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I dedicate this thesis to my mother, who always loved and supported me. I miss you every day.

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Abstract

The development of new and better methods in agriculture, to increase crop yield, has become more and more important in the last years, as the world's population is growing, and the demand for more food production increases. The traditional use of chemicals is damaging to the environment, and the focus on more eco-friendly methods has escalated in the last decades. One of these methods is the use of plant growth-promoting bacteria (PGPB). By inoculation of these bacteria to the soil, or other growth substrates, they may have a positive effect on the growth of the plants.

In this thesis, seven bacteria, isolated by another research group, from roots of tomatoes grown in Italy; a well-known PGPB, *Pseudomonas simiae* WCS417; and a *Paenibacillus* sp. isolated from *Solanum pennellii* here at the University of Stavanger (UiS), were all used in different root growth assays with *Arabidopsis thaliana*. Mutated *A. thaliana* was also used to investigate whether some genes, which are important regulators of protein phosphatase 2A (PP2A), are involved in the interaction between the plant and the PGPB. This was done by looking at the effect on the root system, i.e. the effect on the primary root, lateral roots, and root hairs. The genes investigated were Phosphotyrosyl Phosphatase Activator (*PTPA*), Leucine Carboxyl Methyl Transferase (*LCMT1*), and Protein phosphatase 2A Methylesterase 1 (*PME-1*). Four different *A. thaliana* mutants were used; a *PTPA* over-expressor ($ptpa_{ox}$), a *PTPA* knock-down ($ptpa_{kd}$), a *LCMT1* knockout (*lcmt1*), and a *PME-1* knockout (*pme1*), in addition to wild type (WT).

The *Paenibacillus* sp. did not appear to have a positive effect on the root system of *A. thaliana* plants, and the effects of the seven bacteria isolated by another research group were variable.

Experiments with the known PGPB *P. simiae* WCS417 gave a similar effect on the root system of *A. thaliana* WT plants, previously described by others, with inhibition of primary root, increase of the numbers of lateral roots, and increase of root hair formation, compared to the control. This was also observed for $ptpa_{ox}$, and $ptpa_{kd,}$ but $ptpa_{kd}$ appeared to have a lower percent increase of lateral roots, compared to WT and $ptpa_{ox}$. However, this experiment was not repeated, and a definite conclusion of PTPA involvement in the interaction between *A. thaliana* and PGPB cannot be made.

In addition, *P. simiae* WCS417 and the isolated *Paenibacillus* sp., were used for a "real-life" experiment. *Solanum lycopersicum* cv. Heinz and Moneymaker, grown in Vermiculite, were inoculated with the bacteria. This experiment did not show any effect of either bacteria.

These experiments show that results obtained by others may be difficult to reproduce, and even though some bacteria show plant growth-promoting traits *in vitro*, many factors, e.g. competing microorganisms and conditions of the growth substrate, will influence their ability to implement these traits on the plants in the field.

Abbreviations

16S rRNA	16S ribosomal ribonucleic acid
ACC	1-aminocylopropane-1-carboxylate
Ami-RNA	Artificial micro RNA
bp	Base pairs
DNA	Deoxyribonucleic acid
IAA	Indole acetic acid
ISR	Induced systemic resistance
LB	Luria-Bertani
LCMT1	Leucine carboxyl methyl transferase 1
LR	Lateral roots
MM	Moneymaker
MS	Murashige and Skoog
N	Sample size
NCBI	National Centre for Biotechnology Information
OA	Organic acid
OD	Optical density
PCR	Polymerase chain reaction
PGPB	Plant growth-promoting bacteria
PGP traits	Plant growth-promoting traits
PME-1	Protein phosphatase 2A methylesterase 1
PP2A	Protein phosphatase 2A
PR	Primary root
PTPA	Phosphotyrosyl phosphatase activator
RH	Root hairs
SD	Standard deviation
Ser	Serine
SNP	Single nucleotide polymorphism
Suc	Sucrose
Thr	Threonine

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1 Introduction

This thesis has been a part of a cooperation between the University of Stavanger (UiS), Norwegian Institute of Bioeconomy Research (NIBIO), and Hasselt University in Belgium. It has been a subproject of an ongoing project, the "BioFresh" project, which is managed by Dr. Michel Verheul (NIBIO, Saerheim). The BioFresh project's goal is to "produce fresh vegetables year-round without the use of fossil energy, chemical plant protection, and without emission of CO_2, fertilizer or other waste fractions".

1.1 Background

The world's population is growing rapidly, and thus an increasing demand for more food production (Glick 2012). The traditionally way, in agriculture, to increase production and crop yield, has been with the use of chemical fertilizers and pesticides. As these practices often damage the environment, it has been a larger focus in the later years to grow more sustainable crops, and to find other environmentally safe methods to increase productivity (Abbamondi et al. 2016).
One method that is of great interest is microbial inoculation of plant growth-promoting bacteria (PGPB). Many PGPB have been isolated in the last decades, and one of the most important work ahead will be to transfer the use of these from the laboratory to the fields, or green houses.
Tomato is one of the most important crop species in the world (Bergougnoux 2014), and as Rogaland is Norway's largest producer of tomatoes (Opplysningskontoret for frukt og grønt), the research of new methods for improving crop yield should be of great interest in this area.

1.2 Plant growth promoting bacteria

The soil contains high numbers of microorganisms, including bacteria and fungi. The microorganisms growing near the roots of plants are called the root microbiome (Zamioudis et al. 2013).
Plants secrete some of their products from the photosynthesis into the rhizosphere. These metabolites stimulate a higher density of microorganisms in the rhizosphere than in the bulk soil. However, there is less microbial diversity in the rhizosphere, and even less in the internal compartments of the plants, due to the ability of the plants to select their own microbiome, as the exudates may stimulate or repress specific microorganisms (Berendsen et al. 2012). Several factors are important for the composition of the microbiome, i.e. soil type, species, and genotype (Berendsen et al. 2012; Lundberg et al. 2012). Pathogen attack of the plant may also lead to a change in the microbial community in the rhizosphere (Berendsen et al. 2012).

The bacteria in the root microbiome are divided into two distinct groups; rhizospheric and endophytic bacteria. The rhizospheric bacteria colonize the rhizosphere, while the endophytic bacteria colonize the internal parts of the plant (Abbamondi et al. 2016).
These bacteria may either be beneficial or harmful to the plant, and affect the health and growth of the plants. The bacteria that have a positive effect on the plants health and growth are called PGPB. They provide hormones that stimulate plant growth, help improve the uptake of nutrition, and protect plants from infections. (Abbamondi et al. 2016; Zamioudis et al. 2013). For example, many PGPB are known to increase the numbers of lateral roots (LR), and root hairs (RH), which increases the root's capacity to take up water and minerals (Zamioudis et al. 2013).
Thousands of PGPB have been isolated in the last decades, but the step from artificial laboratory experiments to field experiments have proven to be difficult (Bulgarelli et al. 2013). Even if one bacterium has shown plant growth-promoting (PGP) traits in the laboratory, it might be difficult to reproduce these results in the field, due to several variables. Competing microorganisms in the soil may affect the inoculation (Berendsen et al. 2012; Bulgarelli et al. 2013). Temperature, pH and salinity, are some of the other factors that will affect the success of the inoculation (Grady et al. 2016).

1.2.1 Bacterial strains isolated from tomato rhizosphere and roots.

Abbamondi and collaborators (2016), isolated 11 rhizospheric strains and 12 root endophytes from roots of different tomato cultivars and new tomato hybrids, grown in Italy. In addition to analysing for different PGP traits, the cultivable isolates were also inoculated on agar plates with *Arabidopsis thaliana* seedlings, to see if they had any effect on root growth. The PGP traits analysed were the production of organic acid (OA), indole acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase. In addition, siderophore production were analysed (Abbamondi et al. 2016). Bacterial strains 15, 16, and 18 were found to produce OA, IAA, ACC, and siderophore. Bacterial strains 5, 6, and 10 produced IAA and ACC, but for these, OA and siderophore were not analysed. Strain 9 produced all, except OA (Abbamondi et al. 2016).
These seven isolated strains mentioned above and in table 1.1, were provided from Jaco Vangronsveld's research group at Hasselt University (Abbamondi et al. 2016), with the aim of testing genes of interest that might be involved in the interaction between plants and PGPB. All these strains had in their experiments given significant ($0.001 < p < 0.05$), or relevant differences ($0.05 < p < 0.1$) in the primary root (PR) length of *A. thaliana* compared to the control. All strains provided had also shown inhibition of the total lateral root length of *A. thaliana*, but only for strain 10 was this

difference significant (0.01 < p >0.05). All provided strains increased root hair development of *A. thaliana* (Abbamondi et al. 2016).

Table 1.1: Bacterial strains isolated from tomato roots (Abbamondi et al. 2016)

	Strain no	Genus
Rhizospheric strains	5	Unknown[1]
	6	Unknown[1]
Endophytic strains	9	*Pseudomonas* sp.
	10	Unknown[1]
	15	*Agrobacterium* sp.
	16	*Rhizobium* sp.
	18	*Agrobacterium* sp.

[1] **Strain not cultivable, and is unidentified.**

1.2.2 *Pseudomonas* WCS417

One of the most abundant genera in root microbiomes are *Pseudomonas* spp. (Bulgarelli et al. 2012; Lundberg et al. 2012). The plant-beneficial *Pseudomonas fluorescence* strain WCS417 (hereafter called *Pseudomonas* WCS417 or WCS417) was isolated from wheat roots at the Willy Commelin Scholten Phytopathological Laboratory (Lamers et al. 1988). After genome sequencing, the strain was later renamed *Pseudomonas simiae* WCS417 (Berendsen et al. 2015). Originally found to suppress take-all disease in soil (Lamers et al. 1988), the WCS417 strain has also other well-known PGP traits. *Pseudomonas* WCS417 is known to trigger induced systemic resistance (ISR) in Arabidopsis, and is also found to stimulate shoot fresh weight, inhibit elongation of primary roots, promote lateral roots formation, and root hair development (Zamioudis et al. 2013). These abilities to alter the root system architecture are exploited in this thesis.

1.2.3 *Paenibacillus* (Paene – almost, *Paenibacillus* – almost a *Bacillus*)

Paenibacillus spp. are rod-shaped, aerobic or facultative anaerobic bacteria, that have the ability to form endospores. These are common characteristics they share with *Bacillus subtilis*, and *Paenibacillus* spp. were thus first classified as *Bacillus*. Based on 16S rRNA sequencing, many species of *Bacillus* were later classified in a new family called Paenibacillaceae (Grady et al. 2016). *Paenibacillus* spp. are Gram-positive, but often stain variable or negative. They have peritrichous flagella, and most species are catalase-positive. The colonies are often smooth and translucent, and

colour can be light brown, white, or light pink. The optimal growth temperature is between 28 and 40°C, and at pH 7, but some species are alkaliphilic. Growth of the bacteria is inhibited with 10% NaCl (Vos et al. 2011).

As of 2016 there were around 200 species belonging to the genus *Paenibacillus*, and they have been isolated from very different environments. Many of them have been isolated from soil, in association with plant roots. Some of these are able to promote plant growth, by nitrogen fixation, making phosphorus or iron available to the plant, or producing phytohormones. Others have the capability to act as biocontrol, by triggering ISR (*Paenibacillus polymyxa*, *Paenibacillus alvei*, *Paenibacillus elgii*, and *Paenibacillus lentimorbus* are found to trigger ISR in plants), or producing insecticides or antimicrobial compounds. Their ability to survive for a long time in unfavourable environments, make them of great interest in sustainable agriculture (reviewed by Grady et al. (2016)).

Some *Paenibacillus* species produces antimicrobial peptides that is of interest in medicine and food processing. One example is polymyxins, isolated from *P. polymyxa*, which have been used in, for example, antibiotic creams. Enzymes produced by *Paenibacillus* strains may be of relevance in different process manufacturing, like laundry and dish detergents-, paper-, and food industry, and some species may be used in bioremediation (reviewed by Grady et al. (2016)).

However, not all *Paenibacillus* spp. are beneficial. *Paenibacillus* is known to spoil dairy products, including milk, and some species are pathogenic to other organisms. *Paenibacillus larvae* causes American Foulbrood disease in honeybees, and *Paenibacillus glabratella* causes snail disease. Some *Paenibacillus* species have also been found to be pathogenic to humans, especially to immunocompromised people (reviewed by Grady et al. (2016)).

1.3 *A. thaliana* WT and mutants involved in PP2A signalling.

Protein phosphatase 2A (PP2A) is a group of highly-conserved serine/threonine (Ser/Thr) protein phosphatases in eukaryotes, that is involved in reversible phosphorylation. The phosphatases dephosphorylate proteins by hydrolysing phosphoester bonds, and thus releasing free phosphate (Lillo et al. 2014). PP2A are trimeric holoenzymes, formed by scaffolding subunit A, regulatory subunit B, and catalytic subunit C. In Arabidopsis, there are 3 genes coding for A subunits, 17 genes for subunit B, and 5 for subunit C, giving 255 possible combinations of the PP2A holoenzyme (Lillo et al. 2014). PP2A is involved in plant metabolism, development, stress response and signal transduction (Kataya et al. 2015).

Many factors are involved in the activation of PP2A in Arabidopsis. Among these are three genes that are important regulators of PP2A. These are Phosphotyrosyl Phosphatase Activator (*PTPA*), Leucine Carboxyl Methyl Transferase (*LCMT1*), and Protein phosphatase 2A Methylesterase 1 (*PME-1*).

PTPA is of special interest because this gene is found near a single nucleotide polymorphism (SNP), that is identified as being associated with *Pseudomonas* WCS417 mediated change in lateral root formation (Wintermans et al. 2016). It is suggested that PTPA is a critical regulator of the assembly of PP2A holoenzyme (Chen et al. 2014). In the assembly of the holoenzyme in Arabidopsis, subunits A and C first form a AC dimer. Then the C unit interacts with PTPA. This makes the C unit able to form the trimeric holoenzyme with the B unit, or to be able to be methylated by LCMT1. A methylated C unit has higher activity than an un-methylated C unit. This methylation takes place at the Leu-309 at the carboxyl end of the PP2A subunit C (Chen et al. 2014). PME-1 is conserved from yeast to human, and is found to reverse the methylation of the PP2A C subunit (Ogris et al. 1999; Xing et al. 2008). There is one *PME-1* orthologue in Arabidopsis (At4g10050) (Lillo et al. 2014). Even though there has been no research of PME-1 in Arabidopsis, it is suggested that it has the same function as in yeasts and humans (Lillo et al. 2014).

Two different *A. thaliana* PTPA mutants have been used for this thesis; *ptpa* with 5x higher expression ($ptpa_{ox}$), and *ptpa* that is downregulated 50% by using artificial microRNA (amiRNA) technique ($ptpa_{kd}$). In addition, knockouts of *lcmt1* and *pme1* have been used.

1.4 Tomato

The tomato, *Solanum lycopersicum*, belongs to the genus *Solanum*, which is the largest genus in the Solanaceae family. Containing around 1500 species, it is one of the most diverse plant genus. The genus *Solanum* contains in addition to tomato, potato and eggplant, and is of great economic importance (Weese and Bohs 2007). Tomato is rich with nutrients, and has become one of the most important crop species in the world (Bergougnoux 2014). Tomato is also of great significance in research, as a model organism.

Wild tomato is native to South- and Central America. The tomato was first domesticated in Peru or Mexico, and was imported to Europe in the 16th century. At first it was only used for decoration, as it was thought to be poisonous as some other Solanum species. Still, after being introduced as an edible vegetable, it took a long time before the tomato became domesticated, which started in the 19th century. Since then numerous of cultivars and hybrids have been produced, with the goal to improve agronomical traits. Cultivation of tomato has resulted in large variety in physiology and morphology, but has also led to reduced genetic diversity (Bergougnoux 2014).

After revised phylogenetic classification, the cultivated tomato (*S. lycopersicum*), and 12 wild relatives were placed in the *Lycopersion* section of *Solanum* (Peralta et al. 2008). One of these wild relatives of *S. lycopersicum*, *Solanum pennellii*, was used in this thesis for isolation of endophytic bacteria. *S.*

pennellii, with its green fruit, is native to Andean regions in South America. Due to its dry habitat, it has developed resistance to drought (Bergougnoux 2014).

Two different tomato cultivars have been used for growth experiments in the thesis; Heinz and Moneymaker (MM). *S. lycopersicum* Heinz 1706 was the first tomato to has its genome sequenced (The Tomato Genome Consortium 2012). Moneymaker is an old English cultivar.

Considering 85 % of Norwegian tomatoes are produced in Rogaland (Opplysningskontoret for frukt og grønt), finding improved methods for growing tomatoes are of great importance for the producers here.

1.5 16S rRNA sequencing of bacteria

16S ribosomal ribonucleic acid (16S rRNA) are genes that are evolutionary conserved amongst bacteria (D'Amore et al. 2016). In addition to conserved regions, the 16S rRNA has 9 hypervariable regions, V1-V9 (Van de Peer et al. 1996). These hypervariable regions can be used for the identification of bacteria, and the conserved regions can be used for designing universal primers (Ghyselinck et al. 2013). The universal primers are used for amplification of the hypervariable regions in a polymerase chain reaction (PCR).

1.6 The aim of this project

The aim of the project was to try to identify some of the genes in *A. thaliana* involved in the interaction between the plant and PGPB. Bacteria isolated from tomato roots by a research group at Hasselt University, Belgium (Abbamondi et al. 2016), have been used for *in vitro* root growth assays with *A. thaliana* wild type (WT) plants and 4 different mutant plants ($ptpa_{ox}$, $ptpa_{kd}$, *lcmt1* and *pme1*). A well-known PGPB, *Pseudomonas* WCS417, has also been used for *in vitro* root growth assays with *A. thaliana* WT and $ptpa_{ox}$, and $ptpa_{kd}$ plants.

In addition, 16S rRNA from endophytic bacteria isolated from *S. pennellii*, have been sequenced. One of the isolated bacteria, a *Paenibacillus* sp., has been used for *in vitro* root growth assays, to see if it had any effect on the root system of *A. thaliana*.

Pseudomonas WCS417 and the *Paenibacillus* sp. isolated from *S. pennellii* have been used for experiments with Heinz and Moneymaker tomatoes sown in Vermiculite, to see if *in vitro* experiments can be transferred to "real-life" experiments.

2 Materials and methods

2.1 Plant and bacteria material

2.1.1 *A. thaliana*

A. thaliana wild-type seeds (Col-0, "Spain"), and four *A. thaliana* mutants altered in PP2A signalling (*ptpa*$_{ox}$, *ptpa*$_{kd}$, *lcmt1*, and *pme1*) were used for root growth assays.
Arabidopsis thaliana single mutant T-DNA insertion lines *lcmt-1* (SALK_079466) (Alonso et al. 2003), *pme1* (GK_804C11), and *ptpa* (over-expressor, GABI_606E07) (Kleinboelting et al. 2012) were obtained from the European Arabidopsis Stock Centre in Nottingham, UK. Mutant selections had already been done by others in the lab (Maria Creighton and Amr Kataya (Creighton 2013)).
ptpa$_{kd}$ was downregulated about 50 % by using artificial microRNA (amiRNA) technique (Creighton 2013).
All *A. thaliana* seeds for root growth assays were surface sterilized with 0.1 % Ca-hypochlorite-ethanol-solution with Tween-20, for 4 minutes. The seeds were then washed several times with ethanol, and left to dry in sterile hood.

2.1.2 Tomato

Roots from *S. pennellii* were used for isolation of endophytic bacteria.
S. lycopersicum cultivars Heinz and Moneymaker were used for growth experiment in Vermiculite.

2.1.3 Bacteria

Seven bacteria isolated from roots of tomatoes grown in Italy (table 1.1) were provided from Jaco Vangronsveld's research group at Hasselt University, in Belgium (Abbamondi et al. 2016).
Pseudomonas simiae WCS417 was obtained from Corné M.J. Pieterse at the Centre for BioSystems Genomics, in the Netherlands.

2.2 Isolation and sequencing of endophytic bacteria in *S. pennellii*

2.2.1 Isolation

Roots from the wild tomato species *S. pennellii* were harvested in a 50 ml Falcon tube with 25 ml P-buffer (6.33 g/l $NaH_2PO_4 \cdot H_2O$, 16.5 g/l $Na_2HPO_4 \cdot 7H_2O$, pH 7.4, added 200 µl Tween 20). The roots were washed with P-buffer 5 times before sterilization with 1 % Ca-hypochlorite for 5 min. After sterilization, the roots were washed 5 times with sterile water. The last washing water was kept for

testing of sterility (sterility control). The roots were placed in a sterile mortar, and cut into smaller fragments before adding 5 ml of 10 mM $MgSO_4 \cdot 7H_2O$. A sterile pestle was used to crush the roots to make a crude extract. From the crude extract, a 5-fold and a 10-fold dilution was made. Aliquots of 100 µl of sterility control, crude extract, and the 5- and 10-fold dilutions were streaked on Luria-Bertani (LB) broth with agar (Luria low salt, Sigma). All plates were incubated for one week at 30°C. Since only one colony appeared, more of the crude extract, that had been stored at 4°C, was streaked out on LB medium (Luria low salt, Sigma). The colonies that appeared were streaked out on new LB-plates, and incubated in room-temperature.

2.2.2 Deoxynucleic acid (DNA) extraction

Overnight cultures were made of two of the isolated bacteria colonies, labelled C and 1, and incubated at 30°C/120rpm.

Total DNA was purified from the isolated bacteria using the DNeasy® Blood & Tissue Kit (Qiagen). Protocol for the kit was followed, with minor modifications. In the last step, the samples were eluted with 2 x 50 µl nuclease free water instead of 200 µl AE buffer. The samples were divided and exposed to different pre-treatments for Gram-positive (samples C (G+) and 1 (G+)), and Gram-negative bacteria (samples C (G-) and 1 (G-)).

2.2.3 PCR and gel electrophoresis

A PCR was run to amplify 16S rRNA. Two different primer combinations were used for each sample:
A: Bacteria specific primer 26F (5'-AGAGTTTGATCCTGGCTCAG-3') + universal primer 1520R (5'-AAGGAGGTGATCCAGCCGGA-3')
B: 26F + universal primer 1492R (5'-GGTTACCTTGTTACGACTT-3').
All primers were obtained from Thermo Fischer Scientific (Appendix A1).
The PCR was run according to protocol for Thermo Scientific DreamTaq DNA Polymerase (Thermo Scientific), with the components listed in table 2.1, and the thermal conditions listed in table 2.2.

Table 2.1: Components used to run PCR with Thermo Scientific DreamTaq DNA Polymerase

Sample	C (G+), µl	C (G-), µl	1 (G+), µl	1 (G-), µl	Control, µl
10X DreamTaq Buffer	5.0	5.0	5.0	5.0	5.0
dNTP Mix, 2 mM each	5.0	5.0	5.0	5.0	5.0
Forward primer, 10 µM	2.5	2.5	2.5	2.5	2.5
Reverse primer, 10 µM	2.5	2.5	2.5	2.5	2.5
Template DNA	1.5	5.0	2.0	2.0	0.0
DreamTaq DNA polymerase	0.4	0.4	0.4	0.4	0.4
Water, nuclease-free	33.1	29.6	32.6	32.6	34.6
Total volume	50.0	50.0	50.0	50.0	50.0

Table 2.2: Thermal cycling conditions for PCR with Thermo Scientific DreamTaq Polymerase

Step	T, °C	Time	Number of cycles
Initial denaturation	95	3 min	1
Denaturation	95	30 s	30
Annealing	60	30 s	
Extension	72	1.5 min	
Final Extension	72	10 min	1
End	4	∞	

The PCR products were run on a 1 % agarose gel in 1xTAE (Tris-acetate-EDTA) buffer. PCR product (10 µl) was loaded with GelRed (1.5 µl), and loading buffer (1.5 µl). HyperLadder™ I (Bioline) was used as molecular weight marker (5 µl + 1 µl GelRed).
The gel was run with 90 V for 40 min.

2.2.4 DNA extraction from agarose gel

The gel electrophoresis did not give single bands. To isolate and purify the wanted DNA fragment (16S rRNA) of around 1500 base pair (bp), DNA was extracted from an agarose gel. A new 1 % agarose gel was run with the same conditions as described above, and the bands at 1500 bp was cut out. The extraction was done using the DNA extraction kit NucleoSpin® Gel and PCR clean-up (Macherey-Nagel).

2.2.5 Sequencing of 16S rRNA

The samples of extracted DNA from the agarose gel were premixed with primer (12 µl sample, 3 µl primer), with a concentration of DNA template of 22.5 ng per 100 bases in a volume of 15 µl, as instructed by SeqLab. All samples were sent for sequencing at SeqLab in Göttingen, Germany (Barcode Economy Run Service).

2.3 Root growth assay with bacteria isolated by Abbamondi et al. (2016)

Surface-sterilized *A. thaliana* seeds used in these experiments were WT (Col-0), $ptpa_{ox}$, $ptpa_{kd}$, *lcmt1* and *pme1*.

Bacterial strains used in the experiments were strains 5, 6, 9, 10, 15, 16, and 18 (table 1.1) isolated from tomatoes, grown in Italy, by another research group (Abbamondi et al. 2016). In addition, a bacterial strain labelled CL8, isolated from *S. lycopersicum* cv. Heinz, in Lillo's laboratory, was used for some of the experiments.

Basic medium used for experiments was 1/50 Gamborg medium (Macronutrients: KNO_3 (0.5 mM), $CaCl_2 \cdot 2H_2O$ (0.02 mM), $MgSO_4 \cdot 7H_2O$ (0.02 mM), $(NH_4)_2SO_4$ (0.02 mM), $NaH_2PO_4 \cdot 2H_2O$ (0.02 mM). Micronutrients: KI (90nM), H_3BO_3 (0.97 µM), $MnSO_4 \cdot H_2O$ (1.18 µM), $ZnSO_4 \cdot 7H_2O$ (0.14 µM), $Na_2MoO_4 \cdot 2H_2O$ (20.7 nM), $CuSO_4 \cdot 5H_2O$ (2 nM), $CoCl_2 \cdot 6H_2O$ (2.1 nM), and Fe-EDTA (2.1 µM)). In addition, the medium was added MES (4 mM), and 1 % plant agar (Duchefa Biochemie).

The bacteria suspensions were prepared as described by Abbamondi et al. (2016). Overnight cultures of each strain were made in low salt LB-broth (Luria low salt, Sigma), and incubated at 30°C on shaker (120 rpm), to a wanted optical density at a wavelength of 600 nm (OD_{600}) of about 0.5. The overnight cultures were centrifuged (4000 rpm/20 min), supernatant removed, and pellets washed twice with 1 ml of 10 mM $MgSO_4 \cdot 7H_2O$. After washing, the pellets were resuspended in 650, or 1000 µl of 10 mM $MgSO_4 \cdot 7H_2O$, and evenly distributed on the medium.

The seeds were sown on 1/50 Gamborg medium supplemented with 0.5 % sucrose. After 3 d of stratification in 4 °C, the plates were placed vertically in a growth chamber (16 h light/ 8 h dark, 22°C with a light intensity of 100 µmol m^{-2} s^{-1}). After 6 d in the growth chamber, the seedlings were transferred to 1/50 Gamborg medium without sucrose, that was inoculated with bacteria suspension, or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Plates were put back vertically in the same growth chamber, and all measurements were performed 6, or 7 d after inoculation.

2.4 Root growth assay with *Pseudomonas* WCS417

Three root growth assays have been performed with the PGPB *Pseudomonas* WCS417. Surface-sterilized *A. thaliana* seeds used in these experiments were WT (Col-0), $ptpa_{ox}$, and $ptpa_{kd}$. Basic medium used for experiments was 1xMS medium (table 2.3) (Murashige and Skoog 1962). The medium was added 0.7 % Agar-agar (Merck), and for some experiments supplemented with 0.5 % sucrose.

Table 2.3: MS medium (Murashige and Skoog 1962)

Chemicals for preparing stock solutions for MS-medium, and volumes needed of stock solutions and vitamins to make 1xMS medium.

Stock solutions		**For 1 l 1xMS medium**
A: KNO_3	95 g/l	20 ml
B: NH_4NO_3	120 g/l	13 ml
C: $MgSo_4 \cdot 7H_2O$	37 g/l	10 ml
D: KH_2PO_4	17 g/l	20 ml
E: $CaCl_2 \cdot 2H_2O$	44 g/l	10 ml
Fe/EDTA (1 l):		50 ml
$Na_2 \cdot EDTA$	0.373 g/l	
$FeSO_4 \cdot 7H_2O$	0.278 g/l	
Minor I (1 l):		10 ml
$ZnSo_4 \cdot 4H_2O$	0.920 g/l	
H_3BO_3	0.620 g/l	
$MnSO_4 \cdot 4H_2O$	2.230 g/l	
Minor II (1 l):		10 ml
$Na_2MoO_4 \cdot 2H_2O$	0.025 g/l	
$CuSO_4 \cdot 5H_2O$	0.003 g/l	
$CoCl_2 \cdot 6H_2O$	0.003 g/l	
KI	0.083 g/l	
Vitamins, conc. 1000X (M7150 – Murashige and Skoog vitamin powder, Sigma-Aldrich)		1 ml

For each assay, bacterial suspension of *Pseudomonas* WCS417 was prepared as described by Wintermans et al. (2016). *Pseudomonas* WCS417 was streaked onto King's B medium (Sigma-

Aldrich), supplemented with 50 μg/ml rifampicin (Sigma), and incubated at 28°C/24 h. After incubation, 5 ml of 10 mM $MgSO_4 \cdot 7H_2O$ was added to the Petri dish and left for about 5 min, while occasionally shaking the dish. The bacteria suspension was pipetted off the dish, and an additionally 5 ml of $MgSO_4 \cdot 7H_2O$ was added to the dish. This was also pipetted off, to an end volume of 10 ml of bacteria suspension. The bacterial suspension was added to Eppendorf tubes (1 ml to each), and centrifuged (3200xg/5min). After discarding the supernatant, 1 ml of 10 $MgSO_4 \cdot 7H_2O$ was added to each of the tubes, and centrifuged (3200xg/5 min). This last step was repeated once more. After washing, the pellets were resuspended in 1 ml of 10 mM $MgSO_4 \cdot 7H_2O$. OD_{600} of the bacteria suspensions was adjusted with 10 mM $MgSO_4 \cdot 7H_2O$, before inoculation of the medium.
All seeds for the root growth assays were sown on 1xMS medium supplemented with 0.5 % sucrose. After 2 d of stratification in 4°C, the plates were placed vertically in a growth chamber (16 h light/8 h dark, 22°C, and a light intensity of 100 μmol m^{-2} s^{-1}). After 5 d in the growth chamber, the seedlings were transferred to inoculated 1xMS medium and placed back in the growth chamber. All measurements were performed 8 d after inoculation.

2.4.1 Root growth assay 1 *Pseudomonas* WCS417; bacteria inoculated 5 cm under root tip on medium without sucrose.

For the first root growth assay with *Pseudomonas* WCS417, OD_{600} of the bacteria suspension was adjusted to 0.004 ($2x10^6$ cells/ml). 5 d old *A. thaliana* WT seedlings were transferred to 1xMS medium without sucrose; 5 seedlings per plate, 4 plates for each treatment. A volume of 300 μl of bacteria suspension, or 10 mM $MgSO_4 \cdot 7H_2O$ for control, was inoculated on the plates in spots about 5 cm from the root tips.

2.4.2 Root growth assay 2 *Pseudomonas* WCS417; bacteria suspension spread on medium without sucrose.

For a second assay with *Pseudomonas* WCS417, two different bacteria suspensions were prepared, in which the OD_{600} was adjusted to 0.004 and 0.5 ($2x10^6$ and $2x10^8$ cells/ml, respectively). 1xMS medium without sucrose were inoculated with 450 μl of bacteria suspension, or 10 mM $MgSO_4 \cdot 7H_2O$ for control, spread evenly on the medium. 5 d old *A. thaliana* WT, $ptpa_{ox}$ and $ptpa_{kd}$ seedlings were transferred to these plates; 5 seedlings per plate, 2 plates for each treatment. The control plates were used for both this experiment and an experiment with a *Paenibacillus* sp.

2.4.3 Root growth assay 3 *Pseudomonas* WCS417; bacteria suspension spread on medium supplemented with sucrose.

In the third root growth assay with *Pseudomonas* WCS417, OD_{600} for the bacteria suspension was adjusted to 0.005 ($2.34x10^6$ cells/ml). 1xMS medium supplemented with 0.5 % sucrose was inoculated with 450 µl of bacteria suspension, or 10 mM $MgSO_4 \cdot 7H_2O$ for control, spread evenly on the medium. 5 d old *A. thaliana* WT, $ptpa_{ox}$ and $ptpa_{kd}$ seedlings were transferred to the inoculated medium; 5 seedlings per plate, 4 plates for each treatment.

2.5 Root growth assay with a *Paenibacillus* sp. isolated from *S. pennellii*

Three root growth assays have been performed with a *Paenibacillus* sp., isolated from *S. pennellii*. Surface-sterilized *A. thaliana* seeds used in these experiments were WT (Col-0), $ptpa_{ox}$, and $ptpa_{kd}$. Basic medium used for experiments was 1xMS medium (table 2.3).

For all assays, overnight cultures of the *Paenibacillus* sp. were made by dissolving a colony into LB-broth (Luria low salt, Sigma). The culture was incubated at 30°C/220 rpm.

Eppendorf tubes were added 1 ml of the overnight culture each, and centrifuged (3200xg/5 min). After discarding the supernatant, 1 ml of 10 mM $MgSO_4 \cdot 7H_2O$ was added to each of the tubes, and the tubes were centrifuged (3200xg/5 min). This washing step was repeated one more time. After the last washing, the pellets were resuspended in 1 ml of 10 mM $MgSO_4 \cdot 7H_2O$. The OD_{600} of the bacteria suspension was adjusted with 10 mM $MgSO_4 \cdot 7H_2O$.

All seeds used in the experiments were sown on 1xMS medium supplemented with 0.5% sucrose. After 2 d stratification in 4°C, the plates were placed vertically in a growth chamber (16 h light/8 h dark, 22°C, with a light intensity of 100 µmol m^{-2} s^{-1}). After 5 d in the growth chamber, seedlings were transferred to inoculated medium, and placed back vertically in the growth chamber. All measurements were taken 8 d after inoculation.

2.5.1 Root growth assay 1 *Paenibacillus;* bacteria suspension spread on medium without sucrose.

For the first root growth assay with the *Paenibacillus* sp., OD_{600} of the bacteria suspension was adjusted to 0.6 ($3x10^8$ cells/ml). 1xMS medium without sucrose was inoculated with 450 µl of the bacteria suspension, or 10 mM $MgSO_4 \cdot 7H_2O$ for control, spread evenly on the medium. 5 d old *A. thaliana* WT, $ptpa_{ox}$ and $ptpa_{kd}$ seedlings were transferred to the inoculated medium; 5 seedlings per plate, 2 plates for each treatment. Control plates were used for both this experiment and an experiment with *Pseudomonas* WCS417.

2.5.2 Root growth assay 2 *Paenibacillus*; bacteria suspension spread on medium supplemented with sucrose.

In the second root growth assay with the *Paenibacillus* sp., OD_{600} of the bacterial suspension was adjusted to 0.3 ($1.5x10^8$ cells/ml). 1xMS medium supplemented with 0.5 % sucrose was inoculated with 450 µl of the bacteria suspension, or 10 mM $MgSO_4 \cdot 7H_2O$ for control, spread evenly on the medium. 5 d old *A. thaliana* WT seedlings were transferred to the inoculated medium; 5 seedlings per plate, 4 plates for each treatment.

2.5.3 Root growth assay 3 *Paenibacillus*; bacteria suspension spread on medium supplemented with sucrose.

For the third root growth assay with the *Paenibacillus* sp., OD_{600} of the bacteria suspension was adjusted to 0.3 ($1.5x10^8$ cells/ml). 1xMS medium supplemented with 0.5 % sucrose was inoculated with 450 µl of the bacteria suspension, or 10 mM $MgSO_4 \cdot 7H_2O$ for control; spread evenly on the medium. 5 d old *A. thaliana* WT, $ptpa_{ox}$, and $ptpa_{kd}$ seedlings were transferred to the inoculated medium; 5 seedlings per plate, 4 plates for each treatment.

2.6 Growth experiment with Heinz and Moneymaker tomatoes inoculated with *Pseudomonas* WCS417, or a *Paenibacillus* sp.

Pots with Agra-vermiculite were added 250 ml 1/5xSuperba™ (Felleskjøpet, Rogaland). After 3 h of soaking, 2-week-old seedlings of Heinz and Moneymaker tomatoes, sown on 1/2xMS medium, were planted in the pots, 12 pots for each type. The plants were placed in a plant room with 24 h light, under plastic, which was removed after 3 d. After a week, the pots were added 150 ml bacteria suspension of *Pseudomonas* WCS417 (OD = 0.027, $1.37x10^7$ cells/ml), *Paenibacillus* (OD = 0.026, $1.32x10^7$ cells/ml), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. The bacteria suspensions were prepared as described in sections 2.3 and 2.4. After inoculation, the plants were not given any nutrients solution, only water. Four weeks after inoculation, the stems were measured with a ruler, and the shoots were cut off and weighed.

3 Results

3.1 Isolation and sequencing of endophytic bacteria from *S. pennellii*

3.1.1 Isolation

Endophytic bacteria were isolated from the roots of *S. pennellii*. After 2 d of incubation on LB agar at 30°C, one yellow colony was found on the plate with crude extract. Since no other colonies formed after 1 week of incubation, 200 µl aliquots of the crude extract, that had been stored at 4 °C were streaked on 2 new plates. On these plates, a total of 3 new colonies appeared. The four bacteria colonies were streaked onto new plates, incubated in room temperature, and then stored at 4°C.

3.1.2 DNA extraction

Two of the bacteria colonies, isolated from *S. pennellii*, was selected for sequencing. DNA was extracted from bacteria colonies labelled C and 1. Bacteria C is the first yellow colony that appeared from the crude extract, while bacteria 1 is one of the bacteria that appeared after streaking the crude extract on new plates. Bacteria 1 were white/translucent of colour. As the identity was not known, the samples were divided and exposed to both Gram-positive (G+) and Gram-negative (G-) pre-treatment before extraction. After DNA extraction, the concentration and purity of the extracts were measured with NanoDrop One (table 3.1)

Table 3.1: Measurements of concentration and purity of DNA, extracted from two different bacteria isolated from roots of *S. pennellii*, measured with NanoDrop One.

Sample	ng/µl	A260/A280	A260/A230
C (G+)	34.5	1.86	1.08
1 (G+)	20.2	1.74	0.84
C (G-)	6.2	1.56	0.87
1 (G-)	15.1	1.81	1.22

G+: Samples exposed to Gram-positive pre-treatment.

G-: Samples exposed to Gram-negative pre-treatment.

3.1.3 PCR and gel electrophoresis

16S rRNA was amplified by running a PCR (Thermo Scientific DreamTaq DNA Polymerase), with two different primer combinations; A and B. To check if the amplification was successful, the PCR products were run on a 1 % agarose gel (fig. 3.1 and 3.2).

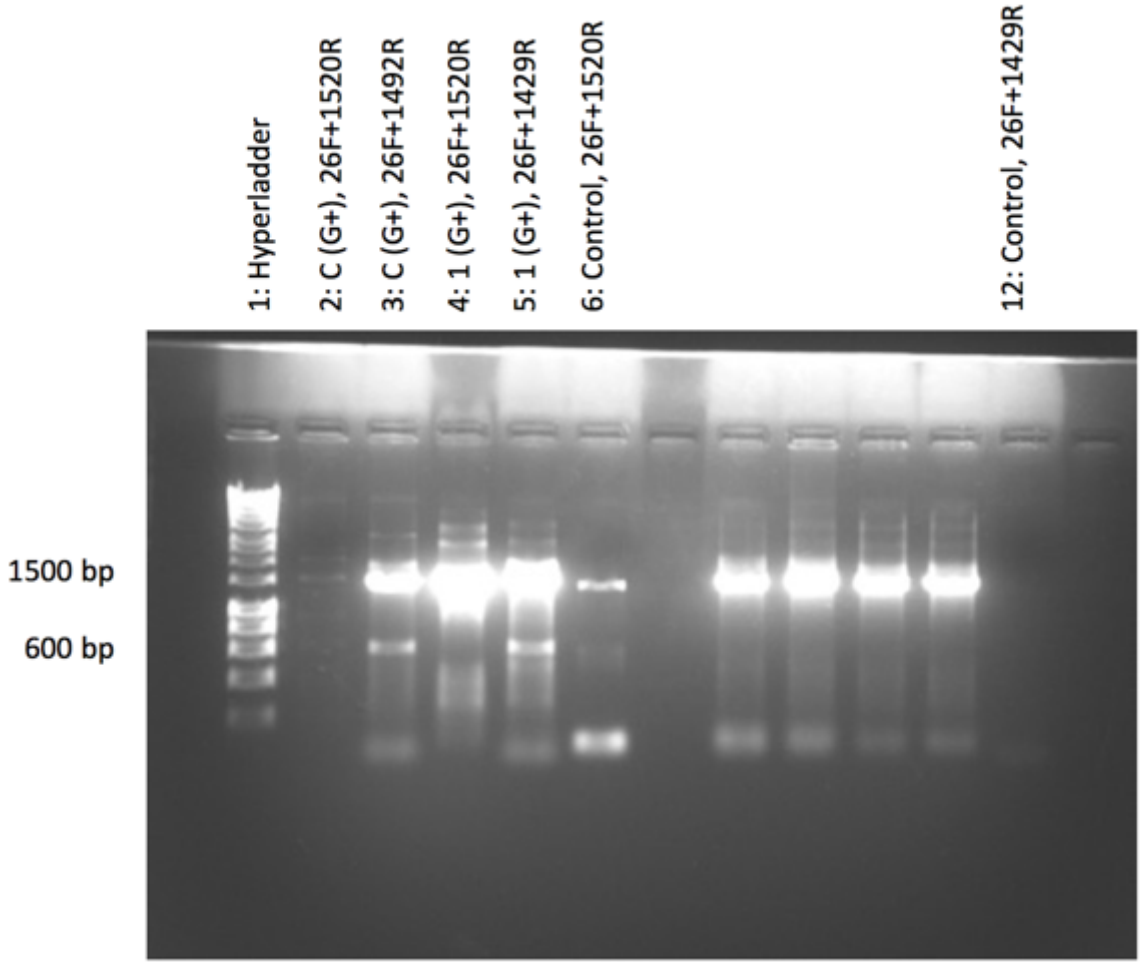

Figure 3.1: Image of 1 % agarose gel of PCR product of amplified 16S rRNA, G+ pre-treatment.

Amplification of the 16S rRNA from two different bacteria colonies, labelled C and 1, isolated from S. pennellii. DNA extracted using DNeasy® Blood and Tissue Kit, bacteria exposed to gram-positive pre-treatment before DNA extraction. The controls are not added any DNA. The lanes are loaded as follows: 1. HyperLadder I™. 2: C (G+) + A. 3: C (G+) + B. 4: 1 (G+) + A. 5: 1 (G+) + B. 6: Control + A. 12: Control + B. A and B is different primer combinations; A: 26F + 1520R, B: 26F + 1492R. Lanes 8-11 are samples from another student, lane 7 is empty.

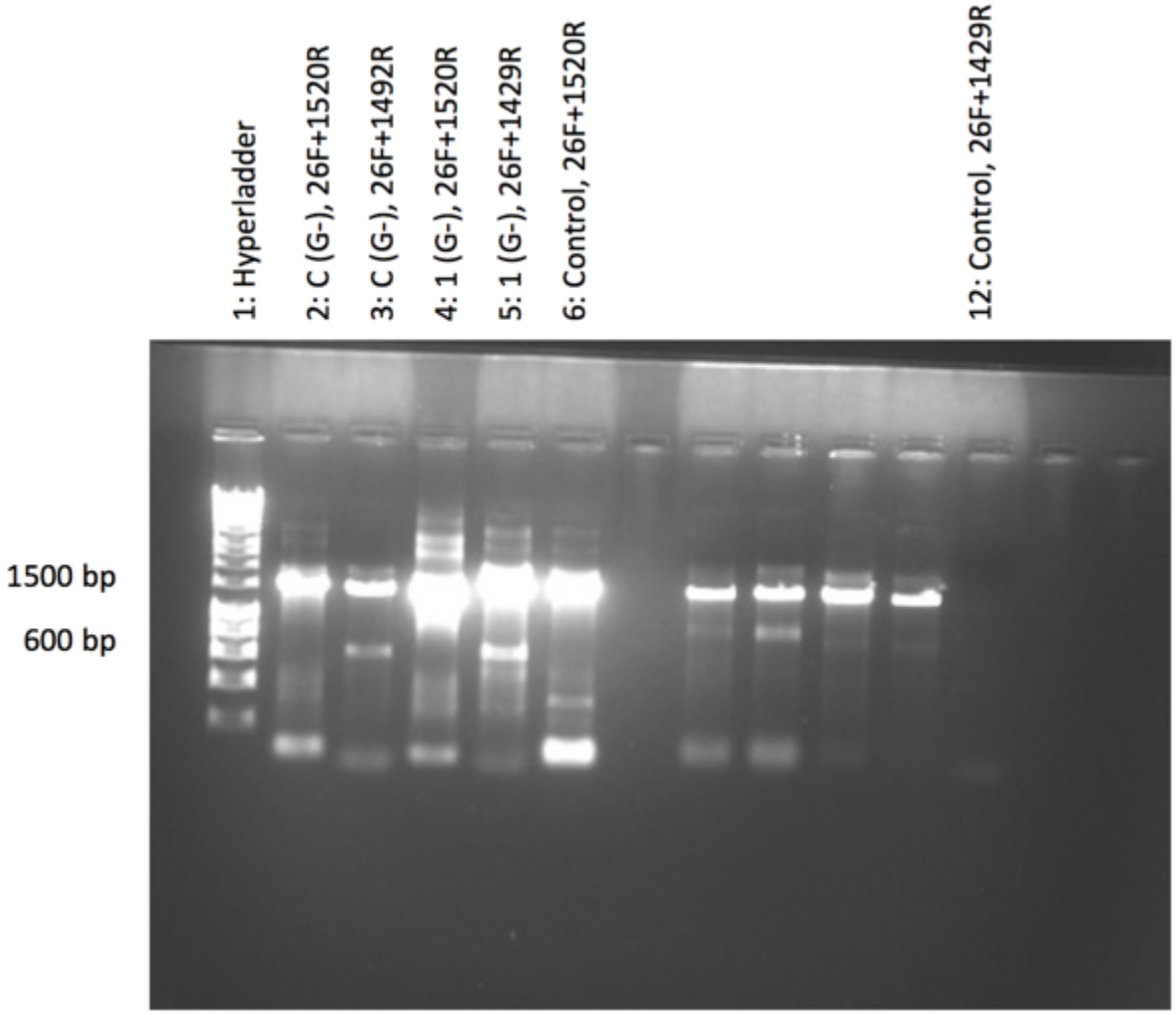

Figure 3.2: Image of 1% agarose gel of PCR product of amplified 16S rRNA, G- pre-treatment.

Amplification of the 16S rDNA from two different bacteria colonies, labelled C and 1, isolated from S. pennellii. DNA extracted using DNeasy® Blood and Tissue Kit, bacteria exposed to gram-negative pre-treatment before DNA extraction. The controls are not added any DNA. The lanes are loaded as follows: 1. HyperLadder I™. 2: C (G-) + A. 3: C (G-) + B. 4: 1 (G-) + A. 5: 1 (G-) + B. 6: Control + A. 12: Control + B. A and B is different primer combinations; A: 26F + 1520R, B: 26F + 1492R. Lanes 8-11 are samples from another student, lane 7 is empty.

All samples, except sample C (G+) + A (lane 2, fig. 3.1) have strong bands at around 1500 base pairs (bp) (fig. 3.1 and 3.2). As 16S rRNA is about 1500 bp, the amplification seems to be successful. However, in both gels, there are some bands in the lane with primer A that should not be there (lane 6, fig. 3.1 and 3.2). This might be a contamination from the previous well, or that the well itself is contaminated, or the stock solution prepared of 1520R could have become contaminated. The amplification with the reverse primer 1429R, gave a by-product of about 600 bp, and the PCR products were cleaned before sending for sequencing.

3.1.4 DNA extraction from agarose gel

After running the PCR product on a 1 % agarose gel, the bands of 1500 bp were cut out, and DNA extracted from the gel. The concentrations and purity of the extracts were measured with NanoDrop One (table 3.2).

Table 3.2: Concentration and purity of DNA extracted from agarose gel, measured with NanoDrop One.

Sample	ng/µl	A260/A280	A260/A230
C (G+), B	38.2	1.86	1.68
1 (G+), A	125.6	1.83	2.04
1 (G+), B	49.1	1.86	1.69
C (G-), A	34.6	1.90	0.94
C (G-), B	23.2	1.85	0.75
1 (G-), A	81.5	1.87	1.69
1 (G-), B	66.8	1.86	1.70

A: Amplification with primer combination 26F + 1520R

B: Amplification with primer combination 26F + 1429R

3.1.5 Sequencing of 16S rRNA

The concentrations measured with NanoDrop One (table 3.2), were used to calculate the volume needed for the acquired concentration of DNA template of 22.5 ng per 100 bases in a volume of 15 µl, as instructed by SeqLab. The samples were premixed with primer (3 µl). All samples were sequenced at SeqLab in Göttingen, Germany. Since the amplification of sample C (G+) + A (lane 2, fig. 3.1) was not successful, this sample was not sent for sequencing.

The nucleotide sequences received from SeqLab, were compared to reference strains in the National Centre for Biotechnology Information (NCBI) database with the help of the BLASTn program (Appendix A2, only sequences of samples exposed to G+ pre-treatment is shown, as results indicated both bacteria are gram-positive.)

For the bacterium labelled 1, the sequencing result for forward and reverse primers, all had highest score for a partial sequence of *Paenibacillus typhae* strain xj7 (Kong et al. 2013), with an identify of 99 % (Appendix A2, fig. A.1-A.3). All the sequences were around 900 bp, and the sum of forward and reverse exceeded the around 1500 bp of 16S rRNA for all samples. To confirm that there is an overlap in the middle of the forward and reverse sequences, local alignments of the forward and reverse

sequences were performed with Emboss Water, that uses the Smith-Waterman algorithm (appendix A3, fig. A.6 and A.8). The BLASTn program (NCBI) was used to compare the sequences with reference strains (appendix A3, fig. A.7 and A.9). The four first reference sequences, *Paenibacillus salinicaeni* strain LAM0A28, *Paenibacillus typhae* strain xj7, *Paenibacillus jilunlii* strain Be17, and *Paenibacillus wynnii* strain LMG 22176 (all partial sequences), had all equal scores, and an identity of 99%. This confirms that bacterium 1 is a *Paenibacillus* sp., and of the obtained reference strains, it has the highest identity with the *P. typhae* strain.

For bacterium labelled C, the sequencing result for forward and reverse primer had highest score for different strains of *Micrococcus*. The forward primer (26F) had the highest score for two *Micrococcus luteus* strains, while the reverse primer (1429R) had highest score for *Micrococcus yunannenensis* strain YIM 65004 (Appendix A2, fig. A.4 and A.5). A local alignment was done for the forward and reverse sequences (Appendix A3, fig. A.10), and the obtained sequence compared to reference strains in the NCBI database (Appendix A3, fig A.11). All the three *Micrococcus* strains mentioned above were among the reference strains with highest scores, but it was not possible to determine the exact strain for bacterium C.

After a quick literature search of the two bacteria, the *Paenibacillus* sp. was considered to be of greatest interest, and it was decided to use this for some root growth assays, to see if this isolated bacterium has some PGP traits.

3.2 Root growth assay with bacteria isolated by Abbamondi et al. (2016)

3.2.1 Root growth assay with *A. thaliana* WT and all bacterial strains

A. thaliana WT seeds were sown on 1/50 Gamborg medium supplemented with 0.5 % sucrose. After 3 days of stratification in 4°C, the plates were placed vertically in a growth chamber (16 h light/8 h dark). After 5 d in the growth chamber, the seedlings were transferred to 1/50 Gamborg medium without sucrose, inoculated with bacterial suspension ($OD_{600} \approx 0.5$), or 10 mM MgSO4·7H2O for control. Two different assays have been performed, one where plates were inoculated with 1 ml (assay 1) of bacterial suspension, and one with 650 µl (assay 2). In both assays, there were 1 plate with 5 seedlings for each bacterium, and one 1 plate for control. Plates were put back vertically in the growth chamber after inoculation.

After 7 d (assay 1), and 6 d (assay 2), the plates were taken out of the growth chamber and photographed (fig. 3.3 and 3.4). Images of the root tips were taken using a Leica microscope (fig. 3.5 and 3.6). Primary roots were measured using ImageJ, and lateral roots were visually counted (table

3.3). Data in table 3.3 were used to make graphical illustrations (fig. 3.7 and 3.8). An unpaired student's t-test was performed with a t-test calculator (GraphPad QuickCalcs Web Site), to see if there were any significant differences between the control plants, and plants inoculated with bacteria. Table 3.3 includes the p-values from this test.

Figure 3.3: Pictures of *A. thaliana* WT plants (assay 1), 7 d after inoculation with different bacterial strains.

A. thaliana WT plants, 7 d after inoculation with 1 ml suspension of different bacterial strains ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: WT control. B: WT + bacterial strain 5. C: WT + bacterial strain 6. D: WT + bacterial strain 9. E: WT + bacterial strain 10. F: WT + bacterial strain 15. G: WT + bacterial strain 16. H: WT + bacterial strain 18. I: WT + bacterial strain CL8.

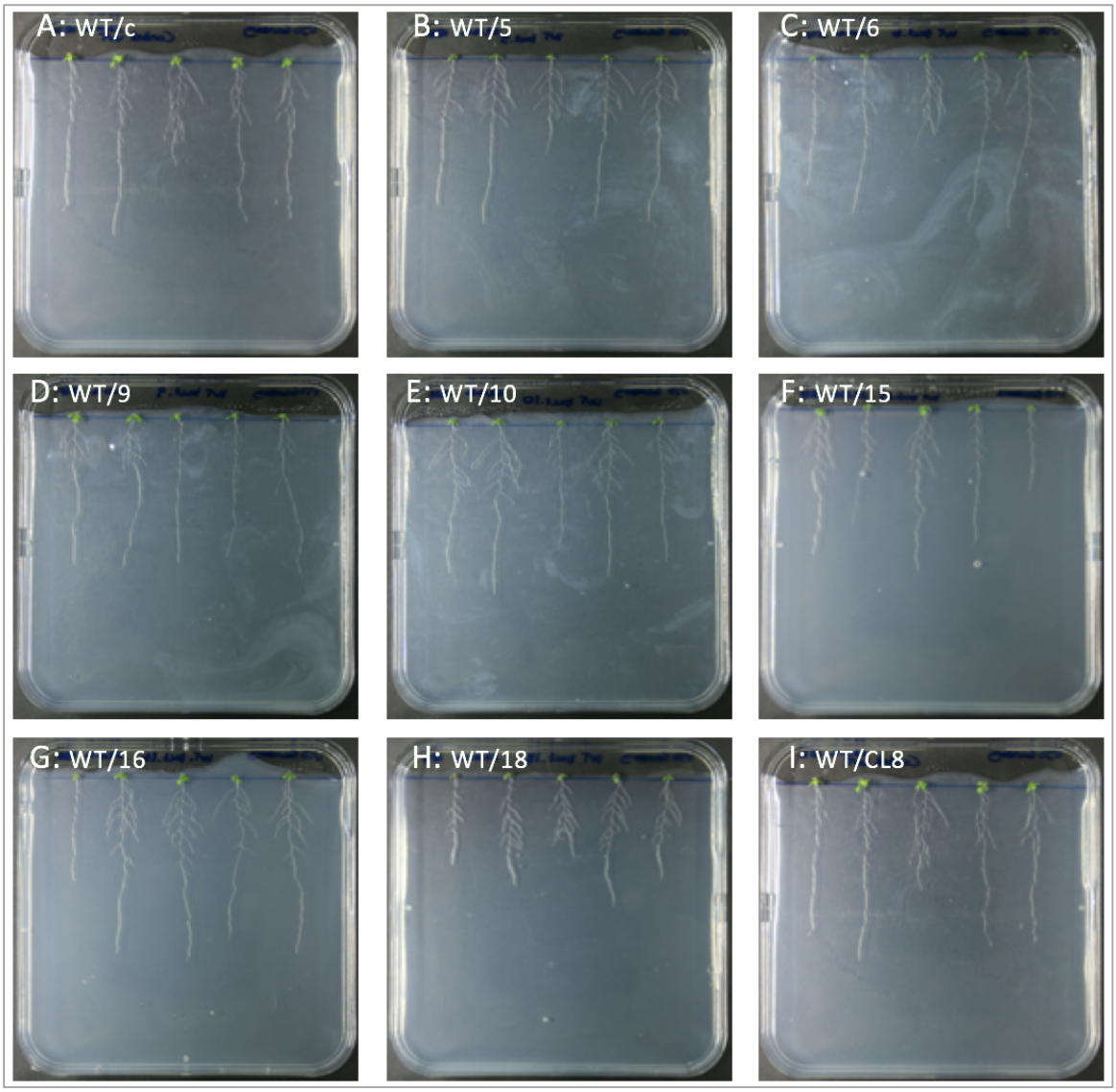

Figure 3.4 Pictures of *A. thaliana* WT plants (assay 2), 6 d after inoculation with different bacterial strains.

A. thaliana WT plants, 6 d after inoculation with 650 µl suspension of different bacterial strains ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: WT control. B: WT + bacterial strain 5. C: WT + bacterial strain 6. D: WT + bacterial strain 9. E: WT + bacterial strain 10. F: WT + bacterial strain 15. G: WT + bacterial strain 16. H: WT + bacterial strain 18. I: WT + bacterial strain CL8.

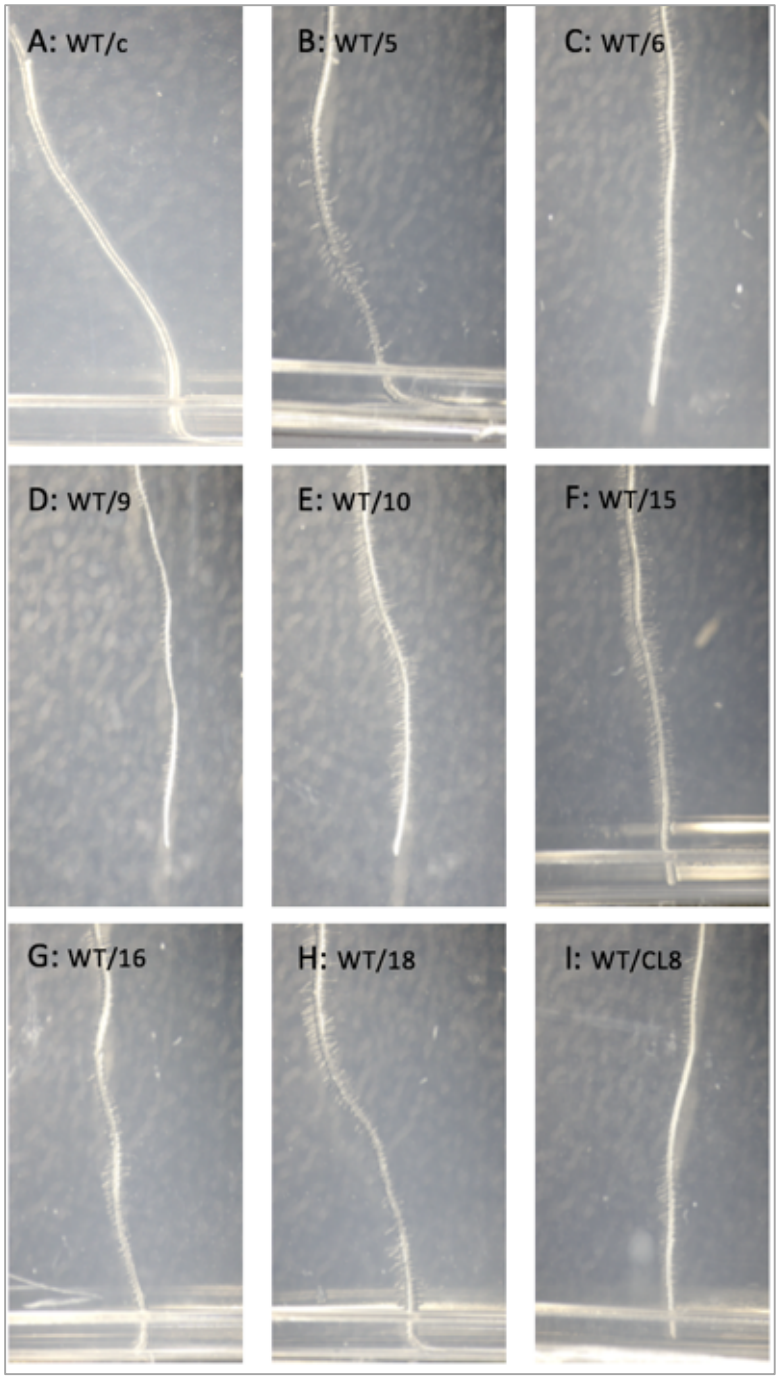

Figure 3.5: Representative images of root tips of *A. thaliana* WT plants (assay 1), 7 d after inoculation with different bacterial strains.

A. thaliana WT root tips, 7 d after inoculation with 1 ml suspension of different bacterial strains ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: WT control. B: WT + bacterial strain 5. C: WT + bacterial strain 6. D: WT + bacterial strain 9. E: WT + bacterial strain 10. F: WT + bacterial strain 15. G: WT + bacterial strain 16. H: WT + bacterial strain 18. I: WT + bacterial strain CL8. Images were taken with a Leica microscope.

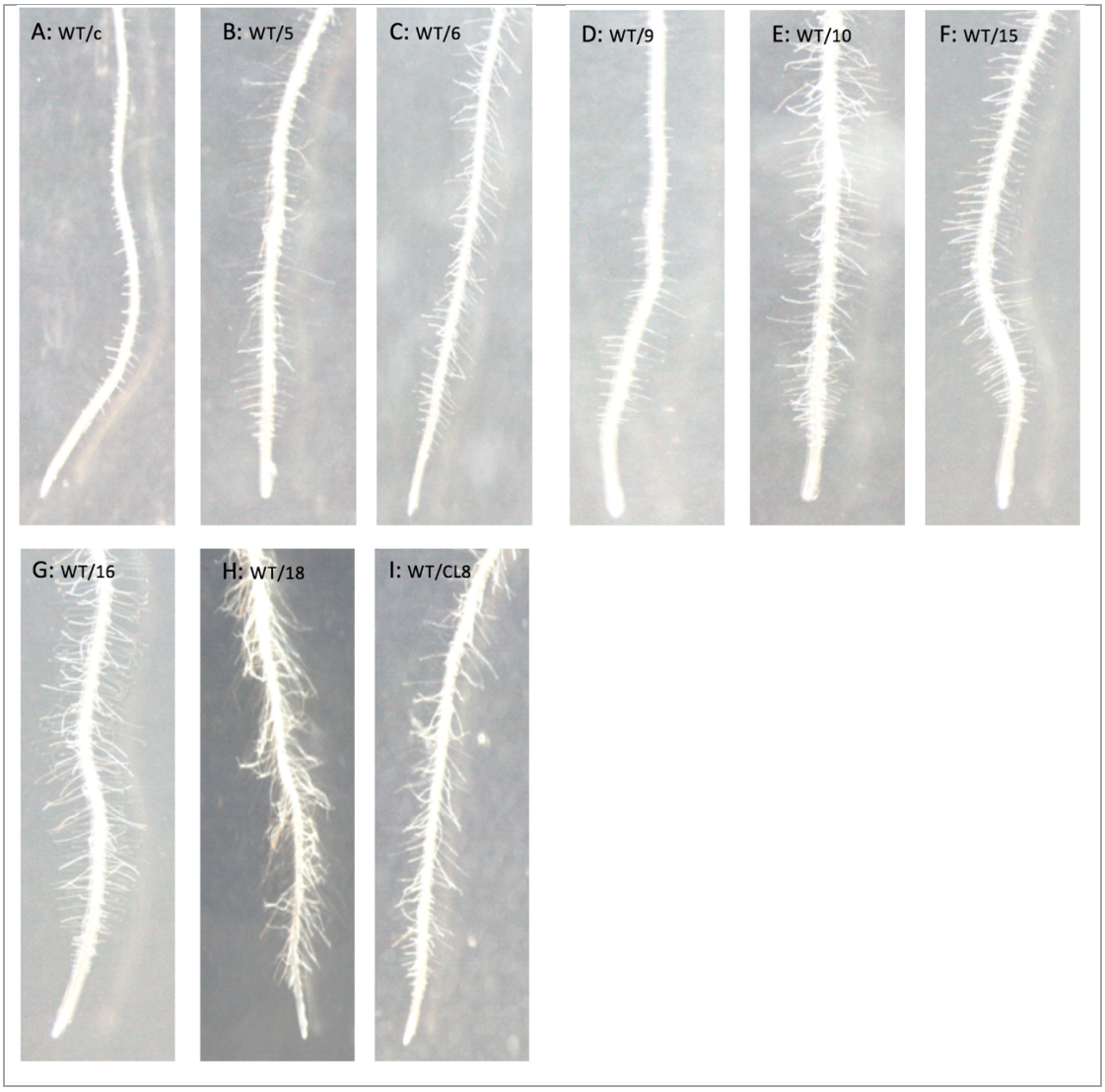

Figure 3.6 Representative images of root tips of *A. thaliana* WT plants (assay 2), 6 d after inoculation with different bacterial strains.

A. thaliana WT root tips, 6 d after inoculation with 650 µl suspension of different bacterial strains ($OD_{600} \approx 0.5$), or 10 mM MgSO4·7H2O for control. A: WT control. B: WT + bacterial strain 5. C: WT + bacterial strain 6. D: WT + bacterial strain 9. E: WT + bacterial strain 10. F: WT + bacterial strain 15. G: WT + bacterial strain 16. H: WT + bacterial strain 18. I: WT + bacterial strain CL8. Images were taken with a Leica microscope.

Table 3.3 Data for root growth assay performed with *A. thaliana* WT plants and different bacterial strains.

Mean length of primary root, and mean numbers of lateral roots for A. thaliana WT plants, 7 d (assay 1), or 6 d (assay 2) after inoculation of different bacterial suspensions ($OD_{600} \approx 0.5$, 1 ml for assay 1, 650 µl for assay 2), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, with corresponding standard deviations (SD). N is number of plants for calculations. An unpaired Student's t-test has been performed to find the p-values.

Type of plants and treatment	Mean length of primary root, cm	SD, primary root	Mean numbers of lateral roots	SD, lateral roots	N	p-values, primary root (compared to control	p-values, lateral roots (compared to control)
WT control (1)	8.34	0.24	19.50	2.69	4		
WT control (2)	5.92	0.34	9.75	1.48	4		
WT bact. 5 (1)	8.98	0.53	20.80	3.66	5	0.0622	0.5732
WT bact. 5 (2)	5.94	0.26	10.25	1.48	4	0.9286	0.6497
WT bact. 6 (1)	7.72	0.54	13.20	2.04	5	0.0725	0.0051*
WT bact. 6 (2)	5.74	0.65	9.50	2.29	4	0.6410	0.8605
WT bact. 9 (1)	8.60	0.58	17.50	4.82	4	0.4391	0.4959
WT bact. 9 (2)	5.64	0.33	8.60	1.36	5	0.2520	0.2643
WT bact. 10 (1)	7.67	0.70	12.40	3.50	5	0.1133	0.0126*
WT bact. 10 (2)	5.87	0.47	10.25	2.38	4	0.8688	0.7334
WT bact. 15 (1)	8.82	0.17	18.80	1.72	5	0.0097*	0.6481
WT bact. 15 (2)	5.23	0.59	11.75	1.79	4	0.0891	0.1358
WT bact. 16 (1)	9.05	0.32	20.00	3.08	4	0.0121*	0.8150
WT bact. 16 (2)	6.40	0.31	12.50	1.80	4	0.0820	0.0563
WT bact. 18 (1)	8.67	0.21	23.00	1.87	4	0.0839	0.0765
WT bact. 18 (2)	3.89	0.63	13.40	2.42	5	0.0007*	0.0340*
WT bact. CL8 (1)	8.12	0.52	17.40	4.96	5	0.4639	0.4745
WT bact. CL8 (2)	5.50	0.48	11.40	2.65	5	0.1848	0.3055

* Statistically significant compared to control, $p < 0.05$.

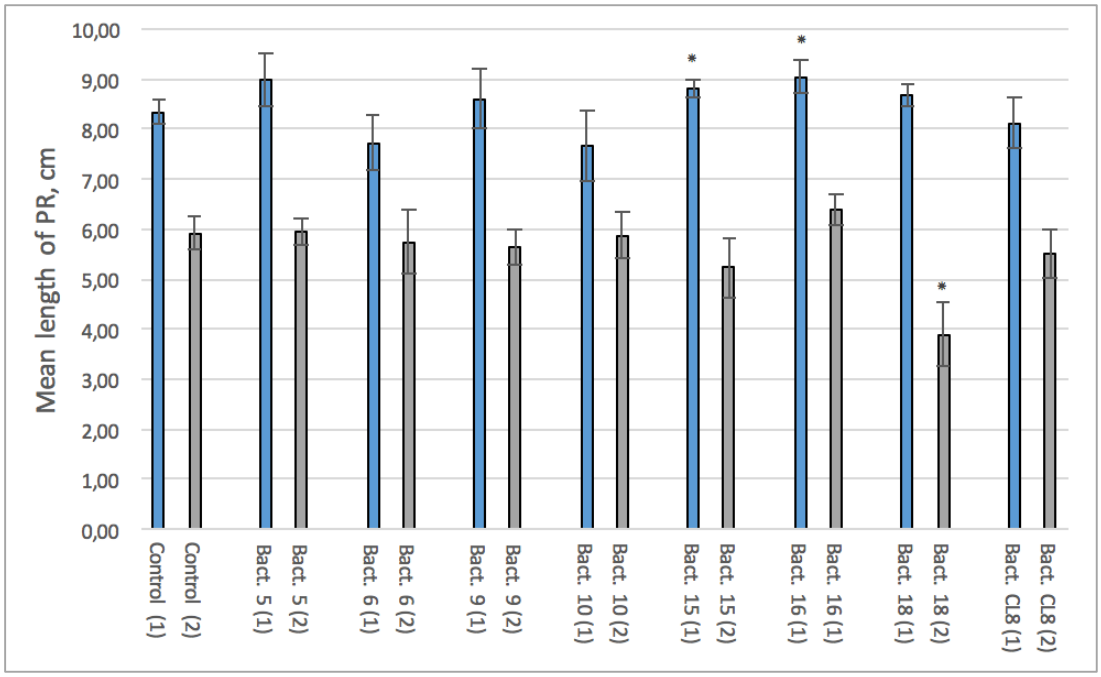

Figure 3.7: Primary root results from root growth assay performed with *A. thaliana* WT plants, and different bacterial strains.

Mean primary root length of A. thaliana WT plants, 7 d (assay 1), or 6 d (assay 2) after transfer to 1/50 Gamborg medium (-suc), inoculated with 1 ml (assay 1) or 650 µl (assay 2) of different bacterial suspensions ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Length of primary roots were measured using ImageJ. Bars show mean of 4-5 plants ± SD, plants grown much shorter than others on the same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

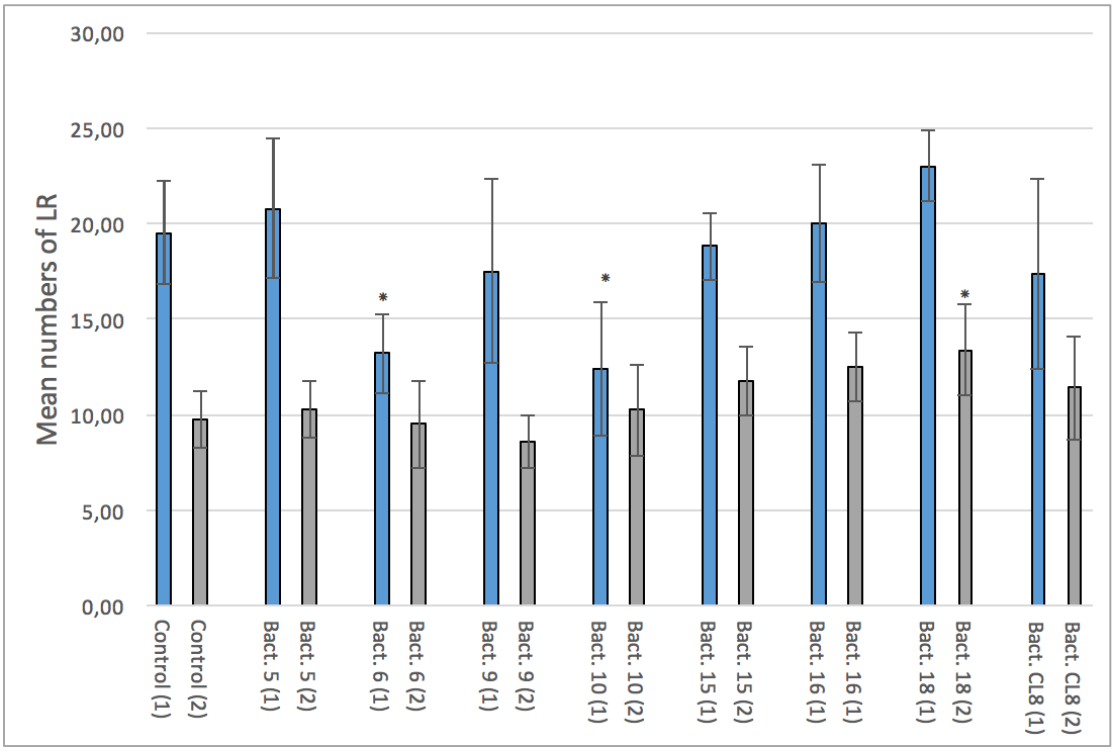

Figure 3.8: Lateral roots results from root growth assay performed with *A. thaliana* WT plants, and different bacterial strains.

Mean numbers of lateral roots on A. thaliana WT plants, 7 d (assay 1), or 6 d (assay 2) after transfer to 1/50 Gamborg medium (-suc) inoculated with 1 ml (assay 1), or 650 µl (assay 2) of different bacterial suspensions ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Lateral roots were counted visually. Bars show mean of 4-5 plants ± SD, plants grown much shorter than others on the same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

In the first assay, none of the tested bacteria significantly inhibited the growth of primary roots on the *A. thaliana* WT plants. Plants inoculated with bacterial strains 15 and 16 had significant longer primary roots, compared to the control (p values = 0.0097 and 0.0121, respectively, table 3.3 and fig. 3.7), unlike earlier results obtained by others, showing significant shorter primary roots (Abbamondi et al. 2016). As for lateral roots, none of the bacteria significantly promoted development of more lateral roots for the plants in assay 1, compared to the control (table 3.3, fig. 3.8). However, inoculation with bacteria 6 and 10 gave significant less lateral roots compared to control (p values = 0.0051 and 0.0126, respectively, table 3.3, fig. 3.8), which is in accordance with Abbamondi et al. (2016).

In the second assay, only one of the bacteria, strain 18, gave significant difference in primary roots on the plants compared to the control, with shorter roots (p value = 0.0007, table 3.3, fig. 3.7). This strain also significantly promoted growth of more lateral roots, compared to the control (p value = 0.0340, fig. 3.8). The testing of this strain by Abbamondi et al (2016), also showed that inoculation

with this bacterium gave shorter primary roots on *A. thaliana* WT plants, compared to control, but the plants had less lateral roots, though none of these results were quite significant. All bacterial strains promoted the formation of root hairs on *A. thaliana* (fig. 3.5 and 3.6), which were also the results for Abbamondi et al. (2016).

Only 5 plants for each treatment were tested, and some of the plants were not taken into calculations because they had grown much shorter than the others on the same plate (fig. 3.3 and 3.4), thus sample sizes were small. In addition, some of the plates were contaminated by an unknown source, which may have affected the results. The results were variable in these two assays, and none of the bacteria tested stood out as having a very positive effect on the root system. Bacterial strain 18 did significant inhibit the primary root, and increase the numbers of lateral roots in the second assay, but this was not seen in the first assay. However, after discussions with supervisor and other students working with the same bacteria, it was decided to use strains 9, 15 and CL8 for further experiments.

3.2.2 Root growth assay with *A. thaliana* WT, *lcmt1*, *pme1*, $ptpa_{ox}$, and $ptpa_{kd}$, with bacterial strain 9, 15, and CL8

A. thaliana WT, *lcmt1*, *pme1*, $ptpa_{ox}$, and $ptpa_{kd}$ seeds were sown on 1/50 Gamborg medium supplemented with 0.5 % sucrose. After 3 days of stratification in 4°C, the plates were placed vertically in a growth chamber (16 h light/8 h dark). After 5 d in the growth chamber, the seedlings were transferred to 1/50 Gamborg medium without sucrose, that were inoculated with 650 μl of suspension of bacterial strain 9, 15, CL8 ($OD_{600} \approx 0.5$), or 10 mM MgSO4·7H2O for control; 5 seedlings per plate, 1 plate for each treatment. The plates were placed vertically back in the growth chamber after inoculation.

After 6 d, the plates were taken out and photographed (fig. 3.9). Images of the root tips were taken using a Leica microscope (fig. 3.10). Primary roots were measured using ImageJ, and lateral roots were visually counted (table 3.4). Data in table 3.4 were used to make graphical illustrations (fig. 3.11 and 3.12). An unpaired student's t-test was performed with a t-test calculator (GraphPad QuickCalcs Web Site), to see if there were any significant differences between the control plants, and plants inoculated with bacteria. Table 3.4 includes the p-values from this test.

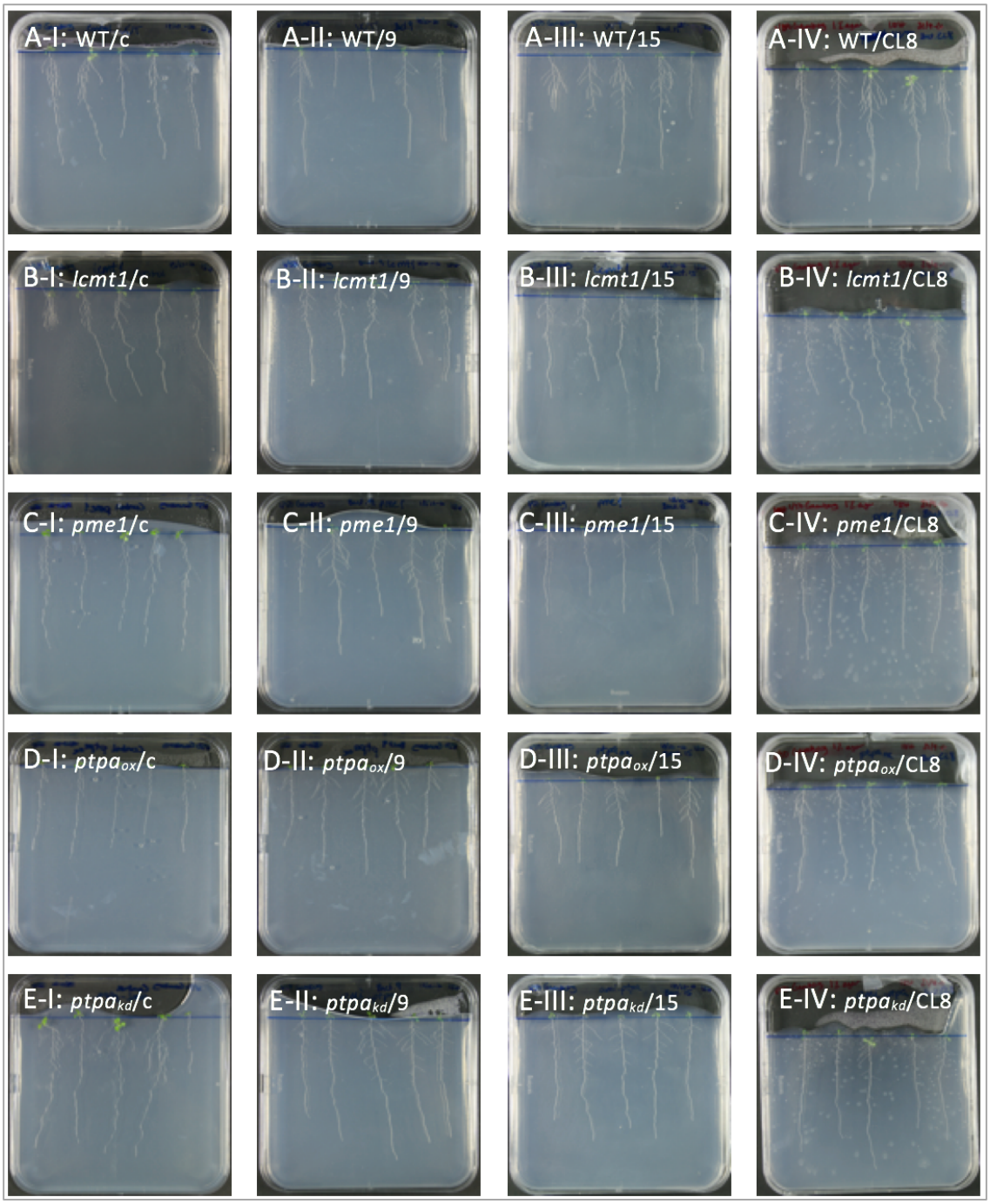

Figure 3.9: Pictures of plants from root growth assay with *A. thaliana* WT, *lcmt1*, *pme1*, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, 6 d after inoculation with bacterial strain 9, 15 or CL8.

Pictures of A. thaliana WT, lcmt1, pme1, ptpa$_{ox}$, and ptpa$_{kd}$ plants, 6 d after inoculation with 650 µl suspension of bacterial strain 9, 15, CL8 (OD$_{600}$ ≈ 0.5), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A-I: WT control. A-II: WT + bacterial strain 9. A-III: WT + bacterial strain 15. A-IV: WT + bacterial strain CL8. B-I: lcmt1 control. B-II: lcmt1 + bacterial strain 9. B-III: lcmt1 + bacterial strain 15. B-IV: lcmt1 + bacterial strain CL8. C-I: pme1 control. C-II: pme1 + bacterial strain 9. C-III: pme1 + bacterial strain 15. C-IV: pme1 + bacterial strain CL8. D-I: ptpa$_{ox}$ control. D-II: ptpa$_{ox}$ + bacterial strain 9. D-III: ptpa$_{ox}$ + bacterial strain 15. D-IV: ptpa$_{ox}$ + bacterial strain CL8. E-I: ptpa$_{kd}$ control. E-II: ptpa$_{kd}$ + bacterial strain 9. E-III: ptpa$_{kd}$ + bacterial strain 15. E-IV: ptpa$_{kd}$ + bacterial strain CL8.

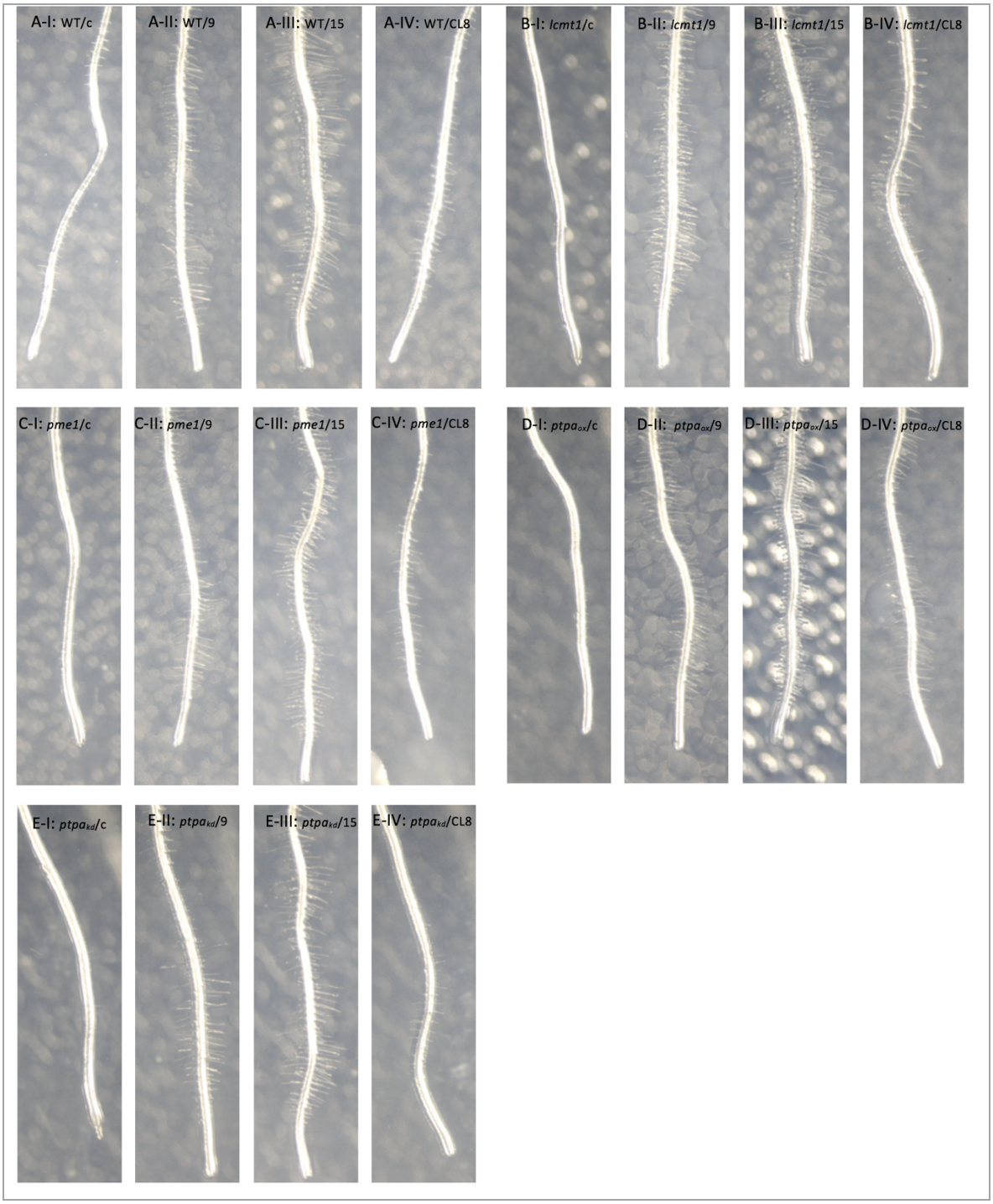

Figure 3.10: Representative images of root tips of *A. thaliana* WT, *lcmt1*, *pme1*, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, 6 d after inoculation with bacterial strain 9, 15, or CL8.

A. thaliana WT, lcmt1, pme1, ptpa$_{ox}$, and ptpa$_{kd}$ plants 6 d after inoculation with 650 µl suspension of bacterial strain 9, 15, CL8 (OD_{600} ≈ 0.5), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A-I: WT control. A-II: WT + bacterial strain 9. A-III: WT + bacterial strain 15. A-IV: WT + bacterial strain CL8. B-I: lcmt1 control. B-II: lcmt1 + bacterial strain 9. B-III: lcmt1 + bacterial strain 15. B-IV: lcmt1 + bacterial strain CL8. C-I: pme1 control. C-II: pme1 + bacterial strain 9. C-III: pme1 + bacterial strain 15. C-IV: pme1 + bacterial strain CL8. D-I: ptpa$_{ox}$ control. D-II: ptpa$_{ox}$ + bacterial strain 9. D-III: ptpa$_{ox}$ + bacterial strain 15. D-IV: ptpa$_{ox}$ + bacterial strain CL8. E-I: ptpa$_{kd}$ control. E-II: ptpa$_{kd}$ + bacterial strain 9. E-III: ptpa$_{kd}$ + bacterial strain 15. E-IV: ptpa$_{kd}$ + bacterial strain CL8. Images were taken with a Leica microscope.

Table 3.4: Data for root growth assay performed with *A. thaliana* WT, *lcmt1*, *pme1*, $ptpa_{ox}$, and $ptpa_{kd}$ plants, and bacterial strain 9, 15, and CL8.

Mean length of primary root, and mean numbers of lateral roots for A. thaliana WT, lcmt1, pme1, $ptpa_{ox}$, and $ptpa_{kd}$ plants, 6 d after inoculation with 650 µl bacterial suspension of bacterial strain 9, 15, CL8 ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, with corresponding standard deviations (SD). N is number of plants for calculations. An unpaired Student's t-test has been performed to find the p-values.

Type of plants and treatment	Mean length of primary root, cm	SD, primary root	Mean numbers of lateral roots	SD, lateral roots	N	p-values, primary root (compared to control)	p-values, lateral roots (compared to control)
WT control	5.98	0.63	9.40	3.14	5		
WT bact. 9	6.13	0.88	9.00	4.32	3	0.7859	0.8833
WT bact. 15	4.89	1.61	11.50	1.66	4	0.2028	0.2695
WT bact. CL8	6.53	0.69	12.40	3.88	5	0.2245	0.2158
***lcmt1* control**	6.12	0.33	10.00	1.41	4		
***lcmt1* bact. 9**	5.80	0.69	9.80	3.71	5	0.4261	0.9224
***lcmt1* bact. 15**	5.29	0.53	8.60	1.58	5	0.0298*	0.2093
***lcmt1* bact. CL8**	6.86	0.38	13.50	1.12	4	0.0259*	0.0081*
***pme1* control**	6.04	0.52	12.40	3.93	5		
***pme1* bact. 9**	7.01	0.30	14.33	2.05	3	0.0275*	0.4690
***pme1* bact. 15**	4.67	0.47	5.80	1.47	5	0.0024*	0.0079*
***pme1* bact. CL8**	5.92	0.40	10.20	2.56	5	0.6933	0.3249
$ptpa_{ox}$ control	4.76	0.46	5.20	1.94	5		
$ptpa_{ox}$ bact. 9	5.41	0.74	8.80	2.99	5	0.1339	0.0538
$ptpa_{ox}$ bact. 15	4.88	0.38	7.50	2.06	4	0.6882	0.1289
$ptpa_{ox}$ bact. CL8	5.51	0.37	8.40	2.87	5	0.0218*	0.0727
$ptpa_{kd}$ control	7.62	0.29	14.25	3.11	4		
$ptpa_{kd}$ bact. 9	7.37	0.58	12.50	2.87	4	0.4699	0.4399
$ptpa_{kd}$ bact. 15	6.64	0.53	9.20	3.54	5	0.0132*	0.0602
$ptpa_{kd}$ bact. CL8	6.12	0.83	7.80	1.83	5	0.0113*	0.0059*

*Statistically significant compared to control, $p < 0.05$.

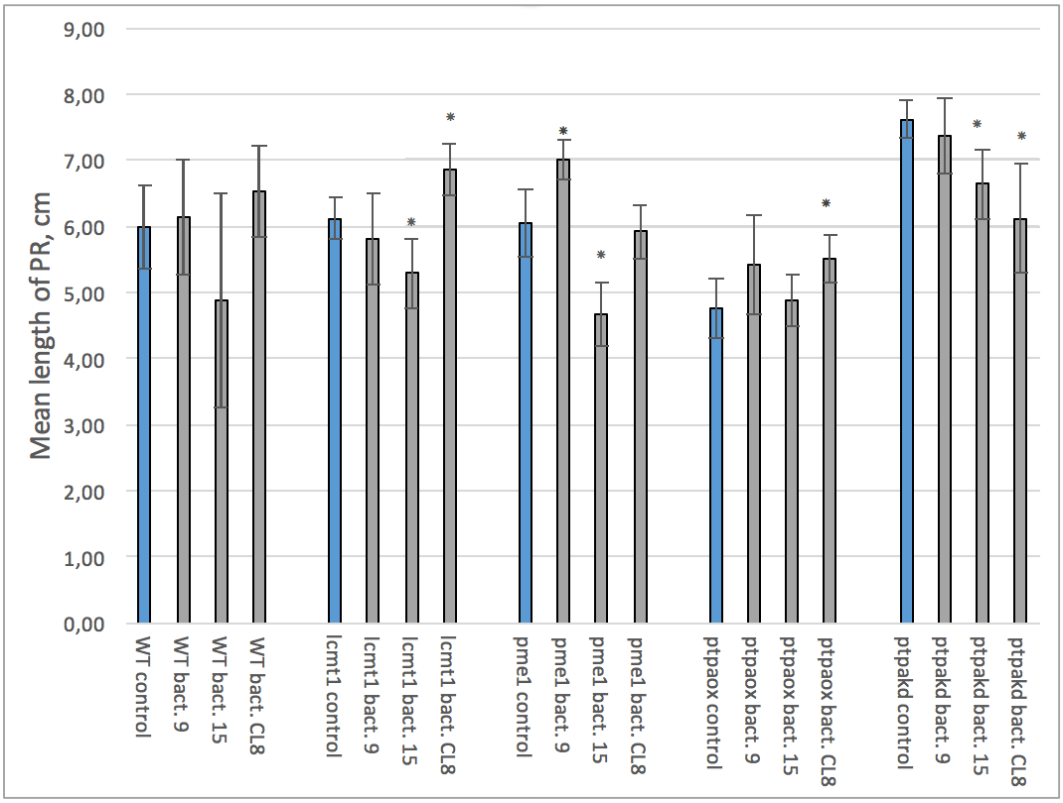

Figure 3.11: Primary root results from root growth assay performed with *A. thaliana* WT, *lcmt1*, *pme1*, $ptpa_{ox}$, and $ptpa_{kd}$ plants, inoculated with bacterial strain 9, 15 or CL8.

Mean primary root length of A. thaliana WT, lcmt1, pme1, $ptpa_{ox}$, and $ptpa_{kd}$ plants, 6 d after transfer to 1/50 Gamborg medium (-suc) inoculated with 650 µl of bacterial suspension of bacterial strain 9, 15, or CL8 ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Length of primary roots were measured using ImageJ. Bars show mean of 3-5 plants ± SD, plants grown much shorter than others on the same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

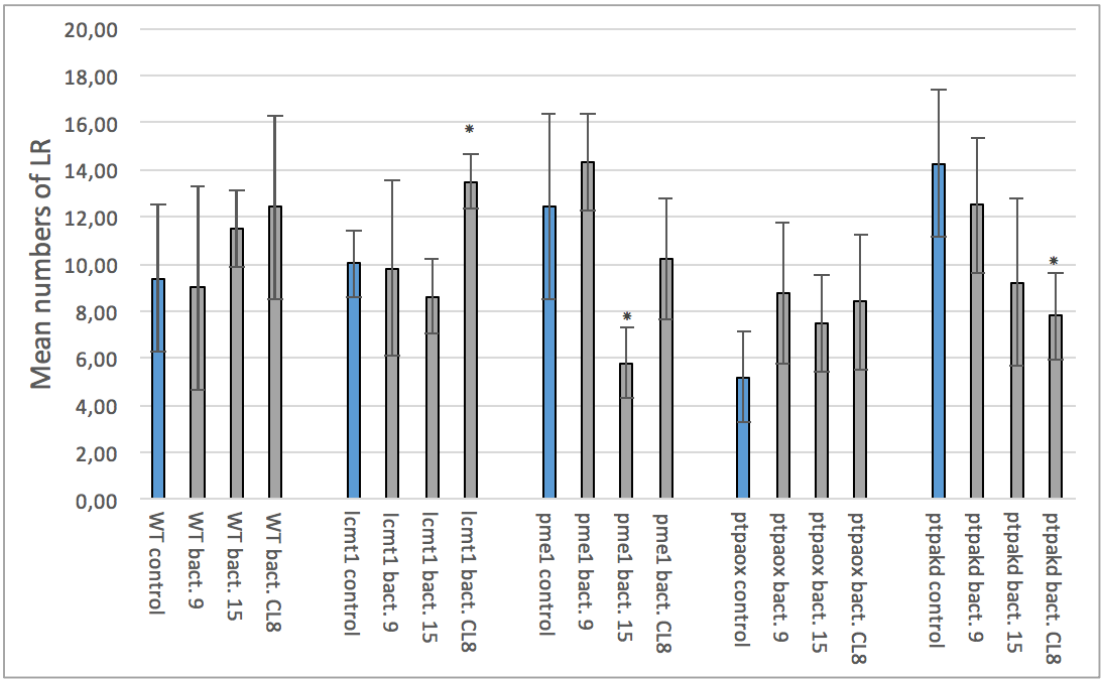

Figure 3.12: Lateral roots results from root growth assay performed with *A. thaliana* WT, *lcmt1*, *pme1*, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, inoculated with bacterial strain 9, 15, or CL8.

Mean numbers of lateral roots on A. thaliana WT, lcmt1, pme1, ptpa$_{ox}$, and ptpa$_{kd}$ plants, 6 d after transfer to 1/50 Gamborg medium (-suc), inoculated with 650 µl of bacterial suspensions of bacterial strain 9, 15, or CL8 ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Lateral roots were counted visually. Bars show mean of 3-5 plants ± SD, plants grown much shorter than others on the same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

Inoculation with bacterial strain 9 significantly affected the primary roots of the *A. thaliana pme1* plants, resulting in elongated primary roots, compared to the control (p value = 0.0275, table 3.4, fig. 3.11). No significant effect was seen for neither primary or lateral roots for the other mutant plants or WT with bacterial strain 9 (fig. 3.11 and 3.12).

Bacterial strain 15 significantly inhibited the growth of primary roots for *A. thaliana lcmt1*, *pme1* and *ptpa$_{kd}$* plants, compared to the controls (p values = 0.0298, 0.0024 and 0.0132, respectively), but no significant effect was seen for the primary roots of WT or *ptpa$_{ox}$* plants (table 3.4, fig. 3.11). Bacterial strain 15 also significantly inhibited promotion of lateral roots for *pme1* plants, compared to the control (p value = 0.0079, but no significant differences compared to the controls were found for the other plants (table 3.4, fig. 3.12).

Inoculation with bacterial strain CL8 promoted elongation of the primary roots on *A. thaliana lcmt1* and *ptpa$_{ox}$* plants, compared to the controls (p values = 0.0259 and 0.0218, respectively, table 3.4, fig. 3.11), and inhibited the primary roots of ptpa$_{kd}$ plants compared to its control (p value = 0.0113, table 3.4, fig. 3.11). Bacterial strain CL8 promoted development of lateral roots for *A. thaliana lcmt1*

plants (p value = 0.0081, table 3.4, fig. 3.12), and inhibited promotion of lateral root for *ptpa$_{kd}$* plants (p value = 0.0059, table 3.4, fig 3.12), compared to the controls. As for previous experiments, all bacterial strains promoted root hair development (fig. 3.10).

Only 5 plants for each treatment were used in this experiment, and some of them grew much shorter than the others, and were taken out of the calculations. Thus, there were too few samples to definite say something about significance, the results were only a guideline for further experiments. In addition, some of the plates were contaminated (fig. 3.9), which may have affected the results. Through discussions with supervisor and other students working with the same bacteria, it was decided to continue with bacteria 15 for the next experiment. To be able to increase the numbers of plants for each treatment, it was also decided to decrease the numbers of mutant plants.

3.2.3 Root growth assay with *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, inoculated with bacterial strain 15.

A. thaliana WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* seeds were sown on 1/50 Gamborg medium supplemented with 0.5 % sucrose. After 3 days of stratification in 4°C, the plates were placed vertically in a growth chamber (16 h light/ 8 h dark). After 5 d in the growth chamber, the seedlings were transferred to 1/50 Gamborg medium without sucrose, that were inoculated with 650 µl of bacterial strain 15 ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control; 5 seedlings per plate, 4 plates for each treatment. After additional 6 d in the growth chamber, the plates were taken out and photographed (fig. 3.13). Images of the root tips were taken using a Leica microscope (fig. 3.14). Primary roots were measured using ImageJ, and lateral roots were visually counted (table 3.5). The plates were then put back in the growth chamber for 2 d, when the fresh weight of roots and shoots were measured (Appendix A4). Data in table 3.5 were used to make graphical illustrations (fig. 3.15 and 3.16). An unpaired student's t-test was performed with a t-test calculator (GraphPad QuickCalcs Web Site), to see if there were any significant differences between the control plants, and plants inoculated with bacteria. Table 3.5 includes the p-values from this test.

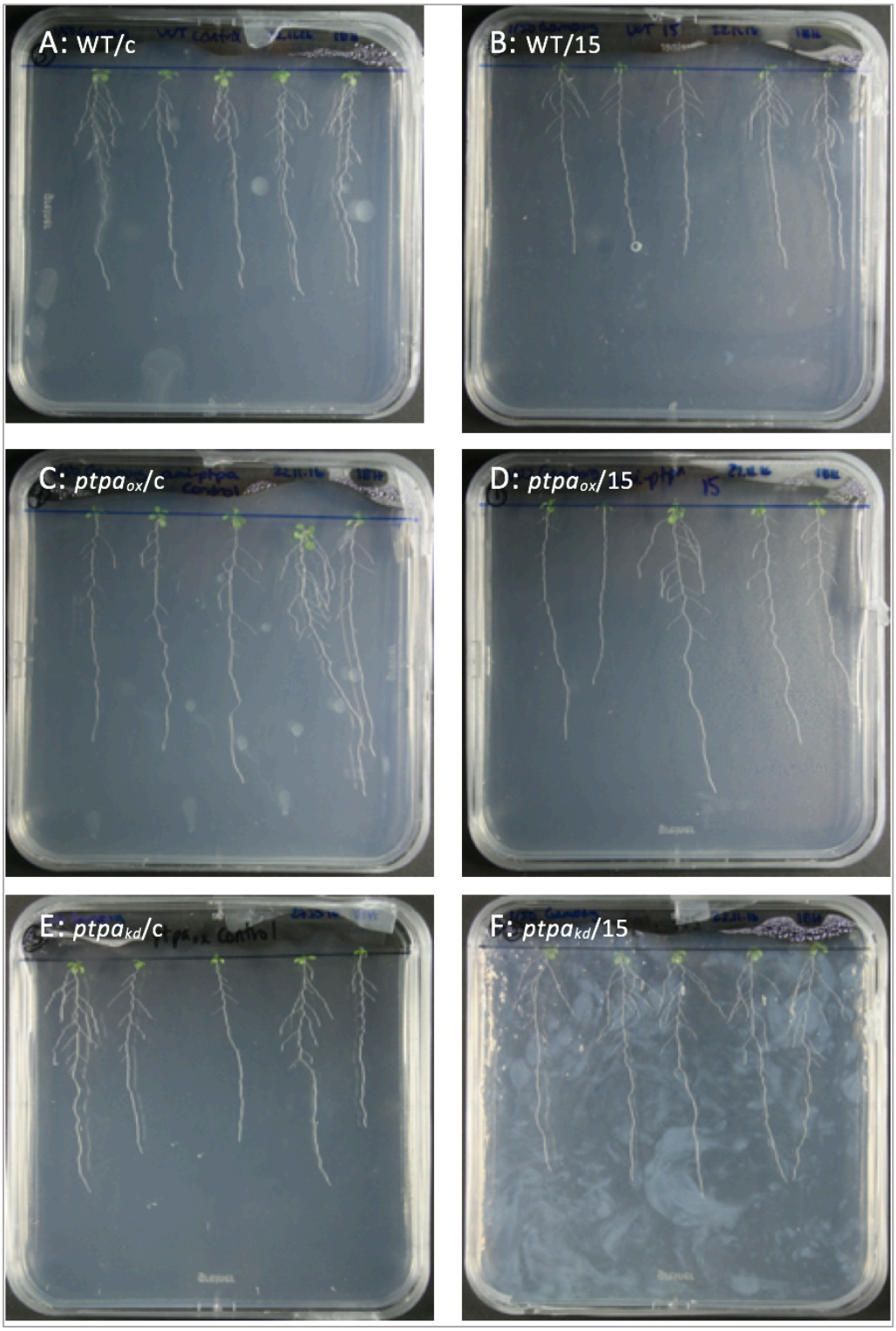

Figure 3.13 Representative pictures of plants from root growth assay with *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, 6 d after inoculation with bacterial strain 15.

A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ plants on 1/50 Gamborg medium (-suc), 6 d after inoculation with 650 µl suspension of bacterial strain 15 (OD ≈ 0.5), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: WT control. B: WT + bacterial strain 15. C: ptpa$_{ox}$ control. D: ptpa$_{ox}$ + bacterial strain 15. E: ptpa$_{kd}$ control. F: ptpa$_{kd}$ + bacterial strain 15.

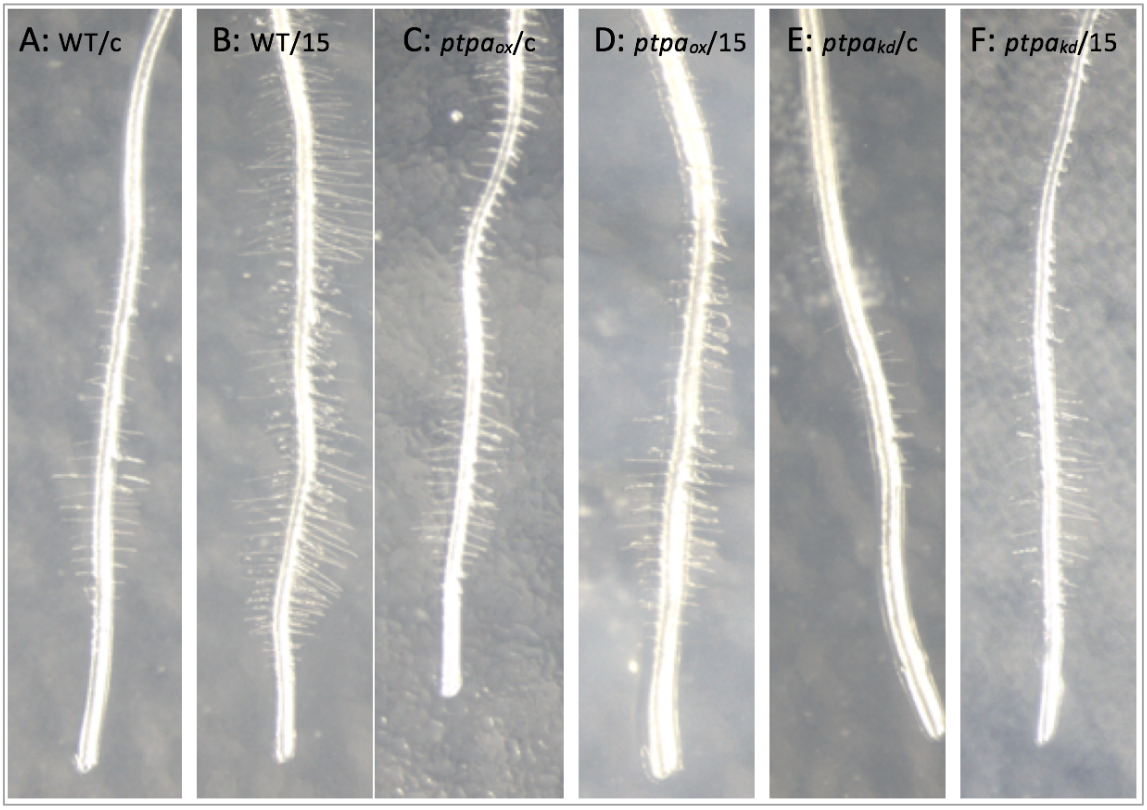

Figure 3.14: Representative images of root tips of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, 6 d after inoculation with bacterial strain 15.

A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ root tips, 6 d after inoculation with bacterial strain 15 (OD_{600} ≈ 0.5), or 10 mM $MgSo_4 \cdot 7H_2O$ for control. A: WT control. B: WT + bacterial strain 15. C: ptpa$_{ox}$ control. D: ptpa$_{ox}$ + bacterial strain 15. E: ptpa$_{kd}$ control. F: ptpa$_{kd}$ + bacterial strain 15.

Table 3.5: Data for root growth assay performed with *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, inoculated with bacterial strain 15.

Mean length of primary root, and mean numbers of lateral roots for A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ plants, 6 d after inoculation with 650 µl suspension of bacterial strain 15 ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, with corresponding standard deviations (SD). N is number of plants for calculations. An unpaired Student's t-test has been performed to find the p-values.

Type of plants and treatment	Mean length of primary root, cm	SD, primary root	Mean numbers of lateral roots	SD, lateral roots	N	p-values, primary root (compared to control)	p-values, lateral roots (compared to control)
WT control	5.86	0.67	10.12	3.74	17		
WT bact. 15	5.26	0.57	8.61	2.52	18	0.0073*	0.1684
***ptpa$_{ox}$* control**	6.59	0.60	12.18	3.36	17		
***ptpa$_{ox}$* bact. 15**	6.42	0.64	10.17	3.37	18	0.4240	0.0866
***ptpa$_{kd}$* control**	6.22	0.81	7.13	3.74	16		
***ptpa$_{kd}$* bact. 15**	6.37	0.71	7.85	3.57	13	0.6048	0.6031

* Statistically significant compared to control, $p < 0.05$.

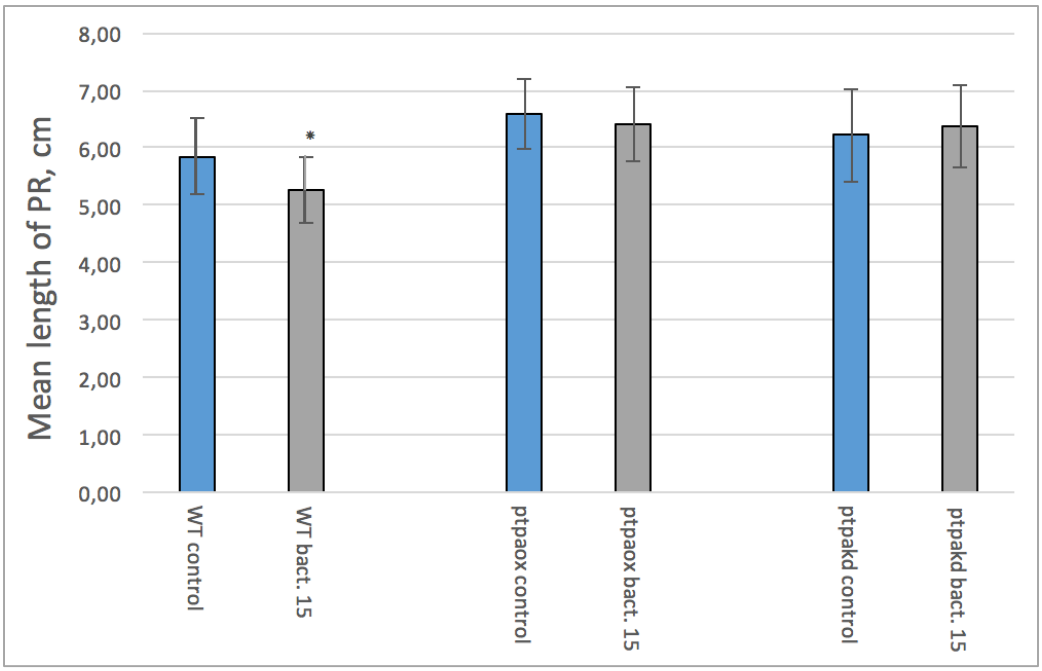

Figure 3.15: Primary root results from root growth assay with *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, 6 d after inoculation with bacterial strain 15.

Mean primary root length of A. thaliana WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, 6 d after transfer to 1/50 Gamborg medium (-suc), inoculated with 650 µl suspension of bacterial strain 15 ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Length of primary roots were measured using ImageJ. Bars shows mean of 13-18 plants ± SD, plants growing much shorter than others on the same plate were not taken into calculations.
** Statistically significant compared to control, $p < 0.05$.*

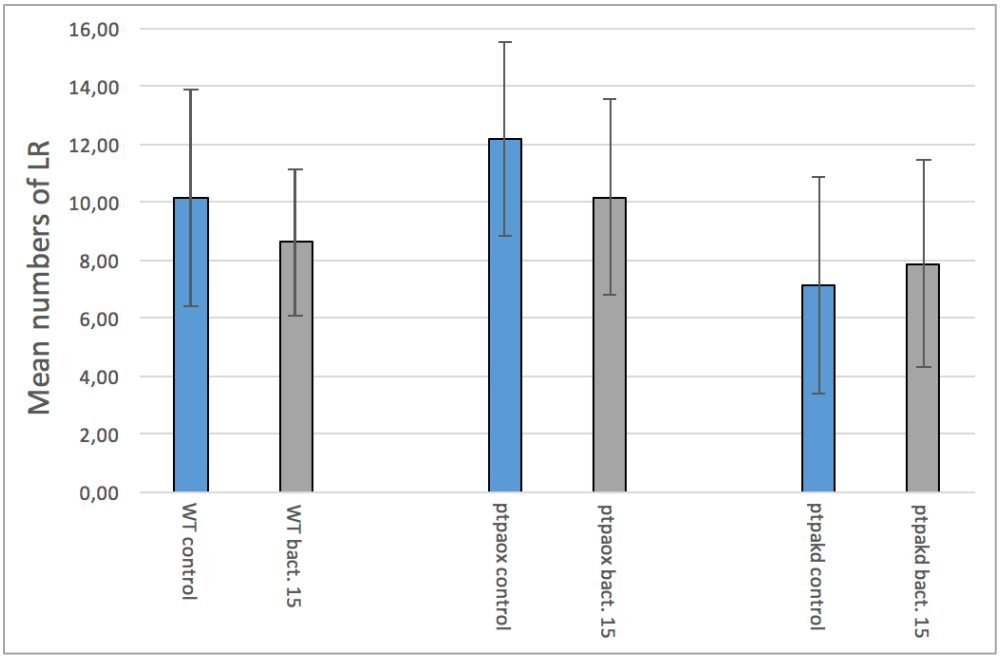

Figure 3.16: Lateral roots results from root growth assay with *A. thaliana* WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, inoculated with bacterial strain 15.

Mean numbers of lateral roots on A. thaliana WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, 6 d after transfer to 1/50 Gamborg medium (-suc) inoculated with 650 µl suspension of bacterial strain 15 ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Lateral roots were counted visually. Bars shows mean of 13-18 plants ± SD, plants grown much shorter than others on the same plate were not taken into calculations.

Inoculation of bacterial strain 15 to *A. thaliana* WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, significantly inhibited the development of primary roots for the WT plants, compared to the control (p value = 0.0073). No significant effect on the primary root was seen for the $ptpa_{ox}$ or $ptpa_{kd}$ plants (table 3.5, fig. 3.15). There was no significant difference for the numbers of lateral roots, neither for WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, compared to the controls (fig. 3.16). The bacteria promoted development of root hairs (fig. 3.14). As for the previous experiments, there were some unknown contamination on some of the plates (fig. 3.13), which may have affected the results.

For this experiment, the fresh weight of roots and shoots were measured. There seemed to be a small positive effect on the WT and $ptpa_{kd}$ shoots, and a negative effect on the $ptpa_{ox}$ shoots when inoculated with the bacterial strain 15. The fresh weight of roots for WT and $ptpa_{ox}$ was lower for

plants inoculated with bacteria, compared to the control. However, SD and p-values could not be calculated, due to how the plants were measured, and the experiment was not repeated (Appendix 4, table A.1, fig A.12 and A.13).

When considering bacterial strain 15, that was used for all experiments, the effects on *A. thaliana* WT were variable. A significant difference in the length of primary roots compared to the controls, was seen in assay 1 in the first experiment with all bacteria, and in the last experiment with only bacterial strain 15. However, in the first experiment bacteria 15 increased the primary roots of WT, and in the last experiment the primary roots were inhibited. For the effect on the lateral roots, none of the experiments with bacterial strain 15 showed any significant difference compared to the control for the WT. In one of the experiments did bacterial strain 15 significantly inhibit the length of primary roots for $ptpa_{kd}$, but no other effects on the mutants were seen for this bacterial strain. Since the effects on the root system were variable, it was decided to use a more well-known PGPB, *Pseudomonas* WCS417, for further experiments.

3.3 Root growth assay with *Pseudomonas* WCS417

3 different root growth assays have been performed using *Pseudomonas* WCS417.

3.3.1 Root growth assay 1 *Pseudomonas* WCS417; bacteria suspension inoculated 5 cm under root tip on medium without sucrose.

A. thaliana WT seeds were sown on 1xMS medium supplemented with 0.5 % sucrose. After 2 days of stratification in 4°C, the plates were placed vertically in a growth chamber (16 h light/8 h dark). After 5 d in the growth chamber, the seedlings were transferred to 1xMS medium without sucrose; 5 seedlings per plate, 4 plates for each treatment. Afterwards, 300 µl of *Pseudomonas* WCS417 suspension (OD_{600} = 0.004, $2x10^6$ cells/ml), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, were inoculated in spots 5 cm under the root tips. After additional 8 d in the growth chamber, the plates were taken out and photographed (fig. 3.17). Primary roots were measured using ImageJ, and lateral roots were counted visually (table 3.6). Data in table 3.6 were used to make graphical illustrations (fig. 3.18 and 3.19). An unpaired student's t-test was performed with a t-test calculator (GraphPad QuickCalcs Web Site), to see if there were any significant differences between the control plants, and plants inoculated with bacteria. Table 3.6 includes the p-values from this test.

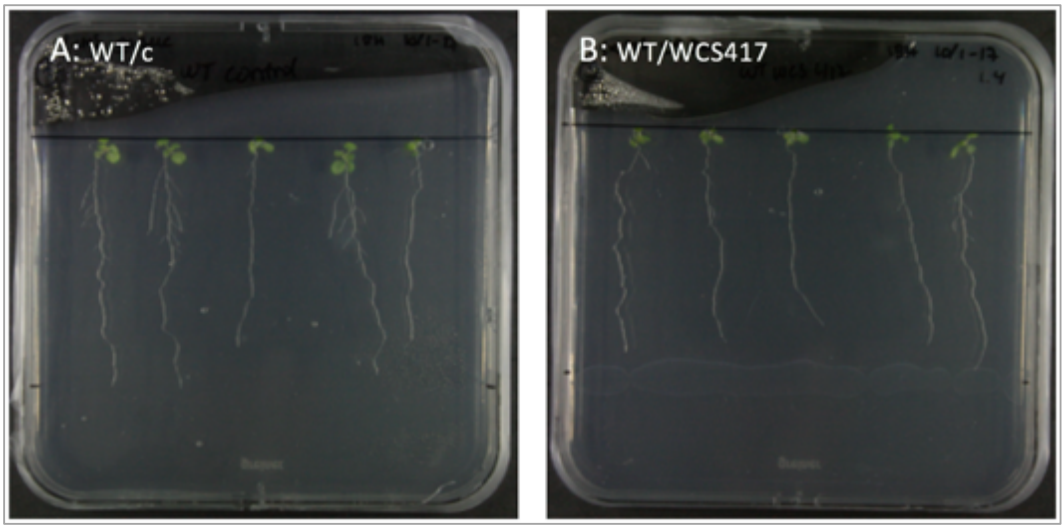

Figure 3.17: Representative pictures of *A. thaliana* WT plants, 8 d after inoculation with *Pseudomonas WCS417.*

A. thaliana WT plants, 8 d after inoculation with 300 µl of Pseudomonas WCS417 suspension (OD_{600} = 0.004), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, in spots 5 cm under the root tips. A: WT control. B: WT + Pseudomonas WCS417.

Table 3.6 Data for root growth assay 1 *Pseudomonas WCS417*.

Mean length of primary root, and numbers of lateral roots for A. thaliana WT plants, 8 d after inoculation with 300 µl of Pseudomonas WCS417 suspension (OD_{600} = 0.004), or 10 mM $MgSO_4.7H_2O$ for control, with corresponding standard deviations (SD). N is number of plants for calculation. Student's t test has been performed to find the p-values.

Type of plants and treatment	Mean length of primary root, cm	SD, primary roots	Mean numbers of lateral roots	SD, lateral roots	N	p-value, primary root (compared to control)	p-value, lateral roots (compared to control)
WT control	5.37	0.55	4.50	4.06	18		
WT WCS417	5.85	0.60	6.50	4.95	17	0.0189*	0.1192

* Statistically significant compared to control, $p < 0.05$.

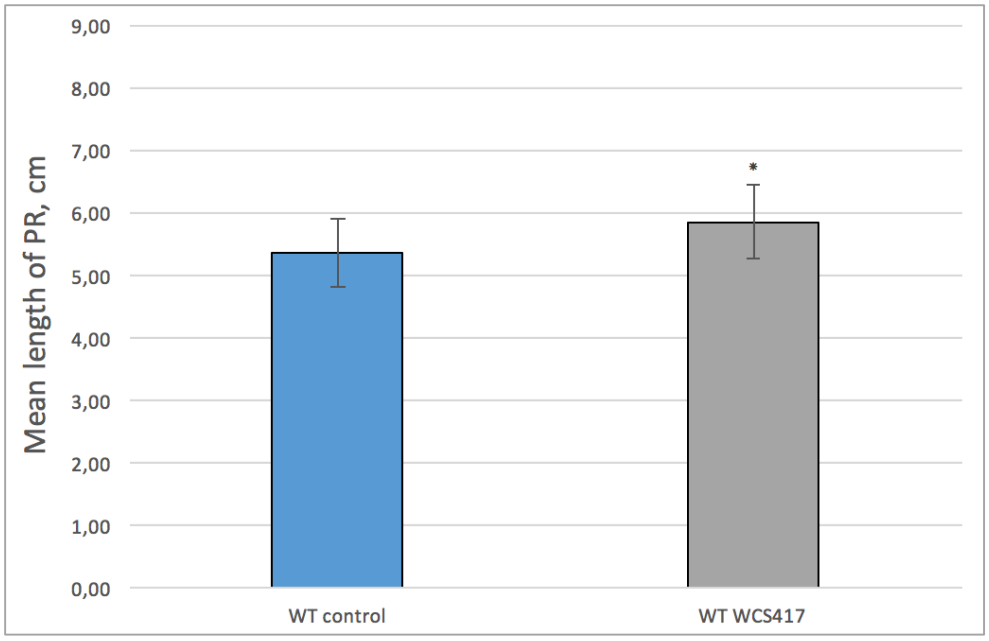

Figure 3.18: Primary root results from root growth assay 1 *Pseudomonas* WCS417.

Mean primary root length of A. thaliana WT plant, 8 d after inoculation. 300 µl of Pseudomonas suspension (OD_{600} = 0.004), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, were inoculated in spots 5 cm under the root tips. Length of primary roots were measured using ImageJ. Bars show mean of 17-18 plants ± SD, plants grown much shorter than others on the same plate were taken into calculations.

** Statistically significant compared to control, p < 0.05.*

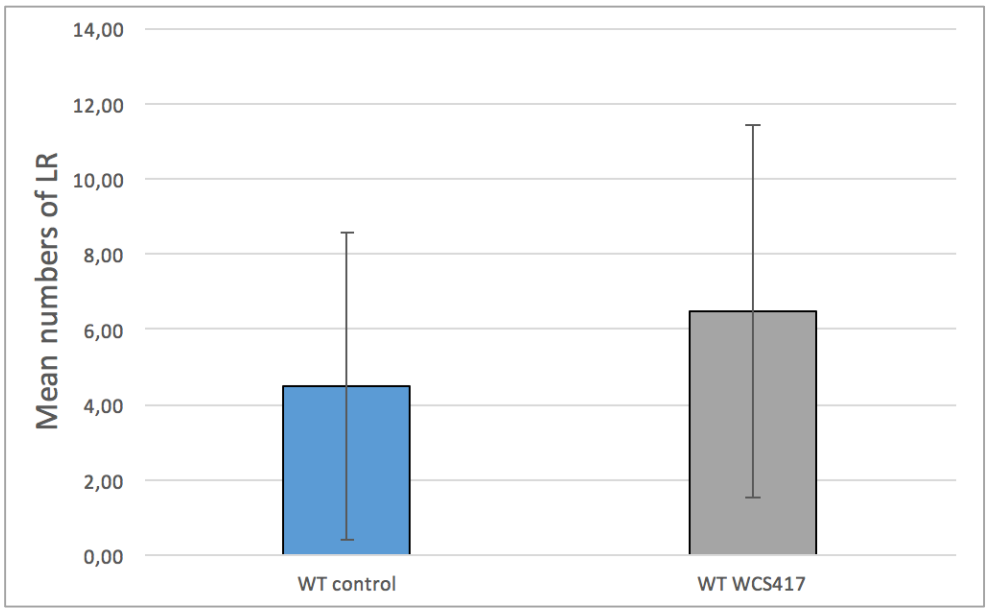

Figure 3.19 Lateral roots results from root growth assay 1 *Pseudomonas* WCS417.

Mean numbers of lateral roots on A. thaliana WT plants, 8 d after inoculation of 300 µl of bacteria suspension (OD_{600} = 0.004), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, in spots 5 cm under root tips. Lateral roots were counted visually. Bars show mean of 17-18 plants ± SD, plants grown much shorter than others were not taken into calculations.

The length of primary root was significantly longer for *A. thaliana* WT plants inoculated with *Pseudomonas* WCS417, compared to the control (p value = 0.0189, table 3.6, fig 3.18). There was no significant difference in the numbers of lateral roots between the control plants, and plants inoculated with bacteria (fig. 3.19). This is not in accordance with results obtained by others (Wintermans et al. 2016; Zamioudis et al. 2013), where inhibition of primary root and increase of lateral roots of *A. thaliana* WT plants were seen. Since the effect was not as expected, it was decided to investigate whether the density of the bacteria would affect the results by using two different densities for the next experiment. The bacteria suspension would also be spread evenly on the medium, as done in previous experiments, instead of in spots under the root tips.

3.3.2 Root growth assay 2 *Pseudomonas* WCS417; bacteria suspension spread on medium without sucrose.

A. thaliana WT, $ptpa_{ox}$, and $ptpa_{kd}$ seeds were sown on 1xMS medium supplemented with 0.5 % sucrose. After 2 days of stratification in 4°C, the plates were placed vertically in a growth chamber (16 h light/8 h dark). After 5 d in the growth chamber, the seedlings were transferred to 1xMS medium without sucrose, inoculated with 450 µl of *Pseudomonas* WCS417 suspension (OD_{600} = 0.004, 10^6 cells/ml or OD_{600} = 0.5, 10^8 cells/ml), or 10 mM $MgSO_4 \cdot 7H_2O$ for control; 5 seedlings per plate, 2 plates per treatments. After additional 8 d in the growth chamber, the plates were taken out and photographed (fig. 3.20). Images of the root tips were taken with a Leica microscope (fig. 3.21). Primary roots were measured using ImageJ, and lateral roots were counted visually (table 3.7). Fresh shoot and root weights were measured (Appendix A4). Data in table 3.7 were used to make graphical illustrations (fig. 3.22 and 3.23). An unpaired student's t-test was performed with a t-test calculator (GraphPad QuickCalcs Web Site), to see if there were any significant differences between the control plants, and plants inoculated with bacteria. Table 3.7 includes the p-values from this test.

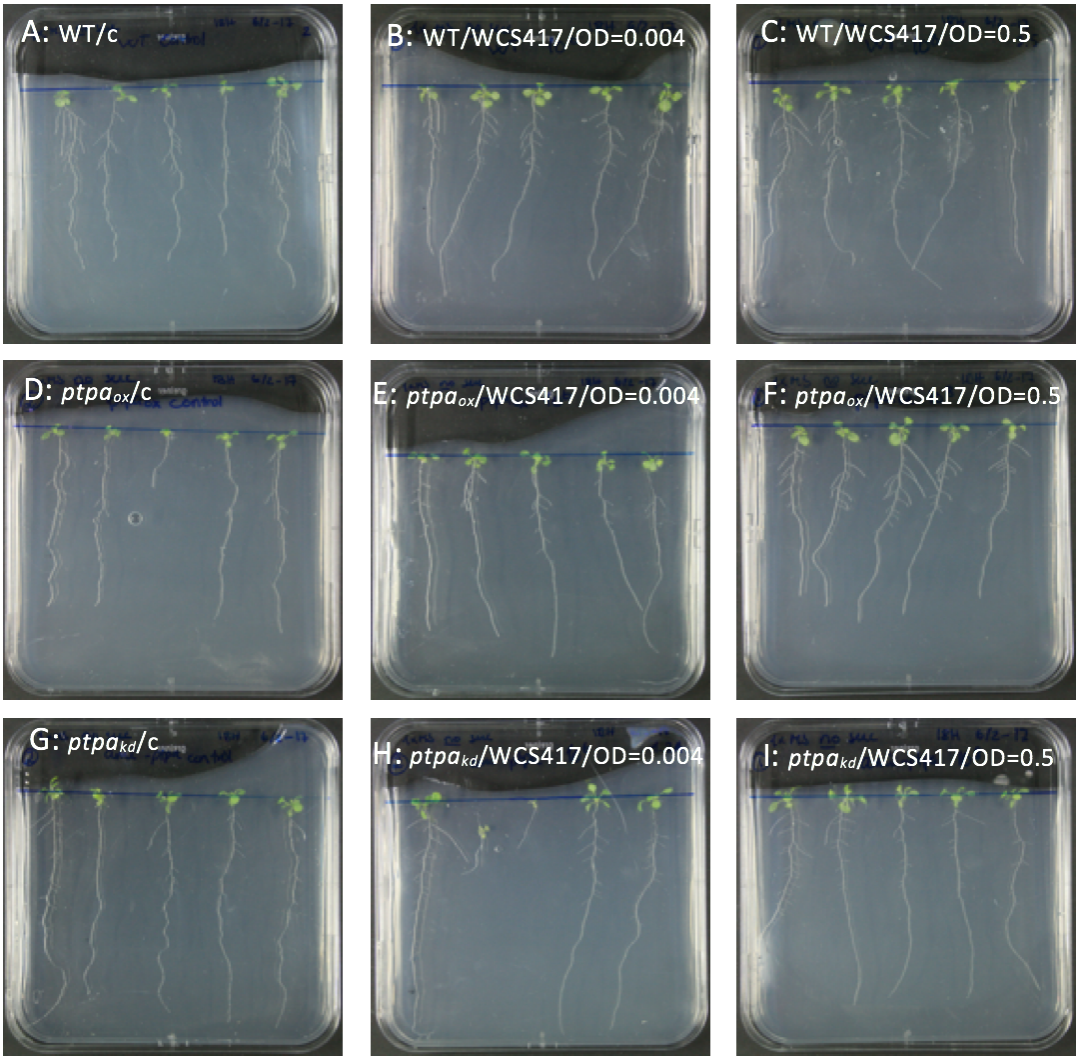

Figure 3.20: Representative pictures of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, 8 d after inoculation with *Pseudomonas* WCS417.

A. thaliana WT, ptpa$_{ox}$ and ptpa$_{kd}$ plants, 8 d after inoculation with 450 µl of Pseudomonas suspension (OD$_{600}$ = 0.004 or 0.5), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: WT control. B: WT + Pseudomonas WCS417 (OD$_{600}$ = 0.004). C: + Pseudomonas WCS417 (OD$_{600}$ = 0.5). D: ptpa$_{ox}$ control. E: ptpa$_{ox}$ + Pseudomonas WCS417 (OD$_{600}$ = 0.004). F: ptpa$_{ox}$ + Pseudomonas WCS417 (OD$_{600}$ = 0.5). G: ptpa$_{kd}$ control. H: ptpa$_{kd}$ + Pseudomonas WCS417 (OD$_{600}$ = 0.004). I: ptpa$_{kd}$ + Pseudomonas WCS417 (OD$_{600}$ = 0.5).

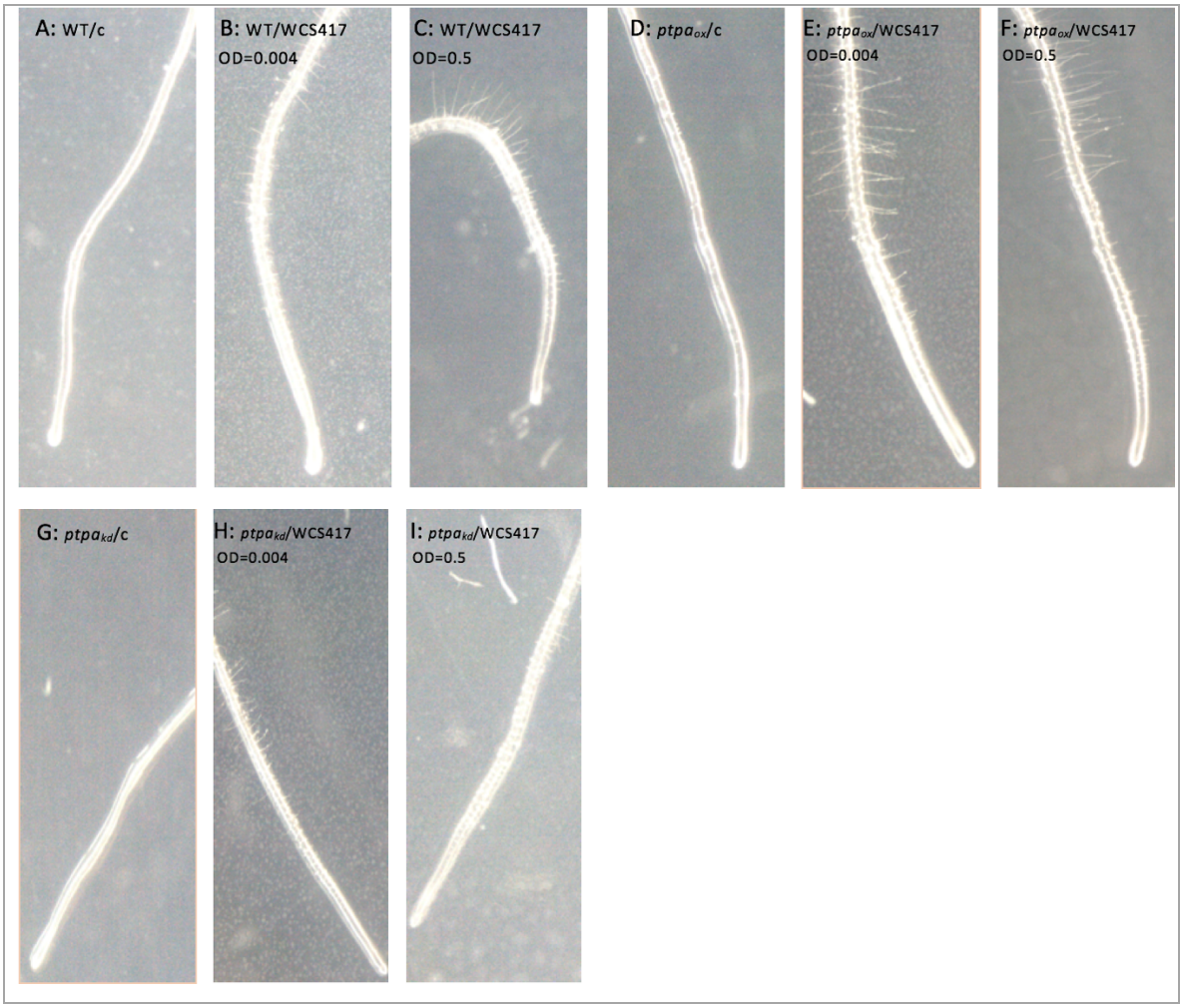

Figure 3.21: Representative images of root tips of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, 8 d after inoculation with *Pseudomonas* WCS417.

A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ root tips, 8 d after inoculation with Pseudomonas WCS417 (OD$_{600}$ = 0.004 or 0.5), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: WT control. B: WT + Pseudomonas WCS417 (OD$_{600}$ = 0.004). C: WT + Pseudomonas WCS417 (OD$_{600}$ = 0.5). D: ptpa$_{ox}$ control. E: ptpa$_{ox}$ + Pseudomonas WCS417 (OD$_{600}$ = 0.004). F: ptpa$_{ox}$ + Pseudomonas WCS417 (OD$_{600}$ = 0.5). G: ptpa$_{kd}$ control. H: ptpa$_{kd}$ + Pseudomonas WCS417 (OD$_{600}$ = 0.004). I: ptpa$_{kd}$ + Pseudomonas WCS417 (OD$_{600}$ = 0.5). Images were taken with a Leica microscope.

Table 3.7: Data for root growth assay 2 *Pseudomonas* WCS417.

Mean length of primary root, and numbers of lateral roots for A. thaliana WT, $ptpa_{ox}$ and $ptpa_{kd}$ plants, 8 d after inoculation with 450 µl of Pseudomonas WCS417 suspension (OD_{600} = 0.004 or 0.5), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, with corresponding standard deviations (SD). N is number of plants for calculation. A student's t test has been performed to find the p-values.

Type of plants and treatment	Mean length of primary root, cm	SD, primary root	Mean numbers of lateral roots	SD, lateral roots	N	p-values, primary root (compared to control	p-values, lateral roots (compared to control)
WT control	7.19	0.75	14.67	5.25	9		
WT WCS417 (OD=0.004)	7.48	0.27	20.71	2.55	7	0.3490	0.0140*
WT WCS417 (OD=0.5)	7.42	0.46	19.63	2.55	8	0.4651	0.0926
***$ptpa_{ox}$* control**	6.88	0.64	7.78	4.49	9		
***$ptpa_{ox}$* WCS417 (OD=0.004)**	7.00	0.63	14.70	5.95	10	0.6859	0.0114*
***$ptpa_{ox}$* WCS417 (OD=0.5)**	7.00	0.61	18.10	3.96	10	0.6809	0.0001*
***$ptpa_{kd}$* control**	8.64	0.47	15.88	5.56	8		
***$ptpa_{kd}$* WCS417 (OD=0.004)**	8.61	0.58	23.00	4.97	6	0.9165	0.0291*
***$ptpa_{kd}$* WCS417 (OD=0.5)**	7.82	0.44	20.22	6.05	9	0.0021*	0.1461

* Statistically significant compared to control, $p < 0.05$.

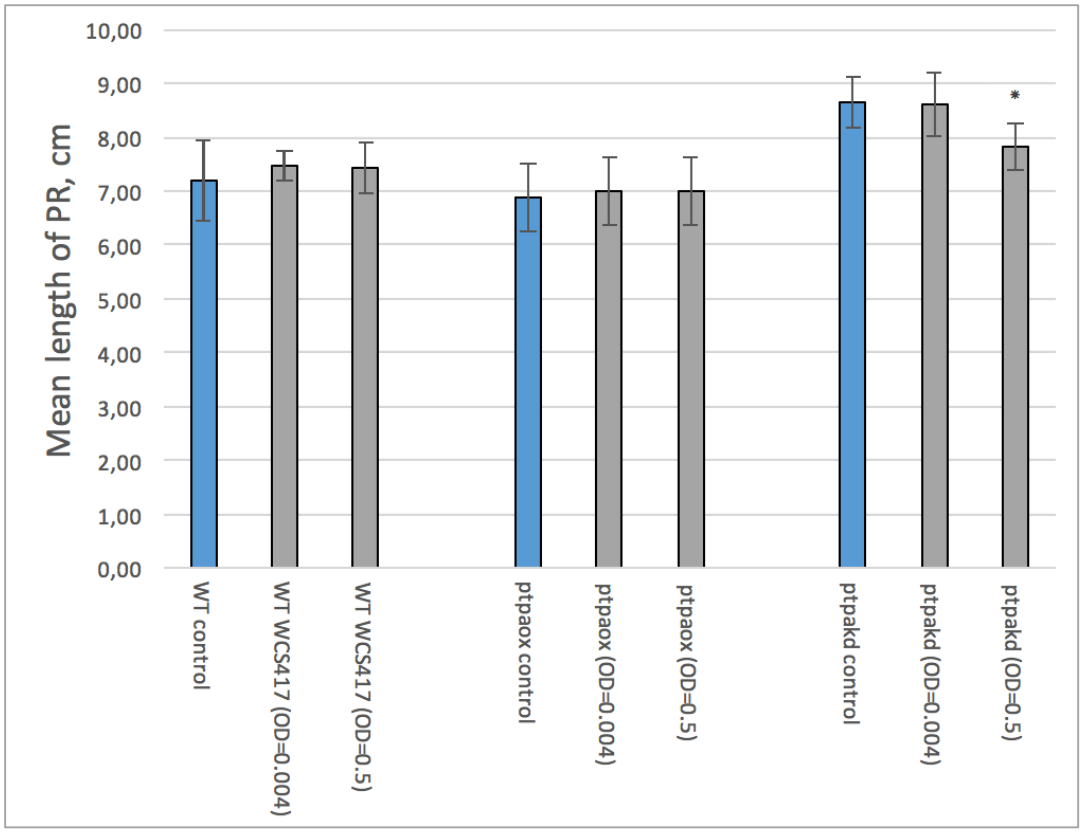

Figure 3.22: Primary root results from root growth assay 2 *Pseudomonas* WCS417.

Mean primary root length of A. thaliana WT, $ptpa_{ox}$ and $ptpa_{kd}$ plants, 8 d after transfer to 1xMS medium (-suc), inoculated with 450 µl of Pseudomonas suspension (OD_{600} = 0.004 or 0.5), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Length of primary roots were measured using ImageJ. Bars show mean of 6-10 plants ± SD, plants grown much shorter than others on the same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

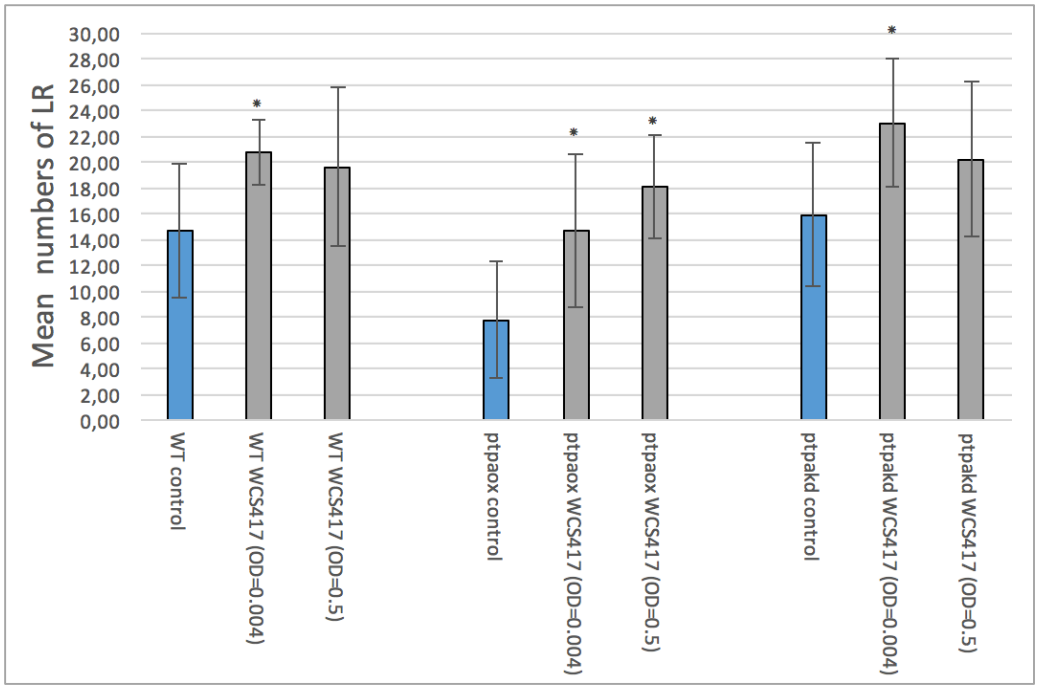

Figure 3.23: Lateral roots results from root growth assay 2 *Pseudomonas* WCS417.

Mean numbers of lateral roots on A. thaliana WT, $ptpa_{ox}$ and $ptpa_{kd}$ plants, 8 d after transfer to 1xMS medium (-suc), inoculated with 450 µl of Pseudomonas suspension (OD_{600} = 0.004 or 0.5), or 450 µl 10 mM $MgSO_4 \cdot 7H_2O$ for control. Lateral roots were counted visually. Bars show mean of 6-10 plants ± SD, plants grown much shorter than others on the same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

Neither of the *Pseudomonas* WCS417 bacteria suspensions with different OD_{600} gave significant difference in the length of primary roots of the *A. thaliana* WT plants, compared to the WT control (fig. 3.22). Both densities promoted more lateral roots for the WT plants, compared to the WT control, but only for the lowest density (OD_{600} = 0.004), was this difference significant (p value = 0.0140, table 3.7, fig. 3.23).

Inoculation with *Pseudomonas* WCS417 on the *A. thaliana* $ptpa_{ox}$ plants, did not promote any significant difference in the length of primary roots compared to the $ptpa_{ox}$ control (fig. 3.22). However, both densities significantly promoted more lateral roots compared to control (p values = 0.0114 and 0.0001, respectively, table 3.7, fig. 3.23).

Inoculation of the highest density (OD_{600} = 0.5) of *Pseudomonas* WCS417, significantly inhibited the growth of primary roots on the *A. thaliana* $ptpa_{kd}$ plants compared to the $ptpa_{kd}$ control (p value = 0.0021, table 3.7, fig. 3.22), but did not significantly affect the lateral root formation (fig. 3.23). The lowest density (OD_{600} = 0.004) significantly promoted lateral root formation (p value = 0.0291, table 3.7, fig. 3.23), but there was no difference in the length of primary root, compared to the $ptpa_{kd}$ control (fig. 3.22).

For the fresh shoot and root weight, an increase in the fresh weight was highest for *ptpa$_{ox}$*, but the significance of these results could not be calculated (Appendix 4, table A.2, fig. A.14 and A.15). Promotion of root hairs were seen for both densities (fig.3.21).
Since there were no special differences in how the different densities effected the roots, it was decided to continue with a low density, as in the previous experiment. The effect of the *Pseudomonas* WCS417 was not quite as expected, and after personal communication with supervisor of Bachelor students, working with the same bacteria, it was decided to transfer the seedlings to medium supplemented with sucrose.

3.3.3 Root growth assay 3 *Pseudomonas* WCS417; bacteria suspension spread on medium supplemented with sucrose.

A. thaliana WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* seeds were sown on 1xMS medium supplemented with 0.5 % sucrose. After 2 days of stratification, the plates were placed vertically in a growth chamber (16 h light/8 h dark). After 5 d in the growth chamber, the seedlings were transferred to 1xMS medium, supplemented with 0.5 % sucrose, inoculated with 450 µl of *Pseudomonas* suspension (OD_{600} = 0.005, $2.32x10^6$ cells/ml), or 10 mM $MgSO_4 \cdot 7H_2O$ for control; 5 seedlings per plate, 4 plates per treatments. After additional 8 d in the growth chamber, the plates were taken out and photographed (fig. 3.24). Images were taken of the root tips with a Leica microscope (fig. 3.25). Primary roots were measured using ImageJ, and lateral roots were visually counted (table 3.8). Fresh shoot and root weight were measured (Appendix A4). Data in table 3.8 were used to make graphical illustrations (fig. 3.26 and 3.27). An unpaired student's t-test was performed with a t-test calculator (GraphPad QuickCalcs Web Site), to see if there were any significant differences between the control plants, and plants inoculated with bacteria. Table 3.8 includes the p-values from this test.

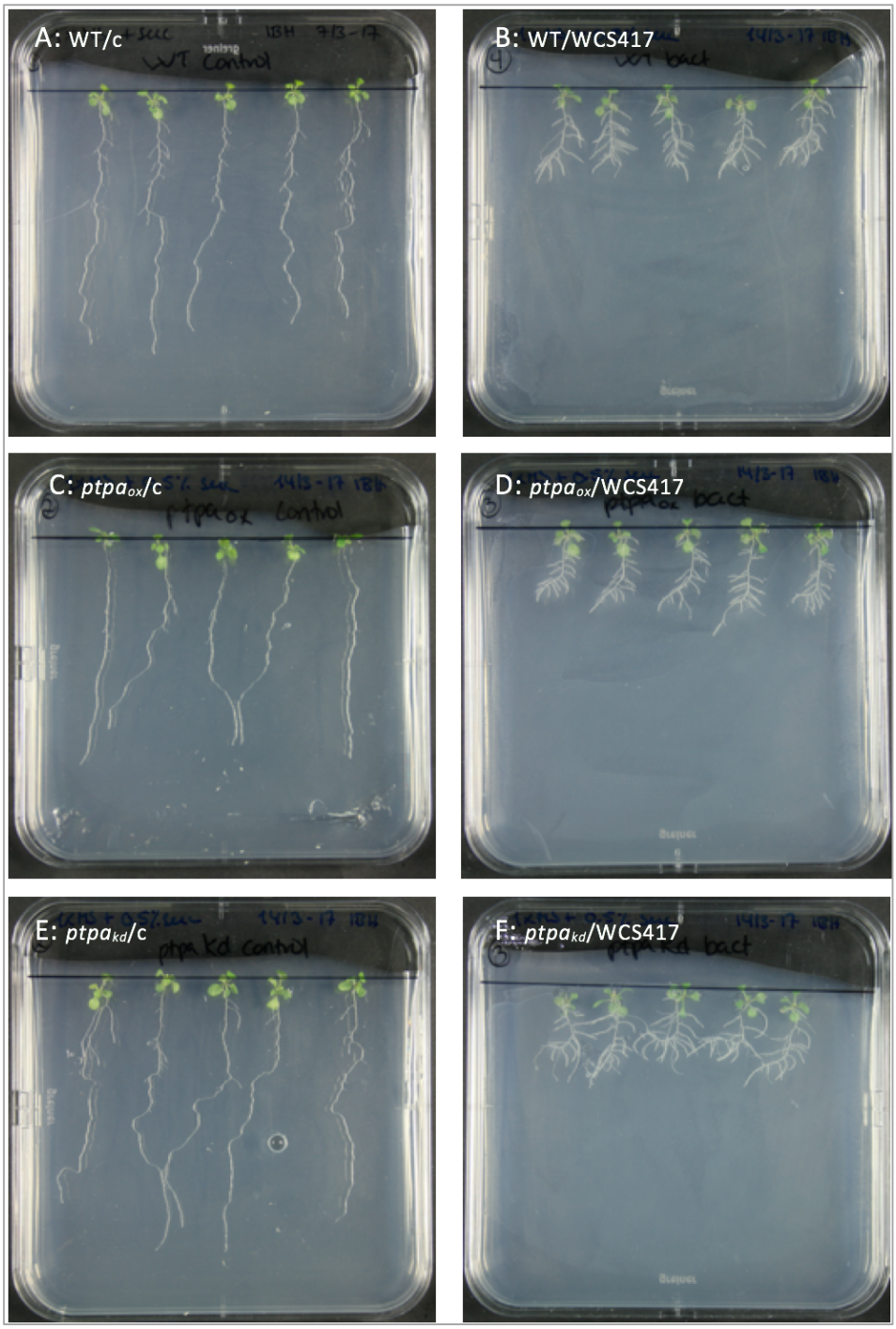

Figure 3.24 Representative pictures of *A. thaliana* WT, *ptpa*$_{ox}$, and *ptpa*$_{kd}$ plants, 8 d after inoculation with *Pseudomonas* WCS417.

A. thaliana WT, ptpa$_{ox}$ and ptpa$_{kd}$ plants, 8 d after inoculation with 450 µl of Pseudomonas suspension (OD = 0.005), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: WT control. B: WT + Pseudomonas WCS417. C: ptpa$_{ox}$ control. D: ptpa$_{ox}$ + Pseudomonas WCS417. E: ptpa$_{kd}$ control. F: ptpa$_{kd}$ + Pseudomonas WCS417.

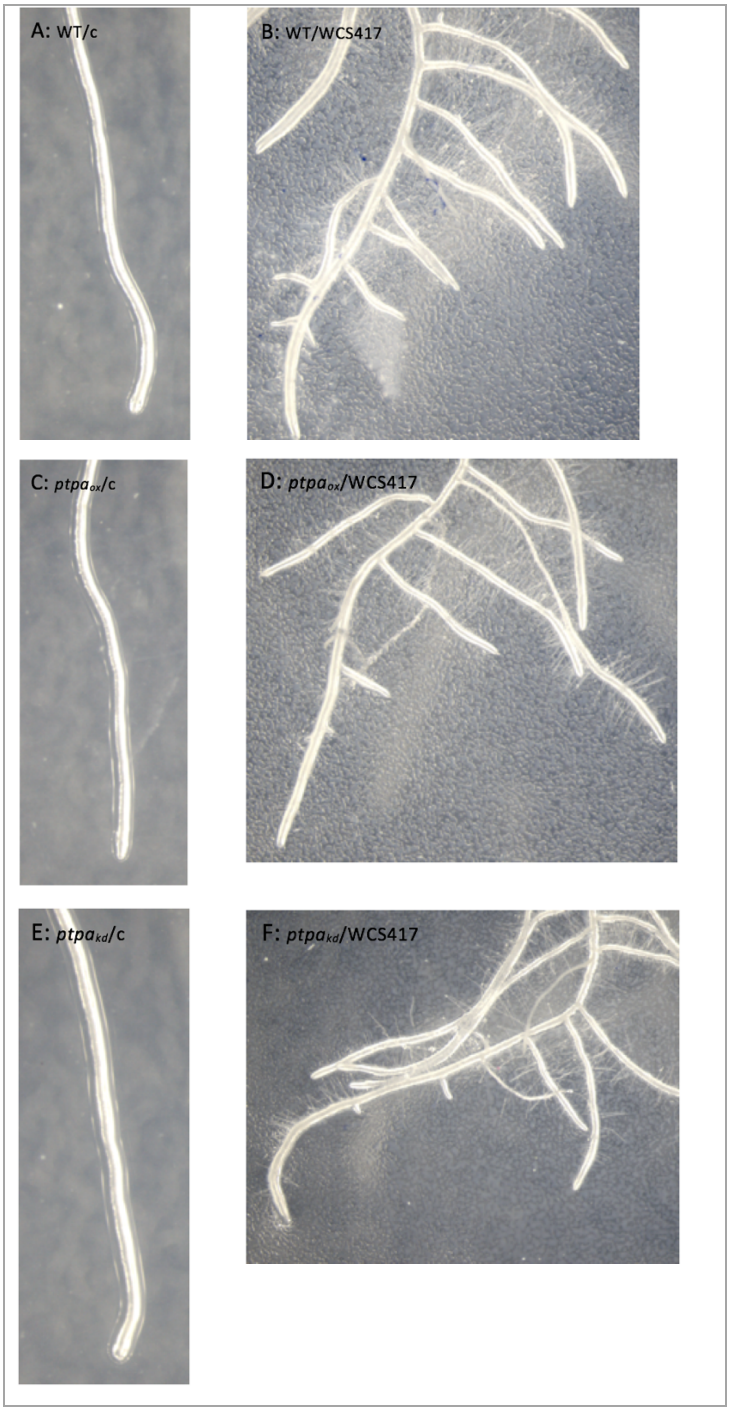

Figure 3.25: Images of root tips of *A. thaliana* WT, *ptpa*$_{ox}$, and *ptpa*$_{kd}$ plants, 8 d after inoculation with *Pseudomonas* WCS417.

A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ root tips, 8 d after inoculation with Pseudomonas WCS417 (OD_{600} = 0.005), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: WT control. B: WT + Pseudomonas WCS417. C: ptpa$_{ox}$ control. D: ptpa$_{ox}$ + Pseudomonas WCS417. E: ptpa$_{kd}$ control. F: ptpa$_{kd}$ + Pseudomonas WCS417. Images were taken with a Leica microscope.

Table 3.8: Data for root growth assay 3 *Pseudomonas* WCS417.

Mean length of primary root, and numbers of lateral roots for A. thaliana WT, $ptpa_{ox}$ and $ptpa_{kd}$ plants, 8 d after inoculation with 450 µl of Pseudomonas WCS417 suspension (OD_{600} = 0.005), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, with corresponding standard deviations (SD). N is number of plants for calculation. An unpaired Student's t test has been performed to find the p-values.

Type of plants and treatment	Mean length of primary root, cm	SD, primary root	Mean numbers of lateral roots	SD, lateral roots	N	p-values, primary root (compared to control)	p-values, lateral roots (compared to control)
WT control	7.34	0.71	11.75	5.22	20		
WT WCS417	2.61	0.22	21.60	3.47	20	0.0001*	0.0001*
***$ptpa_{ox}$* control**	7.00	0.73	9.89	4.29	18		
***$ptpa_{ox}$* WCS417**	2.70	0.28	20.33	2.81	18	0.0001*	0.0001*
***$ptpa_{kd}$* control**	8.71	0.61	15.30	4.12	20		
***$ptpa_{kd}$* WCS417**	3.24	0.38	20.00	3.14	18	0.0001*	0.0001*

*** Statistically significant compared to control, $p < 0.05$.**

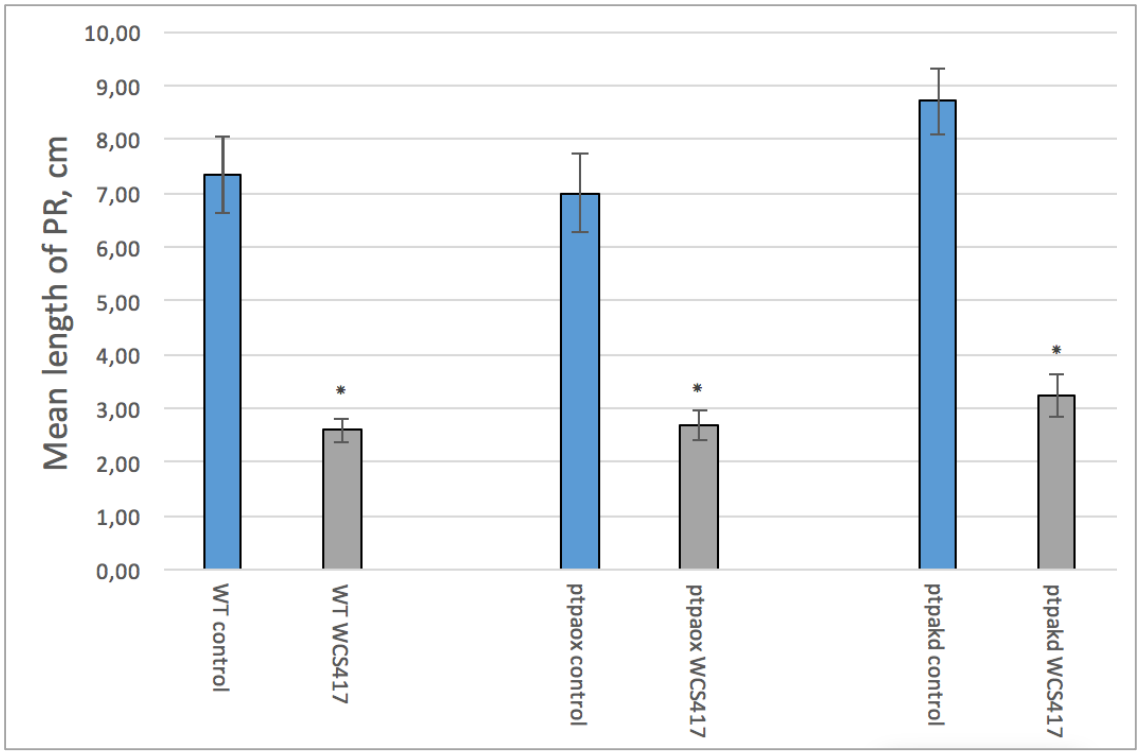

Figure 3.26: Primary root results from root growth assay 3 *Pseudomonas* WCS417.

Mean primary root length of A. thaliana WT, $ptpa_{ox}$ and $ptpa_{kd}$ plants, 8 d after transfer to 1xMS medium (+ 0.5 % suc), inoculated with 450 µl of Pseudomonas suspension with (OD_{600} = 0.005), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Length of primary roots were measured using ImageJ. Bars show mean of 18-20 plants ± SD, plants grown much shorter than the others on same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

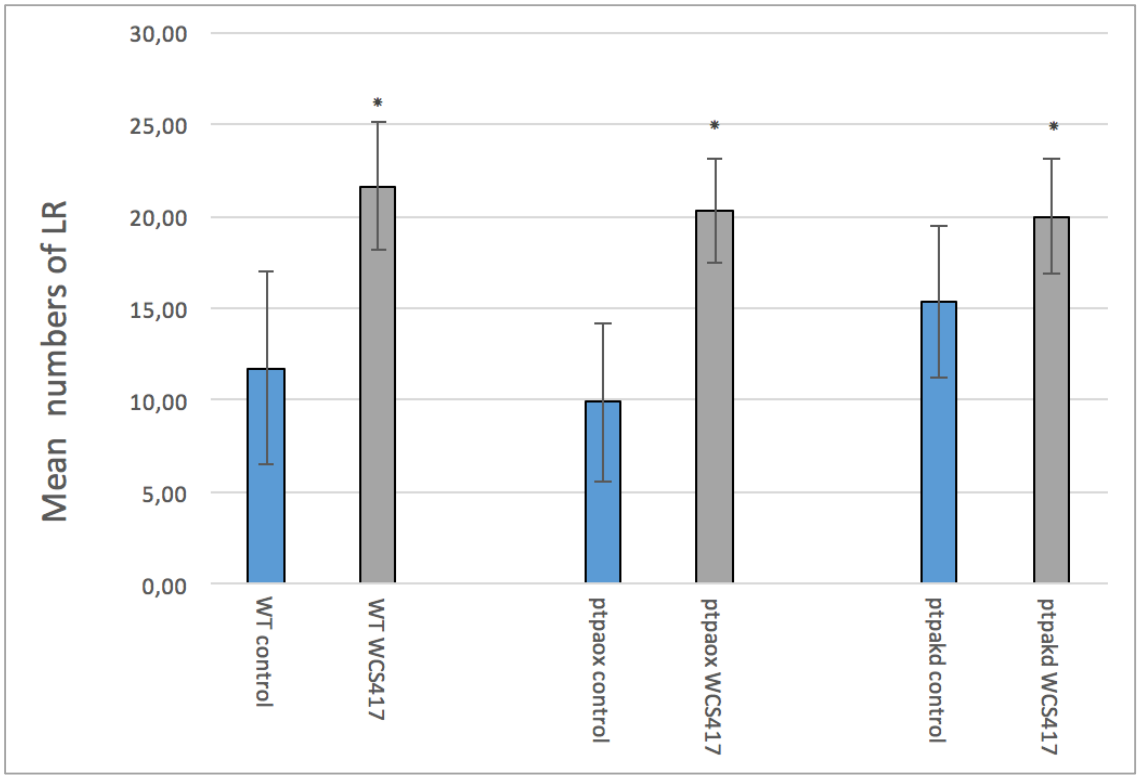

Figure 3.27: Lateral roots results from root growth assay 3 *Pseudomonas* WCS417.

Mean numbers of lateral roots on A. thaliana WT, $ptpa_{ox}$ and $ptpa_{kd}$ plants, 8 d after transfer to 1xMS medium (+ 0.5 % suc), inoculated with 450 µl of Pseudomonas suspension (OD_{600} = 0.005), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Lateral roots were counted visually. Bars show mean of 18-20 plants ± SD, plants grown much shorter than the others on the same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

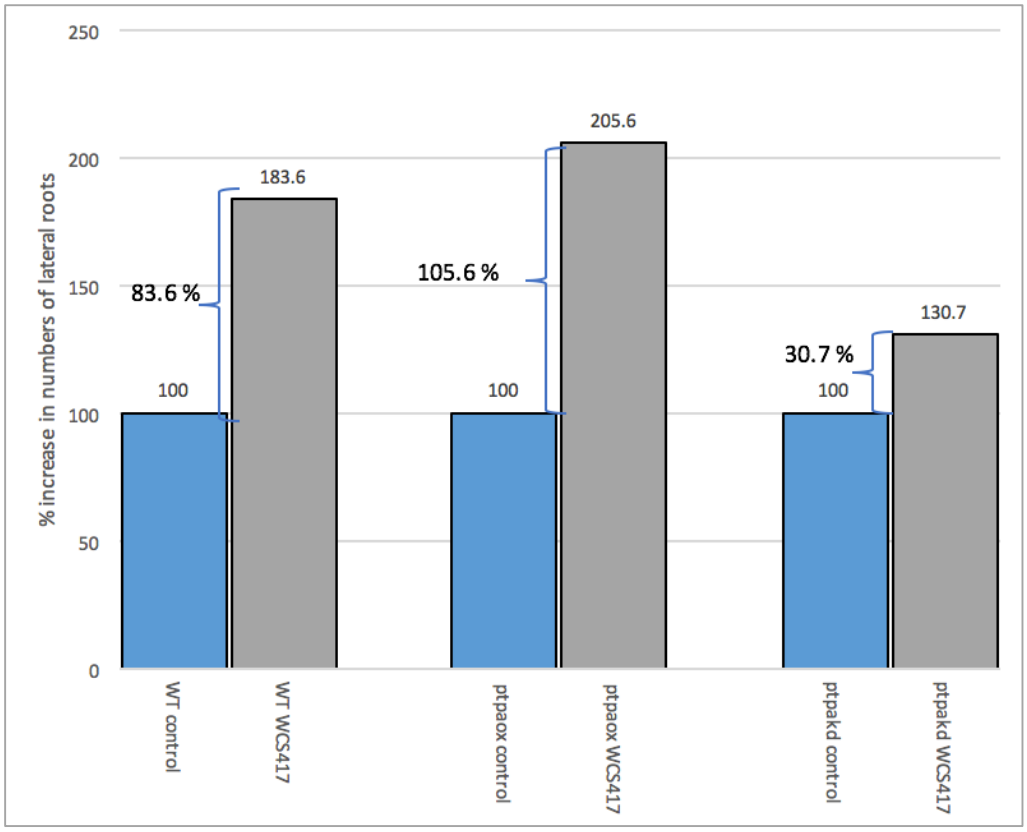

Figure 3.28: Percent increase in numbers of lateral roots.

By setting the controls as 100 %, the percent increase in numbers of lateral roots for plants inoculated with Pseudomonas WCS417 could be calculated.

For this assay the seedlings were transferred to 1xMS medium supplemented with 0.5 % sucrose, inoculated with *Pseudomonas* WCS417 with OD_{600} = 0.005 ($2x10^6$ cells/ml). This time the effect on the root system could easily be seen, with clearly inhibition of the primary roots, and promotion of the lateral roots (fig. 3.24). There was also a clear promotion of root hairs (fig. 3.25). *Pseudomonas* WCS417 significantly inhibited the length of primary roots, and significantly increased the number of lateral roots on *A. thaliana* WT, $ptpa_{ox}$, and $ptpa_{kd}$ compared to the controls (All p values = 0.0001, table 3.8, fig. 3.26 and 3.27). For WT and $ptpa_{ox}$ there was a significant increase in the mean fresh root weight per plant (p values = 0.0041 and 0.0002, respectively). There was a small increase seen in the fresh root weight for the $ptpa_{kd}$ plants, compared to the control, but this was not significant (Appendix 4, table A.3, fig. A.17). The results for the *A. thaliana* WT are in accordance with results obtained by Zamioudis et al. (2013) and Wintermans et al. (2016). However, there was no significant increase in the fresh shoot weight for the plants inoculated with *Pseudomonas* WCS417, compared to the control (Appendix 4, table A.3, fig. A.16).

A. thaliana $ptpa_{ox}$ plants inoculated with *Pseudomonas* WCS417 had a 30.7 % increase in the numbers of lateral roots, compared to the control, which was much lower compared to WT and

ptpa$_{ox}$ (fig. 3.28). This effect was not seen for the primary roots. It appears that when PTPA is lower expressed than normal, the promotion of lateral roots by the *Pseudomonas* WCS417 is not as effective as for WT and ptpa$_{ox}$, that has normal or higher expression of PTPA. As this experiment was not repeated, a definite conclusion of the PTPA involvement in the interaction between *A. thaliana* and PGPB cannot be made.

3.4 Root growth assay with a *Paenibacillus* sp. isolated from *S. pennellii*

3.4.1 Root growth assay 1 *Paenibacillus*; bacteria suspension spread on medium without sucrose.

A. thaliana WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* seeds were sown on 1xMS medium supplemented with 0.5 % sucrose. After 2 days of stratification in 4°C, the plates were placed vertically in a growth chamber (16 h light/ 8 h dark). After 5 d in the growth chamber, the seedlings were transferred to 1xMS medium without sucrose, that were inoculated with 450 μl of *Paenibacillus* suspension (OD_{600} = 0.6, $3x10^8$ cells/ml), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, 5 seedlings per plate, 2 plate for each treatment. After additional 8 d in the growth chamber, the plates were taken out and photographed (fig. 3.29). Images of the root tips were taken using a Leica microscope (fig. 3.30). Primary roots were measured using ImageJ, and lateral roots were visually counted (table 3.9). Fresh shoot and root weight were measured (Appendix A4). Data in table 3.9 were used to make graphical illustrations (fig 3.31 and 3.32). An unpaired student's t-test was performed with a t-test calculator (GraphPad QuickCalcs Web Site), to see if there were any significant differences between the control plants, and plants inoculated with bacteria. Table 3.9 includes the p-values from this test.

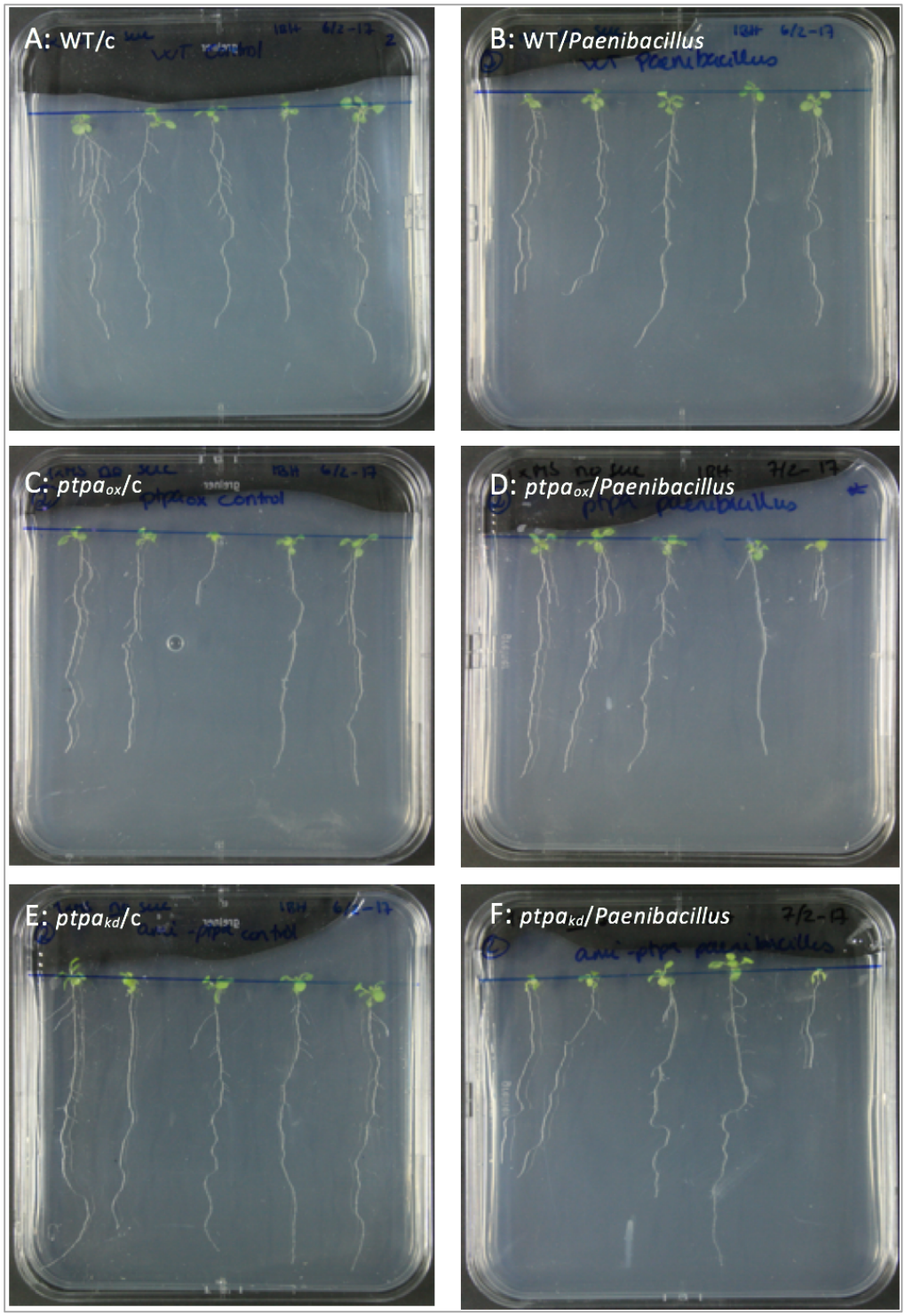

Figure 3.29: Representative pictures of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, 8 d after inoculation with a *Paenibacillus* sp.

A. thaliana WT, ptpa$_{ox}$ and ptpa$_{kd}$ plants, 8 d after inoculation with 450 µl of a Paenibacillus suspension (OD$_{600}$ = 0.6), or 10 mM MgSO$_4$·7H$_2$O for control. A: WT control. B: WT + Paenibacillus C: ptpa$_{ox}$ control. D: ptpa$_{ox}$ + Paenibacillus. E: ptpa$_{kd}$ control. F: ptpa$_{kd}$ + Paenibacillus

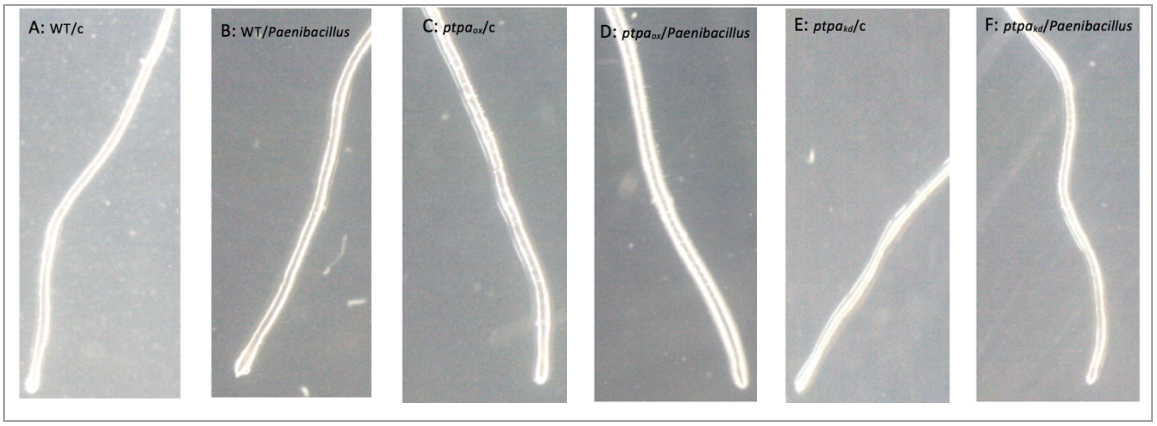

Figure 3.30: Representative images of root tips of *A.* thaliana WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, 8 d after inoculation with a *Paenibacillus* sp.

Root tips of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ plants, 8 d after inoculation with Paenibacillus (OD_{600} = 0.6), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: WT control. B: WT + Paenibacillus. C: ptpa$_{ox}$ control. D: ptpa$_{ox}$ + Paenibacillus. E: ptpa$_{kd}$ control. F: ptpa$_{kd}$ + Paenibacillus. Images were taken with a Leica microscope.

Table 3.9 Data for root growth assay 1 *Paenibacillus.*

Mean length of primary root, and numbers of lateral roots for A. thaliana WT, ptpa$_{ox}$ and ptpa$_{kd}$ plants, 8 d after inoculation with 450 µl of a Paenibacillus suspension (OD_{600} = 0.6), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, with corresponding standard deviations (SD). N is number of plants for calculation. An unpaired Student's t-test has been performed to find the p-values.

Type of plants and treatment	Mean length of primary root, cm	SD, primary root	Mean numbers of lateral root	SD, lateral roots	N	p-values, primary roots (compared to control)	p-values, lateral roots (compared to control)
WT control	7.19	0.75	14.67	5.25	9		
WT *Paenibacillus*	6.67	0.48	8.67	3.94	9	0.0989	0.0145*
***ptpa$_{ox}$* control**	6.88	0.64	7.78	4.49	9		
ptpa$_{ox}$ Paenibacillus	6.73	0.62	8.71	4.27	7	0.6447	0.6811
***ptpa$_{kd}$* control**	8.64	0.47	15.88	5.56	8		
ptpa$_{kd}$ Paenibacillus	6.53	1.39	6.50	6.30	8	0.0012*	0.0070*

* Statistically significant compared to control, $p < 0.05$.

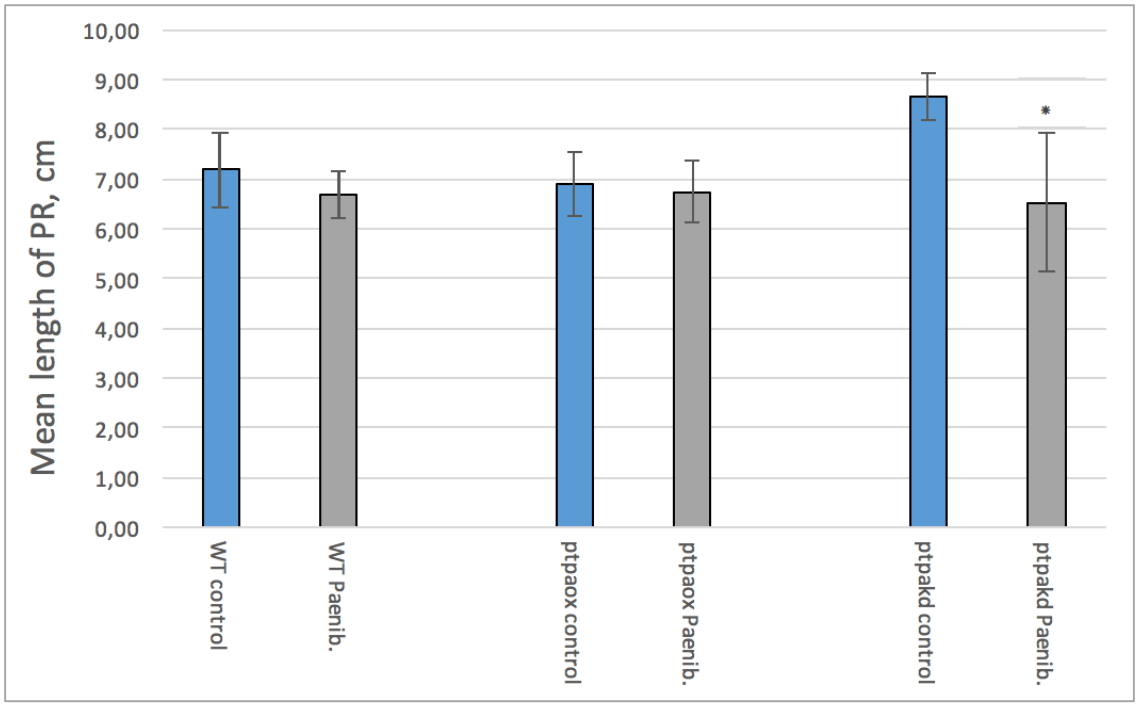

Figure 3.31: Primary root results for root growth assay 1 *Paenibacillus*

Mean primary root length of A. thaliana WT, $ptpa_{ox}$ and $ptpa_{kd}$ plants, 8 d after transfer to 1xMS medium (- suc), inoculated with 450 µl of a Paenibacillus suspension (OD_{600} = 0.6), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Length of primary roots were measured using ImageJ. Bars show mean of 7-9 plants ± SD, plants grown much shorter than the others on the same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

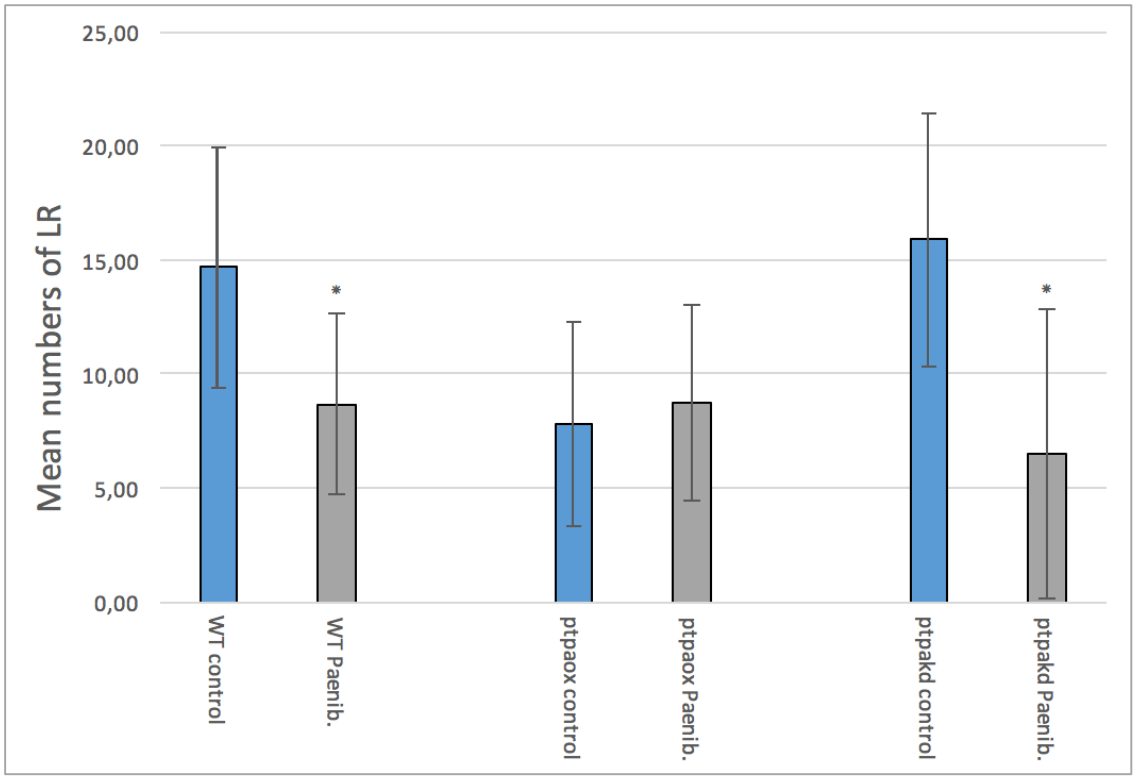

Figure 3.32: Lateral roots results for root growth assay 1 *Paenibacillus*

Mean numbers of lateral roots on A. thaliana WT, $ptpa_{ox}$, and $ptpa_{kd}$ plant, 8 d after transfer to 1xMS medium (-suc), inoculated with 450 µl of a Paenibacillus suspension (OD = 0.6), 10 mM $MgSO_4 \cdot 7H_2O$ for control. Lateral roots were counted visually. Bars show mean of 7-9 plants ± SD, plants grown much shorter than others on the same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

The *Paenibacillus* sp. did not give any significant difference in the length of primary roots for *A. thaliana* WT plants, compared to the WT control (fig.3.31). However, it did significantly inhibit the numbers of lateral roots on the WT plants, compared to the control (p value = 0.0145, table 3.9, fig. 3.32). There seemed to be a reduction in fresh shoot and root weight of WT plants, when inoculated with the bacteria, compared to the control, but the significance of these results could not be calculated (Appendix 4, table A.4, fig A.18 and A.19).

For the *A. thaliana* $ptpa_{ox}$ plants, there were no significant difference, neither in the length of primary root, or the numbers of lateral roots, between plants inoculated with bacteria, and the control (table 3.9, fig. 3.31 and 3.32). The fresh shoot and root weight seemed to increase when inoculated with bacteria, but the significance of these results was not calculated (Appendix 4, table A.4, fig. A.18 and A.19).

The *Paenibacillus* sp. significantly inhibited the primary roots on the *A. thaliana* $ptpa_{kd}$ plants compared to the $ptpa_{kd}$ control (p value = 0.0012, table 3.9, fig 3.31). In addition, there were significantly fewer lateral roots on the *A. thaliana* $ptpa_{kd}$ plants inoculated with bacteria, compared

to the control plants (p value = 0.0070, table 3.9, fig. 3.32). A decrease in the fresh shoot weight, and a small increase in the fresh root weight were seen, but the significance of these results could not be calculated (Appendix 4, table A.4, fig. A.18 and A.19).

The *Paenibacillus* sp. did not promote growth of root hairs, neither for WT or the mutant plants. (fig. 3.30).

To see if sucrose would have any effect, as this appeared to have a positive effect for the *Pseudomonas* WCS417 root growth assay, it was decided to transfer the seedlings to medium supplemented with sucrose for the next experiment.

3.4.2 Root growth assay 2 *Paenibacillus*; bacteria suspension spread on medium supplemented with sucrose.

A. thaliana WT seeds were sown on 1xMS medium supplemented with 0.5 % sucrose. After 2 days of stratification in 4°C, the plates were placed vertically in a growth chamber (16 h light/8 h dark). After 5 d in the growth chamber, the seedlings were transferred to 1xMS medium, supplemented with 0.5 % sucrose, that were inoculated with 450 µl of *Paenibacillus* suspension (OD_{600} = 0.3, $1.5x10^8$ cells/ml), or 10 mM $MgSO_4 \cdot 7H_2O$ for control; 5 seedlings per plate, 4 plates for each treatment. After additional 8 d in the growth chamber, the plates were taken out and photographed (fig. 3.33). Images were taken of the root tips using a Leica microscope (fig. 3.34). Primary roots were measured using ImageJ, and lateral roots were visually counted (table 3.10). Fresh shoot and root weight was measured (Appendix A4). Data in table 3.10 were used to make graphical illustrations (fig. 3.35 and 3.36). An unpaired student's t-test was performed with a t-test calculator (GraphPad QuickCalcs Web Site), to see if there were any significant differences between the control plants, and plants inoculated with bacteria. Table 3.10 includes the p-values from this test.

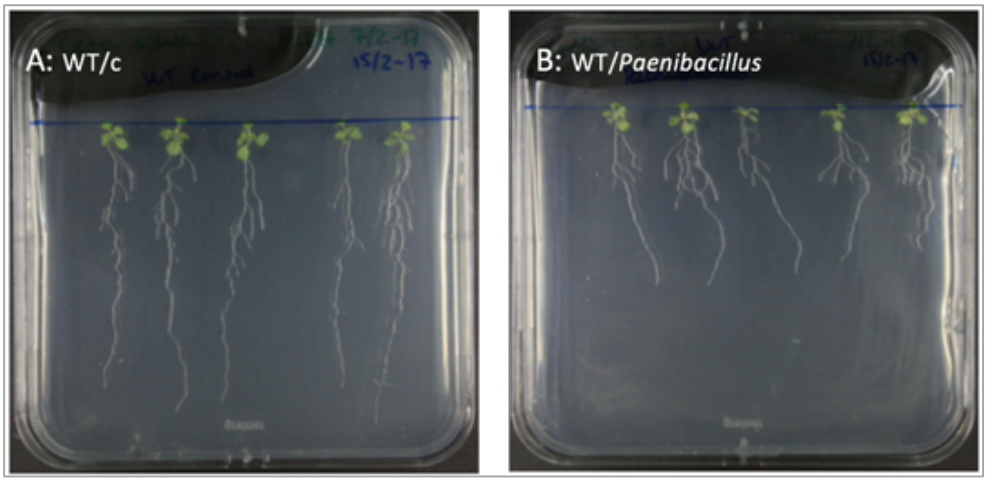

Figure 3.33: Representative pictures of *A. thaliana* WT plants, 8 d after inoculation with a *Paenibacillus* sp.

A. thaliana WT plants, 8 d after inoculation with 450 µl of a Paenibacillus suspension (OD_{600} = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: WT control. B: WT + Paenibacillus.

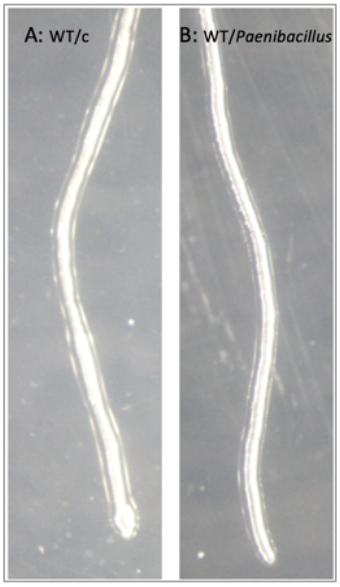

Figure 3.34: Representative images of root tips of *A. thaliana* WT plants, 8 d after inoculation with a *Paenibacillus* sp.

A. thaliana WT root tips, 8 d after inoculation with Paenibacillus (OD_{600} = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: WT control. B: WT + Paenibacillus. Images were taken with a Leica microscope.

Table 3.10: Data for root growth assay 2 *Paenibacillus*

Mean length of primary root, and numbers of lateral roots for A. thaliana WT plants, 8 d after inoculation with 450 µl of a Paenibacillus suspension (OD_{600} = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, with corresponding standard deviations (SD). N is number of plants for calculation. An unpaired Student's t test has been performed to find the p-values.

Type of plants and treatment	Mean length of primary root, cm	SD, primary root	Mean numbers of lateral roots	SD, lateral roots	N	p-value, primary root (compared to control)	p-value, lateral roots (compared to control)
WT control	7.47	0.43	13.05	4.24	20		
WT *Paenibacillus*	5.27	0.60	10.15	3.07	20	0.0001*	0.0178*

* Statistically significant compared to control, p < 0.05.

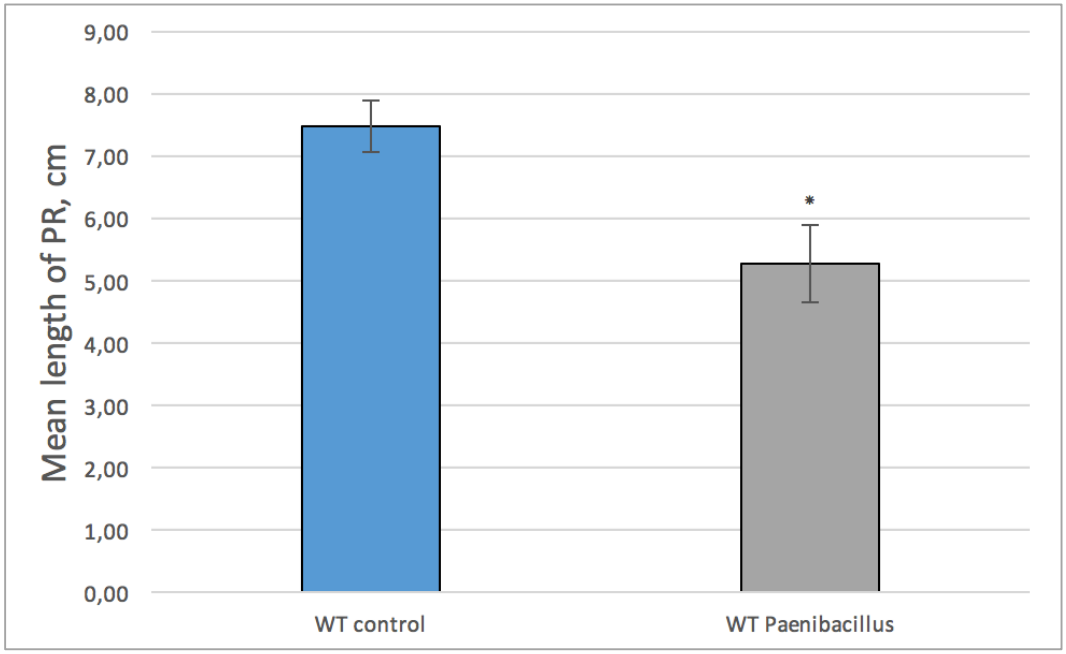

Figure 3.35: Primary root results from root growth assay 2 Paenibacillus

Mean length of primary roots of A. thaliana WT plants, 8 d after transfer to 1xMS medium (+ 0.5 % suc), inoculated with 450 µl of Paenibacillus suspension (OD600 = 0.3), or 10 mM MgSO4·7H2O for control. Length of primary roots were measured using ImageJ. Bars show mean of 20 plants ± SD.

** Statistically significant compared to control, p < 0.05.*

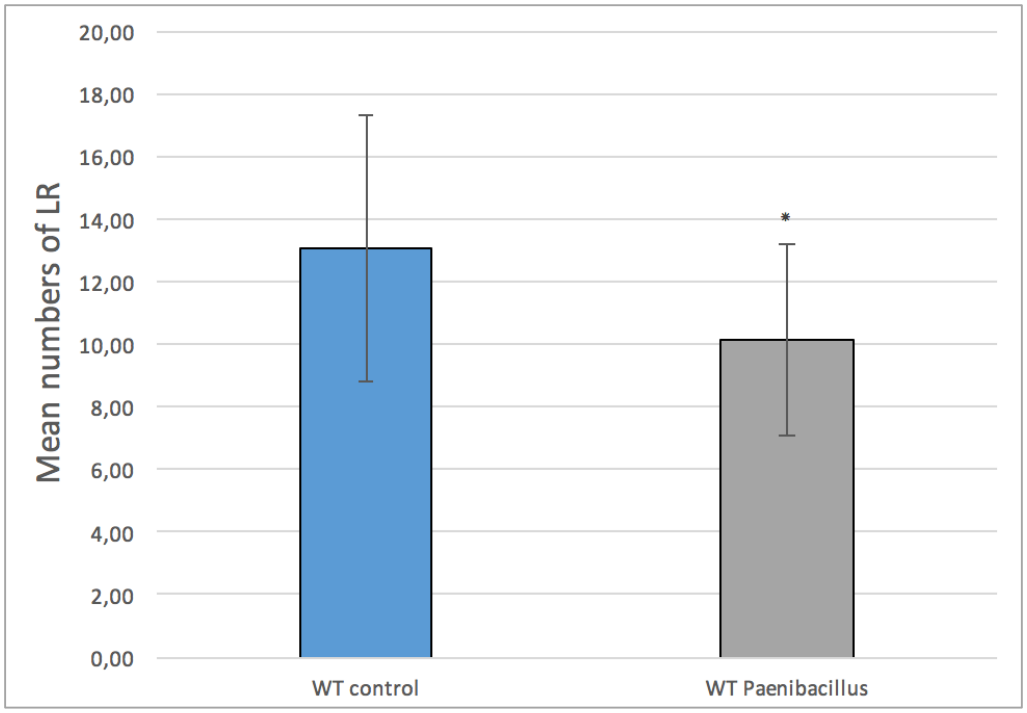

Figure 3.36: Lateral root results from root growth assay 2 *Paenibacillus* (lateral roots)

Mean numbers of lateral roots on A. thaliana WT plants, 8 d after transfer to 1xMS medium (+ 0.5 % suc), inoculated with 450 µl of bacteria suspension (OD_{600} = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Lateral roots were counted visually. Bars show mean of 20 plants ± SD.

** Statistically significant compared to control, p < 0.05.*

A. thaliana WT plants inoculated with the *Paenibacillus* sp. had significantly shorter primary roots compared to the control (p value = 0.0001, table 3.10, fig. 3.35). They had also significant less lateral roots compared to the control (p value = 0.0178, table 3.10, fig. 3.36). The fresh root and shoot weights were significant lower, compared to control (p values = 0.0362 and 0.0075, respectively, Appendix 4, table A.5, fig. A.20 and A.21). Inoculation with the *Paenibacillus* sp. did not promote growth of root hairs (fig. 3.34). Since there were significant differences between control plants and plants inoculated with the *Paenibacillus* sp., it was decided to do another experiment to try to reproduce the results. In addition, $ptpa_{ox}$ and $ptpa_{kd}$ plants were added to the next experiment.

3.4.3 Root growth assay 3 *Paenibacillus*; bacteria suspension spread on medium supplemented with sucrose.

A. thaliana WT, $ptpa_{ox}$, and $ptpa_{kd}$ seeds were sown on 1xMS medium supplemented with 0.5 % sucrose. After 2 days of stratification in 4°C, the plates were placed vertically in a growth chamber (16 h light/ 8 h dark). After 5 d in the growth chamber, the seedlings were transferred to 1xMS medium supplemented with 0.5 % sucrose, that were inoculated with 450 µl of Paenibacillus

suspension (OD = 0.3, $1.5x10^8$ cells/ml), or 10 mM $MgSO_4 \cdot 7H_2O$ as control, 5 seedlings per plate, 4 plates for each treatment. After additional 8 d in the growth chamber, the plates were taken out and photographed (fig. 3.37). Images were taken of the root tips with a Leica microscope (fig. 3.38). Primary roots were measured using ImageJ, and lateral roots were visually counted (table 3.11). Fresh shoot and root weight was measured (Appendix A4). Data in table 3.11 were used to make graphical illustrations (fig. 3.39 and 3.40). An unpaired student's t-test was performed with a t-test calculator (GraphPad QuickCalcs Web Site), to see if there were any significant differences between the control plants, and plants inoculated with bacteria. Table 3.11 includes the p-values from this test.

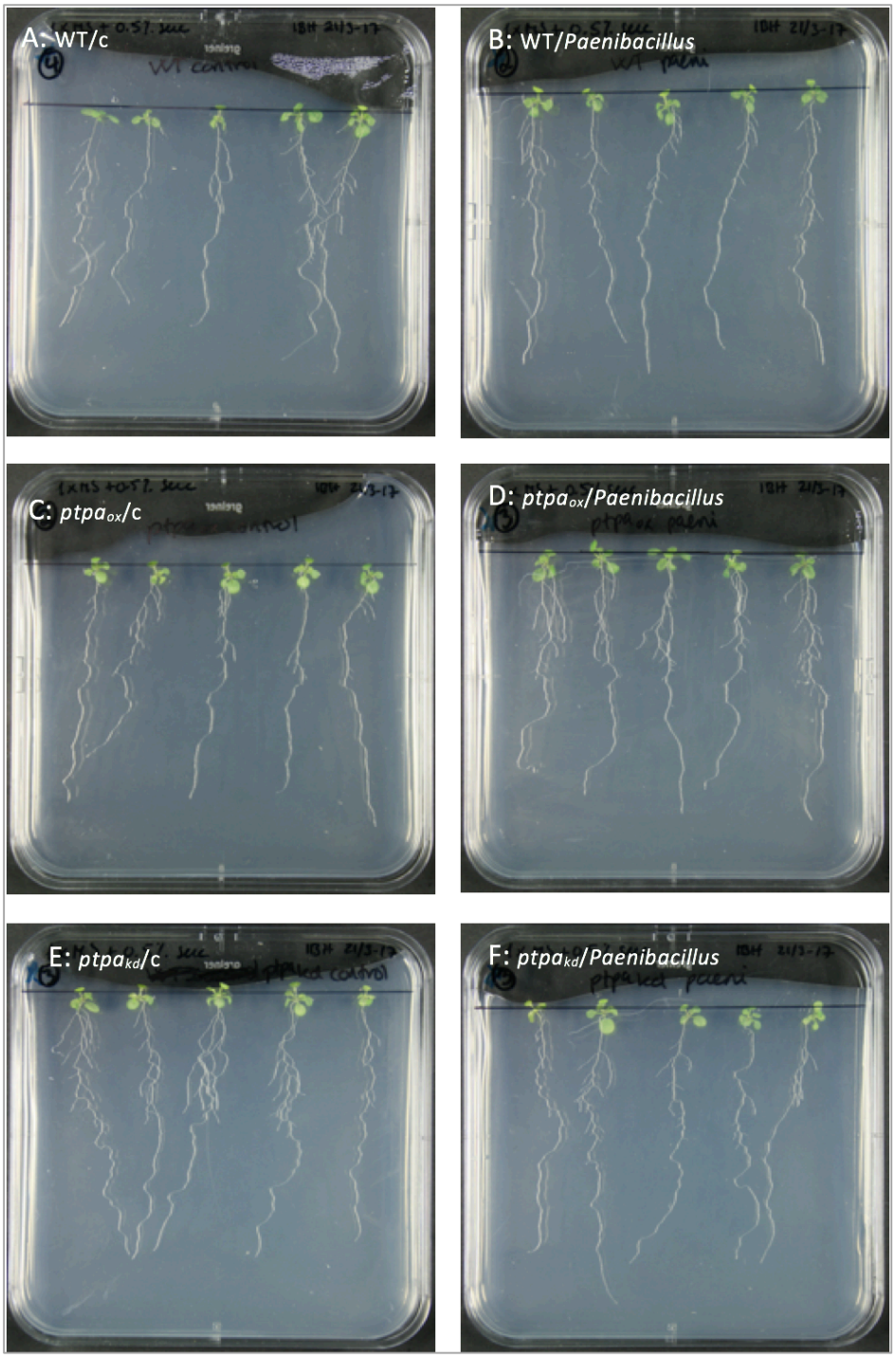

Figure 3.37: Pictures of *A. thaliana* WT, *ptpa*$_{ox}$, and *ptpa*$_{kd}$ plants, 8 d after inoculation with a *Paenibacillus* sp.

A. thaliana WT, ptpa$_{ox}$ *and ptpa*$_{kd}$ *plants, 8 d after transfer to 1xMS medium (+0.5 % suc), inoculated with 450 µl of a Paenibacillus suspension (OD*$_{600}$ *= 0.3), or 10 mM* $MgSO_4 \cdot 7H_2O$ *for control. A: WT control. B: WT + Paenibacillus. C: ptpa*$_{ox}$ *control. D: ptpa*$_{ox}$ *+ Paenibacillus. E: ptpa*$_{kd}$ *control. F: ptpa*$_{kd}$ *+ Paenibacillus.*

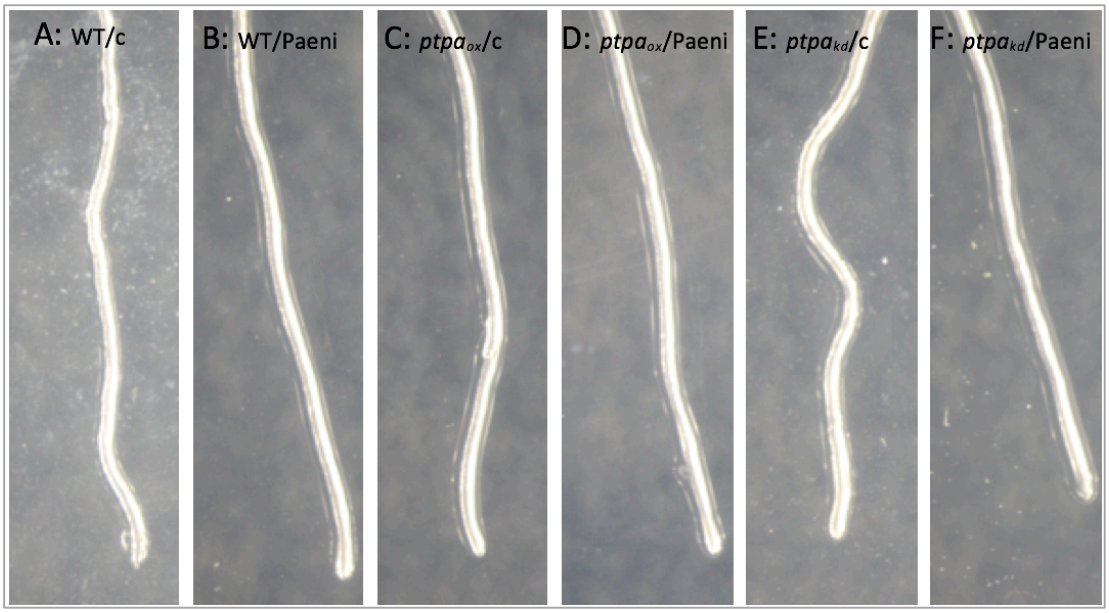

Figure 3.38: Representative images of root tips of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, 8 d after inoculation with a *Paenibacillus* sp.

Root tips of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ plants, 8 d after inoculation with 450 µl of Paenibacillus (OD$_{600}$ = 0.3), or 10 mM MgSO$_4$·7H$_2$O for control. A: WT control. B: WT + Paenibacillus. C: ptpa$_{ox}$ control. D: ptpa$_{ox}$ + Paenibacillus. E: ptpa$_{kd}$ control. F: ptpa$_{kd}$ + Paenibacillus.

Table 3.11: Data for root growth assay 3 *Paenibacillus*

Mean length of primary root, and numbers of lateral roots for A. thaliana WT, ptpa$_{ox}$ and ptpa$_{kd}$ plants, 8 d after transfer to 1xMS medium (+ 0.5 % suc), inoculated with 450 µl a Paenibacillus suspension (OD$_{600}$ = 0.3), or 10 mM MgSO$_4$·7H$_2$O for control, with corresponding standard deviations (SD). N is number of plants for calculation. An unpaired Student's t test has been performed to find the p-values.

Type of plants and treatment	Mean length of primary root, cm	SD, primary root	Mean numbers of lateral root	SD, lateral roots	N	p-values, primary root (compared to control)	p-values, lateral roots (compared to control)
WT control	7.20	0.74	12.56	5.59	18		
WT *Paenibacillus*	7.45	0.65	13.05	3.58	20	0.2749	0.7470
***ptpa$_{ox}$* control**	6.78	0.71	8.26	4.52	19		
ptpa$_{ox}$ Paenibacillus	7.66	0.49	13.84	4.52	19	0.0001*	0.0005*
***ptpa$_{kd}$* control**	9.16	0.68	16.06	4.86	18		
ptpa$_{kd}$ Paenibacillus	8.93	0.72	13.94	4.48	17	0.3381	0.1896

* Statistically significant compared to control, $p < 0.05$.

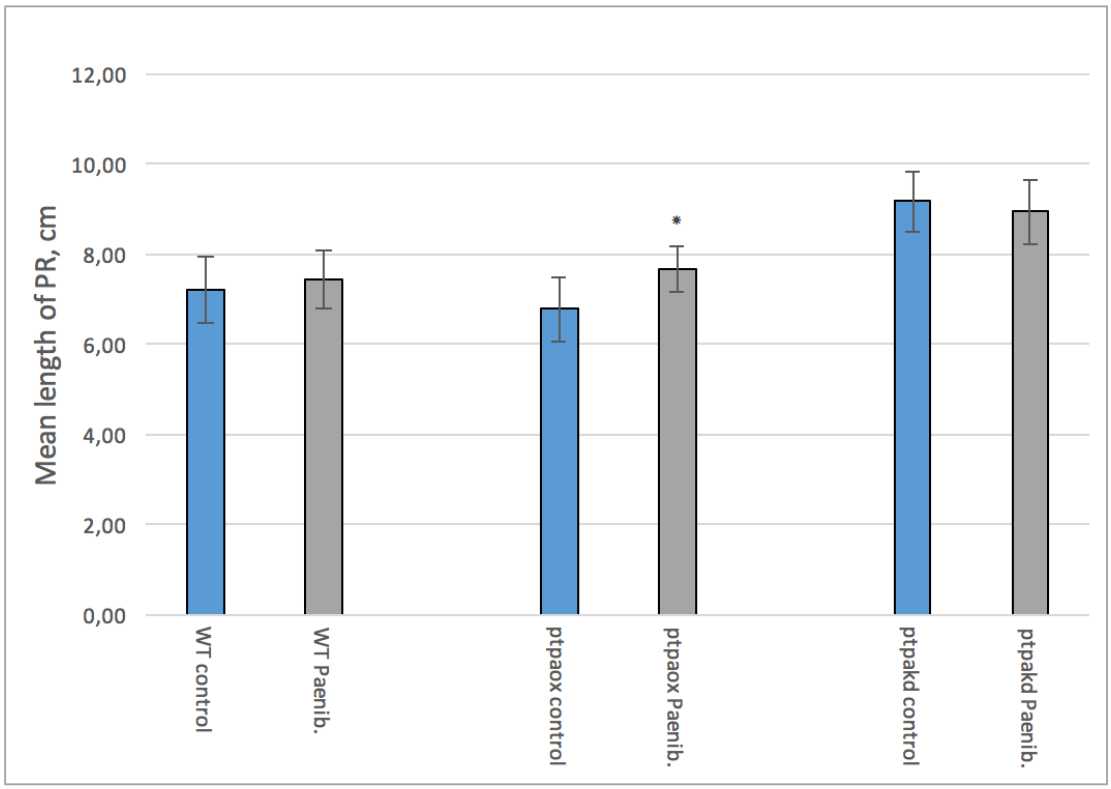

Figure 3.39: Primary root results for root growth assay 3 *Paenibacillus*

Mean length of primary root of A. thaliana WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, 8 d after transfer to 1xMS medium (+ 0.5 % suc), inoculated with 450 µl of a Paenibacillus suspension (OD_{600} = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Length of primary roots were measured using ImageJ. Bars show mean of 17-20 plants ± SD, plants grown much shorter than others on the same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

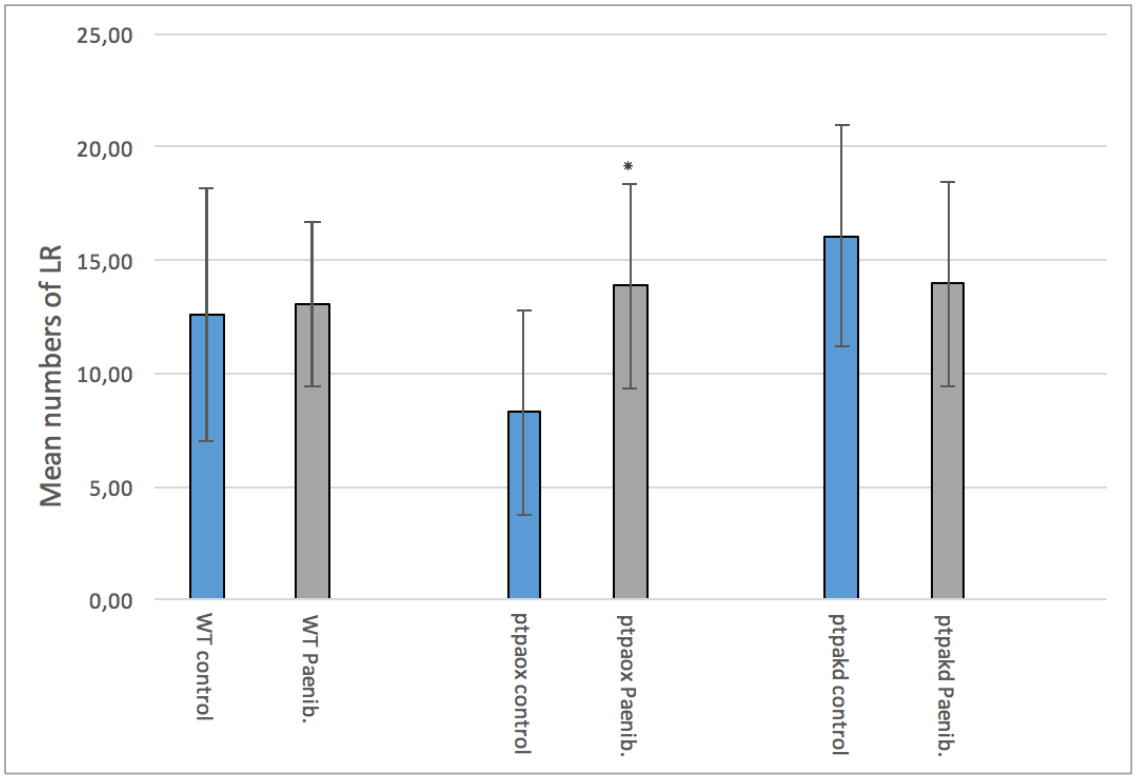

Figure 3.40: Lateral roots results for root growth assay 3 *Paenibacillus*

Mean numbers of lateral roots on A. thaliana WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, 8 d after transfer to 1xMS medium (+ 0.5 % suc), inoculated with 450 µl of a Paenibacillus suspension (OD_{600} = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Lateral roots were counted visually. Bars show mean of 17-20 plants ± SD, plants grown much shorter than others on the same plate were not taken into calculations.

** Statistically significant compared to control, $p < 0.05$.*

The *Paenibacillus* sp. had no significant effect on the length of primary roots, or the numbers of lateral roots, for the *A. thaliana* WT and $ptpa_{kd}$ plants, compared to the control plants (fig. 3.39 and 3.40). The fresh weight of *A. thaliana* $ptpa_{kd}$ shoots inoculated with the bacterium was significant lower compared to control (p value = 0.0381, Appendix 4, table A.6, fig. A.22).

A. thaliana $ptpa_{ox}$ plants inoculated with the *Paenibacillus* sp. had significant longer primary roots compared to $ptpa_{ox}$ control plants (p value = 0.0001, table 3.11, fig. 3.39). They also had more lateral roots compared to the control (p value = 0.0005, table 3.11, 3.40). This led to an increase in the fresh root weight, and there was also an increase in the fresh shoot weight for the $ptpa_{ox}$ plants inoculated with the bacterium (p values =0.0151 and 0.0038, respectively, Appendix 4, table A.6, fig A.22 and A.23).

As for previous experiments with the *Paenibacillus* sp., no effect on the root hairs were seen (fig. 3.38), The results from the previous experiment were not repeated in this experiment, the results were variable. However, it does not appear that the *Paenibacillus* sp. has a positive effect on the root system of *A. thaliana.*

3.5 Growth experiment with tomato

3.5.1 Heinz and Moneymaker tomatoes inoculated with *Pseudomonas* WCS417 or a *Paenibacillus* sp.

Heinz and Moneymaker plants (around 3-weeks old), were inoculated with *Pseudomonas* WCS417 (OD_{600} = 0.027), *Paenibacillus* (OD_{600} = 0.026), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Pictures of the plants were taken once a week (fig. 3.41-3.44). Four weeks after inoculation, the shoots were weighed, and the primary stem measured (fig. 3.45, table 3.12). The data in table 3.12 were used to make graphical illustrations (fig. 3.46 and 3.47). An unpaired student's t-test was performed with a t-test calculator (GraphPad QuickCalcs Web Site), to see if there were any significant differences between the control plants, and plants inoculated with bacteria. Table 3.12 includes the p-values from this test.

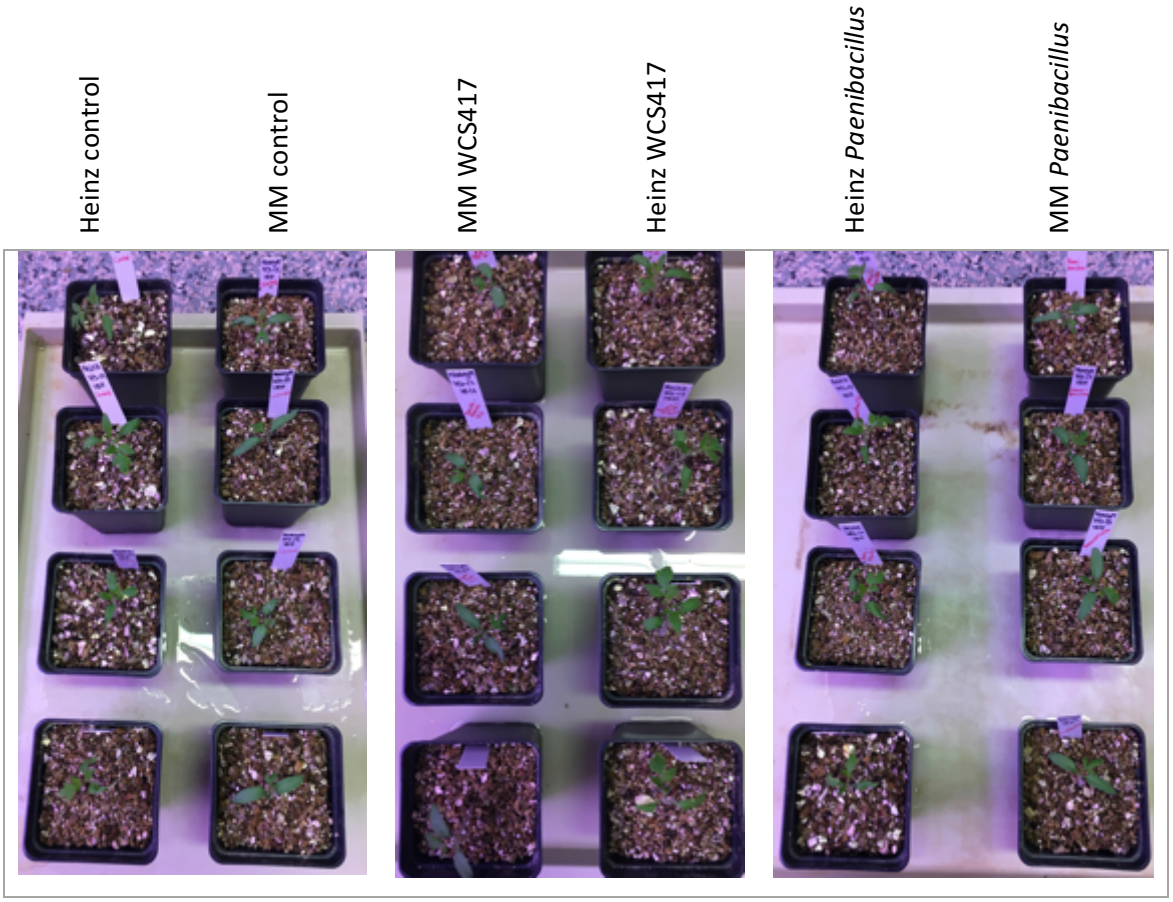

Figure 3.41: Heinz and Moneymaker plants on day of inoculation with bacteria.

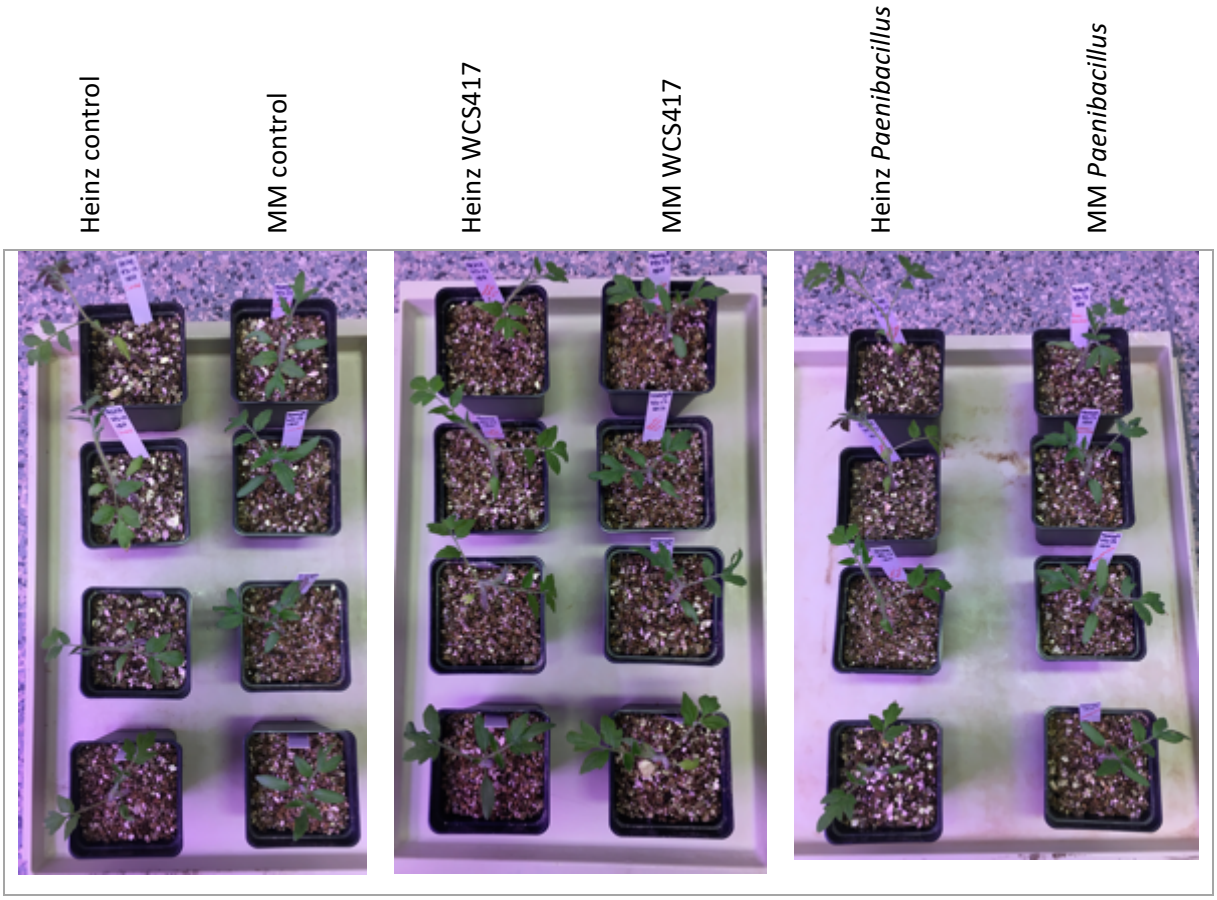

Figure 3.42: Heinz and Moneymaker plants one week after inoculation with bacteria.

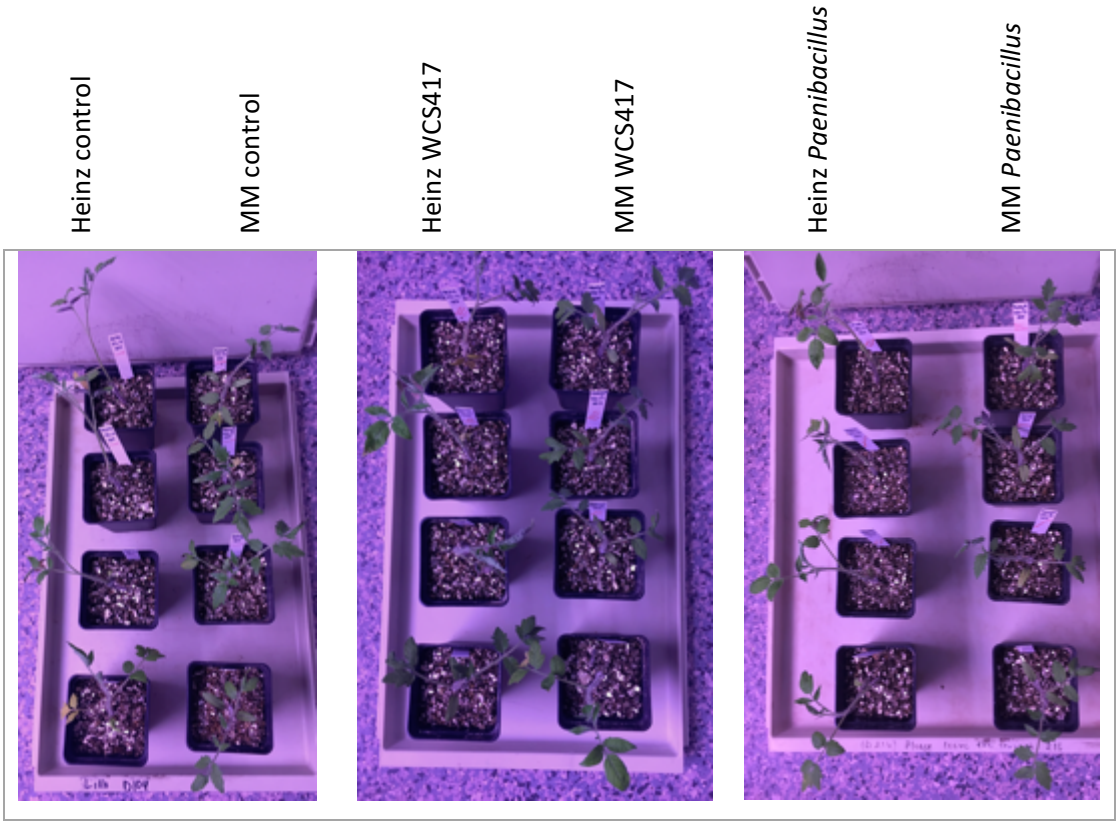

Figure 3.43: Heinz and Moneymaker plants two weeks after inoculation with bacteria.

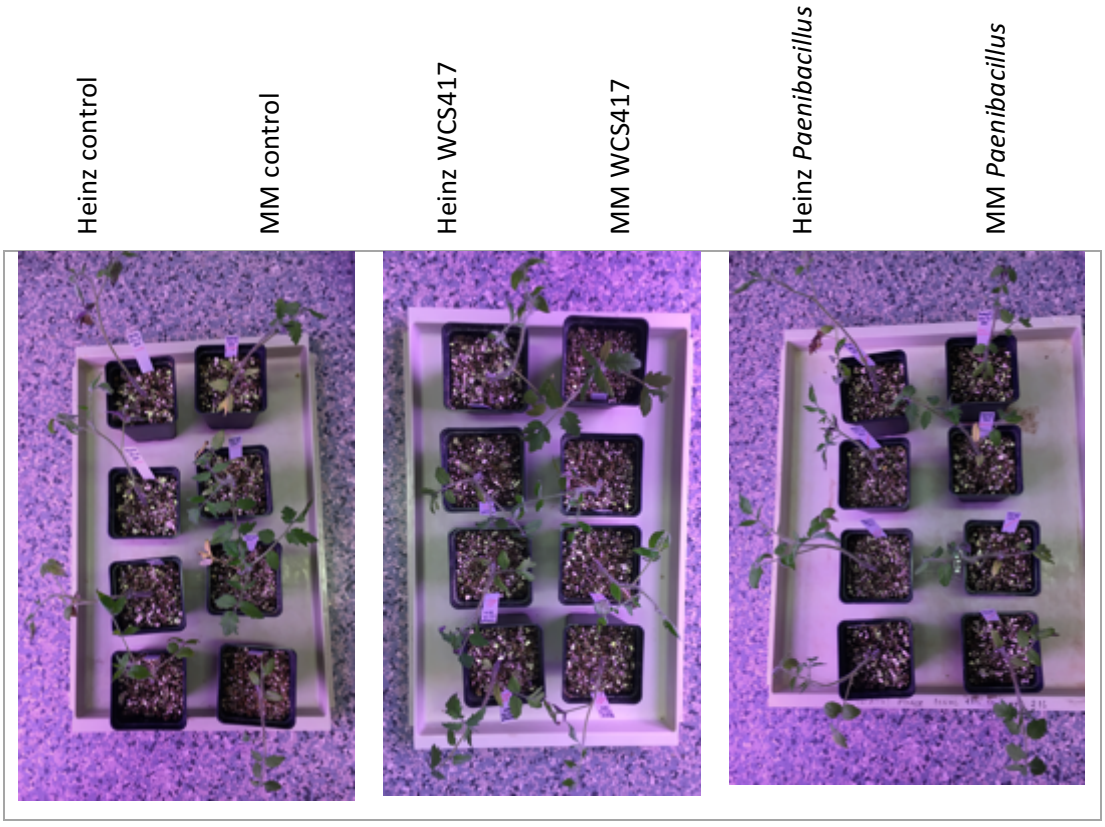

Figure 3.44: Heinz and Moneymaker plants three weeks after inoculation with bacteria.

Figure 3.45: Representative pictures of shoots, 4-weeks after inoculation with bacteria.

Shoots of Heinz and Moneymaker plants, 4-weeks after inoculation with Pseudomonas WCS417 (OD_{600} = 0.027), Paenibacillus (OD_{600} = 0.026), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. A: Heinz control. B: Heinz + Pseudomonas WCS417. C: Heinz + Paenibacillus. D: MM control. E: MM + Pseudomonas WCS417. F: MM + Paenibacillus.

Table 3.12: Data for growth assay with Heinz and Moneymaker tomato plants.

Mean length of primary stems, and mean weight of shoots of Heinz and Moneymaker, 4 weeks after inoculation with Pseudomonas WCS417 (OD_{600} = 0.027), Paenibacillus (OD_{600} = 0.026), or 10 mM $MgSO_4 \cdot 7H_2O$ for control, with corresponding standard deviations (SD). N is number of plants for calculation. An unpaired Student's t test has been performed to find the p-values.

Type of plants and treatment	Mean length of primary stem, cm	SD, primary stem	Mean weight of shoots, g	SD, shoots	N	p-values primary stem (compared to control)	p-values shoots (compared to control)
Heinz, control	21.50	0.50	0.3866	0.1002	4		
Heinz, *Pseudomonas*	23.00	2.92	0.4048	0.1326	4	0.3503	0.8339
Heinz, *Paenibacillus*	24.75	5.89	0.4948	0.0357	4	0.3137	0.0881
Moneymaker, control	14.00	1.22	0.7412	0.3164	4		
Moneymaker, *Pseudomonas*	14.50	1.12	0.8853	0.1763	4	0.5681	0.4565
Moneymaker, *Paenibacillus*	14.50	1.12	0.8014	0.1565	4	0.5681	0.7447

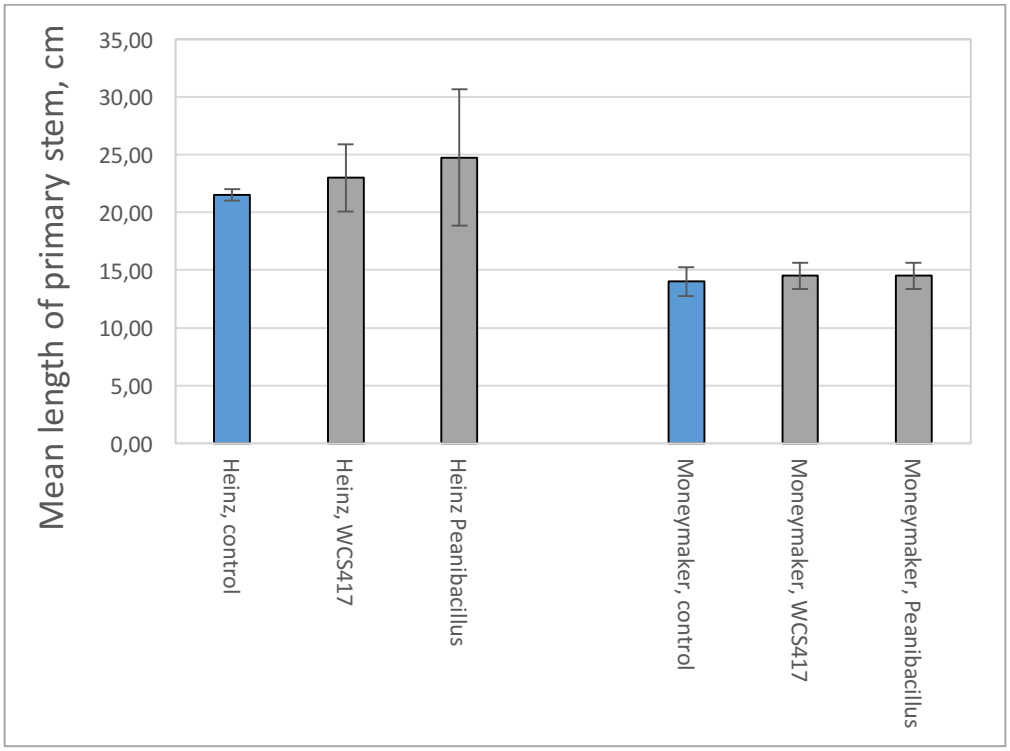

Figure 3.46: Results (primary stem) from growth assay with Heinz and Moneymaker tomato plants

Mean length of primary stem of Heinz and Moneymaker tomato plants, 4 weeks after inoculation with Pseudomonas WCS417 (OD_{600} = 0.027), Paenibacillus (OD_{600} = 0.026), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Bars show mean of 4 plants ± SD.

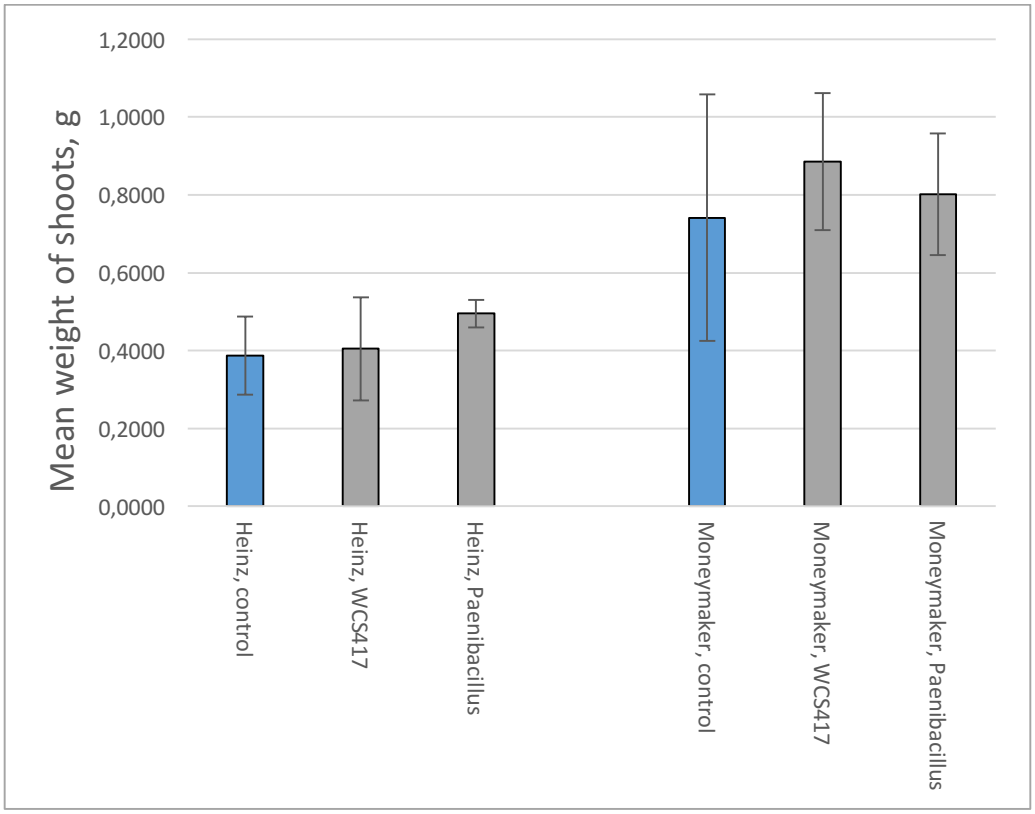

Figure 3.47: Results (shoots) of growth assay with Heinz and Moneymaker tomato plants

Mean weight of shoots of Heinz and Moneymaker tomato plants, 4 weeks after inoculation with Pseudomonas WCS417 (OD_{600} = 0.027), Paenibacillus (OD_{600} = 0.026), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Bars show mean of 4 plants ± SD.

No visually effect of *Pseudomonas* WCS417 and the *Paenibacillus* sp. could be seen in this experiment (fig. 3.41-4.45). This was also reflected in the measurements.

The mean length of the stem for Heinz tomato plants was slightly longer on the plants inoculated with bacteria, but the differences were not significant (table 3.12, fig 3.46). The mean weight of shoots of Heinz plants was higher for plants inoculated with the *Paenibacillus* sp., compared to the control, but the difference was not significant (table 3.12, fig. 3.47).

No significant differences were found for the mean length of the stem for the Moneymaker plants inoculated with bacteria, compared to the control (table 3.12, fig. 3.46). The mean weight of shoots of the Moneymaker plants was higher for the plants inoculated with bacteria, compared to the control, but these differences were not significant (table 3.12, fig. 3.47).

Pseudomonas WCS417 and the *Paenibacillus* sp. did not seem to affect the growth of the tomato plants. However, they did not seem to harm the plants in any way either, all control plants and plants inoculated with bacteria showed sign of stress (fig. 4.45), most likely due to mineral deficiency, as the plants were not added any nutrients solution after inoculation.

4 Discussion

4.1 Isolation and sequencing of endophytic bacteria from *S. pennellii*

Thousands of bacteria with PGP traits have been isolated. An important reason for this work has been to find alternative, environmentally safer methods to increase productivity in agriculture, instead of the use of chemicals (Abbamondi et al. 2016). The PGPB, both endophytic and rhizopheric bacteria, have been isolated from a vast amount of different plants and environments. Here two endophytic bacteria were isolated from the wild tomato *S. pennellii* (labelled 1 and C). By sequencing the 16S rRNA, bacteria C was identified as a *Micrococcus* sp., and bacteria 1 as a *Paenibacillus* sp. As several reference strains had the same scores and identity, it was not possible to determine the exact strain for the *Micrococcus* sp. The *Paenibacillus* sp. had the highest identity with *P. typhae* strain xj7 (Kong et al. 2013), with a 99 % identity. As only parts of the 16S rRNA gene were sequenced, it was not possible to determine the exact strain. Further identification of the *Paenibacillus* sp. can possible be done by using a highly specific primer for *Paenibacillus*, PAEN515F (Shida et al. 1997), in combination with the universal primer 1377R (Shida et al. 1996).

4.2 Root growth assay with bacteria isolated by (Abbamondi et al. 2016)

The seven bacterial strains isolated from tomatoes, grown in Italy, by Abbamondi et al. (2016), which they had tested for different PGP traits, were provided for further investigation. In addition, a bacterium already isolated at this lab, labelled CL8, were tested.
This was done by applying the bacteria to *A. thaliana* WT plants, and different mutants, in different root growth assays.
First the bacteria were applied only on *A. thaliana* WT plants, in two different root growth assays. This was done to see if the effects on the root system found by Abbamondi et al. (2016) could be reproduced in this lab, and to decide which bacteria it would be interesting to test on mutant plants. The results from these two root growth assays were variable, and it was difficult to reproduce results previously obtained by (Abbamondi et al. 2016). In assay 2 there seemed to be a small positive effect with the plants inoculated with bacterial strain 18. This strain was also one of the strains with highest activity of all the PGP traits tested (table 2 Abbamondi et al. 2016). However, when deciding which bacteria to go ahead with in further experiments, results from three students, working simultaneously, were compared (personal discussions with fellow students and supervisor). After these discussions, it was decided to use bacterial strains 9, 15 and CL8 for further experiments with WT and the four mutants. In the next experiment, none of the bacteria had a significant effect on the

WT plants, and the effect on the mutants were variable. As in the previous experiments, the sample sizes were too low to definite draw any conclusions about the results. With five different plant types, including WT, and several bacterial strains, it was difficult to increase the sample size for each treatment. Thus, it was decided to reduce the numbers of mutants and bacterial strains. PTPA was of special interest, since this gene was found near a SNP associated with *Pseudomonas* WCS417 mediated change in lateral root formation (Wintermans et al. 2016). As two of the mutants were an over-expressor and a knockdown of this gene, $ptpa_{ox}$ and $ptpa_{kd}$ respectively, it was decided that these were the most interesting to use for further experiments. Also, comparing results in discussion with supervisor and other students, resulted in the decision to only use bacterial strain 15 for the next experiment.

Bacterial strain 15 did, in this experiment, significantly inhibit the growth of the primary root of WT plants, compared to control. It also seemed to inhibit the numbers of lateral roots, but this result was not significant. No significance differences were seen for $ptpa_{ox}$ and $ptpa_{kd}$, neither for primary root or lateral roots. From these results, bacterial strain 15 does not appear to have any positive effect on the root system of *A. thaliana*.

The plan was to follow the methods described in Abbamondi et al. However, OD_{600} was measured on the overnight cultures, not after washing and resuspending in 10 mM $MgSO_4 \cdot 7H_2O$. The OD_{600} in the final inoculations might therefore not be exactly 0.5. The amount of inoculation also differed among some of the experiments, and was different from the volume stated by Abbamondi et al. (2016). The thought was that more inoculum was needed because larger Petri dishes were used. However, when applying 1 ml in the first experiment, it took very long time to dry, and for the next experiments, the amount was decreased to 650 µl. There were also differences in the days of growth after inoculation, due to the roots growing down to the bottom of the dish, making it difficult to do measurements. Some of the plates were contaminated, by unknown sources, and the growth was variable, and some of the plants had to be taken out of the calculations. For the first experiments, only 5 plants for each treatment were applied, and when plants had to be taken out of the calculations, the sample sizes (N) were small. In later experiment, the numbers of bacterial strains and mutants were reduced, and the sample size increased to 20 for each treatment.

The problems with the density and contamination, in addition to small sample sizes, makes it difficult to compare these results with certainty to the results obtained by Abbamondi et al. (2016). The experiments performed here also demonstrated the difficulties of reproducing results obtained by others. The seven bacterial strains isolated by Abbamondi et al. (2016) had promising PGP traits, i.e. production of OA, IAA, ACC, and siderophore, but the results obtained here implied that the effects on the roots system were variable. However, all the bacterial strains promoted root hair formation.

To be able to easily see if the genes of interest were associated with the interaction between the plant and PGPB, it would be more helpful to use bacteria that properly promoted the desirable traits; an increase of the root system. Since these traits were essential for further work, other possibilities had to be considered.
In further experiments, *Pseudomonas* WCS417, which is well-known to inhibit elongation of primary roots, and promotion of lateral roots and root hair (Zamioudis et al. 2013), was used as inoculum.

4.3 Root growth assay with *Pseudomonas* WCS417

The first experiment with *Pseudomonas* WCS417 was performed with *A. thaliana* WT plants to see if the results, regarding primary root, lateral roots, and root hairs, obtained by Zamioudis et al. (2013), could be reproduced. To follow their methods, bacteria suspension was inoculated in spots 5 cm under the root tips. The clearly inhibition of primary root elongation, and the promotion of lateral roots, obtained by Zamioudis et al. (2013), was not reproduced in this experiment. The reason for this could possibly be problems with the bacteria suspension. The OD_{600} stated by Zamioudis et al. (2013) was very low (0.002), and there were some difficulties measuring this low OD with the machine in the lab. As for the bacteria, no growth could be seen in areas inoculated, which could easily be seen in previous experiment by Zamioudis et al. (2013).
For the next experiment, the bacteria suspension was spread on the medium, as done in the root growth assays with the seven bacteria isolated by Abbamondi et al. (2016). The seedlings, WT, $ptpa_{ox}$, and $ptpa_{kd}$, were transferred to 1xMS medium without sucrose, and two different ODs (0.004 and 0.5) were tested. This assay did, as the previous, not show the desired effects of the *Pseudomonas* WCS417 as described by Zamioudis et al. (2013).

In personal conversation with supervisor of Bachelor students, working parallel with the same bacteria, a conclusion was drawn that to get the wanted effects, the seedlings should be transferred to inoculated 1xMS medium supplemented with sucrose. In previous assays, the seedlings had been transferred to 1xMS medium without sucrose.
By transferring the seedlings to medium supplemented with sucrose, the effects described by (Zamioudis et al. 2013) could be seen on the plants. There was significantly inhibition of the primary roots, and an increase in the numbers of lateral roots.
There were no large differences found between WT, $ptpa_{ox}$, and $ptpa_{kd}$ when comparing the inhibition of primary roots, all had around a 60 % decrease in the length of primary roots when inoculated with *Pseudomonas* WCS417 (data not shown). When comparing the differences in the

increase of the numbers of lateral roots, when inoculated with bacteria, there was a small difference between WT and *ptpa*$_{ox}$. However, while the numbers of lateral roots increased with around 80% for WT, and around 100% for *ptpa*$_{ox}$ plants inoculated with bacteria, compared to the controls, the numbers of lateral roots for *ptpa*$_{kd}$ only increased with 30% *ptpa*$_{kd}$ is downregulated with around 50 % (Creighton 2013). From this result, it seems that when PTPA is downregulated, the effect of the *Pseudomonas* WCS417 on the lateral roots, is smaller than for WT and *ptpa*$_{ox}$, where the PTPA is normal expressed or over-expressed. Since this effect on the lateral roots was not repeated in other root growth assays, a definite conclusion of the involvement of PTPA in the interaction between *A. thaliana* and PGPB cannot be made.

4.4 Root growth assay with a *Paenibacillus* sp. isolated from *S. pennellii*

A *Paenibacillus* sp. was isolated from *S. pennellii*, and used as inoculum in several root growth assays to see if it has any effect on the growth of primary root, lateral roots, or root hairs.
In the first root growth assay with *Paenibacillus*, *A. thaliana* WT, *ptpa*$_{ox}$, and *ptpa*$_{kd}$ seedlings were transferred to 1xMS medium without sucrose. In the second assay with the *Paenibacillus* sp., the seedlings were transferred to 1xMS medium supplemented with 0.5 % sucrose, since experiments with *Pseudomonas* WCS417 showed that sucrose might have a positive impact. Only WT plants were tested in this assay. In the third root growth assay, WT, *ptpa*$_{ox}$, and *ptpa*$_{kd}$ seedlings were transferred to inoculated 1xMS medium supplemented with sucrose.

There was no consistency in the results from assay to assay, but all in all does the *Paenibacillus* sp. not appear to increase the root system of *A. thaliana* plants. For WT, most of the results indicated that the *Paenibacillus* sp. both inhibits primary root and lateral roots formation, thus reducing the plants ability to absorb water and nutrients. Only for *ptpa*$_{ox}$ in one of the assays, did the *Paenibacillus* sp. seem to increase the root system, but this was not reproduced. The *Paenibacillus* sp. did not have any effect on the root hairs in any of the assays performed.

However, there are many other PGP traits that could be investigated; species of *Paenibacillus* have been found to fix nitrogen, some are able to make phosphorus and iron available for the plants, and others produce phytohormones. For example, over 20 species of *Paenibacillus* are found to be nitrogen fixing bacteria. By growing the isolated bacteria on nitrogen-free medium, this ability could be identified (Grady et al. 2016). Other abilities found among *Paenibacillus* spp., are the ability to trigger IRS, produce insecticides or antimicrobial compounds. Even though the isolated bacterium was not found to increase the root system, it might have other beneficial traits, under other growth

conditions, that can be exploited in the aim of finding new and eco-friendly methods for improving crop size.

4.5 Growth experiment with tomato

A growth experiment with tomato and *Pseudomonas* WCS417 was previously performed at the laboratory, where *Pseudomonas* WCS417 seemed to promote the growth of shoots/leaves (personal communication). To see if this result could be repeated, two different types of tomato plants were inoculated with *Pseudomonas* WCS417. In addition, the isolated *Paenibacillus* sp. was also inoculated on tomato plants, to see if this had any effect on the growth. The effect on the tomato plants, previously seen with *Pseudomonas* WCS417, was not repeated in this experiment. However, even if *Pseudomonas* WCS417, or the *Paenibacillus* sp., did not seem to improve the growth of the plant, neither bacteria seemed to harm the tomato plants in any way. There were visually stress on all plants, with pale yellow/green and purple/reddish leaves, due to mineral deficiency, but this was also seen on the control plants. There were also withered, and curled leaves on most of the plants. However, altogether there were no large visually difference on the leaves on the plants inoculated with bacteria compared to the control.

4.6 Summary and outlook

The main aim of the study has been to investigate whether the selected *A. thaliana* genes are involved in the interaction between the plant and the PGPB. Since there were problems in the use of the provided bacterial strains isolated by Abbamondi et al. (2016), it was not possible to use these for this aim. The *Paenibacillus* sp. isolated from *S. pennellii*, was also applied to the WT, $ptpa_{ox}$, and the $ptpa_{kd}$ plants. However, this bacterium did not seem to promote an increase of the root system, and could not be used for the investigation of the genes.

By using the well-known PGPB *Pseudomonas* WCS417, the effect with inhibition of primary root, increased lateral roots, and promotion of root hair were seen in experiment with WT, and two of the mutant plants, $ptpa_{ox}$ and $ptpa_{kd}$. The increase in number of lateral roots appeared to be much lower for $ptpa_{kd}$ plants than for WT and $ptpa_{ox}$, indicating that a low expression of PTPA negatively effects the promotion of lateral roots, when inoculated with bacteria. However, the experiment was not repeated, and a conclusion of whether PTPA is involved in the interaction between *A. thaliana* and PGPB, or not, cannot be made without further experiments. Further investigation of *lcmt1* and *pme1* should also be performed with *Pseudomonas* WCS417, as this was not done. It would also be interesting to further investigate the *Paenibacillus* sp., isolated here, for other PGP traits.

Many factors, like competing microorganisms, soil type, pH and temperature, influences the ability of a specific bacterium. Even though some bacteria are found to have PGP traits, these experiments performed here show that there is a long way from isolating PGPB in the lab, to the practical use of them in agriculture.

5 References

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Opplysningskontoret for frukt og grønt Tomat er grønnsaken med høyeste omsetningsverdi i Norge. https://www.frukt.no/presse/pressemeldinger/tomat-er-gronnsaken-med-hoyeste-omsetningsverdi-i-norge/. Accessed 20 April 2017

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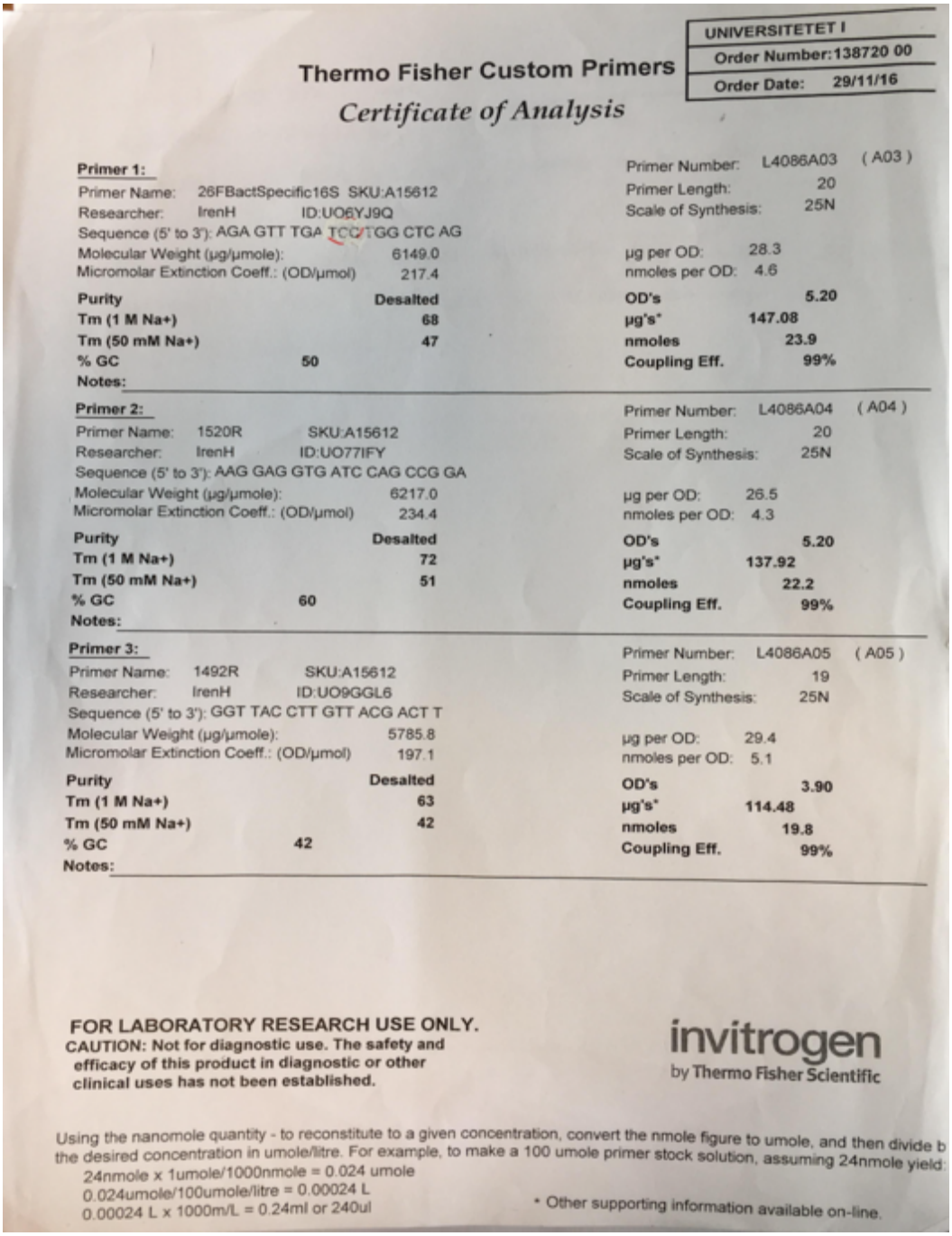
Appendices

Appendix 1: Thermo Fisher Custom Primers *Certificate of Analysis*



UNIVERSITETET I
Order Number:138720 00
Order Date: 29/11/16

Thermo Fisher Custom Primers

Certificate of Analysis

Primer 1:
Primer Name: 26FBactSpecific16S SKU:A15612
Researcher: IrenH ID:UO6YJ9Q
Sequence (5' to 3'): AGA GTT TGA TCC TGG CTC AG
Molecular Weight (µg/µmole): 6149.0
Micromolar Extinction Coeff.: (OD/µmol) 217.4

Purity	**Desalted**
Tm (1 M Na+)	**68**
Tm (50 mM Na+)	**47**
% GC	**50**
Notes:	

Primer Number: L4086A03 (A03)
Primer Length: 20
Scale of Synthesis: 25N
µg per OD: 28.3
nmoles per OD: 4.6

OD's	**5.20**
µg's*	**147.08**
nmoles	**23.9**
Coupling Eff.	**99%**

Primer 2:
Primer Name: 1520R SKU:A15612
Researcher: IrenH ID:UO77IFY
Sequence (5' to 3'): AAG GAG GTG ATC CAG CCG GA
Molecular Weight (µg/µmole): 6217.0
Micromolar Extinction Coeff.: (OD/µmol) 234.4

Purity	**Desalted**
Tm (1 M Na+)	**72**
Tm (50 mM Na+)	**51**
% GC	**60**
Notes:	

Primer Number: L4086A04 (A04)
Primer Length: 20
Scale of Synthesis: 25N
µg per OD: 26.5
nmoles per OD: 4.3

OD's	**5.20**
µg's*	**137.92**
nmoles	**22.2**
Coupling Eff.	**99%**

Primer 3:
Primer Name: 1492R SKU:A15612
Researcher: IrenH ID:UO9GGL6
Sequence (5' to 3'): GGT TAC CTT GTT ACG ACT T
Molecular Weight (µg/µmole): 5785.8
Micromolar Extinction Coeff.: (OD/µmol) 197.1

Purity	**Desalted**
Tm (1 M Na+)	**63**
Tm (50 mM Na+)	**42**
% GC	**42**
Notes:	

Primer Number: L4086A05 (A05)
Primer Length: 19
Scale of Synthesis: 25N
µg per OD: 29.4
nmoles per OD: 5.1

OD's	**3.90**
µg's*	**114.48**
nmoles	**19.8**
Coupling Eff.	**99%**

FOR LABORATORY RESEARCH USE ONLY.
CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

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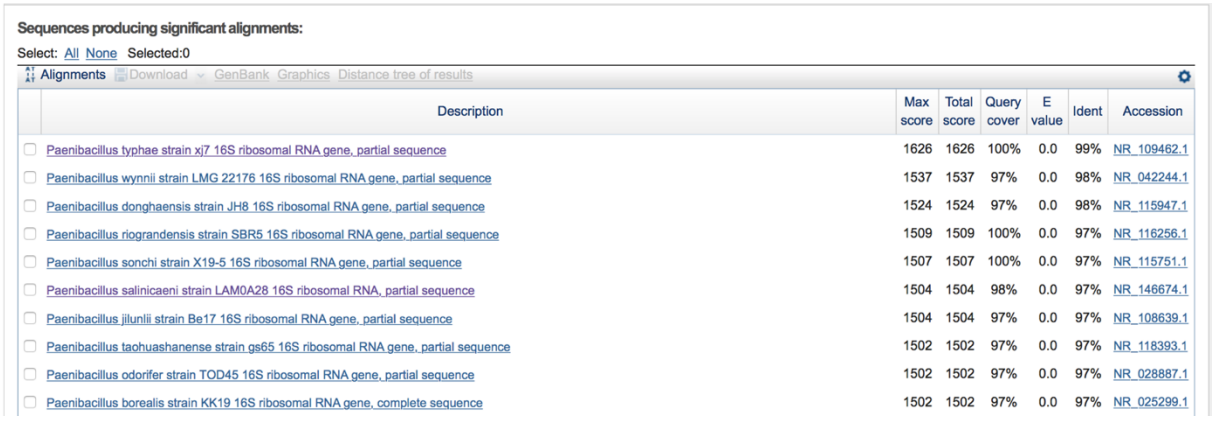
Using the nanomole quantity - to reconstitute to a given concentration, convert the nmole figure to umole, and then divide b the desired concentration in umole/litre. For example, to make a 100 umole primer stock solution, assuming 24nmole yield:
24nmole x 1umole/1000nmole = 0.024 umole
0.024umole/100umole/litre = 0.00024 L
0.00024 L x 1000m/L = 0.24ml or 240ul

* Other supporting information available on-line.

Appendix 2: BLASTn NCBI) results of forward and reverse primer sequences



Sequences producing significant alignments:

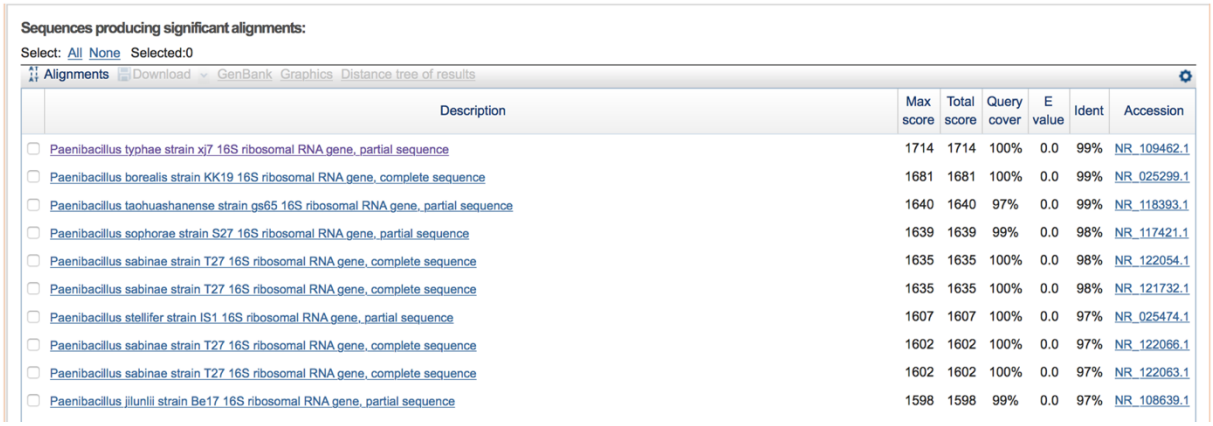
Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Paenibacillus typhae strain xj7 16S ribosomal RNA gene, partial sequence	1626	1626	100%	0.0	99%	NR_109462.1
Paenibacillus wynnii strain LMG 22176 16S ribosomal RNA gene, partial sequence	1537	1537	97%	0.0	98%	NR_042244.1
Paenibacillus donghaensis strain JH8 16S ribosomal RNA gene, partial sequence	1524	1524	97%	0.0	98%	NR_115947.1
Paenibacillus riograndensis strain SBR5 16S ribosomal RNA gene, partial sequence	1509	1509	100%	0.0	97%	NR_116256.1
Paenibacillus sonchi strain X19-5 16S ribosomal RNA gene, partial sequence	1507	1507	100%	0.0	97%	NR_115751.1
Paenibacillus salinicaeni strain LAM0A28 16S ribosomal RNA, partial sequence	1504	1504	98%	0.0	97%	NR_146674.1
Paenibacillus jilunlii strain Be17 16S ribosomal RNA gene, partial sequence	1504	1504	97%	0.0	97%	NR_108639.1
Paenibacillus taohuashanense strain gs65 16S ribosomal RNA gene, partial sequence	1502	1502	97%	0.0	97%	NR_118393.1
Paenibacillus odorifer strain TOD45 16S ribosomal RNA gene, partial sequence	1502	1502	97%	0.0	97%	NR_028887.1
Paenibacillus borealis strain KK19 16S ribosomal RNA gene, complete sequence	1502	1502	97%	0.0	97%	NR_025299.1

Figure A. 1: Result of BLASTn (NCBI) analysis of forward primer (27F) for bacterium 1.

Query length for analysis was 901. The 10 first sequences producing significant alignments are shown here. The bacterium was exposed to G+ pre-treatment.



Sequences producing significant alignments:

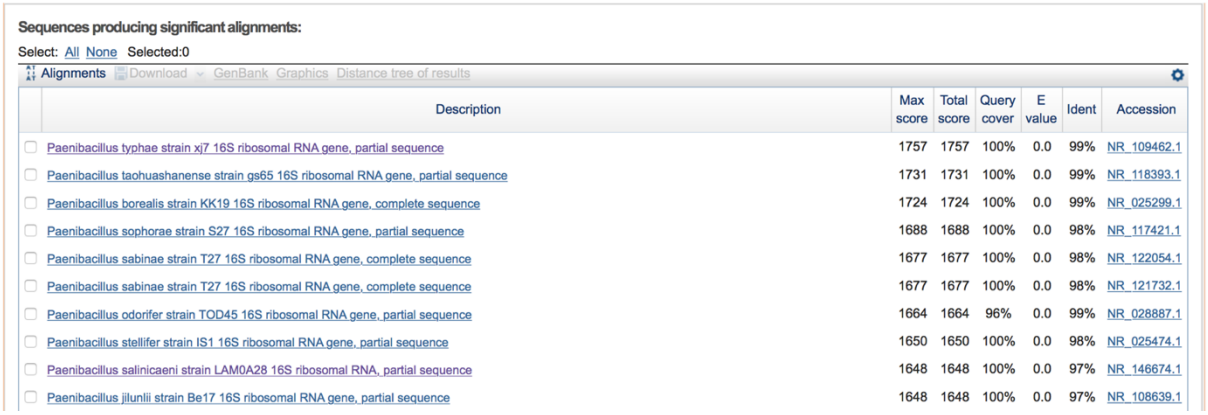
Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Paenibacillus typhae strain xj7 16S ribosomal RNA gene, partial sequence	1714	1714	100%	0.0	99%	NR_109462.1
Paenibacillus borealis strain KK19 16S ribosomal RNA gene, complete sequence	1681	1681	100%	0.0	99%	NR_025299.1
Paenibacillus taohuashanense strain gs65 16S ribosomal RNA gene, partial sequence	1640	1640	97%	0.0	99%	NR_118393.1
Paenibacillus sophorae strain S27 16S ribosomal RNA gene, partial sequence	1639	1639	99%	0.0	98%	NR_117421.1
Paenibacillus sabinae strain T27 16S ribosomal RNA gene, complete sequence	1635	1635	100%	0.0	98%	NR_122054.1
Paenibacillus sabinae strain T27 16S ribosomal RNA gene, complete sequence	1635	1635	100%	0.0	98%	NR_121732.1
Paenibacillus stellifer strain IS1 16S ribosomal RNA gene, partial sequence	1607	1607	100%	0.0	97%	NR_025474.1
Paenibacillus sabinae strain T27 16S ribosomal RNA gene, complete sequence	1602	1602	100%	0.0	97%	NR_122066.1
Paenibacillus sabinae strain T27 16S ribosomal RNA gene, complete sequence	1602	1602	100%	0.0	97%	NR_122063.1
Paenibacillus jilunlii strain Be17 16S ribosomal RNA gene, partial sequence	1598	1598	99%	0.0	97%	NR_108639.1

Figure A. 2: Result of BLASTn (NCBI) analysis of reverse primer (1520R) for bacterium 1.

Query length for analysis was 941. The 10 first sequences producing significant alignments are shown here. The bacterium was exposed to G+ pre-treatment.



Sequences producing significant alignments:

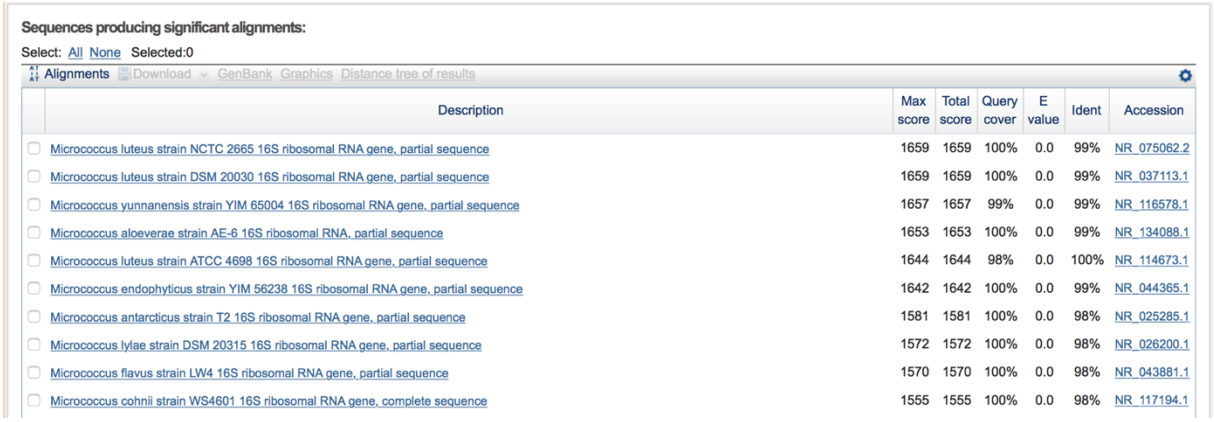
Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Paenibacillus typhae strain xj7 16S ribosomal RNA gene, partial sequence	1757	1757	100%	0.0	99%	NR_109462.1
Paenibacillus taohuashanense strain gs65 16S ribosomal RNA gene, partial sequence	1731	1731	100%	0.0	99%	NR_118393.1
Paenibacillus borealis strain KK19 16S ribosomal RNA gene, complete sequence	1724	1724	100%	0.0	99%	NR_025299.1
Paenibacillus sophorae strain S27 16S ribosomal RNA gene, partial sequence	1688	1688	100%	0.0	98%	NR_117421.1
Paenibacillus sabinae strain T27 16S ribosomal RNA gene, complete sequence	1677	1677	100%	0.0	98%	NR_122054.1
Paenibacillus sabinae strain T27 16S ribosomal RNA gene, complete sequence	1677	1677	100%	0.0	98%	NR_121732.1
Paenibacillus odorifer strain TOD45 16S ribosomal RNA gene, partial sequence	1664	1664	96%	0.0	99%	NR_028887.1
Paenibacillus stellifer strain IS1 16S ribosomal RNA gene, partial sequence	1650	1650	100%	0.0	98%	NR_025474.1
Paenibacillus salinicaeni strain LAM0A28 16S ribosomal RNA, partial sequence	1648	1648	100%	0.0	97%	NR_146674.1
Paenibacillus jilunlii strain Be17 16S ribosomal RNA gene, partial sequence	1648	1648	100%	0.0	97%	NR_108639.1

Figure A. 3: Result of BLASTn (NCBI) analysis of reverse primer (1429R) for bacterium 1.

Query length for analysis was 964. The 10 first sequences producing significant alignments are shown here. The bacterium was exposed to G+ pre-treatment.



Sequences producing significant alignments:

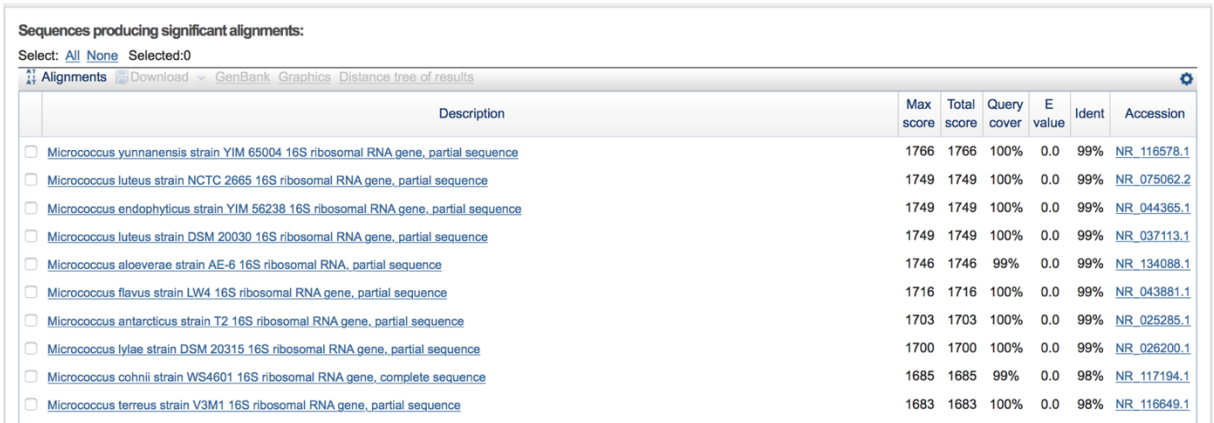
Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Micrococcus luteus strain NCTC 2665 16S ribosomal RNA gene, partial sequence	1659	1659	100%	0.0	99%	NR_075062.2
Micrococcus luteus strain DSM 20030 16S ribosomal RNA gene, partial sequence	1659	1659	100%	0.0	99%	NR_037113.1
Micrococcus yunnanensis strain YIM 65004 16S ribosomal RNA gene, partial sequence	1657	1657	99%	0.0	99%	NR_116578.1
Micrococcus aloeverae strain AE-6 16S ribosomal RNA, partial sequence	1653	1653	100%	0.0	99%	NR_134088.1
Micrococcus luteus strain ATCC 4698 16S ribosomal RNA gene, partial sequence	1644	1644	98%	0.0	100%	NR_114673.1
Micrococcus endophyticus strain YIM 56238 16S ribosomal RNA gene, partial sequence	1642	1642	100%	0.0	99%	NR_044365.1
Micrococcus antarcticus strain T2 16S ribosomal RNA gene, partial sequence	1581	1581	100%	0.0	98%	NR_025285.1
Micrococcus lylae strain DSM 20315 16S ribosomal RNA gene, partial sequence	1572	1572	100%	0.0	98%	NR_026200.1
Micrococcus flavus strain LW4 16S ribosomal RNA gene, partial sequence	1570	1570	100%	0.0	98%	NR_043881.1
Micrococcus cohnii strain WS4601 16S ribosomal RNA gene, complete sequence	1555	1555	100%	0.0	98%	NR_117194.1

Figure A. 4: Result of BLASTn (NCBI) analysis of forward primer (27F) for bacterium C.

Query length for analysis was 901. The 10 first sequences producing significant alignments are shown here. The bacterium was exposed to G+ pre-treatment.



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Micrococcus yunnanensis strain YIM 65004 16S ribosomal RNA gene, partial sequence	1766	1766	100%	0.0	99%	NR_116578.1
Micrococcus luteus strain NCTC 2665 16S ribosomal RNA gene, partial sequence	1749	1749	100%	0.0	99%	NR_075062.2
Micrococcus endophyticus strain YIM 56238 16S ribosomal RNA gene, partial sequence	1749	1749	100%	0.0	99%	NR_044365.1
Micrococcus luteus strain DSM 20030 16S ribosomal RNA gene, partial sequence	1749	1749	100%	0.0	99%	NR_037113.1
Micrococcus aloeverae strain AE-6 16S ribosomal RNA, partial sequence	1746	1746	99%	0.0	99%	NR_134088.1
Micrococcus flavus strain LW4 16S ribosomal RNA gene, partial sequence	1716	1716	100%	0.0	99%	NR_043881.1
Micrococcus antarcticus strain T2 16S ribosomal RNA gene, partial sequence	1703	1703	100%	0.0	99%	NR_025285.1
Micrococcus lylae strain DSM 20315 16S ribosomal RNA gene, partial sequence	1700	1700	100%	0.0	99%	NR_026200.1
Micrococcus cohnii strain WS4601 16S ribosomal RNA gene, complete sequence	1685	1685	99%	0.0	98%	NR_117194.1
Micrococcus terreus strain V3M1 16S ribosomal RNA gene, partial sequence	1683	1683	100%	0.0	98%	NR_116649.1

Figure A. 5: Result of BLASTn (NCBI) analysis of reverse primer (1429R) for bacterium C.

Query length for analysis was 959. The 10 first sequences producing significant alignments are shown here. The bacterium was exposed to G+ pre-treatment.

Appendix 3: Local alignment performed by Emboss Water

```
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# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EDNAFULL
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# Extend_penalty: 0.5
#
# Length: 416
# Identity:     416/416 (100.0%)
# Similarity:   416/416 (100.0%)
# Gaps:           0/416 ( 0.0%)
# Score: 2080.0
#
#
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EMBOSS_001         1 GTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCTACTTAAGTCTGG     50

EMBOSS_001       535 TGTTTAAACCTTGGGCTCAACCTGAGGTCGCACTGGAAACTGGGTGGCTT    584
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EMBOSS_001       101 GAGTACAGAAGAGGAAAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAG    150

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EMBOSS_001       151 AGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGGCTGTAACTGA    200

EMBOSS_001       685 CGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAG    734
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                     ||||||||||||||||||||||||||||||||||||||||||||||||||
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EMBOSS_001       785 GTGCCGAAGTTAACACAGTAAGCACTCCGCCTGGGGAGTACGGTCGCAAG    834
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EMBOSS_001       835 ACTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCAGTGGAGTATGT    884
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       351 ACTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCAGTGGAGTATGT    400

EMBOSS_001       885 GGTTTAATTCGAAGCA    900
                     ||||||||||||||||
EMBOSS_001       401 GGTTTAATTCGAAGCA    416
```
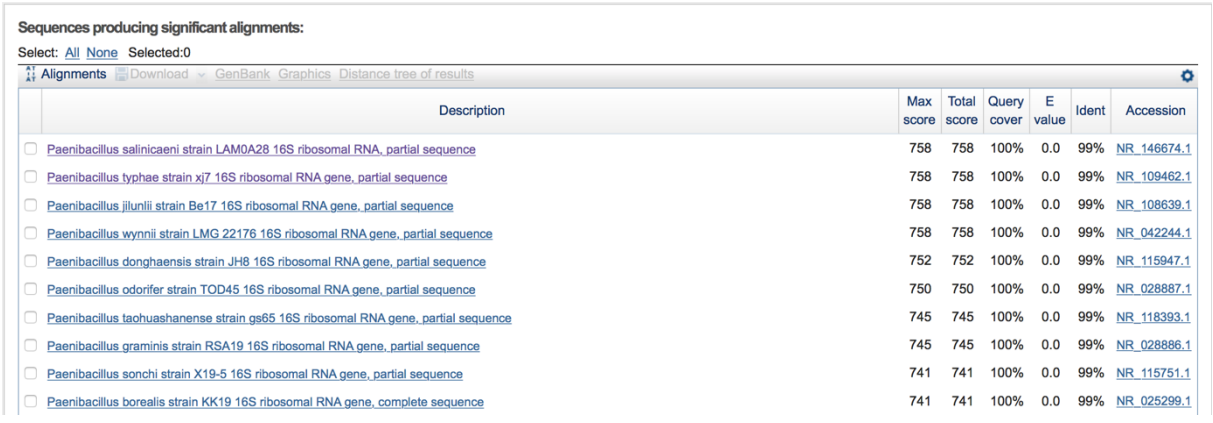

Figure A. 6: Local alignment of 27F (first sequence) and 1520R (second sequence) for bacterium 1.

Local alignment performed with Emboss water.



Sequences producing significant alignments:

Select: All None Selected:0

Alignments | Download | GenBank | Graphics | Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Paenibacillus salinicaeni strain LAM0A28 16S ribosomal RNA, partial sequence	758	758	100%	0.0	99%	NR_146674.1
Paenibacillus typhae strain xj7 16S ribosomal RNA gene, partial sequence	758	758	100%	0.0	99%	NR_109462.1
Paenibacillus jilunlii strain Be17 16S ribosomal RNA gene, partial sequence	758	758	100%	0.0	99%	NR_108639.1
Paenibacillus wynnii strain LMG 22176 16S ribosomal RNA gene, partial sequence	758	758	100%	0.0	99%	NR_042244.1
Paenibacillus donghaensis strain JH8 16S ribosomal RNA gene, partial sequence	752	752	100%	0.0	99%	NR_115947.1
Paenibacillus odorifer strain TOD45 16S ribosomal RNA gene, partial sequence	750	750	100%	0.0	99%	NR_028887.1
Paenibacillus taohuashanense strain gs65 16S ribosomal RNA gene, partial sequence	745	745	100%	0.0	99%	NR_118393.1
Paenibacillus graminis strain RSA19 16S ribosomal RNA gene, partial sequence	745	745	100%	0.0	99%	NR_028886.1
Paenibacillus sonchi strain X19-5 16S ribosomal RNA gene, partial sequence	741	741	100%	0.0	99%	NR_115751.1
Paenibacillus borealis strain KK19 16S ribosomal RNA gene, complete sequence	741	741	100%	0.0	99%	NR_025299.1

Figure A. 7: Result of BLASTn (NCBI) analysis of sequence obtained from local alignment of 27F and 1520R sequences for bacterium 1 (fig. A.6).

Query length for analysis was 416. The 10 first sequences producing significant alignments are shown here. The bacterium was exposed to G+ pre-treatment.

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# 2: EMBOSS_001
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# Extend_penalty: 0.5
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# Similarity:   468/468 (100.0%)
# Gaps:           0/468 ( 0.0%)
# Score: 2340.0
#
#
#=======================================

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EMBOSS_001         1 CCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCG     50

EMBOSS_001       484 TTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCTACTTAAGTCT    533
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001        51 TTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCTACTTAAGTCT    100

EMBOSS_001       534 GGTGTTTAAACCTTGGGCTCAACCTGAGGTCGCACTGGAAACTGGGTGGC    583
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       101 GGTGTTTAAACCTTGGGCTCAACCTGAGGTCGCACTGGAAACTGGGTGGC    150

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EMBOSS_001       151 TTGAGTACAGAAGAGGAAAGTGGAATTCCACGTGTAGCGGTGAAATGCGT    200

EMBOSS_001       634 AGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGGCTGTAACT    683
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       201 AGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGGCTGTAACT    250

EMBOSS_001       684 GACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGT    733
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
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EMBOSS_001       734 AGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGGGTTTCGATACCCT    783
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       301 AGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGGGTTTCGATACCCT    350

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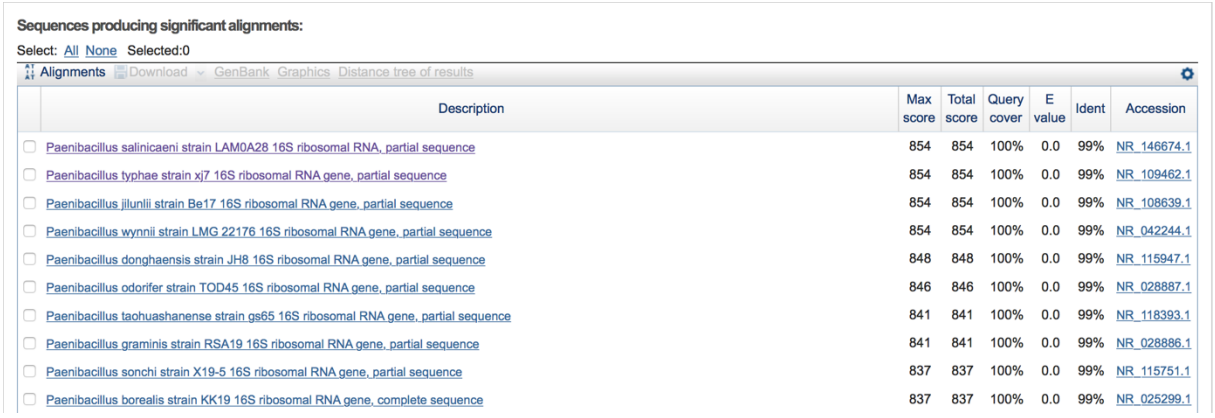
EMBOSS_001       884 GTGGTTTAATTCGAAGCA    901
                     ||||||||||||||||||
EMBOSS_001       451 GTGGTTTAATTCGAAGCA    468
```

Figure A. 8: Local alignment of 27F (first sequence) and 1429R (second sequence) for bacterium 1.

Local alignment performed with Emboss Water.



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Paenibacillus salinicaeni strain LAM0A28 16S ribosomal RNA, partial sequence	854	854	100%	0.0	99%	NR_146674.1
Paenibacillus typhae strain xj7 16S ribosomal RNA gene, partial sequence	854	854	100%	0.0	99%	NR_109462.1
Paenibacillus jilunlii strain Be17 16S ribosomal RNA gene, partial sequence	854	854	100%	0.0	99%	NR_108639.1
Paenibacillus wynnii strain LMG 22176 16S ribosomal RNA gene, partial sequence	854	854	100%	0.0	99%	NR_042244.1
Paenibacillus donghaensis strain JH8 16S ribosomal RNA gene, partial sequence	848	848	100%	0.0	99%	NR_115947.1
Paenibacillus odorifer strain TOD45 16S ribosomal RNA gene, partial sequence	846	846	100%	0.0	99%	NR_028887.1
Paenibacillus taohuashanense strain gs65 16S ribosomal RNA gene, partial sequence	841	841	100%	0.0	99%	NR_118393.1
Paenibacillus graminis strain RSA19 16S ribosomal RNA gene, partial sequence	841	841	100%	0.0	99%	NR_028886.1
Paenibacillus sonchi strain X19-5 16S ribosomal RNA gene, partial sequence	837	837	100%	0.0	99%	NR_115751.1
Paenibacillus borealis strain KK19 16S ribosomal RNA gene, complete sequence	837	837	100%	0.0	99%	NR_025299.1

Figure A. 9: Result of BLASTn (NCBI) analysis of sequence obtained from local alignment of 27F and 1429R sequences for bacterium 1 (fig. A.8).

Query length for analysis was 468. The 10 first sequences producing significant alignments are shown here. The bacterium was exposed to G+ pre-treatment.

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# 2: EMBOSS_001
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EMBOSS_001       469 GCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCG    518
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EMBOSS_001        51 GCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCG    100

EMBOSS_001       519 TCTGTCGTGAAAGTCCGGGGCTTAACCCCGGATCTGCGGTGGGTACGGGC    568
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EMBOSS_001       101 TCTGTCGTGAAAGTCCGGGGCTTAACCCCGGATCTGCGGTGGGTACGGGC    150

EMBOSS_001       569 AGACTAGAGTGCAGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGGAAT    618
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EMBOSS_001       151 AGACTAGAGTGCAGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGGAAT    200

EMBOSS_001       619 GCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCTGT    668
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EMBOSS_001       669 AACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACCC    718
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EMBOSS_001       301 TGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGACCATTCCAC    350

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EMBOSS_001       351 GGTTTCCGCGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGG    400

EMBOSS_001       819 CCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGG    868
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       401 CCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGG    450

EMBOSS_001       869 AGCATGCGGATTAATTCGATGCAACGCGAAGAA     901
                     |||||||||||||||||||||||||||||||||
EMBOSS_001       451 AGCATGCGGATTAATTCGATGCAACGCGAAGAA     483
```

Figure A. 10: Local alignment of 27F (first sequence) and 1429R (second sequence) for bacterium C.

Local alignment performed with Emboss Water.

Sequences producing significant alignments:

Select: All None Selected:0

Alignments | Download | GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Micrococcus luteus strain NCTC 2665 16S ribosomal RNA gene, partial sequence	893	893	100%	0.0	100%	NR_075062.2
Micrococcus aloeverae strain AE-6 16S ribosomal RNA, partial sequence	893	893	100%	0.0	100%	NR_134088.1
Micrococcus yunnanensis strain YIM 65004 16S ribosomal RNA gene, partial sequence	893	893	100%	0.0	100%	NR_116578.1
Micrococcus endophyticus strain YIM 56238 16S ribosomal RNA gene, partial sequence	893	893	100%	0.0	100%	NR_044365.1
Micrococcus luteus strain ATCC 4698 16S ribosomal RNA gene, partial sequence	893	893	100%	0.0	100%	NR_114673.1
Micrococcus luteus strain DSM 20030 16S ribosomal RNA gene, partial sequence	893	893	100%	0.0	100%	NR_037113.1
Micrococcus flavus strain LW4 16S ribosomal RNA gene, partial sequence	887	887	100%	0.0	99%	NR_043881.1
Micrococcus cohnii strain WS4601 16S ribosomal RNA gene, complete sequence	876	876	100%	0.0	99%	NR_117194.1
Micrococcus antarcticus strain T2 16S ribosomal RNA gene, partial sequence	870	870	100%	0.0	99%	NR_025285.1
Micrococcus lylae strain DSM 20315 16S ribosomal RNA gene, partial sequence	870	870	100%	0.0	99%	NR_026200.1

Figure A. 11: Result of BLASTn (NCBI) analysis of sequence obtained from local alignment of 27F and 1429R sequences for bacterium C (fig. A.10).

Query length for analysis was 483. The 10 first sequences producing significant alignments are shown here. The bacterium was exposed to G+ pre-treatment.

Appendix 4: Fresh shoot and root weight

Root growth assay with *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, inoculated with bacterial strain 15.

Table A. 1 Data for fresh shoot and root weight measurements for root growth assay performed with *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, inoculated with bacterial strain 15.

Mean weight of fresh shoots and roots for A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ plants, 8 d after inoculation with 650 µl suspension of bacterial strain 15 ($OD_{600} \approx 0.5$), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. N is number of plants per treatment.

Type of plants and treatment	Mean weight of fresh shoot per plant, g	Mean weight of fresh root per plant, g	N
WT control	0.0011	0.0016	5[1]
WT bact. 15	0.0014	0.0009	5[1]
***ptpa$_{ox}$* control**	0.0025	0.0021	20
***ptpa$_{ox}$* bact. 15**	0.0023	0.0018	20
***ptpa$_{kd}$* control**	0.0007	0.0019	13[1]
***ptpa$_{kd}$* bact. 15**	0.0009	0.0019	13[1]

[1]Due to contamination, only 5 WT plants, and 13 ptpa$_{kd}$ plants could be weighed.

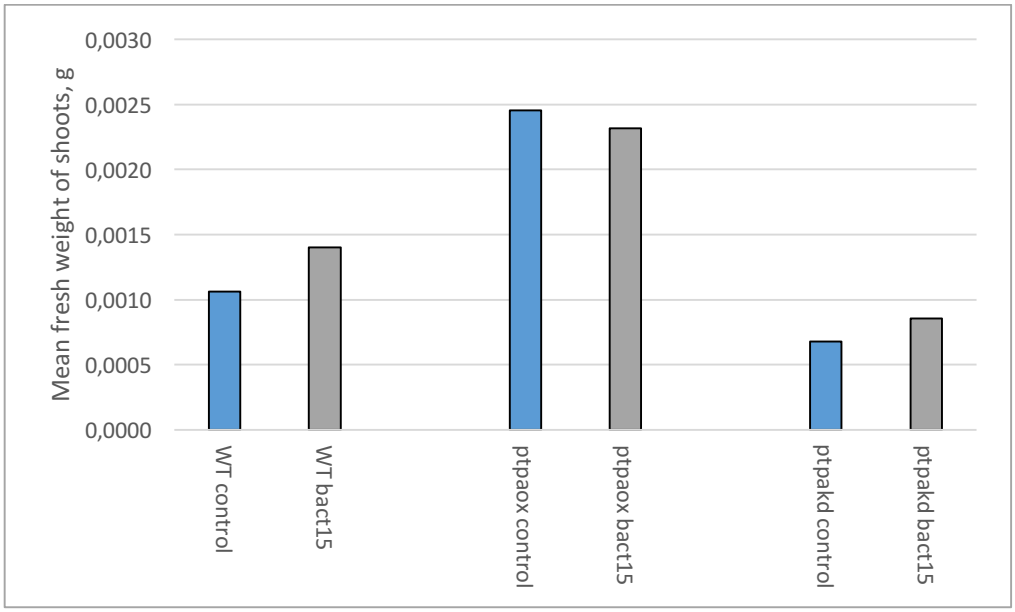

Figure A. 12: Results from measurement of fresh weight of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* shoots, 8 d after inoculation with bacterial strain 15.

Mean fresh weight of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ shoots, 8 d after inoculation with bacterial strain 15 (OD ≈ 0.5), or 10 mM for control.

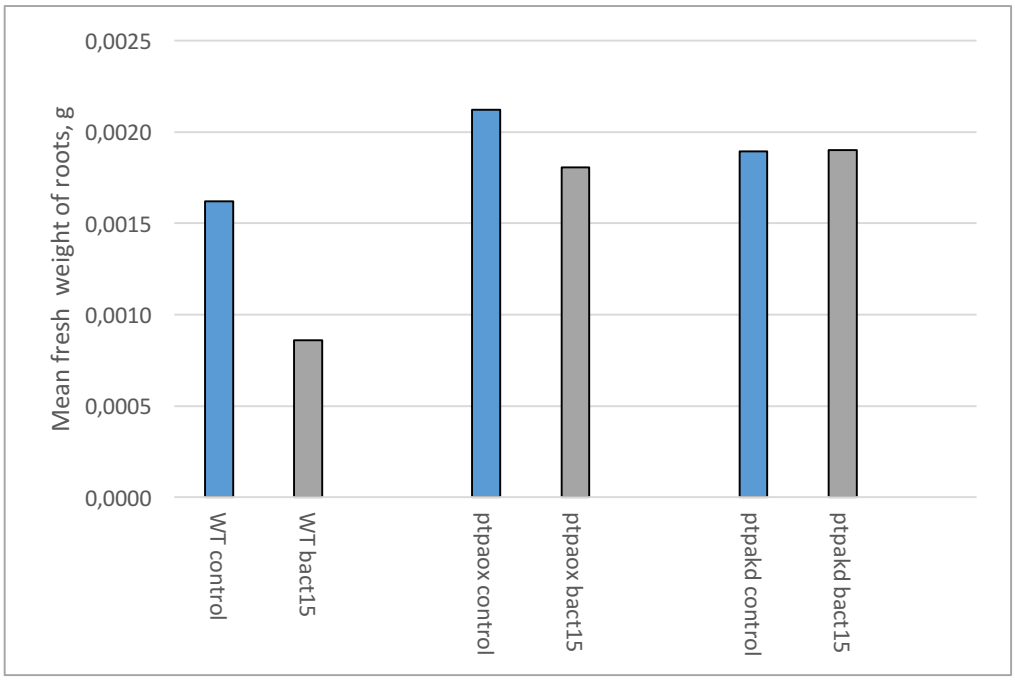

Figure A. 13: Results from measurement of fresh weight of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* roots, 8 d after inoculation with bacterial strain 15.

Mean fresh weight of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ shoots, 8 d after inoculation with bacterial strain 15 (OD ≈ 0.5), or 10 mM for control.

Root growth assay 2 *Pseudomonas* WCS417

Table A. 2: Data for fresh shoot and root weight measurements for root growth assay performed with *A. thaliana* WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, inoculated with *Pseudomonas* WCS417.

Mean weight of fresh shoots and roots for A. thaliana WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, 8 d after inoculation with 450 µl suspension of Pseudomonas WCS417 (OD_{600} = 0.004 or 0.5), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. N is number of plants per treatment.

Type of plants and treatment	Mean weight of fresh shoot per plant, g	Mean weight of fresh root per plant, g	N
WT control	0.0061	0.0015	10
WT *Pseudomonas* WCS417 (OD_{600} = 0.004)	0.0062	0.0016	10
WT *Pseudomonas* WCS417 (OD_{600} = 0.5)	0.0065	0.0015	10
***$ptpa_{ox}$* control**	0.0043	0.0009	10
***$ptpa_{ox}$ Pseudomonas* WCS417 (OD_{600} = 0.004)**	0.0066	0.0017	10
***$ptpa_{ox}$ Pseudomonas* WCS417 (OD_{600} = 0.5)**	0.0066	0.0018	10
***$ptpa_{kd}$* control**	0.0065	0.0014	10
***$ptpa_{kd}$ Pseudomonas* WCS417 (OD_{600} = 0.004)**	0.0068	0.0016	8
***$ptpa_{kd}$ Pseudomonas* WCS417 (OD_{600} = 0.5)**	0.0068	0.0017	9

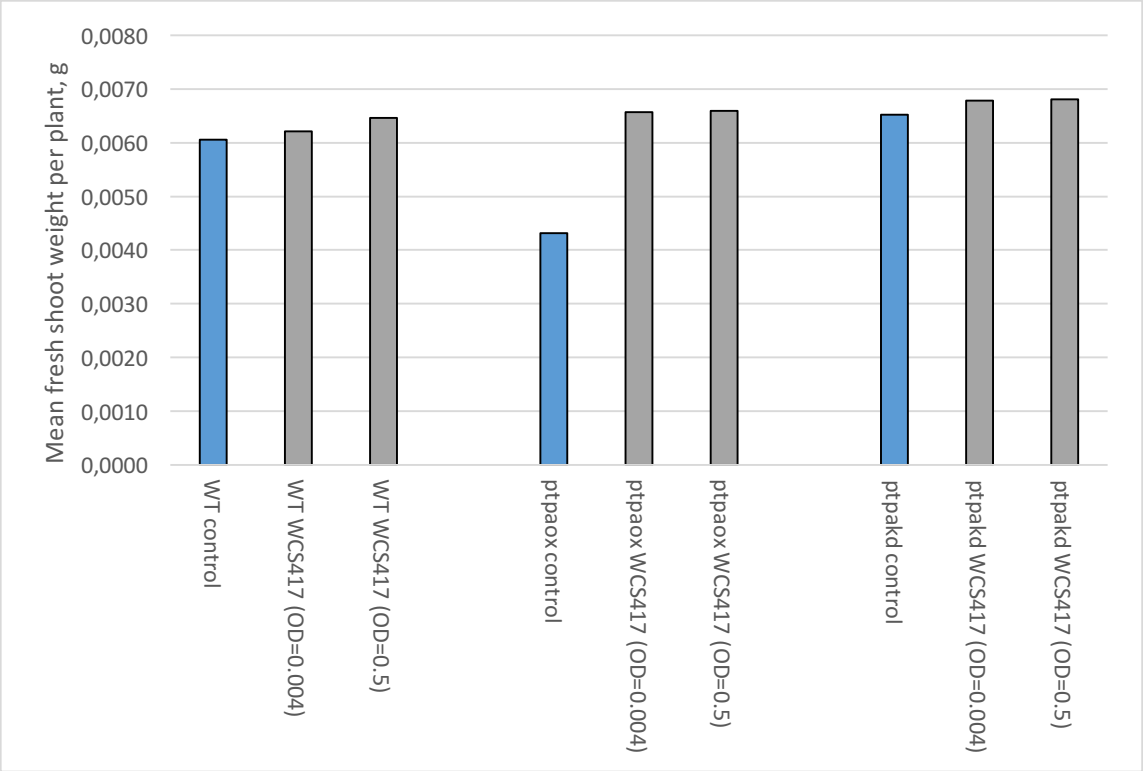

Figure A. 14: Results from measurement of fresh weight of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* shoots, 8 d after inoculation with *Pseudomonas* WCS417.

Mean fresh weight of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ shoots, 8 d after inoculation with Pseudomonas WCS417 (OD$_{600}$ = 0.004 or 0.5), or 10 mM $MgSO_4 \cdot 7H_2O$ for control.

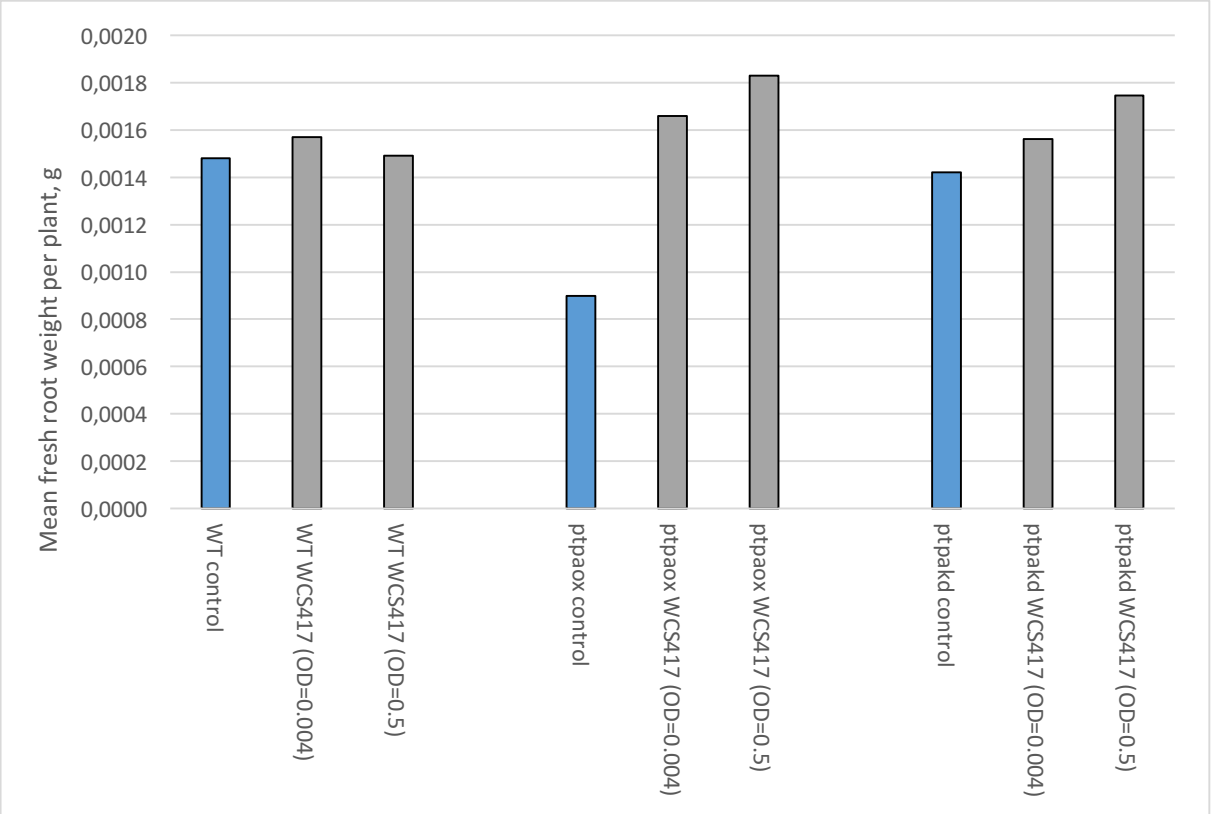

Figure A. 15: Results from measurement of fresh weight of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* roots, 8 d after inoculation with *Pseudomonas* WCS417.

Mean fresh weight of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ roots, 8 d after inoculation with Pseudomonas WCS417 (OD$_{600}$ = 0.004 or 0.5), or 10 mM $MgSO_4 \cdot 7H_2O$ for control.

Root growth assay 3 *Pseudomonas* WCS417

Table A. 3: Data for fresh shoot and root weight measurements for root growth assay performed with *A. thaliana* WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, inoculated with bacterial strain 15.

Mean weight of fresh shoots and roots for A. thaliana WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, 8 d after inoculation with 450 µl suspension of Pseudomonas WCS417 (OD_{600} = 0.005), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. The calculations are means of 4 plates with corresponding standard deviations (SD), a total of 18-20 plants per treatment.

Type of plants and treatment	Mean weight of fresh shoot per plant, g	SD, fresh shoot	Mean weight of fresh root per plant, g	SD, fresh root	p-values for fresh shoot weight (compared to control	p-values for fresh root weight (compared to control)
WT control	0.0064	7.48×10^{-4}	0.0013	2.49×10^{-4}		
WT *Pseudomonas* WCS417	0.0055	3.37×10^{-4}	0.0019	9.42×10^{-5}	0.0707	0.0041*
$ptpa_{ox}$ control	0.0054	7.12×10^{-4}	0.0012	1.28×10^{-4}		
$ptpa_{ox}$ *Pseudomonas* WCS417	0.0053	6.01×10^{-4}	0.0019	1.08×10^{-4}	0.8371	0.0002*
$ptpa_{kd}$ control	0.0071	9.43×10^{-4}	0.0017	2.17×10^{-4}		
$ptpa_{kd}$ *Pseudomonas* WCS417	0.0060	1.02×10^{-3}	0.0019	4.95×10^{-4}	0.1649	0.4873

* Statistically significant compared to control, $p < 0.05$.

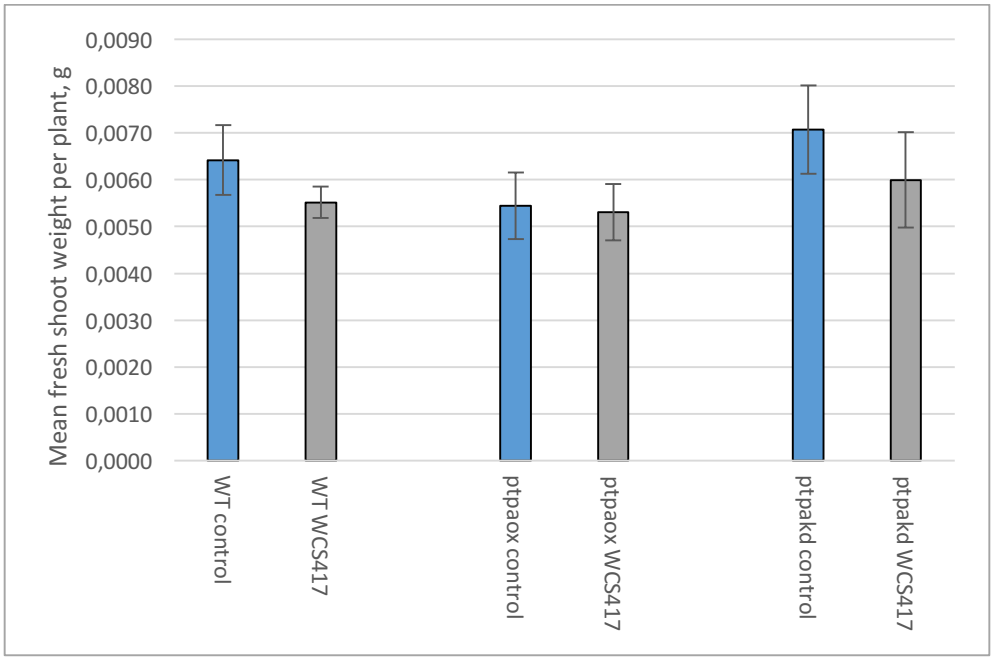

Figure A. 16: Results from measurement of fresh weight of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* shoots, 8 d after inoculation with *Pseudomonas* WCS417.

Mean fresh weight of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ shoots, 8 d after inoculation with Pseudomonas WCS417 (OD_{600} = 0.005), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Bars show mean of 4 plates ± SD, a total of 18-20 plants per treatment.

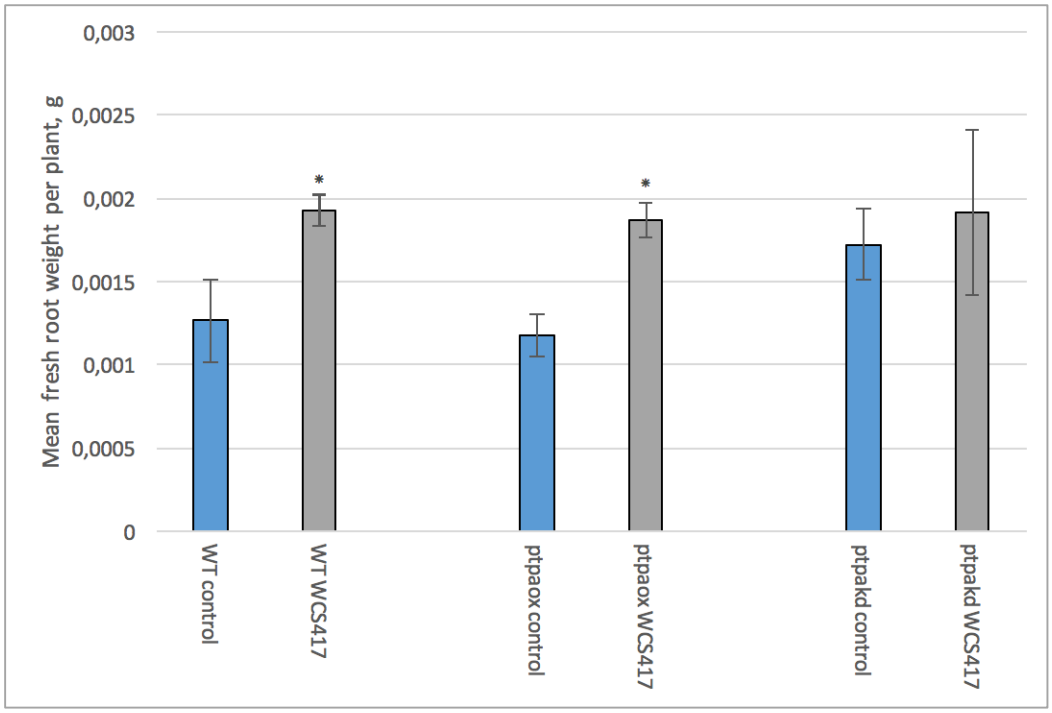

Figure A. 17: Results from measurement of fresh weight of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* roots, 8 d after inoculation with *Pseudomonas* WCS417.

Mean fresh weight of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ roots, 8 d after inoculation with Pseudomonas WCS417 (OD_{600} = 0.005), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Bars show mean of 4 plates ± SD, a total of 18-20 plants per treatment.

** Statistically significant compared to control, $p < 0.05$.*

Root growth assay 1 *Paenibacillus*

Table A. 4: Data for fresh shoot and root weight for root growth assay 1 performed with *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* plants, inoculated with a *Paenibacillus* sp.

Mean weight of fresh shoots and roots for A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ plants, 8 d after inoculation with 450 µl suspension of Paenibacillus (OD_{600} = 0.6), or 10 mM $MgSO_4 \cdot 7H_2O$ for control.

Type of plants and treatment	Mean weight of fresh shoot per plant, g	Mean weight of fresh root per plant, g	N
WT control	0.0061	0.0015	10
WT *Paenibacillus*	0.0047	0.0010	10
***ptpa$_{ox}$* control**	0.0043	0.0009	10
ptpa$_{ox}$ Paenibacillus	0.0057	0.0013	7
***ptpa$_{kd}$* control**	0.0065	0.0014	10
ptpa$_{kd}$ Paenibacillus	0.0048	0.0016	8

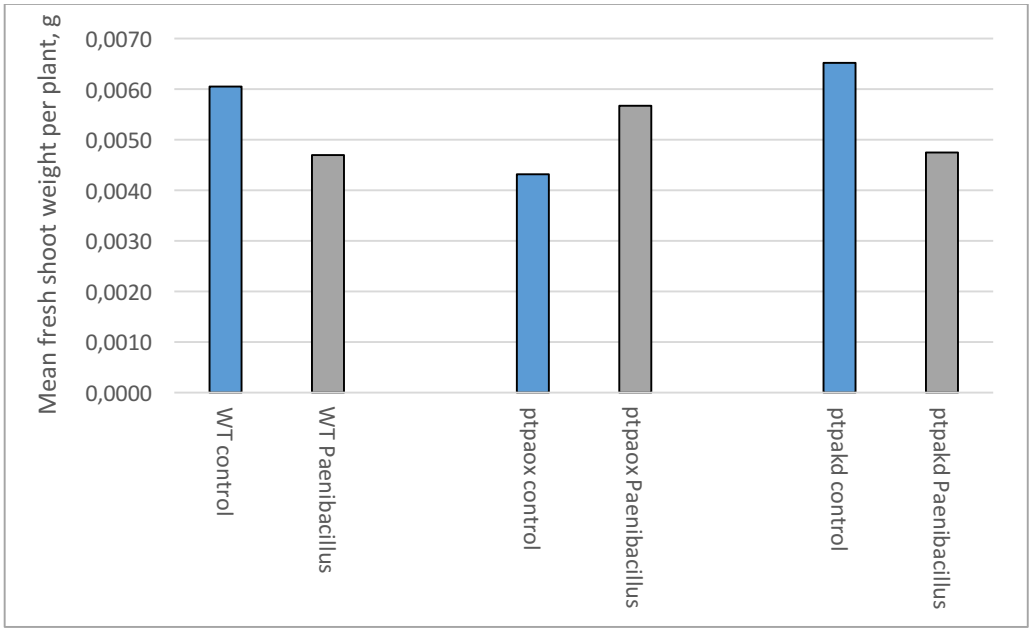

Figure A. 18: Results from measurement of fresh weight of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* shoots, 8 d after inoculation with a *Paenibacillus* sp.

Mean fresh weight of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ shoots, 8 d after inoculation with Paenibacillus (OD_{600} =0. 6), or 10 mM $MgSO_4 \cdot 7H_2O$ for control.

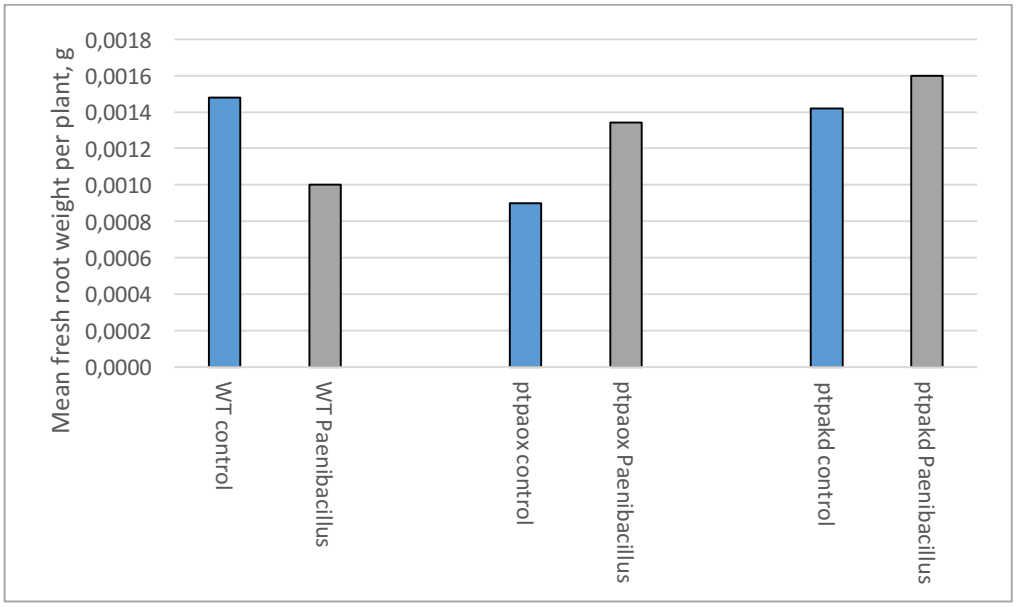

Figure A. 19: Results from measurement of fresh weight of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* roots, 8 d after inoculation with a *Paenibacillus* sp.

Mean fresh weight of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ roots, 8 d after inoculation with Paenibacillus (OD$_{600}$ = 0.6), or 10 mM $MgSO_4 \cdot 7H_2O$ for control.

Root growth assay 2 *Paenibacillus*

Table A. 5: Data for fresh shoot and root weight for root growth assay 2 performed with *A. thaliana* WT plants, inoculated with a *Paenibacillus* sp.

Mean weight of fresh shoots and roots for A. thaliana WT plants, 8 d after inoculation with 450 µl suspension of Paenibacillus (OD$_{600}$ = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. The calculations are means of 4 plates with corresponding standard deviations (SD), a total of 20 plants per treatment.

Type of plants and treatment	Mean weight of fresh shoot per plant, g	SD, fresh shoot	Mean weight of fresh root per plant, g	SD, fresh root	P value, Fresh shoot weight (compared to control)	P value, Fresh root weight (compared to control)
WT control	0.0077	0.00059	0.0017	3.84×10^{-5}		
WT *Paenibacillus*	0.0060	0.00063	0.0014	2.21×10^{-4}	0.00075*	0.0362*

* Statistically significant compared to control, $p < 0.05$

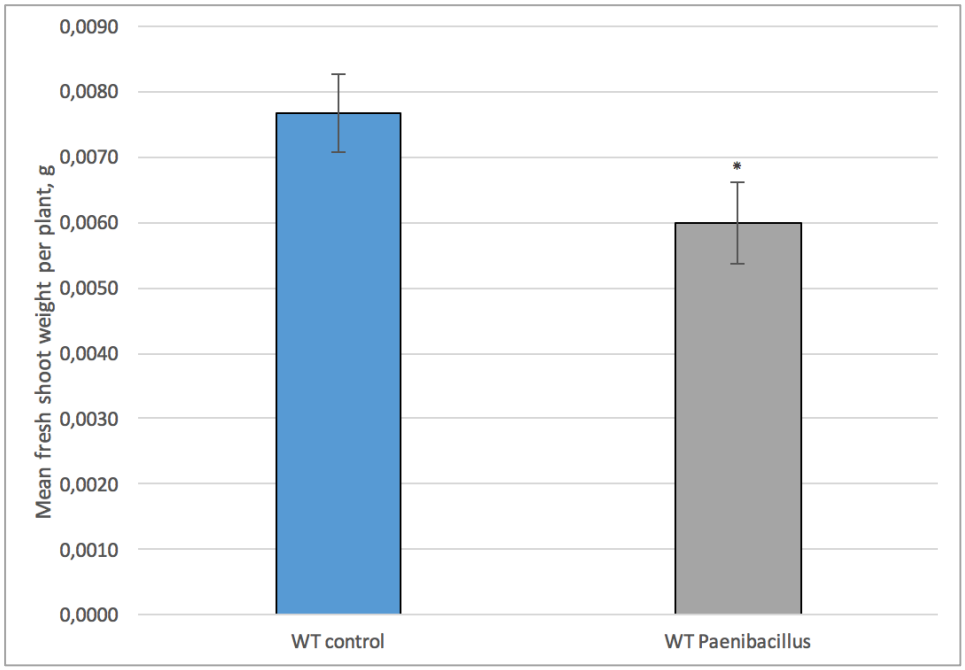

Figure A. 20: Results from measurement of fresh weight of *A. thaliana* WT shoots, 8 d after inoculation with a *Paenibacillus* sp.

Mean fresh weight of A. thaliana WT, $ptpa_{ox}$, and $ptpa_{kd}$ shoots, 8 d after inoculation with Paenibacillus (OD_{600} = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Bars show mean of 4 plates ± SD, a total of 20 plants per treatment.

** Statistically significant compared to control, $p < 0.05$.*

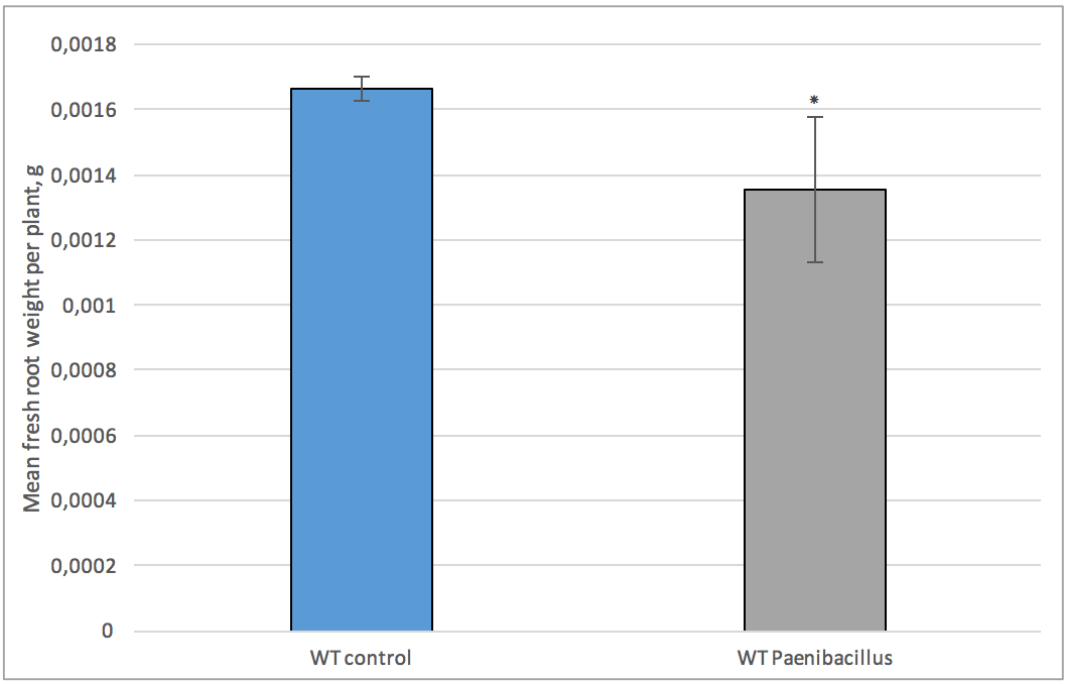

Figure A. 21: Results from measurement of fresh weight of *A. thaliana* WT roots, 8 d after inoculation with a *Paenibacillus* sp.

Mean fresh weight of A. thaliana WT roots, 8 d after inoculation with Paenibacillus (OD_{600} = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Bars show mean of 4 plates ± SD, a total of 20 plants per treatment.

** Statistically significant compared to control, $p < 0.05$.*

Root growth assay 3 *Paenibacillus*

Table A. 6: Data for fresh shoot and root weight for root growth assay 3 performed with *A. thaliana* WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, inoculated with a *Paenibacillus* sp.

Mean weight of fresh shoots and roots for A. thaliana WT, $ptpa_{ox}$, and $ptpa_{kd}$ plants, 8 d after inoculation with 450 µl suspension of Paenibacillus (OD_{600} = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. The calculations are means of 4 plates with corresponding standard deviations (SD), a total of 17-20 plants per treatment.

Type of plants and treatment	Mean weight of fresh shoot per plant, g	SD, fresh shoot weight	Mean weight of fresh root per plant, g	SD, fresh root weight	p-values for fresh shoot weight (compared to control)	p-values for fresh root weight (compared to control)
WT control	0.0069	$1.32x10^{-3}$	0.0016	$5.15x10^{-4}$		
WT *Paenibacillus*	0.0063	$7.61x10^{-4}$	0.0016	$3.18x10^{-4}$	0.4607	1.0000
$ptpa_{ox}$ control	0.0051	$8.94x10^{-4}$	0.0011	$1.85x10^{-4}$		
$ptpa_{ox}$ *Paenibacillus*	0.0074	$4.58x10^{-4}$	0.0018	$3.72x10^{-4}$	0.0038*	0.0151*
$ptpa_{kd}$ control	0.0089	$1.50x10^{-4}$	0.0022	$3.43x10^{-4}$		
$ptpa_{kd}$ *Paenibacillus*	0.0068	$5.18x10^{-4}$	0.0021	$3.27x10^{-4}$	0.0381*	0.6879

* Statistically significant compared to control, $p < 0.05$.

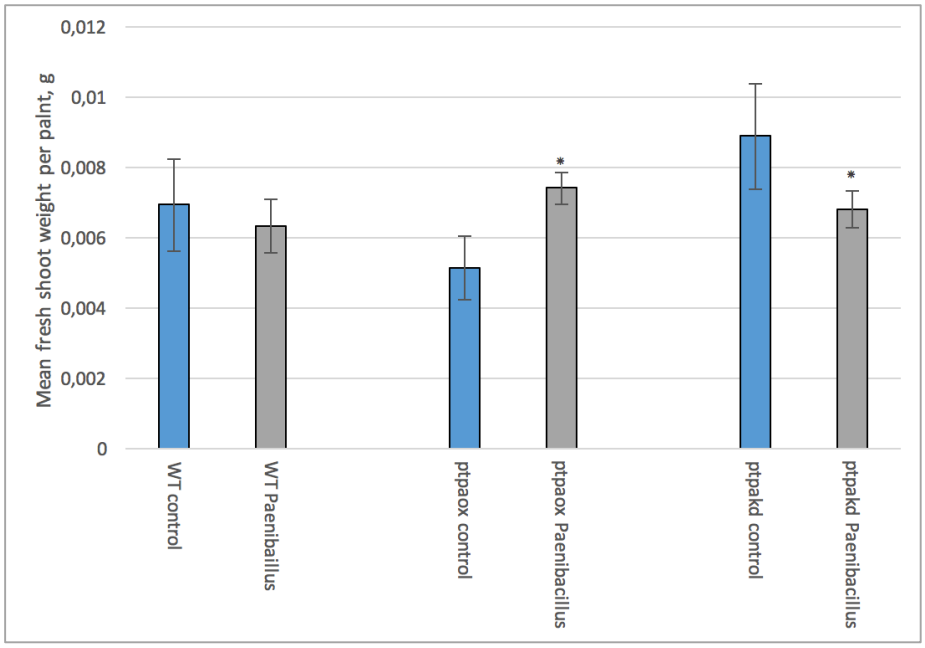

Figure A. 22: Results from measurement of fresh weight of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* shoots, 8 d after inoculation with a *Paenibacillus* sp.

Mean fresh weight of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ shoots, 8 d after inoculation with Paenibacillus (OD_{600} = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Bars show mean of 4 plates ± SD, a total of 17-20 plants per treatment.

** Statistically significant compared to control, $p < 0.05$.*

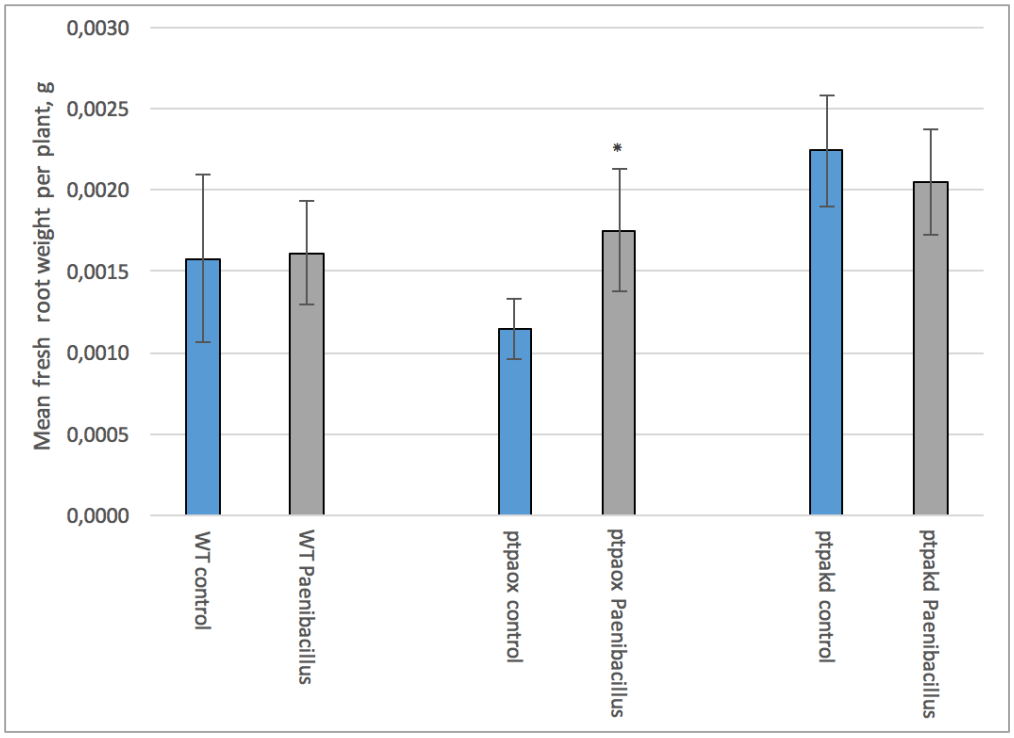

Figure A. 23: Results from measurement of fresh weight of *A. thaliana* WT, *ptpa$_{ox}$*, and *ptpa$_{kd}$* roots, 8 d after inoculation with *Paenibacillus* spp.

Mean fresh weight of A. thaliana WT, ptpa$_{ox}$, and ptpa$_{kd}$ roots, 8 d after inoculation with Paenibacillus (OD_{600} = 0.3), or 10 mM $MgSO_4 \cdot 7H_2O$ for control. Bars show mean of 4 plates ± SD, a total of 17-20 plants per treatment.

** Statistically significant compared to control, $p < 0.05$.*