Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences

Impact of plant ecotype on *Bacillus*-mediated growth promotion

– extending beyond the standard model plant

Staffan Matzén

Department of Plant Biology
Master´s thesis • Independent Project / Degree course 30 hec • Second cycle, A2E
Degree of Master of Science in Agriculture and Degree of Master of Science with a Major in Biology
Examensarbete • Nr: 164
Uppsala, 2018

Impact of plant ecotype on *Bacillus*-mediated growth promotion
- extending beyond the standard model plant

Staffan Matzén

Supervisor: Prof. Johan Meijer, Swedish University of Agricultural Sciences Department of Plant Biology, Uppsala

Assistant Supervisor: Dr. Sarosh Bejai, Swedish University of Agricultural Sciences Department of Plant Biology, Uppsala

Examiner: Prof. Anders Kvarnheden, Swedish University of Agricultural Sciences, Department of Plant Biology, Uppsala

Credits: 30 hec
Level: Second cycle A2E
Course title: Independent project/ Degree course in Biology
Course code: EX0565
Programme/education: DEGREE OF MASTER OF SCIENCE IN AGRICULTURE and DEGREE OF MASTER OF SCIENCE with a major in Biology

Place of publication: Uppsala
Year of publication: 2018
Cover picture: Upper left: *A. thaliana* on sucrose medium showing heavy colonization by *B. amyloliquefaciens* UCMB 5113 at 14 days post-inoculation (included for artistic purposes), Upper right: *Bacillus*-treated plants of L*er*-0 ecotype, Bottom: main root of *A. thaliana* ecotype Ler-0 treated with *B. amyloliquefaciens* UCMB 5113 . Staffan Matzén, photos.
Title of series: Examensarbete / Institutionen för växtbiologi, SLU
Number of part of series: 164
Online publication: http://stud.epsilon.slu.se

Keywords: Bacillus, Growth promotion, Root exudates, Volatiles

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Plant Biology

En tillväxtfrämjande bakterie och dess inverkan på modellväxter

-Växtens ursprung påverkar utfallet

Staffan Matzén

En jordlevande bakterie från Karpaterna i Ukraina (*Bacills amyloliquefaciens* UCMB 5113) har visat sig kunna skydda oljeväxter mot skadegörande svampar och i vissa fall öka växters tillväxt. Bakterien har potential att ersätta kemiska bekämpningsmedel och konstgödning i jordbruket vilket kan ge minskad miljöbelastning. Mycket är dock okänt kring hur bakterien kan påverka grödor. Med bättre kännedom om hur bakterien samspelar med växter kan användningen av den bli mer effektiv. Detta kan till exempel innebära att bakterien eller dess exudat appliceras på rätt sätt och används med rätt grödor samt mot rätt skadegörare. I jordbruksforskning används ofta modellväxter för att enklare kunna studera samspelet mellan bakterier och växter. backtrav, eller *Arabidopsis thaliana* som den heter på latin, är en populär modellväxt inom forskningen.

I den här studien visades bland annat att en modellväxts geografiska ursprung (ekotyp) kan påverka utfallet av samspelet mellan denna och den tillväxtstimulerande jordbakterien *Bacillus amyloliquefaciens* UCMB 5113. Det är en kunskap som kan vara värdefull vid val av modellväxt för fortsatt forskning. Att använda en modellväxt i stället för de olika jordbruksgrödorna har flera fördelar då många forskare använder samma växt och det därmed finns mycket information som kan delas mellan forskargrupperna. Inom arten backtrav finns det dock en viss variation som beror på lokala förutsättningar i det område varianten kommer från. Backtrav från en visst område benämns ofta ekotyp. Vanligtvis används en ekotyp av backtrav som kommer från Columbia i USA och förkortas Col-0. För att studera vissa samspel mellan växter och bakterier kan det dock vara fördelaktigt att använda en ekotyp av backtrav som har utvecklats i en annan miljö som gör den mer lik den gröda som forskningen i slutänden ska användas på. För att undersöka om det förekommer en skillnad i behandlingseffekt mellan olika ekotyper av backtrav odlades flera olika backtrav-ekotyper som härstammar från olika delar av världen på ett geléaktigt näringsmedium och behandlades med bakterien *Bacillus amyloliquefaciens* UCMB 5113. Rötternas tillväxt studerades efter behandlingen genom att bilder av växternas rotsystem analyserades med datorprogram för bildanalys. Mätningen innefattade längden av huvudrot samt sidorötter samt längd och antal rothår på en bit av roten. Datan från bildanalysen tolkades sedan statistiskt för att upptäcka möjliga skillnader i behandlingseffekt mellan ekotyper av backtrav. Rotexudat utsöndrade från de olika backtrav-ekotypernas rötter användes även för att odla *B. amyloliquefaciens* UCMB 5113 bakterier i flytande lösning för att undersöka om rotexudaten från de olika ekotyperna skiljde sig i sin förmåga att påverka *Bacillus* tillväxt. Även bakteriens möjlighet att påverka växten genom gasformiga ämnen studerades. Växterna odlades avskilt från bakterierna så att inga lösliga ämnen kunde transporteras mellan bakterien och växten, utan endast gasformiga sådana. Resultaten visade att näringsämnena som bakterien hade tillgängliga under sin tillväxt påverkade dess effekt på växten. Eftersom kontakt mellan *Bacillus*-bakterien och växten endast kunde ske genom luften är det rimligt att tro att sammansättningen eller koncentrationen av de gasformiga ämnena som bakterien utsöndrar skiljer sig beroende på vilken näring bakterien har tillgänglig.

Abstract

This study investigated how bacterial treatment modulates plant growth. *Bacillus amyloliquefaciens* UCMB5113 was used for treatment of several different natural accessions (ecotypes) as well as mutants of *Arabidopsis thaliana*. Treatment effect on plant root architecture was studied in particular.

Increased quantitative and qualitative production has long been an underlying motivation for agricultural development. We are currently in a phase of environmental awareness that calls for agricultural practices that cause less strain on natural ecosystems. However, increased production and lower environmental impact are two goals that blend poorly. Bio-fertilization and bio-control have gained increased attention lately as approaches to reducing the use of synthetic pesticides and fertilizers in agriculture, thus lowering environmental impact. In short, the terms describe the use of natural organisms to combat pests and increase yield in crops. Soil-borne rhizobacteria have been found to host a number of candidates for biocontrol and biofertilization. Among these is the *Bacillus amyloliquefaciens* subsp. *plantarum* strain UCMB5113 which has been shown to possess the ability of promoting growth and increasing pest resistance in plants. The mode of action is largely unknown and most of the work concerning it is undertaken using *A. thaliana*, and particularly the natural accession Colombia (Col-0). In this thesis, a number of natural *A. thaliana* accessions (ecotypes) as well as mutants were treated with *B. amyloliquefaciens* in order to contribute to a better understanding of the properties underlying a fruitful interaction between these two organisms. Such knowledge could prove valuable in the identification or development of agricultural crops able to be cultivated effectively with UCMB 5113 bacteria.

Treatment with *B. amyloliquefaciens* UCBM 5113 proved to significantly increase root hair growth in half of the tested *A. thaliana* accessions. The increase in total root hair length was a result of either higher number of root hairs, increased length of individual root hairs or a combination of both. The root hair-deficient mutant N2259 showed formation of root hairs following treatment with *Bacillus*, which could indicate an ability to restore calcium ion influx to root hair cells. When incubating UCMB 5113 with *A. thaliana* root exudates, some of the *A. thaliana* root exudates could be differentiated from one another based on the growth kinetics of *Bacillus*. Root exudates from Ag-0, Stw-0 and Cvi-0 led to the highest endpoint growth of *Bacillus*, while Col-0, L*er*-0 and Edi-0 resulted in the lowest *Bacillus* growth. The importance of plant accession for volatile organic compound (VOC)-mediated bacterial plant interaction was studied in a concluding set of experiments, using five accessions. Can-0 increased total root growth as a result of exposure to VOC emitted by *B. amyloliquefaciens* UCBM 5113 while Mt-0 was repressed in total root growth. It was also observed that the substrate supporting the *Bacillus* growth affected the outcome of the interaction, with LB medium generally resulting in repression of root growth.

Sammanfattning

Denna studie undersökte vikten av växt-ekotyp för interaktion mellan *Arabidopsis thaliana* och den tillväxtstimulerande bakterien *Bacillus amyloliquefaciens* UCMB5113. Effekter på rotarkitekturen har varit i fokus under försöken. Ökning av såväl kvalitativ som kvantitativ avkastning har alltid varit en strävan inom jordbruket. På senare tid har kraven på ett miljömässigt hållbart jordbruk bidragit till ett ökat intresse för biologisk bekämpning och gödsling. Det finns ett antal bakterier som undersöks för deras användning som produktionshöjare inom jordbruket. *B. amyloliquefaciens* subsp. *plantarum* UCMB5113 är en rhizobakterie som visat sig ha förmåga att främja tillväxt och öka sjukdomsresistensen i växter. Mekanismerna som ligger till grund för effekterna är till stor del okända och det mesta av arbetet som bedrivs på området sker med hjälp av modellväxten backtrav (*Arabidopsis thaliana*) och främst med ekotypen Colombia (Col-0). Inom ramen för den här studien har ett antal naturliga *A. thaliana* ekotyper samt mutanter behandlas med *B. amyloliquefaciens* för att studera eventuella skillnader i behandlingseffekt. I förlängningen kan sådan kunskap vara av vikt för att identifiera och utveckla jordbruksgrödor som kan behandlas med tillfredställande resultat. Behandling med *B. amyloliquefaciens* UCBM 5113 visade sig signifikant öka rothårstillväxt i hälften av de testade ekotyperna av *A. thaliana*. Ökningen i total rothårslängd var ett resultat av antingen ökat antal rothår, ökad längd på rothår eller en kombination av båda.

Den rothårsdefekta *Arabidopsis*-mutanten N2259 bildade rothår vid behandling med *Bacillus*, möjligtvis på grund av återställande av kalciumjontransporten i rothårsceller. Vid inkubation av *Bacillus* med rotexudat från *A. thaliana* kunde några av rotexudaten differentieras från varandra utifrån tillväxtens karaktär. Rotexudat från Ag-0, Stw-0 och Cvi-0 ledde till den högsta absoluta tillväxten av *Bacillus*, medan Col-0, L*er*-0 och Edi-0 gav lägst bakterietillväxt. Avslutningsvis studerades påverkan av rottillväxt hos olika *Arabidopsis*-ekotyper då dessa utsattes för flyktiga ämnen, utsöndrade av *Bacillus*. Can-0 visade ökad total rottillväxt som ett resultat av behandlingen medan Mt-0 visade minskad rottillväxt. Substratet för bakterietillväxten visade sig vara av vikt för utfallet av interaktionen mellan bakterie och växt där LB medium gav genomgående en hämmande effekt.

Table of Contents

Introduction

Ever since the dawn of human agriculture, there has been a strive to increase the quantity and quality of crop production. During the course of the last century great progress has been achieved, especially in the increase of production per unit area of farmland. Aside from crop development, a major part of the advances made can be attributed to increased mechanization and use of synthetic fertilizers and pesticides (Tilman 1998). The advances have undoubtedly been rapid, but have at the same time imparted a heavy strain on natural resources (Pimentel 1995, Tilman et al. 2002). During the last decade however, plant growth promoting rhizobacteria (PGPR) have proved to be a viable alternative to synthetic fertilizers and pesticides (Talboys et al. 2014). There is a number of PGPR that are available on the market today, mainly belonging to the *Bacillus* genus. The appeal of the *Bacillus* strains is mainly attributed to their ability of forming endospores that have a longer shelf-life and have a wider range of permissible storage conditions than vegetative bacteria (Qiao et al. 2014). The PGPR species *Bacillus amyloliquefaciens* can be used as biocontrol and growth promotion agent and could as such reduce the use of chemicals in agriculture. Research on PGPR-plant interaction is depending heavily on the use of the model plant *Arabidopsis thaliana*. *Arabidopsis* was originally thought to be native to Euroasia and North Africa, but has more recently been found over large parts of the world. Although the species has great homogeneity in conserved genes coding for complex traits, it is able to produce vastly different phenotypes by tailoring its gene expression (Weigel 2012). Natural variants of *A. thaliana* are commonly called ecotypes and represent accessions of different evolutionary background, often with distinct features attributed to them. Such variation in aspects, such as pest resistance or flowering has been used for ecotype screening to identify the molecular basis for the traits (Weigel 2012). The overarching purpose of the study was to further enhance the understanding of the growth-promoting aspects of the biocontrol agent *Bacillus amyloliquefaciens* subsp. *plantarum* UCBM 5113 (recently also denoted as *B. methylotroficus* or *B. velezensis*) on *A. thaliana*. It has been observed that *B. amyloliquefaciens* can alter *Arabidopsis* and *Brassica napus* root morphology (Sarosh et al. 2009, Asari et al. 2017). Presumably this is through stimulation of production of hormones by *Bacillus* and/or the plant (Asari et al. 2017). Recent evidence from a study using *Bacillus* sp. LZR216 suggests that cytokinin signalling is involved in the change of root morphology (Wang et al. 2017). Water and nutrient uptake are both processes that are associated with root architecture and may act as limitations in crop productivity in most agricultural systems (Lynch 1995). Preliminary experiments have suggested that *B. amyloliquefaciens* activates jasmonic acid, ethylene and brassinosteroid dependent signalling in host plants. In an attempt to gain further insight into the mechanisms of these interactions, two main areas of the interaction between plant and bacteria were studied:

- Plant genotype dependence for root colonization of *B. amyloliquefaciens* 5113 as well as root growth modulation through either direct interaction or through volatiles.

- Factors involved in *Bacillus*-based growth promotion.

The information obtained was used to develop an understanding of which conditions with respect to plant properties and *Bacillus* application that would result in a more beneficial plant-bacterial interaction.

Because of the different nature of aspects studied in this plant-bacterial interaction, the experimental section of this thesis is divided into five major parts. These subdivisions are largely independent and contain their own introductory material. Where methodology or results coincide, cross-references are made between the sections in order to provide the necessary information. The interrelation between the experiments is addressed in a finalizing discussion at the end, followed by general conclusions. For more information on the *Arabidopsis* accessions used, such as characteristics and geographical origin, visit www.*Arabidopsis*.org. Large scale sequencing of *Arabidopsis* accessions also provides genome and gene information that can help to identify common features and correlate phenotype with genotype (Weigel and Nordborg 2015, 1001 Genomes Consortium 2016).

In short, the first experiment aimed at identifying possible differences in root growth promotion between different accessions of *A. thaliana* when treated with *B. amyloliquefaciens* UCMB 5113. The second experiment aimed at studying differences in root colonization pattern of the same bacteria on two accessions with extreme growth response in an attempt to link it to morphological treatment response. The third experiment aimed, by the use of *A. thaliana* mutants, to identify which factors may be involved in the phenotypic responses observed in the first experiment. The fourth experiment sought to investigate whether root exudates from different *A. thaliana* accessions differed in their ability to promote *Bacillus*-growth. The fifth experiment intended to investigate the effect of *Bacillus* derived volatile organic compounds (VOCs) on root architecture of *A. thaliana*. Gnotobiotic experiments were used exclusively because of the complexity and large degree of uncertainties related to the studied interactions, that would likely be amplified if a more natural system was used.

1. Plant accession dependence on root phenotype alteration when treated with *B. amyloliquefaciens* 5113

Introduction

Some of the microbes that provide stress tolerance to plants can also promote plant growth. These plant growth-promoting rhizobacteria/bacteria (PGPR/PGPB) can stimulate plant growth directly and indirectly by production of plant hormones, by improving nutrient uptake or deterrence of pathogens (Beneduzi et al. 2012). Treatment of plants with the PGPR UCMB 5113 has been observed to enhance growth of *A. thaliana* Col-0 accession (Niazi et al. 2014). Possible differences in root growth between accessions of *A. thaliana* in their response to growth promotion by *Bacillus* species in general and UCBM 5113 in particular have not been extensively studied. Differences in promotion of rosette and stalk growth between two *A. thaliana* accessions have been observed when these were treated with different strains of *B. amyloliquefaciens* (Schwachtje et al. 2012). The root architecture of plants is closely linked to plant productivity through nutrient and water uptake (Lynch 1995). It may therefore be of great agricultural interest to identify accessions of *A. thaliana* that respond positively to

Bacillus treatment. So far, a number of PGPB effects on root architecture have been observed in the Col-0 accession. Observations include stunted main root growth coupled with increased number and length of lateral roots as well as development of longer root hairs (López-Bucio et al. 2007, Niazi et al. 2014, Asari et al. 2017). In *A. thaliana*, root hairs have a diameter of approximately 10 µm and can reach a length of more than 1 mm which increases the root surface area substantially (Grierson et al. 2014). As root hairs increase, the area of the root increases enabling increased nutrient acquisition and surfaces for microbe interactions (Grierson et al. 2014). The plants own exudation plays a role in lateral root formation, L*er*-0 being more sensitive than Col-0 (Caffaro et al. 2011). The PGPB *Bacillus megaterium* has been shown to inhibit main root growth, increase lateral root growth and lateral root number as well as increase root hair length in Col-0 (López-Bucio et al. 2007).

Aim

The aim of the experiment was to determine the possible differences in phenotypic root growth response among a number of *A. thaliana* accessions in response to *B. amyloliquefaciens* UCMB 5113 treatment.

Hypothesis

As a result of genotype differences in the PGPR response due to different life history in widely different habitats there will be a difference in root growth among different *A. thaliana* accessions following *B. amyloliquefaciens* UCMB 5113 treatment.

Materials and methods

Twelve *A. thaliana* accessions were used for the experiment, the geographical origin of which can be seen in appendix 1. The accessions were grown in batches of four at a time for practical reasons and were randomly selected. All seeds were surface sterilized before use, and the entire exposed handling process was conducted in sterile flow hood. In preparation of the sterilization, all seeds were packaged in envelopes made from Miracloth (22-25 µm pores, Andwin Scientific) and sealed with colour-coded plastic paper clips. This was done in order to simplify the handling of the seeds and facilitate uniform treatment. The sterilization procedure consisted of a 10 second dip in 70% analytical grade ethanol followed by a 5 minute soak in 10 % bleach (volumetric dilution of Colgate brand household bleach containing sodium hypochlorite). The seeds were then rinsed in 100 ml autoclaved ultrapure water for 15 minutes, with the water being changed every 5 minutes. The seeds were then spread onto Petri dishes containing a 5 mm thick layer of 0.5x MS substrate with 0.6 % plant agar. This was done with the help of a 1 ml pipette that was used to distribute the seeds evenly across the surface using sterile water, which was then removed to the greatest extent possible in order not to have the seeds submerged. The plates were sealed with micropore (3M™ Micropore™) tape to avoid contamination of the plates while still maintaining aeration.

The plates were kept in a growth chamber for 7-12 days (N13, Ta-0, Edi-0, Mt-0, Col-0, Ta-0: 7 days; Shadara, Can-0, L*er*-0, Col-0, Ms-0: 8 days; Stw-0, Ws-0 12 days). The growth conditions were 16/8h day/night, 22 ^{o}C and 200 µmol photons m^{-2} s^{-1}. The time of incubation was dependent on the slowest germinating/growing accession of the batch so as to have a sufficient number of seedlings for transplantation. Seedlings were transplanted to rectangular 12 x 12 cm Petri dishes containing 0.5x MS and 0.8 % plant agar in numbers of four seedlings per plate and four plates per accession. The seedlings were transferred by using a flame-sterilized pair of tweezers, modified not to allow full closure in an effort to reduce the risk of

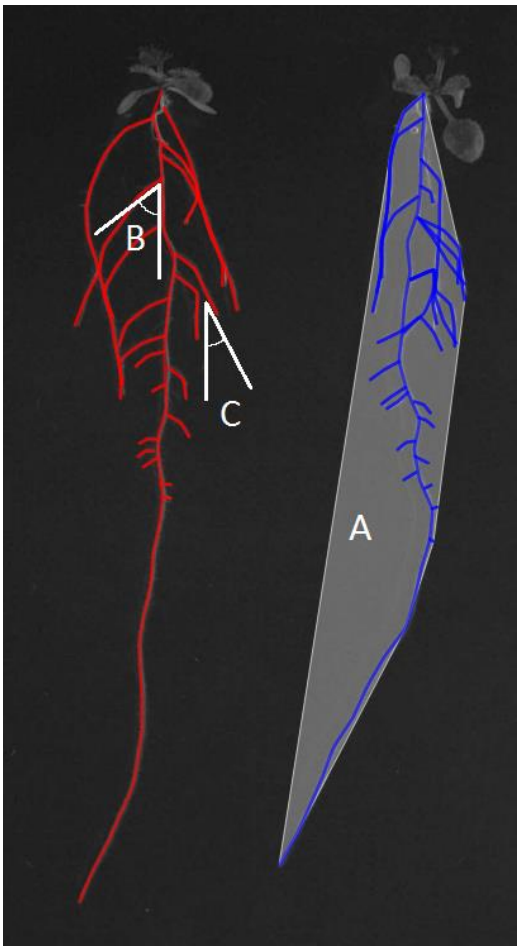

Figure 1. Image output from root image analysis software RootNav showing illustration of recorded traits. Boundary for hull area (A), lateral root insertion angle (B), lateral root tip angle (C). The coloured lines represent the root system as the software recognizes it.

accidental tissue damage. The seedlings were positioned in a linear pattern ca 20 mm from the top of the petri dish and with 25 mm spacing in between. The root tips were arranged to point towards the far bottom edge, parallel to each other. With all plants transplanted, two plates from each accession were selected to be inoculated with a suspension of *B. amyloliquefaciens* UCMB 5113. The two remaining plates were mock inoculated with sterile water. The bacterial suspension used was prepared by taking a single colony of UCMB 5113 from a LB agar plate and inoculating liquid LB broth, which was then incubated at 28 ^{o}C for 18 h in a shaker set to 180 rpm. A 100 µl sample was then taken and analyzed for OD at 1:10 dilution using a spectrophotometer set at 600 nm. Based on the reading, the bacterial suspension was adjusted to a concentration representing OD 0.4 by diluting it with autoclaved water. An aliquot (20 ml) of the resulting suspension was centrifuged at 5,000 rpm for 10 minutes using an Eppendorph 5804R centrifuge. The supernatant was discarded and 100 µl of sterile water added to the pellet, which was then vortexed. The volumes were adjusted according to the inoculation. An aliquot (10 µl) of the resulting bacteria slurry was added 30 mm below each root tip, and the petri dish subsequently sealed with micropore tape. The control plants were mock inoculated with 10 µl of sterile water. The plates were left for 20 minutes in a horizontal position for the bacteria to adhere to the growth medium, this so that the plates could be flipped upside down in the photo studio and photographs acquired from the bottom, through the medium. The plates were placed in a custom-made stand holding the plates at a 75 degree angle from the horizontal plane, allowing ca 40 mm spacing between the plates. The treatment and accessions were shuffled 2 steps in a circular pattern every other day and the stand rotated 180 degrees along the vertical axis in an effort to eliminate the potential effect of environmental differences within the growth chamber. At two day intervals the plates were photographed with a digital camera (Canon DS126151 with EFS18-58 objective) and the resulting images analyzed with RootNav software (Pound et al. 2013), a semi-automated image analysis software for recording root phenotypes. A flatbed scanner (Cano scan 4400F) was used momentarily, based on findings by Slovak et al. (2014). The acquired images were resized and contrast enhanced using Photoshop (Adobe) before being imported to the software. Recorded traits included main root length, lateral root length, number of lateral roots as well as insertion angle and hull area (the area defined by the outer perimeters of the root system, as shown in 1).

Generally, the standard *Arabidopsis* plate script was used as supplied with the software, but for certain images where conditions (light, contrast etc.) demanded, the patch size was modified as well as the threshold limit. For the accessions Stw-0, Ws-0, N13, Shadara, Col-0, L*er*-0, Ms-0 the progression of root growth was recorded after 4 days as well as after 8 days, but for the second set of measurements ImageJ (Schneider et al. 2012**)** was used for the analysis instead of RootNav. For the accessions Mt-0, Can-0 and L*er*-0, a period of 5 days of growth was used preceding the first measurement, Ta and Edi were measured after 6 days of growth. The differences in time derived from image quality issues in early batches and only days where images of all plants could be accurately analyzed were used. Root area measurements were produced for all roots using a grayscale threshold and particle analysis. Total growth was divided with the time of treatment until measurement, in an effort to make the results comparable.

For analysis of root hairs, microscopic images were taken after four days using a stereo microscope (Nikon SMZ 1500). Images were analyzed with ImageJ software to determine the length of root hairs. A length of 0.9 mm was analyzed for each root, starting 4.6 mm from the root tip. All root hairs within the focal range were measured and counted using ImageJ.

All experiments involving the screening of accessions had a common design with two independent variables: *accession* and *treatment* and a number of dependent variables. These conditions necessitated the use of multiple comparison statistics. R-statistics was used for all statistical analysis (R Core Team 2014). The first step of making the multiple comparisons involved plotting the data in a Cleveland plot for identification of extreme outliers, indicating typing or measurement error. A linear model was then fitted to the data. It was then assessed whether the data could be treated as being of normal distribution, an assumption that is made when doing analysis of variance. The normality of the dataset was determined by plotting measured and theoretical quantiles against each other (QQ-plot) and assessing the shape of the plot as described by Zuur et al. (2010). In cases where the data was not properly described by a linear model, variables were transformed with logarithmic or square root transformation to make the data better comply with the normality assumption made. A two-way ANOVA was used to identify possible treatment effects induced by the two independent variables *accession* and *treatment*. If there was any significant main effect induced by the independent variables or interaction between the two, a Tukey post-hoc test was performed for pairwise comparison of all treated vs. control groups of all accessions. The R-statistics plugin package: *Lattice-Multivariate Data Visualization with R* (Sarkar 2008) was used for the Tukey post-hoc test. The groups of accessions with significant differences between treated group mean μ_Y, and control group mean μ_X, underwent calculation of relative treatment effect:

$$Relative\ treatment\ effect\ (\mu_X, \mu_Y) = (\mu_Y - \mu_X)/\mu_X)$$

The relative treatment effects were compared using a one-way ANOVA. Approximations of standard deviation for the relative treatment effects were made using the delta method, based on a first-order Taylor series expansion:

$$SD.\left(\frac{\mu_Y - \mu_X}{\mu_X}\right) \approx \sqrt{\frac{Var(y)\mu_X^2 + Var(x)\mu_Y^2}{\mu_X^4}}$$

$Var(y)$ is the variance of the *Bacillus* treated group and $Var(x)$ the variance of the control.

Results

Root hair analysis

Treatment with *B. amyloliquefaciens* UCBM 5113 proved to significantly increase root hair growth for six out of ten analyzed *A. thaliana* accessions (Figure 2A). The increase in total root hair length was a result of either higher number of root hairs, increased length of individual root hairs or a combination of both. The accessions Shadara, Ta-0, Ms-0, n13, Stw-0 and Ler-0 showed a statistically significant increase in total root hair length as a result of treatment with *B. amyloliquefaciens* UCBM 5113.

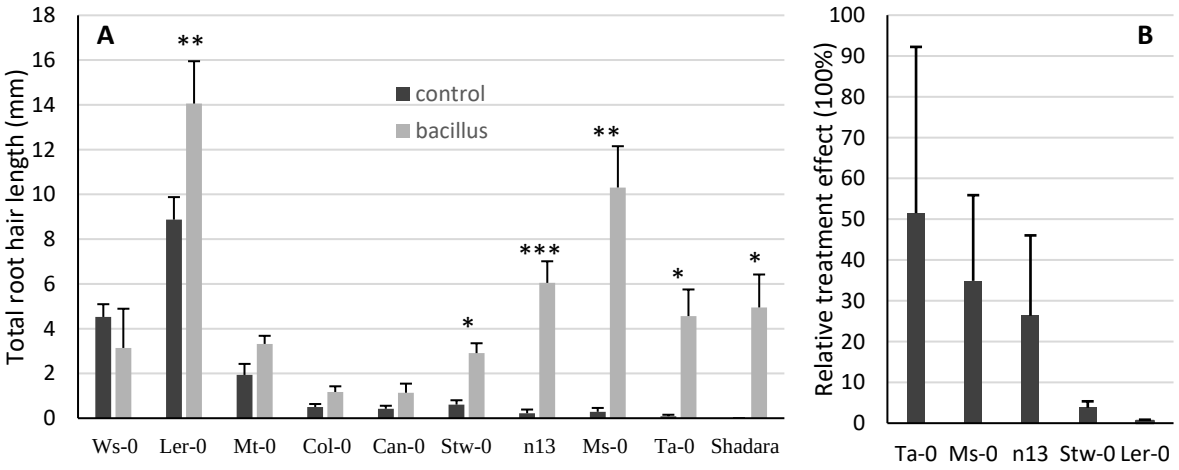

Figure 2. Combined length of all root hairs within a 0.9 mm segment of the main root with its centre 4.6 mm from the root tip of UCBM5113-treated and mock-inoculated plants, 2 days after start of treatment (A). Accessions having significant treatment effect plotted according to magnitude of relative difference between the treated group and negative control (B). Error bars represent SE and asterisks represent significance levels of differences between control and treated groups. n = 8 for all treatments. A Tukey HSD post-hoc test was performed after a multi-way ANOVA, significance levels in absolute treatment responses are represented by the following codes: '*' P ≤ 0.001 '**' P ≤ 0.01 '*' P ≤ 0.05. n = 8 for all treatments.**

The means and standard errors for total root hair length are presented in Figure 2A. A two-way ANOVA test was made to determine if there was any general treatment effect. The test showed a significant main effect for the accession factor, $F(9.1) = 24.37$, $p < 0.001$; significant main effect for the treatment factor, $F(1,9) = 104.36$, $p < 0.001$; and the interaction between accession and treatment was significant, $F(1,9) = 6.37$, $p < 0.001$. A Tukey's post-hoc test identified the six accessions where the treated group had higher total root hair length than the negative control group. Accessions showing significant treatment effect after *Bacillus* treatment with respect to total root hair length were, as earlier mentioned Ms-0, Stw-0, Ta-0, Shadara, Ler-0 and n13. The relative treatment effects of these accessions were compared with an ANOVA using the variances for the relative treatment effect that was calculated with the delta method as described on page 5. The ANOVA results for difference in relative treatment effect for total root hair length showed no significant difference among relative differences $F(4) = 0.99$, $P = 0.43$ (Figure 2B). The Shadara accession was not included in the comparison of relative treatment effects as all negative control plants belonging to said accession lacked root hairs on the studied section of the root, which would mean division with zero.

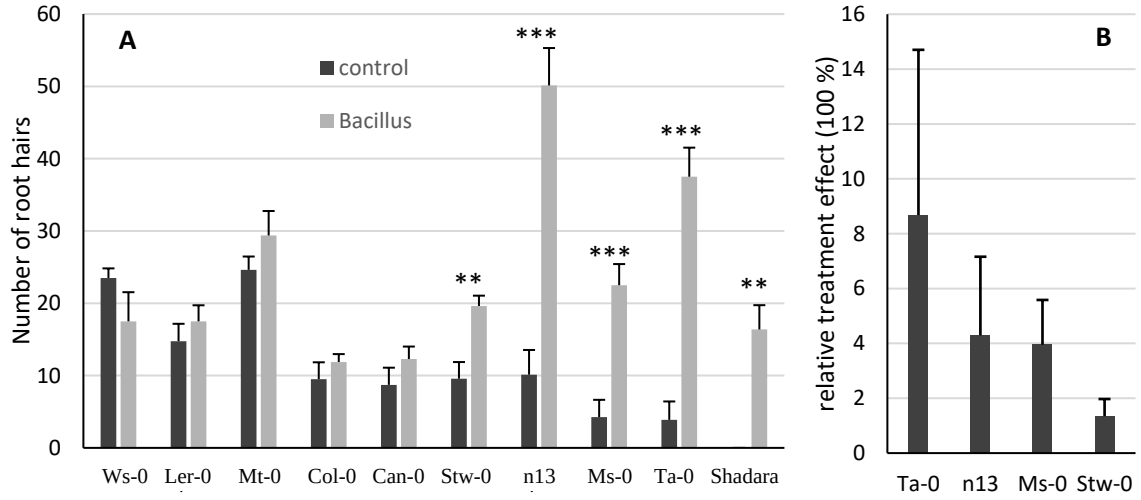

Figure 3. Number of root hairs within a 0.9 mm segment of the main root with its centre 4.6 mm from the root tip of UCBM5113-treated and mock-inoculated plants 2 days after start of treatment (A). Accessions having significant treatment effect plotted according to magnitude of relative difference between the treated group and negative control (B). Error bars represent SE and asterisks represent significance levels of differences between control and treated groups. n = 8 for all treatments. A Tukey HSD post-hoc test was performed after a multi-way ANOVA, significance levels in absolute treatment responses are represented by the following codes: '*' P ≤ 0.001 '**' P ≤ 0.01 '*' P ≤ 0.05. n = 8 for all treatments.**

The means and standard errors for the number of root hairs are presented in Figure 3A. A two-way ANOVA test was used to determine the presence of any general treatment effect. The two-way ANOVA showed a significant main effect for the *accession* factor, $F(9,1) = 15.00$, $p < 0.001$; significant main effect for the treatment factor, $F(1,9) = 166.51$, $p < 0.001$; and the interaction between accession and treatment was significant, $F(1,9) = 15.82$, $p < 0.001$. A Tukey's post-hoc test identified the accessions Shadara, Ta-0, n13, Ms-0 and Stw-0 as having formed significantly more root hairs in the treated group than in the negative control group. Analysis of possible differences between relative treatment effects on the number of root hairs was made on these accessions using a one-way ANOVA using variances derived via the delta method as described on page 5. No significant difference between relative treatment effects for number of root hairs could be seen, $F(3) = 0.78$, $P = 0.51$ (Figure 3B).

When visually inspecting plant roots it appeared that roots tended to become aerial when approaching the *Bacillus* patch and also formed more/longer root hairs when leaving the growth medium. No quantification of these observations was made as the event would take place after microscopic images had already been acquired and the three-dimensional growth pattern of the roots at this stage made it hard to take comparative photographs.

Root system architecture

The total root growth as plotted in Figure 4 was analyzed for general treatment by applying a two-way ANOVA test. There was a significant difference between accessions for root growth $F(10,1) = 8.69$, $p < 0.001$, however the effect of treatment was not significant, $F(1,10) = 0.02$, $p > 0.05$. There was no interaction effect between accession and treatment $F(10,1) = 0.6436$, $p > 0.05$. No post-hoc test was performed as there was no significant treatment effect.

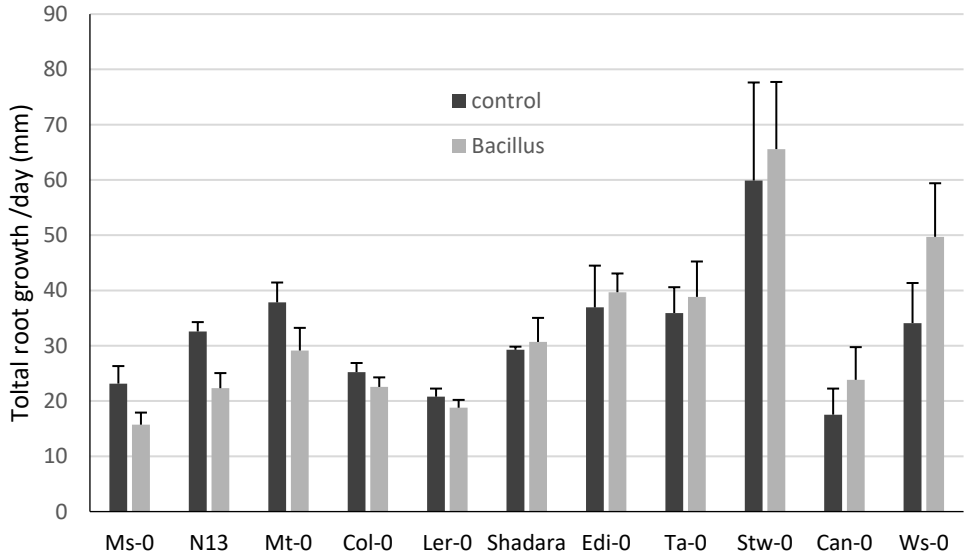

Figure 4. Average increase in total root length, including the length of main root and all lateral roots of UCBM5113-treated and mock-inoculated plants. Total root growth is given as an average growth per day during the treatment period in order for scaling reasons since length of treatment differed slightly between accessions. Error bars show standard error of the mean. A two-way ANOVA revealed no significant treatment effect on total root length. n= 8 for all accessions and treatments except: Col-0 and Ta-0 control and treated n=16, Mt-0 control n=4.

Figure 5. Photographic images of the accessions Mt-0 (A), and Col-0 (B) treated with a suspension of *B. amyloliquefaciens* UCMB 5113 visible as light streaks across the centre of the Petri dish. Mock inoculated plants of the accessions Mt-0 (C) and Col-0 (D) can be seen at the bottom. Pictures were taken at 8 days after the start of treatment.

Two accessions treated with *Bacillus* are shown in Figure 5 with stunted main root growth and longer lateral roots in the *Bacillus*-treated plants. A diversion of the main root around the *Bacillus*-patch could be seen in two of the treated Col-0 plants, a pattern that was seen to varying degree in most accessions coupled with less noticeable diversion of lateral roots (note that these observations were not quantified).

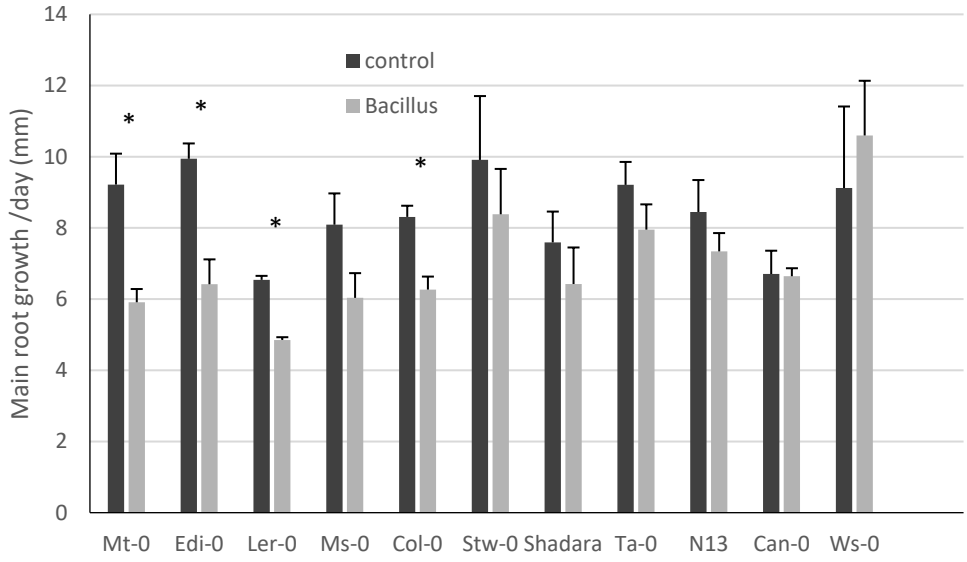

Figure 6. Average increase in main root length of UCBM5113-treated and mock-inoculated plants. Main root growth is given as an average growth per day during the treatment period in order for scaling reasons since length of treatment differed slightly between accessions. Error bars are standard error of the mean. A multi-way ANOVA revealed a significant treatment effect on total root length. Group sizes: n= 8 for all accessions and treatments except: Col-0 and Ta-0 control and treated n = 16, Mt-0 control n = 4. Significance levels in treatment responses are represented by the following codes: '*' P ≤ 0.001 '**' P ≤ 0.01 '*' P ≤ 0.05.**

Growth of the main root differed significantly between accessions and treatment with UCBM 5113 had a significant effect (Figure 6). A two-way ANOVA showed significance for the accession factor, $F(10,1) = 3.56$, $p < 0.001$, and a significant effect of the treatment factor, $F(1,10) = 18.89$, $p < 0.001$. There was no significant interaction effect between accession and treatment $F(10,1) = 1.01$, $p > 0.05$. A Tukey post-hoc test revealed that there was a significant inhibition of main root growth in five of the accessions as a result of *B. amyloliquefaciens* UCBM 5113 treatment.

Five accessions grown in parallel were analyzed after eight days of treatment. The capabilities of RootNav to trace roots at such an advanced growth were found insufficient and ImageJ was used to measure the combined area of all root parts instead of doing length measurements with RootNav. When the area of root colonization was analyzed with a Tukey's post-hoc test following a one-way ANOVA, the L*er*-0 accession showed a significantly larger area of root colonization in plants of the treated group than in the negative control group while the four other accessions, although not statistically significant appeared to show a tendency towards smaller root area in the treated groups (Figure 7).

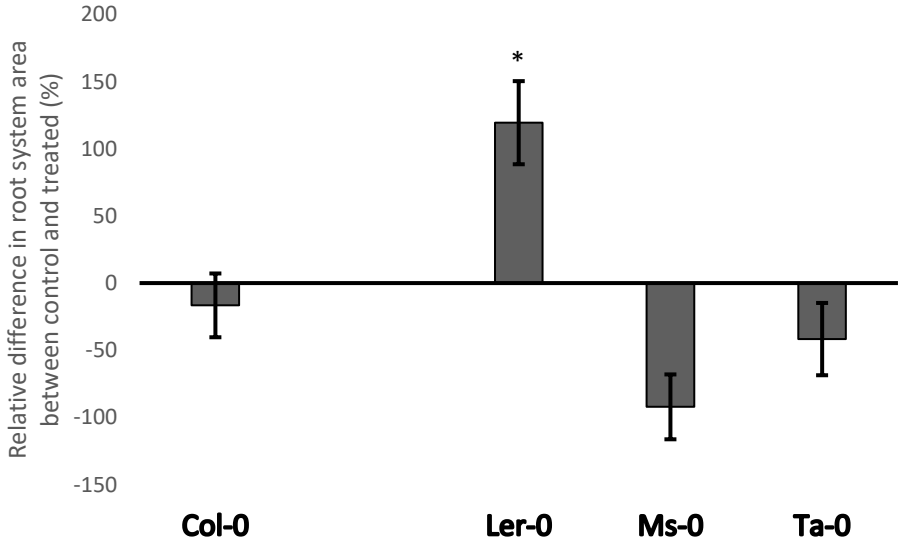

Figure 7. Relative difference in root area between negative control and UCBM5113-treated plants at 8 days after start of treatment. Positive percentages indicate higher growth in the treated group than in the control group and vice versa. Treatment effect was determined by one-way ANOVA and pairwise comparison was done with Tukey HSD post-hoc test. n = 8 for all treatments.

Discussion

The prospect of altering root architecture in crop plants in order to more efficiently harvest nutrients from the soil is without doubt appealing. The results from the conducted experiments in conjunction with previous research does make it clear that *B. amyloliquefaciens* UCMB 5113 treatment of plants may well establish a more beneficial root architecture. Differences in effect on root architecture by *Bacillus* treatment between the accessions were observed. The most spectacular treatment effect of *Bacillus* treatment was that on root hair growth, it will therefore have to be the key feature for discussing accessions in possession of advantageous properties. The accessions Shadara, Ta-0, Ms-0, n13, stw-0, and Ler-0 were topping the scale in relative difference between negative control plants and *Bacillus* treated plants when it came to total root hair length. The same accessions that showed increased total root hair growth appeared to also have a significant increase in the number of root hairs, except for the L*er*-0 accession, for which the increase in total root hair length was solely an effect of longer individual root hairs. When studies on phosphate acquisition efficiency have been performed, a high root hair density in combination with long root hairs have been seen to be beneficial to the plant (Narang et al. 2000). The importance of the increased root hair growth can be emphasized by a recent cost-benefit modelling on root traits conducted by Brown et al. (2013), where they concluded that root hair is the most interesting trait for increasing soil phosphorous uptake. The same study also indicated that increasing the length of root hairs would be more cost effective than increasing root hair density. From that perspective, the L*er*-0 accession appears to possess the most beneficial properties for potential *Bacillus*-mediated enhancement of phosphorus uptake.

To further investigate the response of root hair growth to *Bacillus* treatment in different accessions, a more complete phenotyping of the whole root system could be made. This could be done by selecting certain areas which are consistent between accessions, e.g., lateral root insertions, lateral root tips etc. Image recognition coupled with automatic microscopic image

acquisition would greatly increase the throughput if this was to be undertaken. Attempts were made to automate the image analysis part of the root hair phenotyping, but it proved hard to generate images of sufficient photographic quality to allow for reliable measurements without the need for extensive manual input. The main problem was that two-dimensional images were used to estimate length and density of root hairs, which are spatially distributed in three dimensions. The naked eye could with relative ease identify which root hairs that were attached perpendicular to the root, and within the relatively narrow focal plane. The image analysis software tended to track blurred root hairs outside the focal plane and typically traced the shortest distance to the root which did not necessarily coincided with the real length of angled root hairs. An attempt to classify the diameter of the root hairs was also made as this would greatly affect the surface area. It did, however prove difficult to get consistency between plants as optical distortion induced by varying thickness of water film around the roots reduced accuracy. If contamination was not an issue, a drop of water added to all roots before image acquisition could potentially remedy this phenomenon.

Some words of caution should probably be mentioned concerning the accuracy of root hair measurements. There could be a possibility that an equal number of root hairs had been formed in both control and *Bacillus*-treated groups at the time of image acquisition, but that the treatment initiated formation closer to the root tips. Another consideration to be made is that the studied area may not necessarily be representative of the whole root system of the plant, hence conclusions based on the data would only be applicable to the area described in the methods section.

A few words on the subject of main effect from the *Bacillus* treatment could be mentioned despite its subordinate role when compared to the treatment-accession interaction effect. The main root was shorter in plants treated with *Bacillus*. The growth stagnated when approaching the patch of bacteria and was seemingly interrupted in its vertical growth for a matter of days (as can be seen in Figure 5), before continuing. Roots respond to zones with elevated nutrient concentrations by proliferating in their vicinity (Robinson, 1994; Hodge, 2004). With this knowledge as base, one could argue that nutrients released by the bacteria themselves could lead to stunted main root growth which in practice would simply be a strategy for increased lateral root growth in order to maximize root length in the general area.

The tendency to diverge around the *Bacillus* patches and continue downwards can be seen in the case of Col-0 seedlings in Figure 5. Even though the inoculated plants had significantly shorter main roots in most cases, the increased number of lateral roots and their increased length seems to have evened out the differences in total root area, giving the two roughly the same area. A reoccurring pattern that was observed for almost all accessions was that of the main root to outflank the patch of *Bacillus*, while the lateral roots seemingly unaffected grew right through them. This could be a result of the main root having receptors for a repelling compound excreted by the bacteria, while the lateral roots may lack such function. Or it could be that the main root is navigating in close proximity to nutrient rich areas while the lateral roots are left to develop a dense infrastructure for nutrient extraction in those areas. If the interpretation of the observations would be made more elaborate, some conclusions could possibly be made regarding accession differences. Image analysis for spatial lateral root density and angular properties of the main root growth with well-defined thresholds would make such a comparison more meaningful.

The idea of measuring the length of roots was based on the perception that root length would be coupled with the ability to interact with the surrounding soil. The surface area of the roots may have a greater impact on the soil interaction, but in much the same manner as discussed for the root hair measurements, some difficulties were encountered when measuring these cylindrical organs. The light setting during photography in combination with varying amounts of water adhering to the roots made the diameter measurements unreliable. For accurate measurements a destructive technique might be necessary.

Finally, the issue of comparing differently aged plants would also have to be discussed. In this experiment the accessions were grown in batches because of space limitations in the growth facilities. Slight time differences between seed plating and start of treatment occurred because of differences in time of germination. That inevitable leads to the question of whether absolute age or developmental stage of growth should be prioritized when making comparative experiments involving different accessions. Increased rate of root elongation has been observed during the first 14 days after germination in *Arabidopsis* and is thought to be related to an elongation of the growth zone, giving a larger number of dividing cells (Beemster and Baskin 1998). If accessions of similar age but different growth stage are compared they could well be in different sections of their respective growth rate curves. The initial plan of accession comparison involved establishment of growth curves for the separate accessions in an attempt to mitigate such effects, however analyzing the staggering amount of photographs for multiple time points and treating the data in a statistically sound manner proved overwhelming.

2. Plant accession dependence on *B. amyloliquefaciens* UCMB 5113 spatial colonization pattern

Introduction

Efficient rhizosphere colonization is of great importance for the formation of successful plant-PGPR interaction (Chin-A-Woeng et al. 2000). Comparatively little is known about the colonization pattern exhibited by gram-positive bacteria in contrast to that of gram-negative bacteria (Dietel et al. 2013). Root hairs of *Arabidopsis* as well as maize have been seen to be favourable habitats for gram-positive bacteria, including *B. amyloliquefaciens* FZB42 (Fan et al. 2012a). The colonization of the root hairs as well as preference for primary root tips has been attributed to the abundance of root exudates in their vicinity (Fan et al. 2012a). Groves between epidermal cells have also been found to be a preferred habitat (Fan et al. 2011). Although unknown in its possible role of shaping PGPR colonization, differences in root exudate metabolite profiles have been shown between several *A. thaliana* accessions (Mönchgesang et al. 2016). The importance of plant accession for *B. amyloliquefaciens* colonization is uncharted territory at the time of writing, and would hence benefit from investigation.

Aim

To study the *Bacillus* colonization pattern for two *A. thaliana* accessions selected based on the level of treatment effect by *Bacillus* on root phenotype.

Hypothesis

There will be similar colonization pattern for the two accessions Ta-0 and Ms-0 since the physiology of the root exudation is expected to be similar even though the exudate composition may differ to some degree.

Materials and methods

Ms-0 and Ta-0 seeds were sterilized as described for experiment 1 and spread on two types of 0.5 % MS agar plates, with 1.5 % sucrose or without sucrose. GFP-tagged UCBM 5113 cells were incubated in LB at 28°C for 18 hours. After incubation, the OD was adjusted to 0.4 by washing and diluting with sterile water. A sample of the suspension was tested in a microscope equipped with a UV-lamp to ensure positive fluorescence function. The seedlings were dipped in the *Bacillus* suspension before being transplanted to 12 x 12 cm square petri dishes containing 0.5 x MS medium with 0.8 % agar. After 7 days of incubation at 16 h photoperiod, 22°C and 200 µmol photons m^{-2} s^{-1}, two roots for each accession and treatment were cut and placed on a microscope slide, followed by the application of a few drops of water. The roots were observed using a Zeiss 780 Inverted Axio observer microscope with a supersensitive GaASp detector. The images were acquired using a C-Apochromat 40x/1.20 W Korr FCS M27 objective using water immersion. Samples were illuminated with 488 nm neon laser at 2.0 % power, and filters allowing for a spectral detection range of 493-598 nm were used. Pixel dwell time was set to 1.58 µs. Pinhole diameter was 90 µm and master gain set to 955.

Results

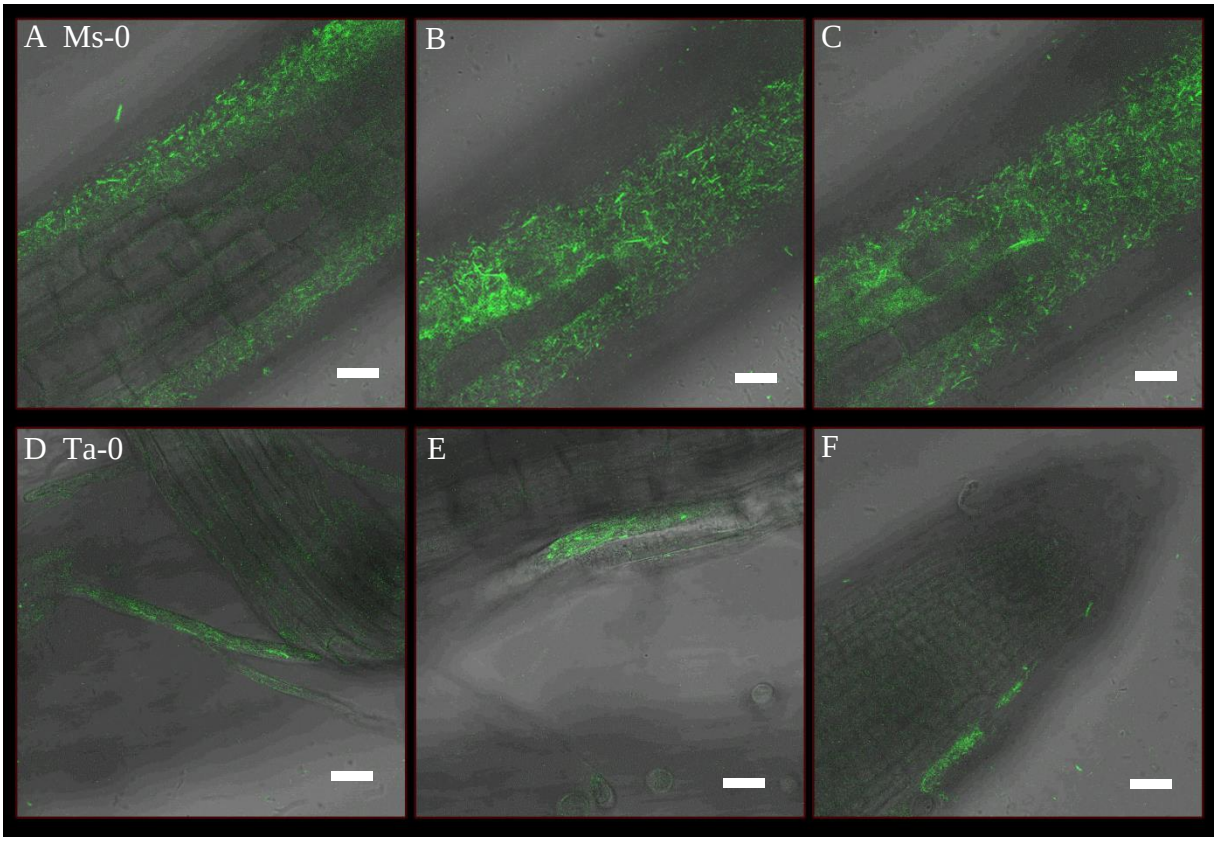

Figure 8. Microscopic images of Ms-0 accession roots grown on sucrose-containing medium and colonized by GFP-tagged *B. amyloliquefaciens* UCMB 5113 (green) at 40x magnification and three different focal lengths to illustrate the colonization pattern 0.5 mm from the root tip (A,B,C). Ta-0 accession grown on sucrose-containing medium, showing colonization along root hair (D,E) and root tip (F). Scale bars = 25 µm.

Ta-0 plants grown on plates without sucrose showed few bacteria attached to the roots, while Ta-0 plants grown on agar plates containing sucrose showed higher bacterial colonization, primarily on and in close proximity to the root hairs (Figure 8D-E). Slight colonization was also noticed close to the root tip (Figure 8F). The Ms-0 plants that were cultivated on sucrose medium showed dense colonies of *Bacillus* (Figure 8A), enveloping major parts of the root close to the root tip (Figure 8B-C). The colonization was not as dense on the actual root tips. Ms-0 plants that had been cultivated on sucrose-free growth medium showed similar levels of colonization as for Ta-0 on sucrose-free medium.

Discussion

Among the plants that were studied, the tendency did seem consistent in that roots of the Ms-0 accession had *Bacillus* colonization close to the root tip, while the roots of Ta-0 plants had root hair colonization to a greater extent. It must be noted though, that due to time constraints a statistically satisfying foundation of observations on the colonization patterns could not be made. The colonization patterns that were observed could be interpreted as having contrasting effects on the plant-microbe interaction. The pattern seen for Ms-0, with dense surface colonization, strongly resembled a biofilm, and would as such likely have a positive effect on the defence towards soil-borne pathogens, as discussed by Bais et al. (2004) among others. The colonization pattern of *Bacillus* on Ta-0 plants, with seemingly a preference for root hairs, has been observed in other studies. It has been observed that *B. amyloliquefaciens* FZB42 tend to adapt the colonization to external features of the root and position themselves

in depressions formed in the boundary zones between adjacent epidermal cells. This bacterial growth effect has been speculated to aim towards maximizing extraction of root exudates originating from these regions (Fan et al. 2012a). Incubation of UCMB 5113 with Ms-0 and Ta-0 exudates may shed some light on whether there is a significant difference in exudation between the two accessions that could account for the difference in colonization pattern on the roots. Better yet would be an *in situ* spatial analysis of exudation. It cannot be excluded that some bacteria may have been washed of the root surface when preparing the samples for microscopy. Free floating bacteria in the water surrounding the roots suggest that at least some bacteria have been relocated from their original niches. A plan for immobilizing the bacteria and binding them to the root surface was devised but never realized. Gene expression alteration mediated by root exudates may affect the colonization preference/ability of *Bacillus*. Fan et al. (2012) suggested that root exudates mainly effect *Bacillus* proliferation through their sugar content. Even so, suppression of the transcription regulator AbrB has been seen to facilitate a denser colonization of cucumber roots (Weng et al. 2013). The same mechanism might well be at play in this case.

3. Factors involved in *Bacillus* root growth promotion

Introduction

PGPB, including *Bacillus,* are able to produce an array of phytohormones that have the potential to alter plant growth in different ways (Glick 2012). The hormones include indole-3-acetic acid (IAA) which is known to stimulate root development by increasing growth and inducing lateral root formation (Pilet and Saugy 1987). Ethylene is the simplest hormone in term of molecular structure, and is known to act in root growth initiation as well as inhibition of root elongation with vastly different outcomes being achieved depending on dosage (Burg 1973, Růžička et al. 2007). Apart from altering root growth through means of hormonal control, PGPB are able to modulate gene expression associated with nutrient uptake and cell division within the host plant (Camilios-Neto et al. 2014). The mechanisms at play in *Bacillus* mediated growth promotion are largely unknown, but are thought to involve elements of auxin/ethylene signalling, as well as direct increase of nutrient availability through various processes (López-Bucio et al. 2007, Niazi et al. 2014, Asari et al. 2017).

In order to identify possible factors involved in *B. amyloliquefaciens* UCMB 5113-derived alteration of root architecture, a number or root mutants were treated as described in experiment 1 and subsequently phenotyped. The mutants had deficiencies in root hair formation, lateral root formation, or main root formation (Table 1).

Two lateral root-deficient mutants were used. The D6012 has been assumed to miss lateral roots as a result of function loss in the *d6pkl2* gene, but lateral root formation can be reestablished by treatment with the auxin 1-naphthalene acetic acid (1-NAA) (Zourelidou et al. 2009). The N2260 mutant is deficient in the At3g13870 gene, required for regulated cell expansion and normal root hair development (Chowdhary et al. 2012). The mutant with PSY-2 defect has a non-functioning phytoene synthase which is a transferase enzyme involved in the biosynthesis of carotenoids, which as a result affects lateral root formation (Norman et al. 2014). López-Bucio et al. (2007) found in their study of *B. megaterium* treatment of *A. thailiana* that the treatment induced increased number of lateral roots and root hair length in *aux1-7* and *eir1* auxin/ethylene mutants, and also concluded that auxin/ethylene independent

mechanisms could be involved. In order to investigate whether *B. amyloliquefaciens* UCMB 5113 is also capable of similar feats, the following experiment was made.

Aim

To test if *B. amyloliquefaciens* UCMB 5113 possesses the ability to alter the root phenotype of root mutants.

Hypothesis

B. amyloliquefaciens UCMB 5113 will be able to revert some of the root phenotype deficiencies characteristic for certain root mutants because of its ability to produce growth stimulatory factors.

Material and methods

Six mutants with root-related defects were used in the experiment. The experiment was divided into two batches with wildtype Col-0 used as a reference. The seeds were surface sterilized as described above, but were only given a quick dip in ethanol not to weaken a potentially already poor germination. The seeds were grown on 0.5 x MS, 0.6 % agar plates for 8 days in a growth chamber with 16 h photoperiod, 22 ^{o}C and 200 µmol photons m^{-2} s^{-1} . For *psy-2* mutants, 1.5 % sucrose was added to the medium because of the slow growth of the phenotype. Seedlings were subsequently transplanted to rectangular 12x12 cm petri dishes with 0.5x MS, 0.8 % agar, and inoculated with 10 µl *Bacillus* suspension prepared as described in experiment 1 (page 4). *psy-2* mutants were inoculated with 5 µl *Bacillus* suspension directly at the root tip. Sixteen plants of each mutant were used, of which 8 were *Bacillus* treated and 8 were mock inoculated with an equal amount of sterile water. For *psy-2*, 8 plants were included in total, 4 negative control and 4 treated plants. The plates were then sealed with micropore tape. Microscopic images of the root hair analysis were acquired after 4 days as described for experiment 1 and photographs for root architecture analysis were taken 5 days after the start of treatment as described for experiment 1.

Table 1. *A. thaliana* mutants with description of the defect and the responsible gene

Name	Defect	Gene ID
N2259	Glabra (gl1), root hair defective (*rhd2*)	At5g51060/At3g27920
N8059	*eto2*, short root	At5g65800
N2260	root hair defective, *rhd3*	At3g13870
N2261	root hair defective, *rhd4*	
D6012	lateral root defective	D6pkl02
PSY-2	lateral root defective	PSY-2

Results

The mutant N2259 did show significantly higher total root hair length in the treated group than in the control group when comparing the two with an independent sample Students t-test (Figure 9A) ($p<0.05$, $n = 8$). A reversal of the stunted root hair growth of N2259 control plants (Figure 9C) appears to have taken place in the *Bacillus* treated-plants (Figure 9D). Mutant 2261 did not show any increase in the number of root hairs as a result of *Bacillus* treatment (Figure 9B) ($P>0.05$, $n = 8$). The root hairs in mutant N2260 appered to have a brush-like apperance and were too dense and curled in both control and treated plants to make measurement of them reliable by conventional means. As such, no sucessful attempt to

analyze effects on N2260 by measuring and counting individual root hairs were made, other than visually and with area analysis in ImageJ, none of which identified any treatment effect.

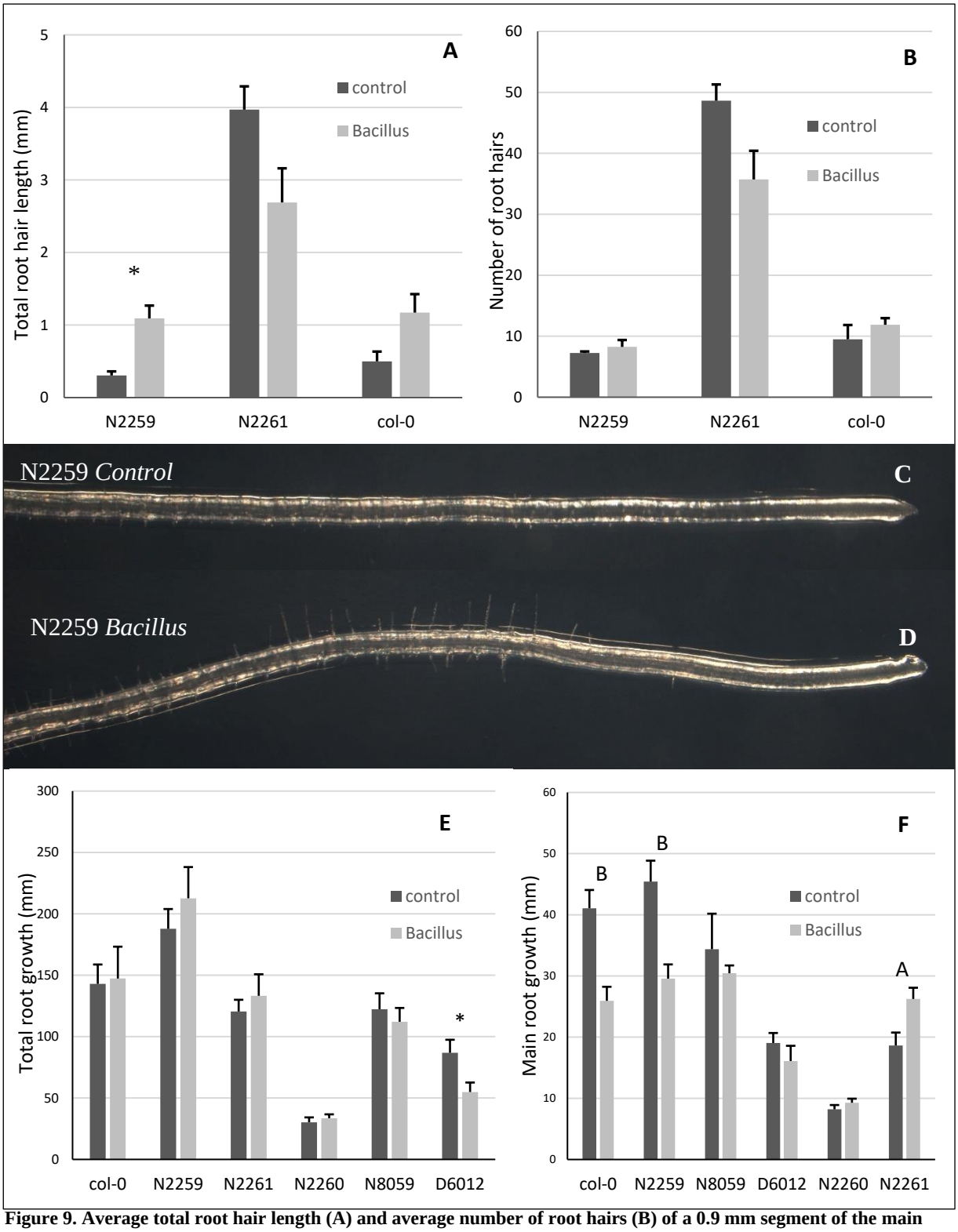

Figure 9. Average total root hair length (A) and average number of root hairs (B) of a 0.9 mm segment of the main root with its centre 4.6 mm from the root tip of 12-day old Arabidopsis treated with UCMB 5113 for 4 days, n=8. Microscopic image showing 7.25 mm long root segments from the N2259 mutant that have undergone mock treatment (C) or *B. amyloliquefaciens* UCMB 5113 treatment (D) for 4 days. Total root length (including main root and lateral roots) of mutants listed in table 1 is shown in (E). Error bars are SEM and asterisk represents a significant difference between treatment and control corresponding to a P <0.05 when using Tukey´s HSD post-hoc test for multiple comparison. Main root growth is plotted for *Bacillus*-treated and control plants, letters signify ranking of significant treatment effects (F). Error bars are showing standard error of the mean in all figures.

Contrary to the natural accession used as reference, the mutants N8059 and D6012 did not show any inhibition of main root growth, while N2261 showed an increased main root growth as a result of *Bacillus* treatment (Figure 9F). Mutant N2259 just as Col-0 suffered a repression of main root growth following the *Bacillus* treatment (Figure 9F). Total root growth only showed a significant treatment response in mutant D6012, with a repressed growth following *Bacillus* treatment (Figure 9E). *Bacillus* treatment did not lead to any significant effect on either the number of lateral roots (Figure 10A) or the hull area in any of the mutants (Figure 10B).

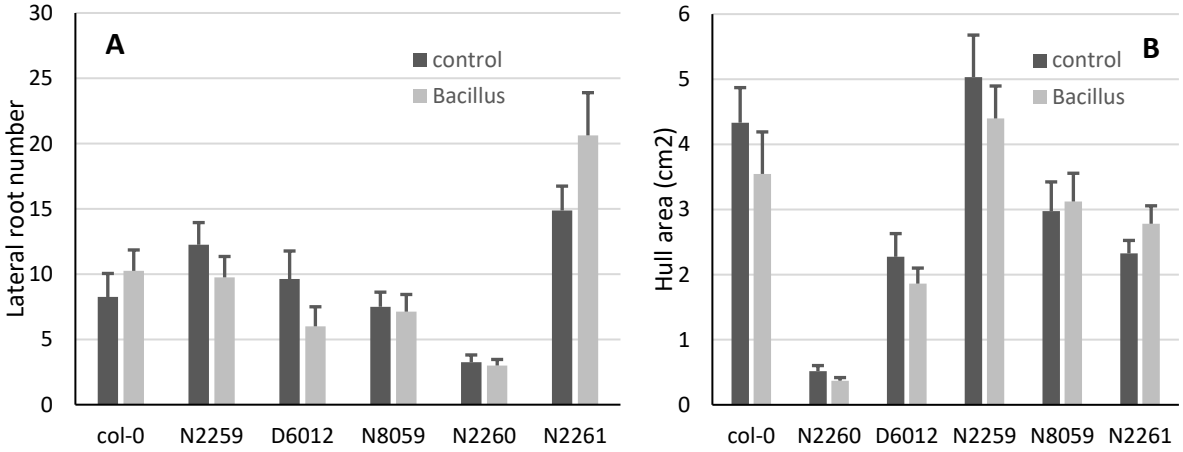

Figure 10. Increase in number of lateral roots (A) and hull area (B) of mutants of *A. thaliana* during 5 days of treatment with UCBM 5113. Col-0 accession was included for reference. Plants were 12 days old when data was gathered. Figure bars represent group means and error bars indicate standard error of the mean. n=8 for all treatments.

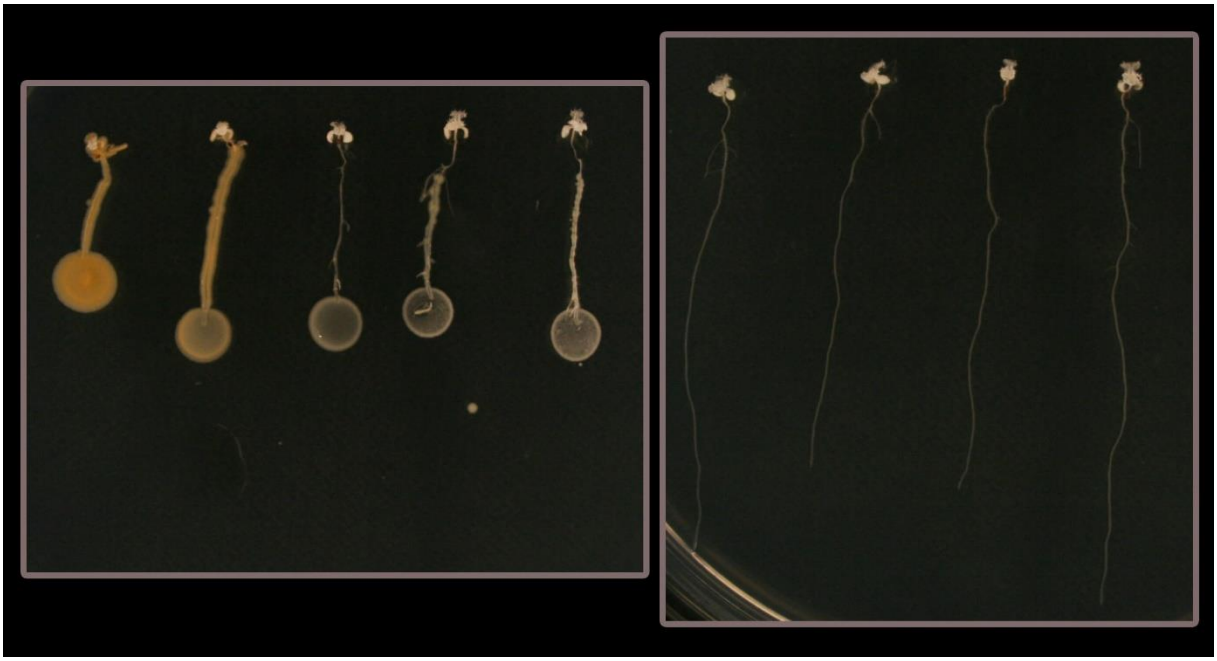

Figure 11. The images show 14-day old *psy-2* mutants, treated with *B. amyloliquefaciens* UCMB 5113 at 5 days post start of treatment (left) or mock inoculated (right).

The PSY-2 deficient mutants showed a stunted root growth in all of the *Bacillus* treated plants, as well as heavy colonization on most of the roots. Three of the four control plants as well as three out of four inoculated plants showed formation of lateral roots (Figure 11).

Discussion

One of the results that could be seen as more interesting and therefore worthy of receiving the initial attention of the discussion is that of mutant N2259. N2259 did show significantly higher total root hair length in the *Bacillus*-treated group than in the mock-treated control, which is intriguing since the control roots were virtually lacking developed root hairs. N2259 appears to have initiated root hair growth, but not yet undergone root hair tip growth. As described by Schiefelbein et al. (1992), root hair growth requires Ca^{2+} influx to the root hair cells. In the N2259 mutant the Ca^{2+} uptake is limited and appears to result from a lack in the production of reactive oxygen species (ROS) (Foreman et al. 2003). In addition, treatment with ROS appeared to suppress the mutant phenotype of short root hairs to some extent (Foreman et al. 2003). *B. amyloliquefaciens* UCBM 5113 is known to possess numerous defences against ROS (Niazi et al. 2014). *Bacillus subtilis*-derived surfactin has been reported to trigger early defence events such as ROS production coupled with Ca^{2+} effects in tobacco (Jourdan et al. 2009). If similar events unfold in the interaction between UCBM5113 and *A. thaliana* they could potentially account for the increased root hair growth in the N2259 root hair-defective mutant. It would in that case be required that ROS production was re-established by the interaction.

Shi et al. (2010) as well as López-Bucio et al. (2007) reported inhibited main root growth and increased lateral root growth as a result of rhizobacterial interaction with *A. thaliana*, which they partly attributed to both auxin-dependent and independent pathways. The formation of sites capable of forming lateral roots are spatially and temporally dependent on periodic changes in gene expression close to the root tip (Norman et al. 2014). The establishment of sites that can develop lateral roots appear to be dependent on carotenoid biosynthesis (Norman et al. 2014). It would appear that the treatment with *B. amyloliquefaciens* UCMB 5113 could initiate lateral root formation as there are indications that the bacteria produce an orange pigment that very well could be some form of carotenoid supported by gene homology and annotation studies (Niazi et al. 2014). The inoculated Psy-2 defective mutants did not seem to show any reversal of their deficiencies as a result of *Bacillus* treatment. If this had been the case it might have indicated the ability of *Bacillus* to restore lateral root formation by restoring or bypassing carotenoid synthesis in the plant. As UCMB 5113 has a gene most likely related to phytoene synthase (Niazi, 2014), the idea of it being able to affect the PSY-2 deficient mutant through exudation of cofactors related to carotenoid biosynthesis was deemed possible. Possible uncertainties in the experiments could be the use of sucrose-MSA substrate for the *psy-2* cultivation. It is possible that it aided the colonization of the roots. A sucrose-free MS medium may give other conditions for the interaction and could possibly produce another result, so further investigation on this subject would be needed.

4. Plant accession role in *B. amyloliquefaciens 5113* proliferation

Introduction

The rhizosphere is a stage for an array of complex interactions between plants and micro-organisms. One of the services provided to the microbial community by the plants, is the excretion of exudates by plant roots (Rovira 1969). Root exudates have been found to play important roles in the interaction with soil bacteria, and for the fruitfulness of the plant-microbe interaction (Fan et al. 2012a). High performance liquid chromatography (HPLC) analysis of root exudates has revealed that different accessions of *A. thaliana* possess unique root exudates with respect to composition and concentration of constituents. The exudates of accessions Cvi-0 and Ws-0 appear to be closely related in their composition, while Col-0 show some difference and L*er*-0 being very different (Micallef et al. 2009). In addition to providing nutrients, the root exudates are able to affect gene regulation in bacteria, among them *B. amyloliquefaciens* FZB42. The root exudates are known to contain carbohydrates, amino acids and organic acids among other compounds (Doornbos et al. 2012).

Aim

To determine the possible differences in growth response of UCMB 5113 to root exudates isolated from different *A. thaliana* accessions.

Hypothesis

Different *A. thaliana* accessions produce unique exudate compositions differing in their ability to support bacterial growth, hence giving a measurable difference in bacterial growth response between them.

Materials and methods

Seeds of nine accessions (Col-0, L*er*-0, Mt-0, Ws-0, Stw-0, Gre-0, Cvi-0, Edi-0, Ag-0) were surface sterilized and plated on 0.5x MS, 0.6% agar plates supplemented with 1.5% sucrose, as described above. The seedlings were transplanted after 7 days to rectangular 12x12 cm petri dishes containing 0.5x MS with 0.8 % agar and 1.5 % sucrose. Plants were lined up tightly, 2 cm from the upper edge of the agar surface, and the plates were put back in to a growth chamber, positioned vertically and incubated for another 14 days with periodic shuffling of position within the chamber to equalize possible heterogeneity in growth conditions.

At the age of three weeks, 20 plants from each accession were transplanted to pipette tip boxes that had been autoclaved for 15 minutes at 125 ^{o}C. The pipette boxes had the rack still installed. An improvised metal hook and a pair of soft tweezers were used to thread the roots through the wells in the tip rack. The plants were left hanging suspended on their rosette, and had the roots protruding down towards the bottom of the box. The boxes were subsequently sealed with micro-pore tape after being filled with 200 ml of liquid 0.5x MS solution. After 2 weeks the liquid in the boxes was concentrated by first freezing the solution at -20°C and then being lyophilized in high vacuum (Edwards Modulyo freeze dryer). The resulting precipitate was suspended in 100 ml of K_2HPO_4/KH_2PO_4 buffer, pH 7. The resulting solution was

filtered using 25 mm diameter, 0.22 µm HPLC grade nylon syringe filters for removal of debris, and then aliquoted and stored at -20^{o}C until the start of the incubation experiment.

For the incubation a 96-well plate was loaded in the following manner: 20 µl of root exudate was added to 70 µl of M9 minimal medium and 10 µl of washed *B. amyloliquefaciens* UCMB 5113 adjusted to OD 0.4 in sterile water. Eight replicates were used for each root exudate and 90 µl M9 with 10 µl of *Bacillus* suspension was used as negative control. Exudates were heated to 65^{o}C for 1 min using a thermomixer, vortexed and cooled to 22 ^{o}C before loading the 96 well plate. M9 medium was prepared as described (Cold Spring Harbor Protocols 2010). It must be noted that $CaCl_2$ was the first salt added when preparing the final concentration of M9 minimal medium, and the final stock was kept at room temperature until use. The loaded 96 well plate was incubated in a FLUOstar Omega microplate reader (BMG labtec) for 17 h 26 min and optical density was measured at intervals. The following program parameters were used: incubation, 28 ^{o}C with continuous double orbital shaking at 200 rpm, measurement: 600 nm wavelength with 479 s cycle time, 132 cycles and 150 flashes/well. The statistical significance of difference between the growth curves was assessed with a permutation test developed for the specific purpose to compare groups of growth curves. The test used is described in further detail by Elso et al. (2003), but is in short a pairwise t-test with a high number of permutations in which the P-value is the proportion of permutations. The statistical test method was designed by Russell Thompson and Gordon Smyth, and is contained within R-package version 1.2.4. http://www.statsci.org/r.

Results

The growth dynamics expressed by *B. amyloliquefaciens* UCMB 5113 incubation with root exudates of different accession origin had a number of differentiating features. The visual assessment of growth curves showed that exudates of Ag-0 stimulated the earliest rise in *Bacillus* growth, starting at 250 minutes. Bacillus growth in all other exudate treatments did not enter an exponential phase until the 800 min mark (Figure 12). *Bacillus* growing with Cvi-0 and Gre-0 exudates took longer time to enter an exponential growth phase than did *Bacillus* growing in exudates of Mt-0, Ws-0, Edi-0 L*er*-0, and Col-0. The latter transitioned from exponential growth to linear growth at 900 minutes after start of incubation. The *Bacillus* growing with exudates of Mt-0, Ws-0, Edi-0, L*er*-0, and Col-0 continued their linear growth pattern until the end of the incubation. *Bacillus* growing with exudates from Stw-0, Gre-0 and Cvi-0 reached a higher OD than all treatments, except the *Bacillus* treatments with exudates from the accession Ag-0 (Figure 12).

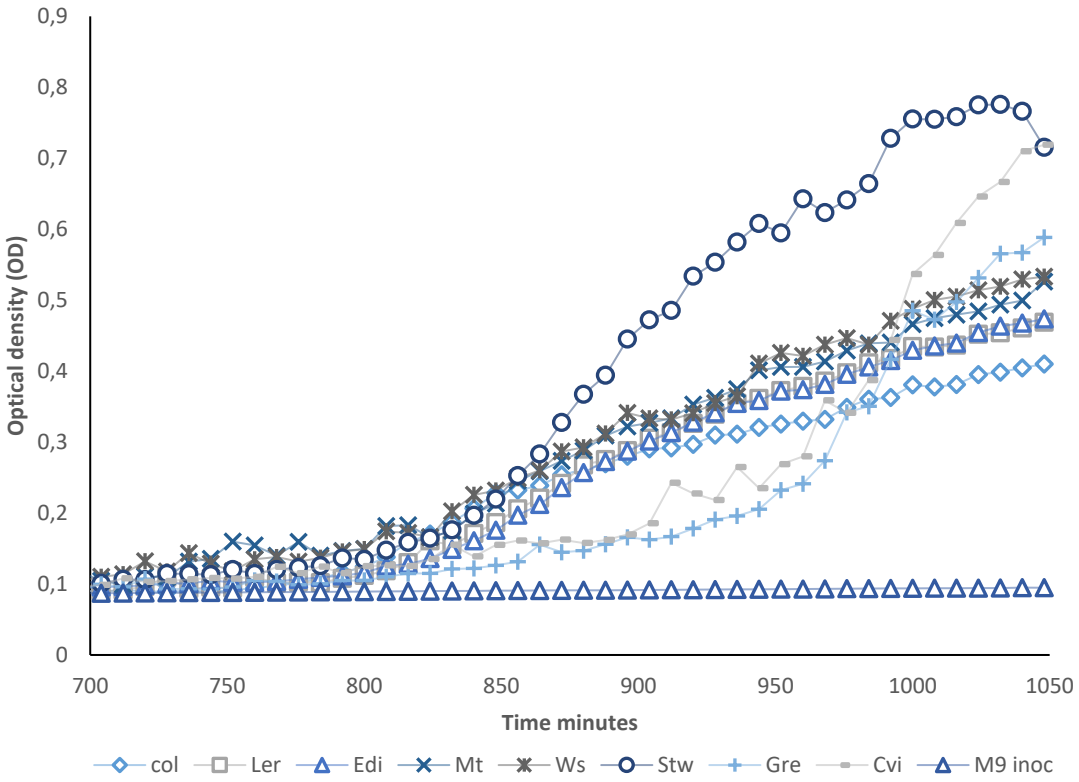

Figure 12. The absorbance curves of UCMB 5113 incubated in M9 medium and 20% root exudates from 8 different *A. thaliana* accessions for a duration of 17.5 h in a 96-well plate. The reference treatment labeled: *M9 inoc* was UCMB 5113 incubated with only M9 medium, no root exudates added. Ag-0 is not plotted for scaling reasons. The mean OD and SE of the mean are shown as a function of time. Each data point represents 8 replicates, except for the M9 control for which 6 replicates were used. Extreme outliers have been excluded automatically using a custom function where measurements higher than 25 % from data points before and after were removed as these were concluded to represent aggregates blocking the light beam giving an unobtainable high OD.

Table 2. UCBM 5113 growth curves showing significant differences in pairwise comparison using a permutation test (*P* = 0.0000–0.012, *n* = 8-8)

Group1	Ag	Ag	Cvi	Cvi	Cvi	Edi	Gre	Ler	Mt	Stw	Stw
Group2	Cvi	Stw	Edi	Ler	Stw	Stw	Stw	Stw	Stw	Ws	Col

Table 3. Endpoint OD ranked from highest to lowest. The values are an average of the 3 last measurements for each group

Exudate	Ag	Stw	Cvi	Gre	Ws	Mt	Edi	Ler	Col
OD	1.32	0.75	0.70	0.57	0.53	0.51	0.47	0.46	0.40

Results showed statistically significant differences based on (*P* = 0.0000–0.012, *n* = 8-8) between a number of accessions (Table 2). The OD at the end of the incubation, 17.5 hours after incubation start showed the highest value for *Bacillus* grown with Ag-0 exudates and the lowest for *Bacillus* grown with Col-0 exudates (Table 3).

Discussion

It appears that *B. amyloliquefaciens* UCMB 5113 is capable of proliferating using a wide range of compounds that are likely present in root exudates (Niazi et al. 2014). With all other conditions being constant between the exudate treatments in this experiment, the difference between the shape in growth curves must be a function of either different nutrient composition and/or concentration, affecting the bacterial growth directly, or it might be a more indirect mode of action, in which the root exudates are able to change the rate of growth by altering gene expression. It has been found that a number of genes related to nutrient utilization, bacterial chemotaxis and motility can be strongly induced in *B. amyloliquefaciens* FZB42 by incubation with root exudates (Fan et al. 2012b). If metabolic processes are altered by different gene expression, the cells may accumulate biomass faster or divide faster resulting in the increased OD values.

The level of bacterium aggregation may also be altered resulting in different optic properties in solution, or the ability to form biofilm on well walls. Regardless, the results seem to be very consistent between the repetitions of the samples. The sudden lowering in growth rate during the exponential phase in *Bacillus* treated with Col-0, L*er*-0 and Edi-0 exudates could be a result of aggregation by the bacterial cells, giving a seemingly lower OD value since both the size and number of particles in a suspension contribute to its OD (Bateman et al. 1959). Another possibility would be that the bacteria sense there is a limited availability of nutrients in these exudates and therefore deliberately slow down growth in order to benefit the population. Such cooperative actions with a seemingly high cost at an individual level have been seen to provide significant gains at the population level for a number of protobacteria (An et al. 2014).

The exudates from *Arabidopsis* accessions in treatments that gave a fast transition of *Bacillus* growth to an exponential phase could have been easy to metabolize but available in lower concentrations, giving an early start but slow progression of growth. The late starters, treatments with Cvi-0 and Gre-0 exudates, on the other hand could have the bacteria being forced to convert precursors to compounds that were metabolically active. This may have had to be done by means of enzyme secretion, in which case the availability of nutrients would increase with time, and if these were of good properties for rapid growth it would account for a rapid growth for some of the accessions that entered exponential growth at a later stage. Time lag for change of gene expression may also account for a delay of entering exponential growth. The process could in addition be coupled with a quorum sensing or metabolite concentration trigger mechanisms to postpone growth until a sufficient amount of precursors have been transformed to a usable form.

A number of inconveniences were encountered during initial trials for this experiment. Highly erratic measurements were obtained from the exudate treatments. The consistency of the measurements was greatly enhanced by treating the M9 medium preparation slightly differently and heating of exudates before loading. At first, heating of the exudates was avoided for the potential risk of destroying active compounds but upon re-evaluation it was found unlikely that a brief heating to 65ºC would cause any denaturation. The cause of the inconsistent readings might have been a combination of precipitation from the exudates and M9 medium, forming crystals that blocked the light beam from reaching the sensor. Some dispersion in measurements were still observed at later stages of the incubation, and the fact that the OD reading declined in one of the treatments suggests that there might be formation

of biofilm or accumulation of bacteria in aggregates, lowering the concentration in suspension. A more vigorous stirring could potentially prevent this from happening.

5. Plant accession response to *B. amyloliquefaciens* 5113 volatiles

Introduction

Plants are known to use volatile organic compounds (VOCs) for signalling in both plant to plant and plant to insect signalling (Heil and Karban 2010, Hare 2011). Plants can also perceive bacterially derived VOC signals, which induce plant defence towards pathogens (Ryu et al. 2004). VOCs from *B. amyloliquefaciens* NJN-6 have been found to have anti-fungal properties and as such repress the germination of *Fusarium* spores (Jun Yuan 2012). There is an additional VOC-related bacterium-plant interaction that revolves around the growth alteration of plants resulting from bacterium-released VOCs, as first described by Ryu et al. (2003).

When it comes to effects of bacterial derived VOCs on plant growth, the ecological relevance as well as the mechanisms at play are not yet well understood. Studies have found that ethylene signalling and sulfur nutrition are both means by which VOCs may alter plant growth (Hofmann 2013). When a large-scale screening involving 42 bacterial strains investigated the growth modulating effect of VOCs on *A. thaliana*, all of them proved to have a significant effect. Some interactions were reported to lead to as much as a six-fold increase in plant biomass while other led to plants dying from VOCs released by bacteria (Blom et al. 2011). More than 300 candidate molecules that could be responsible for the growth alterations have been identified (Bailly and Weisskopf 2012). Among these compounds are acetoin and 2,3-butanediol that have both been seen capable of triggering plant growth (Ryu et al. 2003, Ryu et al. 2004). A number of plant growth properties has been studied, including plant biomass and leaf area (Blom et al. 2011). The effect on plant root growth by bacterial VOCs has been studied to a lesser degree than above ground growth, although effects on main root growth and number of lateral roots per unit length of main root have been seen in a few cases (Gutiérrez-Luna et al. 2010, Meldau et al. 2013). When it comes to the study of the plant accession role for the effect of bacterial VOCs on root growth the published research is by the time of writing nonexistent.

Most research on VOCs in plant-bacteria interactions has been performed using split/bipartite petri dishes. These are of a limited size however and as such do not allow for more than a few cm of vertical root growth. The methodology opted for in this experiment, featuring an insert containing bacteria, allowed for 100 mm of root growth. Primarily the effect on different *Arabidopsis* accessions was of interest during the experiment.

The purpose of the experiment was to examine the effect of *Bacillus*-emitted VOCs on the root development of *A. thaliana*. Interaction between plant and bacteria will be limited to VOCs by means of a physical barrier between respective growth medium of the two organisms. However, volatile substances had access to both of these. Root growth was studied specifically in order to complement previous experiments not focused on the volatile interaction.

Aim

To investigate the effect of volatiles in the interaction between *Bacillus* and a number of *A. thaliana* accessions with respect to root development.

Hypothesis

Different accessions of *A. thaliana* will respond differently to volatiles emitted by *Bacillus* due to genetic differences in reception of the involved molecules.

Materials and methods

Seeds of the accessions Col-0, Can-0, Mt-0 and Ta-0 were surface sterilized as described on page 3, and spread on Petri dishes containing 0.5x MS with 0.6 % agar. Plates were kept in a growth chamber for 7 days at 16/8h day/night, 22 ºC and 200 µmol photons m^{-2} s^{-1}. Seedlings of uniform development were then selected and transferred to rectangular Petri dishes containing 0.5x MS with 0.8% agar. The plates had been prepared in two ways, either by submerging a 15 ml sterilized falcon tube lid containing 1.6 ml LB agar in the newly poured MS agar securing it 5 mm from the bottom centre of the plate, or by using the same type of lid as a hole punch and removing the solidified MS agar in the same position and pouring melted LB agar in the recess formed in the medium. The LBA was inoculated with 20 µl of *B. amyloliquefaciens* UCMB 5113 in a water suspension at an OD600 value of 1. The plates were kept close to vertical in a growth chamber, and shuffled around at regular intervals to even out possible variations in growth conditions. The plates were photographed 8 and 11 days after the start of treatment and analyzed with RootNav software. All roots from the plants and a strip of agar collected 1 cm from the bacteria patch were placed on LB plates and incubated for 3 days in order to detect root colonization in case the bacteria had traversed from the inoculated LB to the roots. A preliminary experiment used two LBA filled lids/plugs per plate and only used the accession Col-0. An additional experiment was performed using the same methodology as above, but with an impermeable barrier between the inoculated LB and the plants, still allowing for gaseous exchange between the compartments. The barrier was constructed from plastic with the dimensions 120x8 mm and was secured to the Petri dish with hot-glue ca 20 mm from one wall. This was followed by a rinsing in sterile water and a 24 h leak test with sterile water on one side of the barrier to evaluate the integrity of the seal between barrier and Petri dish. The plates were then poured with MSA and LBA as follows: negative control; MSA on both sides of the barrier, MSA+LBA inoculated; MSA on both sides of the barrier, but two circular patches in the MSA of the smaller compartment replaced with LBA, LBA inoculated; MSA in the larger compartment and LBA in the smaller compartment. Only Col-0 plants were used with the barrier plates, with four being placed on each plate towards the top of the larger compartment. The treatment duration was 7 days for this experiment, terminated when the main root of the control treatment reached the previously described barrier.

Results

For the two accessions Can-0 and Ta-0 with the treatment where *Bacillus* was grown on LB medium in direct contact with the surrounding MS medium, an increased total root growth could be observed when compared to the treatment where the LBA was physically isolated from the MSA (Figure 13). For Can-0, the medium contact treatment resulted in a higher total root growth than that of the control. Mt-0 showed lower total root growth for both *Bacillus*

treatments as compared to the control. No *B. amyloliquefaciens* colonization of roots could be seen when roots of all accessions were incubated on LB agar for 3 days after the end of the experiment.

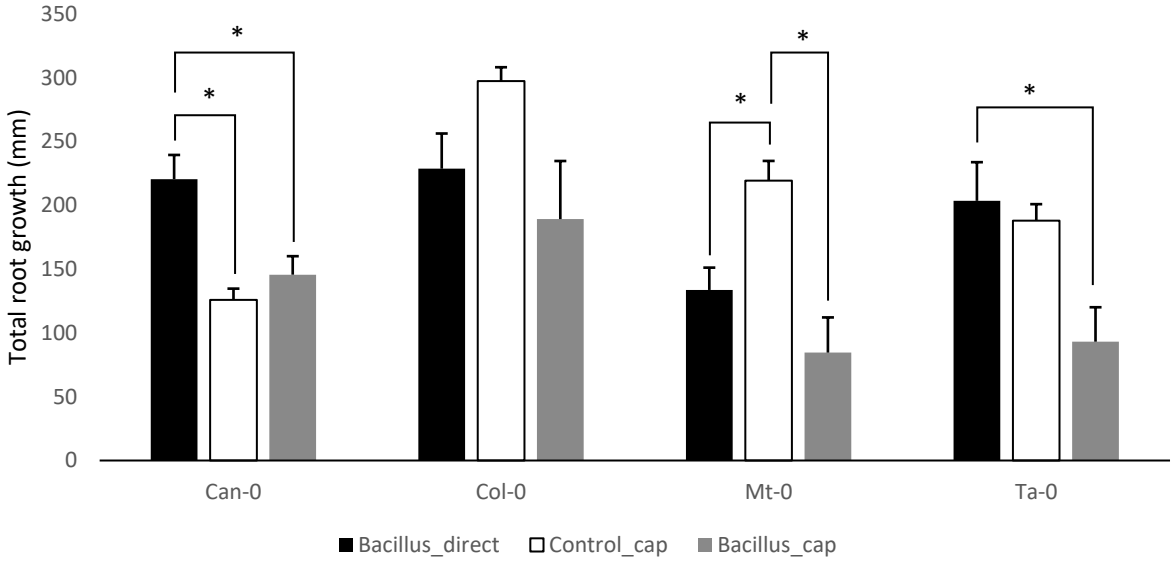

Figure 13. Average total root during during an 8 day period of four *A. thaliana* accessions treated with *B. amyloliquefaciens* in two different ways: Direct, meaning the bacteria were inoculated on a patch of LB agar cast in the 0.5x MS agar the plants were growing on. Isolated, meaning the bacteria were growing on LB in a plastic cap isolating them from the 0.5 x MS medium the plants were growing on. Error bars are standard error of the mean and asterisks show significant pairwise comparisons using Students t-test ($p<0.05$). Can-0 showed significantly higher total root growth in *Bacillus* direct treatment than both control and *Bacillus* isolated, Mt-0 showed significantly higher total root growth in the control than in the *Bacillus* direct and *Bacillus* isolated.

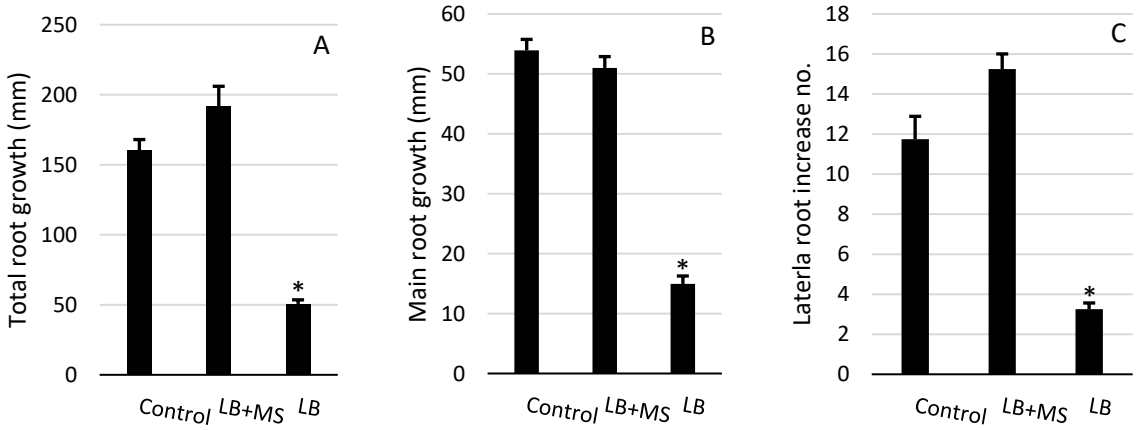

Figure 14. Graphs A through C show different aspects of root growth in Col-0 in response to treatment with *Bacillus amyloliquefaciens* UCBM 5113 VOCs. Treatment "LB+MS" had *A. thaliana* Col-0 subjected to VOCs formed by *Bacillus* growing with access to LB-agar and MS-agar and with only means of contact with the plants through gaseous compounds. Treatment "LB" had *A. thaliana* Col-0 subjected to VOCs formed by *Bacillus* growing with access only to LB-agar and with only means of contact with the plants through gaseous compounds. The treatment "control" had the same design as treatment "LB" but without inoculation of the LB with *Bacillus*. Average total root length growth of Col-0 accession during seven days of treatment (A). Average main root length growth of Col-0 accession during seven days of treatment (B). Average lateral root count increase in Col-0 accession during seven days of treatment (C). Error bars show standard error of the mean. Asterisk represents significant difference with Students t-test ($p<0.05$). n = 8 for all treatments.

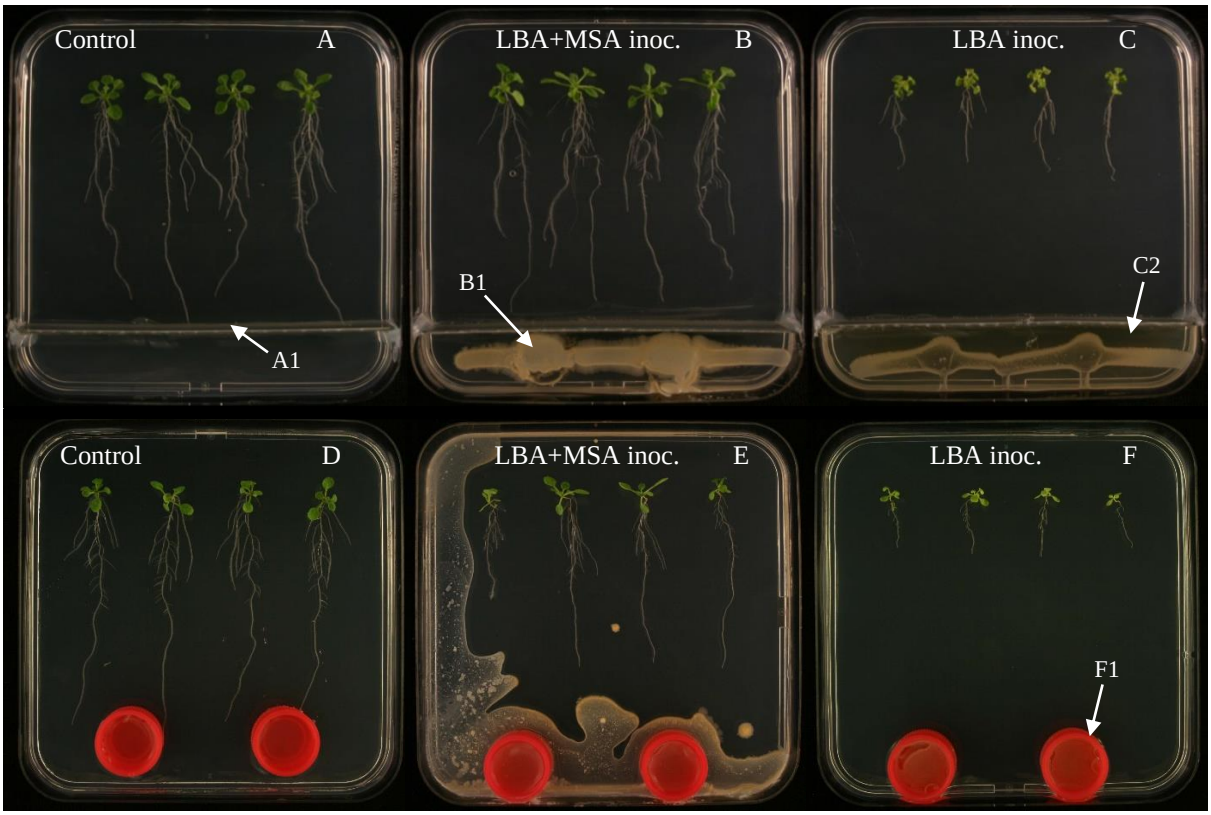

Figure 15. The photographs above depict the effect of bacterial VOCs on *A. thaliana* Col-0. Images A through C show an experimental setup where the nutrient medium supporting plant growth was separated from the nutrient medium supporting bacterial growth by a barrier (A1) while images D through F depict an experimental setup where the nutrient medium that was supporting bacterial growth was contained within an open top plastic cap. Split Petri dish with two sections divided by a barrier (A1) containing MS-agar on each side of the barrier and four Col-0 accession plants on the upper section (A). Petri dish of the same design as A but with two round patches (B1) in the MS-agar in the lower partition replaced with LB-agar, and having been inoculated with UCBM 5113 seven days earlier (B). Petri dish of the same design as plate A but with the entire portion below the partition barrier concisting of LB-agar (C2), having been inoculated with UCBM 5113 seven days earlier (C). Petri dish filled with 0.5% MS-agar with two LB-agar filled caps positioned towards the bottom edge, four Col-0 accession plants are growing on the plate (D). Petri dish with the same layout as D, but with the LB-agar in the caps as well as the MS-agar inoculated with UCBM 5113 six days earlier (E) Petri dish with the same layout as D, but with the LB-agar in the caps (F1) inoculated with UCBM 5113 six days earlier (F).

The VOC-experiment where *Bacillus* was grown on LB and a combination of LB and MS, but with partitions between the bacteria and plant appear to reveal that the difference in growth modulating effect seen between the two incubation methods was possible only using volatile signalling. When using an impermeable barrier between the inoculated LB-agar and the MS-agar supporting the plants, the total root length as well as main root length and number of lateral roots were significantly higher in the treatment where the inoculated LB was in direct contact with MS medium in comparison to that of LB isolated from MS medium (Students t-test: $p<0.05$ $n=8,8$) (Figure 14). The area colonized by *Bacillus* appeared to be similar in the treatment where *Bacillus* was allowed to access only LB-agar (Figure 15C) as it was when the bacteria could access both LB-agar and MS-agar (Figure 15B), but the colour and density of colonization may differ, although this was not quantified. Figure 15E shows the unintentional inoculation of both the LB-agar in the plastic caps and the MS-medium as appeared in a plate in a preliminary VOC experiment. The plants then appeared to be less stunted than when only the LB-agar was inoculated (Figure 15F).

Discussion

As was mentioned in the introduction, the existing information is scarce on plant accession response to VOCs produced by bacteria, especially so when it comes to root growth modulation by bacterial VOCs. Although most research has involved the *A. thaliana* accession Col-0, different plant species have been subjected to VOCs and those findings could serve as a foundation for elucidating the reasons to accession differences. The importance of plant species seems to be sub-ordered that of bacterial growth conditions, as found by Bailly and Weisskopf (2012) in their review on growth-modulating volatiles. The differences seen between the accessions in this experiment could be a result of differing number or sensitivity of receptors able to sense the volatiles emitted by bacteria. It could also be a result of difference in signalling induced by perception of VOCs, perhaps dependent on the individual accessions´ evolutionary dependence on external factors associated with said VOCs. Natural variation in transcriptome response to defence-related signalling molecules has been seen among different accessions of *A. thaliana* (Leeuwen et al. 2007, Ahmad et al. 2011). Aside from the role of plant accession in root growth, the growth conditions of *Bacillus* clearly had an effect on the outcome of the interaction. The treatments having *Bacillus* growing on isolated LB had all lower total root growth than both the control and medium contact treatments, except for the accession Can-0. The results from the first VOC experiment made it clear that either the growth substrate available to the bacteria, the ability to affect the plant through the media or the combination of both could be held responsible for the difference in outcomes. A research review performed by Blom et al. (2011) showed that when bacteria were grown on LB, the effect of volatiles was often repression of plant growth as opposed to other growth media such as MS where unaltered or increased growth ensued. The MS and LB media are very different, with LB being a complex medium consisting mainly of hydrolyzed proteins and being slightly alkaline whereas MS is an acidic mineral medium with either no carbon source or with sucrose. Blom et al. (2011) credited the differing composition of the media for allowing the bacteria to produce different VOCs.

When studying the images from the experiment with partition between plant and bacteria, there appears to be some visual cues that could be associated with the differing VOC formation. As the area colonized seemingly did not differ between the treatment with LB-agar in contact with MS-agar and the treatment with only LB-agar available for bacterial growth, the differing effect on the plants may not be dependent upon the surface area of the colonization. Difference in colour and density of said colonization could be of importance for figuring out why the VOC effect on the plants differed. The more opaque appearance of the bacterial colonization for the LB-agar + MS-agar treatment could indicate that the *Bacillus* is in another growth phase than those growing on pure LB and thus emitting different levels or blends of VOCs.

An analysis of the volatile compounds emitted from *B. amyloliquefaciens* UCMB 5113 growing on different media has shown that the VOC profile is dependent upon growth conditions (Asari et al. 2016). The existing literature describes a number of bacterial volatiles that appear to be involved in plant growth modulation. The most abundant compounds found in bacterial volatiles include aldehydes, ketones and alcohols (Gutiérrez-Luna et al. 2010). Indole, 1-hexanol and pentadecane were found by Blom et al. (2011) to be associated with increased plant growth. Experiments conducted by Hofmann (2013) in which labelled sulfur

was used in the bacterial growth medium, suggested that bacterial volatiles can contribute to plant sulfur nutrition, and hence increase growth.

A few concluding remarks can be made regarding the VOC experiments. The VOC experiment presented here was the result of multiple pilot experiments performed on Col-0 in order to find a workable method for comparison of accessions. The methodologies used in the VOC experiments were by no means perfected since there is a number of variables to be controlled, all of which can affect the outcome. The greatest challenge was to find and decide upon what form of interaction to study. Only effects by VOC would be the simplest, since contamination and other modes of contact could be excluded. However, in a natural system such a distinct isolation would be unlikely, which led to the decision to include a treatment where the bacteria were not physically isolated from the growth media supporting the plant.

One finding that shaped the experimental design was that *Bacillus* does not easily traverse vertically over sucrose-free MS agar, meaning the effect on root growth is not facilitated by the bacteria themselves, even though they are growing on LB without physical barriers between plant root and bacterium.

MS with sucrose can, however, easily be traversed by the *Bacillus* and efforts are necessary to contain the bacteria in order to exclude the effect from bacteria directly interacting with the plants and/or possible different characteristics of VOCs produced when the bacteria are growing on the MS medium.

For future research it could be of interest to block the bacteria from the MS medium through the use of a membrane in order to allow for interaction both through the growth media and through volatiles while ensuring that the bacteria only are colonizing the LB. Conversely limiting VOC effects and only having contact through the growth medium could yield yet different results. The latter could mimic water saturation in the soil. Attempts at these experimental setups were made but proved unsuccessful in containing the bacteria despite using 0.45 μm membranes. In order to produce a more realistic experimental set-up an aeroponic growth system, such as described by Vaughan et al. (2011), could be used to study the interaction between *Bacillus* and *A. thaliana* roots in soil while maintaining gas exchange.

Conclusions: VOCs affect root growth differently dependent upon the mode of interaction between bacteria and plant.

Bacillus surface colonization of MS in combination with LB colonization results in increased root growth in comparison to LB alone.

It could potentially be a very complex interaction, with exudates contacting the bacteria determining the nature of volatiles production by bacteria as well as their production of liquid bound compounds.

The results strongly indicate that the growth-modulating effect on plant roots is highly dependent upon the substrate the bacteria are growing on.

General discussion

For the purpose of connecting the individual experiments, which were conducted for this thesis, common matters and converging results will be examined in a concluding discussion. Specific matters regarding individual experiments are, however, discussed in their respective section. The focus of this section is on merging the subsections and identifying topics for continuing research. During the exploration of possible approaches to the accession screening, a number of interaction properties were discovered. The mode of *Bacillus*-plant interaction was found to be extremely important for the outcome of the interaction, as was depicted by the differing results of volatile versus direct interaction between bacteria and plant. In a field cultivation scenario differences in the mode of interaction may be brought about by variable properties such as soil pore volume, hydraulic conductivity, capillary transport, saturation level and organic soil matter content, all of which could potentially affect the fluid and gaseous transport between bacteria and plants. The above mentioned conditions may be altered to certain extents by varying the cultivation practices.

More effort should probably be spent investigating the fundamentals of the interaction between plants and bacteria before involving different accessions, as there are so many variations that can result in different outcomes. The conducted experiments indicate that accession differences in *Bacillus*-induced growth modulation of root architecture do exist, and a task for future research would be to investigate if these are linked to actual qualitative or quantitative changes in the harvested parts of the plants, which ultimately is of greatest interest. The relative importance of each trait in the specific interaction between *Arabidopsis* accessions and *B. amyloliquefaciens* UCMB 5113 also remains to be investigated, e.g., does longer total root hair length compensate for shorter total root length? An interesting candidate for continued research into *Bacillus* treatment response would be the Ms-0 accession. The Ms-0 accession showed a significant increase in root hair growth as a result of *Bacillus* treatment and supported dense root-surface colonization. As such it would seem that Ms-0 is a good candidate for finding properties beneficial for the plant-bacteria interaction, both from a growth-promotion and crop-protection perspective.

Results from the VOC experiment suggest there was a multiple way feedback system between exudates and volatiles. An in depth study of the dependence of the plant response on gaseous/aqueous signal interaction may be of crucial importance for perfection of *in situ* application. Further topics for investigation related to the signalling complex would include variables such as pest resistance and tolerance to abiotic stress.

A few words would have to be said about the experimental conditions and how the results would relate to field conditions. As the primary setting for *B. amyloliquefaciens* as a PGPR in agriculture would likely be in open fields, the value of the results would benefit from having experimental setups closely resembling field conditions. Using soil cultivation was considered in a few cases, but discarded in favour of gnotobiotic systems as the number of unknown factors was expected to increase drastically. A study by Xu et al. (2013) questions the commonly used method of *Arabidopsis in vitro* cultivation in transparent Petri dishes as plant roots are exposed to light. Similar experiments as performed within the frame of this thesis have been performed in soil, for example studies on bacterial colonization pattern of *A. thaliana* have been performed in a soil system, although admittedly painstaking as described

by the authors (Fan et al. 2012a). The effect of volatiles on root growth has also been studied in soil using special aeroponic cultivation systems (Vaughan et al. 2011). Techniques such as x-ray tomography or 3D scanning could also strengthen the analysis by allowing for more natural growth conditions, not practical when using simple 2D photographic data acquisition.

A final suggestion for future attempts at large-scale root phenotyping would be to perfect the image acquisition and integration of image analysis software. There is a number of software programs aimed at facilitating the analysis of root architecture however, few of these are suited for large-scale analysis as substantial manual input may be required for accurate measurements. This is especially true for more advanced growth stages with intermingled lateral rots. The timeframe during which the plants were studied in the experiments conducted within this thesis could have been expanded if images of more advanced growth could be analyzed, and may in turn have yielded different results.

Another issue that would have to be treated as common for all experiments was the preparation of homogenous bacterial suspensions for plant treatment. High density aggregation may have caused underestimation of final suspension concentration due to OD measurement being done with such a small sample. Aggregate size in a suspension affects the OD value as measured by spectrophotometry (Malik et al. 2003). The aggregation index could be examined by phase-contrast microscopy and adjusted for in order to ensure equal bacterial concentration for treatments.

A more theoretical matter concerning the experiments within this thesis was the statistical challenges encountered in the comparison of multiple treatments of multiple plant accessions. Choosing an appropriate test was indeed more challenging than initially anticipated. At the foundation of this challenge lays the fact that most multiple comparison tests deal with one control and many experimental groups whereas in this case every group had to have its own control group. The difference in the difference between the pairs of groups with their respective controls had to be determined. A multi-way ANOVA test could give answers to whether there were interaction effects between treatment and accession but would not give answers to which of the accessions had statistical differences in their relative difference between treatment and negative control. ANOVA and Kruskal-Wallis tests can only tell whether there is a difference between two or more of your groups and not which ones. That was why the tests were run on the difference of relative differences, which reduced the ability to extract significant differences since the possible variation greatly increased by performing this operation. The statistical approach to the analysis of data sparked some intense debate when an online statistics forum was consulted. The delta method of estimating variance for relative difference between groups is common practice in several branches of science, although some consider it too conservative and requiring exceptionally large sample sizes to generate significant results. The condensed summary of the debate can be interpreted as there being a multitude of ways of analyzing the data, of which other methods may or may not be able to extract more/other results, and each with their own tradeoffs. In continuing screening experiments with the same design, the strength of the statistics would benefit from larger group sizes if the difference of relative treatment effects are to be determined with any satisfactory confidence.

General conclusions

B. amyloliquefaciens UCMB 5113 treatment can have different effects on *A. thaliana* root-hair growth depending on the plant accession (genotype).

Some *A. thaliana* root exudates differ in their abilities to promote *B. amyloliquefaciens* proliferation.

Volatiles emitted by *B. amyloliquefaciens* UCMB 5113 have the ability to modulate *A. thaliana* root growth and the outcome of said interaction is dependent upon the bacterial growth substrate as well as the plant accession.

Use of *Arabidopsis* ecotypes is a good system to address mechanisms involved in beneficial plant-microbe interactions.

References

1001 Genomes Consortium. 2016. 1,135 Genomes reveal the global pattern of polymorphism in *Arabidopsis thaliana*. Cell 166: 481-491.

Ahmad, S., M. Van Hulten, J. Martin, C. M. J. Pieterse, S. C. M. Van Wees, and J. Ton. **2011**. Genetic dissection of basal defence responsiveness in accessions of *Arabidopsis thaliana*. Plant Cell Environ. 34: 1191–1206.

An, J. H., E. Goo, H. Kim, Y.-S. Seo, and I. Hwang. **2014**. Bacterial quorum sensing and metabolic slowing in a cooperative population. Proc. Natl. Acad. Sci. U. S. A. 111: 14912–14917.

Asari, S., D. Matzén, S. Petersen, M. Bejai, S. Meijer, J. 2016. Multiple effects of Bacillus amyloliquefaciens volatile compounds: plant growth promotion and growth inhibition of phytopathogens. FEMS Microbiol. Ecol. 92:fiw070

Asari, S., D. Tarkowská, J. Rolčík, O. Novák, D. V. Palmero, S. Bejai, and J. Meijer. **2017**. Analysis of plant growth-promoting properties of *Bacillus amyloliquefaciens* UCMB5113 using *Arabidopsis thaliana* as host plant. Planta. 245:15-30.

Bailly, A., and L. Weisskopf. **2012**. The modulating effect of bacterial volatiles on plant growth: current knowledge and future challenges. Plant Signal. Behav. 7: 79–85.

Bais, H. P., R. Fall, and J. M. Vivanco. **2004**. Biocontrol of *Bacillus subtilis* against infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant Physiol. 134: 307–319.

Bateman, J. B., E. J. Weneck, and D. C. Eshler. **1959**. Determination of particle size and concentration from spectrophotometric transmission. J. Colloid Sci. 14: 308–329.

Beemster, G. T. S., and T. I. Baskin. **1998**. Analysis of cell division and elongation underlying the developmental acceleration of root growth in *Arabidopsis thaliana*. Plant Physiol. 116: 1515–1526.

Beneduzi, A., A. Ambrosini, and L. M. P. Passaglia. **2012**. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genet. Mol. Biol. 35: 1044–1051.

Blom, D., C. Fabbri, E. C. Connor, F. P. Schiestl, D. R. Klauser, T. Boller, L. Eberl, and L. Weisskopf. **2011**. Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. Environ. Microbiol. 13: 3047–3058.

Brown, L. K., T. S. George, L. X. Dupuy, and P. J. White. **2013**. A conceptual model of root hair ideotypes for future agricultural environments: what combination of traits should be targeted to cope with limited P availability? Ann. Bot. 112: 317–330.

Burg, S. P. 1973. Ethylene in plant growth. Proc. Natl. Acad. Sci. U. S. A. 70: 591–597.

Caffaro, M. M., J. M. Vivanco, F. H. Gutierrez Boem, and G. Rubio. **2011**. The effect of root exudates on root architecture in *Arabidopsis thaliana*. Plant Growth Regul. 64: 241–249.

Camilios-Neto, D., P. Bonato, R. Wassem, M. Z. Tadra-Sfeir, L. C. Brusamarello-Santos, G. Valdameri, L. Donatti, H. Faoro, V. A. Weiss, L. S. Chubatsu, F. O. Pedrosa, and E. M. Souza. **2014**. Dual RNA-seq transcriptional analysis of wheat roots colonized by *Azospirillum brasilense* reveals up-regulation of nutrient acquisition and cell cycle genes. BMC Genomics. 15: 1–13.

Chin-A-Woeng, T. F., G. V. Bloemberg, I. H. Mulders, L. C. Dekkers, and B. J. Lugtenberg. **2000**. Root colonization by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato foot and root rot. Mol. Plant-Microbe Interact. 13: 1340–1345.

Chowdhary, G., A. R. Kataya, T. Lingner, and S. Reumann. **2012**. Non-canonical peroxisome targeting signals: identification of novel PTS1 tripeptides and characterization of enhancer elements by computational permutation analysis. BMC Plant Biol. 12: 142.

Cold Spring Harbor Protocols. **2010**. M9 minimal medium (standard). Cold Spring Harb. Protoc. 2010: pdb.rec12295.

Dietel, K., B. Beator, A. Budiharjo, B. Fan, and R. Borriss. **2013**. Bacterial traits involved in colonization of *Arabidopsis thaliana* roots by *Bacillus amyloliquefaciens* FZB42. Plant Pathol. J. 29: 59–66.

Doornbos, R. F., L. C. van Loon, and P. A. H. M. Bakker. **2012**. Impact of root exudates and plant defense signalling on bacterial communities in the rhizosphere. A review. Agron. Sustain. Dev. 32: 227–243.

Elso, C. M., L. J. Roberts, G. K. Smyth, R. J. Thomson, T. M. Baldwin, S. J. Foote, and E. Handman. **2003**. Leishmaniasis host response loci (lmr1–3) modify disease severity through a Th1/Th2-independent pathway. Genes Immun. 5: 93–100.

Fan, B., R. Borriss, W. Bleiss, and X. Wu. **2012a**. Gram-positive rhizobacterium *Bacillus amyloliquefaciens* FZB42 colonizes three types of plants in different patterns. J. Microbiol. Seoul Korea. 50: 38–44.

Fan, B., L. C. Carvalhais, A. Becker, D. Fedoseyenko, N. von Wirén, and R. Borriss. **2012b**. Transcriptomic profiling of *Bacillus amyloliquefaciens* FZB42 in response to maize root exudates. BMC Microbiol. 12: 116.

Fan, B., X. H. Chen, A. Budiharjo, W. Bleiss, J. Vater, and R. Borriss. **2011**. Efficient colonization of plant roots by the plant growth promoting bacterium *Bacillus amyloliquefaciens* FZB42, engineered to express green fluorescent protein. J. Biotechnol. 151: 303–311.

Foreman, J., V. Demidchik, J. H. F. Bothwell, P. Mylona, H. Miedema, M. A. Torres, P. Linstead, S. Costa, C. Brownlee, J. D. G. Jones, J. M. Davies, and L. Dolan. **2003**. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature. 422: 442–446.

Glick, B. R. 2012. Plant Growth-Promoting Bacteria: Mechanisms and applications. Scientifica. 2012: e963401.

Grierson, C., E. Nielsen, T. Ketelaarc, and J. Schiefelbein. **2014**. Root hairs. Arabidopsis Book, Am. Soc. Plant Biol. Grierson C, Nielsen E, Ketelaarc T, Schiefelbein J. Root

Hairs. The Arabidopsis Book / American Society of Plant Biologists. doi:10.1199/tab.0172.

Gutiérrez-Luna, F.M., López-Bucio, J., Altamirano-Hernández, J., Valencia-Cantero, E., Reyes de la Cruz, H., and L. Macías-Rodríguez. 2010. Plant growth-promoting rhizobacteria modulate root-system architecture in *Arabidopsis thaliana* through volatile organic compound emission. Symbiosis. 51: 75–83.

Hare, J. D. 2011. Ecological role of volatiles produced by plants in response to damage by herbivorous insects. Annu. Rev. Entomol. 56: 161–180.

Heil, M., and R. Karban. **2010**. Explaining evolution of plant communication by airborne signals. Trends Ecol. Evol. 25: 137–144.

Hodge A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytologist 162: 9–24.

Hofmann, N. R. 2013. Volatile organic compounds: A bacterial contribution to plant sulfur nutrition. Plant Cell. 25: 2381–2381.

Jourdan, E., G. Henry, F. Duby, J. Dommes, J. P. Barthélemy, P. Thonart, and M. Ongena. **2009**. Insights into the defense-related events occurring in plant cells following perception of surfactin-type lipopeptide from *Bacillus subtilis*. Mol. Plant. Microbe Interact. 22: 456–468.

Jun Yuan, W. R. 2012. Antifungal activity of *Bacillus amyloliquefaciens* NJN-6 volatile compounds against *Fusarium oxysporum* f. sp. cubense. Appl. Environ. Microbiol. 78: 5942–4.

Leeuwen, H. van, D. J. Kliebenstein, M. A. L. West, K. Kim, R. van Poecke, F. Katagiri, R. W. Michelmore, R. W. Doerge, and D. A. St.Clair. **2007**. Natural variation among *Arabidopsis thaliana* accessions for transcriptome response to exogenous salicylic acid. Plant Cell. 19: 2099–2110.

López-Bucio, J., J. C. Campos-Cuevas, E. Hernández-Calderón, C. Velásquez-Becerra, R. Farías-Rodríguez, L. I. Macías-Rodríguez, and E. Valencia-Cantero. **2007**. *Bacillus megaterium* rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signalling mechanism in *Arabidopsis thaliana*. Mol. Plant-Microbe Interact. 20: 207–217.

Lynch, J. 1995. Root architecture and plant productivity. Plant Physiol. 109: 7–13.

Malik, A., M. Sakamoto, S. Hanazaki, M. Osawa, T. Suzuki, M. Tochigi, and K. Kakii. **2003**. Coaggregation among nonflocculating bacteria isolated from activated sludge. Appl. Environ. Microbiol. 69: 6056–6063.

Meldau, D. G., S. Meldau, L. H. Hoang, S. Underberg, H. Wünsche, and I. T. Baldwin. **2013**. Dimethyl disulfide produced by the naturally associated bacterium *Bacillus* sp B55 promotes *Nicotiana attenuata* growth by enhancing sulfur nutrition. Plant Cell. 25: 2731–2747.

Micallef, S. A., M. P. Shiaris, and A. Colón-Carmona. **2009**. Influence of *Arabidopsis thaliana* accessions on rhizobacterial communities and natural variation in root exudates. J. Exp. Bot. 60: 1729–1742.

Mönchgesang, S., N. Strehmel, S. Schmidt, L. Westphal, F. Taruttis, E. Müller, S. Herklotz, S. Neumann, and D. Scheel. **2016**. Natural variation of root exudates in *Arabidopsis thaliana*-linking metabolomic and genomic data. Sci. Rep. 6: 29033.

Narang, R. A., A. Bruene, and T. Altmann. **2000**. Analysis of phosphate acquisition efficiency in different Arabidopsis accessions. Plant Physiol. 124: 1786–1799.

Niazi, A., S. Manzoor, S. Asari, S. Bejai, J. Meijer, and E. Bongcam-Rudloff. **2014**. Genome analysis of *Bacillus amyloliquefaciens* subsp. *plantarum* UCMB5113: A rhizobacterium that improves plant growth and stress management. PLoS ONE. 9: e104651.

Norman, J. M. V., J. Zhang, C. I. Cazzonelli, B. J. Pogson, P. J. Harrison, T. D. H. Bugg, K. X. Chan, A. J. Thompson, and P. N. Benfey. **2014**. Periodic root branching in Arabidopsis requires synthesis of an uncharacterized carotenoid derivative. Proc. Natl. Acad. Sci. U.S.A. 111: E1300–E1309.

Pilet, P.-E., and M. Saugy. **1987**. Effect on root growth of endogenous and applied IAA and ABA. Plant Physiol. 83: 33–38.

Pimentel, D. 1995. Amounts of pesticides reaching target pests: Environmental impacts and ethics. J. Agric. Environ. Ethics. 8: 17–29.

Pound, M. P., A. P. French, J. A. Atkinson, D. M. Wells, M. J. Bennett, and T. Pridmore. **2013**. RootNav: Navigating images of complex root architectures. Plant Physiol. 162: 1802–1814.

Qiao, J.-Q., H.-J. Wu, R. Huo, X.-W. Gao, and R. Borriss. **2014**. Stimulation of plant growth and biocontrol by *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 engineered for improved action. Chem. Biol. Technol. Agric. 1: 1–14.

R Core Team. **2014**. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Robinson D. 1994. The responses of plants to non-uniform supplies of nutrients. New Phytologist 127: 635–674.

Rovira, A. D. 1969. Plant root exudates. Bot. Rev. 35: 35–57.

Růžička, K., K. Ljung, S. Vanneste, R. Podhorská, T. Beeckman, J. Friml, and E. Benková. **2007**. Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. Plant Cell. 19: 2197–2212.

Ryu, C.-M., M. A. Farag, C.-H. Hu, M. S. Reddy, J. W. Kloepper, and P. W. Paré. **2004**. Bacterial volatiles induce systemic resistance in Arabidopsis. Plant Physiol. 134: 1017–1026.

Ryu, C.-M., M. A. Farag, C.-H. Hu, M. S. Reddy, H.-X. Wei, P. W. Paré, and J. W. Kloepper. **2003**. Bacterial volatiles promote growth in Arabidopsis. Proc. Natl. Acad. Sci. 100: 4927–4932.

Sarkar, D. 2008. Lattice - multivariate data visualization with R. Springer, New York. ISBN 978-0-387-75968-5.

Sarosh, B. R., J. Danielsson, and J. Meijer. **2009**. Transcript profiling of oilseed rape (*Brassica napus*) primed for biocontrol differentiate genes involved in microbial interactions with beneficial *Bacillus amyloliquefaciens* from pathogenic *Botrytis cinerea*. Plant Mol. Biol. 70: 31–45.

Schiefelbein, J., A. Shipley, and P. Rowse. **1992**. Calcium influx at the tip of growing root-hair cells of *Arabidopsis thaliana*. Planta. 187:455-459

Schneider, C. A.; Rasband, W. S. & Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature methods 9: 671-675.

Schwachtje, J., S. Karojet, S. Kunz, S. Brouwer, and J. T. van Dongen. **2012**. Plant-growth promoting effect of newly isolated rhizobacteria varies between two Arabidopsis ecotypes. Plant Signal. Behav. 7: 623–627.

Shi, C.-L., H.-B. Park, J. S. Lee, S. Ryu, and C.-M. Ryu. **2010**. Inhibition of primary roots and stimulation of lateral root development in *Arabidopsis thaliana* by the rhizobacterium *Serratia marcescens* 90–166 is through both auxin-dependent and -independent signalling pathways. Mol. Cells. 29: 251–258.

Slovak, R., C. Göschl, X. Su, K. Shimotani, T. Shiina, and W. Busch. **2014**. A scalable open-source pipeline for large-scale root phenotyping of Arabidopsis. Plant Cell. 26: 2390-2403.

Talboys, P. J., D. W. Owen, J. R. Healey, P. J. Withers, and D. L. Jones. **2014**. Auxin secretion by *Bacillus amyloliquefaciens* FZB42 both stimulates root exudation and limits phosphorus uptake in *Triticum aestivum*. BMC Plant Biol. 14: 51.

Tilman, D. 1998. The greening of the green revolution. Nature. 396: 211–212.

Tilman, D., K. G. Cassman, P. A. Matson, R. Naylor, and S. Polasky. **2002**. Agricultural sustainability and intensive production practices. Nature. 418: 671–677.

Vaughan, M. M., D. Tholl, and J. G. Tokuhisa. **2011**. An aeroponic culture system for the study of root herbivory on *Arabidopsis thaliana*. Plant Methods. 7: 5.

Wang, J. Zhang, Jin, J. Li, Q. Zhao, C. Nan, W. Wang, X. Ma, Bi, Y. 2017. An intact cytokinin-signalling pathway is required for Bacillus sp. LZR216-promoted plant growth and root system architecture alteration in Arabidopsis thaliana seedlings. Plant Growth Regul. 84:1-12.

Weigel, D. 2012. Natural variation in Arabidopsis: From molecular genetics to ecological genomics. Plant Physiol. 158: 2–22.

Weigel D, Nordborg M. 2015. Population genomics for understanding adaptation in wild plant species. Annu. Rev. Genet. 49: 315-338.

Weng, J., Y. Wang, J. Li, Q. Shen, and R. Zhang. **2013**. Enhanced root colonization and biocontrol activity of *Bacillus amyloliquefaciens* SQR9 by abrB gene disruption. Appl. Microbiol. Biotechnol. 97: 8823–8830.

Xu, W., G. Ding, K. Yokawa, F. Baluška, Q.-F. Li, Y. Liu, W. Shi, J. Liang, and J. Zhang. **2013**. An improved agar-plate method for studying root growth and response of *Arabidopsis thaliana*. Sci. Rep. 3:1273; DOI:10.1038/srep01273

Zourelidou, M., I. Müller, B. C. Willige, C. Nill, Y. Jikumaru, H. Li, and C. Schwechheimer. **2009**. The polarly localized D6 PROTEIN KINASE is required for efficient auxin transport in *Arabidopsis thaliana*. Development. Camb. Engl. 136: 627–636.

Zuur, A. F., E. N. Ieno, and C. S. Elphick. **2010**. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1: 3–14.

Appendix 1

A. thaliana accessions with country of origin and coordinates of isolation. Source: arabidopsis.org.

Acession	Country	Lat	Long	Other
Col-0	USA	38.3	-92.3	
Edi-0	UK	55.9	-3.2	
Ta-0	Czech Republic	49.5	14.5	
Mt-0	Libya	32.3	22.46	
Ms-0	Russia	55.7	37.6	
Cvi-0	Cape Verde	15.1	-23.6	
Ws-0	Russia	52.6	30	
Ler-0	Germany	48.0	10.9	
Shadara	Tadjikistan	-	-	3400m alt.
Stw-0	Russia	52	36	
Can-0	Spain	29.2	13.5	
Gre-0	USA	43.2	85.2	
N-13	Russia	61.4	34.2	
Ag-0	France	45	1.3	

Nr 164

Uppsala 2018

Department of Plant Biology SLU

Box 7080

75007 Uppsala, Sweden