

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DETERMINATION OF INTERACTION BETWEEN
***Arabidopsis thaliana* AND PUTATIVE ENDOPHYTIC**
BACTERIUM ASSOCIATED WITH FRASER PHOTINIA

MARIA BATOOL
A THESIS SUBMITTED FOR THE DEGREE OF
MASTERS OF SCIENCE
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

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ALEVAĞACI İLE İLİŞKİLİ OLASI
ENDOFİTİK BAKTERİNİN *Arabidopsis thaliana*
BİTKİSİ İLE ETKİLEŞİMİNİN
BELİRLENMESİ

MARIA BATOOL
YÜKSEK LİSANS TEZİ
MOLEKÜLER BİYOLOJİ VE GENETİK ANABİLİM DALI

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SUMMARY

Plant growth promoting bacteria (PGPB) are normally beneficial to the plants in several ways such as increase in overall plant mass or root growth or sometimes an increase in chlorophyll contents. Recently, a putatively endophytic PGPB was isolated from long term *in vitro* cultured Fraser photinia microshoots [Şeker, et al., 2017]. This thesis aimed to analyze possible positive effects of this putative endophytic bacterium on the overall *in vitro* growth of model plant, *Arabidopsis thaliana*. A simple flood inoculation technique was performed at two different physiological stages of *in vitro* germinated *Arabidopsis* i.e. seed and one week-old seedling. Seeds and seedlings were inoculated with 10^7 and 10^8 cfu/mL Active (A) and Inactive (In) (at 95° C for 20 min.) endophytic bacterial suspensions along with their mix compositions (A+In) and suspended in MPYE broth along with controls (untreated ones). Biological replicates were subjected to plant growth conditions at 22°C with 16/8 h photoperiod. Two week-old seedlings were evaluated for various plant growth parameters like fresh shoot weight (FSW), shoot and root fresh weight (FW), shoot length (SL), dry shoot weight (DSW), shoot and root dry weight (DW), root length (RL) and photosynthetic pigments including chlorophyll *a*, *b* and carotenoids of plantlets as well as endophytic and rhizospheric nature of bacteria. Positive effects of various inactive and active bacterium suspensions were determined on fresh and dry root and shoot biomass and photosynthetic pigments for 7 day inoculated seedlings whereas an increase in photosynthetic contents for germination stage inoculations was observed. Verification of rhizospheric and endophytic colonization of the bacteria was done using conventional Gram staining method and PCR was used to show the presence of *virD1* gene which is previously determined to be existed in the plasmid of the bacterium. Overall findings demonstrated that this putative endophytic bacterium is beneficial in active and even more useful in inactive form for *A. thaliana* model plant if it is used in optimum concentrations and conditions and has potential to be used as bio-fertilizer along with various other beneficial PGPRs. Moreover, presence of putative *virD1* gene is also a positive sign for future evaluations of this bacterium for potential plant transformation purposes.

Keywords: *Arabidopsis thaliana*, Endophytic bacteria, Rhizospheric interaction, *virD1* gene.

ÖZET

Bitki büyümesini teşvik eden bakterilerin (BBTB) genellikle bitki biyokütlesini ve kök büyümesini ve bazı durumlarda da klorofil içeriğini arttırdığı bilinmektedir. Son zamanlarda, uzun süre *in vitro* koşullarda kültürlenmiş alev ağacı mikrogövdelelerinden olası endofitik bir BBTB adayı bakteri izole edilmiştir [Şeker, et al., 2017]. Tez çalışmasında, sözkonusu bu bakterinin *Arabidopsis thaliana* (*A. thaliana*) model bitkisinin büyümesine olan etkilerinin belirlenmesi amaçlanmıştır. Denemeler basit pipetleme inokülasyonu tekniğiyle, *in vitro* koşullarda çimlendirilen *A. thaliana* bitkisinin tohumu ve bir haftalık fidesi olmak üzere iki farklı fizyolojik aşamasında gerçekleştirilmiştir. Tohumlar ve fideler, Aktif (A) ve İnaktif (İn) endofitik bakteri süspansiyonu ve ayrıca bunların karışımlarıyla (A+İn) inoküle edilerek bakteriyle inoküle edilmeyen kontrol gruplarıyla karşılaştırılmıştır. Biyolojik tekrarlar; 22°C'de 16/8 saatlik ışık periyodu koşullarında bitki büyüme odasına kaldırılmıştır. İki haftalık fidelerin yaş gövde ağırlığı (YGA), gövde ve kök yaş ağırlığı (YA), gövde uzunluğu (GU), kuru gövde ağırlığı (KGA), gövde ve kök kuru ağırlığı (KU), kök uzunluğu (KU) ve klorofil *a*, *b* ve karotenoidleri içeren fotosentetik pigmentlerin miktar tayini gibi çeşitli bitki büyüme parametreleri ve bakterilerin endofitik ve rizosferik nitelikleri değerlendirilmiştir. 7 günlük *Arabidopsis* fidelerinin inaktif ve aktif bakteri süspansiyonları ile inokülasyonu, kök ve sürgünlerin yaş ve kuru ağırlıklarını ve fotosentetik pigmentlerin miktarlarını olumlu yönde etkilenmişken, bakteri inokülasyonun tohum aşamasında uygulanması sadece fotosentetik pigmentlerin miktarlarını arttırmıştır. Bakterilerin rizosferik ve endofitik kolonizasyonunun doğrulanması için Gram boyama yöntemi kullanılırken, bakterinin plazmitinde *virD1* geni varlığı PCR yöntemi ile moleküler olarak da doğrulanmıştır. Elde edilen sonuçlara göre; tez çalışması kapsamında çalışılan olası endofitik bakteri, optimum konsantrasyonlarda ve koşullarda kullanıldığında, aktif olarak bazı olumlu etkiler göstermişse de, inaktif formda inoküle edilen bakteri süspansiyonlarının bitkiye daha faydalı olduğunu önermektedir. Sonuç olarak, tez kapsamında çalışılan olası endofitik bakteri izolatının çeşitli bitki büyümesini teşvik eden yararlı bakteriler (BBTB) ile birlikte biyo-gübre olarak kullanılabileceğine dikkat çekilmektedir.

Anahtar kelimeler: *Arabidopsis thaliana*, Endofitik bakteri, Rizosferik etkileşim, *virD1* geni.

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LIST of ABBREVIATIONS and ACRONYMS

<u>Abbreviations and Acronyms</u>	<u>Explanations</u>
µg	: microgram
µl	: microliter
g	: gram
ng	: nanogram
A	: active bacteria
BAP	: benzyl amino purine
BCA	: biological control agents
C	: control
cfu	: colony forming unit
<i>Chl</i>	: chlorophyll
dH ₂ O	: distilled water
DNA	: deoxyribonucleic acid
dpi	: days post inoculation
DW	: dry weight
DSW	: dry shoot weight
E1	: endophytic active bacteria
E2	: endophytic inactive bacteria
E3	: endophytic control
EtOH	: ethyl alcohol
FW	: fresh weight
FSW	: fresh shoot weight
GA	: gibberellic acid
GE	: gel electrophoresis
HCl	: hydrochloric acid
IAA	: indole-3-acetic acid
IM	: infiltration media
In	: inactive bacteria
M	: molar
MES	: 4-morpholine ethane sulfonic acid

mL	: milliliter
mM	: millimolar
MPYE	: mineral-peptone-yeast extract
MS	: murashige skoog
NaOCl	: sodium hypochlorite
NaOH	: sodium hydroxide
nm	: nanometer
PCR	: polymerase chain reaction
PGPB	: plant growth promoting bacteria
PGPC	: plant growth promoting characteristics
PGPR	: plant growth promoting rhizobacteria
pmol	: picomol
PSW	: physiological saline water
R1	: rhizospheric active bacteria
R2	: rhizospheric inactive bacteria
R3	: rhizospheric control
RL	: root length
SW	: shoot weight
SL	: shoot length
Ti	: tumor-inducing
UV	: ultraviolet

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1. INTRODUCTION

Arabidopsis thaliana stands alone, of all the known flowering plants, as the most thoroughly studied species so far. Being quite useful for genetic experiments, *Arabidopsis* was originally adopted as a model organism. There are some principle attributes that make this plant different and feasible to study such as short life cycle, high seed fertility rate because of self-pollination, small size that make it more economical to study as limited growth facilities are required. Other than that, some more features, that were unforeseen 40 years ago include compliance to detailed analysis, definitive genetic map and accessibility for mutation analysis etc. have kept *Arabidopsis thaliana* a primary model for plant studies. [Koornneef and Meinke, 2010].

There are generally two types of soil bacteria described to have potential to act as plant growth promoting bacteria (PGPB); those which are found around the roots of termed as rhizospheric, and which reside inside plants are termed endophytic [Lacava and Azevedo, 2013]. Usually, if not always, rhizospheric and endophytic bacteria follow the same kind of mechanisms to support plant growth. Only endophytic differs due to the fact that once inside the plant tissues, they remain no longer to exposed to environmental stresses and limiting factors such as soil, pH, water accessibility or other biotic factors etc. which may compete with the PGPB residing around the rhizosphere and limit their growth [Glick, 2012].

Hallmann et al. (1997) defined endophytic bacteria as “visibly unharmed bacteria isolated from surface disinfested plant tissue or extracted from inside the plant”. It is thought that these endophytic bacteria follow the same mechanisms of action as does the plant growth promoting rhizobacteria (PGPR) [Vessey, 2003].

Almost 300 000 plant species have been reported to live on this planet out of which of which the percentage of plants tested against these endophytic bacteria is very low which accordingly provide the likelihood of subsequent discovery of further more important and novel metabolites which may have possible applications in medicine, biotechnology, agriculture, environmental protection and in the field of pharmaceuticals [Ryan et al., 2008; Strobel et al., 2004].

A vast level of diversity has been shown for the bacterial endophytes not only in respective plant hosts but also among the bacterial phylum [Bacon and Hinton, 2006]. There is a huge number of Gram negative and positive bacterial species found among

the bacterial populations isolated from endophytic and rhizospheric habitats. There were 71 different genera of 219 endophytic bacteria species reported by Hallmann and Berg (2006) out of which mostly belong to common soil bacteria such as *Pseudomonas*, *Enterobacter*, *Bacillus*, *Burkholderia* and *Azospirillum*.

It is never known which species or groups of endophytic or rhizospheric bacteria are beneficial to plant growth and which is harmful; nevertheless, this thesis presents the interaction of putative endophytic bacterium, which is recently isolated from long term *in vitro* cultures of Fraser photinia microshoots [Şeker, et al., 2017], with model plant *Arabidopsis thaliana*.

1.1. Aim and Scope of the Study

Plant-microbe interaction is a very important subject that draw considerable interest when it comes to the factor of plant growth promotion. Normally, microbial contamination is considered as harmful when it comes to plant tissue culture and preferred to be eliminated to prevent loss of the culture. But sometimes, among these contaminants, there can be some beneficial microorganisms that have growth promoting features and can become potential bio-inoculants. Therefore, it is striking for the scientists to isolate and characterize these contaminants that can become potential plant growth promoting agents, in order to study their effects and produce biologically active compounds from the plants. With this approach, our research group [Şeker, et al., 2017] recently isolated and identified a putative endophytic bacterial contaminant that was detected in Fraser photinia long term *in vitro* cultures of microshoots. The bacterium was analyzed for phenotypic and biochemical activities as well as its potential as plant growth promoting endophytic bacteria. The results indicated that this bacterium possessed nitrogen-fixing ability and also rendered positive for gibberellic acid (GA₃) and indole-3-acetic acid (IAA) production. It was identified as Gram negative putative endophytic bacterium while phylogenetic tree construction by Neighbour Joining method and 16S rRNA sequencing showed that this bacterium had striking resemblance with uncultured bacteria and Rhizobiales. So concludingly, this bacterium was regarded as potential beneficial microorganism to promote overall growth of Fraser photinia. Thus, the aim of the study is to determine the possible interaction of this bacterium by using a model plant such as *Arabidopsis thaliana* to understand whether this bacterium is i) a host specific one, ii) endophytic

one, iii) and it has a beneficial effect on *Arabidopsis thaliana in vitro* seed germination and seedling growth. Moreover, several other factors (population density (cfu/mL, infiltration media) of the above mentioned bacterium were also optimized to study plant and bacterium interaction.

2. PLANT GROWTH PROMOTING RHIZO-BACTERIA (PGPR)

Plant growth-promoting rhizobacteria (PGPR) are among the beneficial interactions between plant and microorganisms which are found to exist and colonize inside many plant species as endophytes and encourage the induction of plant growth by positively effecting the plant physiology as well as increased resistance toward abiotic stresses (salinity, water logging, soil pH etc.) and biotic factors such as microbial diseases [Poupin, et al., 2013]. So, it can be stated that PGPRs are free living or root colonizing soil borne bacteria, which provide overall support to plant growth when plant or seed is vulnerable to soil borne diseases and stresses [Kloepper et al., 1980]. As these microorganisms are beneficial to plant growth and development, they can promote plant growth by direct or indirect mechanisms [Kloepper et al., 1989]. PGPRs directly promote plant growth by facilitating the uptake of water and nutrients from the soil and environment or by synthesizing the essential substances which are otherwise unavailable to plants [Glick et al., 1999]. While indirectly, these microorganisms may provide defense or tolerance against harmful effects of plant pathogens or by producing such substances that cause an increase in natural resistance of plants against these pathogens [Cartieaux et al., 2003; Nehl et al., 1996; Handelsman and Stabb, 1996].

It is essential for the PGPRs to contain certain inherent abilities which define them. These might include i) ability to actively colonize the roots surface, ii) ability to thrive and compete against the other potentially harmful microbiota in the surrounding and simultaneously carry out the plant growth activity and iii) ability to promote plant growth directly or indirectly [Kloepper, 1994]. So the plant growth promoting rhizobacteria are actually that 2-5% of soil microbiota, which when isolated from a rhizosphere and again introduced by plant inoculation in a potentially competitive soil microflora, promote the growth and overall development of plant [Kloepper, Schroth, 1978].

2.1. PGPRs Mechanisms of Action

Somers et al. (2004) characterized PGPR according to their operational activities as i) bioinoculants (biofertilizers, which can make the nutrients available to the plant),

ii) phytostimulators or plant growth promoters (produce phytohormones), iii) rhizoremediators (degrade and recycle organic contaminations surrounding roots) and iv) biopesticides, producing antifungal and antibiotic metabolites [Antoun and Prevost, 2005]. Moreover, it is customary that even a single PGPR can exhibit more than one, often several, modes of actions including biocontrol [Vessey, 2003; Ryu et al., 2003]. The direct mechanisms that PGPR exhibit might also include nitrogen fixation, siderophores production, phosphate solubilization, phytohormones production such as cytokinins, auxins, gibberellins and also reducing ethylene concentration [Glick 1995; Glick et al. 1999; Kloepper et al. 1989]. Antibiotic production is exhibited by many PGPRs and also metabolite production is one of several indirect features which also has antimicrobial activities. Iron depletion from rhizosphere is also a feature of PGPRs whereas many also produce certain enzymes that cause lysis of fungal cell walls, thus acting as biological control agents. Competition in rhizosphere and inducing systemic resistance in plants are also key features of PGPRs [Dunne et al., 1993; Glick et al., 1999; Kloepper et al., 1988; Liu et al., 1995; Weller and Cook, 1986].

2.1.1. Nitrogen fixation

Nitrogen is among the most essential nutrients needed to promote the yield and growth of plant as it is essentially integrated with nucleic acids, biomolecules and proteins [Bøckman, 1997]. Atmosphere is composed of 79% of nitrogen but unfortunately, it is not in available form for the plants and other eukaryotic organisms and thus required to be converted to soluble or available forms such as ammonia or others [Ladha et al., 1997]. Among the most analyzed and studied diazotrophs, *Azospirillum* sp. is best studied and it was isolated by Beijerinck in 1925 from nitrogen depleted soils [Holguin et al., 1999]. This bacterial genus has capability of plant growth promotion by fixing atmospheric nitrogen to available forms. In 1987, Lifshitz with his colleagues isolated certain bacterial strains from different native plants growing in Canadian High Arctic which possessed the capability to reduce acetylene to ethylene. These strains turned out to belong to *Pseudomonas* genus and had acquired ability of colonizing canola cv. Tobin rhizosphere under field conditions. However, these bacteria didn't followed the path of nitrogen fixation to promote plant growth as

the amount of nitrogen fixed by them was very low and it was predicted that these bacteria may produce certain other factors such as phytohormones or may induce enhanced nutrient uptake instead of nitrogen fixation [James and Olivares, 1997].

2.1.2. Phytohormones

Phytohormones or plant growth promoters' production, as discussed above, is one of the most direct modes of action which PGPRs exhibit to facilitate plant growth [Glick, 1995]. Auxins, gibberellins and cytokinins, ethylene and abscisic acids when applied to plants increase the growth and yield of the plant and this has been reported by Frankenberger and Arshad (1995) in detail. Over the last two decades, phytohormones production by microbial population has been a hot topic and studied and reviewed by various authors [Barbieri and Galli 1993; Hartmann et al., 1983; Pilet et al., 1979; Patten and Glick 1996; Fallik and Okon 1989; Patten and Glick, 2002].

There are certain features that show the response of plant towards auxins such as enlargement of cell, root initiation, accelerated growth rate, apical dominance, elevated cell division, root initiation, geotropism and phototropism etc. [Frankenberger and Arshad, 1995]. Most (80%) of the rhizospheric microorganisms isolated from soils of various crops are capable of auxin production [Kampert et al., 1975; Loper and Schroth, 1986]. These may include *Pseudomonas*, *Acetobacter*, *diazotrophicus*, *Azospirillum*, *Xanthomonas*, *Rhizobium*, *Bradyrhizobium japonicum* as well as *Alcaligenes faecalis* [Patten and Glick, 1996].

Cytokinins are N⁶-substituted aminopurines which are capable of effecting both physiology and development of plants [Salisbury and Ross, 1992]. They constitute another group of phytohormones which are produced by certain microorganisms [Cartieaux et al., 2003]. Non-pathogenic populations of microorganisms residing in the rhizosphere of the plant have also been reported to effect the overall development and physiology of the plant [Arshad and Frankenberger, 1993]. Bioynthesis of cytokinins so far, has not been studied very often as in the case of auxins production by the microorganisms [Frankenberger and Arshad, 1995]. Plants when inoculated by cytokinins *in vitro* result in enhanced root growth, inhibited root elongation and enhanced root hair formation, elevated cell division and various other physiological responses [Frankenberger and Arshad, 1995]. The percentage of bacterial populations

isolated from rhizosphere of plants demonstrating the ability of cytokinins production is higher (90%) as compared to auxin production by these microorganisms [Barea et al., 1976].

Some examples of rhizospheric PGPB are like *Micrococcous*, *Arthrobacter*, *Caulobacter*, *Azotobacter*, *Flavobacterium*, *Bacillus*, *Burkholderia*, *Agrobacterium*, *Azospirillum*, *Erwinia*, *Chromobacterium*, *Pseudomonas* and *Serratia* etc. [Bhattacharyya and Jha, 2012]. Similarly, some examples of the endophytic PGPB are *Mesorhizobium*, *Allorhizobium*, *Bradyrhizobium*, *Azorhizobium*, and *Rhizobium* of the family Rhizobiaceae. Rhizobacteria belonging to these groups are mostly Gram negative rod shaped while a lower percentage of cocci, Gram positive or pleomorphic is recorded [Bhattacharyya and Jha, 2012]. A major portion of rhizosphere based microorganisms contains various species of actinomycetes which have potential to exhibit remarkable plant growth promotion features [Bhattacharyya and Jha, 2012; Merzaeva and Shirokikh, 2006]. Among them, *Micromonospora* sp., *Streptomyces* spp., *Streptosporangium* sp., and *Thermobifida* sp., exhibiting a great potential as biological control agents against various root bacterial and fungal pathogens are worth mentioning [Bhattacharyya and Jha, 2012; Franco-Correa et al., 2010].

It appears that the nature has adapted a mechanism of natural selection for bacteria best suitable to reside inside plants. There is a large population of rhizospheric or free living bacteria from where the endophytes are mobilized inside the plants. A study on bacterial populations associated with potato indicated that versatility and enrichment of bacteria antagonistic to fungal pathogens was low within the roots in comparison to the ones surrounding the potato rhizosphere [Berg et al., 2005]. Similar kind of results were reported by Germida and associates (1998) where rhizospheric species were more diverse and endophytic populations seemed to appear from rhizospheric populations. For example, *Rhizobium etli* strains found residing inside the stems of maize were observed to be the fraction of population originated from the *Rhizobium etli* pool present in nodules of *Phaseolus vulgaris*, roots or around the roots [Rosenblueth and Martínez-Romero, 2004]. There were certain competition experiments carried out which showed that some endophytic bacteria are more vigorous colonizers and displace other populations easily. For instance, Verma et al. in 2004 reported out competing of *Ochrobactrum* sp by *Pantoea* sp. in rice and Rosenblueth and Martínez-Romero in (2004) with different *Rhizobium etli* strains in maize.

2.2. Endophytic Bacteria and Plant-Microbe Interaction

Endophytes are generally considered as relatively nature's untapped source of important natural products and therefore biological studies of endophytes still remains of interest. There is a continuous progress in reporting various kinds of endophytes in the process of discovering more and more diverse endophytic bacteria associate with plant tissues and roots [Rosenblueth and Martinez-Romero, 2006; Hallmann and Berg, 2006; Ryan et al., 2008]. Endophytes contribution towards growth and development of various plant varieties has been reported [Hardoim et al., 2008; Sessitsch et al., 2004].

Normally, the main point of entry of endophytic bacteria residing inside plant tissues is thought to be primarily through root cracks, considered as "hotspots" for bacteria colonization, formed in lateral junction of roots [Hardoim et al., 2008]. Although this appears to be the most evident entry site, other different sites of entry have also been proposed such as aerial parts of plants and seeds. [Lodewyckx et al., 2002].

All plants, ranging from monocotyledons to dicotyledons and from woody trees to succulent crops, have been reported to contain endophytic bacteria inside [Lodewyckx et al., 2002]. Not only this but also structures and organs of plants such as stems, roots, leaves, tissues, fruits and shoots etc. have been observed to be consisting of endophytic bacteria [Hallmann et al., 1997].

2.2.1. Mechanisms of Action for Endophytic Bacteria

Endophytic bacteria in a single plant host are not restricted to a single species but comprise several genera and species. No one knows if communities inside plants interact, and it has been speculated that beneficial effects are the combined effect of their activities. There can be various factors of endophytes that can help or stimulate to benefit the plant such as nitrogen fixation, siderophore production, biocontrol of root zone phytopathogens, production of phytohormones, enhancement of availability of nutrients etc. [Sessitsch et al 2002; Sturz et al., 2000]. A newly discovered mechanism is production of volatile substances such as 2-3 butanediol and aceotin by bacteria which seems responsible for plant-growth promotion [Ryu et al., 2003].

Endophytes produce adenine ribosides that stimulate growth and mitigate browning of pine tissues [Pirttilä et al., 2004]. Bacterial endophytes may benefit other crops in rotation with the host plants as they are capable of suppressing nematode proliferation [Rosenblueth and Martínez-Romero, 2006].

Sturz in 1995 provided direct evidence for plant growth-promoting activity by endophytic bacteria. His studies revealed the recovery of approximately 10% of bacterial isolates from within potato tubers that were shown to promote plant growth. In other experiments with clover and potatoes in a crop rotation 21% of the isolated endophytic bacteria promoted plant growth, showing 63% increased shoot height, 66% shoot wet weight and 55% increased root wet weight [Sturz et al., 1998]. Endophytic actinobacteria are effective antagonists of the pathogenic fungus *Gaeumannomyces graminis* in wheat [Coombs et al., 2004], and several endophytes showed antagonism against *Rhizoctonia solani* [Parmeela and Johri, 2004]. Some of the endophytic isolates produced antibiotics and siderophores *in vitro* [Sessitsch et al., 2004]. Remarkably, *Salmonella* strains were been detected as endophytes in alfalfa sprouts. Since 1995, outbreaks with these bacteria in alfalfa sprouts have been recorded in North America, Asia and Europe [Ponka et al., 1995]. It has been suggested that alfalfa plants and seeds should be colonized with safe bacteria to out-compete human pathogens. For example, *Salmonella enterica* and the enterohemorrhagic *Escherichia coli* were found to be eliminated by *Enterobacter absuriae* from *Arabidopsis thaliana* seeds [Cooley et al., 2003]. It is a matter of concern that there may be human or opportunistic pathogens among plant endophytes.

We currently have not much information about any specific form of communication between the endophytic bacteria and plants that serves in colonization of plant tissues internally, as compared to the mechanisms used by Rhizobia spp. [Spaink, 2000]. Rhizobia spp. are endophytic bacteria that internally colonize the plant tissues and serve in nodule formation that results in nitrogen fixation [Bonafante and Anca, 2009] in return of fixed carbon [Hong et al., 1991]. *Gluconacetobacter diazotrophics* can be thought the best example of endophytic bacterial diazotrophs which was first isolated from sugarcane in Brazil about two decades ago and has continued to be isolated all over the world. It has a wide range of host crops such as corn, sugarcane and wetland rice [Saravanan et al., 2008]. Other examples of bacterial diazotrophs isolated from crops include *Klebsiella* spp. from maize, *Herbaspirillum* spp. from maize, sugarcane and rice and *Azoarcus* spp. from kallar grass [Dobbelaere

et al., 2003]. Endophytic bacteria are capable of fixing N₂ in various crops without forming nodule-like structures with plants. They colonize the interiors of plant tissues such as roots, stem and leaves and also found to be beneficial for plant growth as they are able to fix nitrogen [James and Olivares, 1997]. These endophytes residing inside the plants are in direct contact with the host to get supply of nutrients directly from their host plant as well as protection from competition with other microorganisms. In return, the inner environment of plant rich in carbon and low in O₂ provides favorable conditions for fixation of N₂ by bacteria and feasible transfer to their hosts [Ladha and Reddy, 2003]. Some examples of endophytic diazotrophs of rice, maize and sugarcane are *Azotobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Azoarcus* spp., *Enterobacter asburiae* and some strains of *Burkholderia* spp. which serve as nitrogen fixers when other available sources of nitrogen are limited [Reinhold et al., 1986, Kirchhof et al., 1997].

Phosphorus deficiency is thought as the limiting factor for legume plants to attain a higher yield as it is important in nodulation and N₂ fixation processes. There are various soil bacterial and fungal genera that have the ability to solubilize insoluble inorganic P mixes and make them accessible for plant take-up. Such microorganisms are alluded to as phosphate solubilizing microorganisms (PSM) [Rodriguez and Fraga, 1999]. Among bacterial genera that are thought to be individuals from PSM are *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium* and *Erwinia*. But most oftenly, *Pseudomonas*, *Bacillus*, and *Rhizobium* are the prevalent P solubilizers [Richardson, 2001]. There was a significant enhancement in dry weight of root, shoot, nodule and nodule number of soybean when co-inoculated with *Bradyrhizobium japonicum* and *Pseudomonas putida*, a good P solubilizer, in comparison to inoculation with *B. japonicum* alone [Rosas et al., 2006]. In a relatively similar case, a significant increase in dry weight of root and nodule, shoot N content and total biomass in both laboratory and field conditions was reported, when chickpea was co-inoculated with *Rhizobium leguminosarum* and *Bacillus megatherium* (M-3), a P-solubilizer [Elkoca et al., 2008].

Iron (Fe) availability can be increased for plants using siderophore-producing bacteria which can enhance the overall growth of host plants by their biocontrol activities (by making iron unavailable for pathogens) or by acting as biofertilizers (by making Fe available to plant from its rhizosphere) [Glick, 1995]. According to a report [Marek-Kozaczuk et al., 1996] when clover was co-inoculated with siderophore

producing *Pseudomonas fluorescens* and *Rhizobium leguminosarum* bv. *Trifolii* under gnotobiotic conditions, positive effect on plant growth along with symbiotic N₂ fixation activity was observed.

Endophytic bacteria also are reported to be able to synthesize indole-3-acetic acid (IAA) [Sessitsch et al., 2004]. Molla et al. in (2001) observed a significant increase of root length, root number, root hair density, root dry matter, fresh weight and number of nodules when soybean was co-inoculated with *Azospirillum brasilense* with *Bradyrhizobium japonicum* either by *Azospirillum* alone or its co-inoculum.

Many Biological Control Agents (BCA) are known to reduce the incidence and severity of plant disease, for example, *Pseudomonas* spp. and *Bacillus* spp. are some of the prominent bacterial BCAs, whereas *Trichoderma* spp. is the most important for fungus inhibition and antagonism [Gerhardson, 2002]. Bacterial endophytes also effectively antagonise a broad spectrum of fungal pathogens [Berg and Hallmann, 2006], majority of which are Gram-negative bacteria and are dominated by the fluorescent pseudomonads, followed by predominant antagonistic group members of the genus *Bacillus* being among Gram-positive bacteria [Berg and Hallmann, 2006].

A review article suggested that as the interest with endophytic bacterial endophytes increases, there is a bright possibility of using them in real world applications which may include industrial and medical applications (e.g. antibiotic production), for plant protection from phytopathogens, as biofertilizers and growth stimulators and for environmental cleansing from organic contaminants by phytoremediation [Ryan et al., 2008]. A near-future application may consider the use of genetically engineered endophytes with biological control potential in agricultural crops. [Rosenblueth and Martínez-Romero, 2006]. Examples of positive effects were the growth promotion of endophytic bacteria during acclimatization of strawberry and pineapple tissue cultures. In woody plants, inoculation of poplar tissue cultures with a *Paenibacillus* isolate led to a higher number of and longer roots on microcuttings. [Quambusch, et al., 2016].

Endophytes are thought to be present in almost all plant species and their presence in *in vitro* plant-endophytes interaction study has been investigated, and along with their beneficial effects, often they are observed to be having detrimental effects too. [Schulz and Boyle 2006; Hardoim et al., 2015, Zaspel et al., 2008; Dias et al., 2008; Almeida et al., 2009; Abreu-Tarazi et al., 2010; Donnarumma et al., 2011; Marino and Gaggi`a, 2015]. Certain members of the fluorescent Pseudomonad group

have been shown to be potential agents for the biocontrol of plant root diseases, and also serve as potential PGPB's as mentioned before. But the bacterium or the group of pathovars of *Pseudomonas syringae* is among the top disease causing bacterium which causes economically important plant diseases [Mansfield et al., 2012]. In the late 1980s, several strains belonging to pathovars *tomato*, *maculicola*, *pisi*, and *atropurpurea* of *Pseudomonas syringae* were discovered to infect the model plant *Arabidopsis thaliana* [reviewed by Crute et al., 1994]. Depending on *P. syringae* strains, chlorosis can also be seen along with necrotic lesions on the tissue. Some strains of *P. syringae* also observed to cause cankers and galls [Agrios, 1997]. *Ralstonia solanacearum* is probably among the most destructive and pathogenic bacterium for agronomically important crops worldwide. Because of its wide geographical distribution and host range, it is really damaging and is responsible for an estimated US\$1 billion in losses each year worldwide on potato alone. [Elphinstone, 2005]. *R. solanacearum* is a model system for the study of bacterial pathogenicity as both root and a vascular pathogen and this was one of the first plant pathogen genomes to be entirely sequenced [Salanoubat et al., 2002]. *Agrobacterium tumefaciens* is used for *A. tumefaciens*-mediated genetic transformation in biotechnology because of the transferrable tumour-inducing (Ti) plasmid presence [Gelvin, 2010; Pitzschke and Hirt, 2010; Tzfira and Citovsky, 2002; Zupan et al., 2000] while on the other hand, it also cause crown gall disease in tomato and various other important crop species worldwide. In the near future, therefore, *A. tumefaciens* will continue to serve not only as a powerful tool for plant genetic engineering, but also as an excellent model organism to decipher host-pathogen interactions [Zaltsman et al., 2010]. Other important plant bacterial pathogens causing root, stem or foliar diseases may include *Pantoea stewartii* subsp. *stewartii* [Koutsoudis et al., 2006], *Xanthomonas campestris* pv *compestris*, *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), *Erwinia amylovora*, *Xylella fastidiosa*, *Dickeya* spp., *Pectobacterium carotovorum*, *Pectobacterium atrosepticum* [Mansfield et al., 2012] and *Clavibacter* etc. [Agrios, 1997].

2.3. *Arabidopsis thaliana* and Putative Endophytic Bacteria

Arabidopsis is an angiosperm flowering plant. Flowering plants originated relatively recently and the last common ancestor of all Angiosperms lived around 180 million years ago. It is an easily grown model plant at high density in glasshouse or

culture room, has a diploid genome, making analysis of recessive mutations easy. It self-fertilizes, so it is possible to isolate seed from a single plant without need to cross fertilize. A single plant produces hundreds or thousands of seeds. Transformation of *Arabidopsis* is now highly reproducible and not that genotype dependent, allowing for the generation of thousands of transformants when needed. The dramatic improvements in high efficiency of plant transformation, an important genetic tool, enhanced the stature and utility of *Arabidopsis* as an experimental organism, leading Koornneef and Meinke (2010) to conclude that adopting *Arabidopsis* as a model organism is indeed a fortunate choice.

Putative endophyte is a terminology often used for those microorganisms which have not yet been validated microscopically. Criteria to recognize “true” endophytic bacteria have been published [Reinhold-Hurek and Hurek, 1998] which requires not only the isolation of microorganism from surface-disinfected tissues but also microscopic evidence to visualize “tagged” bacteria inside plant tissues. [Rosenblueth and Martínez-Romero, 2006].

There are various variables that effect the plant endophytes such as the growth stage of plant, type of plant tissue analyzed, plant health, nutritional condition of plant, condition and type of soil including pH and moisture content, temperature, altitude etc. and sometimes even the type of plant under consideration [in case of testing one endophyte on another plant] [Hardoim et al., 2008]. Therefore, in order to have more understanding about various types of endophytes found in a single plant and their associations with plant, it is important to carry out broad screening techniques, whether culture based or not to determine the genotype-specific associations of endophytes. Such knowledge provides base for the future development of more efficient biological control agents including those specific from plant to plant. For this purpose, determination of endophyte core microbiome would be quite helpful especially in agronomically important plants. As a forward step in this reference, a recent study by Lundberg et al. in 2012 reported about the core root-associated microbiome in the model organism *Arabidopsis thaliana*. Poupin et al. in (2013) studied the effects of the PGPR bacterial model *Burkholderia phytofirmans* PsJN on the whole life cycle of *Arabidopsis thaliana*. The studies revealed that at different physiological stages of plant, strain PsJN colonized the roots of *A. thaliana* both rhizospherically and endophytically while early ontogeny also showed increased several growth parameters and accelerated growth rate of the plants by strain PsJN. Inoculations of *A. thaliana*

with *Paenibacillus yonginensis* DCY84 (T) and *Micrococcus yunnanensis* PGPB7 indicated that *P. yonginensis* DCY84 (T)-inoculated plants were more resistant to salinity, drought and heavy metal stresses than control plants [Sukweenadhi, et al., 2015]. A recent study documented that PGPB strains *Pseudomonas simiae*, *Chryseobacterium polytrichastri* and *Burkholderia ginsengiterrae* co-inoculated with aluminum stressed *Arabidopsis thaliana* were able to support the overall growth of plant by exhibiting plant growth promoting activities e.g. auxins and siderophores production and phosphate solubilization [Farh, et al., 2017].

3. MATERIAL AND METHODS

3.1. Materials

Table 3.1. The list of the kits and chemicals used in this thesis.

<i>Arabidopsis thaliana</i> Col-0 seeds		• Grown by Doğa Selin Kayıhan at Plant Biotechnology Lab greenhouse, Gebze Technical University, Kocaeli.	
PCR KIT		• DreamTaq Green PCR Mastermix® [5X] • Forward primer for rhizogene 15955 <i>virD1</i> gene • Reverse primer for rhizogene 15955 <i>virD1</i> gene • PCR 1ml eppendorfs • 100µl, 10µl micropipettes and tips	
Buffers, Solutions and Media	MPYE (Mineral-Peptone-Yeast Extract) Agar	• Peptone 3g/L • Yeast Extract 3g/L • Agar 15g/L • 1M MgCl₂ 1ml/L • 1M CaCl₂ 1ml/L	pH was adjusted as7.00 using 0.1M NaOH or 0.1M HCl. For MPYE broth, agar is not added
	MS (Murashige Skoog) Medium	• MS salt 4.405/L • Sucrose 10g/L • Gelrite 3g/L	For ½ MS, we used half of the MS medium g/L amount. pH was adjusted as 5.7 using 0.1M NaOH or 0.1M HCl.
	Infiltration Media 1 (final concentration 50ml) (IM1)	• BAP (Benzyl Amino Purine) 200 µl from 0.2 M stock • MS 0.11g • Tween 20 0.07% • Glucose 5%	pH adjusted as 5.7. Autoclaved and stored at 4°C
	Infiltration Media 2 (final concentration as 50mL) (IM2)	• MES [4-MorpholineEthane Sulfonic acid] 5mL from 500mM stock • Na₃PO₄.12H₂O 5 mL from 20mM stock • Glucose 5ml from 50g/L stock	IM2 is always prepared fresh from stock solutions. Stock solutions were stored at 4°C.
	Infiltration Media 3 (Final concentration as 50mL) (IM3)	• IM1+ 0.1% MES	
	Gram staining	• Crystal violet • Gram’s iodine • EtOH 96% • Lugol	
	Gel Electrophoresis	• 1x TAE • Agarose A [0.3 g for 30 mL gel [1%]] • Redsafe [1.5 µl for 30 mL] • Gel tank • Casting tray • Gel comb • Electric potential	
	Nucleospin® Plasmid/Plasmid Extraction Kit		

3.2. Methods

3.2.1. Surface Sterilization Protocol of *Arabidopsis thaliana* Seeds

A simple protocol for surface sterilization of *A. thaliana* seeds was followed. *A. thaliana* Columbia (Col-0) WT-02 seeds were used for all the experiments performed for this study. Seeds were first weighed according to the quantity required (120 seeds~0.033gm) in a clean eppendorf tube. Sterilization protocol was performed in sterile laminar flow cabinet (pretreatment with UV for 20 minutes followed by cleaning with Zefiran (1% v/v) and EtOH (70% v/v). Seeds were treated with 70% v/v EtOH for 1-2 minutes with seeds kept in continuous motion by gentle patting of tube. EtOH was drained out followed by rinsing of seeds with 15% v/v bleach (NaOCl) for 10 minutes. Again, it was ensured that the seeds stay in continuous motion during the whole time. Following bleach, seeds were washed 3 times for 5 minutes each with sterile distilled water and then transferred to 9mm Petri plates containing ½ MS solid medium, which is optimum medium for *A. thaliana* seedlings growth. Seeds were placed on the ½ MS containing petri plates in a horizontal line in the upper half of each plate with 10 seeds/petri plate. Sheer care was taken to keep the seeds in all the plates at the same level so that root length measurement and growth of seedling could be monitored easily at any time. Petri plates containing seeds were placed at 4°C in dark for~3 days to synchronize germination (the cooling of seed during germination in order to accelerate flowering when it is planted). After 3 days, Petri plates were transferred to plant growth chamber and kept vertically at 22°C with a photoperiod of 16 h of light and 8 h of dark.

3.2.2. Quantative Optimization (cfu/mL) of Bacteria

Quantative optimization (cfu/mL) of Fraser photinia associated putative endophytic bacteria was done and McFarland optical density was optimized accordingly. Normally, for PGPB, 10⁸ is considered as optimum cfu/mL for inoculation to the host plants. 100µl of putative endophytic bacteria was taken from -80°C stored in glycerol [Şeker, et al., 2017] and grown in 10mL of MPYE broth medium for 5-7 days as pre-culture. A main culture was started from this pre-culture

in 250 mL of flasks containing 100 mL of MPYE broth. After 7-10 days, classical serial dilution procedure was followed (Fig.1). 100µl of bacterial MPYE suspension was spread on 9mm Petri plates containing MPYE agar using spread plate technique. This putative endophytic bacterium is slow growing and it is required to use slightly thick layer of agar medium (about 23-25ml/Petri plate). Serial dilutions of upto 10^{-6} cfu/mL were inoculated to agar plates using spread plate technique. 2 replicates were made for each dilution on MPYE agar plates and petriplates were incubated at 30°C for 7-10 days until the colonies appeared. Colony counting was done for the plates with 100-300 colonies (statistically significant) by using a colony counter and multiplied by the dilution factor to obtain original cfu/mL for the parent culture. Initial number of bacteria was calculated according to the formula indicated below.

$$\text{Initial number of bacteria} = \frac{\text{dilution factor} \times \text{number of bacteria on Petri plates}}{\text{inoculation volume in mL}} \quad (3.1)$$

$$\text{Dilution factor} = \frac{1}{\text{dilution rate}} \quad (3.2)$$

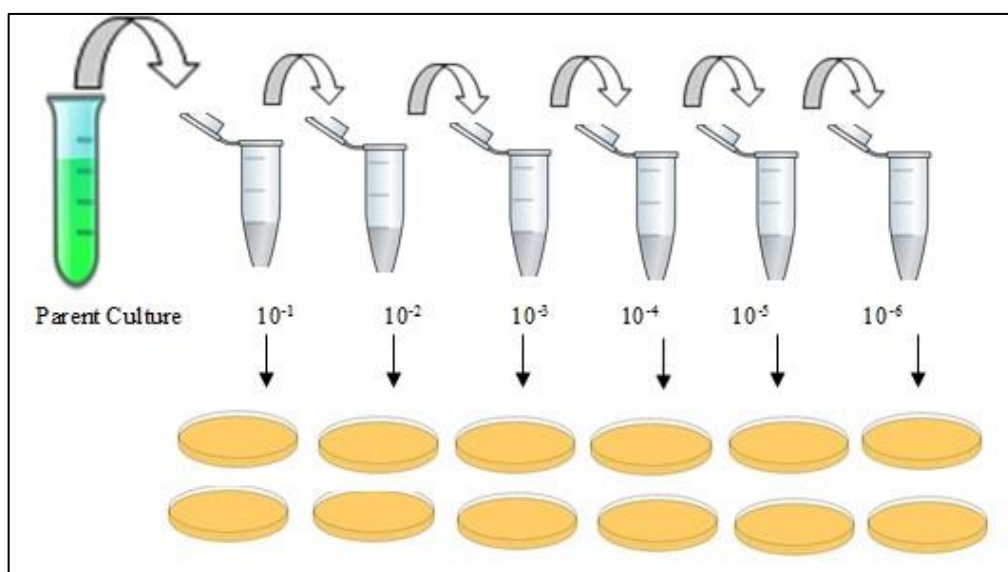


Figure.3.1. Schematic diagram of serial dilution method for optimization of cfu/mL of putative endophytic bacteria.

3.2.3. Infiltration Media Optimization

Determination of a suitable infiltration media to suspend putative endophytic *Fraser photinia* associated bacterium, in order to inoculate it to *A. thaliana* seedlings and seeds, was to be done. Various infiltration media were tested including IM1

suggested in literature to be best so far for interaction of bacteria with *A. thaliana* [Das, P. and Joshi, N., 2011], IM2, a media suggested in literature for tobacco transformation [Sparkes, et al., 2006], IM3, a solution prepared based on MES positive effects reported on *A. thaliana* [Kagenishi, et al., 2016] and MPYE which is a bacterial media for *Rhodobacter* [Öztürk, et al., 2008]. One week old seedlings and seeds of *A. thaliana* grown on ½ Murashige and Skoog agar plates were inoculated with Infiltration Media 1, 2, 3, and MPYE along with control (no media inoculation) using flood inoculation technique [Ishiga, et al., 2011] and kept vertically in plant growth room at 22°C with 16:8 light and dark period, seedlings for one week and seeds for 14 days, respectively. 14 days old seedlings (in both cases) were evaluated for fresh weight (FW), dry weight (DW), root length (RL), shoot weight (SW), chlorophyll *a*, *b* and carotenoids.

3.2.4. Inoculation of Putative Endophytic Bacterium to Seeds and Seedlings

Bacterial inoculation was conducted on one-week old seedlings and seeds of *A. thaliana*, in order to assess the effects of bacteria on two different physiological stages of the plant i.e. seeds and seedlings. Seeds and seedlings were inoculated with two different concentrations of Active (A) putative endophytic bacteria, i.e. 10^7 cfu/mL and 10^8 cfu/mL using flood inoculation technique [Ishiga, et al., 2011]. To assess the effect of inactivated bacteria, inoculums of same concentrations were heated separately at 95°C for 20 minutes and this was named Inactive (In) bacteria throughout the experiment. There were two other suspensions of bacteria, i.e. (A+In) 10^7 cfu/mL and (A+In) 10^8 cfu/mL tested for the possible effects on the seedlings and seeds (Table 3.2). Plantlets on ½ MS plates were inoculated with 1ml of inoculants under sterile conditions of laminar flow (20 minutes of pre-experiment UV sterilization) and 3 replicates (10 seeds or seedlings each) were done for each inoculation. Plates were placed in plant growth chamber vertically at 22°C with a photoperiod of 16 h of light and 8 h of dark for evaluation at 14 days after seed germination.

Table 3.2. Putative endophytic bacterium concentrations and suspensions

Concentrations Suspensions	10^7 cfu/mL	10^8 cfu/mL
Active (A)		
Inactive (In)		
Active + Inactive (A+ In)		
Control (no bacteria)	-	-

3.2.5. Determination of Putative Endophytic Bacterium effect on Seeds and Seedlings

One-week post inoculation, 7 days inoculated seedlings with bacterial suspensions were assessed for different growth parameters such as fresh shoot weight (FSW), fresh (shoot and root) weight (FW), dry shoot weight (DSW), dry (shoot and root) weight (DW), root length (RL), shoot length (SL) photosynthetic pigments such as *Chl a*, *Chl b* and caretonoids. FSW, FW, SL and RL of individual seedlings were measured the same day, whereas for DW and photosynthetic pigments, at least 24-hour delay was required as seedlings were to be incubated at 65°C for at least 24 hours. For FSW and FW, 4-5 seedlings were selected at random from each plate and weighed separately on weighing balance, first the shoot and then together with root of the seedling. Later, root length was measured for these seedlings followed by incubation of seedlings at 65°C on a filter paper for at least 24 hours before measuring DW. Dry weights were taken for each seedling the next day using sensitive weighing balance in the same way as the fresh weight was measured. For chlorophyll analysis, 2 seedlings from each plate were selected randomly and their roots were discarded, and shoots were kept in acetone in 2ml eppendorfs each at 4°C for at least 24 hours before measurement of photosynthetic pigments using spectrophotometer. After 24-48 hours, chlorophyll pigment analysis was done for wavelengths 661.6nm, 644.8 nm and 470 nm for each shoot. Calculations were made for *Chl a*, *b* and carotenoids using Lambert Beer Law

(equation below) [Lichtenthaler and Buschmann, 2001] and equations for chlorophyll analysis.

$$c_a (\mu g/ml) = 11.24 A_{661.6} - 2.04 A_{644.8} \quad (3.3)$$

$$c_b (\mu g/ml) = 20.13 A_{644.8} - 4.19 A_{661.6} \quad (3.4)$$

$$c_{(x+c)} (\mu g/ml) = (1000 A_{470} - 1.90 c_a - 63.14 c_b)/214 \quad (3.5)$$

The concentration of *Chl a* and *b* in plant material can be quantified with different reference systems. Here we used fresh weight as the reference system (Index). For seeds stage experiment, 14 days post inoculation (dpi); same procedures were repeated as mentioned before.

3.2.6. Determination of Bacterial Existence inside Seedlings

To check endophytic as well as rhizospheric interaction of bacterium with the plantlets, various protocols were followed. Note that we needed to surface sterilize the seedlings which were to be assessed for endophytic interaction. For rhizospheric interaction, plantlets were to be suspended in the bacterial broth medium i.e. MPYE. For this, first the seedlings (3 each) were weighed randomly from plates with active bacteria, inactive bacteria and no bacteria (control), separately each time for both endophytic and rhizospheric assessment. Immense care was taken not to contaminate the seedlings at any step with any other bacteria, because it was the crucial step when seedlings could be contaminated, especially for rhizospheric interaction analysis. For this, immense sterile conditions were provided, where sterile petri plate for weighing was used on surface sterilized weighing balance in UV sterilized laminar flow. Seedlings for endophytic assessment were suspended in 5% of hydrogen peroxide (H₂O₂) for 3 minutes in order to decontaminate rhizosphere associated bacterium. It was assured that seedlings were on orbital shaker during all the sterilization procedure. Later, these seedlings were rinsed with sterile distilled water 3 times for 5 minutes each, to wash away any surface H₂O₂ left. Surface sterilized seedlings for endophytic interaction were crushed in sterile mortar pestle with 1ml of MPYE broth, each separately for endophytic active

bacteria (E1), endophytic inactive bacteria (E2) and endophytic control (E3) seedlings, i.e., in total 3 inoculums for endophytic colonization. Serial dilutions were made from these crushed extracts as 10^0 , 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} in 1.5 ml eppendorf tubes each containing 900 μ l MPYE broth. 100 μ l from each solution was spread on MPYE agar plates using glass rod spread plate technique, making 2 replicates for each dilution. A cross check for validity of correct sterilization protocol was also performed by taking last sterilization water and spreading separately on MPYE agar plates, with two replicates for each. For rhizospheric analysis, seedlings after weighing separately from active, inactive bacteria and control plates, were suspended in MPYE broth and vortexed for 2 minutes to shed any surface bacterium present. Vortexed MPYE broth with seedlings for 3 inoculums (rhizospheric active bacteria (R1), rhizospheric inactive bacteria (R2) and rhizospheric control (R3) seedlings) was used in place of crushed plant and broth mixture and followed the same procedure as mentioned above. All plates were incubated at 30°C until the colonies (normally in 7 days) appear if any and examined under light microscope for identification.

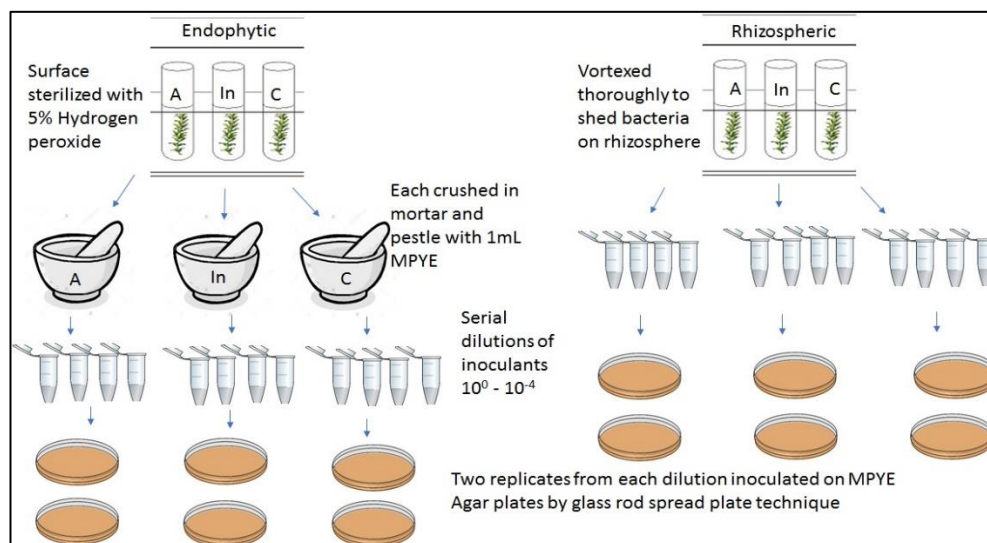


Figure 3.2. Schematic diagram of Methodology to determine the presence of bacterium in and around the roots of *Arabidopsis thaliana*.

3.2.7. Gram Staining of Putative Endophytic Bacteria

Gram staining method was performed for preliminary identification of putative endophytic bacterial strain. A single colony was taken aseptically using a

sterile metal inoculation loop heated on fire and cooled, from MPYE agar plate containing 7-10 days grown colonies of bacteria and smeared on a clean glass slide in a drop of dH₂O. The smear was fixed on slide over a gentle flame while moving the slide in a circular manner to avoid localized heating. Smear was air dried or incubated for 2-3 minutes at 60°C and gram staining was done. First, few drops of a crystal violet stain was added to smear and left to stand for 60s. Stain was gently poured off and rinsed with dH₂O. Iodine was added to the smear, enough to cover the stain area and left to stand for 60s, and again rinsed with dH₂O. Excess distilled water was shaken off from the slide surface. Few drops of 96% alcohol were added to decolorize, and immediately rinsed off by dH₂O, while color trickled down from the slide. A counter strain lugol was added on the smear and left to stand for 60s, then washed off with dH₂O. Gram stained smear was allowed to dry and then observed under a light microscope for identification.

3.2.8. Plasmid DNA Extraction

To confirm that the bacterial colonies found on MPYE agar plates are of *Fraser photinia* associated bacterium, protocol of plasmid DNA extraction was followed. Plasmid extraction from the single colony grown cultures was done both from endophytic and rhizospheric cultures using Macherey-Nagel Nucleospin® Plasmid Extraction Kit. Followed by dry centrifugation at 10000 x g to remove any residual buffer, the NucleoSpin Plasmid/Plasmid (NoLid) column was placed in a clean 1.5 ml collection tube and 25µl of elution buffer was added to the column following incubation at room temperature for 2 minutes. Centrifugation was repeated to elute the plasmid DNA. The extracted DNA was checked on nanodrop for nucleic acid concentration (ng/µl) and OD 260/280 (optical density) and OD 260/230 where elution buffer was used as blank (as plasmid DNA is extracted in elution buffer). To confirm that the plasmid extraction was successful, 100 ng/µL of the reaction solution was mixed with 1 µL loading dye and 4µl of nuclease free dH₂O and run on 1% agarose gel stained with 1.5 µl redsafe Nucleic Acid Stain to see the presence of plasmid for 40 min. on 80volts, using Lambda DNA/Hind III marker. Visualization was done using Gel Logic 200 Imaging system® under U.V. light. (Biotium Inc, Hayward CA, USA). Plasmid DNA was stored at -80°C for later use.

3.2.9. PCR Amplification to Confirm the Presence of Photinia Associated Putative Endophytic Bacterium in *A. thaliana*

The presence of putative *virD1* gene of *Agrobacterium tumefaciens* in isolated Fraser photinia associated bacterium was determined by Şah (2017). Thus the presence of putative endophytic bacterial plasmid DNA isolated from both endophytic and rhizospheric single colony cultures was confirmed by PCR using the GeneAmp® PCR System 9700 [Applied Biosystem, California]. Each amplification mixture contained 4µl of 5x FIREpol® PCR Mastermix (12.5 mM of MgCl₂), 0.4 µl of each primer (0.2µM) and 1µl of template DNA (~100ng/µl) in a total volume of 20µl. All PCR reactions were run under the following conditions: one cycle of initial denaturation at 94°C for 5 min., followed by 25 cycles of denaturation at 95°C for 30 s, annealing for 30 s at 55°C and extension at 72°C for 1 min. The program ended with an additional extension at 72°C for 7 min. followed by a cool down to 4°C. Universal forward and reverse primers, 5-ATGTCGCAAGGCAGTAGGCCACCT-3' and 3'-CTACAAGGCGTCTTTCAGCAGCGAGC-5' for *virD1* were used. All samples were run on a 1% agarose gel stained with GelRedsafe Nucleic Acid Stain (Applied Biological Materials, Inc., B.C., Cat # GO60- 2) on gel electrophoresis for 30 minutes at 100 volts where 24075 SiZer™ 1000bp plus DNA marker (144ng/ µl) was used. Visualization was done using Gel Logic 200 Imaging system® under U.V. light. [Biotium Inc, Hayward CA, USA].

3.2.10. Statistical Analysis

Statistical analysis of *A. thaliana* growth parameters, FW, SW, DW, RL and photosynthetic pigments (*chl a*, *chl b* and carotenoids) ,treated with different infiltration media, was carried out using LSD test. For Fraser photinia associated putative endophytic bacterial inoculations, statistical analysis for FSW, FW, RL, SL, DSW, DW and photosynthetic pigments (*chl a*, *chl b* and carotenoids) of *A. thaliana* was done and LSD test was carried out. The mean difference was significant at the 0.05 level for all the results.

4. RESULTS

4.1. Determination of Plant Growth for Infiltration Media (IM) inoculated Seeds and Seedlings

Evaluation results of 14 days old seedlings for both physiological stages (seedling and seed) revealed that MPYE medium appeared to give the best results for seedlings (Fig. 4.1 & 4.8), with no significant difference or negative effects in growth compared to control (C). The worst results were shown by PSW (not shown) followed by IM1 and IM3. Both seedlings and seeds of *A. thaliana* showed best results for MPYE broth and IM2, giving far better results even at seed level.

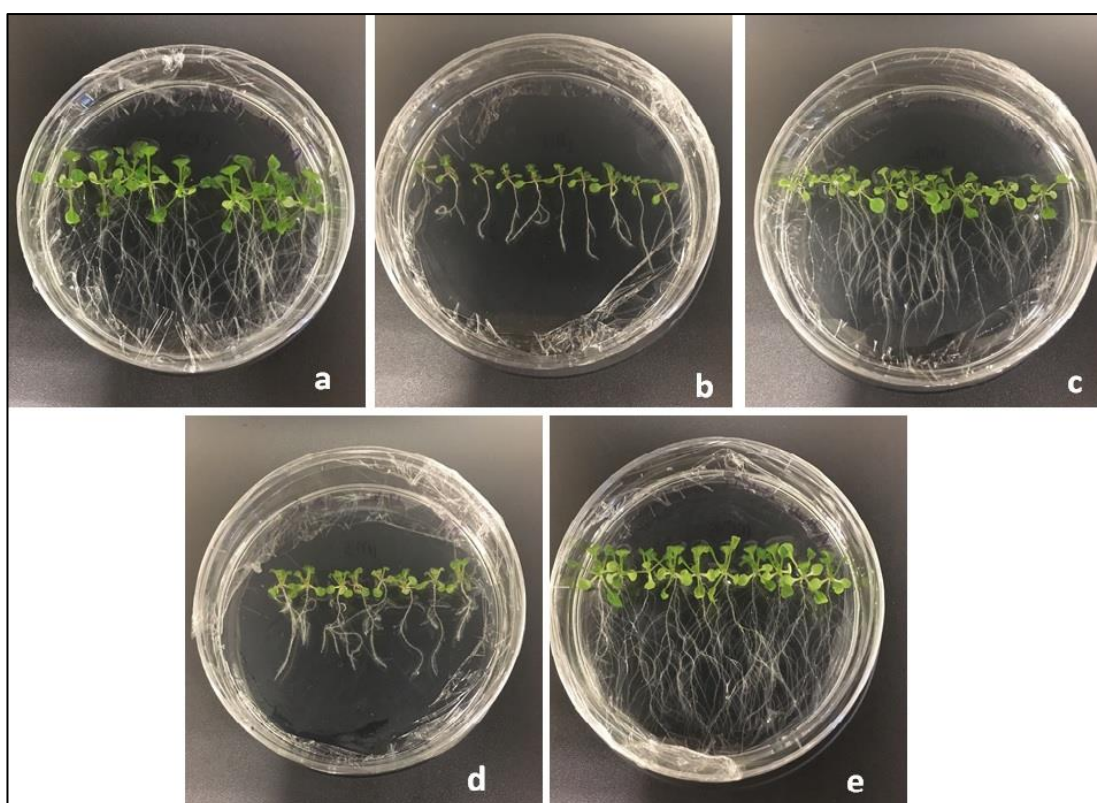


Figure 4.1. Phenotypic results of 14 days old *A. thaliana* seedlings inoculated with different media at 0 days of germination. a) Control, b) IM1, c) IM2, d) IM3, e) MPYE.

MPYE inoculated seedlings phenotypically appeared to be quite similar to C as shoot size, root density and root length was not different from C but only leaves were a little paler. The results appeared consistent for both seedling and germination stage inoculations (4.1 e & 4.8 e).

For IM2, shoot and leaf size was quite near to C, slightly yellowish but greener than MPYE. Roots were less dense but longer as compared to MPYE and C for seedling stage treatment (Fig. 4.8 c). For germination stage, overall appearance of seedlings appeared quite smaller than 'C' with smaller and paler shoots and less dense and diminished roots (Fig. 4.1 c).

IM3 appeared to be quite toxic for seedlings, results being more prominent at germination stage treatment. Smaller leaves and shoots with paler leaves while roots were thin, and had quite a scattered pattern compared to C (Fig. 4.1 d & 4.8 d).

IM1 so far gave the worst phenotypic results. Roots appeared thin and short with less root hair. Shoots and leaves appeared smaller and paler (Fig. 4.8 b), where a little browning could also be observed for germination stage treatments. Overall, the negative effect was more significant for germination stage (Fig. 4.1b).

Photosynthetic pigments were evaluated according to a variable reference system i.e. fresh weight of shoots and a constant reference system i.e. leaf number [Lichtenthaler and Buschmann, 2001] (Fig. 4.6, 4.7, 4.13 & 4.15). The chlorophyll content, as seen by the naked eye, appeared to be produced more in case of MPYE inoculated seedlings for both the inoculations (germination stage and 7-day seedling stage of *A. thaliana*), but the results from the conventional reference system (using fresh weight or dry weight) showed totally different perspective of the photosynthetic contents.

The statistical results from seed stage inoculation were more prominent. MPYE proved to be least toxic for the seedlings for FW, DW and RL (Fig. 4.2, 4.3 and 4.4 respectively) while causing no effect on SW (Fig. 4.5) and a slight decrease in photosynthetic pigments as compared to control (Fig. 4.6) and proved to be suitable infiltration medium for both physiological stages of the plant. Worst results were obtained from IM1 and IM3 where there was a significant decrease in all measured parameters of seedlings, being more prominent for germination stage treatment. IM2, which had no significant toxic effect on seedlings from seedling stage inoculation showed a decrease in FW, DW and photosynthetic pigments of seedlings for germination stage inoculation whereas non-significant decrease in RL. Chlorophyll contents results mentioned above are according to constant reference system (leaf no.) whereas variable reference system showed the reverse results (Fig. 4.7).

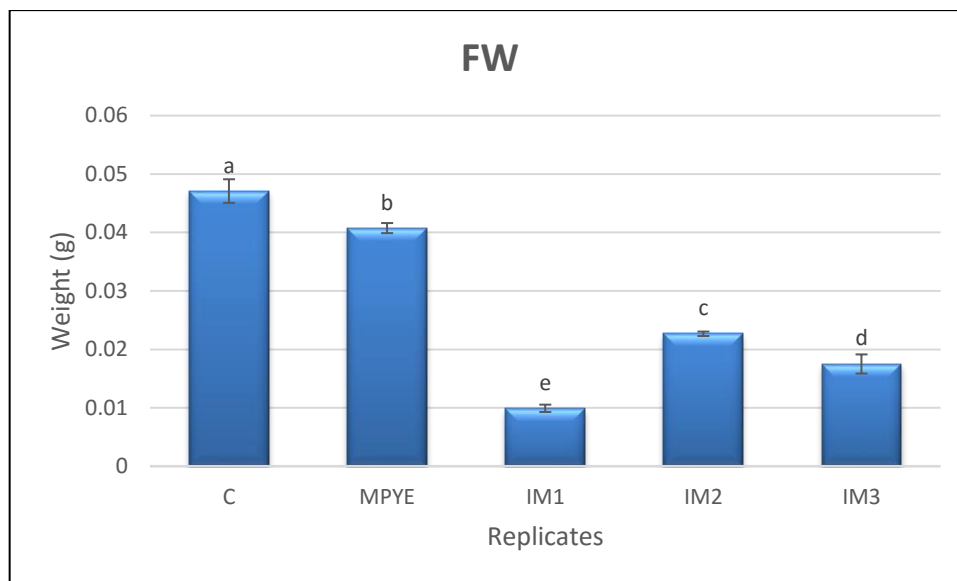


Figure 4.2. Graphical representation of FW of 14 days *A. thaliana* seedlings inoculated at germination stage with different IM's. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

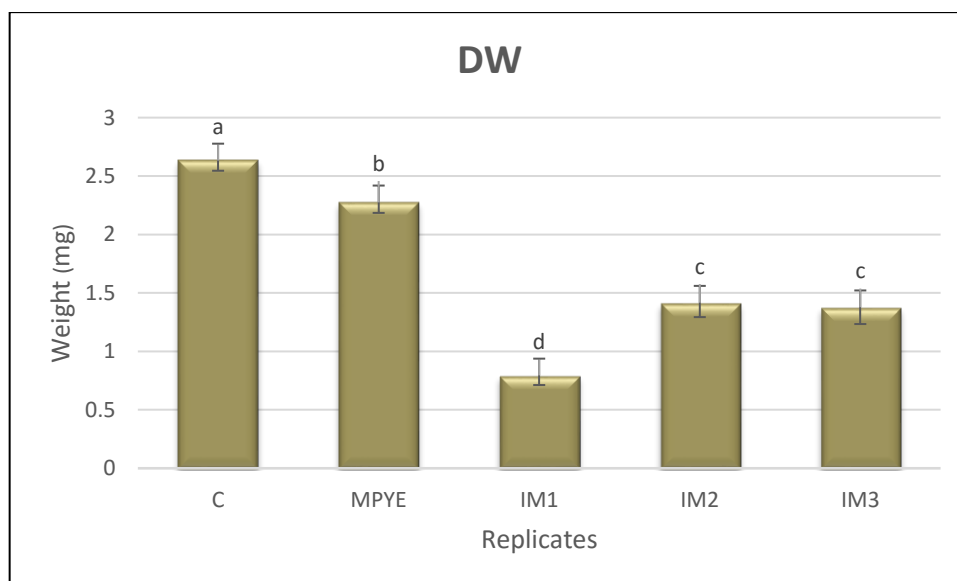


Figure 4.3. Graphical representation of DW of 14 days *A. thaliana* seedlings inoculated at germination stage with different IM's. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

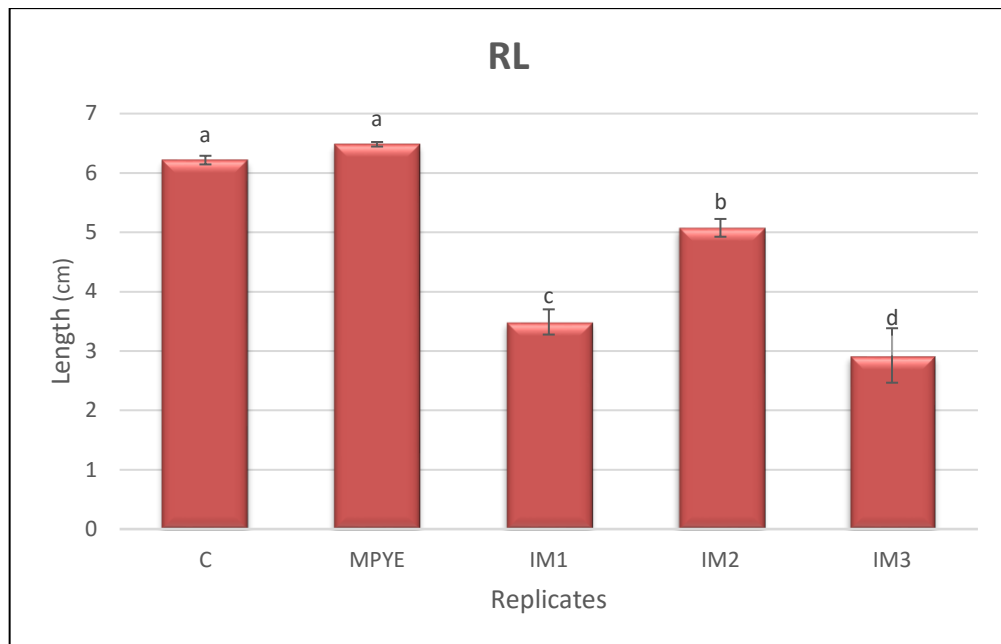


Figure 4.4. Graphical representation of RL of 14 days *A. thaliana* seedlings inoculated at germination stage with different IM's. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

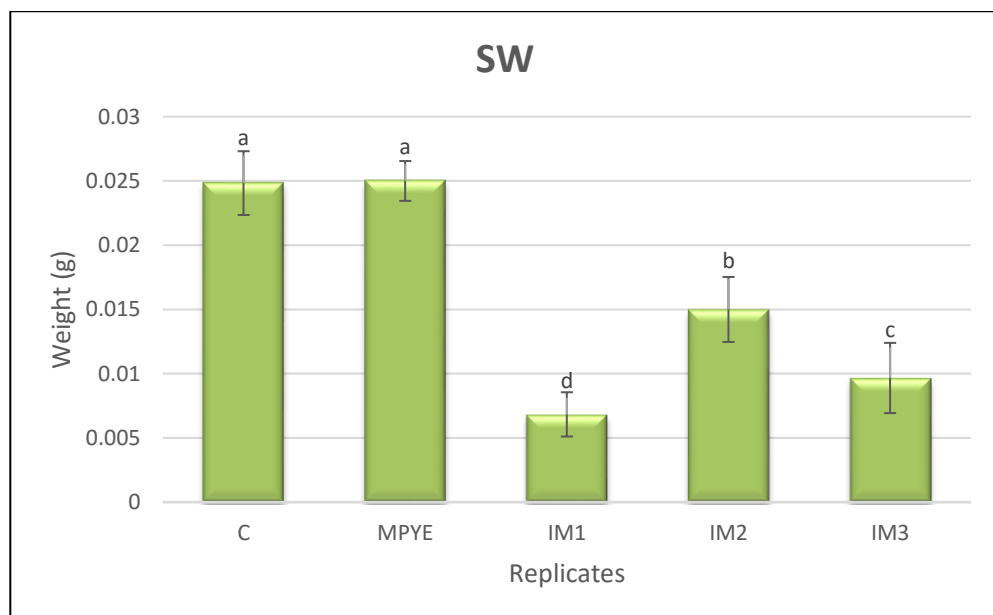


Figure 4.5. Graphical representation of SW of 14 days *A. thaliana* seedlings inoculated at germination stage with different IM's. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

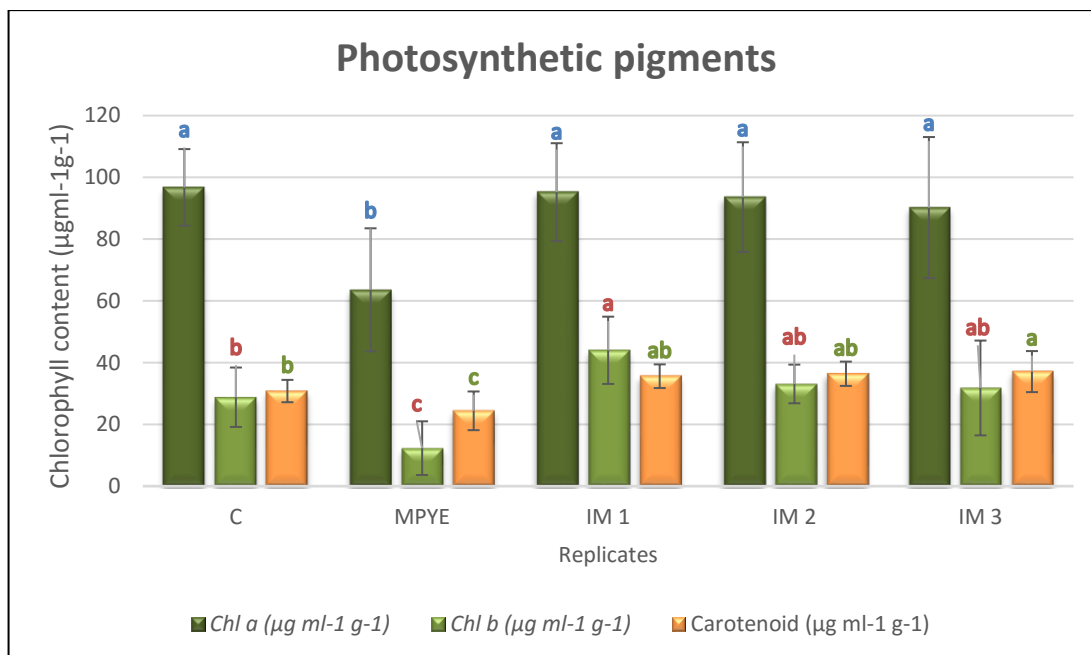


Figure 4.6. Graphical representation of photosynthetic pigments (*Chl a*, *Chl b*, carotenoids) of 14 days seedlings of *A. thaliana* inoculated at germination stage with different IM's according to a variable reference system (fresh weight). The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

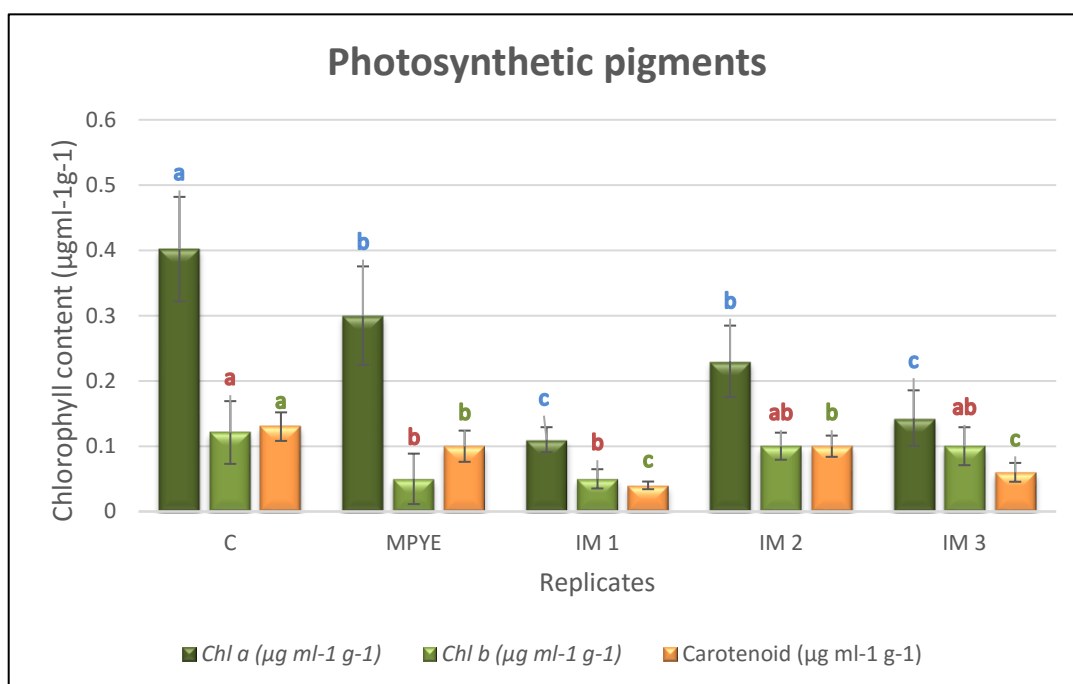


Figure 4.7. Graphical representation of photosynthetic pigments (*Chl a*, *Chl b*, carotenoids) of 14 days seedlings of *A. thaliana* inoculated at germination stage with different IM's according to a constant reference system (leaf no.). The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

For 7 day old seedling stage inoculations with IM's (Fig. 4.8), compared to C, Fig. 4.9 & 4.10 respectively showed that MPYE gave even better results for FW and DW of *A. thaliana* 14 days old seedlings whereas RL and SW had no significant difference from C (Fig. 4.11 & 4.12).

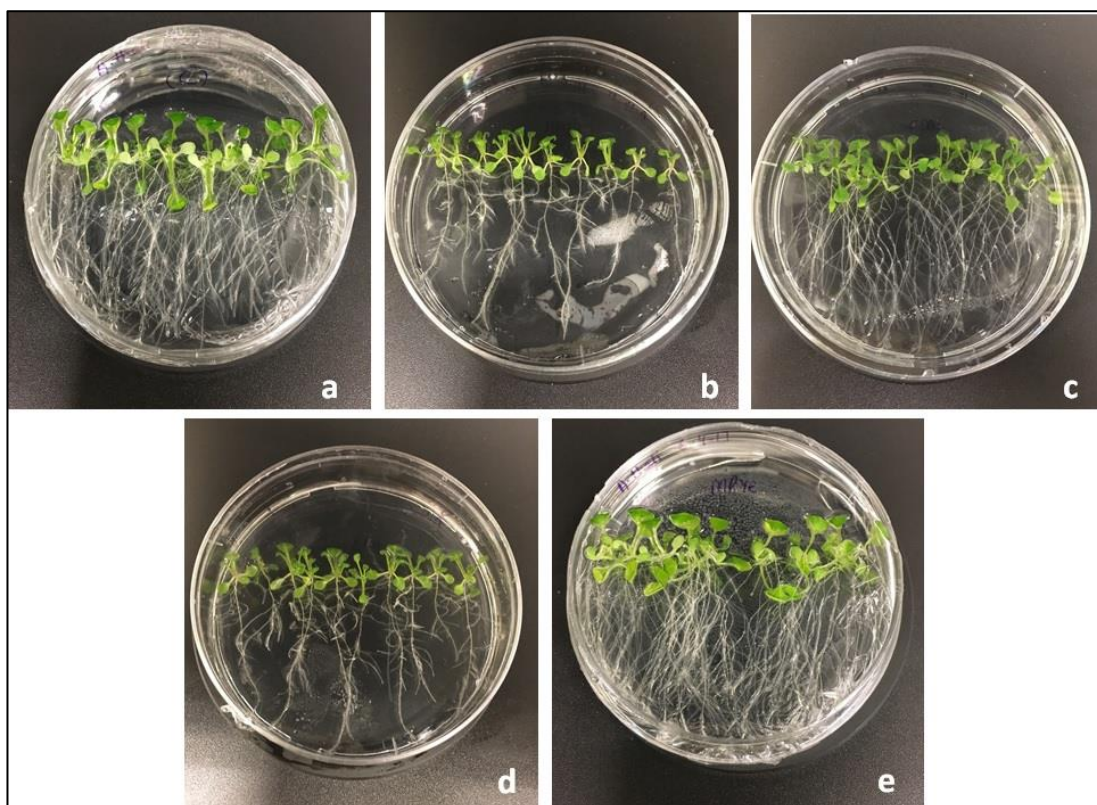


Figure 4.8. Phenotypic results of 14 days old *A. thaliana* seedlings inoculated with different media at 7th day of germination. a) Control, b) IM1, c) IM2, d) IM3, e) MPYE.

For chlorophyll contents, there was a certain decrease in *Chl a*, *Chl b* and carotenoid content for MPYE inoculations (Fig. 4.13 a) according to the constant reference system (leaf no.). Again, variable reference system showed the reverse results (Fig. 4.13 b) (discussion) Overall, MPYE showed a positive influence on plant growth. IM2 followed MPYE, showing no significant difference in any of the parameter as compared to C as indicated by LSD analysis. There was an overall negative effect of IM1 and IM3 on plant growth with significant decrease in all parameters.

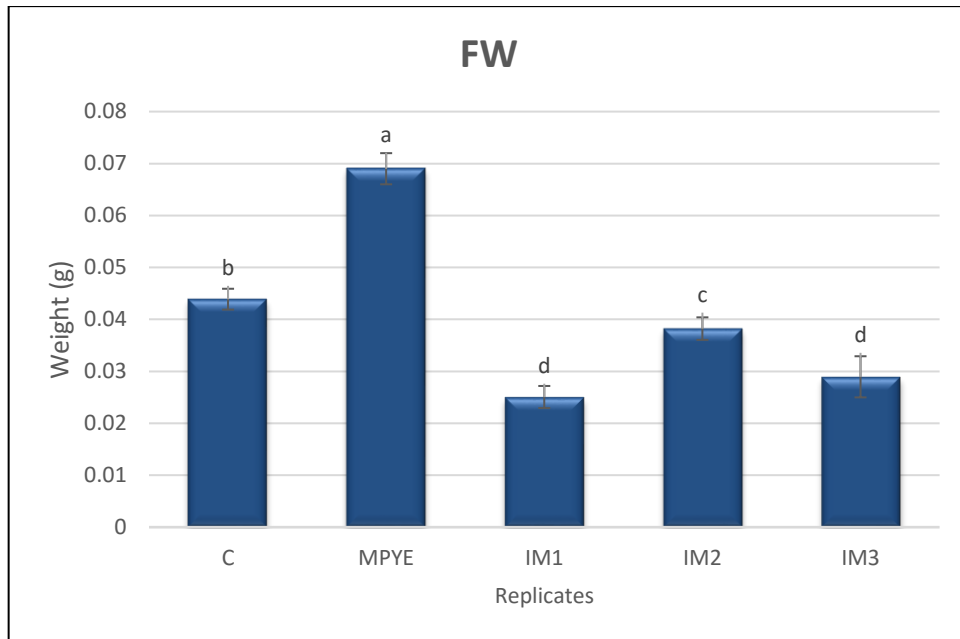


Figure 4.9. Graphical representation of FW of 14 days *A. thaliana* seedlings inoculated at 7th day of germination with different IM's. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

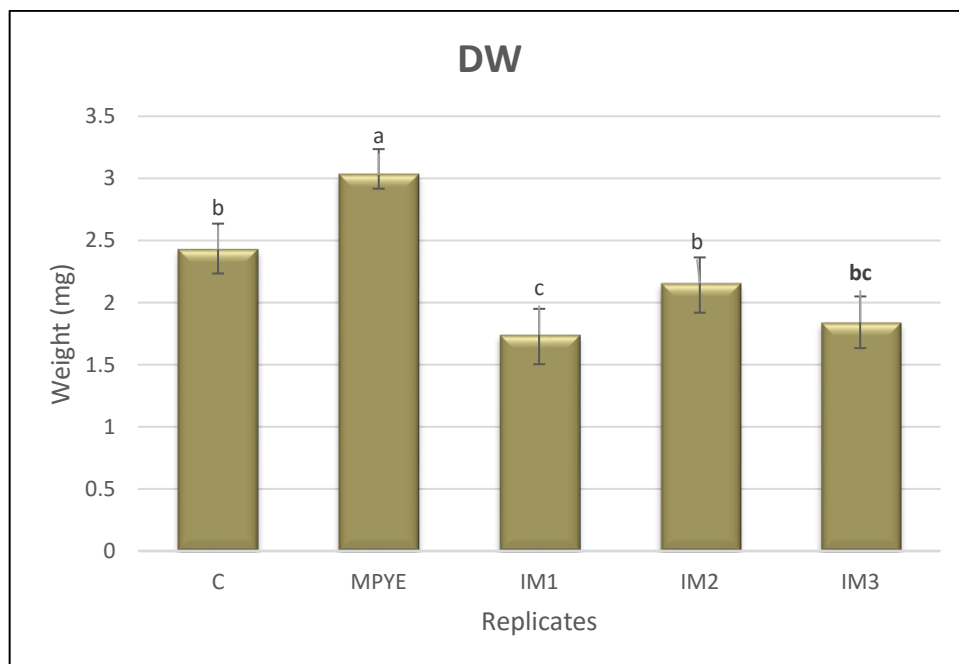


Figure 4.10. Graphical representation of DW of 14 days *A. thaliana* seedlings inoculated at 7th day of germination with different IM's. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

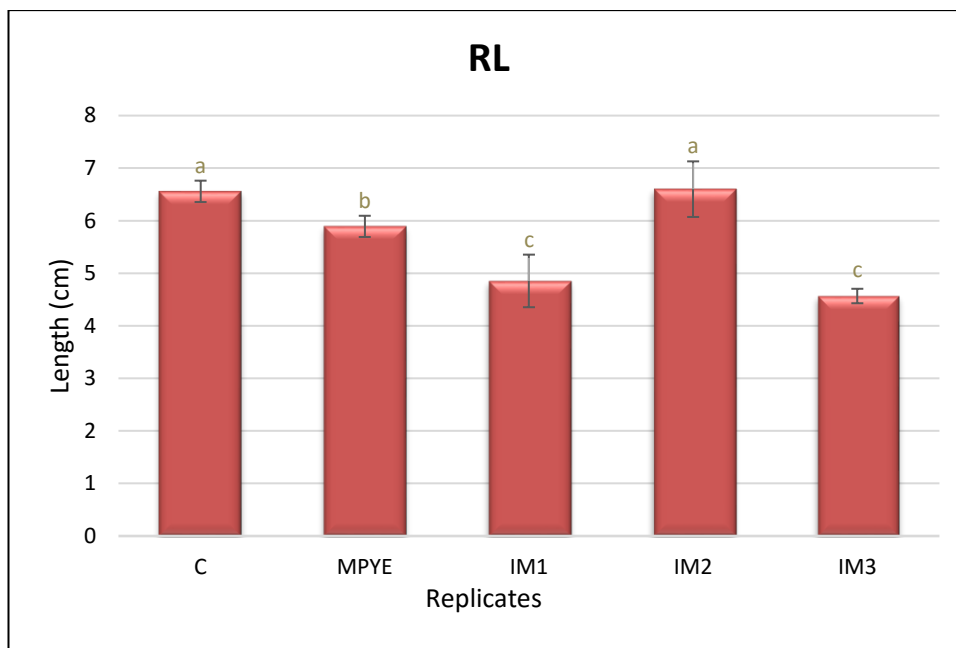


Figure 4.11. Graphical representation of RL of 14 days *A. thaliana* seedlings inoculated at 7th day of germination with different IM's. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

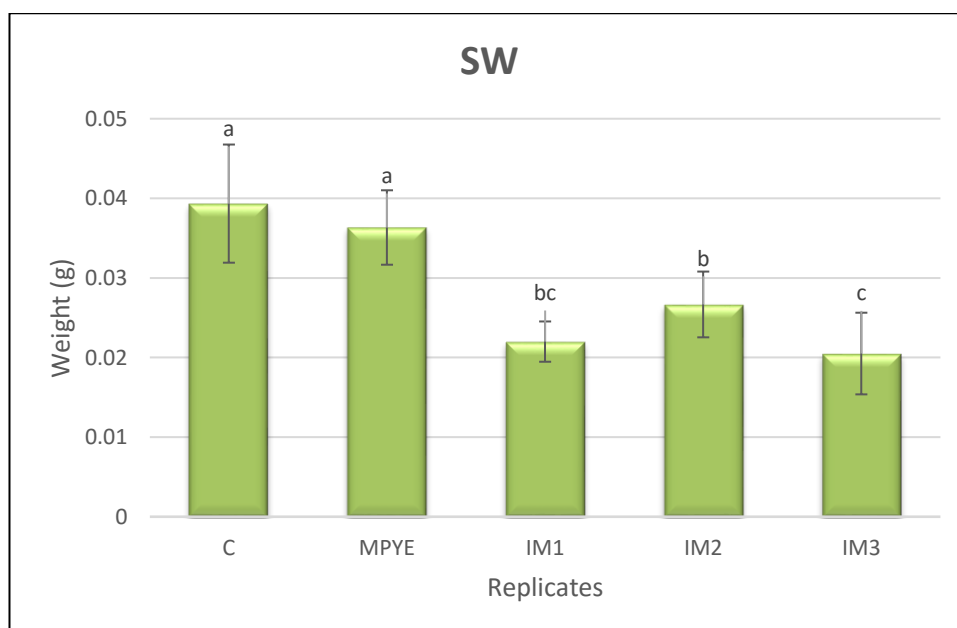


Figure 4.12. Graphical representation of SW of 14 days *A. thaliana* seedlings inoculated 7th day of germination with different IM's. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

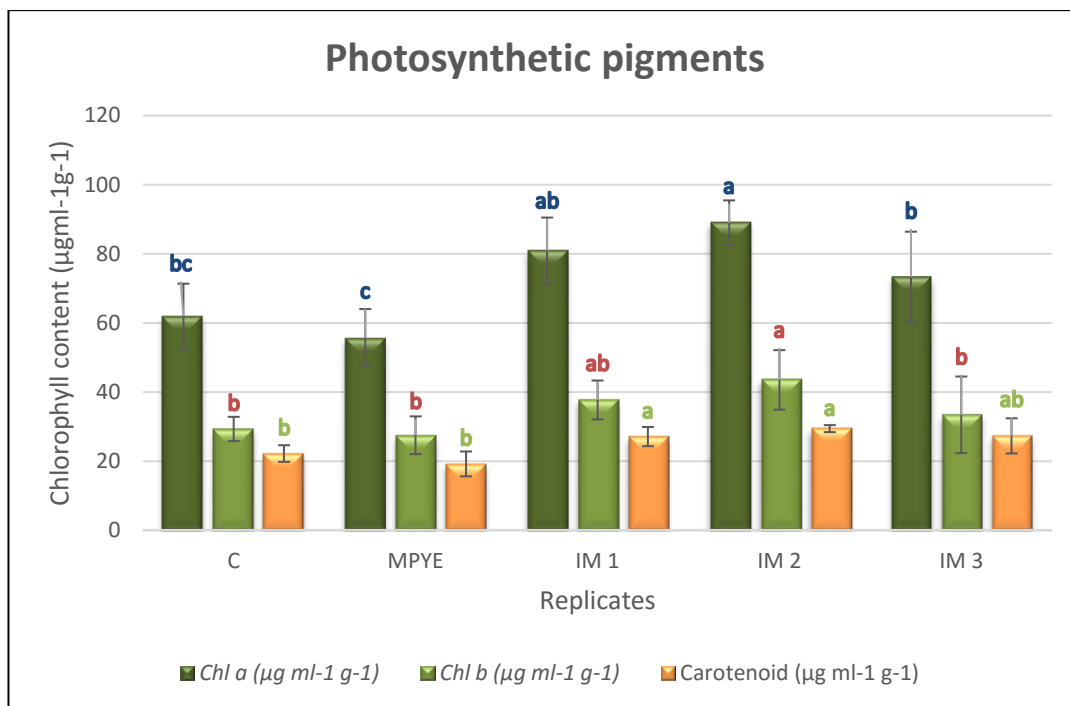


Figure 4.13. Graphical representation of photosynthetic pigments (*Chl a*, *Chl b*, carotenoids) of 14 days seedlings of *A. thaliana* inoculated with different IM's at 7th day of germination according to a variable reference system (fresh weight). The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

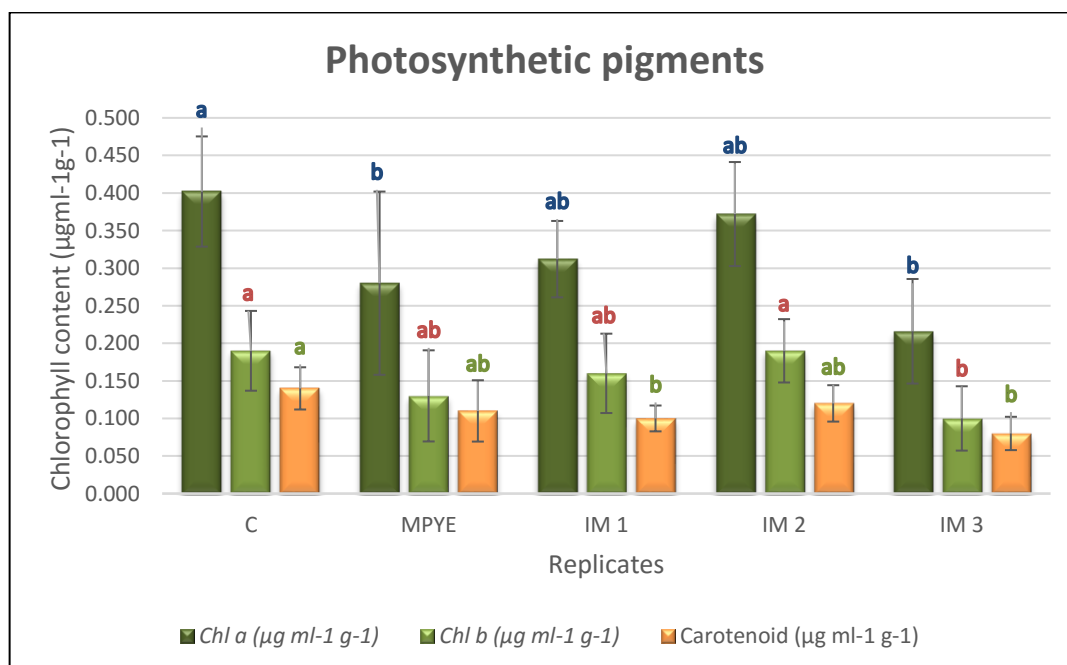


Figure 4.14. Graphical representation of photosynthetic pigments (*Chl a*, *Chl b*, carotenoids) of 14 days seedlings of *A. thaliana* inoculated with different IM's at 7th day of germination according to a constant reference system (leaf no.). The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

4.2. Influence of Putative Endophytic Bacteria on Seed Germination and Seedling Growth

14 days old *A. thaliana* seedlings inoculated with 10^7 and 10^8 cfu/mL of (A) and (In) bacteria and (A+In) compositions for both concentrations were evaluated for FSW, FW, SL, DSW, DW, RL and photosynthetic pigments, *chl a*, *chl b* and carotenoids (Fig. 4.15-4.31). For germination stage inoculations, seedlings appeared very similar for A 10^7 and A 10^8 cfu/mL concentrations such as small leaves and shoots with thinner and shorter roots as compared to C (Fig 4.15. b, e). The roots and shoots appeared normal for (In) suspensions except that In 10^8 cfu/mL replicate had more paler leaves compared to C than In 10^7 cfu/mL bacterial inoculation (4.15.C, f). The same was true for (A+ In) suspensions (Fig. 4.15. d, g).

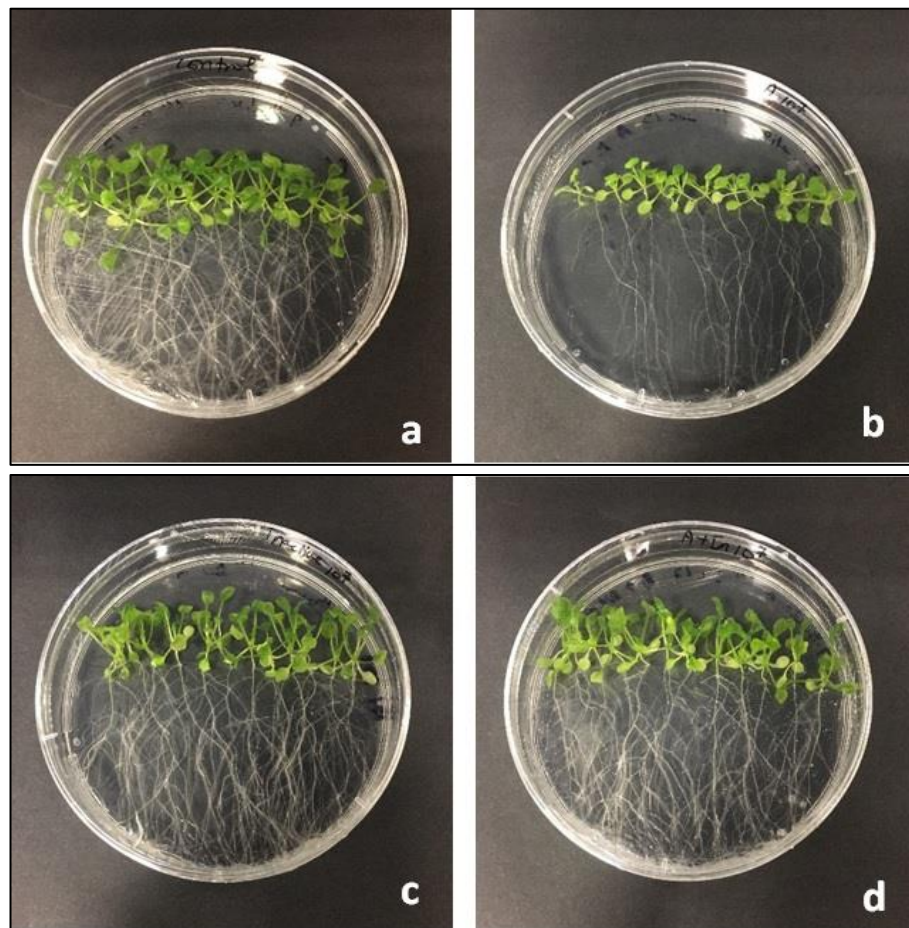


Figure 4.15. Phenotypic results of 14 days old *A. thaliana* seedlings inoculated with 10^7 and 10^8 cfu/mL Active (A), Inactive (In) and (A+In) suspensions of endophytic bacteria at germination stage, a) Control, b) 10^7 cfu/mL (A), c) 10^7 cfu/mL (In), d) 10^7 cfu/mL (A+ In), e) 10^8 cfu/mL (A), f) 10^8 cfu/mL (In) and g) 10^8 cfu/mL (A+ In) bacterial suspension inoculations.

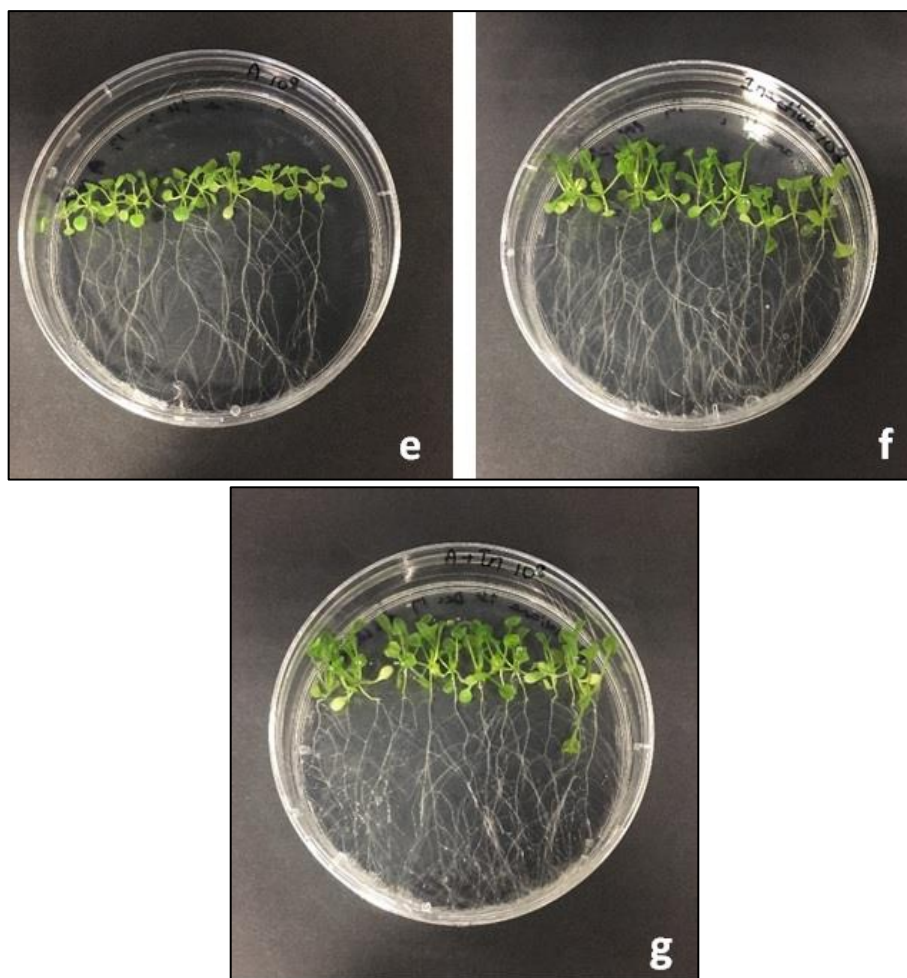


Figure 4.15. Continued.

For germination stage, graphical analysis and LSD analysis indicated that A+In 10^7 cfu/mL inoculum caused an insignificant increase in FSW and FW, In 10^7 cfu/mL had no effect whereas A 10^7 cfu/mL insignificantly reduced the FSW and FW (Fig. 4.16 & 4.17). The 10^8 cfu/mL suspension had no positive affect on fresh weights rather A 10^8 and In 10^8 cfu/mL suspensions significantly reduced fresh biomass of the seedlings. A+In 10^8 cfu/mL suspension had some positive affect towards SL followed by In 10^7 cfu/mL. The rest had no effect on SL except for A 10^8 cfu/mL which caused some reduction in SL (Fig. 4.18). No suspension significantly affected RL of the seedlings (Fig. 4.19). For dry biomass, (DW, DSW) there was no effect of (In) and (A+ In) suspensions of 10^7 and 10^8 whereas A 10^7 and A 10^8 significantly reduced dry weights of seedlings (Fig. 4.20 & 4.21). An overall increase in photosynthetic pigments was observed per fresh weight of shoots (variable reference system) for A 10^7 and A 10^8 suspensions while In 10^7 and A+In 10^7 insignificantly reduced the

photosynthetic contents of shoots. The rest of 10^8 suspensions didn't affect photosynthetic contents of the seedlings (Fig. 4.22). The results were different for the constant reference system (leaf no.) (Fig. 4.23).

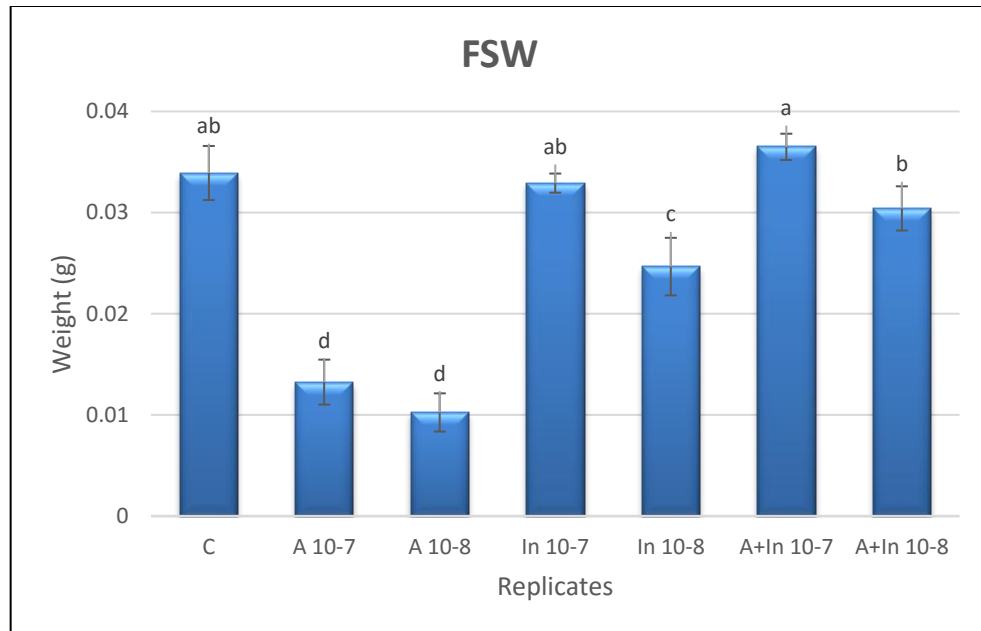


Figure 4.16. Graphical representation of FSW of 14 days old seedlings of *A. thaliana* for germination stage inoculation with 10^7 and 10^8 cfu/mL Active(A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

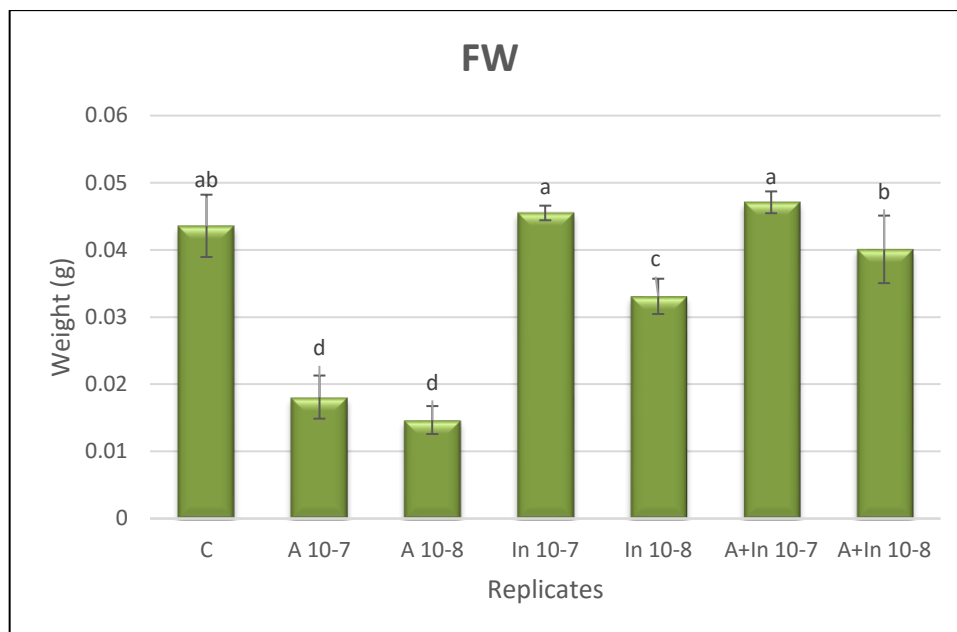


Figure 4.17. Graphical representation of FW of 14 days old seedlings of *A. thaliana* for germination stage inoculation with 10^7 and 10^8 cfu/mL Active(A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

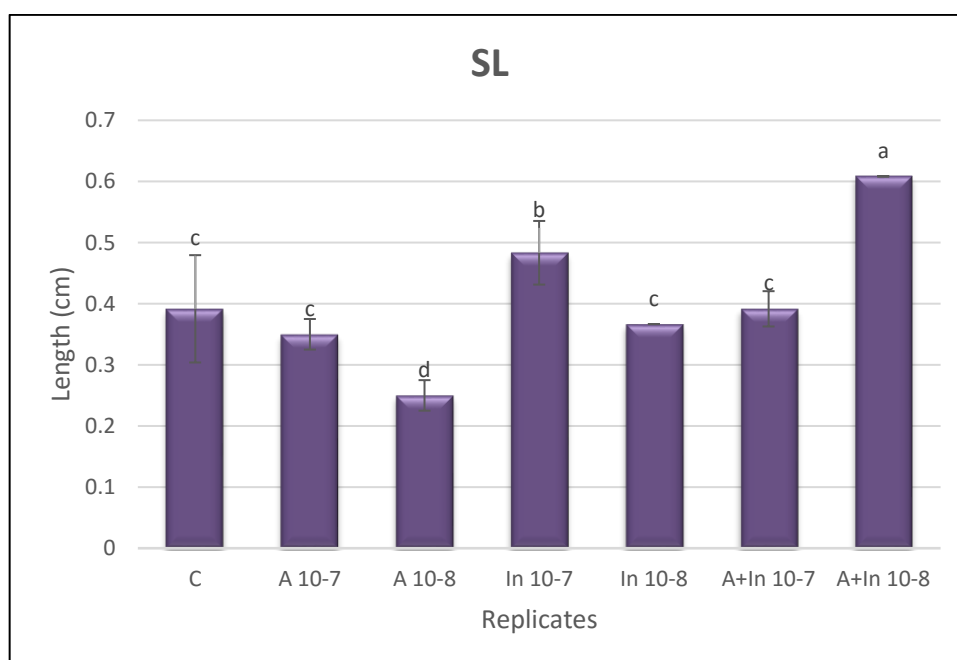


Figure 4.18. Graphical representation of SL of 14 days old seedlings of *A. thaliana* for germination stage inoculation with 10^7 and 10^8 cfu/mL Active(A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

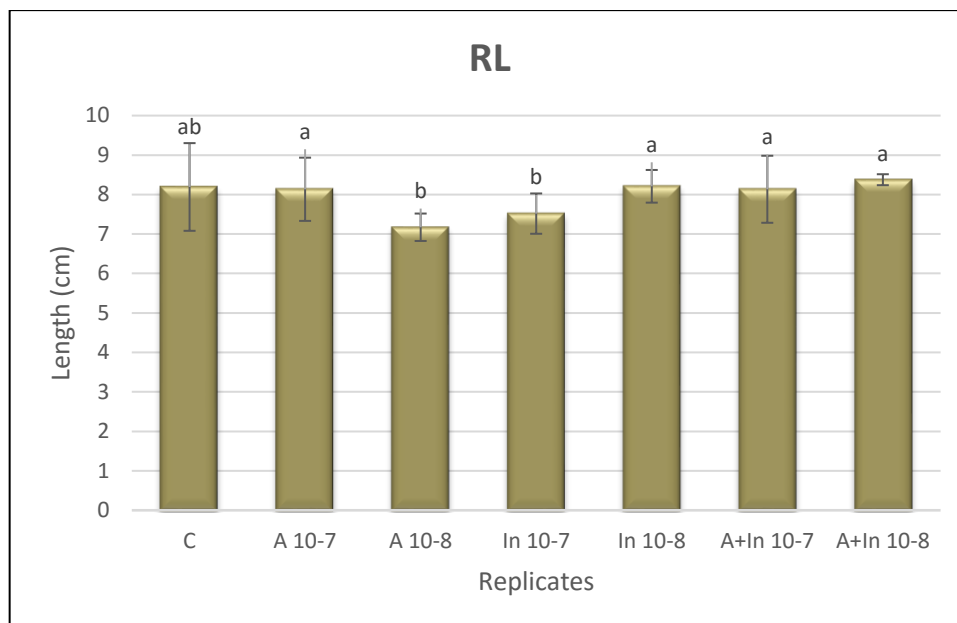


Figure 4.19. Graphical representation of RL of 14 days old seedlings of *A. thaliana* for germination stage inoculation with 10^7 and 10^8 cfu/mL Active(A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

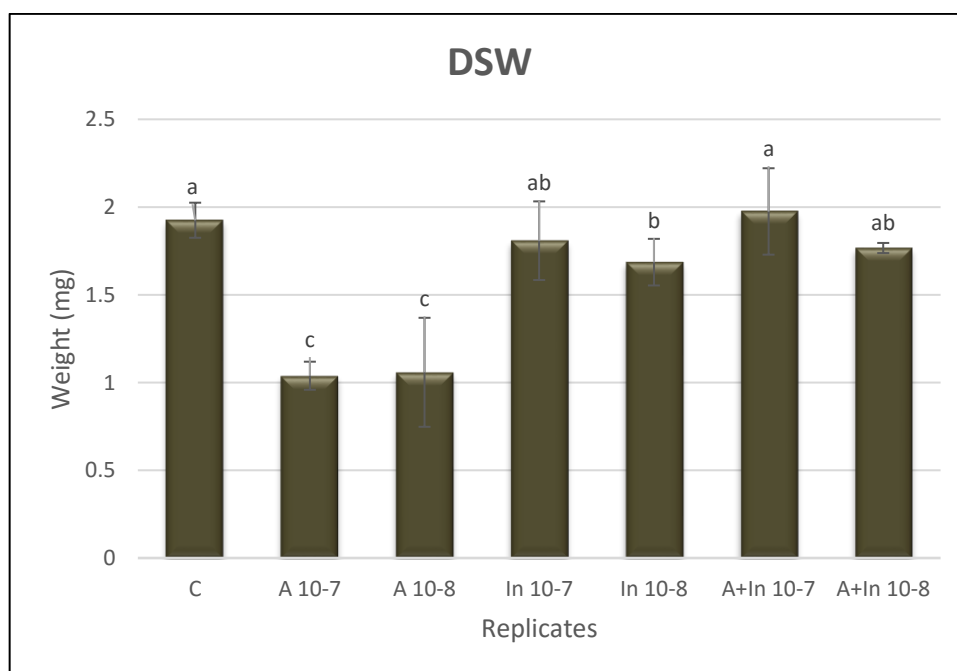


Figure 4.20. Graphical representation of DSW of 14 days old seedlings of *A. thaliana* for germination stage inoculation with 10^7 and 10^8 cfu/mL Active(A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

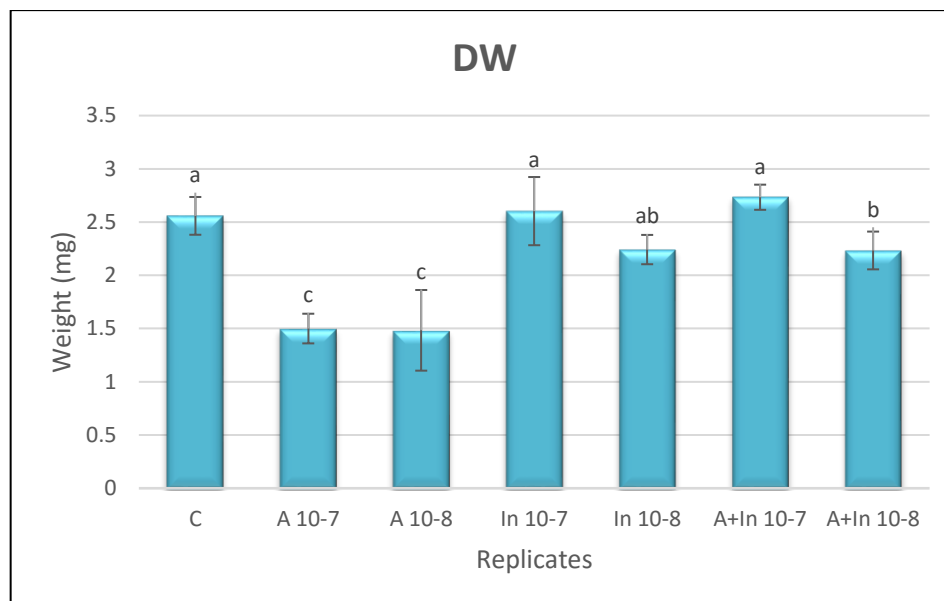


Figure 4.21. Graphical representation of DW of 14 days old seedlings of *A. thaliana* for germination stage inoculation with 10^7 and 10^8 cfu/mL Active(A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

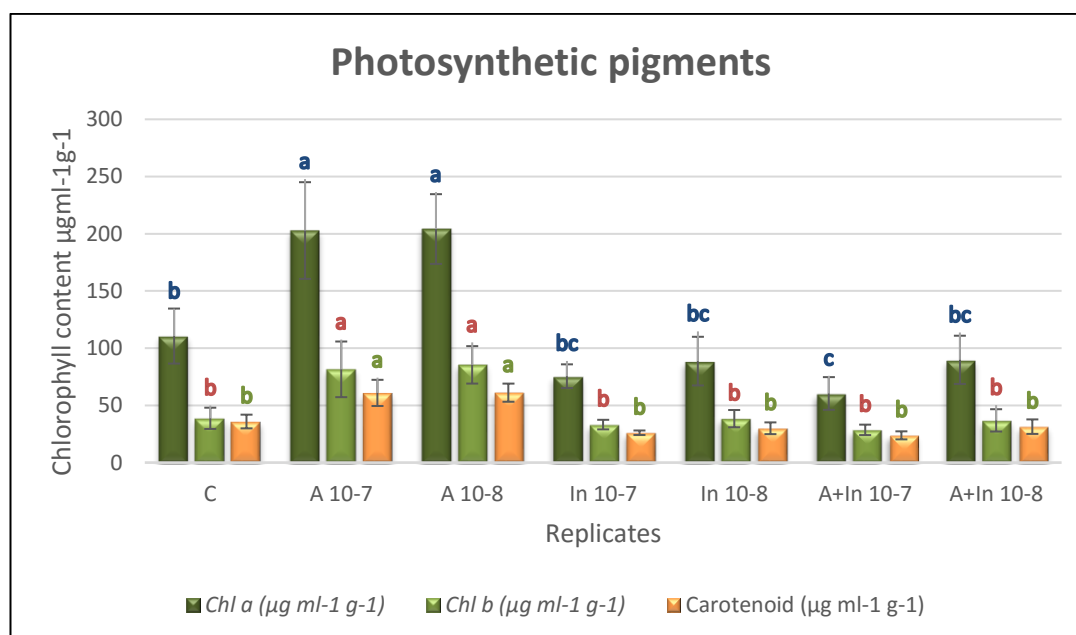


Figure. 4.22. Graphical representation of photosynthetic pigments (*Chl a*, *Chl b*, carotenoids) of 14 days old seedlings of *A. thaliana* for germination stage inoculation with 10^7 and 10^8 cfu/mL Active(A), Inactive (In) and (A+In) bacterial suspensions according to a variable reference system (fresh weight). The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

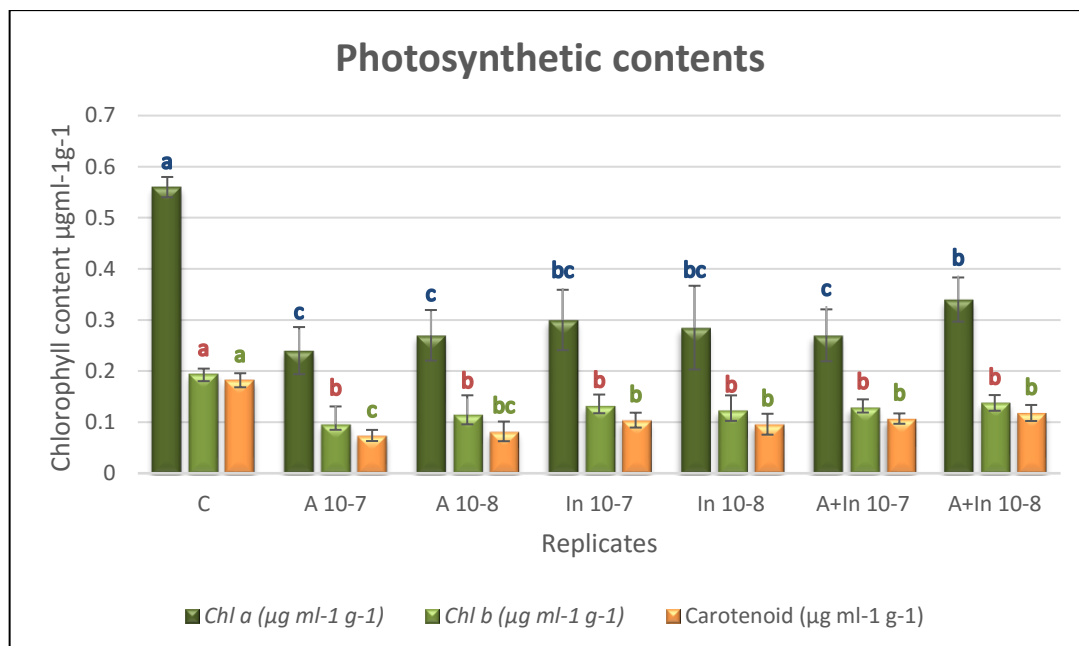


Figure. 4.23. Graphical representation of photosynthetic pigments (*Chl a*, *Chl b*, carotenoids) of 14 days old seedlings of *A. thaliana* for germination stage inoculation with 10^7 and 10^8 cfu/mL Active(A), Inactive (In) and (A+In) bacterial suspensions according to a constant reference system (leaf no.). The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

For 7-day inoculated replications (Fig. 4.24), shoots appeared slightly taller for (A) and (In) suspensions of 10^7 , 10^8 and $A+In10^8$ cfu/mL (Fig. 4.24. b, c, f, g respectively) while similar in appearance to C for $A+In10^7$ and $A10^8$ cfu/mL inoculants (Fig. 4.24. d, e respectively). Leaves appeared paler for $A10^7$ (Fig. 4.24 b), brighter for $A10^8$ and $In10^8$ cfu/mL replicates (Fig. 4.24. e, f) while almost similar to C for the rest of suspensions (Fig. 4.24. c, d, g). Roots were dense and branched for (In) and (A+In) suspensions of both 10^7 and 10^8 cfu/mL concentrations (Fig. 4.24. c, d, f, g) while similar to C for $A10^7$ and $A10^8$ cfu/mL suspensions (Fig. 4.24.b, e).

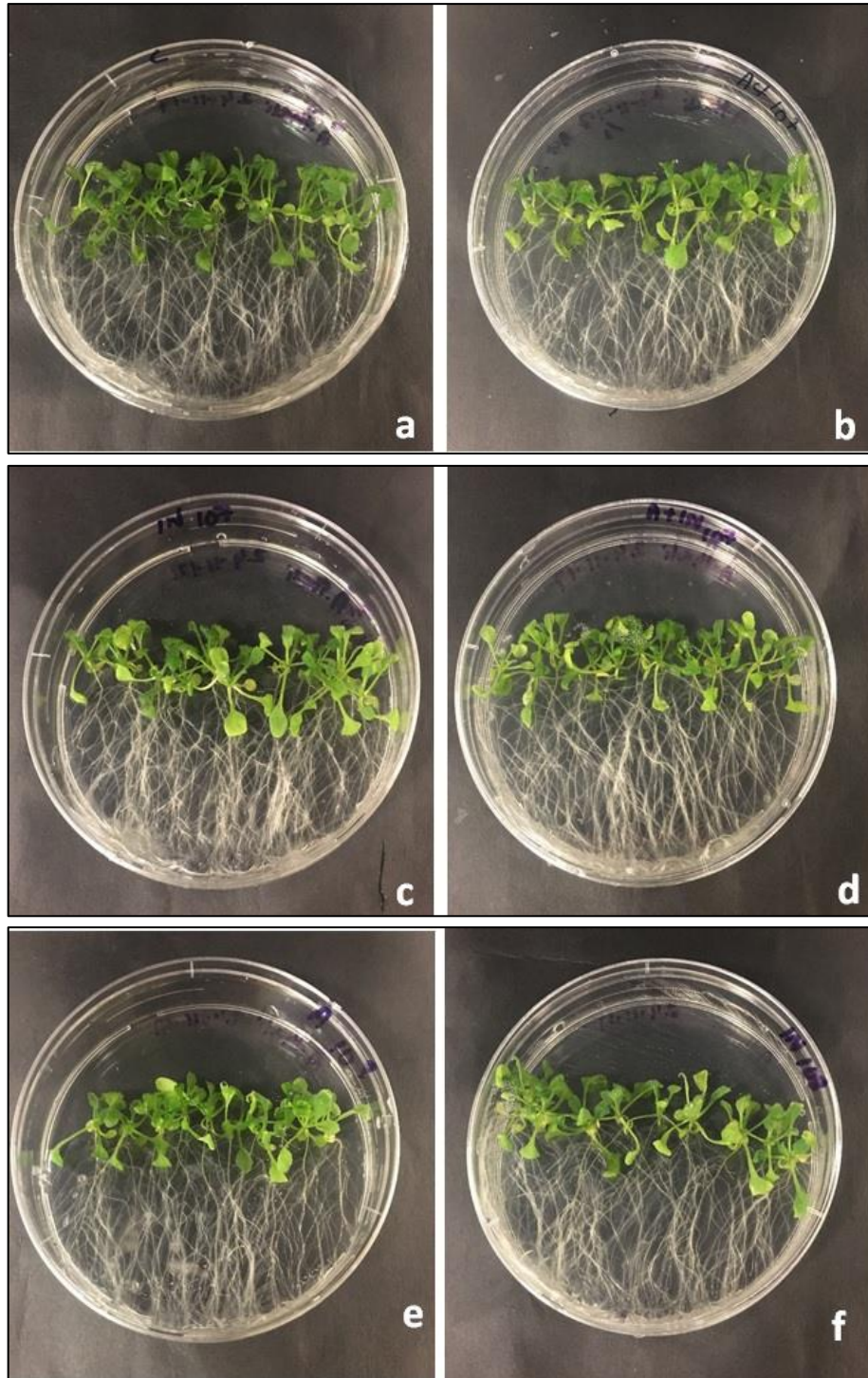


Figure 4.24. Phenotypic results of 14 days old *A. thaliana* seedlings inoculated with 10^7 and 10^8 cfu/mL Active(A), Inactive (In) and (A+In) bacterial suspensions at 7th day of germination. a) Control, b) 10^7 cfu/mL (A), c) 10^7 cfu/mL (In), d) 10^7 cfu/mL (A+ In), e) 10^8 cfu/mL (A) and f) 10^8 cfu/mL (In), g) 10^8 cfu/mL (A+ In) bacterial suspensions inoculations.



Figure 4.24. Continued.

Graphical representation for 7 days inoculated seedlings shows that there is an increase in FSW compared to C for $\text{In}10^7$ cfu/mL suspension, followed by less significant increase for A+ $\text{In}10^8$ cfu/mL, $\text{A}10^7$ cfu/mL, $\text{In}10^8$ cfu/mL and $\text{A}10^7$ cfu/mL bacterial suspensions and almost no effect for $\text{A}10^8$ cfu/mL suspension (Fig. 4.25). FW was significantly increased by A+ $\text{In}10^7$ cfu/mL, followed by $\text{In}10^7$, $\text{In}10^8$ and A+ $\text{In}10^8$ cfu/mL bacterial suspensions, which also showed an increase in FW compared to C, but less significant as compared to A+ $\text{In}10^7$ cfu/mL suspension. $\text{A}10^7$ and $\text{A}10^8$ suspensions had insignificant effects as shown in Figure 4.26.

For SL and RL, no significant effect was seen for any suspension compared to C, except that A+ $\text{In}10^7$ cfu/mL showed some positive increase in RL (Fig. 4.27 & 4.28). $\text{In}10^8$ and A+ $\text{In}10^8$ cfu/mL bacterial suspensions showed significant increase in dry shoot mass (DSW) followed by 10^7 cfu/mL, which showed an insignificant increase in DSW compared to $\text{In}10^8$ cfu/mL suspension but showed some positive increase compared to C. $\text{A}10^8$ cfu/mL didn't show any effect (Fig. 4.29). The results were similar for DW (Fig. 4.30). For photosynthetic pigments, $\text{A}10^8$ and $\text{In}10^8$ cfu/mL suspensions caused a significant increase in *chl a* contents while an insignificant affect in *chl b* and carotenoids was observed whereas A+ $\text{In}10^8$ cfu/mL suspension had no significant affect on any of the three photosynthetic pigments (variable reference i.e. fresh shoot weight). For 10^7 cfu/mL suspensions, (A+ In) bacterial inoculum showed better results for *chl a* content, while the rest pigments being insignificantly affected. $\text{In}10^7$ cfu/mL suspension showed insignificant increase in *chl a* and *chl b* pigments while carotenoids remain insignificantly affected. $\text{A}10^7$ cfu/mL significantly caused

an overall decrease in the photosynthetic pigments (Fig. 4.31). The results were same for constant reference system due to same reasons (Fig. 4.32).

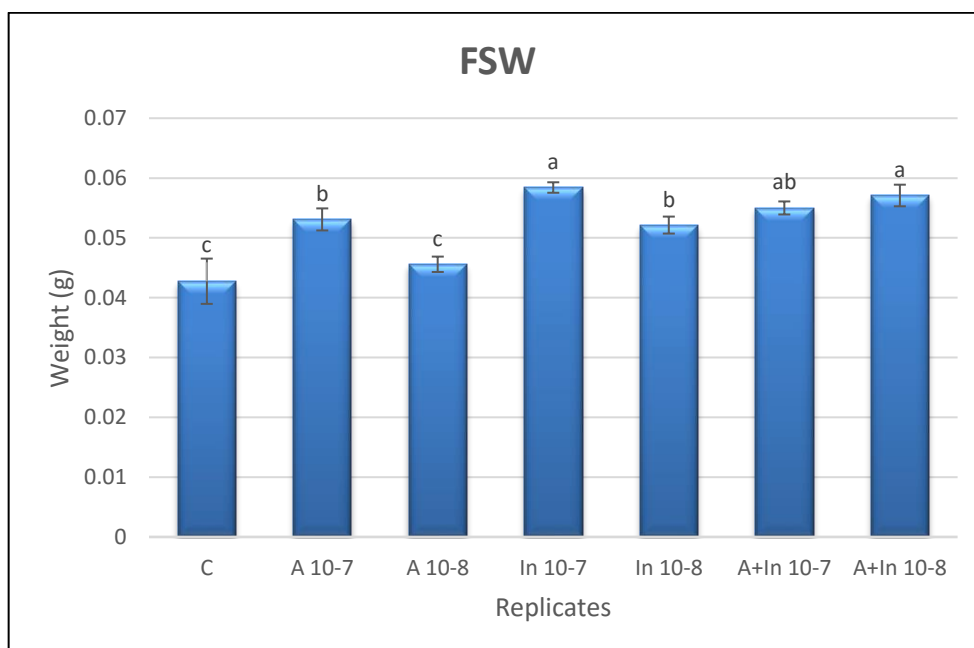


Figure 4.25. Graphical representation of FSW of 14 days old seedlings of *A. thaliana* inoculated at 7th day of germination with 10^7 and 10^8 cfu/mL Active (A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

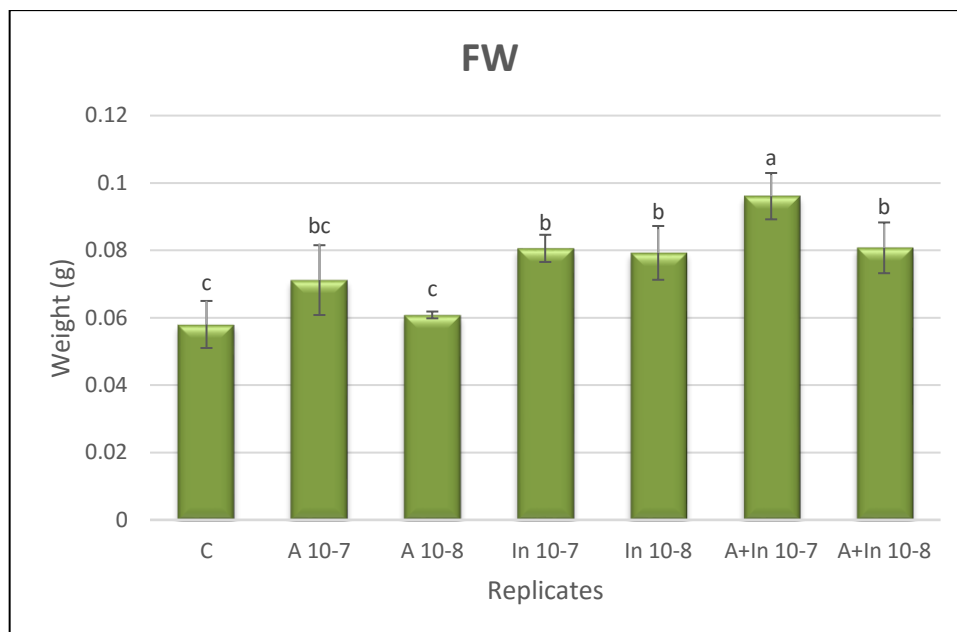


Figure 4.26. Graphical representation of FW of 14 days old seedlings of *A. thaliana* inoculated at 7th day of germination with 10⁷ and 10⁸ cfu/mL Active (A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

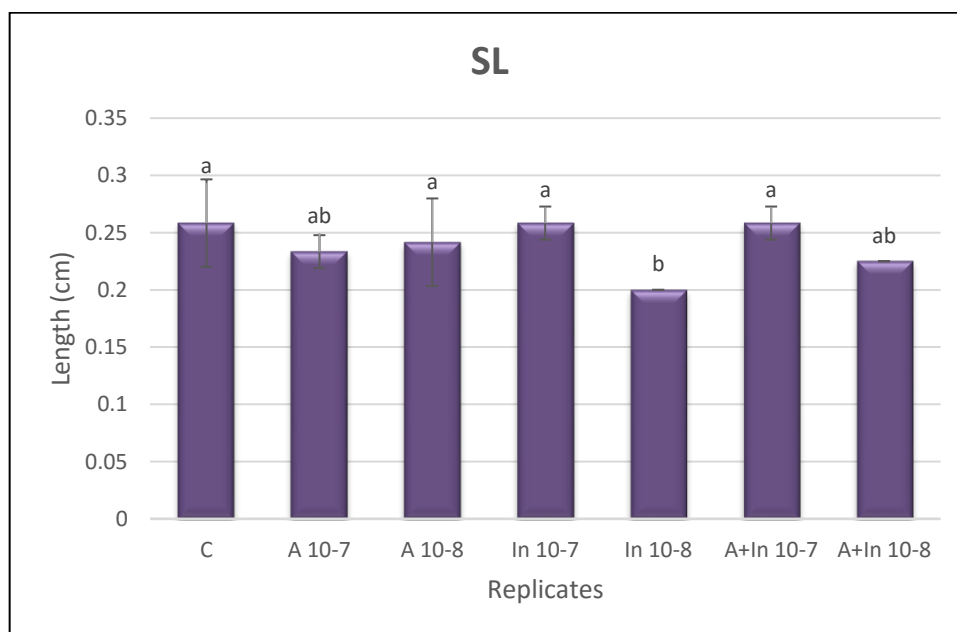


Figure 4.27. Graphical representation of SL of 14 days old seedlings of *A. thaliana* inoculated at 7th day of germination with 10⁷ and 10⁸ cfu/mL Active (A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

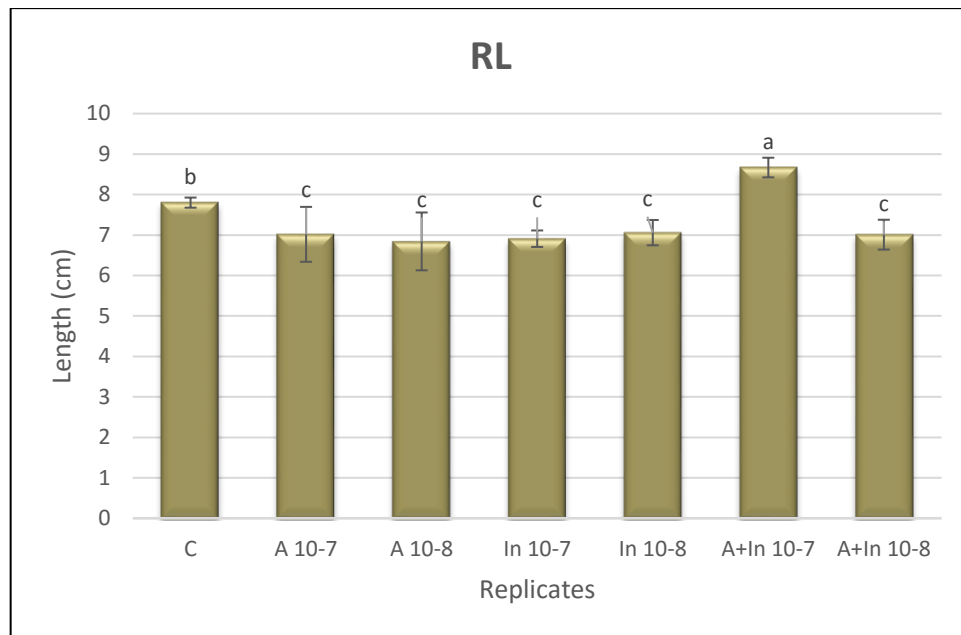


Figure 4.28. Graphical representation of RL of 14 days old seedlings of *A. thaliana* inoculated at 7th day of germination with 10⁷ and 10⁸ cfu/mL Active (A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

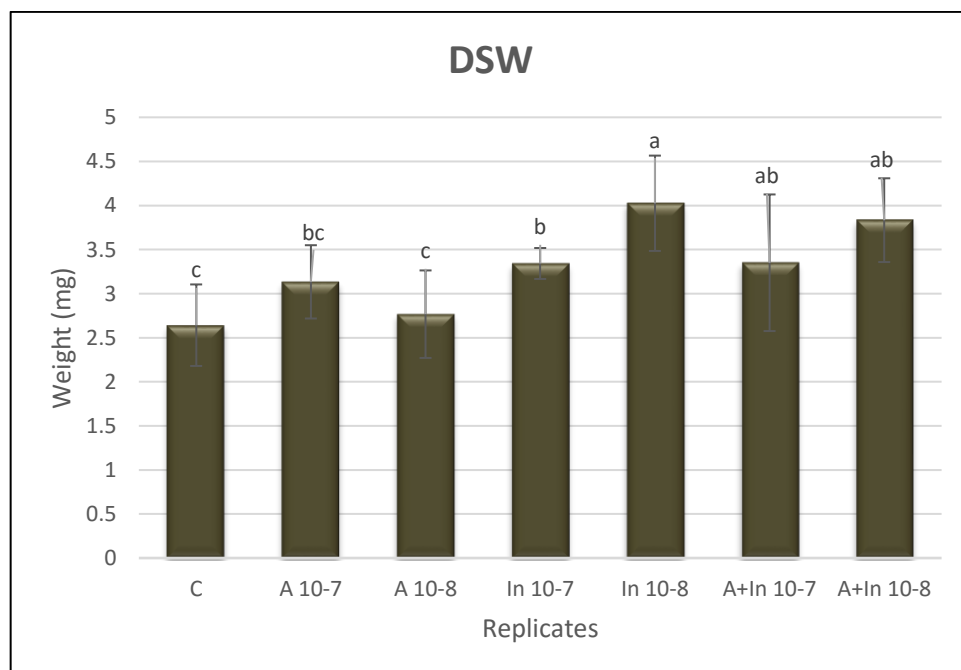


Figure 4.29. Graphical representation of DSW of 14 days old seedlings of *A. thaliana* inoculated at 7th day of germination with 10⁷ and 10⁸ cfu/mL Active (A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

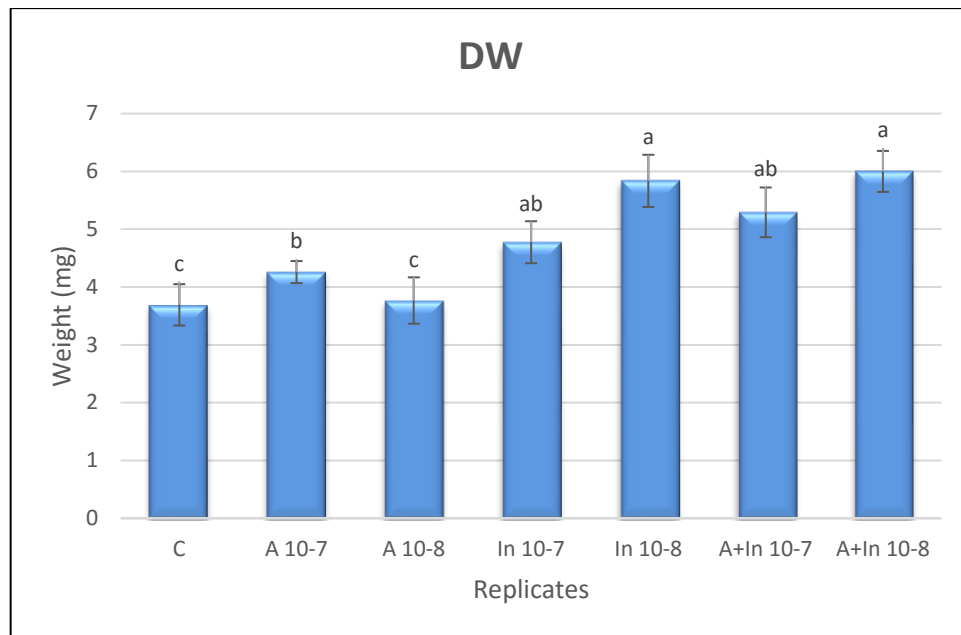


Figure 4.30. Graphical representation of DW of 14 days old seedlings of *A. thaliana* inoculated at 7th day of germination with 10^7 and 10^8 cfu/mL Active (A), Inactive (In) and (A+In) bacterial suspensions. The error bars show upper and lower standard deviation. Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

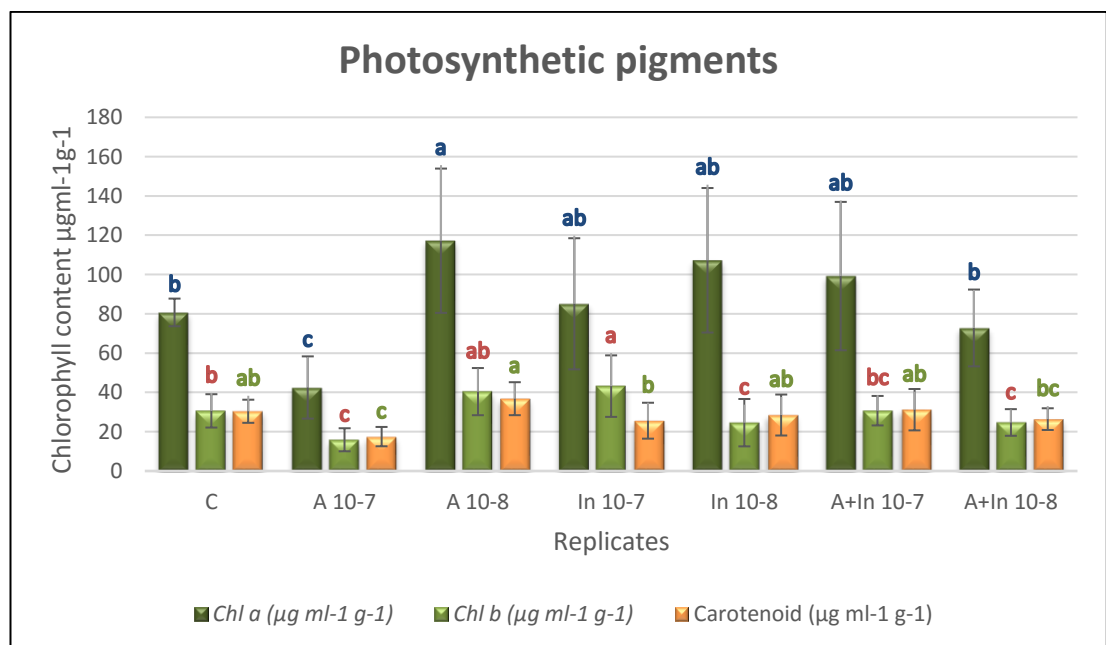


Figure 4.31. Graphical representation of photosynthetic pigments (*Chl a*, *Chl b*, carotenoids) of 14 days old seedlings of *A. thaliana* inoculated at 7th day of germination with 10^7 and 10^8 cfu/mL Active (A), Inactive (In) and (A+In) bacterial suspensions according to a variable reference system (fresh weight). Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

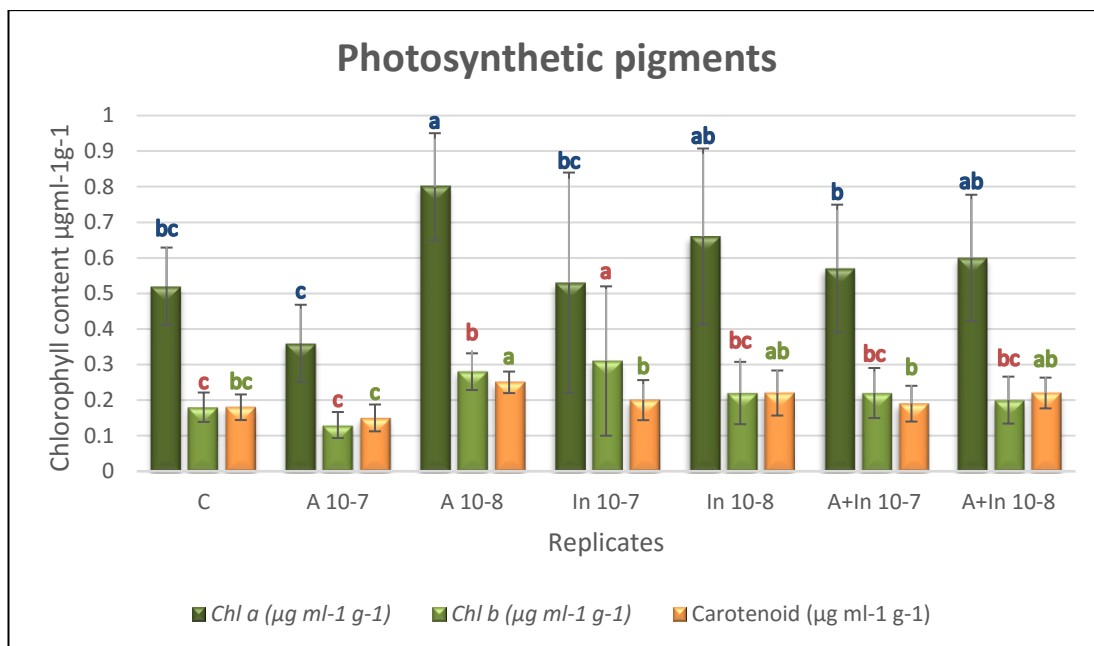


Figure 4.32. Graphical representation of photosynthetic pigments [*Chl a*, *Chl b*, carotenoids] of 14 days old seedlings of *A. thaliana* inoculated at 7th day of germination with 10⁷ and 10⁸ cfu/mL Active (A), Inactive (In) and (A+In) bacterial suspensions according to a constant reference system (leaf no.). Lowercase alphabets show LSD test with mean significant difference, $p \leq 0.05$.

4.3. Phenotypic and Microscopic Examination of Putative Endophytic Bacteria

The MPYE agar Petri plates with experimental inoculants incubated at 30°C in incubator were analyzed for endophytic and rhizospheric detection of bacteria. There was appearance of colonies on E1 and R1 inoculated plates, detecting the presence of bacteria both endophytically and rhizospherically (Fig. 4.33. a, b). There were no colonies at E2, E3, R2, R3 and sterilization control plates, indicating that inactive bacteria inoculation plates (E2, R2) were also bacterial free and experiment was performed under clean and sterilized environment. Gram staining results from both E2 and R2 single colony staining showed the presence of gram negative, non-motile bacterial cells occurring singly or in pairs (Fig. 4.33. c, d), which was in accordance with the characters of *Fraseriella* associated putative endophytic bacteria, isolated and characterized earlier [Şeker, et al., 2017].

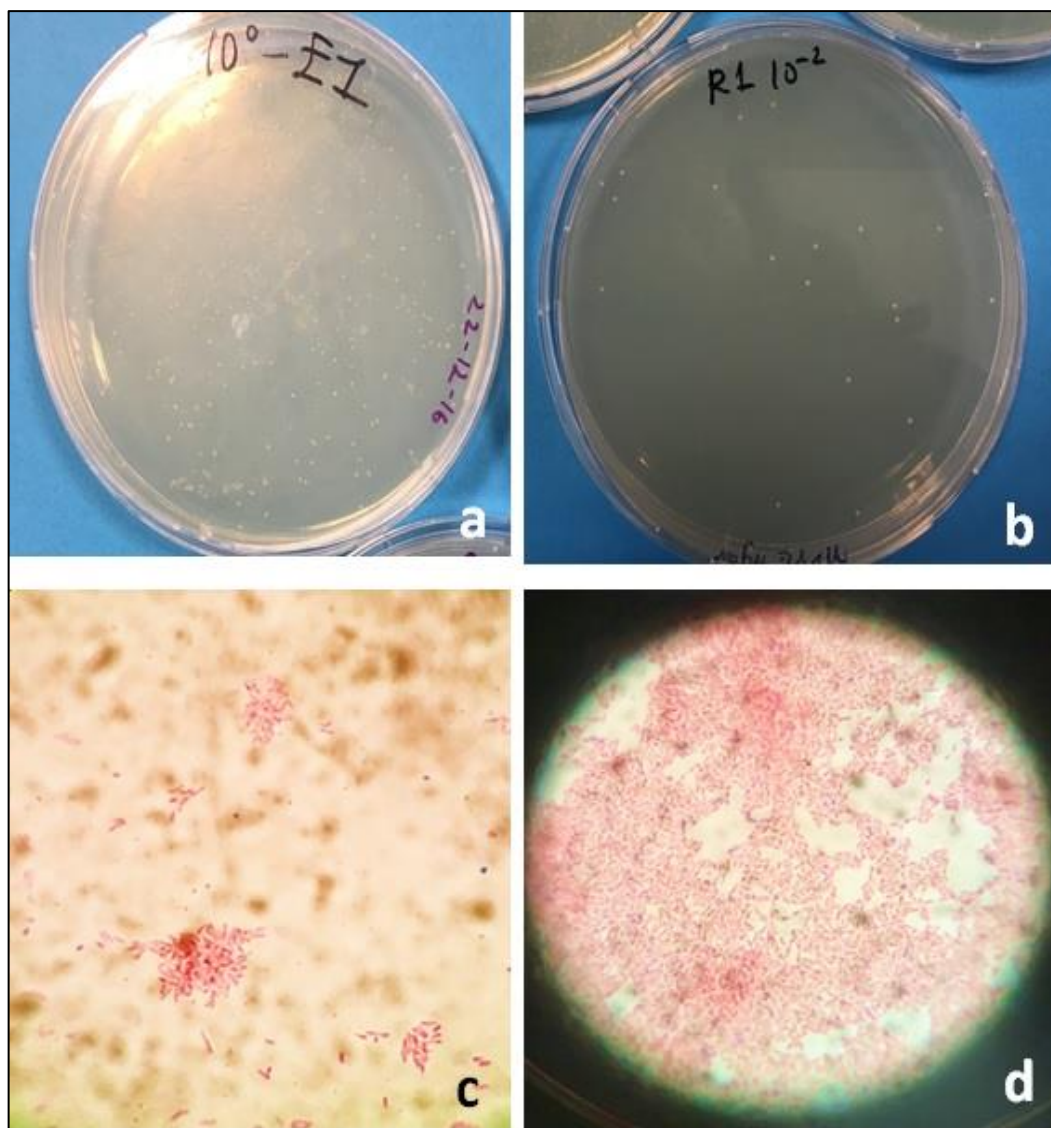


Figure 4.33. MPYE agar petriplates and microscopic pictorial representation of bacterial colonies. a) Bacterial colonies on 10^0 dilution from putative endophytic bacterial inoculant, b) Bacterial colonies on 10^{-2} dilution from putative rhizospheric bacterial inoculant, c) Gram staining of putative endophytic bacteria for E1, d) Gram staining of putative endophytic bacteria for R1.

4.4. PCR Analysis

Gel Electrophoresis of PCR products, obtained with *virD1* universal primers, showed bands appearance near ~550bps size (according to 1kb molecular marker) for both endophytically and rhizospherically isolated bacteria from the seedlings and also from the single colony liquid culture, re-isolated from the inoculated seedlings in the respective experiments. Gel Electrophoresis result has been shown in Figure 4.34 b.

These results indicated that Fraser photinia associated bacterium showed both rhizospheric and endophytic colonization in *A. thaliana*.

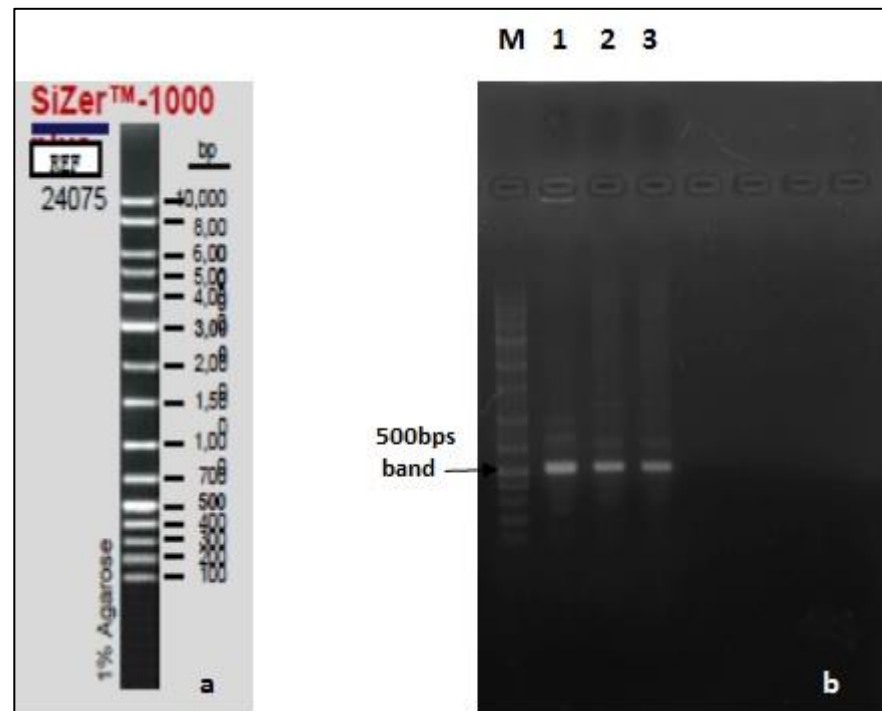


Figure. 4.34. Agarose Gel Electrophoresis results for the PCR done for plasmid DNA of putative endophytic bacteria putative *virD1* gene isolated from endophytic and rhizospheric samples of seedlings. a) 1 kb Molecular Marker used for PCR products (www.intronbio.com), b) Agarose gel bands, Lane M; 1kb Molecular weight marker, Lane 1; single colony culture, Lane 2; endophytically isolated plasmid DNA, Lane 3; rhizospherically isolated plasmid DNA.

5. DISCUSSION AND CONCLUSION

Much research has been made on developing an infiltration media required to transform *A. thaliana*. To optimize an infiltration medium, that would be compatible both for the plant and the bacterium, FW, DW, RL, fresh SW, dry SW and photosynthetic pigments *chl a*, *b* and carotenoids of 14 days old seedlings of *A. thaliana* were measured against 4 different infiltration media including MPYE, which is bacterium's own growth medium also. Infiltration media test results suggested that MPYE medium is suitable for *A. thaliana*, which is interesting. As putative endophytic bacterium is considered as alpha proteobacteria that grow well on MPYE, and at the same time, it has positive endophytic interaction with photinia, so technically plant also can be compatible with the medium on which the bacteria is grown. On the other hand, the results from IM1, which is suggested inoculation medium for *A. thaliana* in literature, showed worst results which is quite surprising as in literature it was devised as best IM for *A. thaliana* transformation with bacteria so far by Das and Joshi in 2011. This can be because of exceeded concentration of BAP from the required amount as BAP is quite basic and elevated amounts can become toxic for the seedlings as can be seen from the figures 4.1 and 4.6. Moreover, the said research suggested 0.004 M [$\sim 4\text{mM}$] amount of BAP to be used in the infiltration medium whereas this amount was rather unsoluble in the solution and as the original paper [Clough, et al., 1998] suggests that 44mM of BAP, which was ~ 10 fold of the amount they used for the medium preparation, was proved to be detrimental, giving transformation rate of *A. thaliana* seedlings 5- 8 times lower than without BAP. We used the amount of BAP in the medium according to the Clough et al. research in 1998. The results were better for 7-day old seedlings stage inoculation than for germination stage inoculation. This can be explained by the fact that seeds are thought to carefully monitor their external environmental parameters like light, temperature and nutrients etc. in order to maintain their protective state until external conditions become favourable for their development process to occur [Karssen 1982; Pritchard et al. 1993; Bungard et al., 1997]. Also, after seed coat imbibition (and bursting) followed by protrusion of testa and embryo exposure, seedling become more sensitive to environmental conditions than when seeds are intact, as reported by Li, et al. in 2005. IM1 wasn't tested for seed

stage in any of the above-mentioned papers, so, this is the first time it has been evaluated for both germination and seedlings stage of *A. thaliana*.

To study the extent to which putative endophytic bacteria affect the growth and health of *A. thaliana* (Col-0) at two different physiological stages, a wide range of parameters were measured in this study towards different concentrations and set of composite inoculants. We specifically observed the effectiveness of 'In' bacteria and 'A+In' bacterial suspensions on the plant growth. It was interesting to see that 'In' and 'A+In' suspensions of 10^7 cfu/mL caused an overall increase in fresh weight of the 7-day inoculated seedlings while same results were observed for 10^8 cfu/mL suspensions in case of dry shoot and root biomass. While active suspensions for both concentrations stayed behind for biomass, they positively affected chlorophyll contents of the seedlings, where again inactive bacteria provided support. For the 0-day inoculated stage, again 'In' suspensions resisted the plant defense mechanisms and didn't reduced any plant growth while 'A' alone stayed vulnerable to plant defense systems. Here, it is important to discuss about the difference of effects for different concentrations of bacterial suspensions. For instance, In 10^7 and In 10^8 cfu/mL suspensions remained more or less positively affective towards all the parameters of the seedlings whether alone or in composition with 'A' while 'A' suspensions (A 10^7 and A 10^8 cfu/mL) kept switching according to the biomass, photosynthetic content ratios and physiological stages of the seedlings.

The positive effects of 'In' bacterial suspensions can be explained by predicting that cell wall of bacterium was some kind of hindrance towards direct interaction with plant surface for some of the remaining active cytoplasmic contents, such as certain root and shoot promoting phyto-hormonal matter. Inactivation might have caused these contents to come in direct contact with the plant surface, thus providing the said results. So we can say that isolate was found positive, or even much better as compared to 'A' for some PGP traits even after high temperature treatments that partially inactivated the bacterium. High temperature tolerance suggests that it can be thinkable to use inactivated form of this putative bacterium with compatible bio-inoculants (bio-fertilizers) for high temperatures, but after a series of various evaluations for different factors. In a similar kind of study, Nehra, et al. in 2016 evaluated *Brevibacillus brevis* as a potential plant growth promoting rhizobacteria for cotton (*Gossypium hirsutum*) crop by treating with high temperatures (46° C) and found that bacterium retained its positive activity. This bacterium partially retained PGP activity as it was subjected to

very high temperature (95° C) and at places, it even enhanced active bacterial effect on seedlings thus providing additive support towards overall growth of plant. Therefore, in future, a temperature tolerance studies can be conducted in detail to learn the potential behavior of this bacterium towards PGP activities. A transcriptome study is needed to be done to actually know the gene expression level of these contents after inactivation of bacterium. The use of 'A+In' in composition has not been seen in researches or studies and therefore, it is another way of finding how a bacterium can be useful in its different forms.

Increase in fresh and dry weight refers to an increased cytokinins and auxins production like IAA and GA₃ since these phytohormones are essential for plant growth and body development while cytokinins also provide primarily for cells growth and differentiation in roots and shoots. These results coincide with the earlier findings for this putative endophytic bacterium [Şeker, M.G., et al., 2017]. These findings are also compatible with the literature for PGPRs and endophytes as these are known to produce phytohormones for plant growth [Mendes et al., 2007]. The putative endophytic bacterium appears to be beneficial for increasing biomass and it is therefore thinkable to use this bacterium with other beneficial and compatible PGPRs or soil microbes for root biomass and chlorophyll contents in right composition to provide an overall increase in plant growth. Valdenegro, et al. in 2001 used a wide range of compatible strains including bacteria, fungi and mycorrhizae to enhance the overall growth of *Medicago arborea* in a semi-arid Mediterranean area.

Interestingly, 'A' suspensions increased chlorophyll content with reference to fresh weight of shoots for seed stage inoculations, which can be related to biomass vs photosynthetic content ratio. Also for seedling stage inoculations, A10⁸ cfu/mL bacterial inoculants caused an increase in photosynthetic contents, while less contributing towards seedling biomass. It was observed that the increase of both fresh weight and photosynthetic contents, as observed through optical density (OD) absorbances at λ 661.6 nm, λ 644.8 nm and λ 470 nm for *chl a*, *chl b* and carotenoids respectively, in direct proportion didn't effect the statistical results for any of the variable or constant reference system. But when the fresh weight decreased, as in the cases of IM experiment (Fig. 4.6, 4.7, 4.13 and 4.14) and also in A10⁷ and A10⁸ cfu/mL bacterial suspensions for germination stage inoculations by bacterium (Fig. 4.16 & 4.17), along with a decrease in photosynthetic content (again according to above mentioned absorbances measured with spectrophotometer and also observed via naked

eye), the data changed (reversed) according to the reference systems. This increase or change in photosynthetic contents as compared to uninoculated seeds can be related to regulatory role of plant symbionts, in this case, Fraser photinia associated putative endophytic bacterium as a study suggested that soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels [Zhang, H., X. Xie, et al., 2008]. The accumulation of sugar content in roots or shoots might be considered as the reason for elevated sugar levels, in particular, induce storage processes and confers feedback inhibition of photosynthesis and vice versa [Jang and Sheen, 1994; Jang et al., 1997; Moore et al., 2003; Rolland et al., 2006; Rook et al., 2001; Sheen, 1994]. So, the bacterium caused both an increase in biomass as well as photosynthetic pigments, especially for the suspensions containing inactivated bacterium. In this regard also, the ‘In’ bacterial suspensions study is very important because of their consistency towards plant growth promotion.

The reason why ‘A’ suspensions didn’t work well with seed stage inoculations might lie in the fact that seeds maintain their protective state until external environment conditions become favorable for their development process and after imbibition and embryo exposure, emerged seedling become even more sensitive to environmental conditions [Karssen 1982; Pritchard et al. 1993; Bungard et al., 1997]. While ‘A’ suspensions caused the seed and later, the seedlings, to activate their plant defense mechanisms, inactive bacteria might have not activated these defense systems, being apparently non-living and so there appeared no negative effect of suspensions containing inactive bacteria for seed stage inoculations. The same can also be considered true for seedling inoculations with the respective bacterium.

The fact that Fraser photinia associated putative endophytic bacterium belongs to family of Rhizobiales [Şeker, et al., 2017], presence of *aux1* and *virD1* gene is not unexpected. Binding of isolated plasmid DNA from bacterium with universal forward and reverse primers of *virD1* 14955 rhizogene confirms the presence of *virD1* gene in the plasmid. As the size of *A. rhizogene virD1* gene is between ~ 417-440bps, appearance of band at ~550 bps suggested that the size of *virD1* gene in this putative bacterium is longer. It is always hard to provide any definitive statement regarding the behavior of a specific or putative bacterial strain’s potential to be a PGPR because of the complex nature of plant-microbe interactions. The difference of results shown by this bacterium towards Fraser photinia and *A. thaliana* suggests that we may not strictly consider the bacterium to be host specific, but this might be a too early

conclusion as very little is known about this bacteria and it can be subjected to various kinds of temperature and biotic and abiotic conditions, a vast range of parameters that are still to be evaluated at both phenotypic and transcriptomic level before reaching a certain conclusion. In this regard, study of this bacterium against different potential hosts will be a handy approach to evaluate its potential as a possible PGPR near future. Moreover, the studies at molecular level for *A. thaliana* using molecular tools such as transcriptome analysis will be quite useful to shed light on plant and bacterium interaction.

REFERENCES

- Agrios G. N., (1997), "Plant Pathology". (San Diego: Academic Press).
- Almeida, C. V., Andreote, F. D., Yara, R., Tanaka, F. A. O., Azevedo, J. L., Almeida, M. (2009), "Bacteriosomes in axenic plants: endophytes as stable endosymbionts". *World Journal of Microbiology and Biotechnology*, 25:1757–1764. doi: 10.1007/s11274-009-0073-8.
- Antoun, H., Prévost, D., (2005), "Ecology of plant growth promoting rhizobacteria". In: Z.A. Siddiqui, Editor, "PGPR: biocontrol and biofertilization". Springer, Dordrecht, 1–38.
- Arshad, M., and Frankenberger, W. T. Jr. (1993), "Microbial production of plant growth Regulators". In: F. B. Metting, Jr. M. Dekker, Editors, "Soil microbial ecology. Applications in agricultural and environmental management". Inc., New York. 307-343.
- Bacon, C. W., Hinton, D. M. (2006), "Bacterial endophytes: the endophytic niche, its occupants, and its utility". In: S.S. Gnanamanickam, Editor, "Plant- Associated Bacteria". Springer, Netherlands, 155-194.
- Barbieri, P., and Galli, E. (1993), "Effect on wheat root development of inoculation with an *Azospirillum brasilense* mutant with altered indole-3-acetic acid production". *Research Microbiology*, 144: 69-75.
- Barea, J.M., Navarro, E., Montoya, E. (1976), "Production of plant growth regulators by rhizosphere phosphate-solubilizing bacteria". *Journal of Applied Bacteriology*, 40: 129-134.
- Berg, G., Hallmann, J. (2006), "Control of plant pathogenic fungi with bacterial endophytes". In: B. J. E. Schulz, C. J. C. Boyle, Th. N. Sieber, Editors, "Microbial Root Endophytes". Springer-Verlag, Berlin, 53-69.
- Berg, G., Krechel, A., Ditz, M., Sikora, R. A., Ulrich, A., Hallmann, J. (2005), "Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi". *FEMS (Federation of European Microbiological Society) Microbial Ecology*, 51: 215-229.
- Bhattacharyya, P. N. Jha, D. K. (2012), "Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture". *World Journal of Microbiology and Biotechnology*, 28: 1327-1350.
- Bonafante, P., Anca, I. A. (2009), "Plants, mycorrhizal fungi, and bacteria: a network of interactions". *Annual Review of Microbiology*, 63, 363-383.
- Bøckman, O. C. (1997), "Fertilizers and biological nitrogen fixation as sources of plant nutrients: perspectives for future agriculture". *Plant and Soil*, 194: 11-14.

Bungard, R. A., Mcneil, D., Morton, J. D. (1997), "Effects of chilling, light and nitrogen-containing compounds on germinations, rate of germination and inhibition of *Clematis vitalba* L". *Annals of Botany*, 79: 643–645.

Cartieaux, F. P., Nussaume, L., Robaglia, C. (2003), "Tales from the underground: molecular plant-rhizobacteria interactions". *Plant Cell and Environment*, 26: 189-199.

Cooley, M. B., Miller, W. G., Mandrell, R. E. (2003), "Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*". *Applied and Environmental Microbiology*, 69: 4915-4926.

Crute I., Beynon J., Dangl J., Huolub E., Mauch-Mani B., Slusarenko A., Staskawicz B. J., Ausubel F. (1994), "Microbial pathogenesis of *Arabidopsis*" in *Arabidopsis*. In: E.M. Meyerowitz and C.R. Somerville, Editors, "Cold Spring Harbor": Cold Spring Harbor Laboratory Press, 1(1):705–747.

Dias, A. C. F., Costa, F. E. C., Andreote, F. D., Lacava, P. T., Teixeira, M. A., Assumpção, L. C., Araújo, W. L., Azevedo, J. L., Melo, I. S. (2008), "Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion". *World Journal of Microbiology and Biotechnology*, 25:189–195. doi: 10.1007/s11274-008- 9878-0.

Dobbelaere, S., Vanderleyden, J. and Okon, Y. (2003), "Plant growth-promoting effects of diazotrophs in the rhizosphere". *Critical Reviews in Plant Sciences*, 22:107-149.

Dunne, C., Crowley, J. J., Loccoz, Y. M., Dowling, D. N., de Bruijn, F., O’Gara, F. (1993), "Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity". *Microbiology*, 143: 3921-3931.

Elkoca, E., Kantar, F., Sahin, F. (2008), "Influence of nitrogen fixing and phosphorous solubilizing bacteria on the nodulation, plant growth, and yield of chickpea". *Journal of Plant Nutrition*, 31:157-171.

Elphinstone, J. G. (2005), "The current bacterial wilt situation: a global overview". In: C. Allen, P. Prior, and A. C. Hayward, Editors, "Bacterial Wilt Disease and the *Ralstonia solanacearum* species Complex, 9–28. St Paul, MN: APS Press.

Fallik, E., Okon, Y. (1989), "Identification and quantification of IAA and IBA in *Azospirillum brasilense* inoculated maize roots". *Soil Biology and Biochemistry*, 21: 147-153.

Farh, M. E.-A., Kim, Y.-J., Sukweenadhi, J., Singh, P., Yang, D. C. (2017), "Aluminium resistant, plant growth promoting bacteria induce overexpression of Aluminium stress related genes in *Arabidopsis thaliana* and increase the ginseng tolerance against Aluminium stress." *Microbiological Research* 200 (Supplement C): 45-52.

Frankenberger, W. T. Jr. and Arshad, M. (1995), "Phytohormones in soils: Microbial production and function". Marcel Dekker, Inc. New York, NY, 503.

Franco-Correa, M., Quintana, A., Duque, C., Suarez, C., Rodríguez, M. X., Barea, J. M. (2010), "Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities". *Applied Soil Ecology*, 45 pp. 209-217.

Gelvin, S. B. (2010), "Plant proteins involved in *Agrobacterium*-mediated genetic transformation". *Annual Review of Phytopathology*, 48: 45–68.

Gerhardson, B. (2002), "Biological substitutes for pesticides". *Trends in Biotechnology*, 20:338- 343.

Germida, J. J., Siciliano, S. D., De Freitas, J. R., Seib, A. M. (1998), "Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.)". *FEMS (Federation of European Microbiological Society)*, *Microbial Ecology*, 26:43-50.

Glick, B. R. (1995), "The enhancement of plant growth by free-living bacteria". *Canadian Journal of Microbiology*, 41:109-117.

Hallmann, J., Berg, G. (2006), "Spectrum and population dynamics of bacterial root endophytes". In: B.J. Schulz, Editor, "Microbial Root Endophytes". Springer-Verlag, Berlin. 15-31.

Hallmann, J., Quadt-Hallman, A., Mahafee, W. F. Kloepper, J. W. (1997), "Bacterial endophytes in agricultural crops". *Canadian Journal of Microbiology*, 43: 895–914.

Handelsman, J. Stabb, E. V. 1996. Biocontrol of soilborne plant pathogens. *The Plant Cell* 8: 1855-1869.

Hardoim, P. R., van Overbeek, L. S., van Elsas, J. D. (2008), "Properties of bacterial endophytes and their proposed role in plant growth". *Trends in Microbiology*, 16:463-471.

Hartmann, A., Singh, M., Klingmüller. (1983), "Isolation and characterization of *Azospirillum* mutants excreting high amounts of indole-acetic acid". *Canadian Journal of Microbiology*, 29: 916-923.

Holguin, G., Patten, C. L., Glick, B. R. (1999), "Genetics and molecular biology of *Azospirillum*". *Biology and Fertility of Soils* 29: 10-23.

Hong, Y., Pasternak, J. J., Glick, B. R. (1991), "Biological consequences of plasmid transformation of the plant growth promoting rhizobacterium *Pseudomonas putida* GR12–2". *Canadian Journal of Microbiology*, 37: 796–799.

Ishiga, Y., Ishiga, T., Uppalapati, S. R., Mysore, K. S. (2011), "*Arabidopsis* seedling flood-inoculation technique: a rapid and reliable assay for studying plant-bacterial interactions." *Plant Methods*, 7(1): 32.

James, E. K., Olivares, F. L. (1997), "Infection of sugarcane and other graminaceous plants by endophytic diazotrophs". *Critical Review in Plant Sciences*, 17: 77-119.

Jang, J. C., Sheen, J. (1994), "Sugar sensing in higher plants". *Plant Cell*, 6: 1665–1679.

Jang, J. C., Leon, P., Zhou, L., Sheen, J. (1997), "Hexokinase as a sugar sensor in higher plants". *Plant Cell*, 9: 5–19.

Kagenishi, T., Yokawa, K., Baluška, F. (2016), "MES Buffer Affects *Arabidopsis* Root Apex Zonation and Root Growth by Suppressing Superoxide Generation in Root Apex." *Frontiers in Plant Science*, 7: 79.

Kampert, M., Strzelczyk, E., and Pokojka, A. (1975), "Production of auxins by bacteria isolated from the roots of pine seedlings (*Pinus silvestris* L.) and from soil". *Acta Microbiologica Polonica*, 7: 135-143.

Karssen, C. M. (1982), "Seasonal patterns of dormancy in weed seeds". In: Khan A. A., Editors, "The Physiology and Biochemistry of Seed Development, Dormancy and Germination". Elsevier Biomedical Press, Amsterdam, Netherlands, 243– 270.

Kirchhof, G., Reis, V. M., Baldani, J. I., Eckert, B., Döbereiner, J., and Hartmann, A. (1997), "Occurrence, physiological and molecular analysis of endophytic diazotrophic bacteria in gramineous energy plants". *Plant and Soil*, 194: 45-55.

Kloepper, J. W., Schroth, M. N., 1978, "Plant growth-promoting rhizobacteria on radishes". In: *Proceedings of the 4th International Conference on Plant Pathogenic Bacteria*, (2) 879–882, Angers, France, August 27 - September 2.

Kloepper, J. W., Leong, J., Teintze, M., Schroth, M. N. (1980), "Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria". *Nature*, 286: 885-886.

Kloepper, J. W., Schroth, M. W. (1981), "Plant growth-promoting rhizobacteria under gnotobiotic conditions". *Phytopathology*, 71: 642-644.

Kloepper, J. W., Hume, D. J., Scher, F. M., Singleton, C., Tipping, B., Laliberté, M., Frauley, K., Kutchaw, T., Simonson, C., Lifshitz, R., Zaleska, I., Lee, L. (1988), "Plant growth-promoting rhizobacteria on canola (rapeseed)". *Plant Disease*, 72: 42- 45.

Kloepper, J. W., Lifshitz, R., Zablotowicz, R. M. (1989), "Free living bacterial inocula for enhancing crop productivity". *Trends in Biotechnology*, 7: 39-44.

Kloepper, J. W. (1994), "Plant growth-promoting rhizobacteria (other systems)" In: Y. Okon Editor, "*Azospirillum*/Plant Associations", CRC Press, Boca Raton, FL, USA. 111-118.

Glick, B. R., Patten, C. L., Holguin, G., Penrose, D. M. (1999), "Biochemical and genetic mechanisms used by plant growth promoting bacteria". Imperial College Press, London, United Kingdom, 267.

Koornneef, M., Meinke, D. (2010), "The development of *Arabidopsis* as a model plant". The Plant Journal, 61 (6): 909-921.

Koutsoudis, M. D., Tsaltas, D., Minogue, T. D., von Bodman, S. B. (2006), "Quorum-sensing regulation governs bacterial adhesion, biofilm development, and host colonization in *Pantoea stewartii* subspecies *stewartii*". Proceedings of the National Academy of Sciences USA, 103, 5983–5988.

Lacava, P. T., Azevedo, J. L. (2013), "Endophytic bacteria: a biotechnological potential in agrobiological system". In: D.K. Maheshwari, M. Saraf, A. Aeron, Editors, "Bacteria in Agrobiological: Crop Productivity", Springer-Verlag, Berlin, Heidelberg, 1-44.

Ladha, J. K., Reddy, P. M. (2003), "Nitrogen fixation in rice systems: state of knowledge and future prospects". Plant Soil, 252:151-167.

Liu, L., Kloepper, J. W., Tuzun, S. (1995), "Induction of systemic resistance in cucumber against bacterial angular leaf spot by plant growth-promoting rhizobacteria". Phytopathology, 85: 843-847.

Li, W., Khan, M. A., Yamaguchi, S., Kamiya, Y. (2005), "Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana*." Plant Growth Regulation, 46(1): 45-50.

Lichtenthaler, H. K. and C. Buschmann (2001), "Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. Current Protocols in Food Analytical Chemistry". John Wiley & Sons, Inc.

Lifshitz, R., Kloepper, J. W. E., Kozłowski, M., Simonson, C., Carlson, J., Tipping, E. M., Zaleska, I. (1987), "Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions". Canadian Journal of Microbiology, 33: 309-395.

Lodewyckx, C. J. Vangronsveld, F. Porteous, E. R. Moore, B., Toghavi, S., Mezgeay, M., Lelie, V. D. (2002), "Endophytic bacteria and their potential applications". Critical Review in Plant Sciences, 21:583-606.

Loper, J. E., Schroth, M. N. (1986), "Influence of bacterial sources of indole-2-acetic acid on root elongation of sugar beet". Phytopathology, 76: 386-389.

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V. Del Rio T. G., Edgar, R. C., Eickhorst, T., Ley, R. E., Hugenholtz, P., Tringe, S. G., Dangl, J. L. (2012), "Defining the core *Arabidopsis thaliana* root microbiome". Nature, 488, 86-90.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., Foster, G. D. (2012), "Top 10 plant pathogenic bacteria in molecular plant pathology". *Molecular Plant Pathology*, 13: 614–629.

Marek-kozaczuk, M., Deryto, M., Skorupska, A. (1996), "Tn5 insertion mutants of *Pseudomonas* sp 267 defective siderophores production and their effect on clover (*Trifolium pretense*) nodulated with *Rhizobium leguminosarum* bv *trifolii*". *Plant Soil*, 179:269-274.

Martínez, J., Martínez, L., Rosenblueth, M., Silva, J., Martínez- Romero, E. (2004), "How are gene sequence analyses modifying bacterial taxonomy? The case of *Klebsiella*". *International Microbiology*, 7:261-268.

Mendes, R., Pizzirani-Kleiner, A. A., Araujo W. L., Raaijmakers, J. M., (2007), "Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of *Burkholderia cepacia* complex isolates". *Applied and Environmental Microbiology*, 73: 7259–7267.

Merzaeva, O. V., Shirokikh, I. G. (2006), "Colonization of plant rhizosphere by actinomycetes of different genera". *Microbiology*, 75, 226-230.

Molla, A. H., Shamsuddin, Z. H. Halimi, M. S. Morziah, M., Putech, A. B. (2001), "Potential for enhancement of root growth and nodulation of soybean co-inoculated with *Azospirillum* and *Bradyrhizobium* in laboratory systems". *Soil Biology and Biochemistry*, 33:457-463.

Moore, B., Zhou, L., Rolland, F., Hall, Q., Cheng, W. H., Liu, Y. X., Hwang, I., Jones, T., Sheen, J. (2003), "Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal signaling". *Science*, 300 (5617): 332–336.

Nehl, D. B., Allen, S. J., Brown, J. F. (1996), "Deleterious rhizosphere bacteria: an integrating perspective". *Applied Soil Ecology*, 5: 1-20.

Nehra, V., Saharan, B. S., Choudhary, M. (2016), "Evaluation of *Brevibacillus brevis* as a potential plant growth promoting rhizobacteria for cotton (*Gossypium hirsutum*) crop." *SpringerPlus* 5(1): 948.

Öztürk, Y., Lee, D. W., Mandaci, S., Osyczka, A., Prince, R. C., Daldal, F. (2008), "Soluble Variants of *Rhodobacter capsulatus* Membrane-anchored Cytochrome c(y) Are Efficient Photosynthetic Electron Carriers." *The Journal of Biological Chemistry*, 283 (20): 13964-13972.

Patten, C., Glick, B. R. (1996), "Bacterial biosynthesis of indole-3-acetic acid". *Canadian Journal of Microbiology*, 42: 207-220.

Patten, C. L., Glick, B. R. (2002), "Regulation of indole-3-acetic acid production in *Pseudomonas putida* GR12-2 by tryptophan and stationary-phase sigma factor RpoS". *Canadian Journal of Microbiology*, 48: 635-642.

Pilet, P. E., Elliott, M. C., Moloney, M. M. (1979), "Endogenous and exogenous auxin in the control of root growth". *Planta*, 146: 405-408.

Pirttilä, A. M., Joensuu, P., Pospiech, H., Jalonen, J., Hohtola, A. (2004), "Bud endophytes of Scots pine produce adenine derivatives and other compounds that affect morphology and mitigate browning of callus cultures". *Physiologia Plantarum*, 121:305-312.

Pitzschke, A., Hirt, H. (2010), "New insights into an old story: *Agrobacterium*-induced tumor formation in plants by plant transformation". *The EMBO Journal*, 29, 1021–1032.

Ponka, A., Andersson, Y., Siitonen, A., de Jong, B., Jahkola, M., Haikala, O., Kuhmonen, A., Pakkala, P. (1995), "*Salmonella* in alfalfa sprouts". *Lancet*, 345:462-3.

Poupin, M. J., Timmermann, T., Vega, A., Zuniga, A., Gonzalez, B. (2013), "Effects of the plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN throughout the life cycle of *Arabidopsis thaliana*." *PLoS One*, 8(7): e69435.

Pritchard, H. W., Wood, J. A., Mjanger, K. R. (1993), "Influence of temperature on seed germination and the nutritional requirements for embryo growth in *Arum maculatum* L". *New phytologist*, 123: 801–809.

Quambusch, M., Brümmer, J., Haller, K., Winkelmann, T., Bartsch, M. (2016), "Dynamics of endophytic bacteria in plant in vitro culture: quantification of three bacterial strains in *Prunus avium* in different plant organs and in vitro culture phases." *Plant Cell, Tissue and Organ Culture (PCTOC)*, 126(2): 305-317.

Richardson, A. E. (2001), "Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants". *Australian Journal of Plant Physiology*, 28:897-906.

Rodriguez, H., Fraga, R. (1999), "Phosphate solubilizing bacteria and their role in plant growth promotion". *Biotechnology Advances*, 17:319-339.

Rolland, F., Baena-Gonzalez, E., Sheen, J. (2006), "Sugar sensing and signaling in plants: conserved and novel mechanisms". *Annual Review of Plant Biology*, 57, 675–709.

Rook, F., Corke, F., Card, R., Munz, G., Smith, C., Bevan, M. W. (2001), "Impaired sucrose-induction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signaling". *The Plant Journal*, 26, 421–433.

Rosas, S. B., Andres, J. A. Rovera, M., Correa, N. S. (2006), "Phosphate-solubilizing *Pseudomonas putida* can influence the rhizobia-legume symbiosis". *Soil Biology and Biochemistry*, 38:3502-3505.

Rosenblueth, M., Martinez-Romero, E. (2006), "Bacterial endophytes and their interactions with hosts". *Molecular Plant-Microbe Interactions Journal*, 19:827-837.

Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J., Dowling, D. N. (2008). "Bacterial endophytes: Recent developments and applications". *FEMS Microbiology Letters*, 278:1-9.

Ryu, C.-M., Farag, M. A., Hu, C. -H., Reddy, M. S., Wei, H. -X., Pare, P. W., Kloepper, J. W. (2003), "Bacterial volatiles promote growth in *Arabidopsis*". *Proceedings of the National Academy of Sciences USA*, 100:4927-4932.

Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., Billault, A., Brottier, P., Camus, J. C., Cattolico, L., Chandler, M., Choise, N., Claudel-Renard, C., Cunnac, S., Demange, N., Gaspin, C., Lavie, M., Moisan, A., Robert, C., Saurin, W., Schiex, T., Siguier, P., Thebault, P., Whalen, M., Wincker, P., Levy, M., Weissenbach, J., Boucher, C. A. (2002), "Genome sequence of the plant pathogen *Ralstonia solanacearum*". *Nature*, 415, 497–502.

Salisbury, F. B., Ross, C. W. (1992), "Plant Physiology". Wadsworth Publishing Company, Belmont, California, U.S.A. 127-138.

Saravanan, V. S., Madhaiyan, M. Osborne, J. Thangaraju, M., T. M. SA. (2008), "Ecological occurrence of *Gluconacetobacter diazotrophics* and nitrogen-fixing *Acetobacteraceae* members: Their possible role in plant growth promotion". *Microbial Ecology*, 55:130-140.

Schulz, B. J. E., Boyle, C. J. C. (2006), "What are endophytes?" In: Schulz, B. J. E., Boyle, C. J. C., Sieber, T. N., Editors, "Microbial root endophytes". Springer, Berlin, 1–13.

Sessitsch, A., Howieson, J. G., Perret, X, Antoun, H., Martínez- Romero, E. (2002), "Advances in Rhizobium research". *Critical Reviews in Plant Sciences*, 21:323-378.

Sessitsch, A., Reiter, B., Berg, G. (2004), "Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities". *Canadian Journal of Microbiology*, 50:239-249.

Sheen, J. (1994), "Feedback control of gene expression". *Photosynthesis Research*, 39: 427–438.

Somers, E., Vanderleyden, J., Srinivasan, M. (2004), "Rhizosphere bacterial signalling: a love parade beneath our feet". *Critical Reviews in Microbiology*, 30, 205-240.

Spaink, H.P. (2000), "Root nodulation and infection factors produced by rhizobial bacteria". *Annual Review. Microbiology*, 54, 257-288.

Sparkes, I. A., Runions, J., Kearns, A., Hawes, C. (2006), "Rapid, transient expression of fluorescent fusion proteins in tobacco plants and generation of stably transformed plants." *Nature Protocols*, 1(4): 2019-2025.

Strobel, G., Daisy, B. Castillo, U., Harper, J. (2004), "Natural products from endophytic microorganisms". *Journal of Natural Products*, 67:257-268.

Sturz, A. V., Christie, B. R., Nowak, J. (2000), "Bacterial endophytes: Potential role in developing sustainable systems of crop production". *Critical Reviews in Plant Sciences*, 19:1-30.

Sturz, A.V. (1995), "The role of endophytic bacteria during seed piece decay and tomato tuberization". *Plant Soil*, 175:257–263.

Sturz, A. V., Christie, B. R., Matheson, B. G. (1998), "Associations of bacterial endophyte populations from red clover and potato crops with potential for beneficial allelopathy". *Canadian Journal of Microbiology*, 44:162–167.

Sukweenadhi, J., Kim, Y. J., Choi, E. S., Koh, S. C., Lee, S. W., Kim, Y. J., Yand, D. C. (2015), "*Paenibacillus yonginensis* DCY84T induces changes in *Arabidopsis thaliana* gene expression against aluminum, drought, and salt stress". *Microbiological Research*, 172(Supplement C): 7-15.

Şeker, M. G., Şah, I., Kırdök, E., Ekinci, H., Çiftçi Y. Ö., Akkaya, Ö. (2017), "A hidden plant growth promoting bacterium isolated from in vitro cultures of fraser photinia (*Photinia* × *fraseri*)". *International Journal of Agriculture and Biology*, 19: 1511–1519.

Şah, I. (2017), "Biochemical and Molecular characterization of plant growth promoting putative endophytic bacterium isolated from in vitro cultures of *Photinia* × *fraseri* dress". PhD thesis, Gebze Technical University.

Tzfira, T., Citovsky, V. (2002), "Partners-in-infection: host proteins involved in the transformation of plant cells by *Agrobacterium*". *Trends in Cell Biology*, 12, 121–129.

Valdenegro, M., Barea, J. M., Azcon, R. (2001), "Influence of arbuscular-mycorrhizal fungi, *Rhizobium meliloti* strains and PGPR inoculation on the growth of *Medicago arborea* used as model legume for re-vegetation and biological reactivation in a semi-arid mediterranean area." *Plant Growth Regulation*, 34(2): 233-240.

Verma, S. C., Singh, A., Chowdhury, S. P., Tripathi, A. K. (2004), "Endophytic colonization ability of two deep-water rice endophytes, *Pantoea* sp. and *Ochrobactrum* sp. using green fluorescent protein reporter". *Biotechnology Letters*, 26:425-429.

Vessey, J. K. (2003), "Plant growth promoting rhizobacteria as biofertilizers". *Plant Soil*, 255:571-586.

Weller, D. M., Cook, R. J. (1986), "Increased growth of wheat by seed treatments with fluorescent pseudomonads, and implications of *Pythium* control". *Canadian Journal of Plant Pathology*, 8: 328-334.

Zaltsman, A., Krichevsky, A., Loyter, A., Citovsky, V. (2010), "Agrobacterium induces expression of a plant host F-box protein required for tumorigenicity". *Cell Host Microbe*, 7, 197–209.

Zaspel, I., Ulrich, A., Boine B., Stauber, T. (2008), "Occurrence of culturable bacteria living in micropropagated black locust cultures (*Robinia pseudoacacia* L.)". *European Journal of Horticultural Science*, 73:231–235.

Zhang, H., Xie, X., Kim, M. S., Korniyev, D. A., Holaday, S., Paré, P. W. (2008), "Soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels in planta." *The Plant Journal*, 56(2): 264-273.

Zupan, J., Muth, T. R., Draper, O., Zambryski, P. C. (2000), "The transfer of DNA from *Agrobacterium tumefaciens* into plants: a feast of fundamental insights". *The Plant Journal*, 23, 11–28.

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