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# Study of *Arabidopsis thaliana* salt stress protection by beneficial bacteria

Presented by :

**BENTRIA Nour ElHoura**

Evaluation Jury:

President Dr. Bouchareb Abdulrazak AMAR TELIDJI University

Examiner Dr. Bennacer Farrok AMAR TELIDJI University

Supervisor Berrabeh Fathi AMAR TELIDJI University

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# Key words

- SA : Salicylic Acid
- SE : Salicylate Esterase
- Npr1 :  Non-Exprsser Of Pathogenesis Related 1
- WT : Wild Type
- CFU : Colonies Forming Unite
- ROS : Reactive Oxygen Species
- PAMP: Pathogen-Associated Molecular Patterns
- PGPM : Plant Growth Promoting Microorganisms
- PGPR : Plant Growth-Promoting Rhizobacteria
- PGPF : Plant Growth Promoting Fungi
- Col-0: Columbia-0
- MS : Murashige and Skoog
- OD : Optical Densities

# figure list

# Summery

Bibliographic Synthesis part

- Gene annotation
- Genomic structural biology

## 3. <u>Results and discussion</u>

3.1. Growth and stress treatment

3.2. Colonies Forming Unite (CFU) analysis

3.3. Gene annotation

3.4. Genomique comparative

## 4. <u>Conclusion</u>

# Abstract

Soil salinity is one of the world's major issues affecting soil quality and agricultural productivity. Some plant growth-promoting bacteria that can thrive in regions with high salt concentrations (NaCl) biotic and abiotic stress have the ability to promote plant growth in salty environments. *Arabidopsis thaliana* is a model plants giving a precious help for understanding the plant sensibility and resistance to the salt stress. In this study we harvest A. thaliana sensibility to salt stress, we assess the role of the defense hormone salicylic acid (SA) in this response by the study of plant overexpression SA-degrading enzyme (*NohG*) or impaired in SA signaling (*nrp1*). Our results indicate that the NaCL at 100mM is sufficient to inhibit the growth of *A. thaliana* cultivated in vitro. Interestingly, *35S::NohG* shows increase of the plant growth in absence of salt stress and increase of sensitivity to the NaCl. By contrast the mutant npr1 show similar behavior with the WT in absence or presence of NaCl. Four *Medicago truncatula* bacterial endophytes (M17, M50, S113 and S116) were tested for their ability to reduce the salt stress on *A. thaliana*. The strain S116 increase the resistance and the growth of *A. thaliana* in response to salt stress, wherase the other strain shows reduction of the impact of the NaCl on the plant growth. Inoculation of *35S::NohG* and npr-1 with the studied strain reveals that the resistance mediated by the bacteria required correct SA synthesis and signaling. Finally comparative genomic analysis reveals the presence of shikimate kinase and salicylate esterase in M50 closest strain suggesting a potential manipulation of the SA pathway for the increase of A. thaliana resistance to the salt stress.



Key words: A. thaliana, SA, salt stress, endophytic bacteria, npr1, NohG

# Résumé

La salinité des sols est l'un des problèmes majeurs affectant la qualité des sols et la productivité agricole. Certaines bactéries favorisant la croissance des plantes, qui peuvent prospérer dans des régions à fortes concentrations de sel (NaCl) et à stress biotique et abiotique, ont la capacité de favoriser la croissance des plantes dans des environnements salés. Arabidopsis thaliana est une plante modèle apportant une aide précieuse pour comprendre la sensibilité et la résistance des plantes au stress salin. Dans cette étude nous récoltons la sensibilité *d'A. thaliana* au stress salin, nous évaluons le rôle de l'hormone de défense acide salicylique (SA) dans cette réponse par l'étude de la surexpression de l'enzyme dégradant le SA (*NohG*) ou de l'altération de la signalisation du SA (*nrp1*). Nos résultats indiquent que le NaCL à 100mM est suffisant pour inhiber la croissance *d'A. thaliana* cultivée in vitro. De manière intéressante, *35S::NohG* montre une augmentation de la croissance de la plante en absence de stress salin et une augmentation de la sensibilité au NaCl. En revanche le mutant *npr1* montre un comportement similaire au WT en absence ou en présence de NaCl. Quatre endophytes bactériens *Medicago truncatula (*M17, M50, S113 et S116) ont été testés pour leur capacité à réduire le stress salin sur *A. thaliana*. La souche S116 augmente la résistance et la croissance *d'A. thaliana* en réponse au stress salin, tandis que l'autre souche montre une réduction de l'impact du NaCl sur la croissance de la plante. L'inoculation de *35S::NohG* et *npr-1* avec la souche étudiée révèle que la résistance médiée par les bactéries nécessite une synthèse et une signalisation SA correctes. Enfin, une analyse génomique comparative révèle la présence de shikimate kinase et de salicylate estérase dans la souche la plus proche de M50 suggérant une manipulation potentielle de la voie SA pour l'augmentation de la résistance *d'A. thaliana* au stress salin.

Mots clés : A. thaliana, SA, stress salin, bactéries endophytes, npr1, NohG

ملخص :

تعتبر ملوحة التربة إحدى المشاكل الرئيسية التي تؤثر على جودة التربة والإنتاجية الزراعية. وتتمتع بعض البكتيريا المعززة لنمو النباتات، والتي يمكن أن تزدهر في المناطق ذات التركيزات الملحية العالية (كلوريد الصوديوم) والإجهاد الحيوي واللاأحيائي، بالقدرة على تعزيز نمو النبات في البيئات المالحة. تعتبر نباتات الأرابيدوبسيس ثاليانا نباتاً نموذجياً يوفر مساعدة قيمة لفهم حساسية النبات ومقاومته للإجهاد الملحي. في هذه الدراسة، قمنا بدراسة حساسية في هذه الاستجابة من نبات أرابيدوبسيس ثاليانا للإجهاد الملحي وتقييم دور هرمون حمض الساليسيليك الدفاعي أو تغيير إشارات حمض (NohG) خلال دراسة الإفراط في التعبير عن الإنزيم المهضِم لحمض الساليسيليك تشير النتائج التي توصلنا إليها إلى أن حمض الصوديوم عند 100 مللي متر كافٍ لتثبيط نمو (nrp1) الساليسيليك النبات المزروع في المختبر

اختُبرت أربع سلالات بكتيرية من بكتيريا ميديكاغو ترونكاتولا لمعرفة قدرتها على تقليل إجهاد الملح على نبات الأرابيدو حيث زادت إحدى السلالات من مقاومة ونمو نبات الأثاليانا استجابةً لإجهاد الملح

بينما قللت السلالة الأخرى من تأثير كلوريد الصوديوم على نمو النبات npr-1 و NohGيكشف تلقيح السلالة أخيرًا، التحليل الجينومي الصحيحة SAوإشارات مع السلالة المدروسة أن المقاومة بوساطة البكتيريا تتطلب تخليق المقارن عن وجود كيناز الشيكيمات كيناز وإستراز الساليسيلات في السلالة الأقرب إلى السلالة الأقرب للإشارة إلى لزيادة مقاومة ألارابيدوبسيس ثاليانا لإجهاد الملح SA إمكانية التلاعب بمسار

الكلمات المفتاحية : A. thaliana, SA, stress salin, bactéries endophytes, npr1, NohG

# Part I. Bibliographical synthesis

**1.Soil microbiome (rhizosphere):**

Rhizosphere, known as the "plant growth-promoting rhizobacteria" (PGPR), which guard the plants against abiotic and biotic stressed environments, and also improve their physiological capacities, these useful soil microbes, however plentiful in the rhizosphere, are generally under-exploited as bio-inoculants for upgrading crop production, particularly under abiotic stress[8,]plant-associated bacteria, fungi, and viruses can enhance stress resistance and cope with the negative impacts of drought through the induction of various mechanisms, which involve plant biochemical and physiological changes[20]

Rhizobacteria's suggests the congregate of rhizospheric microbes around the plant root surfaces, this endophytic are located in intra- and inter-cellular regions or the vascular tissue and colonized aerial parts or roots[7]

Bacteria can live both externally or internally in their host plant. On one hand, the bacteria that live outside their host plants are either epiphytic; those living on the plant leaf surfaces, or rhizospheric; those inhabiting plant roots within the soil[17]. On the other hand, bacteria that live and thrive inside their host plant are called endophytic bacteria[18]
the rhizosphere as the area around a plant root that is inhabited by a unique population of microorganisms influenced[6].

**2. Beneficial microorganisms:**

Plant-beneficial microbial interactions can be roughly divided into three categories. First, those microorganisms that, in association with plants, are responsible for its nutrition[44]in this case, while most may not directly interact with the plant, their effects on soil biotic and abiotic parameters certainly have an impact on plant growth[10]. Second, there is a group of microorganisms that stimulate plant growth indirectly by preventing the growth or activity of pathogens[47]. Such microorganisms are referred to as biocontrol agents, and they have been well documented. A third group involves those microorganisms responsible for direct growth promotion, for example, by production of phytohormones.[37]

PGPR consist of the rhizosphere bacteria that can enhance plant growth and stress resistance by a wide variety of mechanisms[24] PGPR are currently intensively studied due to their properties which are of considerable value both for traditional and sustainable agriculture. PGPR can enhance plant mineral nutrition via associated nitrogen fixation , mobilization of phosphate in the soil siderophore production stimulation of the mycorrhizal symbiosis development and modulation of root architecture [46].

**3. Plant defense molecular signaling:**

Plants have evolved sophisticated and efficient defense mechanisms to cope with biotic stresses and to mount effective defense mechanisms. Reactive oxygen species (ROS) are highly reactive chemical molecules formed via oxygen consumption in a so-called oxidative burst. ROS act as signal molecules in various biological processes in plants, including photorespiration, stomatal movement, and photosynthesis, ROS signaling acts as an early response in plants when combating biotic or abiotic stresses[27]

Production of Phytohormone: unlike animals, plants lack specialized mobile immune cells, so they do not have an adaptive immune system. Instead, plants can launch specific, self-tolerant immune responses and establish immune memory. They possess defense mechanisms that efficiently detect and ward off potentially dangerous microorganisms. These defense mechanisms start with multiple signaling processes responsible for sensation, recognition, signal collection and conveying information between cells[36]. Plant hormones are also implicated in plant defense signaling pathways; salicylic acid, jasmonic acid, and ethylene have been increasingly studied in plant responses to pathogens. These innate immune system components interact with each other and provide protection against invading pathogens[28]

Endophytes produce phytohormone which enhances plant growth promotion and changes the morphology and structure of the plant, as a result of this attribute, endophytes have gained ground in the area of agricultural sustainability [26], Pathogen recognition results in downregulation of growth mediated by phytohormones and upregulation of defense-related genes[29]

**4.Mycorhizal symbiosis in plant Arabidopsis growth**

Plants are sessile organisms whose growth and development depend on its interaction with the soil, environment, and microbes. The fixed nature of plants to the soil during its growth cycle has made its interactions with soil [33]. Due to this positive rhizosphere effect, there are active and passive physical and chemical changes in the root system of the higher plants, which may have a great impact both on their nutrient status and their growth. Among the microbes present in the rhizosphere, the microsymbiont bacteria and fungi are the most important for the plant growth and development. [34]

**5.Bacteria Associated with Plants:**

Plants have a diverse microbiota that includes various microorganisms such as bacteria, fungi, protists, and viruses. These microorganisms colonize the roots, stems, leaves, and seeds of plants, forming a complex community known as the plant microbiota: phytobiome, Bacteroidetes, Proteobacteria Endophytic, Rhizospheric and Epiphytic Bacteria.

**6.Abiotic stress :**

Abiotic stress conditions cause extensive losses to agricultural production worldwide [12] Individually, stress conditions such as drought, salinity or heat have been the subject of intense research , Recent studies have revealed that the molecular and metabolic response of plants to a combination of drought and heat is unique and cannot be directly extrapolated from the response of plants to each of these different stresses applied individually [35] Although reactive oxygen species (ROS) are associated with many different biotic or abiotic stress conditions, different genes of the ROS gene network of Arabidopsis were found to respond differently to different stress treatments [34]

Types of Abiotic Stress:

a) Temperature Stress: One of the most significant environmental elements influencing the growth and development of plants is temperature. Temperatures both high and low can seriously harm plant tissues and cells, which will inhibit growth and yield
b) Drought Stress: One of the worst abiotic stresses that plants can experience is drought. Water scarcity may result from it, which could significantly affect plant growth and production.
c) Flooding Stress: Another abiotic stress that has an impact on the growth and development of plants is flooding. It may result in oxygen deprivation, which can significantly alter the metabolism of plants.

**7- Mechanisms of Plant Responses and types of Abiotic Stresses:**

Abiotic stress, which refers to the effect of non-living environmental variables on plant growth, development, and survival, presents a variety of difficulties to plants. Extreme temperatures, drought, flooding, excessive salinity, heavy metal toxicity, and other factors can all be sources of stress[35]. Different systems are involved in the response to abiotic stresses:

1) Antioxidant defense system: *i*n plants, the antioxidant defense system is a complex network of processes that protect cells from oxidative stress induced by reactive oxygen species (ROS) Plants are continually exposed to many environmental challenges, including both biotic and abiotic
2) Osmoregulation: is a biological process in which organisms regulate the osmotic pressure of their fluids to maintain fluid balance and the concentration of electrolytes and salts within their bodies.
3) Phytohormonal regulation: Phytohormones, often known as plant hormones, are signaling molecules that control many aspects of plant growth and development[1]. At low concentrations, these hormones regulate processes like embryogenesis, organ size determination, pathogen defense, stress tolerance, and reproductive development

**8- Endophyte (microbiota)**

Endophytes are mainly prokaryotic organisms like bacteria found within the healthy host tissue and can benefit the host in several ways, such as biotic and abiotic stress resistance the environmental stresses that affect plants [3].They have an important role in plant growth promotion, as they directly or indirectly promote plant growth. They do it by inhibiting the growth of plant pathogens, and/or by producing various secondary metabolites[4] increased availability of nutrients, degradation of toxic molecules, and production of phytohormones [38]Although endophytic bacteria primarily occupy the intercellular spaces due to their abundance of the carbohydrates, amino acids and other nutrients, and some are also capable of intracellular colonization. Endophytic strains colonize various parts of plants, including the roots, leaves, stems, flowers and seeds[5] Plants respond to all these environmental factors because they are fixed in a inimitable zone

Plants closely associate with complex microbial communities composed of bacteria, fungi, oomycetes, and other microorganisms. This community, or microbiome, colonizes the soil surrounding the roots (rhizosphere), external plant surfaces, and the spaces within plants (endosphere). The microbiome affects plant growth [9], abiotic stress tolerance, and disease resistance [10]

**9- Natural Products from Endophytes**

The production of functional secondary metabolites by endophytes is linked to the improvement of plant fitness [18]. Besides plant-growth promotion, one major function of microbial metabolites is protecting the plant against biotic and abiotic stress, e.g. by inducing resistance inst pathogens [13]. Most of these compounds can be further categorized into four main classes based on their biosynthetic origins: polyketides, peptides, alkaloids, and terpenoids [20] which they play an important role in the defense of plants against herbivores and pathogens, as well as in intercellular communication.

A main group of interesting secondary metabolites is composed of high-molecular compounds, such as peptides and polyketides, encoded by non-ribosomal peptide synthetases and polyketide synthases. This type of metabolites displays complex structural diversity, along with a broad range of biological activities and functions, such as anti-bacterial, antifungal and cytotoxic activity., They also facilitate the way endophytes coexist with their hosts in this way. Fungi and actinobacteria are promising suppliers of these kinds of natural compounds. Endophytic actinobacteria in particular are thought to be a largely untapped source of beneficial secondary metabolites.

**10- Plant protection properties of the Plant Growth-Promoting**

Plants are constantly exposed to various stress factors, such as drought, salinity, pathogens, and nutrient deficiencies, which can significantly reduce crop yield and quality [45],The rhizosphere-inhabiting microorganisms which result in improvement of growth and protection of plant can be collectively defined as plant growth promoting microorganisms (PGPM) plant growth-promoting rhizobacteria (PGPR) improve plant growth and supports the plant to endure abiotic and biotic stresses ,plant growth promoting fungi (PGPF) are known to colonize the region of the root of plants, and they enhance the plant nutrient uptake, can also activate plant pathogen resistance suppress pathogen growth and alleviate the inhibitory effects of abiotic stressors like “drought ,salinity , and heavy metal pollution” Because of growing public concern about the damaging effects of chemical fertilizers and pesticides.

**11- Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants**

Nitrogen is one of the most essential elements for all forms of life; a basic material for synthesizing proteins, nucleic acids and other organic nitrogenous compounds[39]. Unfortunately, no plant species is able to reduce atmospheric dinitrogen into ammonia and use it directly for its growth[43] bacteria known collectively as the ‘‘Rhizobia’’ are famous for their ability to induce nodules on the roots (and occasionally, stems) of legume plants. Within these nodules, the differentiated, ‘‘bacteroid’’ forms fix atmospheric nitrogen and the resultant ammonia being used as a source of fixed nitrogen[19] non-leguminous plants form an extended niche for various species of Nitrogen Fixing bacteria. These bacteria thrive within the plant, successfully colonizing roots, stems and leaves. During the association, the invading bacteria benefit the acquired host with a marked increase in plant growth, vigor and yield. With increasing population, the demand of non-leguminous plant products is growing [42]

**12- Biotic stress response with plant-pathogenic and symbiotic interactions**

Plants regularly interact with both pathogenic and symbiotic microorganisms. Recent research has identified several regulatory mechanisms governing these connections. These include pathogenic bacteria as well as symbiotic bacteria, to which the plant defense system responds by activating. It's interesting to note that shortly after the symbiotic microbe was discovered, the plant defense system was destroyed to encourage symbiotic plant infection [11]

The participation of microorganisms as enhancers is related to their enzymatic activity. For example, potential for nitrogen fixing is a property of some prokaryotes[10]. It is known, that during the growth, the plans interact intimately with microorganisms of soils. The interaction between the microorganisms and the plants can be beneficial, neutral or detrimental.[65]

**13- Endophyte defensive of oxidative stress protection**

Salt stress has led to strong adaptations based on the exclusion, compartmentation, and excretion of ions and oxidative defense system responses that counteract the effects of reactive oxygen species (ROS) overproduced during stresses such as salt or metal stress beneficial effects of plant endophytes on host plants[6]. The current explanation of endophyte protection {defensive mutualism} of host plants is based on the secondary metabolites[14], endophytic bacteria can improve plant health by targeting pests and pathogens with antibiotics, hydrolytic enzymes, nutrient limitation, and by priming plant defenses [15]hydrolytic enzymes are essential enzymes that catalyze the breakdown of various biomolecules into their simpler forms through hydrolysis [16]

There're two types for stress: biotic and abiotic stresses. Abiotic stress includes temperature, ultraviolet radiation, salinity, floods, drought, heavy metals[55], which results in the loss of important crop plants globally, while biotic stress refers to damage caused by insects, herbivores, nematodes, fungi, bacteria, or weeds[2]

**14- Plant growth promoting bacteria in agricultural application**

It is known that the bacteria present in the soil have three different effects on the plants. Among these, beneficial bacteria are called plant growth promoting bacteria [39], (PGPB). These bacteria promote plant growth using different mechanisms. These mechanisms are; direct mechanisms such as modulating plant hormone levels and facilitating the acquisition of resources, and indirect mechanisms such as the production of chemicals inhibiting the effects of certain plant pathogens[47]

Soil is replete with microscopic life forms including bacteria, fungi, actinomycetes, protozoa, and algae. f these different microorganisms, bacteria are by far the most common 23

can also activate plant pathogen resistance suppress pathogen growth and alleviate the inhibitory effects of abiotic stressors like "drought ,salinity , and heavy metal pollution" Because of growing public concern about the damaging effects of chemical fertilizers and pesticides,

## Introduction to the problematic of the study

*Arabidopsis thaliana* has rapid life cycle and high production of seeds, another feature of this model plant, and a crucial reason in the decision to sequence its small genome size, when compared to other plants This initiative intends to explore the interaction between endophytic bacteria who are one of the ecosystem's varied microbial groups, and they are more effective than rhizobacteria in minimizing the detrimental effects of environmental stresses on plants

The aim of this study is:

- ✓ Validation of the endophytic behaviors of the isolated colonies under abiotic stress (salt stress)
- ✓ Find specific genes of endophytic bacteria and their metabolism
- ✓ Analysis of the endophytes on *Arabidopsis thaliana* symbiosis.
- ✓ The role of salicylic acid (SA) in plant growth and resistance

Four strains of endophytic bacteria were analyzed during this study. The following sections describe, the material and method used during the study and the results obtained.

# Part II. Materials and Methods

1. **Plant and bacterial cultures:** Wild-type *Arabidopsis thaliana* Col-0 (WT) and and its corresponding overexpressing 35S::*NohG* and mutant *npr-1* lines, were cultivated *in vitro* in (MS) solidified and previously inoculated with suspension of the followed bacterial endophytes: M50, M17, S113, S116.
2. **Sub-culturing:** Cultures of the studied bacteria M50/M17/S116 /S113 were performed in yeast extract broth (YEB), after 48h of incubation. The cultures were collected in sterile physiological serum and the OD600 was adjusted at 0.1. The plant inoculation is realized by addition of 250µl of the suspension in 25ml of 1/2MS medium.
3. **Sterilization and culture preparation with stress:** Under a hood, the seeds we use are (col0, col0-1, *NohG* and *npr-1*), their sterilization is in few steps, SDS solution (70% ethanol and 0.02% SDS) were seeds will sterilize for 12 min with vortex agitation afterwards we rinse 3 times in (70% ethanol), last rinse in (100% ethanol) and leave the seeds to dry for an hour.
4. **Preparation of culture co-inoculation:** Murachige and Skoog (MS) plant culture medium was prepared as fellow: ½MS is prepared by adding 1.1g/L of MS in distilled water and solidified by addition of 12g/l of agar. The pH is adjusted at 5.7. *Arabidopsis thaliana* growth in round petri dishes 25ml of 1/2 MS with or without (control) the studied bacteria. The plants sterile seeds of *Arabidopsis thaliana* were dropped in the prepared medium and stratified at 4°C, 48h in the dark. Then the seeds were transferred in a growth chamber at 26°C at 60% humidity with a photoperiod of 16h/8h of light/dark respectively for the germination phases for 5 days.
5. **Colonies Forming Unite (CFU)** To determine the CFU, the surface of the roots will be sterilized in 3 steps after cutting all the roots from each petri dish we put them in 5° sodium chlorine solution supplemented with 0.02% SDS for 3min with stirring using a vortex, the 2nd step is to soak it in 70% ethanol solution twice, then in distilled water and last steps to leave it in physiological water, due to the small size of our roots we carry out the procedure using Eppendorf tube, This process is carried out to ensure that only endophyte is present in roots, the suspension is spotted in YEB agar plate for 24hours for colonization

6. **Gene annotation** aims to identify and mark the locations of genes and their various features in a genome sequence. The primary target of gene annotation is to accurately predict the location of protein-coding genes, non-coding RNA genes, regulatory elements, and other functional elements within the genome. This process involves predicting gene structures, including exon-intron boundaries, transcription start sites, and regulatory regions. Additionally, gene annotation targets the identification of specific functional elements within genes, such as protein domains, motifs, and regulatory sequences that control gene expression

- Annotation process for coding gene: is known as the Targeted stage which is followed by the Similarity stage in which proteins from closely related species are used to build transcript structure in regions were a Targeted transcript structure is absent
- Annotation for non-coding RNAs (ncRNAs) are involved in many biological processes and are increasingly seen as important. As is the case with proteins, it is the overall structure of the molecule which imparts function: tRNA transfer RNA, rRNA ribosomal RNA).

Bioinformatics tools:

- STRING: functional protein association networks (string-db.org) In molecular biology, STRING is a biological database and web resource of known and predicted protein–protein interactions.
- RAST Server - RAST Annotation Server (nmpdr.org) which is a powerful tool that provides high-quality genome annotations
- Comparative Genomics - MicroScope - Web Interface System & Specialized Databases for (re)Annotation and Analysis of Microbial Genomes (cns.fr) This interface provides an analysis of the pan-genome and its components (core-genome, variable-genome) for an organism set. It use the MicroScope gene families (MICFAM) which are computed with software

# Part III. Results and discussion:

### 1. NaCl reduce *A. thaliana* growth *in vitro*

To study the salt stress response and resistance of A. thaliana, analysis of the WT plants cultivated *in vitro* was firstly assessed. For that plants cultivated *in vitro* in 1/2MS medium supplemented or not with 100mM of NaCl were studided. The Figure 1 shows the obtained results.

Two days of cultivation the plant length was 1 cm and at slight reduction of the plant growth is observed. After four days a growth of 3 cm is observed for untreated plants and 2.5cm for the plant traded with the salt. Finally, after 6 days the growth the untreated plant was 4 cm, wherase for the treated plants, a growth of 3.5 cm is observed.

These results indicate that in our condition, the addition of 100mM of NaCl for WT plant is sufficient to reduce plant growth after 4 days of incubation.

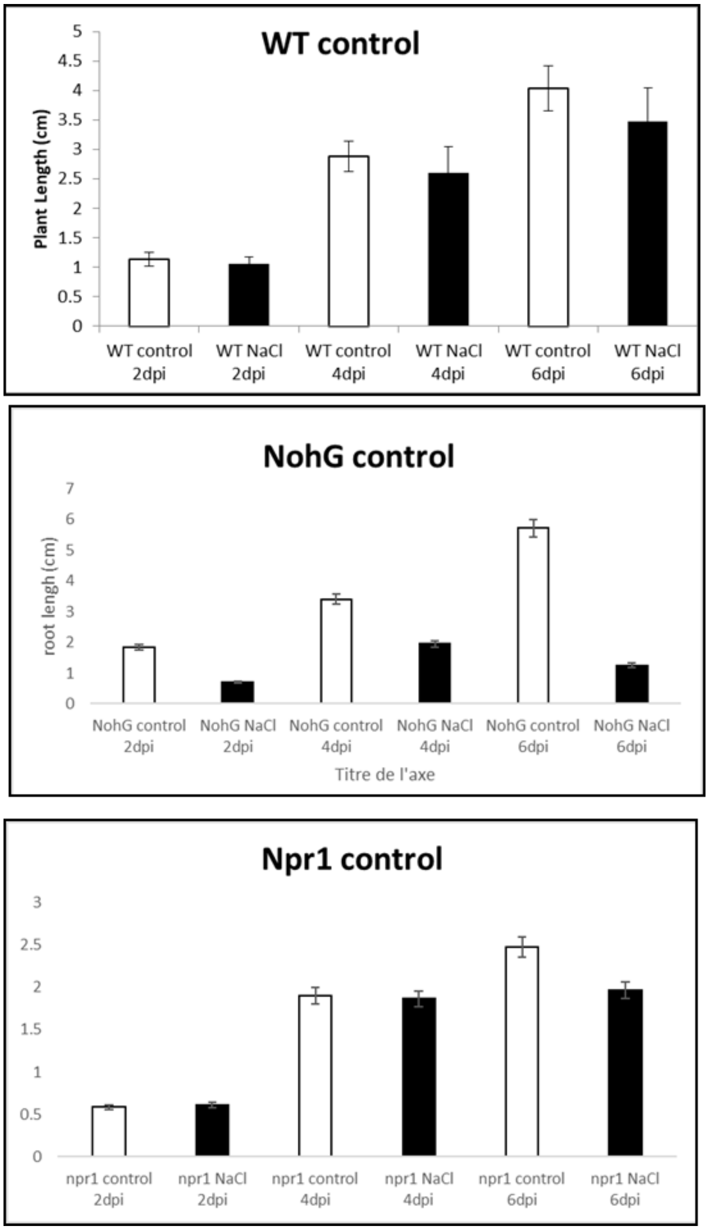


**Figure 1. *A. thaliana* response to salt stress and impact of the SA signaling on its response.** Analysis of the length of WT, *35S:NohG* and *npr-1* roots of plant cultivated 6 days in presence or absence of 100mM of NaCL. In white the control and black plant treated with 100mM of NaCl.

## 2. Reduction of SA content increase the plant sensitivity of the salt stress

To characterize the role of the SA in the resistance of the plants to the salt stress, analysis of the response of A. thaliana plants overexpressing SA-degrading enzyme: NohG were harvested (Figure 1).

After two days of incubation the growth of the plants is around 2 cm, addition of NaCl reduce drastically the growth of studied plants and around 0.8 cm of plant growth is observed. This inhibiting effect is maintained after 4 and 6 days of incubation with a growth of 3 and 6 cm observed for untreated plants and 2 and 1.5 cm for treated plants respectively.

In comparison with the WT (Figure 1), these results indicate that reduction of SA cotenants increase the plant sensitivity to the salt stress of *A. thaliana*. Interestingly, at 6 days, the reduction of SA contenant seems to increase the growth of untreated plants.

## 3. Evaluation of NPR1 signaling protein during salt stress response

NPR1 is the major signaling protein involved in the SA response. This protein is present in plant cell in multimeric form. The uptake of SA by the cell, leads to changes in the redox statut changes and ends by monomerization of NPR1. The monomertic NPR1 is then translocate inside the nucleus, and induce the SA-mediated response. Two other genes NPR homologous genes are present inside *A. thaliana* genome (NPR2 and NPR3).

To harvest the hypothesis of NPR1 mediation during the SA effect during the plants salt stress response, the response of *npr1* mutant to the salt stress was harvested. After 2, 4 and 6 days of growth, npr1 show reduction of plant growth similar to the WT in response to the salt (Figure 1). This observation indicates that npr1 seems to don't play crucial role during the SA-mediate resistance to the salt stress. We can speculate that NPR2 and/or 3 could be key players for the SA-mediate response to the salt stress.

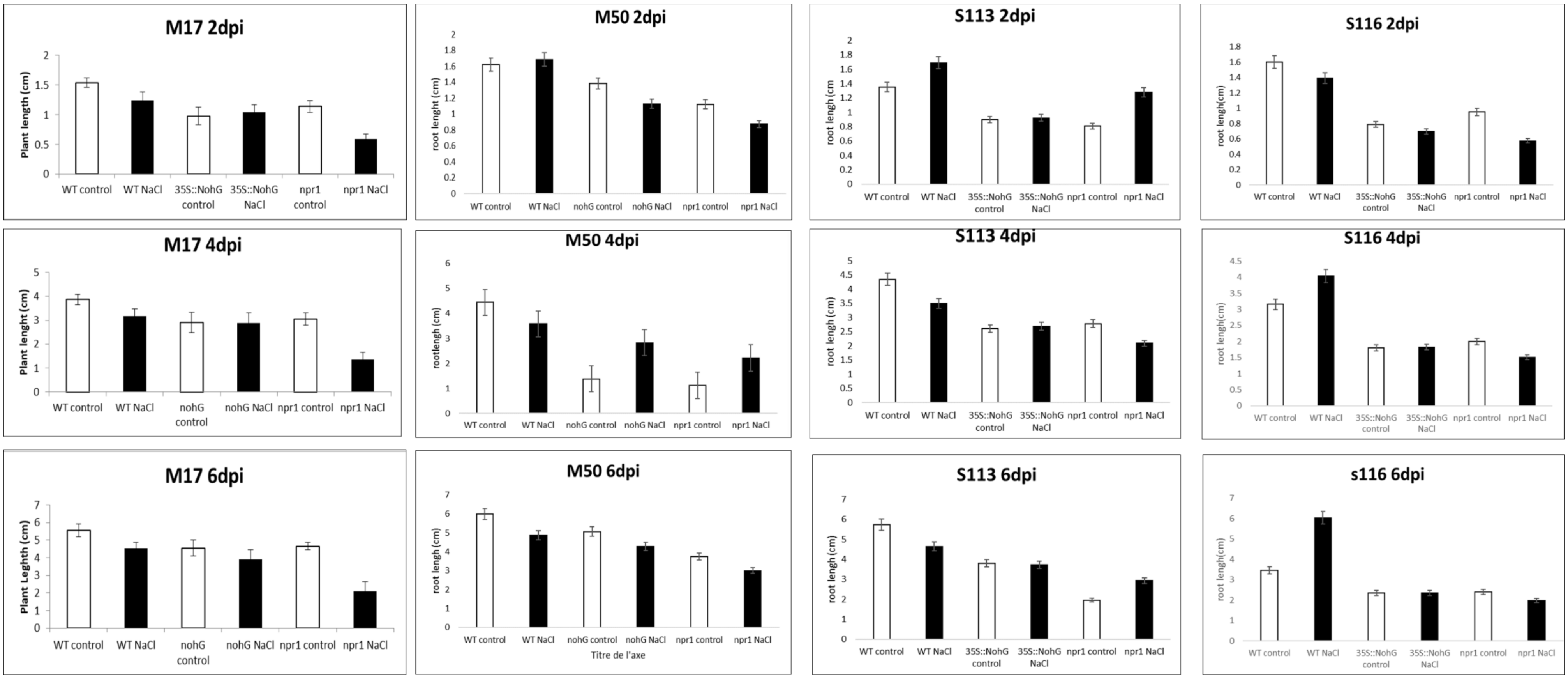


**Figure 2.** ***A. thaliana*** **salt stress protection by bacterial endophytes.** Analysis of the length of WT, *35S:NohG* and *npr-1* roots of plant cultivated 6 days in presence or absence of 100mM of NaCL. Plants are inoculated with M17, M50, S113 and S116 strain of the lab. In white the control and black plant treated with 100mM of NaCl.

**4. *A. thaliana* response to endophytic bacteria**

To study the ability of endophytic bacteria to protect the A. thaliana from salt stress, four endophytic bacteria available in the lab were studied. These endophytes were initially isolated from the model legumes plant *Medicago truncatula* cultivated in wild condition. The Figure 2 shows the obtained results.

In absence of salt stress, at 2 days post inoculation (dpi) M17, M50, S113 and S116 shows respectively growth of 1.5, 1.6, 1.4 and 1.6 cm. This is similar level of growth observed for the no inoculated WT plants (Figure 1). At 4 dpi, M17, M50, S113 and S116 show respectively 4, 4.5, 4.5 and 4 cm of growth respectively. These results are higher than those obtained for the no inoculated WT plants (Figure 1) showing around 3 cm of growth in the same conditions. Analysis of the plant growth at 6 dpi reveals a growth of 5.5, 6, 5.8 and 6 cm for M17, M50, S113 and S116 respectively. These observations indicate an increase of around 1.5 to 2 cm of growth of plants inoculated with endophytes compared to the WT.

These results indicate that the studied bacteria increase the growth of A. thaliana cultivated in vitro. M17, M50, S113 and S116 can be classified as PGPRs microbes.

**5. Endophytes effect on *A. thaliana* salt stress response**

To harvest the potential effect of studied bacteria on A. thaliana response to salt stress, inoculated plants were studied in response to 100mM of NaCl (Figure 2).

After 2 dpi, low or no variation is observed in inoculated plants (Figure 2) compared to no-inoculated WT plants (Figure 1) with slight decrease or increase of plant growth in response to studied bacteria. At 4 dpi, all bacteria shows decrease of plant growth in response to the salt, excepting S116, which show increase of 2 cm of plant treated with NaCl compared to untreated plants. At 6 dpi, similar results with those obtained at 4 dpi are observed, with reduction of the growth of treated plant inoculated with M17, M50, S113 and increase of the growth of treated plants inoculated with S116 compared to untreated plants.

These results indicate that the stain S116 protect *A. thaliana* from salt stress and increase its growth in response to NaCl.

**6. Interplay between endophytes and SA signaling during A. thaliana salt response**

To harvest the potential link between the effect of endophytic bacteria and SA-signaling during *A. thaliana* response to salt stress, the overexpression *NohG* and npr1 mutant lines were inoculated with M17, M50, S113 and S116 strain, in presence of absence of 100mM NaCl. The Figure 2 shows the obtained data. The fallowed results are observed:

- M17Vs *NohG*: the overall growth of the inoculated plants compared to no-inoculated plants is slightly reduced (Figure 1 Vs. Figure 2). No variation of plant growth is observed for plant inoculated with the strain in presence or absence of NaCl (Figure 2).
- M50 Vs *NohG*: Reduction of the growth at 4 dpi plants compared to no-inoculated plants (Figure 1 Vs. Figure 2). A reduction, increase and reduction of plant growth is observed at 2, 4 and 6 dpi of plant treated compared to no-treated plants with NaCl (Figure 2).
- S113 Vs *NohG* : the overall growth of the plants compared to no-inoculated plants shows low variation (Figure 1 Vs. Figure 2). No variation of plant growth is observed for plant inoculated with the strain in presence or absence of NaCl (Figure 2).
- S116 Vs *NohG*: A reduction of plant growth of plant inoculated with S116 compared to no-inoculated plants (Figure 1 Vs. Figure 2). No variation of plant growth is observed for plant inoculated with the strain in presence or absence of NaCl (Figure 2).
- M17 Vs. *npr1*: increase of the overall growth of the inoculated plants compared to no-inoculated plants (Figure 1 Vs. Figure 2). A decrease of plant growth is observed of plant treated compared to no-treated plants with NaCl (Figure 2).
- M50 Vs. *npr1*: the overall growth of the inoculated plants compared to no-inoculated plants is slightly reduced (Figure 1 Vs. Figure 2). A decrease, increase and decrease of inoculated plant in response to NaCl compared to untreated plants at 2, 4 and 6 dpi respectively (Figure 2).
- S113 Vs. *npr1*: increase of the overall growth of the inoculated plants compared to no-inoculated plants (Figure 1 Vs. Figure 2). An increase, decrease, increase of inoculated plant in response to NaCl compared to untreated plants at 2, 4 and 6 dpi respectively (Figure 2).
- S116 Vs. *npr1*: the overall growth of the plants compared to no-inoculated plants shows low variation (Figure 1 Vs. Figure 2). A decrease of inoculated plant in response to NaCl compared to untreated plants is observed (Figure 2).

Together these results that changing in SA contenant and signaling can affect differentially the plant response to NaCl depending the studied strain. As an example the S116 strain, which display increase of plant growth in response to the NaCl (Figure 1), shows alteration of its protection in plants degrading the SA or impaired in SA signaling. These results seem to indicate in this case, that the SA signaling is important for the resistance mediated by S113.

**7. Genomic characterization of selected strain**

To go further in the study, we performed a genomic analysis on the closest strains of M50. The corresponding r16S gene of M50 was previously sequenced. Concerning our findings regarding the

{M50 = Lysinibacillus sp strain 7B } bacterium, which guarantees the growth of the roots both in the presence and absence of salt, and these findings are taken into account , we use the NCBI to gather data that advances our knowledge of molecular biology and genetics : Home - Nucleotide - NCBI (nih.gov) After selecting the strain closest to M50, Lysinibacillus sp 7B, we do an annotation (RAST) to Lysinibacillus fusiformis, M50's ortholog.

The results shows high proportion of genes associated to secondary metabolism (Figure 3). Secondary metabolites function as signaling molecules and plant growth regulators. For example, some alkaloids and terpenoids act as plant hormones or hormone-like substances. Plant hormones play key roles in regulating the biosynthesis of secondary metabolites. They modulate the expression of genes involved in secondary metabolic pathways. The way a plant reacts to both biotic and abiotic stressors is influenced by phytohormones. Phytohormone signaling pathways are responsible for inducing the formation of secondary metabolites as part of the stress response.

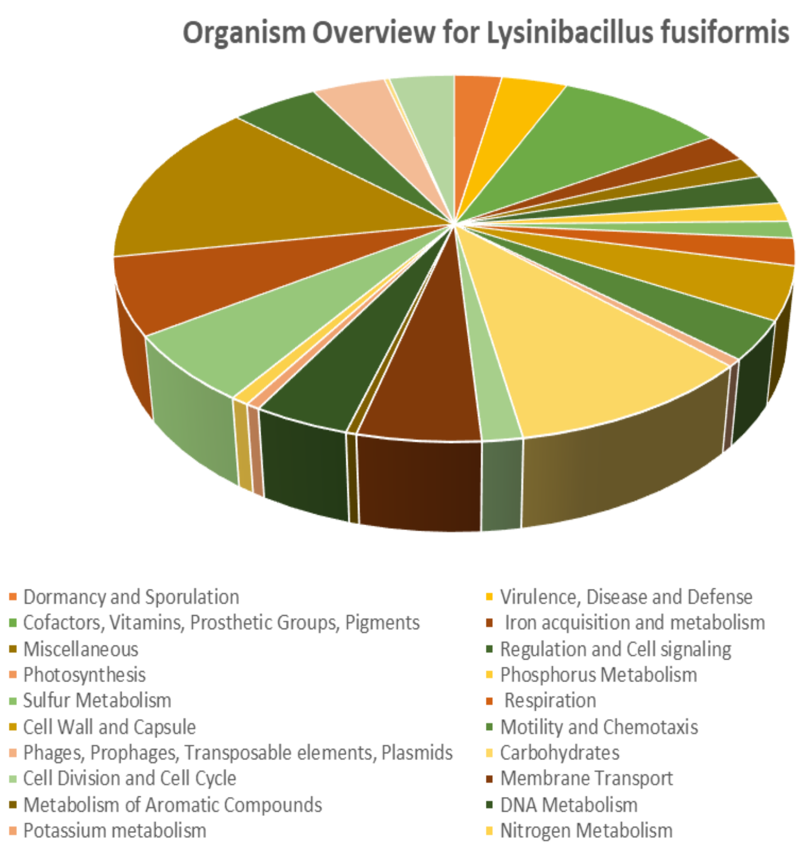


**Figure 3. Gene annotation of *Lysinibacillus fusiformis* strain RB-21**

To further characterize further in the genomic characterization, we then performed comparative genomic analysis between *L. fusiformis* strains available on the online tool available on the MicroScope data base a https://mage.genoscope.cns.fr/microscope/home/index.php.

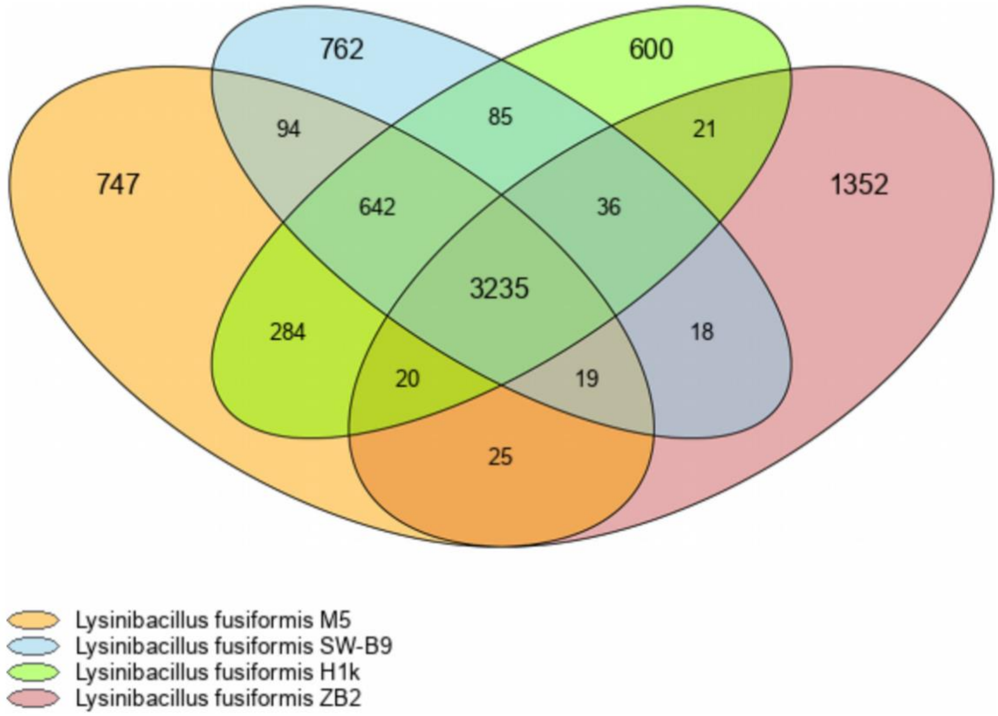


**Figure 5. Comparative genomic analysis between *L. fusiformis* strains**

Comparative analysis of the samples reveals the number of common and unique genes among the samples analyzed. The outcome displays the number of pan- genome and core-genomes all of *Lysinibacillus fusiformis* strains, The core genome may contain contains genes present in all individuals, and one or more homologous, we find the common gene potentially involved in the control of SA mediate response is: shikimate kinase, this essential gene that is present in *Lysinibacillus fusiformis SW-B9* which is a plant-associated is closely related to *lysinibacillus sp. 7b.* During the biosynthesis of salicylic acid (SA) in plants, chorismate is converted to isochorismate, the enzyme isochorismate synthase catalyzes the reaction that converts chorismate to isochorismate. This reaction arises from the chorismic acid and is involved in the biosynthesis of aromatic amino acids.

Pan-Genome describes the entire range of genotypes, or all of the genotypes of all species, salicylate esterase (SE) is also accountable on roots developments in plants. (SE) is essential for controlling the pathway of (SA) biosynthesis in the bacteria *Lysinibacillus* *fusiformis* SW-B9. The SA retroactively prohibits the esterase activity of the (SABP2), allowing for fine-grained regulation of AS levels. Salicylic acid (SA) is responsible for certain plant defense responses and NPR1 is the master regulator of SA perception.

# Conclusion:

The *Arabidopsis thaliana* model organism is widely used in plant biology research because it provides a unique combination of genetic resources, culture ease, and application across various domains. In this study, the plant is used to have a beneficial effect on endophytic in plant growth under salinity stress, specifically in the interactions between bacteria and plant growth through endophytes. In this study, we shows that SA plays a crucial role during *A. thaliana* resistance to the salt stress and that some of the endophytes isolated from another model plant, *M. truncatula* (S116 strain) can confer a resistance to the salt stress. Interestingly, the resistance mediated by S116 is associated to correct synthesis and signaling of SA. Comparative genomics reveals the presence of genes potentially involved in SA synthesis in M50 closest strain, these genes include shikimate kinase, salicylate esterase who have a relationship.

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