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Title

Isolation and screening of antagonistic bacteria against phytopathogenic fungi and evaluation of their potential as biocontrol agents *in vitro* and *in vivo*

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NAIM

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BCAs: Biological Control Agents

ISR: Induced Systemic Resistance

SAR: Systemic Acquired Resistance

PGPR: Plant Growth-Promoting Rhizobacteria

FAWs: Fall ArmyWorms

IPM: Integrated Pest Management

EPNs: EntomoPathogenic Nematodes

VOC: Volatile Organic Compound

IPM: Integrated Pest Management

EPNs: EntomoPathogenic Nematodes

ANOVA: Analysis Of Variance

PBS: Phosphate-Buffered Saline

PCA: Plate Count Agar

CMC: CarboxyMethyl Cellulose

HCN: Hydrogen CyaNide

PDA: Potato Dextrose Agar

NH3: Ammonia

PGI: Percentage of fungal Growth Inhibition

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Introduction

Phytopathogenic fungi represent one of the most challenging threats to global agriculture, with over 8,000 documented species (Fisher *et al*., 2020) causing approximately 85% of all plant diseases (Isleib, 2012). These pathogens cause considerable economic losses and pose severe risks to global food security (Pennisi, 2010). Antifungal control measures are essential to mitigate these impacts, protect crop health, prevent pathogen infections, and maintain agricultural productivity. These methods belong to the category of phytosanitary products, commonly termed pesticides, which also include herbicides and insecticides as part of integrated plant protection strategies (Aumer, 2019).

Growing awareness of the environmental and human health risks associated with chemical pesticides has stimulated substantial research into sustainable alternatives for fungal disease management (Köhl *et al*., 2019). Modern integrated strategies now combine multiple approaches: (1) biological control agents, (2) development of pathogen-resistant cultivars, (3) cultural and prophylactic measures, (4) plant defense stimulators (such as chitin- and algae-derived elicitors), (5) nanofungicides, and (6) fungal phages (Moser *et al*., 2008).

Among these approaches, biological control has emerged as a promising strategy to mitigate plant pathogen damage. Researchers worldwide are actively developing biocontrol solutions to reduce agricultural dependence on chemical pesticides (Panth *et al*., 2020). Biological control involves the application of living organisms classified as Biocontrol Agents (BCAs) including bacteria, fungi, viruses, or their synergistic combinations, to manage plant diseases. These agents are applied to either the plant or soil to prevent pathogen infection or inhibit the establishment of pathogens in the plant. The targeted action of BCAs represents their principal advantage, exhibiting high specificity toward pathogenic organisms while maintaining minimal adverse effects on non-target species and the surrounding ecosystem (O'Brien, 2017).

Potential biocontrol agents include rhizosphere competent fungi and bacteria that not only exhibit antagonistic activity but also promote plant growth by inhibiting pathogens growth or producing growth stimulating compound (Anjaiah *et al*., 1998). Among these microorganisms, *Bacillus* and *Pseudomonas* bacteria isolated from the rhizosphere, have shown significant efficacy against a broad spectrum of fungal pathogens (Sevdalina and Lubka, 2009). Notably, *Bacillus* species display strong antagonistic effects through multiple

mechanisms, including hydrolytic enzyme production and nutrient competition (Panth *et al*., 2020).

Numerous studies have been published in recent years on the application of rhizospheric bacteria to enhance plant health and crop yields. This study aligns with that research direction, aiming to select local bacterial isolates demonstrating potential antagonistic activity against phytopathogenic fungi. Our work evaluates these isolates through both *in vitro* assays and *in vivo* testing on tomato fruits with particular emphasis on their modes of action as biocontrol agents.

The manuscript is divided into three parts:

- The first part is dedicated to a literature review, providing an introduction to phytopathogenic fungi, and biocontrol agents.
- The second part describes the methodology used in this study,
- The third part is devoted to results and discussion.

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

Chapter I: Phytopathogenic fungi

I.1. Generalities

I.1.1. Definition of phytopathogenic fungi

Phytopathology is the scientific study of plant diseases, their causes, and the mechanisms by which these diseases develop, spread, and can be controlled (Sharma, 2021). Phytopathogenic fungi are among the most destructive plant pathogens, causing devastating diseases that lead to massive yield losses in global agriculture (Chandrasekaran *et al*., 2016). They threaten food security and can trigger economic crises, ecosystem disruption, and even famine in vulnerable regions. These fungi employ diverse infection mechanisms that differentially affect host plants (Idnurm and Howlett, 2001). Through evolutionary adaptation, they have developed diverse colonization strategies, leading to outcomes that span mutualistic symbiosis to pathogenic virulence resulting in host mortality (Doehlemann *et al*., 2017). During infection, fungi produce virulent factors including polymer-degrading enzymes and secondary metabolites (Salvatore and Andolfi, 2021). Moreover, researchers have identified genes involved in: (1) infection structure formation, (2) cell wall degradation, (3) host defense evasion, (4) environmental sensing, (5) toxin production, and (6) signaling cascades (Idnurm and Howlett, 2001).

I.1.2. Taxonomy and classification

Mycologists face a daunting challenge: while an estimated 2.2 to 3.8 million fungal species exist globally (Hawksworth & Lücking 2017), only about 150,000 (5% to 7%) have been formally described. At the current discovery rate of approximately 2,000 species per year, completing this taxonomic inventory could require over 1,800 years (Cheek *et al*., 2020). Notably, many newly described species are plant pathogens, including significant crop diseases (Cheek *et al*., 2020). This vast undiscovered diversity ensures that taxonomic classifications and nomenclature will remain dynamic, posing persistent challenges for mycologists and plant pathologists engaged in fungal identification and systematics research.

I.1.3. Diversity and distribution

The global distribution of potential phytopathogenic fungi showed highest prevalence in the Indian Ocean (19.17%) followed by North America (11.51%), and Europe (11.25%). These fungi were also widely distributed across various land cover types, as well as in

specific habitats like air (40.37%), dust (24.16%), and plant shoots (20.42%) (Li *et al*., 2023). In addition, potential phytopathogenic fungi were detected in 12 different habitat types, such as soils, plant shoots and roots, rhizospheres, deadwood, air, sediment, litter, lichen, freshwater, top-soil, and dust (Koljalg, U. *et al*., 2005). Moreover, the global distribution of these fungi was largely influenced by climatic factors (Delgado-Baquerizo, M. *et al.* 2020).

I.2. Impact of phytopathogenic fungi on agriculture and food security

Phytopathogenic fungi significantly threatens global agriculture, causing up to 12% yield losses in total crop production (Crous *et al*., 2021). Their effects extend to food security, ecosystem services, and human health (Li *et al*., 2023). Temperature increases are driving their spread, with projections showing rising diversity and invasive potential by 2100 (Li *et al*., 2023), positioning these fungi as a growing climate-driven threat. (Salvatore *et al*., 2021).

These fungi impact ecosystems by altering abiotic factors including CO_2 levels, temperature, and soil water regimes (Abo Nouh and Abdel-Azeem, 2020). They modify host survival and host-pathogen interactions, potentially causing new diseases and changes in phytopathogen diversity through ecological niche variations. Consequently, they affect species distribution, temporal activity, community structure, host resistance, and mycotoxin production (Gavrilova *et al*., 2016).

A prominent example is *Claviceps purpurea*, the ergot fungus affecting rye, barley, oats, and wheat, which has historically impacted both agriculture and human culture.

I.3. Mechanisms of pathogenicity

Fungal pathogens are among the most significant causes of plant diseases. To successfully infect and colonize their hosts, they employ diverse pathogenic strategies. Necrotrophic fungi kill plant cells and derive nutrients from dead tissue, whereas biotrophic fungi establish long-term infections by growing within living host tissues. Successful infection often depends on the precise regulation of pathogenic development, including the formation of specialized infection structures. Additionally, fungal pathogens produce a range of virulence factors, adapted to their infection strategy, to facilitate host colonization and disease progression (Doehlemann *et al*., 2017).

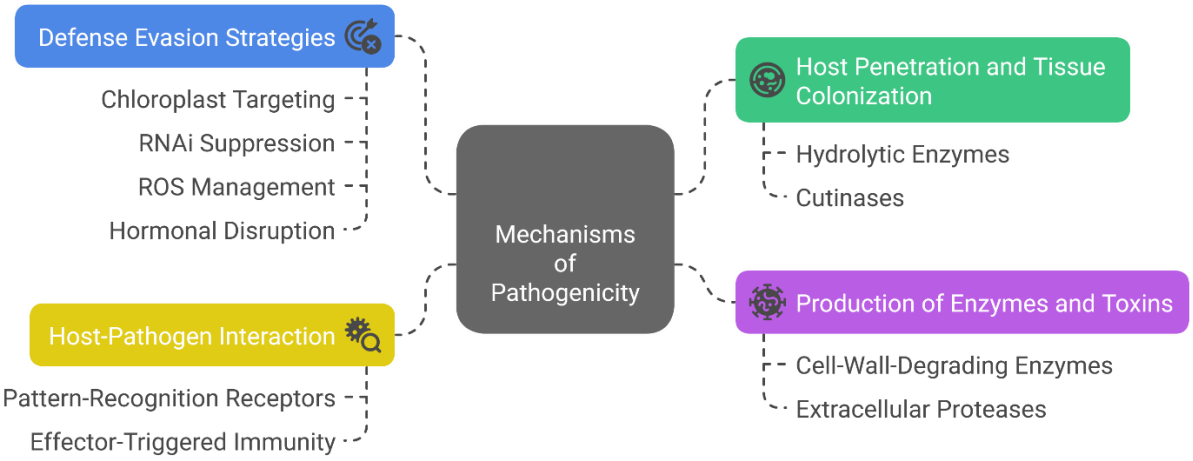

Figure 01: Mechanisms of pathogenicity

I.3.1. Fungal host attachment, penetration and tissue colonization

Fungal spores disperse via wind, water, or insect vectors to access host plants. Once on the surfaces, they adhere firmly through secretion of an adhesive extracellular matrix. This attachment is essential to prevent spore removal before host penetration can occur (Doehlemann *et al*., 2017).

All fungal pathogens disturb the plant's primary defenses, but their mechanisms differ. During infection, fungal pathogens produce various proteins and metabolites to facilitate host colonization (Li *et al*., 2019). Notably, secreted hydrolytic enzymes play a pivotal role by penetrating host tissues, breaking physical barriers, and degrading plant structures to release nutrients for disease progression. Among these, Cutinases, which degrade the plant cuticle, are recognized as key virulence factors in many fungal pathogens (Dickman and Patil, 1986).

I.3.2. Production of enzymes and toxins

The ability of pathogenic fungi to colonize their host plants depends critically on their production of cell wall-degrading enzymes, which are considered major virulence factors (Annis & Goodwin, 1997; Lebeda *et al*., 2001; Juge, 2006; Nakajima & Akutsu, 2014; Peng, 2021). These microorganisms also secrete extracellular enzymes that contribute to: (1) fungal growth, (2) infection structure formation, (3) plant cell wall lysis, (4) modification of pathogenesis-related proteins, and (5) induction of host defenses (Movahedi and Heale, 1990).

Fungal enzymes such as proteases appear crucial for multiple infection stages, including host cell attachment, early penetration of plant cell walls, and tissue colonization (Poussereau *et al*., 2001). The significance of these proteases is underscored by their positive correlation with pathogen aggressiveness and necrotic symptom severity (Doehlemann *et al*., 2017). Furthermore, fungi frequently produce multiple hydrolases that target the same plant substrate. This enzymatic redundancy enhances the efficiency of the hydrolytic complex and provides crucial metabolic adaptability (Riou *et al*., 1991).

I.3.3. Host-pathogen interaction and defense evasion in phytopathogenic fungi

Plants have natural defenses to resist pathogen infection and disease development. Their outer surface, composed of a thick cuticle and strong cell walls, constitutes a physical barrier against pathogenic fungi (Guest and Brown, 1997). When an attack occurs, the plant can detect microbial intruders and activate defense mechanisms. However, certain specialized fungi have evolved sophisticated evasion strategies, including the production of substances that neutralize the plant's defense system by targeting critical organelles like chloroplasts (Kretschmer *et al*., 2020). These attacks allow fungi to block defense mechanisms, prevent cell death in infected tissues, and reprogram host metabolism to favor their growth (biotrophic fungi). Some pathogens even manipulate the plant's hormonal system to create a favorable environment for their growth. These strategies highlight the remarkable adaptability of fungi, which effectively divert normal plant cell functions for their own benefit (Petre *et al*., 2016; Doehlemann *et al*., 2017; Xu *et al*., 2020).

I.4. Plant diseases caused by pathogenic pungi

Plant diseases caused by pathogenic fungi pose severe risks to global crop production, significantly reducing crop productivity and quality. These pathogens utilize multiple infection mechanisms, including the production of phytotoxic secondary metabolites that induce characteristic symptoms such as wilting, chlorosis, necrosis, and leaf spotting (Yin *et al*., 2016; Soyer *et al*., 2015; Li *et al*., 2017) (Figure 2). Among the most damaging manifestations is fruit rot, which particularly affects economically important crops such as melon (*Cucumis melo* L.) and strawberries, leading to substantial economic losses (Hong *et al*., 2022; Suwannarach *et al*., 2024).

Postharvest infections present additional challenges, as fungal pathogens cause rot and spoilage during transit, handling, and storage (Junior *et al.*, 2019). Losses affect 20 to 25% of produce in industrialized nations and even higher percentages in developing countries due to inadequate storage and transportation (Gomes *et al.*, 2015). Fungi dominate as postharvest pathogens, accounting for 80 to 90% of microbial-related losses, with major genera including *Fusarium* (Zakaria, 2023), *Botrytis cinerea*, *Penicillium* spp., *Colletotrichum* spp., and *Alternaria alternata* (Zhang *et al*., 2021).

The dissemination of fungal pathogens occurs through airborne conidia transported via rain splash and irrigation water (Zakaria, 2023). These infections severely degrade produce marketability by impairing quality and visual appeal (Suwannarach *et al*., 2024). Recent advances in molecular research have identified key virulence genes in these pathogens, clarifying their infection mechanisms and regulatory networks (Zhang *et al*., 2021). These findings provide crucial insights for developing novel postharvest disease management strategies.

Figure 02 : Symptoms of gray rot (Aumer, 2019)

Chapter II: Biocontrol agents

II.1. Biocontrol: Basic concepts and definitions

Biological control, or biocontrol, relies on the use of living organisms, including viruses, to suppress the population of damaging pests or weeds, ultimately offering advantages for environmental and human interests (O'Brien, 2017). In entomology, it refers to using living predatory insects or entomopathogenic nematodes to suppress various pathogenic insects. In phytopathology, it involves employing microorganisms to suppress plant diseases and control pathogenic weeds. The living organism used in this approach is termed a "biological control agent" (Pal and Gardener, 2006).

Despite historical divides between pathologists and entomologists, the term biological control has gained broad societal acceptance. Its positive reputation has since spurred industrial and scientific groups to extend the concept to encompass biologically derived agents and products beyond traditional biocontrol definitions (Gray *et al*., 2018; Santos *et al*., 2011). Over the past century, the methods and approaches associated with this practice have evolved, relying on diverse scientific and taxonomic fields (Stenberg, 2021).

The principles of biological control now extend far beyond agricultural applications, serving critical roles in food safety (Jordan *et al*., 2014) and medical innovations (Dedrick *et al*., 2019) and fecal microbiota transplants (van Nood *et al*., 2013), despite variations in discipline-specific terminology. Within agriculture, the global adoption of integrated pest management (IPM) frameworks has significantly increased reliance on biocontrol strategies (Stenberg, 2017). Contemporary approaches have evolved from simple antagonist introductions to active agroecosystem engineering through crop rotation, residue management, and other cultural practices to optimize indigenous beneficial species (Nigam and Mukerji, 2023). The operational unit, now uniformly termed a Biological Control Agent (BCA), reflects the evolution of technology from earlier labels like "Biopesticide" (Sharma, 2023).

II.2. Mechanisms of action of biocontrol agents

II.2.1. Competition for nutrients and space

Ecological competition arises when multiple species compete for limited resources such as nutrients, space, water, or light. In biological control systems, microbial agents often

compete with pathogens for these resources, particularly for nutrients (carbohydrates, nitrogen, oxygen, …) and infection/colonization sites (Howell, 2003; Whipps, 2004; Spadaro *et al*., 2010).

This competitive interaction presents an interesting dynamic with endophytic microorganisms. While endophytes are specifically adapted to thrive within plant tissues, they typically compete less effectively with external microorganisms in non-endophytic environments. However, microbial lifestyles exist on a continuum, and the same organism may function as an endophyte, epiphyte, or pathogen depending on environmental conditions (Collinge *et al*., 2022).

II.2.2. Antibiotic production

Antibiotic compounds represent a diverse class of low-molecular-weight organic molecules synthesized by microorganisms as secondary metabolites. These bioactive substances specifically target and disrupt the growth or metabolic processes of competing microorganisms (Nakatsuji *et al*., 2012).

The microbial world displays particular specialization in antimicrobial production. Filamentous actinomycetes have evolved as the most prolific and chemically diverse antibiotic producers in nature. Among unicellular bacteria, species belonging to the *Bacillus* and *Pseudomonas* genera dominate as the most prolific producers of antimicrobial compounds (Berdy, 2005).

II.2.3. Induction of systemic resistance

Induced systemic resistance (ISR) represents a crucial plant defense strategy exploited by beneficial bacteria in biocontrol applications (Kour *et al*., 2024). Unlike systemic acquired resistance (SAR), ISR is specifically triggered by plant-growth-promoting rhizobacteria (PGPR) through jasmonate- or ethylene-dependent signaling pathways upon root colonization (Mandal and Ray, 2011). This primed defense state enables plants to deploy enhanced physiological and metabolic responses against multiple pathogens, employing diverse structural and biochemical defenses (Messa, 2021).

Biological control agents (BCAs) operate through distinct mechanisms, categorized as either direct antagonism (via antibiosis, competition, or parasitism) or indirect ISR-mediated

protection (Raymaekers *et al*., 2020). The durability of ISR is particularly notable, as induced plants often maintain protection throughout most of their lifespan (Mandal and Ray, 2011).

II.2.4. Production of hydrolytic enzymes

Biological control agents produce a diverse array of hydrolytic enzymes that play crucial roles in pathogen suppression. Bacteria synthesize multiple vital enzymes including chitinases, cellulases, lipases, proteases, glucanases, and amylases (Saberi Riseh *et al*., 2024), while fungi like *Trichoderma harzianum* specifically induce chitinase, β-1,3-glucanase, and protease production when exposed to fungal cell walls (Schirmböck *et al*., 1994). Nematophagous fungi employ a similar enzymatic arsenal, with proteases, chitinases and lipases acting synergistically to degrade pathogen structures (Freitas Soares *et al*., 2023). *Bacillus* species demonstrate particular efficiency in hydrolytic enzyme production during fermentation, generating high enzyme concentrations using cost-effective culture media (Ajuna *et al*., 2023).

II.3. Biocontrol agents

II.3.1. Bacteria

Numerous bacterial genera, including *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Stenotrophomonas*, *Streptomyces*, and *Xanthomonas*, exhibit protective activity against fungal and bacterial plant pathogens (Montesinos *et al*., 2009). These beneficial bacteria employ multiple complementary biocontrol mechanisms (Lugtenberg *et al*., 2009). They actively colonize infection sites and competing with pathogens for space and nutrients. Simultaneously, they produce potent antimicrobial compounds such as antibiotics and cell wall-degrading enzymes, while also being capable of inducing plants' natural defense systems (Berendsen *et al*., 2012). Their broad spectrum of activity enables them to target various pathogens (McSpadden Gardener, 2004), which reduce pathogen populations on plant surfaces (Trias *et al*., 2008).

II.3.2. viruses

As the most abundant biological entities on Earth, viruses play a crucial ecological role in regulating organism populations (Wagemans *et al*., 2022). These obligate pathogens

exclusively replicate within their host insects, providing one of the most effective and sustainable pest control methods (Abd-Alla *et al*., 2020). Beyond insect control, bacteriophages show significant potential as biocontrol agents throughout food production chains, from field to post-harvest stages (Garvey, 2022). Viruses can also combat plant viral diseases, using attenuated virus strains to immunize plants against more virulent strains (Wagemans *et al*., 2022).

II.3.3. Fungi

Beneficial fungi produce a wide array of bioactive compounds that can be utilized as crop protection agents. The development of fungal strains as biocontrol agents has attracted growing interest, as these microorganisms have demonstrated the ability to inhibit various plant pathogens (Thambugala, 2020). Among the most studied, the genera *Trichoderma, Aspergillus* and *Penicillium* rank among the most effective biological fungicides against both bacterial and fungal plant diseases (Farzand *et al*., 2019). Other species such as *Gliocladium* and *Saccharomyces* also exhibit antagonistic properties against several pathogens (Poveda, 2021). *Trichoderma* genus comprises filamentous fungi that are ubiquitous in soils with diverse lifestyles, as saprophytes, plant symbionts or mycoparasites. These organisms have been extensively researched and applied as biocontrol agents in agriculture (Degani *et al*., 2021).

II.3.4. Predatory insects

Numerous studies demonstrate the remarkable efficacy of predatory insects in agricultural pest control. The mirid bugs *Nesidiocoris tenuis* and *Macrolophus pygmaeus*, when established on pepper crops, effectively reduce both adult and immature populations of *Frankliniella occidentalis* and *Bemisia tabaci* (Bouagga *et al*., 2018). Among other biological control agents, parasitoid wasps (Hymenoptera) are widely employed against agricultural and forest pests (Wang *et al*., 2019). Similarly, *Dicyphus maroccanus* reduces *Tuta absoluta*-infested leaves by over 90% compared to control groups (Abbas *et al*., 2014).

Predatory dipterans represent another significant resource, with field studies showing that generalist predators (single species or species assemblages) significantly reduce pest

levels in approximately 75% of cases (Symondson *et al*., 2002). Their omnipresence across all terrestrial ecosystems, including Antarctica, underlines their potential (Burgio *et al*., 2025).

II.3.5. Entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) are highly effective against fall armyworms (FAWs) (Fallet *et al*., 2024). Moreover, various species of EPNs, including *Steinernema spp.* and *Heterorhabditis spp.*, have been discovered to be fairly effective and possess substantial potential to manage cutworms. As bacto-helminth parasites, EPNs share mutualistic relationships with *Xenorhabdus* and *Photorhabdus* bacteria (Tomar *et al*., 2022). Their safety and precision against pathogens make them valuable biocontrol tools (Abd-Elgawad, 2025). Studies highlight its promising potential in managing major insect pests (Zyl and Malan, 2014), with optimized formulations offering a sustainable and economical alternative to pesticides for FAW control (Fallet *et al*., 2024). Currently, EPNs paired with their bacterial symbionts are central to biocontrol and integrated pest management (IPM) (Tomar *et al*., 2022).

II.3.6. Predatory mites

Soil mites represent a highly diverse group, including predators, scavengers, and prey. Among them, predatory soil mites are often polyphagous and prey upon multiple pest species, which makes them promising and adaptable natural enemies (Berndt *et al*., 2004). For instance, the predatory mite *Parasitus bituberosus* Karg surpassed *Hypoaspis aculeifer* in greenhouse trials on onion plants, reducing *Thrips tabaci* populations by nearly 80% when released at high densities (Castro-López and Martínez-Osorio, 2021).

However, a key limitation in utilizing predatory soil mites is the difficulty in studying them. Assessing their population densities is particularly challenging, as current methods produce highly inconsistent results (Sabu *et al*., 2011; Knapp *et al*., 2018).

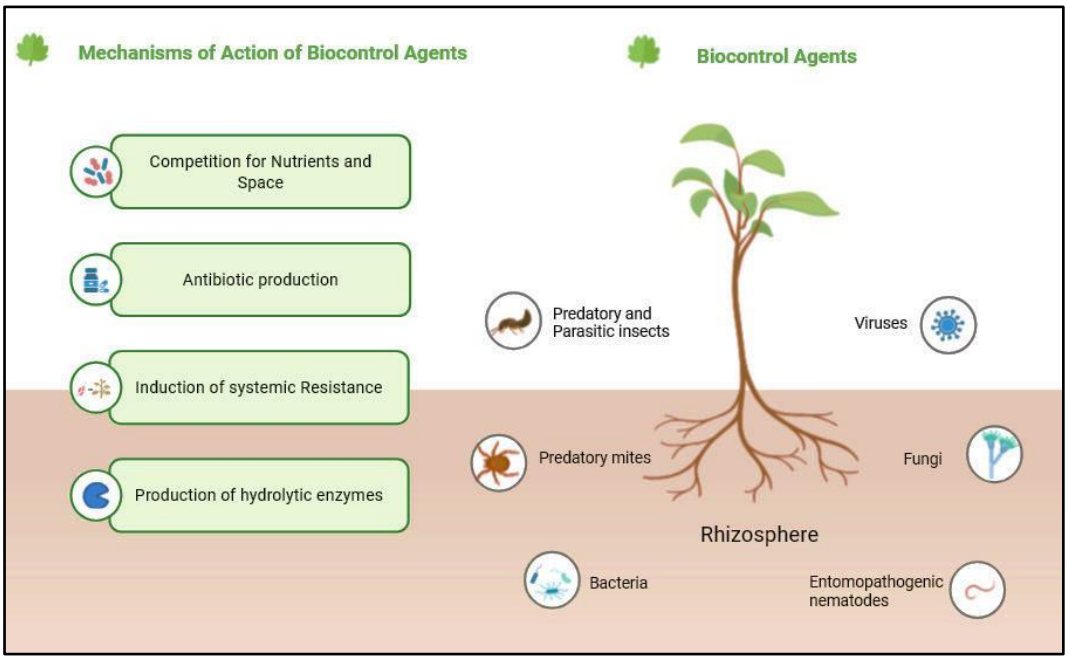

Figure 03: Biocontrol agents

II.5. Applications of biocontrol

Chemical fertilizers (N, P, K) continue to play a vital role in global food production (McGuire *et al*., 2015), yet their environmental consequences highlight the need for sustainable approaches like biocontrol. Biocontrol agents employ four primary mechanisms: enzymatic degradation of pathogen cell walls through chitinases, β-1,3-glucanases, proteases and lipases; iron limitation via siderophores; competitive exclusion in the rhizosphere; and induction of plant systemic resistance (Parani *et al*., 2012; Köhl *et al*., 2019).

Post-harvest rotting of fruits and vegetables is a major source of agricultural losses, with fungal pathogens being particularly problematic due to their ability to produce damaging mycotoxins (Sharma *et al*., 2009; Dwiastuti *et al*., 2021). To combat these problems, biocontrol strategies have emerged as effective solutions. Field application of antagonistic micro-organisms enables early colonisation of fruit surfaces, offering preventive protection against pathogens (Ippolito and Nigro, 2000). These microbial antagonists have a dual function: they control both plant diseases and product contamination (Sellitto *et al*., 2021).

Research over the last three decades has established that post-harvest BCAs are viable alternatives to synthetic pesticides (Droby *et al*., 2016). In plant protection, BCAs offer a promising alternative to chemical inputs, efficiently reducing the severity of many crop

diseases (Ayaz *et al*., 2023). Modern agricultural systems are increasingly incorporating microbial biostimulants, which are more effective and have a lower environmental impact than synthetic agrochemicals (Jaiswal *et al*., 2022). These microbial technologies contribute significantly to biodiversity conservation and ecosystem services, which are fundamental to sustainable development (Mamy *et al*., 2022).

II.6. Advantages and limitations of biocontrol versus chemical control

II.6.1. Advantages

Biocontrol offers significant advantages over conventional chemical methods, considering environmental and human health impacts (Hulot and Hiller, 2021). Its major advantage derives from the total absence of toxic residues in agricultural products (Pertot *et al*., 2017). Unlike chemical pesticides, this approach actively preserves soil microbial biodiversity, thus ensuring the balance of rhizosphere ecosystems (Gerbore *et al*., 2013). Scientific studies confirm the dual performance of biocontrol: effective crop protection and biodiversity enrichment due to the drastic reduction of chemical inputs (Hulot and Hiller, 2021). This sustainable solution is a radical departure from traditional pesticides, whose harmful effects on the environment and public health are widely documented (Gerbore *et al*., 2013).

In addition to biocontrol through bacterial agents, bacteriophages offer innovative perspectives, due to their targeted action, their capacity for self-reproduction, and their synchronized evolution with their pathogenic hosts (Aldayel *et al*., 2019). These unique characteristics position these biological agents as promising solutions for ecosystem-based agriculture (Velivelli *et al*., 2014).

While several effective BCAs are currently available, further research is needed to identify novel microorganisms and their bioactive compounds with broad-spectrum antagonistic effects against phytopathogens (Ayaz *et al*., 2023).

II.6.2. Limitations

The application of BCAs is still limited, due to no-uniform effects under field conditions (Gerbore *et al*., 2013). In addition, Biocontrol agents present technical limitations such as high specificity, sensitivity to environmental conditions and difficulties in stabilizing formulations characteristics that paradoxically reinforce their ecological acceptability (Powell and Jutsum, 1993). Their variable effectiveness in the field, influenced by pedoclimatic contexts and patho-systems (Massart *et al*., 2015; Pertot *et al*., 2017), requires adapted approaches including rigorous testing protocols in real conditions (Velivelli *et al*., 2014) and optimization of formulations. These constraints do not invalidate the potential of biocontrol but highlight the need for a systems approach and in-depth research to reconcile operational efficiency and ecological sustainability.

Experimental part

Materials and methods

I. Methods

I.1. Soil sampling

Two soil samples were collected in February 2025, from agricultural fields located in Bejaia: Akbou (36°27'45"N 4°30'52"E) and Djebira village (36°42'15"N 5°04'34"E).

Figure 04: Sampling locations

The sampling was conducted in the root zones (rhizospheres) of cultivated crops to ensure the collection of root-associated microbial communities. The soil samples were carefully transferred into sterile vials to prevent contamination and immediately transported to the laboratory for further analysis (Figure 06).

Figure 05: Soil samples

I.2. Isolation and purification of soil bacteria

To make a uniform soil solution, one gram of each soil was suspended in nine milliliters of Phosphate-Buffered Saline (PBS) (Appendix 1). One milliliter of each solution was serially diluted from 10^{-1} to 10^{-7} in the medium. To ensure homogeneous microbial dispersion, 1 ml of each dilution was plated onto Plate Count Agar (PCA) (Appendix 2) using the flooding technique. The plates were then incubated at 30°C for 24 hours. After incubation, distinct colonies were selected for purification through successive subcultures (Figure 07), to obtain a pure microbial isolate for further analysis.

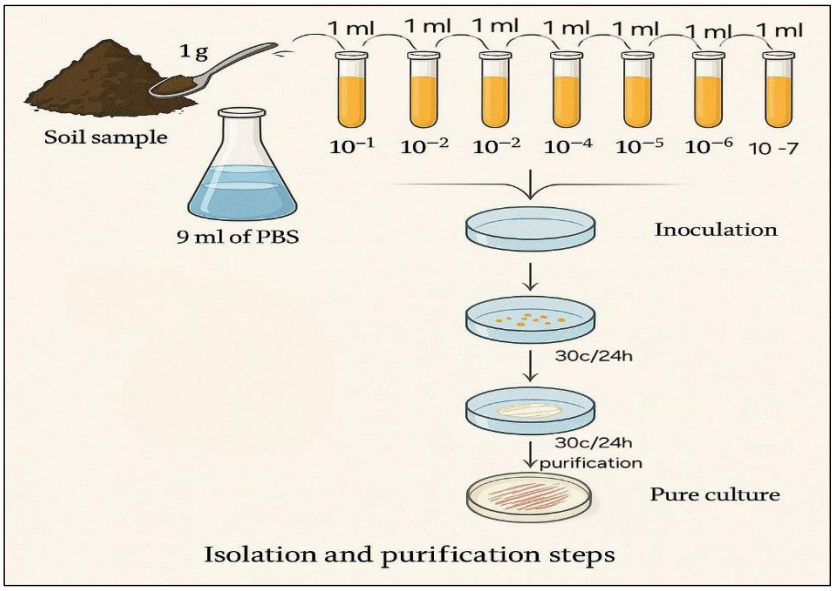

Figure 06: Steps of isolation and purification of rhizobacteria

I.3. Antifungal activity of bacterial isolates

Antifungal activity of bacterial isolates was evaluated using an *in vitro* dual-culture assay adapted from Saddiki (1999). A 7 mm mycelial disc of the target fungus, obtained from a 24-48h old culture, was placed at the center of a Tryptic Soy Agar (TSA) plate (Appendix 2). In each Petri dish, two distinct bacterial isolates were tested simultaneously by inoculating two spots of 24-hour bacterial cultures equidistantly (2.5 cm from the center) around the fungal disc (Figure 08). This square configuration ensured direct interaction between each bacterial strain and the developing fungus.

Negative control plates (fungal disc alone, without bacteria) were prepared in parallel. All plates were incubated at 25 ± 2°C for 5 to 7 days, with duration adjusted according to the fungal species' growth rate. The test was performed in duplicate to ensure reproducibility.

After incubation, fungal growth inhibition was measured using the following formula:

$$PGI(\%) = \frac{KR - R1}{KR} X100$$

Where:

KR: Distance (in mm) from the fungal inoculation point to the colony edge in the control plate.

R1: Distance (in mm) from the fungal inoculation point and the colony edge of the colony in the plate test.

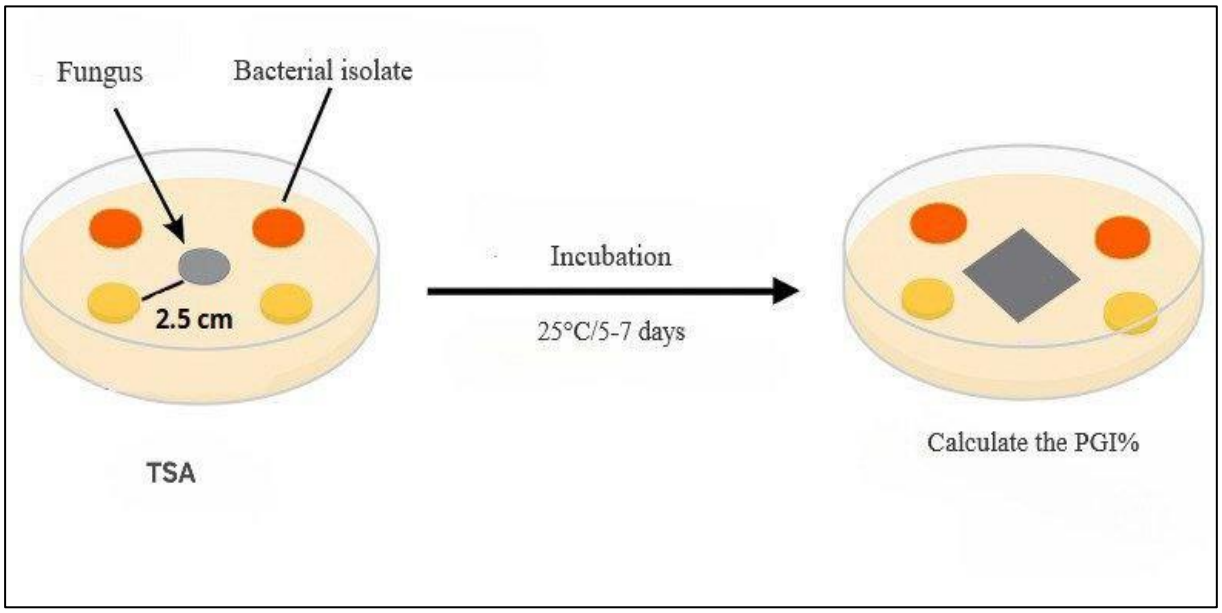

Figure 07: Fungal growth inhibition assay

I.4. Enzymatic tests

I.4.1. Proteolytic activity

The proteolytic activity of bacterial isolates was assessed using a medium containing the following components (in g/L): pancreatic casein (5.0), yeast extract (2.5), glucose (1.0), and agar (15.0), with the pH adjusted to 7.0. Prior to pouring the autoclaved medium into Petri dishes, 100 mL of a sterile 10% (w/v) skim milk solution was aseptically added to the cooled medium (~50°C). Bacterial strains were spot-inoculated onto the solidified milk-casein agar

surface. After 48 hours of incubation at 30°C, proteolytic activity was indicated by the formation of clear hydrolysis zones around bacterial colonies (Bach and Munch, 2000).

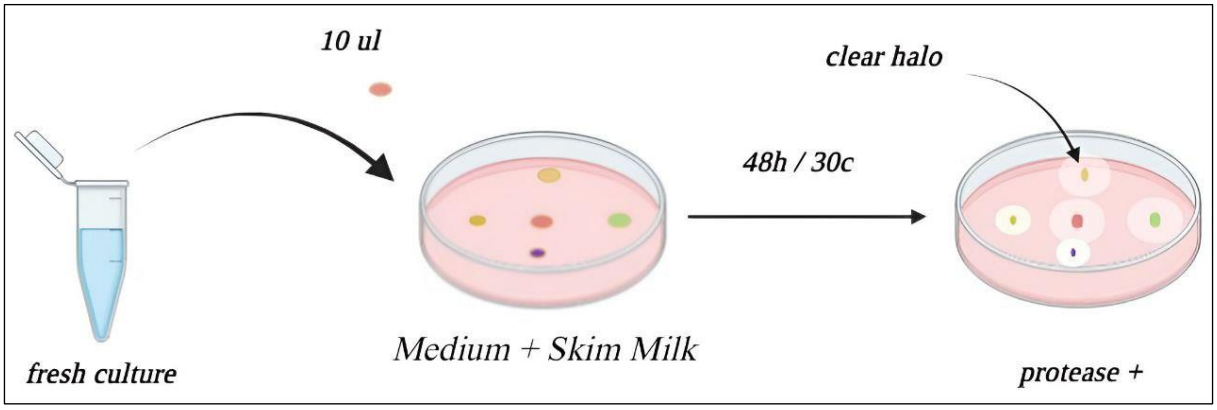

Figure 08: Proteolytic activity assay

I.4.2. Urease activity

The test medium was prepared by combining the following components in 950 mL distilled water: peptone (1 g), glucose (1 g), NaCl (5 g), Na_2HPO_4 (1.2 g), KH_2PO_4 (0.8 g), phenol red (0.012 g), and agar (15 g). The pH was adjusted to 6.8. After sterilization and cooling to approximately 50°C, the medium was supplemented with 50 mL of a sterile 40% (w/v) urea solution. After inoculation and incubation, urease-positive microorganisms were identified by the development of a distinct pink halo around colonies, indicating urea hydrolysis (Christensen, 1946).

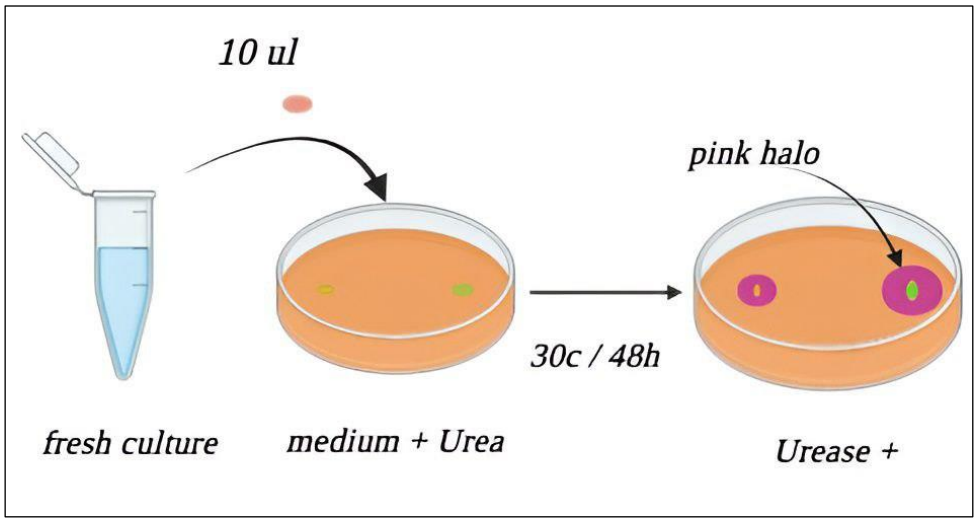

Figure 09: Urease activity assay

I.4.3. Esterase and lipase activity

The methodology used was described by Sierra (1957) and Carrim *et al*. (2006). The isolates were spot inoculated in a medium containing in g/L: peptone (10), NaCl (5), $CaCl_2 \cdot 2H_2O$ (0.1), and agar (18), supplemented with 1% sterilized Tween 80 for esterase activity or Tween 20 for lipase activity. The pH was adjusted to 7.4. After incubation at 30°C for 48 hours, the presence of an opaque halo surrounding the colonies (Figure 11) indicated enzymatic activity.

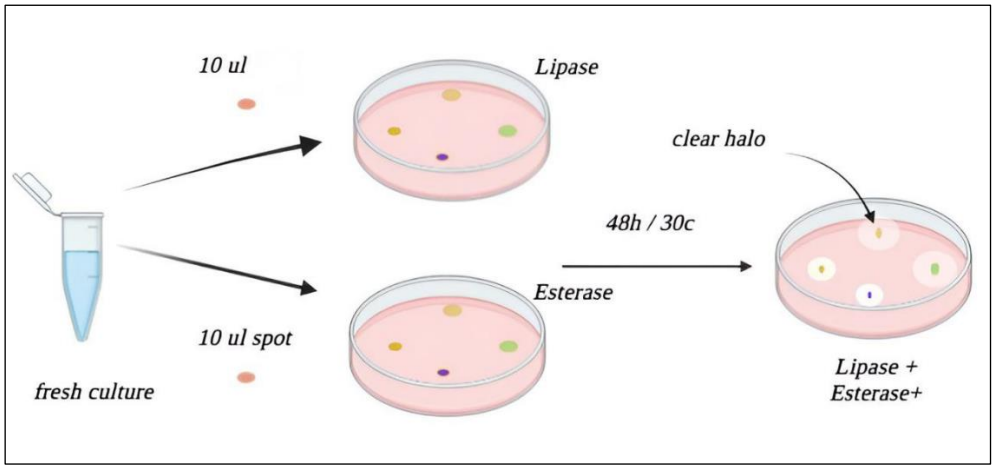

Figure 10: Esterase and lipase activity's assay

I.4.4. Cellulolytic activity

The isolates were inoculated onto carboxymethyl cellulose (CMC) agar containing in g/L: Na_2HPO_4 (6), KH_2PO_4 (3), NaCl (0.5), NH_4Cl (1), yeast extract (3), CMC (7), and agar (15). The cultures were incubated at 30°C for seven days. To visualize the carboxymethyl cellulose hydrolysis zone, the agar medium was flooded with 1% Congo red solution for 20 min then washed with 1 M NaCl and kept overnight. Clear zones around the colonies indicate extracellular cellulase production (Figure 12) (Carder, 1986).

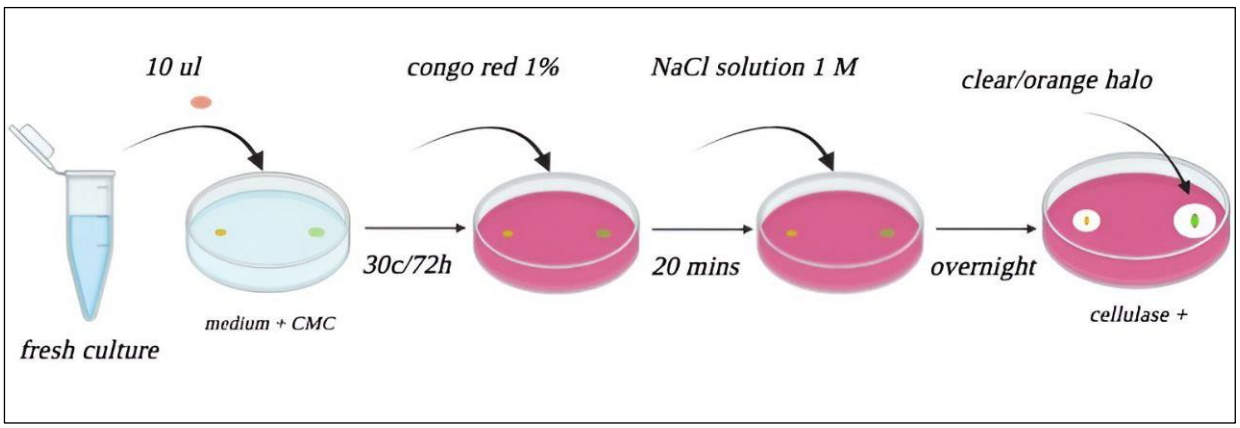

Figure 11: Cellulolytic activity assay

I.4.5. Amylolytic activity

The isolates' ability to hydrolyze soluble starch was assessed using starch agar medium containing the following components (in g/L): KNO_3 (0.5), KH_2PO_4 (1), $MgSO_4$ (0.2), $CaCl_2$ (0.1), $FeCl_3$ (0.001), soluble starch (10), and agar (15), with a final pH of 7.2. After inoculation, the cultures were incubated at 30°C for 72 hours. The medium surface was flooded with Lugol's iodine solution (containing 1 g iodine, 2 g KI, and 300 mL distilled water). After a few minutes, the excess solution was discarded, and the plates were rinsed with distilled water. A clear zone around the colonies indicates starch-degrading activity (Figure 13) (Singh *et al*., 2016).

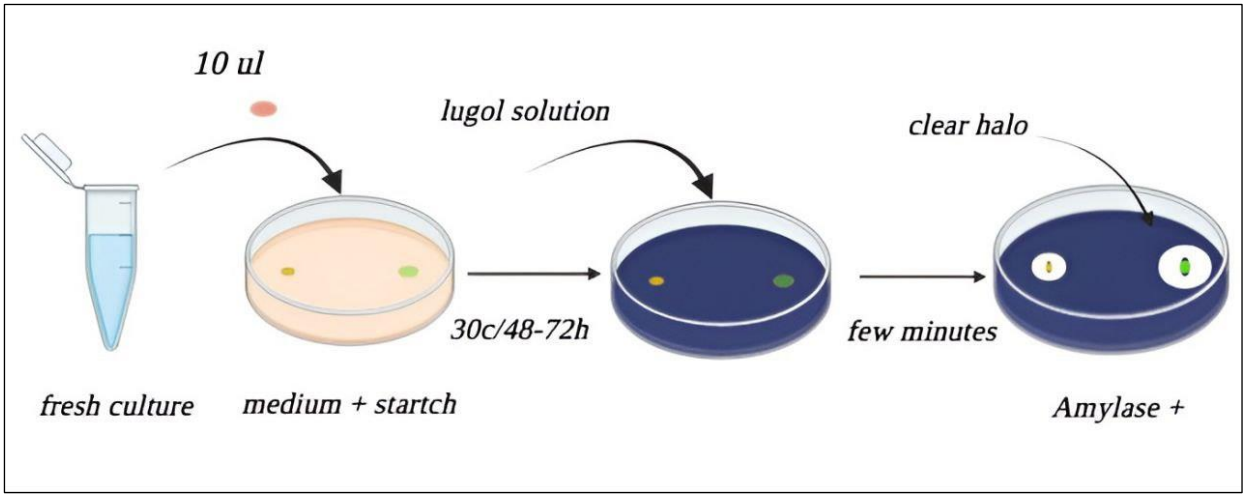

Figure 12: Amylolytic activity assay

I.5. Bioactive compounds with antifungal properties

I.5.1. Production of hydrogen cyanide (HCN)

Hydrogen cyanide (HCN) production by bacterial isolates was investigated using a modified version of Lorck (1948) method. Nutrient agar (Appendix 2) was supplemented with glycine at a concentration of 4.4 g/L. Bacterial isolates were streaked onto the agar surface. To detect HCN emission, a Whatman No. 42 filter paper disc was soaked in a sodium picrate solution (5% picric acid and 2% anhydrous sodium carbonate). The impregnated disc was carefully placed inside the lid of each Petri dish without contacting the agar. The plates were then sealed with parafilm to prevent volatile compound leakage and incubated at 30°C for 96 hours. During incubation, any HCN released by the bacteria reacted with the sodium picrate on the filter paper. A positive reaction was indicated by a color change of the paper from yellow to varying shades of orange, reddish-brown, or dark brown, depending on the HCN concentration produced (Figure 14).

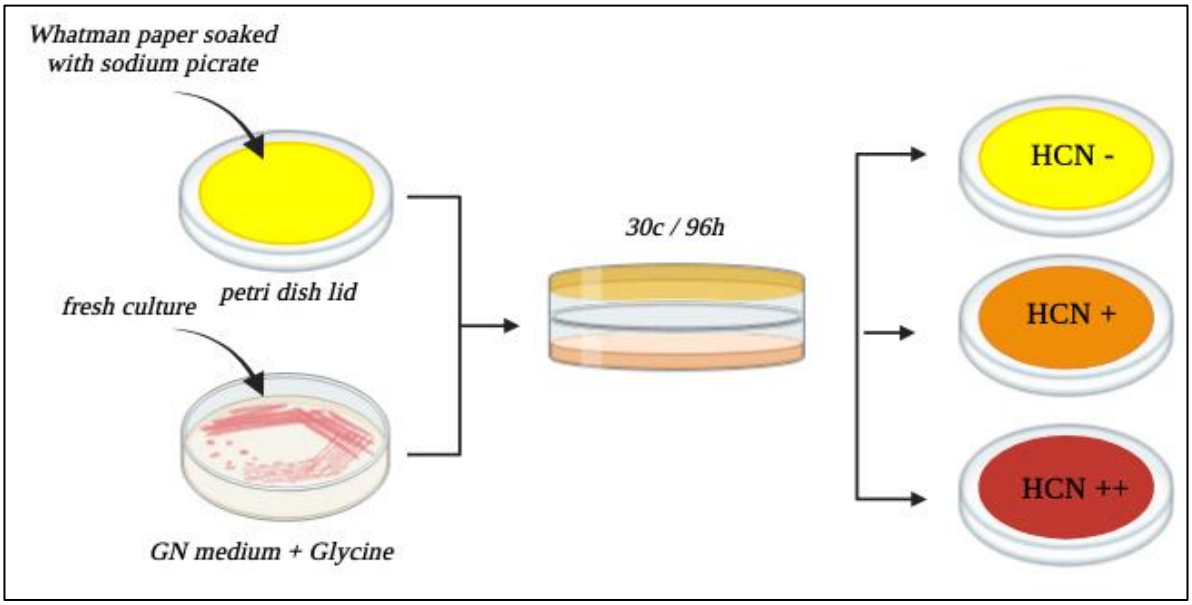

Figure 13: Production of hydrogen cyanide (HCN)

I.5.2. Ammonia (NH3) production

In this test, 1ml of peptone water was inoculated with 10 µl of bacterial suspension. Following 96 hours of incubation at 30°C**,** 25 µl of Nessler's reagent was added to each Eppendorf tube. Ammonia (NH_3) production was indicated by the development of a yellow-to-orange color (Figure 15). This protocol was adapted with minor modifications from Cappuccino and Sherman (1992)**.**

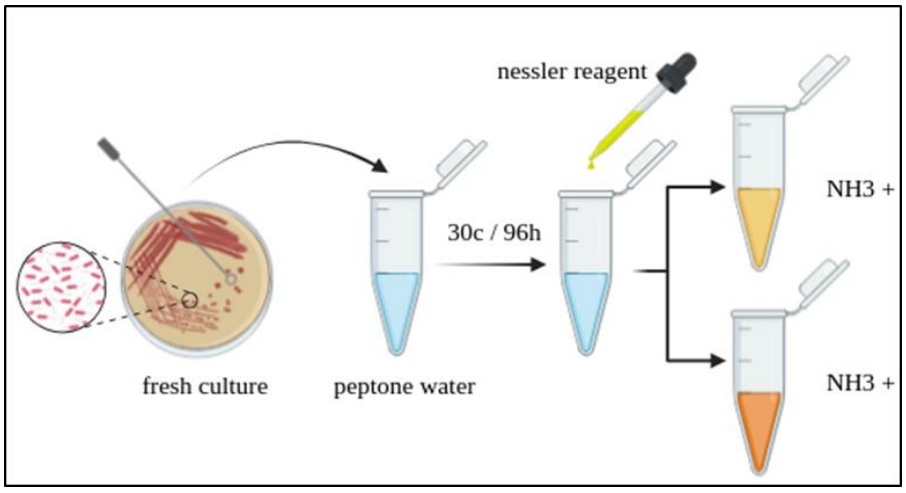

Figure 14: Production of ammonia (NH3)

I.6. Detection of antifungal volatile organic compounds (VOCs)

The antifungal activity of volatile compounds produced by our best bacterial isolates was tested using a dual-plate assay. A 7-mm diameter agar disc of *Aspergillus niger* was placed in the center of a glass Petri dish containing potato dextrose agar (PDA) (Appendix 2). This plate was then placed upside-down over another glass Petri dish (of equal diameter) containing PCA medium (Appendix 2), previously inoculated with bacterial isolates. The two dishes were carefully aligned, sealed with parafilm, and incubated at 25 ± 2°C for 5–7 days (Figure 16) (Fiddaman, 1993).

The percentage of fungal growth inhibition (PGI %) was calculated using the formula:

$$PGI(\%) = \frac{a - b}{a} X100$$

Where:

- **a**: Mean mycelial diameter in the control plate (cm)
- **b**: Mean mycelial diameter in plate containing bacterial isolate (cm)

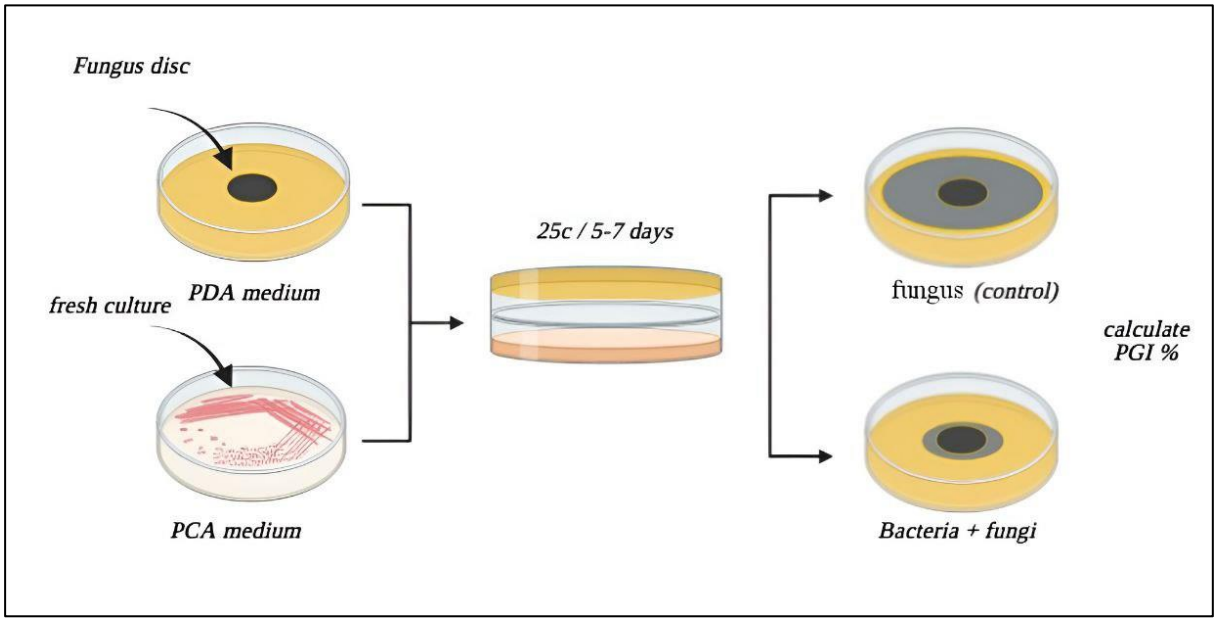

Figure 15: Dual-plate assay for antifungal VOCs detection

I.7. *In vivo* antifungal activity assay

I.7.1. Pretest for host selection

To identify the optimal host for antifungal activity assay, fresh, ripe, and undamaged produce (apples, potatoes, garlic, cucumbers, and tomatoes) were selected. The samples were

rinsed with sterile distilled water to remove residues, then disinfected by immersion in a 1% sodium hypochlorite solution for 2 minutes (Shankar *et al*., 2024), followed by three sterile water rinses. Standardized artificial lesions (2–3 mm deep) were created using a sterile needle (Sanzani *et al*., 2016), then inoculated with 10–20 µL of a spore suspension (DO=0.05) (*Penicillium* sp., *Fusarium sp*., or *Aspergillus niger*). The inoculated produce was incubated at 25°C (to optimize fungal growth) in disinfected, humidified boxes at controlled relative humidity for 4-6 days.

This preliminary test revealed that *Aspergillus niger* exhibited optimal mycelial development on tomatoes, with rapid and uniform lesion colonization. The tomato/*A. niger* combination was therefore selected for subsequent trials, providing a reproducible model to evaluate antifungal treatment efficacy.

I.7.2. *In vivo* antifungal activity assay on tomato fruits

This protocol was adapted from Bautista-Baños *et al*. (2006) and Tabli (2018) to evaluate the biocontrol potential of three bacterial isolates selected based on their in vitro antifungal activity against *Aspergillus niger* (Figure 17).

The test was performed as follows.

- **Host preparation**
    a. Select fresh, ripe, undamaged tomatoes.
    b. Clean with sterile distilled water, disinfect with 1% sodium hypochlorite (2 min), and rinse three times with sterile water.
    c. Create two artificial lesions (2–3 mm deep) per fruit.

- **Inoculation**
    a. Add 30 µL of bacterial suspension (10^8 cells/ml) to each lesion.
    b. Incubate for 2 hours at room temperature (sterile conditions).
    c. Add 15 µL of *Aspergillus niger* spore suspension.

- **Controls**
    a. **Control 1**: Bacterial suspension (30 µL) + sterile saline solution (15 µL)
    b. **Control 2**: Sterile saline solution (30 µL) + fungal spores (15 µL)
    c. **Control 3**: Sterile saline solution (30 µL + 15 µL)

- **Incubation**

Maintain at 25°**C** and high relative humidity in disinfected containers for 4–6 days.

To demonstrate the protective effect of the isolates, tomatoes were photographed and lesion areas were measured using ImageJ software.

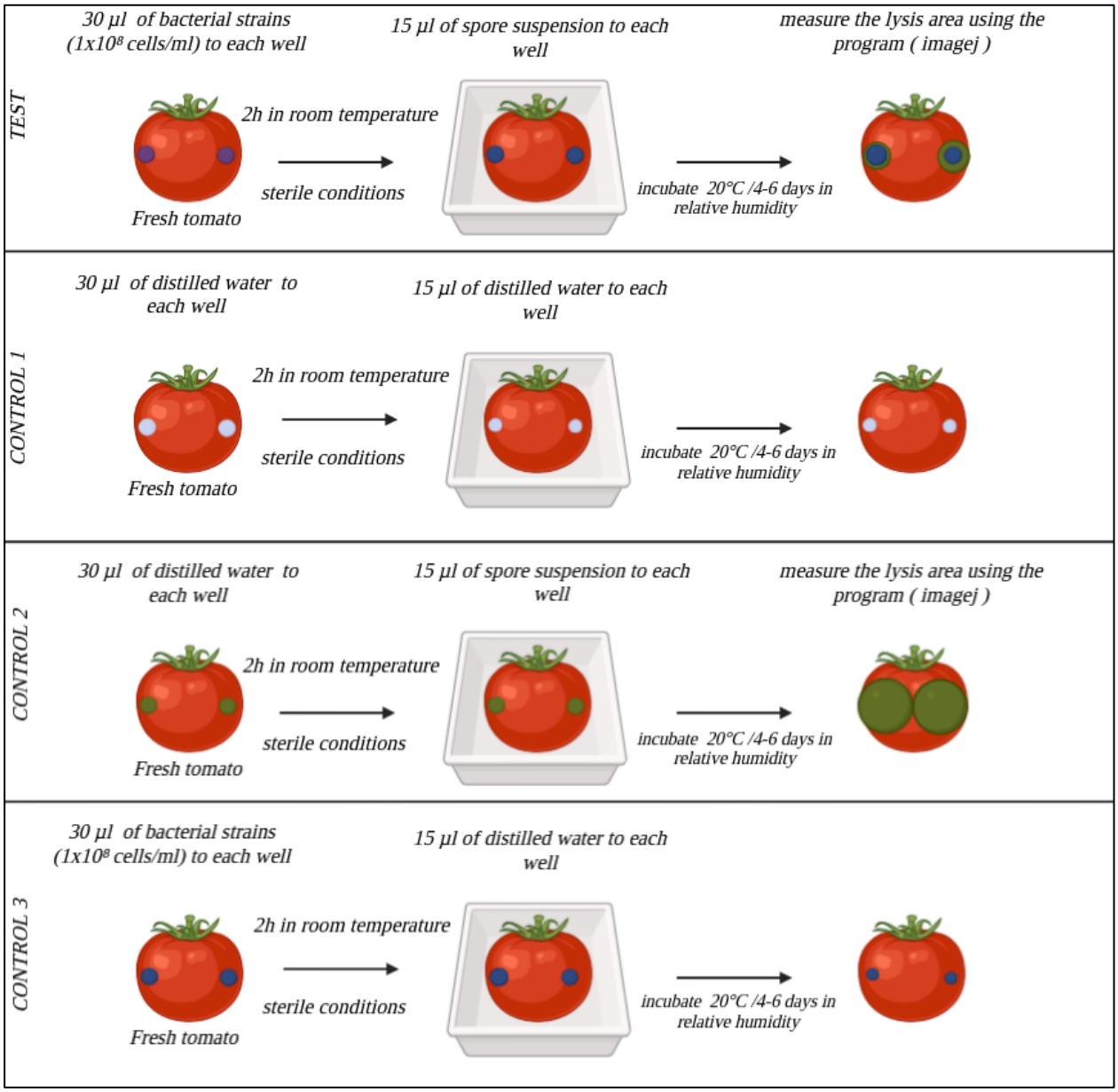

Figure 16: *In vivo* antifungal activity assay

I.8. Statistical analysis

An analysis of variance (ANOVA) is performed using GraphPad Prism® software version 6.01. If significant differences are detected, a multiple comparison test of means (LSD) is performed.

Results and discussion

I. Isolation, purification and screening of rhizobacteria

Bacterial colonies cultured on PCA medium were macroscopically examined, revealing notable morphological diversity in size, shape, color, and texture (Figure 17). From rhizospheric soil samples collected at two sites Akbou and Djebira village, 34 morphologically distinct colonies were isolated and purified. Akbou isolates were designated N1–N12, while those from Djebira were labeled. L1 to L22. To maintain viability for subsequent analyses, each purified isolate was transferred into Eppendorf tubes containing nutrient broth and stored at 4°C.

The significant morphological diversity observed among our isolates reflects the ecological complexity of rhizospheric environments. This finding aligns with established literature demonstrating that root-associated soils are rich in microbial communities, sustained by diverse nutritional inputs from plant root exudates (Andy *et al*., 2020; Verma and Pal, 2020).

According to Güler and Öğütcü (2024), the rhizosphere serves as a hotspot for microbial activity, harboring diverse bacteria with potential plant growth-promoting (PGP) and biocontrol properties.

As demonstrated by Verma and Pal (2020), preliminary morphological characterization as performed in this study is a critical first step in differentiating bacterial isolates prior to biochemical, enzymatic, and antagonistic screening. The substantial morphological diversity observed among our isolates suggests that the studied rhizospheric soils contain functionally versatile bacteria, which may hold promise for sustainable agriculture and biocontrol applications.

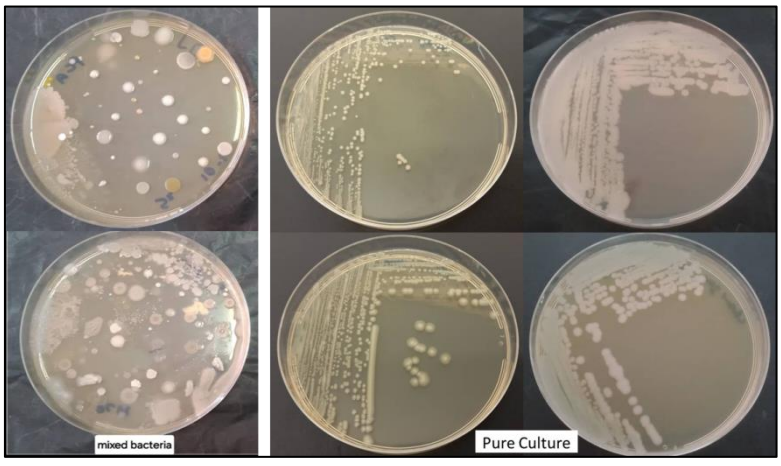

Figure 17: Isolation and purification of Rhizobacteria

II. Antifungal activity of bacterial isolates

Following a 7-day incubation period, most isolates exhibited antifungal activity against the tested fungi (*Aspergillus niger*, *Penicillium* sp. and *Fusarium* sp.), with varying percentages. The results obtained are illustrated in the following graphs and figures.

II.1. Against *Aspergillus niger*

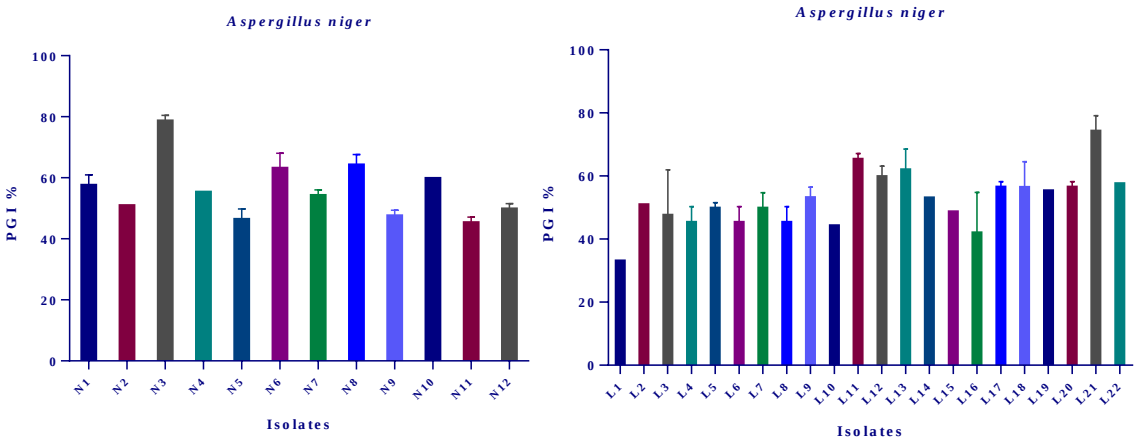

Figure 18: Inhibition percentages of bacterial isolates against *Aspergillus niger*

The results reveal significant variability in antifungal activity among the tested isolats, with inhibition percentages ranging from 33.33% to 80%. Isolates N3 (77.78–80%) and L21 (71.11–77.78%) exhibited the strongest inhibitory effects, demonstrating their potential as highly effective biocontrol agents. Other isolates, such as L1 showed moderate activity (33.33) while N8, N6, L11, L12, and L13 displayed robust antifungal performance, with PGI values reaching up to 60%.

II.2. Against *Fusarium* sp.

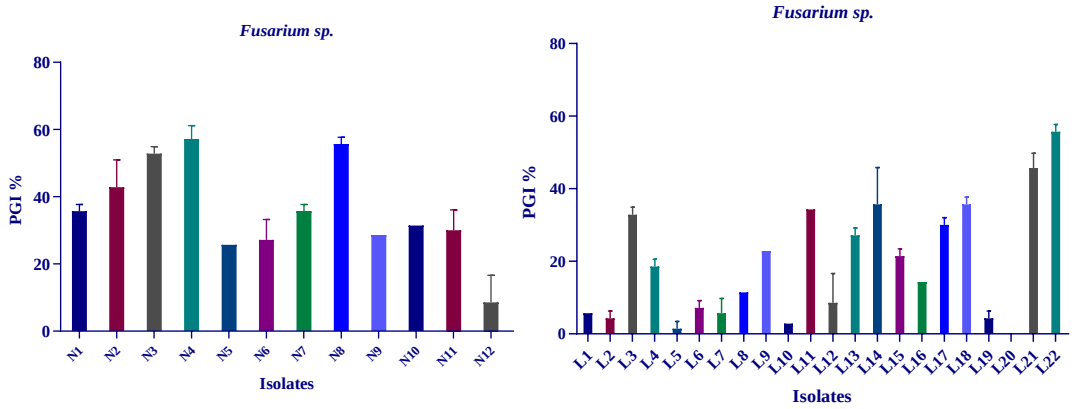

Figure 19: Graphs of antagonistic results against *Fusarium* sp.

The evaluation of the effect of the 34 bacterial isolates on the *in vitro* growth of *Fusarium* sp. during the direct confrontation test highlights variability in their antagonistic activity. While some bacteria showed no inhibitory effect, others induced over 55% inhibition of mycelial growth.

The percentage of growth inhibition (PGI%) varied widely, ranging from 0% to 60%. Notably, isolates N4 (54.29–60%), N8 (54.29–57.14%), N3 (51.43–54.29%), and L22 (54.29–57.14%) displayed the highest antifungal activity. In contrast, isolates such as L1, L2, L5, and L10 showed minimal inhibition (0–6%), while others, including L14, L18, and L21, exhibited moderate activity, with PGI values between 30% and 48%.

II.3. Against *Penicillium* sp.

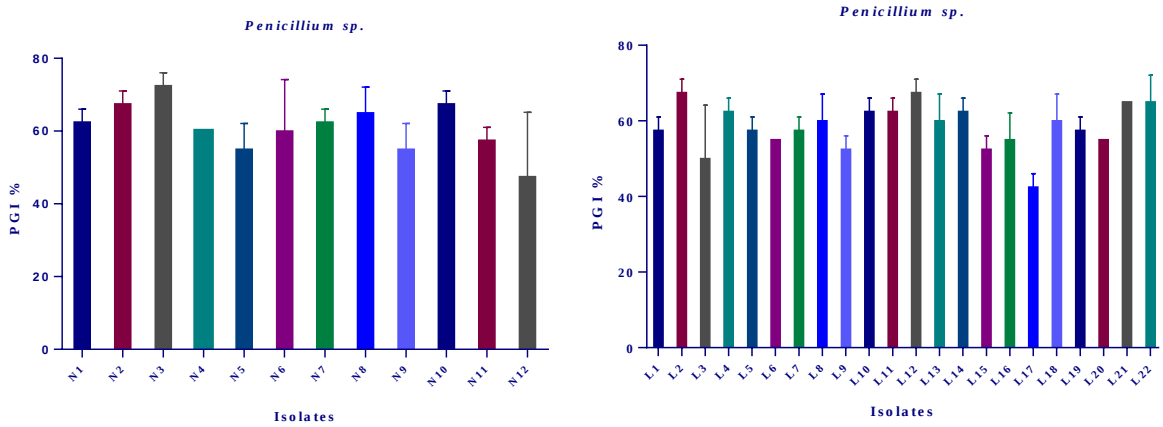

Figure 20: Graphs of antagonistic results against *Penicillium* sp.

The 34 rhizobacterial isolates (N1–N12 and L1–L22) exhibited varying levels of antagonistic activity against *Penicillium* spp., with percent growth inhibition (PGI%) ranging from 35% to 75%. The isolates N3 (70–75%), N2 (65–70%), L12 (65–70%), and L2 (65–70%) showed the highest inhibition percentages. The majority of other isolates, including N10, N8, L4, L10, L11, and L22, also demonstrated strong antifungal activity, consistently achieving PGI values above 60%.

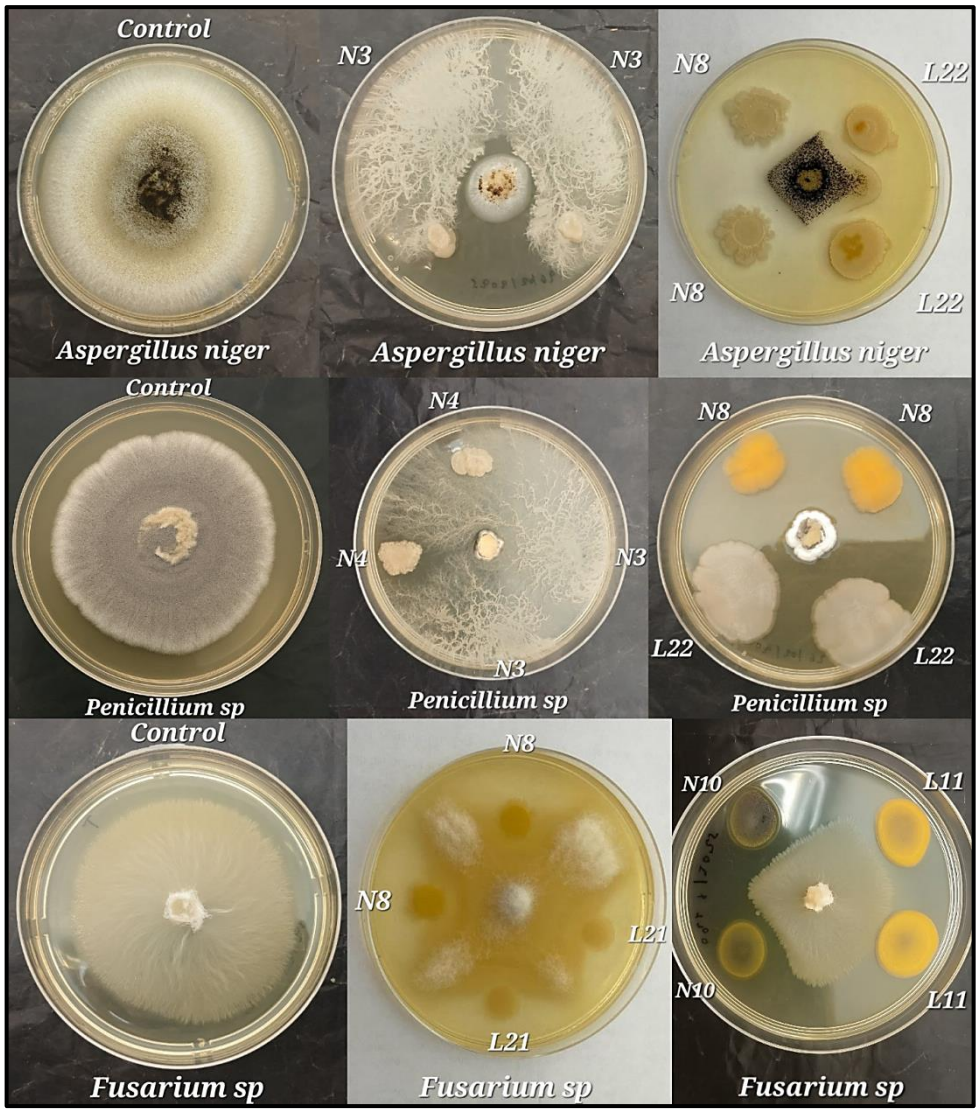

Figure 21: Antagonistic effect of selected isolates on the tested phytopathogenic fungi

Rhizosphere bacteria have demonstrated significant antagonistic activity against phytopathogenic fungi. Studies by Rakotoarimanga *et al.* (2014) revealed that 24 soil-derived actinomycetes suppressed *Fusarium* sp. growth by 14–60%, while Yuttavanichakul *et al.* (2012) identified 11 antifungal rhizobacterial isolates exhibiting 17.98–67.81% growth inhibition against *Aspergillus niger* (PGI%).

Figueiredo *et al.* (2025) have reported that Their greenhouse and field experiments showed that seed treatment with *Bacillus velezensis* CNPMS-22 isolated from rhizospheric soil, reduced *Fusarium* symptoms in plants and increased maize productivity.

A weaker overall antifungal effect was observed against *Fusarium* sp. compared to *Aspergillus niger*, likely due to the higher innate resistance of *Fusarium* species. Our study

revealed deformations and inhibition of pathogenic fungi mycelial growth similar to those reported by Al-Nadabi *et al*. (2021), specifically localized around active biocontrol isolates.

In contrast to our results with *Fusarium* sp., which exhibited generally moderate to weak inhibition (mostly below 55%), the activity against *Penicillium* sp. was significantly stronger. This suggests that the bacterial isolates employ a broader or more effective mode of action against *Penicillium*, likely due to this genus' greater sensitivity to diffusible bioactive compounds.

The observations by Kjeldgaard *et al*. (2022) that certain bacterial isolates exhibit broad-spectrum antifungal activity in plate-based co-inoculation assays are supported by our consistently high Percentage Growth Inhibition (PGI) values against the tested fungi.

The antagonistic action of the isolates does not appear to be pathogen-specific; in some cases, it may be broad-spectrum, simultaneously affecting all tested fungi.

Several mechanisms have been proposed to explain the inhibition of phytopathogenic fungi growth by bacteria including the production of antibiotics, the secretion of hydrolytic enzymes, competition for nutrients and space, or through toxin production, predation, parasitism, and induced host resistance (Compant *et al*., 2005). The production of antifungal compounds and competitive ability against pathogens are the two most frequently cited mechanisms in biocontrol research (Kjeldgaard *et al*., 2022; Soliman *et al*., 2022).

The variation in inhibition intensity among these isolates may be attributed to either: the nature and quantity of compounds produced by the antagonistic bacteria, or the conditions governing antifungal metabolite production (culture medium composition, pH, incubation temperature, and incubation duration).

Based on our recent findings, several bacterial isolates (N3, N4, N8, L21, and L22) demonstrate excellent broad-spectrum antagonistic activity, positioning them as prime candidates for future development as multipurpose biocontrol agents.

III. Enzymatic tests

Following assessment of our rhizobacterial isolates, we observed antagonistic activity against *Aspergillus niger*, *Fusarium* sp., and *Penicillium* sp. using *in vitro* confrontation assays. The percentage growth inhibition (PGI) was calculated to evaluate the antifungal potential. To

elucidate the underlying mechanisms, we focused on the production of extracellular lytic enzymes, known to degrade fungal cell walls and play a crucial role in biological control.

The production of enzymes (amylase, protease, esterase, lipase, urease, and cellulase) was assessed through standardized enzymatic assays. Beyond their established role in biocontrol, these enzymes contribute significantly to biofertilization, nutrient cycling, and plant growth promotion, demonstrating their multifunctional agricultural value.

The enzymatic profiles of the isolates showed significant variation, as demonstrated in Figure 22 and Table 01. This diversity in enzyme production patterns may contribute to their differential biocontrol efficacy against various phytopathogens.

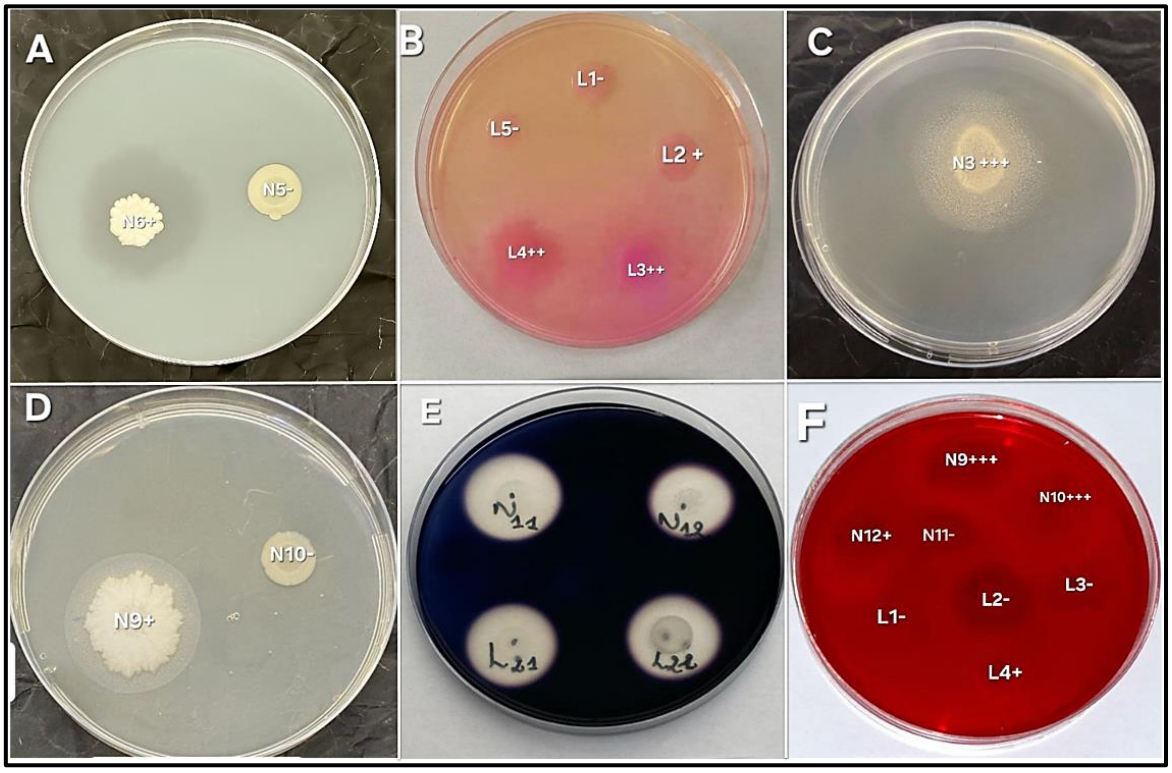

Figure 22: Enzymatic test results

A: Protease; B: Urease; C: Lipase; D: Esterase; E: Amylase; F: Cellulase

Table 01: Results of enzymatic tests

isolates	Amylolytic	Proteolytic	Esterase	Lipase	Ureolytic	cellulolytic
N1	-	+	-	+	++	-
N2	++	+	-	+	-	+
N3	++	++	+	+++	++	-
N4	++	-	-	+	-	+
N5	++	-	+	-	+	-
N6	+	+	+	+	-	-
N7	+	+	-	+	-	+
N8	+++	+	+	+	+	-
N9	+	+	++	+	-	+++
N10	++	+	+	+	-	+++
N11	++	+	+	+	-	-
N12	++	+	+	-	-	+
L1	-	+	-	+	-	-
L2	-	-	-	-	+	-
L3	-	-	-	-	++	-
L4	++++	+	-	+	++	+
L5	-	+	+	+	-	-
L6	-	-	-	+	-	-
L7	-	-	-	-	+	-
L8	+	-	-	-	-	-
L9	-	+	+	+	+	+++
L10	+	-	+	-	-	-
L11	++	+	-	-	-	-
L12	+	-	-	-	-	-
L13	+	+	-	-	+	++
L14	-	+	-	+	-	++
L15	+	-	-	-	-	-
L16	+	+	+	+	-	+++
L17	++	+	-	-	+	+
L18	+	+	-	-	+	+++
L19	+	+	+	+	-	+++
L20	-	-	-	-	-	+++
L21	++	+	+	+	-	-
L22	++	+	+	+	-	+++

-: 0 mm; +: diameter between 1 and 3 mm; ++: diameter between 3 and 6 mm; +++: diameter between 9 and 12 mm; ++++: more than 12 mm .

III.1. Proteolytic activity

A significant proportion of isolates from both bacterial groups (N and L) demonstrated substantial protease activity (Table 01), a trait commonly associated with antagonistic potential and plant growth promotion, as evidenced by our proteolytic enzyme assays. Among the tested isolates, fourteen of twenty-two in group L showed positive results. In the N group, only N4

and N5 tested negative, with 10 of 12 isolates exhibiting protease production, notably isolate N3, which demonstrated particularly strong activity (++).

These results suggest that protease production is a common trait among our rhizobacterial isolates and may contribute to the observed antifungal activity, as evidenced by earlier antagonistic tests and high PGI% values. Proteases are known to enhance biocontrol by degrading fungal cell walls and/or suppressing pathogenicity mechanisms (Zain *et al*., 2019).

Our results align with prior studies highlighting the role of proteolytic enzymes in fungal inhibition. As demonstrated by Nurikhsanti *et al*. (2024), all bacterial isolates from the peanut rhizosphere produced protease, with one isolate exhibiting a notably high proteolytic index, consistent with our observations. However, their work revealed that high protease activity did not always correlate with strong antifungal effects, indicating that antagonistic efficacy depends on multiple factors beyond a single enzyme system. In our study, while elevated protease levels in most isolates likely enhance fungal cell wall degradation, this trait probably acts synergistically with other mechanisms, such as siderophore biosynthesis, antibiotic secretion**,** or the synthesis of additional enzymes, to improve overall biocontrol effectiveness (Nurikhsanti *et al*., 2024).

Furthermore, these protease-producing isolates not only suppress pathogens but also promote plant growth and health. As Plant Growth-Promoting Rhizobacteria (PGPR), they can release proteases and other extracellular enzymes as part of a broader strategy to colonize roots and outcompete pathogenic microorganisms (Khalifa *et al*., 2022). Many of the tested isolates exhibit both protease activity and antagonistic effects against harmful pathogens, reinforcing their potential as biocontrol agents for sustainable agriculture and other applications. Their broad-spectrum efficacy is further highlighted by consistent activity against multiple fungal genera (*Aspergillus*, *Fusarium*, and *Penicillium*), suggesting the need for deeper investigation in both greenhouse and field conditions.

III.2. Urease activity

The urease activity test was conducted to evaluate the soil isolates' ability to hydrolyze urea through urease enzyme production, a process that releases ammonia and modifies pH. The results revealed varying response levels: a strongly positive reaction (++ or +++) indicated by a large pink halo with rapid and intense color change; a moderately or weakly positive reaction

(+) showing a small pink zone with delayed and slight color change; and finally, a negative reaction (-) with no color change to pink.

Several tested isolates exhibited varying levels of urease activity. In the L series, isolates L2, L3, L4, L7, L9, L13, L17, and L18 showed consistently positive reactions, while in the N series, isolates N1, N3, N5, and N8 demonstrated positive responses (++ or +). These urease-positive isolates clearly produced the enzyme, suggesting adaptation to urea-rich environments such as fertilized soils or areas with active organic matter decomposition. However, most isolates in both series were urease-negative (-), indicating either an inability to metabolize urea or enzyme expression below detectable levels.

Urease enzyme is commonly found in numerous bacterial species, including *Proteus*, *Klebsiella*, and specific isolates of *Bacillus* and *Pseudomonas* (Mobley *et al*., 1995). Urease activity often reflects adaptation to ecological niches with urea inputs, such as agricultural soils (Kumar *et al*., 2018). These urease-positive bacteria play a vital role in the nitrogen cycle by converting urea into ammonia, a readily available nitrogen source for plants (Modolo *et al*., 2015).

III.3. Lipase activity

Significant differences in lipase activity were observed among the tested bacterial isolates. In the N group, isolate N3 exhibited the highest activity (+++), while most other isolates (N1, N2, N4, N6–N11) showed moderate activity (+). Only two isolates (N5 and N12) displayed no detectable lipase activity. Similarly, within the L group, isolates L1, L4, L5, L6, L9, L14, L16, L19, L21, and L22 demonstrated significant lipase production, whereas the remaining isolates showed no activity.

These results indicate that the majority of the isolates possess at least some lipolytic capacity, which likely contributes to their antagonistic effects against phytopathogenic fungi. Given the abundance of lipids in fungal cell membranes, lipase-mediated hydrolysis of these lipids may disturb membrane integrity, potentially inhibiting fungal growth and proliferation.

The lipolytic enzymes produced by most of our tested isolates play a significant role in biocontrol activity, as demonstrated by previous studies on endophytic and rhizospheric bacteria. These extracellular enzymes, including lipases, cellulases, chitinases, and proteases substantially enhance antifungal activity by degrading key structural components of fungal cell

walls and membranes. (Admassie *et al*., 2022). These findings support the hypothesis that lipase production serves as a key antagonistic mechanism against the tested phytopathogenic fungi. Our results demonstrate that a substantial proportion of the examined isolates exhibited lipolytic activity, consistent with this proposed mode of action. Furthermore, the ecological relevance of these observations is reinforced by well-documented reports of lipase production by environmental and plant-associated bacteria, a trait known to enhance both environmental adaptation and biocontrol potential (Khan *et al*., 2022).

III.4. Esterase activity

The esterase activity assays revealed a moderate, variable distribution among the tested rhizobacterial isolates from both soil samples. In the N group, isolate N9 exhibited the strongest activity (++), while N3, N5, N6, N8, N10, N11, and N12 showed weaker but detectable (+) activity. The remaining N-group isolates were esterase-negative. Within the L group, esterase production was limited to only seven isolates (L5, L9, L10, L16, L19, L21, and L22), while the majority showing no detectable activity.

These findings suggest that while esterase production is less prevalent than lipase activity among the same isolates, several isolates possess significant esterolytic potential. This enzymatic capability may contribute to their antifungal activity through hydrolysis of ester bonds in fungal cell structures, potentially enhancing their biocontrol efficacy against phytopathogenic fungi.

The production of esterase enzymes by rhizobacteria holds significant ecological importance, as it contributes to both soil organic matter modification and nutrient cycling processes while simultaneously exerting direct antagonistic effects against fungal pathogens (Fashogbon *et al*., 2021).

III.5. Amylolytic activity

The amylolytic assay evaluated the ability of soil bacterial isolates to hydrolyze starch through amylase production, as indicated by clear zones surrounding colonies on iodine-stained agar plates.

Strong amylase production (++ to +++) was observed in N-series isolates: N2-N5, N8, N10 and N12, with N8 exhibiting particularly high activity (+++). Notably, L4 showed exceptional enzymatic capacity (++++), while L11, L17, L21, and L22 displayed moderate

activity (++). Weak starch degradation (+) was detected in N6, N7, N9, L8, L10, L12, L13, L15, L16, and L19. No detectable amylolytic activity (-) was found in N1, L1-L3, L5-L7, L9, L14, or L20.

The diverse patterns of amylolytic activity observed among these soil bacterial isolates reflect significant functional diversity. Particularly in carbohydrate-rich environments like plant rhizospheres or organic matter decomposition sites, highly amylolytic isolates likely play a crucial role in ecosystem carbon cycling (Pandey *et al*., 2020). The ecological strategy of amylase-producing bacteria as key decomposers is fundamentally linked to their prevalence in soil ecosystems. These microorganisms play an essential role in depolymerizing plant-derived polysaccharides, thereby driving nutrient cycling processes (Souza *et al*., 2015).

The absence of detectable amylolytic activity in some isolates may indicate either: a true genetic deficiency in starch-degrading capacity, or conditional expression requiring specific environmental signals not present in assay conditions (DeAngelis *et al*., 2015).

III.6. Cellulolytic activity

Cellulase screening revealed distinct degradation patterns among bacterial isolates, only eleven isolates demonstrating cellulose-depolymerizing capability. Six isolates (N9, N10, L9, L13, L17, and L18) exhibited strong cellulolytic activity (+++ or ++), while five others (N2, N4, N7, N12, and L4) showed moderate to low activity (+). The majority of isolates (N1, N3, N5, N6, N8, N11, L1, L3, L5, L8, L10, L12, L14, L16, L19, and L22) displayed no detectable cellulase production under the tested conditions.

Cellulolytic bacteria play a crucial role in terrestrial carbon cycling through their ability to degrade cellulose, the primary structural component of plant cell walls. This enzymatic capacity is particularly vital in the rhizosphere, a hotspot for plant-derived organic matter (Zhang *et al*., 2023).

Isolates like N9, N10, and L17 with high cellulolytic activity can probably generate effective enzymes that improve cellulose breakdown. The observed variability in activity levels can be explained eather by genetic polymorphisms within cellulase gene clusters or by adaptive regulatory mechanisms influenced by substrate availability (Mattam *et al*., 2021).

These findings have significant ecological implications, as cellulolytic bacteria play a vital role in enhancing soil fertility through organic matter decomposition and subsequent nutrient mineralization (Yang *et al*., 2014).

IV. Hydrogen cyanide (HCN) and ammonia (NH3) production

The results of HCN and ammonia production are shown in the table below.

Table 02: Results of HCN and NH3 tests

Isolate	HCN	NH3
N1	-	+
N2	-	+
N3	+	+
N4	-	-
N5	-	+
N6	-	++
N7	+	+
N8	+++	++
N9	-	-
N10	-	+
N11	-	+
N12	-	+
L1	-	-
L2	+	+
L3	-	++
L4	+	++
L5	-	-
L6	-	+
L7	-	+
L8	-	+
L9	-	-
L10	-	++
L11	-	+
L12	-	+
L13	-	-
L14	-	-
L15	-	-
L16	-	-
L17	-	-
L18	-	-
L19	-	-
L20	-	-
L21	-	++
L22	-	-

-: no production; +: low production; ++: moderate production; +++: very high production

IV.1. Hydrogen cyanide (HCN) production

The hydrogen cyanide (HCN) production assay revealed isolate-dependent variation in cyanogenic capacity (Figure 23). Among the tested isolates, isolates L2, L3, N4, and N8 demonstrated positive HCN production, with N8 exhibiting particularly strong activity (+++). In contrast, the majority of isolates (L1, L3, L5, L22, N1, N2, N4, N7, N9, N12) showed no detectable HCN synthesis under the tested conditions.

Several bacterial species, particularly plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas* spp. and *Bacillus* spp., are known to produce hydrogen cyanide (HCN) as an effective biocontrol mechanism against phytopathogens (Compant *et al*., 2005). The notably high HCN production by isolate N8 suggests strong antimicrobial potential, consistent with previous studies demonstrating HCN-mediated suppression of fungal pathogens including *Rhizoctonia* and *Fusarium* species (Anand *et al*., 2020).

The absence of HCN production in most isolates (L1, L5, and L9 to L22) may indicate non-pathogenic or non-competitive traits, as HCN is typically associated with antagonistic microbial interactions (Blom *et al.*, 2011). Genomic characterization could verify whether these isolates lack the hcnABC biosynthetic cluster essential for HCN production (Laville *et al*., 1998).

Notably, isolate N8 emerged as a particularly promising biocontrol agent due to its robust HCN synthesis. Subsequent research should evaluate its *in vivo* efficacy against phytopathogens, including both greenhouse and field trials to assess its agricultural potential.

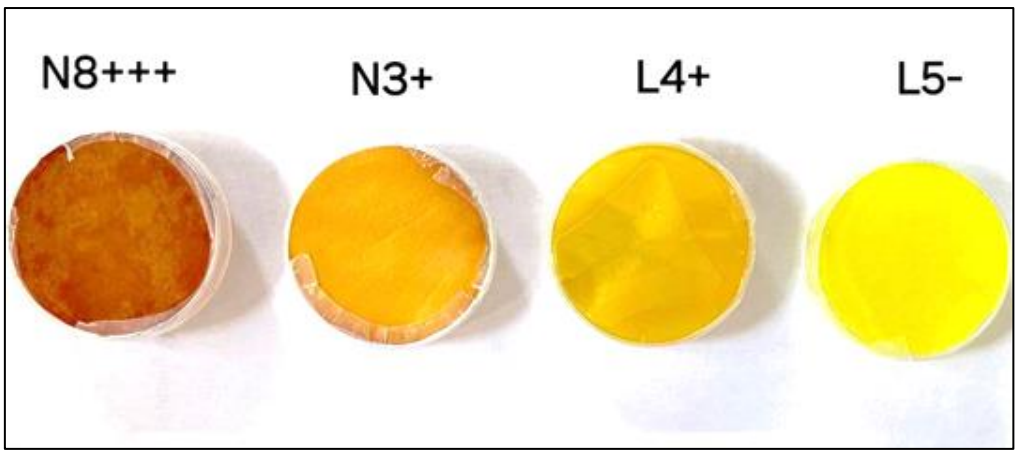

Figure 23: Hydrogen cyanide (HCN) results

IV.2. Ammonia (NH3) production

The rhizosphere-derived bacterial isolates (L and N series) exhibited distinct ammonia production profiles. Among L-series isolates, L3, L4, L10, and L21 demonstrated high production (++), while L2, L6, L7, L8, L11, and L12 showed moderate levels (+). The remaining L-series isolates (L1, L5, L9, L13–L20, and L22) produced no detectable ammonia (−). In contrast, ammonia production was more prevalent in the N series: isolates N6 and N8 displayed robust activity (++), N1, N2, N3, N5, N7, N10, N11, and N12 exhibited moderate production (+). Only N4 and N9 lacked this capability entirely.

The observed differential ammonia production between L and N series isolates suggests distinct ecological strategies and metabolic specializations. The significantly higher prevalence of ammonia-producing isolates in the N series (10/12, 83.3%) compared to the L series (10/22, 45.5%) likely reflects evolutionary adaptations to different nitrogen-cycling niches within the rhizosphere. Consistent with current literature, these ammonia-producing rhizobacteria play a vital role in soil nitrogen mineralization processes, particularly in agricultural ecosystems (Chinthalapudi *et al*., 2025).

The strong ammonia producers (L3, L10, L21, N6, N8) likely possess efficient amino acid deamination pathways or specialized nitrogen metabolism enzymes. Since ammonia is known as crucial and very important source of nitrogen for many plants, these isolates may be especially useful for promoting plant growth (Plunkett *et al*., 2020). Many L series isolates (L1, L5, L9, L13-L20, L22) do not produce ammonia, which supports alternate nitrogen metabolism methods that may concentrate on nitrogen conservation or alternative nitrogen transformation routes (Haskett *et al.*, 2022).

From an ecological perspective, the coexistence of both HCN-producing and non-producing isolates across the two series suggests functional complementarity within soil microbial communities. This metabolic diversity enhances ecosystem resilience, ensuring the maintenance of nitrogen cycle processes under fluctuating environmental conditions (Rijavec and Lapanje, 2016). The great reaction diversity observed in the L-series isolates may reflect adaptive strategies to fluctuating nitrogen availability, whereas the consistently higher ammonia (NH_3) production capacity of the N-series isolates suggests specialization for nitrogen-rich soil environments.

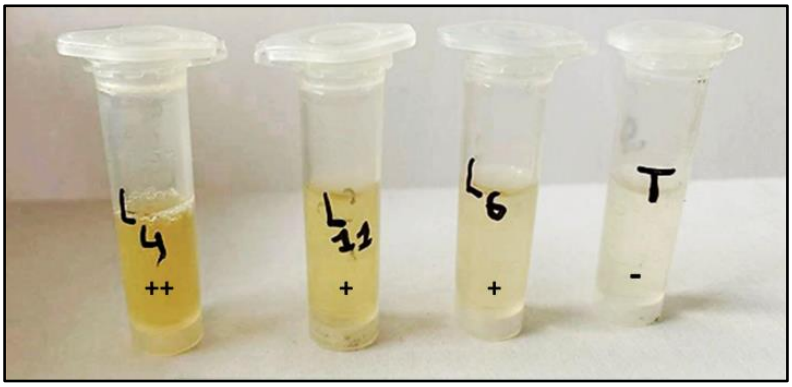

Figure 24: Ammonia (NH3) production results

V. Detection of antifungal volatile organic compounds (VOCs)

Volatile compounds represent one of the key mechanisms for effectively controlling phytopathogenic agents. These bacterial compounds inhibit or suppress fungal growth, impair fungal spores and hyphae, and/or promote plant growth (Weisskopf, 2021).

The results of VOCs production are shown in the graph below.

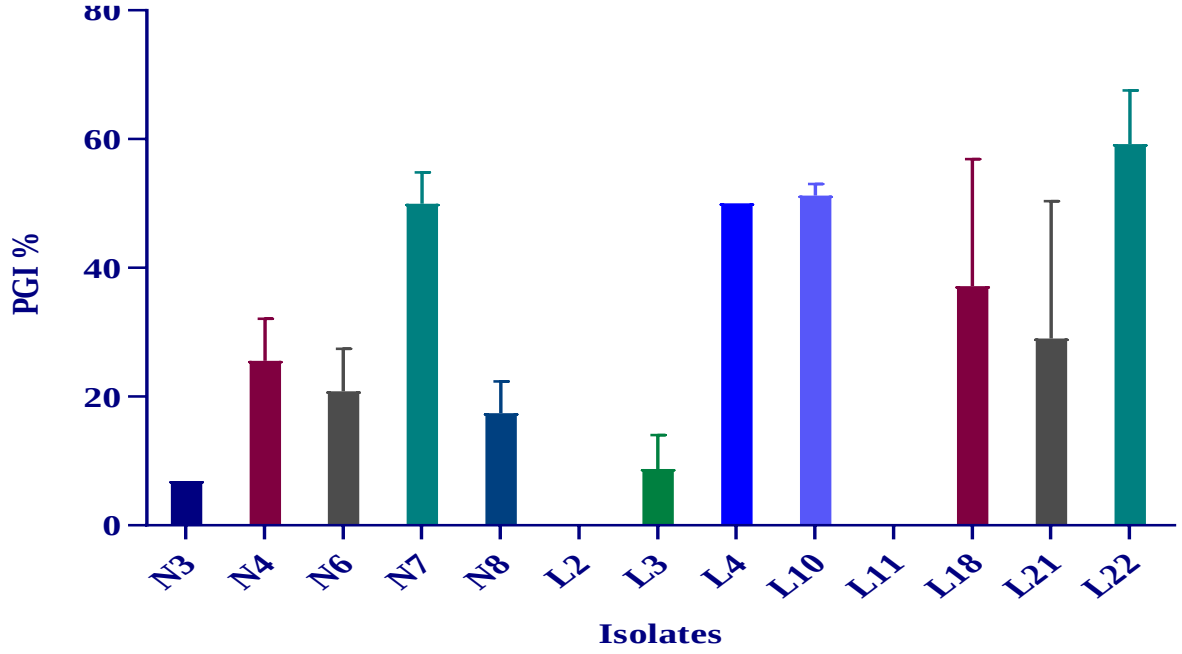

Figure 25: Fungal growth inhibition (%) resulted from bacterial volatile compounds production

The screened bacterial isolates exhibited significant variation in their capacity to synthesize volatile organic compounds, with production levels ranging from 0% to 59.2%. Notably, isolates L22 (59.2%), L10 (54.5%), L4 (50%) and N7 (49.95%), emerged as the most prolific VOC producers, suggesting strong potential for fungal inhibition through volatile-mediated mechanisms. A second tier of moderate producers included L21 (29%), N4 (25.55%),

N6 (21%), and N8 (17.4%), demonstrating intermediate metabolic activity. In contrast, isolates N3 (6.2%), L3 (8.75%), L2 (0%), and L11 (0%) showed minimal or undetectable VOC production, indicating possible reliance on alternative biocontrol strategies.

It has been found that microbial antagonism and plant growth promotion depend highly on volatile organic compounds (VOCs) (Ryu *et al*.2003). The elevated VOC production observed in isolates L22, L4, L10 and N7 suggests a significant potential for antifungal or antibacterial properties, as many VOCs are known for their ability to suppress pathogens (Kanchiswamy *et al*., 2015). A significant reduction in *Aspergillus niger* sporulation was observed in some isolates, particularly in L22 (Figure 26).

Volatile organic compounds (VOCs) produced by antagonistic bacteria can readily diffuse and inhibit pathogen growth both *in vitro* and in soil environments. These compounds efficiently permeate through soil pores to suppress pathogenic activity (Wheatley, 2002). Specific rhizobacterial genera, particularly *Pseudomonas* and *Bacillus* isolates, are known to produce volatile organic compounds (VOCs) that elicit induced systemic resistance (ISR) in plants (Piechulla *et al*., 2017).

The isolates L22, L4, and N7 emerge as highly promising biocontrol candidates due to their robust volatile organic compound (VOC) production profiles. However, further research is needed to: chemically characterize the specific VOC mixtures produced by these isolates and systematically evaluate their effects on both pathogen suppression and plant growth promotion under controlled and field conditions.

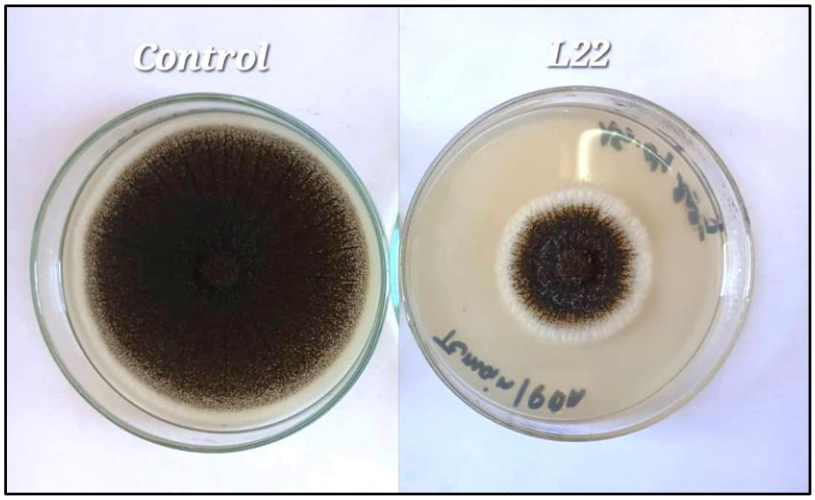

Figure 26: Aspergillus niger growth inhibition resulted from volatile compounds production by the L22 isolate

VI. *In vivo* test

VI.1. Pretest for host selection

In the initial phase of *in vivo* testing, four produce types: apples, tomatoes, garlic, and cucumbers were evaluated for their susceptibility to infection by *Penicillium* sp., *Fusarium* sp., and *Aspergillus niger*. Consistent fungal colonization was observed exclusively on wounded apples and tomatoes (Figure 27), which developed visible mycelial growth and sporulation at inoculation sites. In contrast, garlic and cucumbers exhibited no detectable signs of infection. Based on these results, apples and tomatoes were designated as optimal hosts for subsequent pathogenicity assays. Tomatoes were ultimately selected over apples as the primary host for *in vivo* testing due to their superior fungal susceptibility in preliminary trials and the opportunity to explore a less conventional model system.

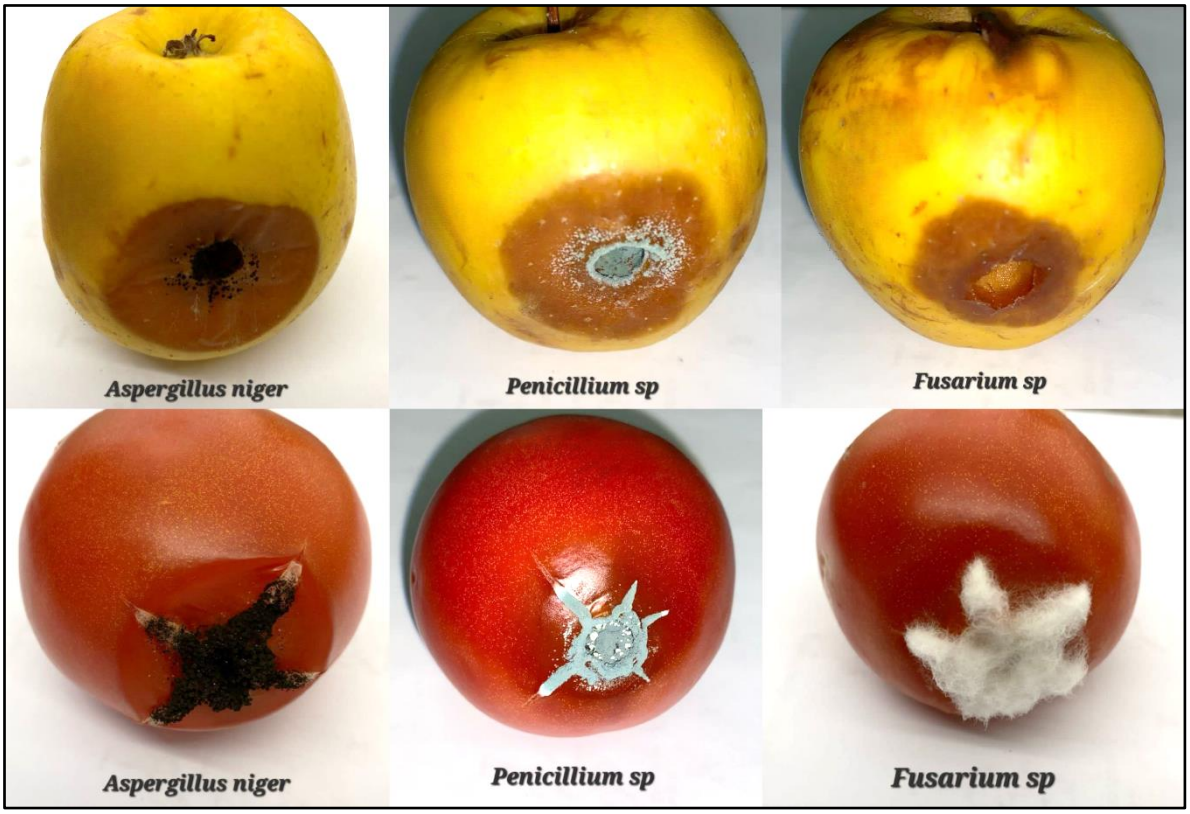

Figure 27: Photographs showing the results of pretest of host selection

VI.2. *In vivo* antifungal assay on tomato fruits

The results obtained from this test are illustrated in the graph below.

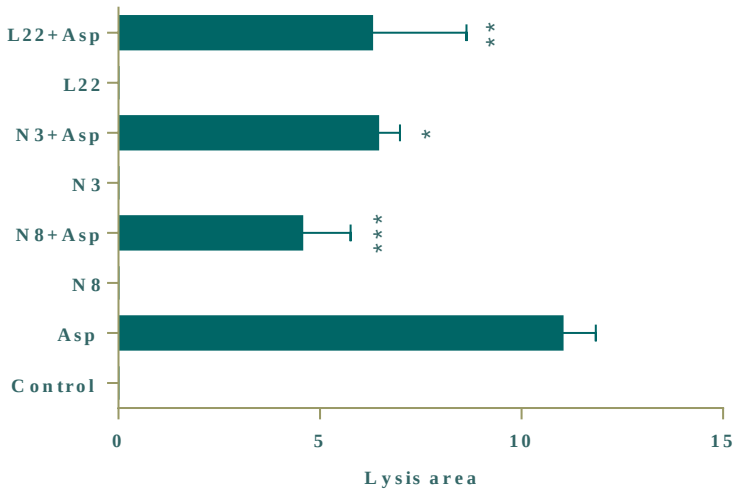

Figure 28: *In vivo* antifungal assay on tomato fruits

*: P<0.05; **: P<0.005; ***: P<0.0005

The three most effective rhizobacterial isolates (L22, N3, and N8), selected based on preliminary screening results, were evaluated for their biocontrol activity against *A. niger* infections in tomato fruits. Each isolate was tested both individually and in co-inoculation with the pathogen. Lesion development was quantified using ImageJ software, revealing significant differences in biocontrol efficacy. Untreated fungal controls developed substantial lesions (11.02 ± 0.825 cm²), while bacterial co-inoculation significantly reduced disease progression: L22 limited lesions to 6.29 ± 2.34 cm² (42.9% reduction), N3 to 6.45 ± 0.542 cm² (41.5% reduction), and N8 showed the strongest suppression at 4.56 ± 1.198 cm² (58.6% reduction). Importantly, none of the bacterial isolates caused any visible damage when inoculated alone, confirming their non-pathogenic nature and safety for potential biocontrol applications. These results demonstrate the promising potential of these rhizobacterial isolates, particularly N8, for controlling postharvest *A. niger* infections in tomatoes (Figure 29).

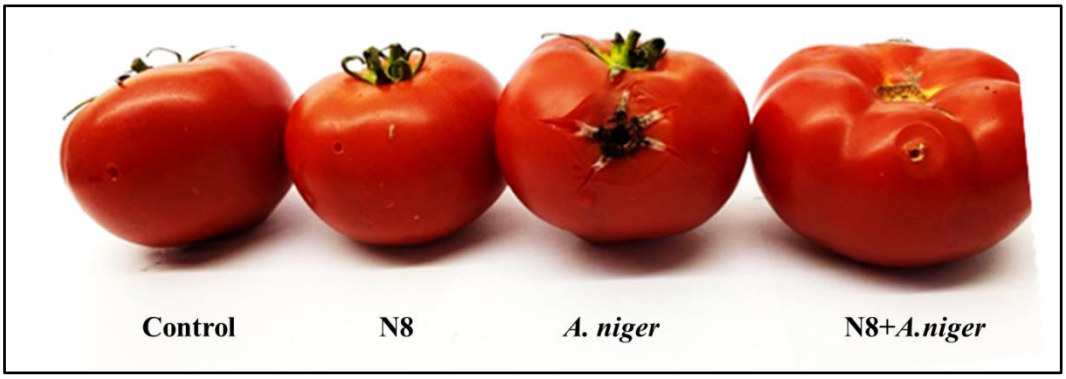

Figure 29: Results of the *In vivo* antifungal activity of N8 isolate on tomato fruits

The observed variations in *Aspergillus niger* inhibition across tomato cultivars suggest that different rhizobacterial isolates employ distinct biocontrol mechanisms. Isolates N8, N3, and L22 likely suppress fungal growth through synergistic combinations of antifungal metabolites (e.g., HCN, NH_3), lytic enzymes, and volatile organic compounds, with production levels varying significantly between isolates, as quantified in prior assays. Furthermore, some rhizobacterial isolates can activate induced systemic resistance (ISR) in host plants in this case, tomatoes, through jasmonic acid and ethylene signaling pathways, thereby enhancing plant immunity (Heo *et al.*, 2022). Isolate N8, which exhibited the strongest and most consistent inhibition, likely produces higher quantities of effective metabolites or elicits a more robust Induced Systemic Resistance (ISR) response, as evidenced by its superior performance.

These results align with the accumulating scientific literature in this field, suggesting that beneficial rhizobacteria can play a central and crucial role in sustainable plant disease management. As highlighted by Zhu *et al*. (2022), enriching the rhizosphere with beneficial microbes can significantly reduce disease incidence and enhance host defense against pathogens. In tomatoes, synthetic microbial communities have demonstrated superior suppression of fungal diseases compared to single-isolate treatments.

Furthermore, numerous tomato-pathogen models have demonstrated the capacity of bacterial antagonists to modulate host biochemical responses during infection. These include mitigating oxidative stress and enhancing phenolic compound and antioxidant enzyme production, particularly in tomato fruits (Abd Alhakim *et al*., 2022). These findings suggest that rhizobacteria such as isolate N8 not only inhibit fungal infections directly but may also enhance the physiological resilience of tomato fruit. This dual action makes them a promising sustainable alternative to chemical fungicides for postharvest disease management.

Conclusion

The use of microbial inoculants in agriculture, particularly rhizobacteria, to control phytopathogens is increasingly gaining recognition as a widespread and interesting practice in agriculture. This practice aims to replace the excessive and uncontrolled use of chemical pesticides.

In this research, rhizosphere soil samples were collected from agricultural soil in Béjaïa province (Akbou and Djebira). After isolation and purification, a total of 34 bacterial isolates were obtained. These isolates were further used to test for antifungal activity against three phytopathogenic fungi: *Aspergillus niger*, *Penicillium* sp. and *Fusarium* sp.

Our results confirm the potential of rhizobacteria as biocontrol agents against tested phytopathogenic fungi. *In vitro* antifungal assay showed that several isolates exhibited significant antifungal activity, highlighting their potential to protect plant crops.

The enzyme production assay revealed that a significant proportion of isolates from both bacterial groups (N and L) exhibited enzymatic activity, indicating their potential to degrade fungal cell walls and contribute to pathogen suppression.

The analysis of volatile antifungal compound production revealed that the selected bacterial isolates exhibited significant variation in their capacity to synthesize volatile organic compounds against *A. niger*, with production levels ranging from 0% to 59.2%. The majority of isolates produced ammonia **(**NH_3**)**, while only five isolates (N3, N7, N8, L2, and L4) demonstrated hydrogen cyanide (HCN) production capacity.

Antagonism test results on tomatoes show that the antifungal activity exhibited by the isolates in the *in vitro* assay is also observed in the *in vivo* test: L22 (42.9% reduction), N3 (41.5% reduction), and N8 (58.6% reduction). These results demonstrate the promising potential of these rhizobacterial isolates, particularly N8, for controlling postharvest *A. niger* infections in tomatoes.

The results of our study suggest that some of our isolates are generally very promising for the biological control of phytopathogenic fungi. The development of bioformulations based on these isolates, for environmentally friendly control of plant diseases, is possible but with more rigorous testing, including:

- *In vitro* antagonism tests against other phytopathogenic microorganisms;
- *In vivo* tests to confirm biocontrol potential;
- Molecular and biochemical identification of isolates;
- Extraction, and identification of molecules involved in biological control;
- Field activity testing.

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Appendices

Appendix I: Composition of Culture Media and PBS (per 1 Litre)

Table 01: Phosphate-Buffered Saline (PBS)

Component	Quantity
NaCl	8 g
KCl	0.2 g
KH_2PO_4	0.24 g
Na_2HPO_4	1.44 g
pH	7.0 ± 0.2

Table 02: Plate count agar (PCA)

Component	Quantity
Glucose	1 g
Tryptone	5 g
Yeast extract	2.5 g
Agar	12 g
pH	7.2 ± 0.2

Table 03: Potato dextrose agar (PDA)

Component	Quantity
Potato (infusion from sliced potatoes)	200 g
Glucose	20 g
Agar	15 g
pH	5.4 ± 0.2

Table 04: Tryptic soy agar (TSA)

Component	Quantity
Tryptone	15 g
Soy peptone	5 g
NaCl	5 g
Agar	15 g
pH	7.3 ± 0.2

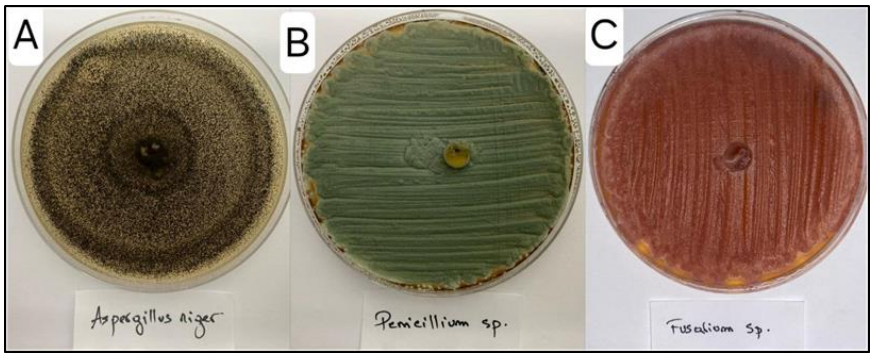
Table 05: Nutrient Agar

Component	Quantity
Peptone	5 g
Meat extract	1 g
Yeast extract	2 g
NaCl	5 g
Agar	7.5 g
pH	7.2 ± 0.2

Appendix II: Materials

1 Target fungal strains

Three fungal strains from the **LMER laboratory collection** were used in this study: ***Aspergillus niger***; ***Penicillium sp.***; ***Fusarium sp.***



Appearance of fungal species on potato dextrose agar (PDA).

A: *Aspergillus niger*; **B**: *Penicillium* sp.; **C**: *Fusarium sp*.

2. Culture media

Culture Media and experimental applications

Culture Media	Abbreviations	Use
Plate Count Agar	PCA	Bacterial flora isolation
Potato Dextrose Agar	PDA	Preparation of fungal pre-culture
Tryptic Soy Agar	TSA	Antifungal activity assay
Protease Test Medium	PTM	Protease activity assay
Urea Agar Base	UAB	urease activity assay
Tween 80 Agar	TW80 Agar	Esterase activity assay
Tween 20 Agar	TW20 Agar	Lipase activity assay
Carboxymethylcellulose	CMC Agar	Cellulase activity assay
Starch Agar Medium	SAM	Amylase activity assay
Peptone Water	PW	Ammonia production assay
Glycine Nutrient Agar	Gly-NA	Hydrogen Cyanide production assay

3. Chemical Reagents

Chemical reagents used in the study

Category	Reagents
Mineral salts	$CaCl_2 \cdot 2H_2O$, KCl, KH_2PO_4, K_2HPO_4, KNO_3, $MgSO_4 \cdot 7H_2O$, NaCl, Na_2CO_3, Na_2HPO_4, NH_4Cl
Indicators/Stains	Congo Red, Lugol's iodine solution, Nessler's reagent, Phenol red, Sodium picrate
Substrates for enzymatic assays	Carboxymethyl cellulose (CMC), Skimmed milk, Pancreatic casein, Soluble starch, Polysorbate 20 (Tween 20), Polysorbate 80 (Tween 80), Glycine, Urea
Organic Additives	Peptone, Yeast extract
Disinfectant	Ethanol (96% or 70%)

4. Equipment

Experimental Equipment List

Equipment	Source
Autoclave	PBINTERNATIONAL
Water Bath	MEMMERT
Refrigerator	ENIEM
Incubator	BINDER
Precision Balance	KERN 770
pH Meter	HANNA Instruments HI2210
hot plate magnetic stirrer	KALSTEIN
Fume Hood	FLOW FAST V

Abstract

Abstract

The use of rhizospheric bacteria to protect plants against phytopathogenic fungi offers a promising strategy to mitigate plant damage and reduce the excessive use of chemical pesticides. The current study focuses on isolating and screening of rhizobacteria from agricultural soils in Béjaïa (Akbou and Djebira regions), and the evaluation of their biocontrol potential against fungal pathogens. 34 bacterial isolates were obtained from two rhizospheric soil samples. All isolates were tested for their ability to inhibit growth of three phytopathogenic fungi (*Aspergillus niger*, *Penicillium* sp. and *Fusarium* sp.) and to produce enzymes and antifungal volatiles compounds (NH3 and HCN). The isolates: N3, N8 and L22 were further evaluated for biocontrol efficiency against *A. niger* infections on Tomato fruits. *In vitro* antifungal assay showed that several isolates exhibited significant antifungal activity with inhibition percentages ranging from 33.33% to 80% against *A. niger*, from 0% to 60% against *Fusarium* sp. and from 35% to 75% against *Penicillium* sp. A significant proportion of our isolates produce extracellular enzymes (Protease, Amylase, Lipase, Esterase, Cellulase and Urease) and ammonia (NH_3)**,** while only five isolates (N3, N7, N8, L2, and L4) demonstrated hydrogen cyanide (HCN) production capacity. In vivo test on tomato fruits, showed that the isolates L22, N3 and N8, reduce significantly the lysis area compared to the positive control (*A. niger*).

Key words:

Rhizobacteria, biological control, phytopathogenic fungi, antifungal activity, hydrolytic enzymes.

Résumé

L’utilisation de bactéries rhizosphériques pour protéger les plantes contre les champignons phytopathogènes constitue une stratégie prometteuse pour atténuer les dommages causés aux plantes et réduire l’utilisation excessive de pesticides chimiques. La présente étude porte sur l’isolement et le criblage de rhizobactéries à partir des sols agricoles situés à Béjaïa (régions d’Akbou et de Djebira), ainsi que sur l’évaluation de leur potentiel de biocontrôle des champignons pathogènes. 34 isolats bactériens ont été obtenus à partir de deux échantillons de sol rhizosphérique. Tous les isolats ont été testés pour leur capacité à inhiber la croissance de trois champignons phytopathogènes (*Aspergillus niger*, *Penicillium* sp. et *Fusarium* sp.) et à produire des enzymes et des composés antifongiques volatils (NH3 et HCN). Les isolats N3, N8 et L22 ont été évalués pour leur efficacité en lutte biologique contre les infections à *A. niger* sur les tomates. Les résultats des tests d´activités antifongiques *in vitro* ont montré que plusieurs isolats présentaient une activité significative, avec des pourcentages d'inhibition allant de 33,33 % à 80 % contre *A. niger*, de 0 % à 60 % contre *Fusarium* sp. et de 35 % à 75 % contre *Penicillium* sp. Une proportion significative de nos isolats produit des enzymes extracellulaires (protéase, amylase, lipase, estérase, cellulase et uréase) et de l'ammoniac (NH3), tandis que seuls cinq isolats (N3, N7, N8, L2 et L4) ont démontré une capacité de production de cyanure d'hydrogène (HCN). Les essais *in vivo* sur les tomates ont montré que les isolats L22, N3 et N8 réduisent significativement la surface de lyse par rapport au témoin positif (*A. niger*).

Mots clés : Rhizobactéries, lutte biologique, champignons phytopathogènes, activité antifongique, enzymes hydrolytiques.