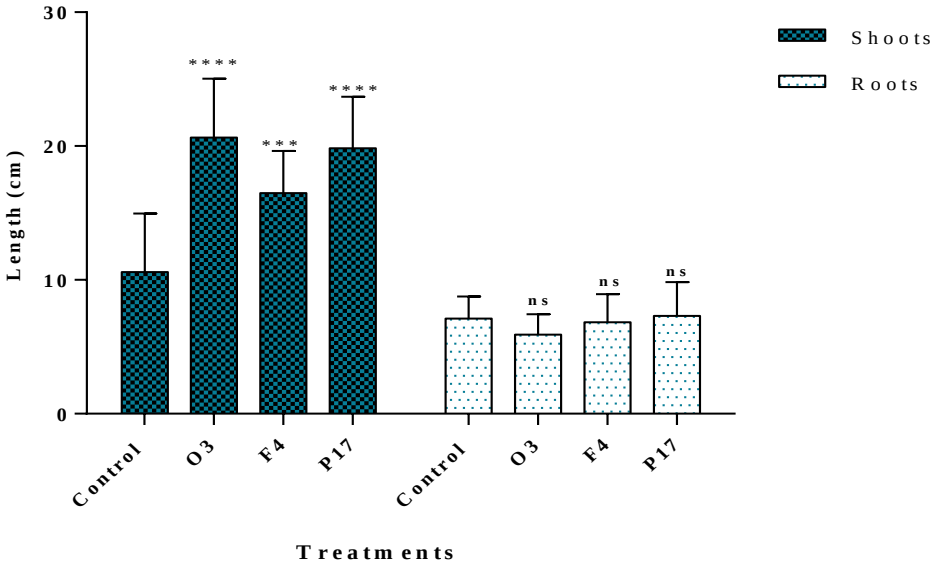


Figure 15: Shoots and root length values for inoculated or non-inoculated wheat

ns : Non significative (p≥0,05) ; *** : p≤0.005 ; **** : p≤0.001

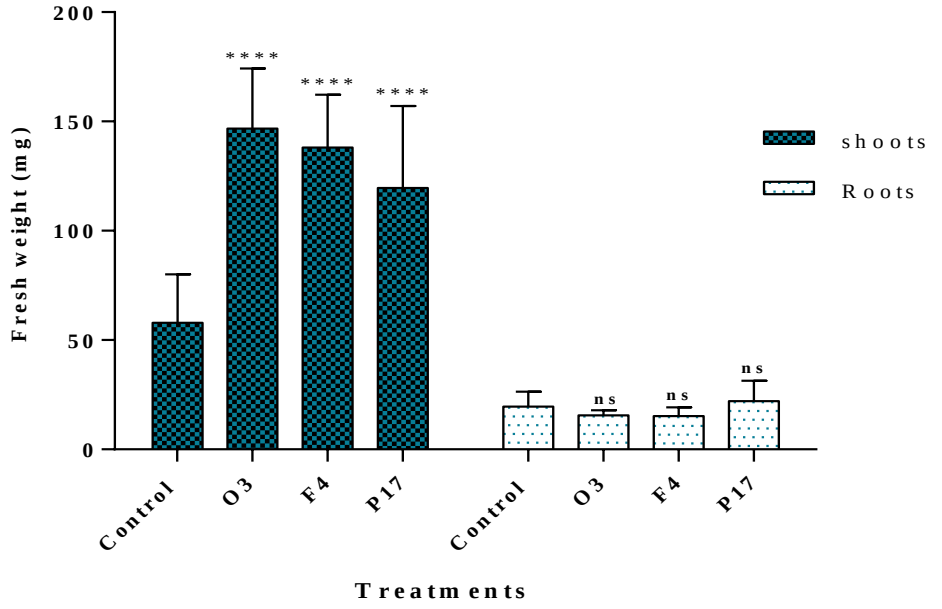


Figure 16: Fresh weight values for shoots and roots of wheat grown treated and control

ns : Non significative (p≥0,05) ; **** : p≤0.001

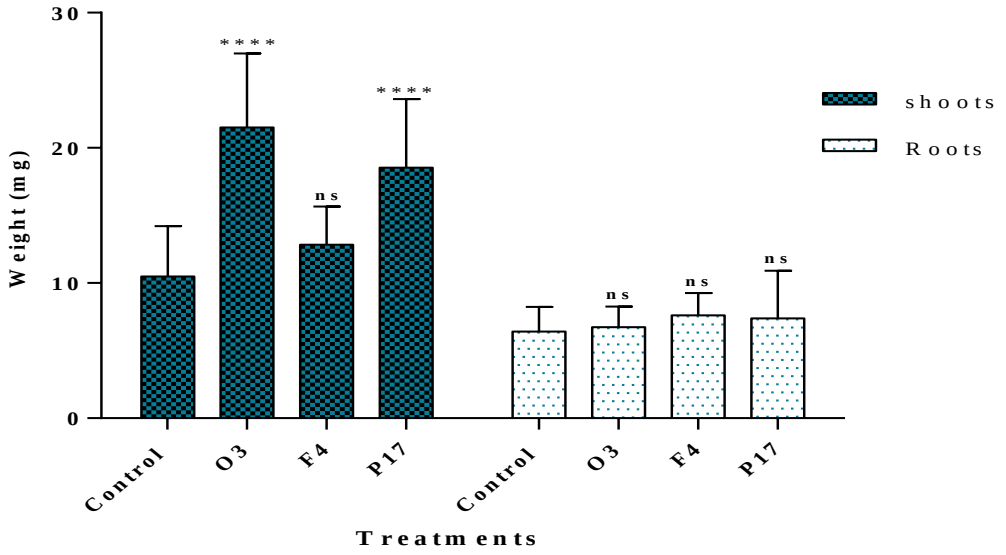


Figure 17: Dry weight values for shoots and roots of wheat grown treated and control

ns : Non significative ($p \geq 0,05$) ; **** : $p \leq 0.001$

The results of the measurements of the various plant growth parameters showed that the O3 and P17 strains significantly ($p \leq 0.001$) stimulated the various growth parameters of the wheat shoots: length (20.63 cm ± 4.4; 19.85 cm ± 3.85), fresh weight (146.85 mg ± 26.75; 19.52 mg ± 37.50) and dry weight (21.51 mg ± 5.7; 18.53 mg ± 5.07) respectively, contrary to F4 isolates which moderately stimulated length (16.5 cm ± 3.16) and fresh weight (138.08 mg ± 24.09) compared to the other two strains mentioned above.

These results are similar to those found by Grobelak and al (2015), who found that PGPR improved length, fresh weight and dry weight of plant stems compared with control plants. Hassan and Bano (2015) found that *Pseudomonas* increased wheat yield by 15 to 25% compared with non-inoculated plants.

On roots parameters, no significant difference was detected ($p \geq 0.05$) between plants-treated and untreated control. We can infer that our strains affect stem growth but not root growth, suggesting they may be specific strains for promoting stem elongation.

Brown and rovira (1999), Khalid et al (2004), Sezen and al (2016) reported that the negative effects on various wheat root growth parameters could be due to the production of a type of phytotoxin. The IAA-producing PGPRs contribute to plant growth and can be considered as a factor in improving plant growth and development as well as yield (Ijaz et al.,

2019; Noureen and al., 2024). In addition, phytohormones such as Cytokinins and gibberellins play a crucial role in cell division and stem elongation (Neshat and al., 2022). Mehrabi and al (2024) have described how PGPRs improve plant performance under difficult environmental conditions. To be effective, a rhizobacteria must be capable of colonizing the rhizosphere at a sufficient population size to exert beneficial effects (Abaid-Ullah and al., 2015).

5.3. Wheat chlorophyll content

The results of chlorophyll a, b and total in wheat treated with the selected isolates are presented in the following graph

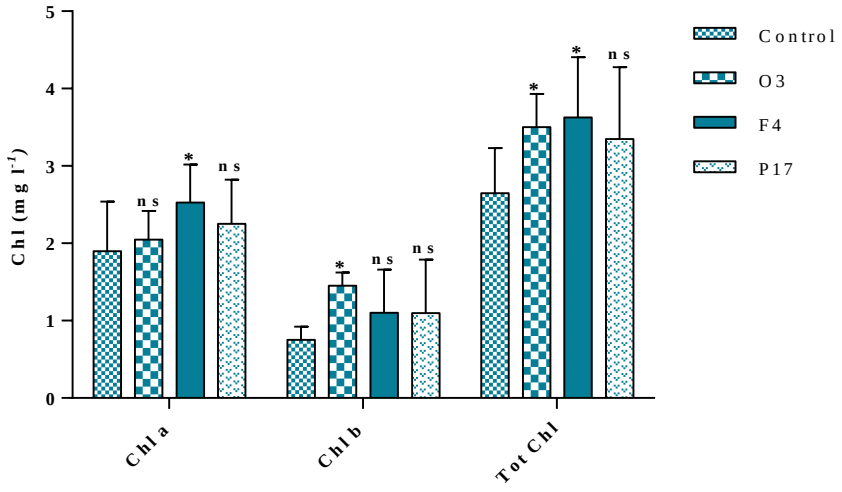


Figure 18: Quantity of chlorophyll a, b and total in treated and control plants.

ns : Non significative ($p \geq 0,05$) ; * : $p \leq 0.05$

In our study, all isolates increase the chlorophyll content of wheat leaves. The highest chlorophyll content was obtained with F4 isolates, which significantly ($p \leq 0.05$) stimulated chlorophyll a and total chlorophyll with (2.52 $mgl^{-1} \pm 0.49$; 3.62 $mgl^{-1} \pm 0.78$) compared to the control (1.9 ± 0.63; 2.65 ± 0.58) respectively.

Strain O3 stimulates significantly ($p \leq 0.001$) chlorophyll b and total with (1.45 $mgl^{-1} \pm 0.17$; 3.5 $mgl^{-1} \pm 0.43$) respectively compared to the control. However, 17 slightly stimulates all three types of chlorophyll, but the difference is not significant ($p \geq 0.05$)

Ray et al (2024) suggest that PGPRs play an important role in green plant growth. According to Purbajanti, 2016 and Muhammad et al. (2021) chlorophyll content is increased by treatment of plants with PGPRs. Mathivanan et al., (2017), showed that consortium treatment (*Rhizobium*, *Pseudomonas*, *Bacillus*) gives higher levels of chlorophyll a, b and total.

Conclusion

Conclusion

The main objectives of our study is the isolation of IAA-producing rhizobacteria and the verification of their potential to promote wheat growth.

Four soil samples were collected from different agricultural fields located in Bejaia. After isolation and purification 61 bacterial isolates were obtained and tested for their ability to produce IAA.

Three isolates were selected and underwent preliminary biochemical identification (Gram staining, catalase, and oxidase tests). The obtained results lead us to suggest that the isolates O3 and F4 belong to the genus *Pseudomonas*, while isolate P17 belongs to either *Moraxella* or *Acinetobacter*

The results indicate that variations in physicochemical parameters such as pH, temperature, and L-tryptophan concentration influence IAA production by the three isolates.

The optimal conditions for IAA production were observed at different temperatures: 30°C for isolate O3 (120 µg/ml) and 35°C for isolates F4 and P17 (120 µg/ml and 105 µg/ml respectively). The pH conditions favoring production were pH 7and 8 and the concentrations of L-tryptophan were 800 µg/ml and 650 µg/ml.

Wheat growth parameters were significantly enhanced with bacterial treatment compared to the control. All the selected isolates enhance significantly shoot growth but not root growth, suggesting they may be specific strains for promoting stem elongation.

The chlorophyll content was significantly higher in the treated wheat than in the control

From this study we conclude that our rhizobacteria have the capacity to produce indole acetic acid (IAA) in significant quantities and can stimulate wheat growth.

At the end of this study, several perspectives appear necessary for better utilization of these isolates:

- Optimization of IAA production
- Testing the effect of isolates on the growth of other plants
- Verification of isolates' ability to produce other agriculturally relevant molecules
- Molecular identification of the isolates

References

References

Abaid-Ullah, M., Hassan, M. N., Jamil, M., Brader, G., Shah, M. K. N., Sessitsch, A., & Hafeez, F. Y. (2015). Plant Growth Promoting Rhizobacteria: An Alternate Way to Improve Yield and Quality of Wheat (*Triticum aestivum*). Int. J. Agric. Biol, 17, 51–60.

Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. Jornal of Kingsaud University-science, 26, 1-20.

Ait Bessai, S., Bensidhoum, L., & Nabti, EL-H. (2022). Optimization of IAA production by telluric bacteria isolated from northern Algeria. Biocatalysis and Agricultural Biotechnology.41, 10.

Ajar, N.Y., Ali, A.R., Neelam, Y., & Divjot, K. Advances in Plant Microbiome and Sustainable Agriculture, Springer Nature Singapore Pte Ltd. 19,39-60.

Alhaithloul, H.A.S., Abu-Elsaoud, A.M., & Soliman, M.H. (2020). Abiotic stress tolerance in crop plants: role of phytohormones. Abiotic stress in plants, 233.

Amara, U., Khalid, R., & Hayat, R. (2015). Soil bacteria and phytohormones for sustainable crop production. Bacterial metabolites in sustainable agroecosystem, 87-103.

Anfang, M., & Shani, E. (2021). Transport mechanisms of plant hormones. Current opinion in plant biology, 63, 102055.

Arnon, D.T. (1949). Copper enzyme in isolated chloroplasts polyphenoloxidase in Beta vulgaris. Plant Physiol, 24, 1-15.

Arzoo, K., Balodi, R., Erayya., Kalmesh, M., & Kumar, J. (2024). Indole Acetic Acid (IAA) Mediated Plant Growth Promotion Activity of *Fluorescent Pseudomonads* and *Bacillus Spp*. Journal of Advances in Biology & Biotechnology, 27, 162-171

Azhar, B.J., Abbas, S., Aman, S., Yamburenko, M.V., Chen, W., Muller, L., & Schaller, G.E. (2023). Basis for high-affinity ethylene binding by the ethylene receptor ETR1 of Arabiodopsis. Proceedings of the National Academy of Sciences, 120.

Baca, B.E., & Elmerich, C. (2007). Microbial production of plant hormones. In: Elmerich, C., Newton, W.E. (Eds). Associative and Endophytic Nitrogen-fixing Bacteria and Cyanobacterial Associations, Springer, Netherlands. Pp. 113-143.

Badr, E.A., Bakhoum, G.S., Amin Howida, G.A., & Khedr, H. (2022). Effect of unconventional fertilizers on Root quality and yield components of sugar beet (Beta vulgaris L.) plants. Plants, 11, 2222.

Bakshi, A., Shemansky, J.M., Chang, C., & Binder, B.M. (2015). History of research on the plant hormone ethylene. Journal of Plant Growth Regulation, 34, 809-827.

Bandick, A.K. & Dick, R.P. (1999) Field management effects on soil enzyme activities. Soil Biol. Biochem. 31, 1471–1479

Bari, R., & Jones, J.D.G. (2009). Role of plant hormones in plant defence responses. Plant Mol Biol, 69, 473- 488.

Bashan, Y., & De-Bashan, L.E. (2005). Plant growth-promoting. Encyclopedia of soils in the environment, 1, 103-115.

Baum, C., Leinweber, P., & Schlichting, A. (2003). Effects of chemical conditions in re-wetted peats temporal variation in microbial biomass and acid phosphatase activity within the growing season. Appl Soil Ecol.22 (2)

Beattie, G. (2007). Plant-associated bacteria: survey, molecular phylogeny, genomics and recent advances. Plant-associated bacteria, 1-56.

Benaissa, A. (2019). Plant growth promoting rhizobacteria a review. Algerian Journal of Environmental Science and Technology, 5, 873-880.

Beneduzi, A., & Passagila, L.M.P. (2011). Genetic and phenotypic diversity of plant growth promoting Bacilli. In: Maheshwari, D.K. (Eds), Bacteria in Agrobiology: Plant growth Responses. Springer-Verlag, Berlin Heidelberg. Pp. 1-20.

Bensidhom, L., & Nabti, El-H. (2020). Biofertilizers and Biopesticides: Microbes for Sustainable Agriculture, In: Naveen KA., Ajar NY., Ali AR., Neelam Y., Divjot K, Advances in Plant Microbiome and Sustainable Agriculture, Vol 19, Springer Nature Singapore Pte Ltd, pp 257-279.

Bent, E., Tuzun, S., Chanway, C.P., & Enebak, S. (2001). Alterations in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. Canadian journal of microbiology, 47, 793-800.

Beneduzi, A., & Passagila, L.M.P. (2011). Genetic and phenotypic diversity of plant growth promoting Bacilli. In: Maheshwari, D.K. (Eds), Bacteria in Agrobiology: Plant growth Responses. Springer-Verlag, Berlin Heidelberg. Pp. 1-20.

Berendsen, R.L., Pieterse, C.M.J., & Bakker, P.A.H.M. (2012). The rhizosphere microbiome and plant health. Trends Plant Sci. 17,478–86.

Bharucha, U., Patel, K., & Trivedi, U.B. (2013). Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteriaon mustard (*Brassica nigra*). Agricultural research, 2, 215-221.

Bhat, M.A., Mishra, A.K., Jan, S., Bhat, M.A., Kamal, M.A., Rahman, S., Shah, A.A., & Jan, A. T. (2023). Plant growth promoting rhizobacteria in plant health: a perspective study of the underground interaction. Plants, 1, 1-21.

Bhattacharyya, P.N., & Jha, D.K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J. Mirobiol. Biotechnol, 28, 1327-1350.

Bloemberg, G.V., & Lugtenberg, B.J. (2001). Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Current opinion in plant biology, 4, 343-350.

Braun, J. C. A. & Colla, L. M. **(2023)** Use of microalgae for the development of biofertilizer and biostimulants. Bioenergy Res. 16, 289–310.

Bric J.M., Bostock R.M., & Silverstone S.E. (1991). Rapid in situ assay for indol acetic acidproduction by bacteria immobilization on a nitrocellulose membrane. Appl Environ. Microbiol, 57, 535-538.

Brown, G. D., & Rovira, A. D. (1999). The rhizosphere and its management to improve plant growth. Advances in Agronomy, 66, 1-102.

Castro-Camba, R., Sánchez, C., Vidal, N., & Vielba, J. M. (2022). Plant development and crop yield: The role of gibberellins. Plants, 11, 1-27.

Chaitanya, K.J., & Meenu, S. (2015). Plant growth promoting Rhizobacteria (PGPR). Journal of Agricultural Research and Development, 5, 108-119.

Chakraborty, T., & Akhtar, N. (2021). Biofertilizers: prospects and challenges for future. Biofertilizers: study and impact, 575-590.

Chandra, S., Askari, K., & Kumari, M. (2018). Optimization of indole acetic acid production by isolated bacteria from rebaudiana rhizosphere and its effects on plant growth. Journal of Genetic Engineering and biotechnology.16, 581-586

Checker, V. G., Kushwaha, H. R., Kumari, P., & Yadav, S. (2018). Role of phytohormones in plant defense: signaling and cross talk. Molecular aspects of plant-pathogen interaction, 159-184.

Chernysheva, E., Korobov, D., Khomutova, T., Fornasier, F., & Borisov, A. (2021). Soil microbiological properties in livestock corrals: An additional new line of evidence to identify livestock dung. J. Archaeol. Sci. Rep.37, 103012

Davies, P.J. (1995). Plant Hormones: Physiology, Biochemistry, and Molecular Biology. Kluwer Academic Publishers, Netherlands

Davies, P.J. (2010). The plant hormones: their nature, occurrence, and functions. Plant hormones: biosynthesis, signal transduction, action! Dordrecht: Springer Netherlands, 1-15.

De Andrade, L.A., Santos, C.H.B., Frezarin, E.T., Sales, L.R., & Rigobelo, E.C. (2023). Plant Growth-Promoting Rhizobacteria for Sustainable Agricultural Production. Microorganisms, 11, 1-16.

Dilly, O., Munch, J. C., & Pfeiffer, E. M. (2007). Enzyme activities and litter decomposition in agricultural soils in northern, central, and southern Germany. Journal of Plant Nutrition and Soil Science, 170(2), 197-204.

Dweipayan, G., Janki, N.T., & Pinakin, C.D. (2016). Portraying mechanics of plant growth promoting rhizobacteria (PGPR). Cogent Food & Agriculture, 2, 1-19.

Egamberdieva, D. (2007). The effect of plant growth promoting and nutrient uptake of maize in two different soils. App Soil Ecolo, 36, 184-189.

Egamberdieva, D., & Kucharova, Z. (2009). Selection for root colonizing bacteria stimulating wheat growth in saline soils. Biol. Fert. Soils, 45, 563-571.

Egamberdieva, D., Berg, G., Lindstrom, K., & Rasanen, L.A. (2010). Co-inoculation of *Pseudomonas* spp. With Rhizobium improve growth and symbiotic performance of fodder galega (Galegaorientalis Lam). European Journal of Soil Biology, 46, 269-272.

Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd_Allah, E. F., & Hashem, A. (2017). Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. Frontiers in microbiology, 8, 1-14.

Fernando, W.G.D., Nakkeeran, S., & Zhang, Y. (2005). Biosynthesis of antibiotics by PGPR and its relation in biocontrol of plant diseases. In: Siddiqui, Z.A. (ed), PGPR: Biocontrol and Biofertilization. Springer, Netherlands, pp. 67-109.

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. Nat. Rev.Microbiol, 15,579.

Gamalero, E., & Glick, R.B. (2011). Mechanisms Used by Plant Growth-Promoting Bacteria. Plant Nutrient Management, 17-46.

Garrido-Sanz, D., Meier-Kolthoff, J. P., Göker, M., Martín, M., Rivilla, R., & Redondo-Nieto, M. (2016). Genomic and genetic diversity within the *Pseudomonas fluorescens* complex. PloS one, 11(2), e0150183.

Gaspar, T., Kevers, C., Penel, C., Greppin, H., Reid, M.D., & Thorpe, T.A. (1996). Plant hormones and plant hormones regulators in plant tissue culture. Society for in Vitro Biology, 32, 272-289.

Ghiasian, M. (2020). Endophytic Microbiomes: Biodiversity, Current Status, and Potential Agricultural Applications. In: Naveen KA., Ajar NY., Ali AR., Neelam Y., Divjot K, Advances in Plant Microbiome and Sustainable Agriculture, Vol 19, Springer Nature Singapore Pte Ltd, pp 61-82.

Glick, B.R. (2012). Plant growth-promoting bacteria: mechanisms and applications. Scientifica, 1-15.

Gomez-Cadenas, A., Vives, V., I Zandalinas, S., Manzi, M., M Sanchez-Perez, A., M Perez-Clemente, R., & Arbona, V. (2015). Abscisic acid: a versatile phytohormone in plant signaling and beyond. Current Protein and Peptide Science, 16, 413-434.

Goswami, D., Thakker, J.N., & Dhandhukia, P.C. (2016). Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. Cogent Food & Agriculture, 2, 1-19.

Götz, M.C.M., Gomes, N., Dratwinski, A., Costa, R., Berg, G., Peixoto, R.,Mendoça-Hagler, L.M. & Smalla, K. (2006). Survival of gfp-tagged antagonistic bacteriain the rhizosphere of tomato plants and their effects on the indigenous bacterial community. FEMS Microbiol. Ecol, 56, 207-218.

Grobelak, A., Napora, A., & Kacprzak, M. (2015). Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. Ecological Engineering, 84, 22-28.

Gutierrez, O. A., Wubben, M. J., Howard, M., Roberts, B., Hanlon, E., & Wilkinson, J. R. (2009). The role of phytohormones ethylene and auxin in plant-nematode interactions. Russian Journal of Plant Physiology, 56, 1-5.

Harikrishnan, H., Shanmugaiah, V., & Balasubramanian, N. (2014). Optimization for production of Indole acetic acid (IAA) by plant growth promoting *Streptomyces* sp VSMGT1014 isolated from rice rhizosphere. Int J Curr Microbiol Appl Sci, 3(8), 158-171.

Hassan, A., Tabassum, B., Hashim, M., & Khan, N. (2023). Role of Plant Growth Promoting Rhizobacteria (PGPR) as a Plant Growth Enhancer for Sustainable Agriculture. Preprints, 1, 1-14.

Hassan, T.U., & Bano, A. (2015). Role of carrier based biofertilizer in reclamation of saline soil and wheat growth. Arch. Agron. Soil. Sci., 61, 1719-1731.

Hiltner, L. (1904). Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berücksichtigung der Gründüngung und Brache. Arbeiten der deutschen landwirtschaftlichen gesellschaft, 98, 59.

Hiscox, J.D. & Tsraelstam, G.F. (1979). A method for the extraction of chlorophyll from leaftissue without maceration. Can. J. Bot. 57: 1332-1334.

Hyder, S., Rizvi, Z.F., los Santos-Villalobos, S.D., Santoyo, G., Gondal, A., Khalid, N., Fatima, S.N., Nadeem, M., Rafique. K., & Rani, A. (2023). Applications of plant growth-promoting rhizobacteria for increasing crop production and resilience. Journal of Plant Nutrition, 46, 1-30.

Igiehon, B.C., Babalola, O.O., & Hassen, A.I. (2024). Rhizosphere competence and applications of plant growth-promoting rhizobacteria in food production-a review. Scientific African, 23, 1-16.

Ijaz, M., Shahzadi, R., Rahman, M. U., & Iqbal, M. (2019). Molecular approaches to study plant growth-promoting rhizobacteria (PGPRs). Plant Growth Promoting Rhizobacteria for Agricultural Sustainability: From Theory to Practices, 215-232.

Javid, M.G., Sorooshzadeh, A., Moradi, F., Modarres Sanavy, S.A. M., & Allahdadi, I. (2011). The role of phytohormones in alleviating salt stress in crop plants. Australian Journal of Crop Science, 5, 726-734.

Joanisse, G. D., Bradley, R. L., & Preston, C. M. (2008). Do late-successional tannin-rich plant communities occurring on highly acidic soils increase the DON/DIN ratio? Biology and Fertility of Soils, 44, 903-907.

Jourdan, E., Ongena, M., & Thonart, P. (2008). Caractéristique moléculaires de l'immunité des plantes induite par les rhizobactéries non pathogènes. Biocontrol. Agron. Soc. Environ. 12, 437-449.

Karnwal, A. (2009). Production of indole acetic acid by fluorescent Pseudomonas in the presence of L-tryptophan and rice root exudates. Journal of plant Pathology, 61-63.

Kaymak, H.C. (2011). Potential of PGPR in agricultural innovations. Plant growth and health promoting bacteria, 45-79.

Kenawy, A., Dailin, D.J., Abo-Zaid, G.A., Malek, R.A., Ambehabati, K.K., Zakaria, K.H. N., … & El Enshasy, H.A. (2019). Biosynthesis of antibiotics by PGPR and their roles in biocontrol of plants diseases. Plant growth promoting rhizobacteria for sustainable stress management. Rhizobacteria in biotic stress management, 2, 1-35.

Khalid, A., Arshad, M., & Zahir, Z.A. (2004). Screening plant growth promoting rhizobacteria for improwing growth and yield of wheat. Journal of Applied Microbiology, 96, 473-480.

Khalil, M.M.R., Fierro-Coronado, R.A., Peñuelas-Rubio, O., Villa-Lerma, A.G., Plascencia-Jatomea, R., Félix-Gastélum, R., & Maldonado-Mendoza, I.E. (2021). Rhizospheric bacteria as potential biocontrol agents against Fusarium wilt and crown and root rot diseases in tomato. Saudi Journal of Biological Sciences, 28(12), 7460-7471.

Khamna, S., Yokota, A., Peberdy, J.F., & Lumyong, S. (2010). Indole-3-acetic acid production by Streptomyces sp. isolated from some Thai medicinal plant rhizosphere soils. EurAsian Journal of BioSciences, 4.

Khan, M. S., Zaidi, A., Wani, P. A., & Oves, M., (2009). Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Enviro. Chem. Lett, 7, 1-19.

Khan, S., Alvi, A. F., & Khan, N. A. (2024). Role of Ethylene in the Regulation of Plant Developmental Processes. Stresses, 4, 28-53.

Kieber, J.J., & Schaller, G.E. (2018). Cytokinin signaling in plant development. Development, 145, 1-7.

Kompała-B ˛aba, A., Bierza, W., Sierka, E., Bło ´nska, A., Besenyei, L., & Wo´zniak, G .(2021). The role of plants and soil properties in the enzyme activities of substrates on hard coal mine spoil heaps. Sci. Rep.11, 5155.

Kouassi, M. (2001). La lutte biologique: une alternative viable à l'utilisation des pesticides. Vertigo La revue électronique en sciences de l'environnement, volume 2, Issue. http:// vertigorevues.org/4101; https://doi.org/10.4000/vertigo4101

Kukreja, K., Suneja, S., Goyal, S., & Narula, N. (2004). Phytohormone production by Azotobacter–a review. Agricultural Reviews, 25, 70-75.

Kumar, A., Kumar, A., Devi, S., Patil, S., Payal, C., & Negi, S. (2012). Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an in vitro study. Recent research in science and technology, 4, 1-5.

Kumar, G.S., Gupta, V., Rahi, R.V., Devki Yadav, M., & Kumar, N.B. (2024). Significant Role of Plant Growth Promoting Rhizobacteria in Agriculture Field. Applied Microbiology, 5, 1-14.

Kwon, H.D., & Song, H.G. (2014). Interactions between indole-3-acetic acid producing Acinetobacter sp. SW5 and growth of tomato plant. Korean Journal of Microbiology, 50(4), 302-307.

Lebrazi, S., Fadil, M., Chraibi, M., & Fikri-Benbrahim, K. (2020). Screening and optimization of indole-3- acetic acid production by Rhizobium sp. strain using response surface methodology. Journal of Genetic Engineering and Biotechnology, 18, 1-10.

Lebrazi, S., Niehaus, K., Bednarz, H., Fadil, M., Chraibi, M., & Fikri-Benbrahim, K. (2020). Screening and optimization of indole-3- acetic acid production and phosphate solubilization by rhizobacterial strains isolated from Acacia cyanophylla root nodules and their effects on its plant growth. Journal of Genetic Engineering and Biotechnology, 18, 1-12.

Lin, Z., Zhong, S., & Grierson, D. (2009). Recent advances in ethylene research. Journal of experimental botany, 60, 3311-3336.

Lugtenberg, B., & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. Annual review of microbiology, 63, 541-556.

Maleki, M., Mostafaee, S., Mokhtarnejad, L., & Farzaneh, M. (2010). Characterization of *Pseudomonas fluorescens* strain CV6 isolated from cucumber rhizospher in Varamin as a potentiel biocontrol agent. A J C S, 4, 676-683.

Martinez-Viveros, O., Jorquera, M.A., Crowley, D.E., Gajardo, G., & Mora, M.L. (2010). Mechanisms and practical consideration involved in plant growth promotion by rhizobacteria. J. Soil Sci. Plant Nutr, 10, 293-319.

Mathivanan, S., Chidambaram, A. A., Amalan Robert, G., & Kalaikandhan, R. (2017). Impact of PGPR inoculation on photosynthetic pigment and protein contents in Arachis hypogaea L. Journal of Scientific Agriculture, 1, 29-36.

Matilla, M.A., & Krel, T. (2018). Plant Growth Promotion and Biocontrol Mediated by Plant-Associated Bacteria Plant Microbiome: Stress Response. In: Dilfuza and Parvaiz. Plant Microbiome: Stress Response. Springer. Vol 5, 45-80.

Mauch-Mani, B., & Mauch, F. (2005). The role of abscisic acid in plant–pathogen interactions. Current opinion in plant biology, 8, 409-414.

Mehrabi, S. S., Sabokdast, M., Bihamta, M. R., & Dedičová, B. (2024). The Coupling Effects of PGPR Inoculation and Foliar Spraying of Strigolactone in Mitigating the Negative Effect of Salt Stress in Wheat Plants: Insights from Phytochemical, Growth, and Yield Attributes. Agriculture, 14(5), 732.

Mekonnen, H., & Kibret, M. (2021). The roles of plant growth promoting rhizobacteria in sustainable vegetable production in Ethiopia. Chem. Biol. Technol. Agric, 18, 1-11.

Minakshi., Sharma, S., Sood, G., & Chauhan, A. (2020). Optimization of IAA production and P-solubilization potential in Bacillus subtilis KA (1) 5r isolated from the medicinal herb *Aconitum heterophyllum*- growing in western Himalaya, India. Journal of Pharmacognosy and Phytochemistry .9, 2008-2015.

Miransari, M., & Smith, D.L. (2014). Plant hormones and seed germination. Elsevier, 99, 110-121.

Misra, M., Sachan, A. & Sachan, S. G. **(2020)** Current Aspects and Applications of Biofertilizer for Sustainable Agriculture. In Plant Microbiomes for Sustainable Agriculture. Sustainable Development and Biodiversity .25, 445–473.

Mohite, B. (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. Journal of soil science and plant nutrition, 13(3), 638-649.

Moore, T.C., & Moore, T.C. (1979). Auxins. Biochemistry and physiology of plant hormones, 32-89.

Muhammad, M., Isnatin, U., Soni, P., & Adinurani, P.G. (2021). Effectiveness of Mycorrhiza, Plant Growth Promoting Rhizobacteria and Inorganic Fertilizer on Chlorophyll Content in Glycine max (L.) cv. Detam-4 Prida. E3S Web of Conferences, 226, 1-8.

Mukherjee, A., Gauvar, A.K., Singh, S., Yadav, S., Bhowmick, S., Abeysinghe, S., & Verma, J.P. (2022). The bioactive potential of phytohormones. Biotechnology Reports, 35, 1-9.

Muller, B., & Sheen, J. (2007). Advance in cytokinin signaling. Science, 318, 68-69. Doi: 10.1126/ science. 1145461.

Mustami, M. K., Hafsan, H., & Asri, A. (2024). The Potential of *Burkholderia* sp. from Zea mays Roots as Plant Growth Promoting Rhizobacteria. Jurnal Pijar Mipa, 19, 464–468.

Nabti, El-H., & Slimane M. (2020). Rhizospheric Microbiome: Biodiversity, Current Advancement and Potential Biotechnological Applications. In: Naveen KA.,

Nadeem, S.M., Ahmad, M., Zahir, Z.A., & Kharal, M.A. (2016). Role of phytohormones in stress tolerance of plants, In: Hakeem K R., Akhtar M S. (eds). Plant Soil and Microbes. vol 2 Springer Nature. Switzerland, 385- 421.

Neshat, M., Abbasi, A., Hosseinzadeh, A., Sarikhani, M. R., Chavan, D. D., & Rasoulnia, A. (2022). Plant growth promoting bacteria (PGPR) induce antioxidant tolerance against salinity stress through biochemical and physiological mechanisms. Physiol Mol Biol Plants, 28, 347–361.

Noureen, S., Iqbal, A., & Muqeet, H. A. (2024). Potential of Drought Tolerant Rhizobacteria Amended with Biochar on Growth Promotion in Wheat. Plants, 13(9), 1183.

Odoh, C.K. (2017). Plant growth promoting rhizobacteria (PGPR): a bioprotectant bioinoculant for sustainable agrobiology. A review. Int J Adv Res Biol Sci, 4, 123-142.

Olanrewaju, O.S., Glick, B.R., & Babalola, O.O. (2017). Mechanisms of action of plant growth promoting bacteria. World Journal of Microbiology and Biotechnology, 33, 1-16.

Panigrahi, S., Mohanty, S., & Rath,C, C. (2020). characterization of endophytic bacteria *Enterobacter cloacae* MG00145 isolated from *Ocimum sanctum* with Indol Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. South African Journal of Botany.134, 17-26.

Panpatte, D.G., Jhala, Y.K., Shelat, H.N., & Vyas, R.V. (2016). Pseudomonas fluorescens: a promising biocontrol agent and PGPR for sustainable agriculture. Microbial inoculants in sustainable agricultural productivity: Research perspectives, 1, 257-270.

Parvin, W., Jahan, Q. S. A., Rahman, M. M., & Wong, M. Y. (2018). In vitro screening and optimization of IAA production from plant growth promoting rhizobacteria *Burkholderia cepacia* UPMB3. Plant Tissue Culture and Biotechnology, 28(1), 25-34.

Parvin, W., Rahman, M. M., Govender, N. T., & Wong, M. Y. (2020). Identification, determination and quantification of indole-3-acetic acid produced by *Pseudomonas aeruginosa* UPMP3 and its effect on the growth of oil palm (*Elaeis guineensis* Jacq). World Journal of Agricultural Research, 8(3), 75-83.

Pathak, R., Shrestha, A., Lamichhane, J., & Gauchan, D.P. (2017). PGPR in biocontrol: mechanisms and roles in disease suppression. International Journal of Advanced Research in Biological Sciences, 11, 69-80.

Patil, N.B., Gajbhiye, M., Ahiwale, S.S., Gunja, A.B., & Kapadnis, B.P. (2011). Optimization of indole 3-acetic acid (IAA) production by *Acetobacter diazotrophicus* Ll isolated from sugarcane. International Journal of Environmental Sciences, 2, 295-302.

Pons, N. (2020). Les hormones dans la symbiose mycorhizienne : étude de la production et des effets d'hormones végétales par les champignons endomycorhiziens. Thèse de doctorat, Université Toulouse 3 - Paul Sabatier, Interactions plantes-microorganismes, 163p.

Praveen Kumar, G., Mir Hassan Ahmed, S. K., Desai, S., Leo Daniel Amalraj, E., & Rasul, A. (2014). In vitro screening for abiotic stress tolerance in potent biocontrol and plant growth promoting strains of *Pseudomonas* and *Bacillus* spp. International Journal of Bacteriology, 2014(1), 195946.

Purbajanti, E. D., Kusmiyati, F., Slamet, W., & Adinurani P. G. (2016). AIP Conference Proceedings. 1755, 1-4.

Raaijimakers, J.M., Leeman, M., Van Oorschot, M.M.P., Van der Sluis, I., Schippers, B., & Bakker, P.A.H.M. (1995). Dose-response relationships in biological control of *Fusarium* wilt of radish by *Pseudomonas* spp. Phytopathology, 85, 1075-1081.

Rahayu, Y.S., Yuliani Y., & Asri, M.T. (2024). The ability of bacteria from legume plant roots grown on former coal mining soil to produce Indole-3-Acetic-Acid (IAA). In E3S Web of Conferences. EDP Sciences. 513, 03003

Rahman, S. U., Li, Y., Hussain, S., Hussain, B., Riaz, L., Ashraf, M. N., Khaliq, A.M., Du, Z., & Cheng, H. (2023). Role of phytohormones in heavy metal tolerance in plants: A review. Ecological Indicators, 146, 1-20.

Raj, M., Kumar, R., Lal, K., Sirisha, L., Chaudhary, R., & Patel, S. K. (2020). Dynamic role of plant growth promoting rhizobacteria (PGPR) in agriculture. Int. J. Chem. Stud, 8, 105-110.

Randiv, V S., Agnihotri, S N., & Bhagat., R B. (2024). Screening and Optimization of IAA Production by PGPR Isolated from Rhizosphere of a *Pterocarpus marsupium* Roxb and their Effect on Plant Growth. Screening. 12.

Raza, A., Charagh, S., Najafi-Kakavand, S., Abbas, S., Shoaib, Y., Anwar, S., & Siddique, K.H. (2023). Role of phytohormones in regulating cold stress tolerance: physiological and molecular approaches for developing cold-smart crop plants. Plant stress, 100152.

Rehman, F., Kalsoom, M., Adnan, M., Toor, M.D., & Zulfiqar, A. (2020). Plant Growth Promoting Rhizobacteria and their Mechanisms Involved in Agricultural Crop Production: A Review. SunText Rev. Biotechnol, 1, 110.

Roopa, S., Mishra, T., Bhattacharya, S., Bhadra, A., Singh, S. R., Shrivastava, R., & Patil, S. J. (2023). Role of IAA In Plant Growth, Development, And Interaction With Other Phytohormones. Eur. Chem. Bull, 12, 5293 – 5297.

Saharan, B.S., & Nehra, V. (2011). Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res, 21, 1-30.

Saleem, M., Hu, J., & Jousset, A. (2019). More than the sum of its parts: microbiome biodiversity as a driver of plant growth and soil health. Annual review of ecology, evolution, and systematics, 50, 145-168.

Saleem, M., Pervaiz, Z.H, & Traw, M.B. (2015). Theories, mechanisms and patterns of microbiome species coexistence in an era of climate change. In Microbiome Community Ecology: Fundamentals and Applications, ed. M Saleem,

Sandur, S.D., & Onkarappa, R. (2023). Optimization for the production of Indole acetic acid by Streptomyces sp. SDSRO-2 isolated from Rhizosphere soil of maize. Scope Journal. 13. 223-230.

Santner, A., Calderon-Villalobos, L.I.A., & Estelle, M. (2009). Plant hormones are versatile chemical regulators of plant growth. Nature Chemical Biology, 5, 301-307.

Sebihi F.Z. (2016). Effet PGPR des souches de *Pseudomonas fluorescens* isolées de la rhizosphère du blé cultivé dans la région de Constantine. Thèse de doctorat, Université des frères Mentouri, Constantine. Faculté des sciences de la nature et de la vie, Constantine, 91 p.

Sezen, A., Ozdal, M., Koc, K., & Algur, O. F. (2016). Isolation and characterization of plant growth promoting rhizobacteria (PGPR) and their effects on improving growth of wheat. Journal of Applied Biological Sciences, 10(1), 41-46.

Sezgin, M., & Kahya, M. (2018). Phytohormones. Bitlis Eren University Journal of Science and Technology, 8, 35-39.

Sharma, S., Kumar, V., & Tripathi, R.B. (2011). Isolation of phosphate solubilizing microorganism (PSMs) from soil. Journal of microbiology and Biotechnology Research, 1, 90-95.

Shen, Z., Zhong, S., Wang, Y., Wang, B., Mei, X., Li, R., & Shen, Q. (2013). Induced soil microbial suppression of banana *fusarium* wilt disease using compost and biofertilizers to improve yield and quality. European Journal of Soil Biology, **57, 1-8.**

Singh, R., Kaur, S., Bhullar, S.S., Singh, H., & Sharma, L.K. (2023). Bacterial biostimulants for climate smart agriculture practices: Mode of action, effect on plant growth and roadmap for commercial products. Journal of Sustainable Agriculture and Environment, 3, 1-20.

Somers, E., Vanderleyden, J., & Srinivasan, M. (2004). Rhizosphere bacterial signaling: a love parade beneath our feet. Crit. Rev. Microbial, 304, 205-240.

Soni, S.K., Manhas, R., Jakhar, Y., Sharma, A., & Soni, R. (**2022)** Biofertilizer for sustainable agriculture: current trends and future perspective. in Genomic, Proteomics, and Biotechnology 331–356.

Su, F., Zhao, B., Dhondt-Cordelier, S., & Vaillant-Gaveau, N. (2024). Plant-Growth-Promoting Rhizobacteria Modulate Carbohydrate Metabolism in Connection with Host Plant Defense Mechanism. International Journal of Molecular Sciences, 25, 1-17.

Suliasih., & Widawati, S. (2020). Isolation of Indole Acetic Acid (IAA) producing *Bacillus siamensis* from peat and optimization of the culture conditions for maximum IAA production. Earth and Environmental Science, 572, 1-11.

Taiz, L., Zeiger, Z., Moller, I.M., & Murphy, A. (2015). Plant physiology and developmen. Sinauer Associates Incorporated, Sunderland, USA. 761Pp.

Timofeeva, A., Galyamova, M., & Sedykh, S. (2022). Prospects for using phosphate-solubilizing microorganisms as natural fertilizers in agriculture. Plants, 11, 1-23.

Turnbull, C.J.N., & Loveys, B.R. (1999). Plant hormones: chemical signaling in plant development. 284-305.

Utami, D.S., Setiawati, M.R., & Simarmata, T. (2024). Synergistic and Competence of Native Plant Growth Promoting Rhizobacteria on Direct Mechanism on Growth Plants: A Review. International Journal of Life Science and Agriculture Research, 3, 103-111.

Uzakbaevna, I.A. (2022). The Effect of Unconventional Fertilizers on the Growth and Development of Cotton. Int. J. Integr. Educ, 5, 226–229

Van Giang, N., Hien, P.H., Diep, V.T.N., Huyen, P.K., & Pylnev, V.V. (2024). Isolation and characterization of Indole Acetic Acid-producing bacteria isolated from rhizospheric soil of paddy rice. In: E3S Web of Conferences. EDP Sciences. 494, 04030.

Van Loon, L.C., Bakker, P.A.H.M. & Pieterse, C.M.J. (1998). Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol, 36, 453-483.

Vandenbussche, F., Vaseva, I., Vissenberg, K., & Van Der Straeten, D. (2012). Ethylene in vegetative development a tale with a riddle. New phycologist, 194, 895-909.

Vincze, É.B., Becze, A., Laslo, É., & Mara, G. (2024). Beneficial Soil Microbiomes and Their Potential Role in Plant Growth and Soil Fertility. Agriculture, 14, 1-23.

Wahyudi, A.T., Astuti, R.I. & Giyanto. (2011). Screening of *Pseudomonas* sp. isolated from Rhizosphere of Soybean Plant as Plant Growth Promoter and Biocontrol Agent. American J. Agril. Biol. Sci. 6(1):134-141.

Wang, K.L.C., Li, H., & Ecker, J.R. (2002). Ethylene biosynthesis and signaling networks. The plant cell, 14, 131-151.

Wang, T., Xu, J., Chen, J., Liu, P., Hou, X., Yang, L., & Zhang, L. (2024). Progress in Microbial Fertilizer Regulation of Crop Growth and Soil Remediation Research. Plants, 13, 346.

Warisman, G.W.G., Damanhuri, D., & Widaryanto, E. (2024). Effect of Gibberellin and Nitrogen Fertilizer on Growth and Zucchini (*Cucurbita pepo* L.) Yield in Tropical Regions. International Journal of Environment, Agriculture and Biotechnology, 9, 90-96.

Wasilewska, A., Vlad, F., Sirichandra, C., Radko, Y., Jammes, F., Valon, C., … & Leung, J. (2008). An update on abscisic acid signalig in plants and more…. Molecular plant, 1, 198-217.

Went, F.W., & Thimann, K.V. (1937). Phytohormones. New York: Macmillan, 294.

Weyers, J.D., & Paterson, N.W. (2001). Plant hormones and the control of physiological processes. New Phytologist, 152, 375-407.

Widawati, S., (2020). Isolation of Indole Acetic Acid (IAA) producing *Bacillus siamensis* from peat and optimization of the culture conditions for maximum IAA production. In IOP Conference Series: Earth and Environmental Science.572, 012025.

Williams, M.E., (2011). Introduction to phytohormones. Teaching Tools in plant biology: lecture Notes. The Plant Cell (online), /10.1105/ . 110.0310.

Yaghoubi Khanghahi, M., Strafella, S., Filannino, P., Minervini, F., & Crecchio, C. (2024). Importance of Lactic Acid Bacteria as an Emerging Group of Plant Growth-Promoting Rhizobacteria in Sustainable Agroecosystems. Applied Sciences, 14, 1798.

YAGÜE, R., Lobo, C., & Garcia, P. (2023). Organic fertilization induces changes in soil nitrogen mineralization and enzyme activities. Plant, Soil & Environment, 69(1).

Yousef, N.M.H. (2018). Capability of plant growth-promoting rhizobacteria (PGPR) for producing indole acetic acid (IAA) under extreme conditions. European Journal of Biological Research.8, 174-182.

Zahir, Z.A., Arshad, M., & Frankenberger, W.T.J. (2004). Plant growth promoting rhizobaceria: Application and prespectives in agriculture. Adv. Agro, 81, 97-198.

Zakharova, E.A., Shcherbakov, A.A., Brudnik, V.V., Skripko, N.G., Bulkhin, N.S., & Ignatov, V.V. (1999). Biosynthesis of indol-3-acetic in *Azospirillium brasilense* insights from quantum chemistry. Eur J Biochem, 259, 572-576.

Annex

Annex

Annex I: The composition of PBS media

- NaCl…….8g
- KCl……0,2g
- KH_2PO_4.....0,24g
- Na_2HPO_4……1,44g

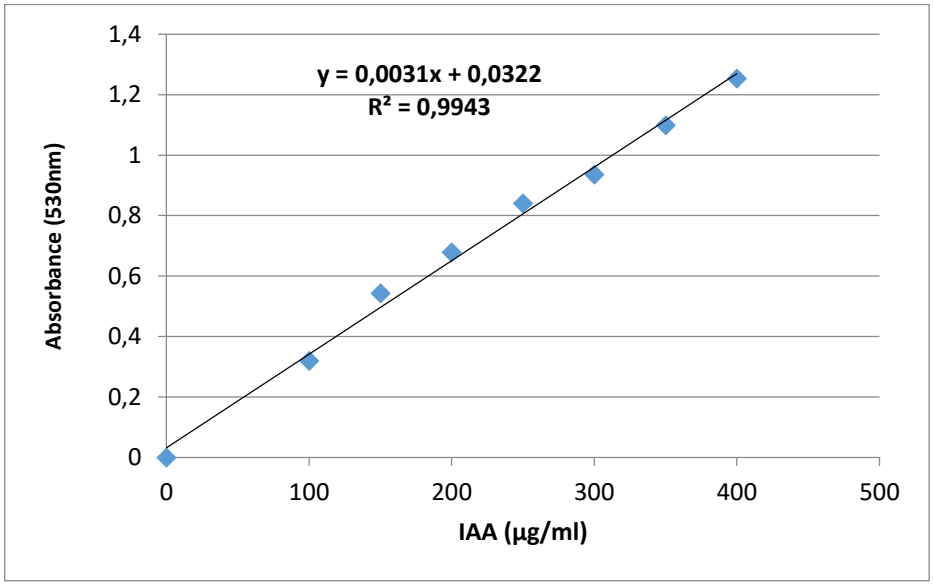
Annex II:

1. The composition of PCA media
   - Glucose……...1g
   - Tryptone…….5g
   - Yeast extract……...2,5g
   - Agar……....12g
   - pH…….....7,0 ± 0,2
2. The composition of LB media
   - Bacto-tryptone…….... 10g
   - Yeast extract…….....5g
   - NaCl……..... 5g
   - pH……7,2 ± 0,2

Annex III: The composition of Salkowsky Reagent

- H_2SO_4 (98%)……...150ml
- H_2O…….250ml
- $FeCl_3$(0,5M)……7,5ml

Annex IV: Calibration curve of IAA



Abstract

Recent studies have highlighted the effect of the phytohormone synthesised by PGPR on plant growth, in particular IAA. The current study focuses on the isolation of IAA-producing rhizobacteria and the verification of their potential to promote wheat growth. From 61 IAA-producing bacteria, four isolates were selected. The influence of temperature, pH and concentration of L-tryptophan on IAA production were verified. The selected isolates were also tested for their ability to promote wheat germination and growth. The parameters: shoots and roots length, fresh and dry weight of shoots and roots as well as chlorophyll content were measured. The results indicated that optimal conditions for IAA production were observed at different temperatures: 30°C for isolate O3 (120 µg/ml) and 35°C for isolates F4 and P17 (120 µg/ml and 105 µg/ml respectively). The pH conditions favoring production were pH 7and 8 and the concentration of L-tryptophan was 800 µg/ml and 650 µg/ml. Wheat growth parameters were significantly enhanced with bacterial treatment compared to the control. All the selected isolates enhance significantly shoot growth but not root growth, suggesting they may be specific strains for promoting stem elongation. In addition, chlorophyll content was significantly higher in the treated wheat than in the control.

Key words: PGPR, phytohormones, Indole 3-acetic acid , optimization. Rhizosphere

Résumé

Des études récentes ont mis en lumière l'effet des phytohormones synthétisées par les PGPR sur la croissance des plantes, en particulier l'AIA. La présente étude se concentre sur l'isolement de rhizobactéries productrices d'AIA et la vérification de leur potentiel dans la promotion de la croissance du blé. Parmi 61 bactéries productrices d'AIA, quatre isolats ont été sélectionnés. L'influence de la température, du pH et de la concentration en L-tryptophane sur la production d'AIA a été vérifiée. Les isolats sélectionnés ont également été testés pour leur capacité à améliorer la germination et la croissance du blé. Les paramètres suivants ont été mesurés : longueur des tiges et des racines, poids frais et sec des tiges et des racines, ainsi que la teneur en chlorophylle. Les résultats ont indiqué que les conditions optimales pour la production d'AIA étaient observées à différentes températures : 30°C pour l'isolat O3 (120 µg/ml) et 35°C pour les isolats F4 et P17 (120 µg/ml et 105 µg/ml respectivement). Les conditions de pH favorisant la production étaient pH 7 et 8, et la concentration de L-tryptophane à 800 µg/ml et 650 µg/ml. Les paramètres de croissance du blé ont été significativement améliorés avec le traitement bactérien par rapport au témoin. Tous les isolats sélectionnés ont significativement amélioré la croissance des tiges mais pas celle des racines, suggérant qu'ils peuvent être des souches spécifiques favorisant l'allongement des tiges. De plus, la teneur en chlorophylle était significativement plus élevée dans le blé traité que dans le témoin.

Mots-clés : PGPR, Phytohormones, Acide Indole-3-Acétique, Optimisation, Rhizosphère