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Abstracts

This study was designed to gain understanding of the roles of plant growth-promoting traits in tomato cultivars using bacteria with the aim to improve plant health and crop productivity based on microbial inoculation. Rhizosphere bacterial from the hydroponic root of *Solanum pennellii* tomato plants were isolated, the gene were investigated using PCR, gel electrophoresis and DNA extraction and the bacteria were identified as *Sphingobium limneticum* and *Acidovorax delafieldii* using 16S rRNA gene sequencing. The study shows the effect of microbial inoculation on model plants of *Arabidopsis thaliana* WT-Columbia and Protein phosphatase 2A catalytic subunit mutants (C2, C2C4, C2C5) and tomato plants (Gemini original) using 1/50 Gamborg medium and 1x MS medium (with sucrose or without sucrose) respectively. To investigate the effect of endospheric and rhizosphere strains (WCS714r, *Sphingobium limneticum* and *Acidovorax delafieldii*) on WT and mutants, it was discovered that WCS714r and *Sphingobium limneticum* and *Acidovorax delafieldii* strains were found to inhibit the primary root length and stimulate the lateral root formation of tomato plants, *Arabidopsis* WT and the mutants either in vivo or in vitro except some of endospheric and rhizosphere bacteria (*Pseudomonas* sp., *Agrobacterium* sp., and *Rhizobium* sp.) obtained from Belgium (Abbamondi et al. 2016) which had little effect on plant growth and development. They are represented as 5, 6, 9, 10, 15, 16, and 18. These results suggest that PGPR stimulate plant growth through the inhibition of plant pathogens.

Abbreviation

ABA - Abscisic Acid Signalling

ACD - *Acidovorax delafieldii*

At - *Arabidopsis thaliana*

ACC - Aminocyclopropane-1-carboxylate (ACC) deaminase

ATP - Adenosine triphosphate

BNF - Biological nitrogen fixation

CFU - Colony -forming units

CO₂ - Carbondioxide

ET - Ethylene

EXP - Experiment

gDNA - genomic DNA

Gem - Gemini

H - HyperLadder

ICs - Indolic compounds

ISR - Induced Systemic Resistance

LF - Lateral Root formation

MS - Murashige and Skoog Medium

N - Nitrogen

P - Phosphorus

PSB - Phosphate-Solubilizing Bacteria

PTP - Phosphotyrosine Phosphatase

PPP - Phosphoprotein Phosphatase

rRNA - Ribosomal RNA

Ser - Serine

SFW - Shoot Fresh Weight

SPH - *Sphingobium limneticum*

T-DNA - Transfer DNA

Thr - Threonine

Tyr - Tyrosine

VOCs - Volatile Organic Compounds

WT - Wild type

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1. INTRODUCTION

1.1 History of Tomato

Tomato originated from Europe in 1500s when Spanish and Portuguese travelers brought unusual vegetables of which tomatoes was one, back to their individual nations (Jones 2008). Early botanists recognized the close connections of tomatoes with genus *Solanum*, and regularly distinguished them as *S. pomiferum* (Razdan 2006). In 1561, Anguillara recognise the tomato as a plant named *Lycopersicon*, which means “wolf peach” by the Greek naturalist Galen fourteen centuries earlier (Razdan 2006). The tomato belongs to the *Solanaceae* family, containing more than 3000 species including many plants of economic significance including potatoes, eggplants, petunias, tobacco, peppers (*Capsicum*) and *Physalis*. *Solanum* is the biggest variety in the *Solanaceae* family, incorporating 1250 to 1700 species (Bergougnoux 2014). Types of the *Solanum* genus are available on all calm and tropical landmasses and are amazing for their morphological and ecological diversity (Bergougnoux 2014). *Solanum* is a major economically important genus, containing crop species and numerous different species delivering poisonous or medicinal compounds (Bergougnoux 2014). The domestication of tomato occurred in Mexico. The name was gotten from ‘tomatl’ in Nahuatl tongue of Mexico (Cheema and Dhaliwal 2005). The cultivars presented from Latin America and largely has exposed stigma facilitating cross pollination. In the last stages, plants with a high rate of fruit set and with short style at the mouth of the anther tube were selected. In 1976, Rick likewise reported the domestication of *Lycopersicon esculentum* has occurred with the transition of exerted to inserted stigma, subsequently of the change of allogamy to autogamy (Cheema and Dhaliwal 2005). Also, Boswell (1949) had reported that prior to 1800, the European cultivars were introduced to the United States. Since 1800, tomato plants are being grown in many part of the world. The tomato plant, though perennial by nature, is almost universally cultivated as annual. The developed species has an herbaceous annual to perennial growth habit (Cheema and Dhaliwal 2005). The tomato is an edible fruit, bright red coloured from the pigment lycopene berry, 1-2 cm diameter in wild plants, commonly much larger in cultivated forms (Heldens et al. 2009). It has a strong tap root, however later adventitious root develops quickly, if the tap root is damaged. The stem is soft, brittle, and hairy when young and hard, woody and copiously branched when develop (Cheema and Dhaliwal 2005).

1.2 *Solanum pennellii*

Solanum pennellii is a wild tomato species categories endemic to Andean regions in South America, where it has developed to flourish in arid habitats (Bolger et al. 2014). The haploid genome size of *S. pennellii* has been evaluated by flow cytometry to be ~1200 Mb, which is fundamentally the same as the ~950 Mb estimated for *S. lycopersicum* (Arumuganathan and Earle 1991). The general genome organization of *S. pennellii* is highly similar to that of *S. lycopersicum*, regardless of their ecologically and morphologically different phenotypes and their being indirectly related taxa in the tomato clade (Rick 1960). Nature has given an incredible abundance of resistance that are available in the wild species (Bai and Lindhout 2007). A significant number of the resistances are essentially inherited, and remarkable successes have been achieved in exchanging disease-resistance genes into cultivated tomato.

One of the first examples was the exploitation of *Cladosporium fulvum* resistance from *S. pimpinellifolium* in 1934 (Bai and Lindhout 2007).

1.3 Economic Importance of Tomato

Tomato is a the major crop of the world economy and supplies basic supplement in human diets (Razdan 2006). Today, tomato is not only sold fresh but as well processed as paste, soup, juice, sauce, powder, concentrate or whole. Tomato stand out amongst the most consumed vegetables in the world, after potatoes and before onions and likely the most preferred garden crop. Tomato is the seventh most important crop species in the world production reaching just about 160 million tons in 2011 after maize, wheat, potatoes, soybeans and cassava (Bergougnoux 2014). During the last 20 years, tomato generation, and additionally as the area devoted to its culture, has multiplied (Bergougnoux 2014). The increasing economic importance of tomato is because of the high nutritious and low energetic value (~20 kilocalories for 100 g of product) of the tomato fruits. This is expected to a high content in water (~95%) while the others are: sucrose and fructose (~3%), proteins (~1%), fats (~0.2%) and strands (~1.8%). Tomato fruits have a profitable micronutrient that display at low concentration. It contains carotenoids that are significant source of vitamin A, C, and E (Abushita et al. 1997).

1.4 Hybrid Seeds of Tomato Plants

The term “hybrid “refers to a plant variety created through a controlled cross of two parent plants. Generally, the parents are compatible varieties inside the similar species (Mattern 2013). This hybridization, or the crossing of compatible varieties, happens naturally in the wild; plant breeders simply control the system to control the outcome (Mattern 2013). Hybrids of tomato demonstrate some heterosis, but this is only chosen for at the most recent phase of the breeding programme, when test hybrids are produced. In prior eras the parent lines, are chosen at a single plant basis yet not for joining capacity or heterosis (Bai and Lindhout 2007). Along these lines, recurrent selection programmes to select parents with the best consolidating capacities, like that utilized as part of field crops, is not a typical practice in tomato breeding (Bai and Lindhout 2007). Hybrid tomato varieties have many advantages compared to open-pollination varieties (Opena et al. 2001). Hybrids deliver higher yields. Many tomato hybrids have better fruit quality, resistance to disease and mature earlier and more uniform in their growth. With all these qualities, many famers prefer to sow hybrid seeds regardless of the higher seed costs. The interest in hybrid tomato seeds open a new market for producers interested in seed production (Opena et al. 2001).

1.5 Plant Growth-promoting Bacteria (PGPB)

Useful organisms in the microbiome of plant roots give important services to the plants as they enhance plant nutrition and give protection against plant pathogens (Wintermans et al. 2016). Soil is loaded with microscopic life forms including bacteria, fungi, actinomycetes, protozoa, and algae. Of these distinctive microorganisms, bacteria are most well-known (i.e., ~95%) (Glick 2012). It has been reported that the soil has a large number of bacteria (often around 10^8 to 10^9 cells per gram of soil) and that the quantity of cultivable bacterial cells in soil is generally around 1% of the aggregate number of cells present (Glick 2012). However, soil are

heterogeneous environments with different element parameters in which any of the parameter can influence microbial growth and survival. Soil is generally nutrient poor; its content of organic matter regularly shifts in concentration from 0.8 to 2.0%. Hence, native soil bacteria always confront nutrient hardship (Timmusk et al. 2011). Both the number and the type of bacteria that are located in different soil are affected by the soil conditions including temperature moisture, presence of salt and other chemicals and in addition by the number and types of plants found in these soils (Glick 2012). More so, bacteria are generally not evenly distributed in soil. This means that concentration of bacteria that is found around root of plant (i.e. in the rhizosphere) is normally significantly more than in the rest of the soil. This as a result of presence of nutrients including amino acids, organic acids, sugar, and other small molecules from plant root exudates that may represent up to 33% of the carbon that is fixed by a plants (Glick 2012). In this thesis, possibility to understand how rhizosphere and endospheric bacterial influence growth of Arabidopsis WT and protein phosphatase 2A catalytic subunit mutants (C2, C4, C2C4, C2C5). Bacteria CL8 was isolated from tomato, *Solanum lycopersicum*, cv. Heinz. Lillo laboratory (University of Stavanger) and bacteria from Belgium (Abbamondi et al. 2016) were used and these rhizosphere bacterial are represented as bacterial strains 5, 6, and endospheric bacterial represented as *Pseudomonas* sp. (number 9), 10, *Agrobacterium* sp. (number 15), *Rhizobium* sp. (number 16), *Agrobacterium* sp. (number 18).

1.6 Plant Growth Promoting Effects

1.6.1 Rhizosphere

The rhizosphere can be referred as the soil region where processes mediated by microorganism are particularly affected by the root system (de Souza et al. 2015), while the rhizobacteria refer to a group of rhizosphere bacteria competent in colonizing the root environment (Ahemad and Kibret 2014). This soil region includes the soil associated to the plant roots and frequently amplifies a few millimeters off the root surface (de Souza et al. 2015). Agricultural production relies on the huge scale utilization of chemical fertilizers. These fertilizers have ended up as segments for modern agriculture since they give vital plant supplements, for example, nitrogen, phosphorus and potassium (de Souza et al. 2015). However, the abuse of fertilizers can bring about unexpected ecological effects. The PGPB-based inoculation technology ought to be used alongside suitable levels of treatment of fertilizers. Also, the utilization of effective inoculants can be viewed as a system for feasible administration and for lessening natural issues by diminishing the utilization of chemical fertilizers (de Souza et al. 2015). Some rhizosphere microorganisms may be neutral or deleterious in response to plant growth, whereas others microbes support their hosts (Compant et al. 2010). Such plant growth-promoting bacteria or plant growth-promoting rhizobacteria can stimulate plant growth, increase yield, decrease pathogen infection, and reduce biotic or abiotic plant stress without giving pathogenicity (Compant et al. 2010). Some examples of bacteria that belong to PGPR are *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Cauloobacter*, *Chromobacterium*, *Erwina*, *Flavobacterium*, *Micrococcous*, *Pseudomonas* and *Serratia* (Bhattacharyya and Jha 2012). This group of rhizobacteria is mostly Gram-negative and rod-shaped with a lower proportion being Gram-positive rods, cocci and pleomorphic (Bhattacharyya and Jha 2012).

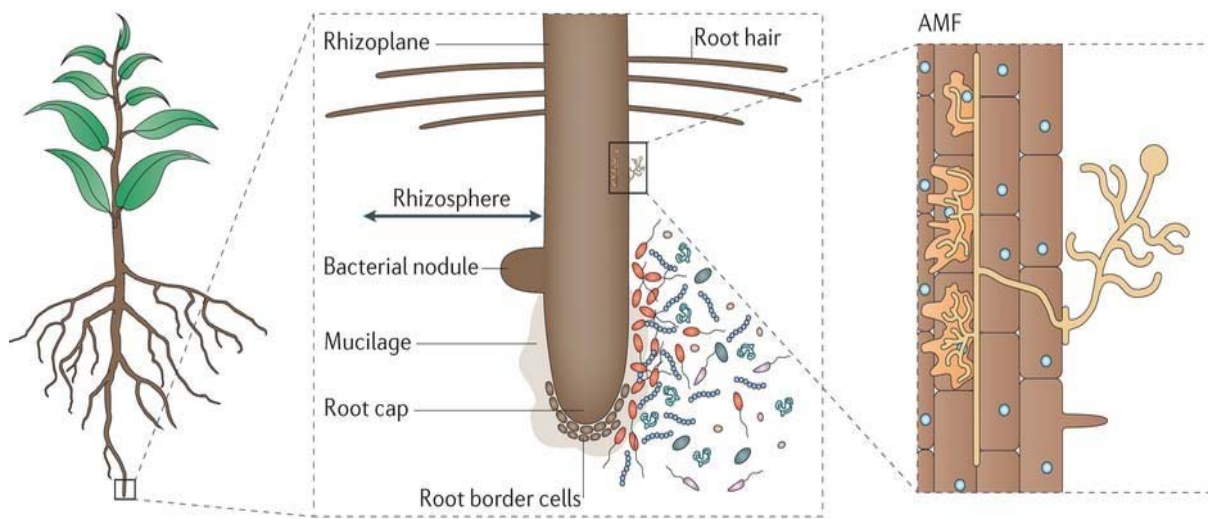


Figure 1-1: The magnified diagram of the rhizosphere, containing saprophytic and symbiotic bacteria and fungi (Philippot et al. 2013).

1.6.2 Endosphere Bacteria

A clear distinction ought to be drawn between bacteria residing in the rhizosphere or phyllosphere (the aerial habitat affected by plants) and bacteria living inside the plants, the so-called endophytes. Endophytic bacteria reside in a tissue of the plant for examples, root cortex or xylem and build up a nearby relationship with the plant, with exchange of nutrients, enzymes (lipase, catalase, oxidase, and so forth), functional agents (siderophores, bio-surfactants, etc.), and “signal” (Abbamondi et al. 2016). Endophytes colonize their plant host tissues in which they hold on without applying the negative impacts of a pathogen such as disruption of respiration, photosynthesis, translocation of supplements, transpiration, etc. Despite what might be expected, the presence of these endophytic bacteria in the host plants prompts beneficial effects on its health and/or growth (Abbamondi et al. 2016). The composition of the bacteria endophytic communication is affected by several factors, for examples, as host plant genotype, seasonal variation, plant growth stage, or geobiochemical conditions (Truyens et al. 2016). Endophyte strains have been isolated from root or stem nodules and stimulates growth either directly or indirectly for examples *Bradyrhizobium*, *Allorhizobium*, *Rhizobium* and *Mesorhizobium* (Wang and Martínez-Romero 2000).

1.6.3 Gram Positive Bacteria

A gram-positive bacteria is a prokaryotic cell consists of peptidoglycan in the cell wall and lacks the outer membrane characteristic of the gram-negative cells (Amils 2011). In gram-positive bacteria, as much as 90% of cell wall comprises of peptidoglycan, and teichoic acid is usually present in small amount (Amils 2011). The most important group of PGPR among positive bacteria are *Bacillus*, *Paenibacillus*, and *Actinomycetes*. Different species of *Paenibacillus* can stimulate plant growth by fixing atmospheric nitrogen and producing auxins (Kadam and Chuan 2016). *Bacillus* strains could likewise repress soil-borne pathogens and stimulate plant resistance to diseases following root colonization. Contrary to *Pseudomonas* and other nonspore-forming bacteria, *Bacillus spp.* can form endospores that enable them to

survive for extended period under unfavorable ecological conditions (Kadam and Chuan 2016). *Bacillus* species have been reported as promoting bacteria in an extensive variety of plants. Different *Bacillus* species were reported to be effective biocontrol agents in greenhouse or field trials (Kadam and Chuan 2016).

1.6.4 Gram Negative Bacteria

The gram-negative cell wall is a multi-layered structure and complex, while the gram-positive cell wall comprises of a single type of molecule, the peptidoglycan, which is frequently considerably thicker (Amils 2011). The most important group of PGPR among gram negative bacteria are the genera *Pseudomonas* (Kadam and Chuan 2016). Strains of fluorescent pseudomonads utilized as a part of biocontrol have contributed incredibly to the understanding of the mechanisms required in disease suppression. Many of these bacteria could prevent plant diseases by various mechanisms: antibiotic, competition, or parasitism. Within the genus *Pseudomonas fluorescens* which are ubiquitous rhizosphere inhabitant bacteria are the most studied group (Kadam and Chuan 2016). They were appeared to have a higher density and activity in the rhizosphere than in bulk soil. At the point when introduced on seed or planting material, they promote plant growth or control plant diseases by suppress deleterious rhizosphere microorganisms. They can compete aggressively for sites in the rhizosphere and prevent proliferation of phytopathogens by niche exclusion, production of antibiotics and siderophores, or inducing systemic resistance; by inducing plant growth by facilitating either take-up of nutrients from soil; or by delivering certain plant growth promoting substances (Kadam and Chuan 2016). Fluorescens Pseudomonads have connected to suppress *Fusarium wilts* of various plant pathogens, *Clavibacter michiganensis* subsp. *Michiganensis*, causal agent of tomato bacterial canker (Kadam and Chuan 2016). It has been revealed by Van Peer et al; protection of carnation from fusariosis due to phytoalexin accumulation upon treatment with pseudomonas strain WCS417. Different works followed including the use of *P. fluorescens* as a stimulating agent to prevent the spread of various plant pathogens. And also inoculation of *A. thaliana* by *P. fluorescens* WCS417r and of rice by WCS374r performed to induced systemic resistance (ISR) respectively to *Pseudomonas syringae* pv. Tomato and to the leaf blast pathogen *Magnaporthe oryzae* (Kadam and Chuan 2016). In this study, the bacteria isolated from tomato root (*Solanum pennellii*) are identified as *Sphingobium lamnesticum* (negative bacteria) which stimulate growth of plants both in vivo and in vitro.

1.6.5 *Sphingobium lamnesticum*

Members from the genus *Sphingobium* are aerobic, chemoorganotrophic, gram-negative, nonmotile or motile rods, and form yellow or whitish-brown colonies, circular, convex colonies on the various agar media (Chen et al. 2013). There two stains formed by *Sphingobium*: strain 301T and 469T were catalase and oxidase-positive, and grew between the temperature of 10 and 40 °C (optimum, 28 °C), and at pH value between 5 and 10 (optimum, pH 7) (Chen et al. 2013). Both strains contained Q-10 as the dominant quinone, sphingoglycolipids and 2-hydroxymyristic acid, whereas 3-hydroxy fatty acids were absent (Chen et al. 2013). Takeuchi et al. firstly in (2001) described the genus *Sphingobium* as a subgroup of the previous genus *Sphingomonas* that was divided into the four genera

Sphingomonas sensu stricto, *Sphingobium*, *Novosphingobium* and *Sphingopyxis* in view of phylogenetic, chemotaxonomic and physiological confirmation (Innerebner et al. 2011). However, the classification and nomenclature of the *Sphingomonads* is under debate (Innerebner et al. 2011).

Sphingomonas spp. are abundant in the phyllosphere and producers of plant growth-stimulating factors (Innerebner et al. 2011). Another way in which plants could profit from their commensal bacteria is by protection against attacking pathogens (Innerebner et al. 2011). It has been reported *Shingobium spp.* prevented severe plant disease and kept pathogen cell numbers lower than those of axenic plants (Innerebner et al. 2011).

1.6.6 *Acidovorax delafieldii*

Acidovorax delafieldii formerly called *Pseudomonas delafieldii* is a member of the subdivision of the class *Proteobacteria* belongs to the 'acidovorans complex,' which involves bacteria closely identified with *Comamonas acidovorans* (Brunen et al. 1991). *A. delafieldii* and *C. acidovorans* seem to possess relatively simple outer membranes, containing just a set number of protein species (Brunen et al. 1991). The acidovorans rRNA complex is a heterogenous group of organisms, many of which ought to be generically renamed (Willems et al. 1990). *Pseudomonas facilis* and *Pseudomonas delafieldii* were described as phenotypically comparable; an important difference was the failure of *P. delafieldii* to oxidize hydrogen (Willems et al. 1990). Most different types of the acidovorans rRNA complex (e.g; *P. facilis*, *P. delafieldii*, *P. avenue*, and *Comamonas testosteroni*) are situated at the branching level of these five rRNA subbranches (Willems et al. 1990).

1.6.7 PGPR *Pseudomonas simiae* WCS417r

Pseudomonas sp. represent 40-80% of the rhizobacterial populace, since their growth is particularly upgraded by root exudates (Persello-Cartieaux et al. 2001). The colonization of the roots by the PGPR *Pseudomonas simiae* WCS417r formerly known as (*Pseudomonas fluorescens* WCS417r) in *Arabidopsis* can stimulate shoot fresh weight by around 30 % when co-cultivated in soil (Wintermans et al. 2016). It was demonstrated that this growth-promoting effect is partly mediated by bacteria VOCs disconnected to the ISR-inducing capacity of WCS417r using-plate system (Wintermans et al. 2016). Interesting, a comparable observation was made when *Arabidopsis* plantlets were inoculated either with the PGPR *Bacillus subtilis* GB03 or with *Pseudomonas fluorescens* WCS417r, which emits VOCs (Vacheron et al. 2013). Colonization of the roots by ISR-inducing rhizobacteria and fungi does not specifically activate the plant immune system but rather primes the aboveground plant parts for accelerated upon pathogen, hence providing a cost-effective protection against plant diseases (Zamioudis et al. 2013). Co-cultivating *Arabidopsis* accession Col-0 with WCS417r result in inhibition of primary root length and promotion of lateral root and root hair formation, bringing about huge changes in the root architecture and increased shoot fresh weight. This bacterially-induced process requires the activity of the plant growth regulator called auxin (Wintermans et al. 2016). The contribution of microbial VOCs in plants growth promotion is regularly studied by co-cultivating plants and PGPR in sealed Petri dishes in which microbial-produced CO₂ can accumulate (Wintermans et al. 2016). Thus, it has been hypothesized that CO₂ produced by the bacteria causes the growth promotion through the improved accessibility of this photosynthesis

substrate (Wintermans et al. 2016). Though there is some confirmation that CO₂ can be partially included, the growth response stimulated by PGPR, is far more prominent than can be explained by elevated CO₂ alone (Wintermans et al. 2016). Elevated CO₂ levels can increase plant biomass by up to 25%, however numerous PGPR effectively surpass this as increase in plant biomass of over eightfold have been reported (Wintermans et al. 2016). It has been confirmed that WCS417r VOCs increases shoot fresh weight of Arabidopsis up to fourfold and stimulate plant growth beyond that caused solely by enhanced CO₂ levels (Wintermans et al. 2016).

1.7 Mechanism of Plant Growth Promotion

Plant growth promoting bacteria promote plant health and growth by three mechanisms: phytostimulation, biofertilization, and biocontrol (Abbamondi et al. 2016). Plant growth promotion by rhizobacteria occur directly and indirectly (Timmusk 2003). As a rule, direct mechanism influenced the balance of plant's growth regulators, upgrading plant's nutritious status and stimulating systemic disease resistance mechanisms (Martínez-Viveros et al. 2010). Indirect mechanisms are known with biocontrol, for example, antibiotic production, chelation of accessible Fe in the rhizosphere, synthesis of extracellular enzymes that hydrolyze the fungal cellular wall and rivalry for niches within the rhizosphere (Martínez-Viveros et al. 2010). The phytopathogenic rhizobacteria produces phytotoxic substances in negative associations, for example, hydrogen cyanide or ethylene can contrarily influence the growth and physiology of the plants (Bhattacharyya and Jha 2012). Counter to these deleterious bacteria, there are some PGPRs that can apply a positive plant growth by direct mechanism such as solubilization of nutrients, nitrogen fixation, production of growth regulator and so on., or by indirect mechanisms for example stimulation of mycorrhizae development, competitive exclusion of pathogens or removal of phytotoxic substances (Bhattacharyya and Jha 2012). Despite the confusion generated by multifunctional PGPR, it is necessary to look at the traits related with each of the three generic descriptors that are utilized to classify PGPR (Martínez-Viveros et al. 2010).

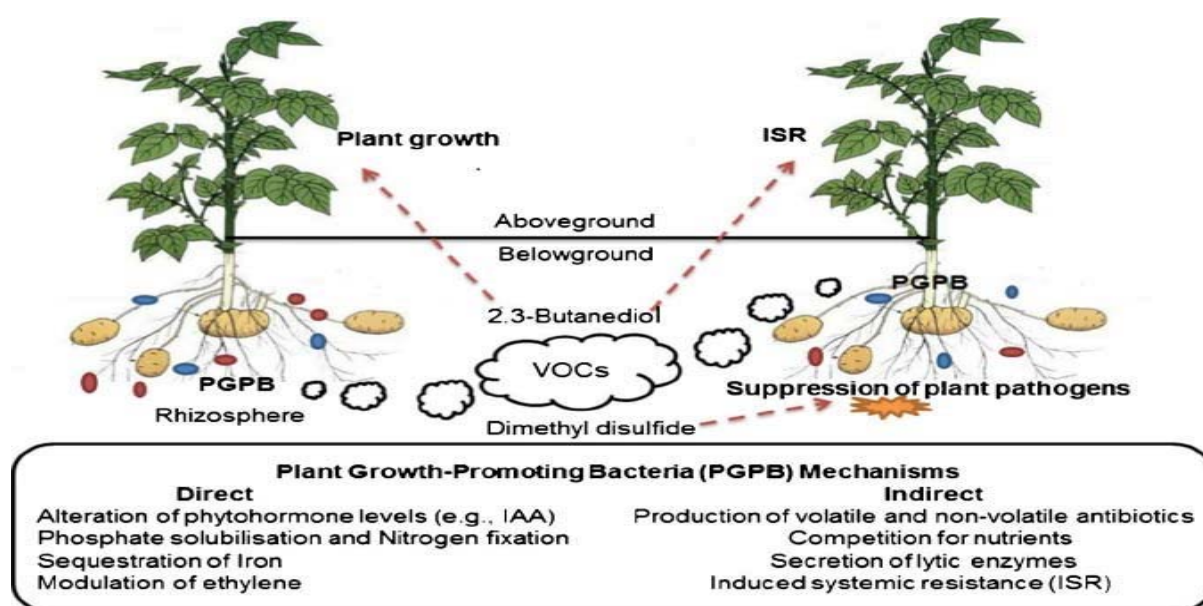


Figure 1-2: Mechanism of plant growth promoting bacteria (PGPB) (Velivelli et al. 2014).

1.8 Biological Nitrogen Fixation

Many associated bacteria can fix N_2 so that they could give nitrogen to the plant. Evidence for the support of the PGPR to the plant N budget has been reported for several plants, especially sugarcane (Vacheron et al. 2013). In addition to *Rhizobia* spp., several free-living bacteria for instance *Azospirillum* spp., are additionally able to fix nitrogen and provide it to plants. It is generally believed that free-living bacteria provide just a small amount of what the fixed nitrogen that the bacterially-associated host plant requires (Glick 2012). Nitrogenase (*nif*) genes required for nitrogen fixation incorporate structural genes, genes required in activation of the Fe protein, iron molybdenum cofactor biosynthesis, electron donation, and regulatory genes required for the synthesis and function of the enzyme (Glick 2012). There are some PGPR that are recognized as diazotrophic bacteria and can develop root associations with various plants including grasses (Santi et al. 2013). When they are found in close association with roots, they are generally designated as associative nitrogen-fixing bacteria. ‘Endophytic’ nitrogen-fixing bacteria have been characterized as bacteria detected inside surface-specialized plants or extracted from inside plants, having no visible harmful consequence on the plants, fixing nitrogen, and proved by microscopic evidence to be situated inside the plant (Santi et al. 2013). In diazotrophic (nitrogen fixing) bacteria, *nif* genes are commonly found in a cluster of around 20-24 kb with seven operons encoding 20 different proteins. Considering of the complexity of this system, genetic strategies to enhance nitrogen fixation have been elusive (Glick 2012). However, the effect of N_2 -fixation by PGPR is debated and is rarely credited for the stimulation of plant growth. In addition, non-fixing rhizobacteria can promote plant growth, demonstrating that N provision is not required for plant growth promotion. For example, *Phyllobacterium brassicacearum* STM196 is probably not going to fix N_2 while it promotes the growth of canola and *Arabidopsis* (Vacheron et al. 2013). Since the procedure of nitrogen fixation requires a large amount of energy in the form of ATP, it would be advantageous if bacterial carbon resources were coordinated towards oxidative phosphorylation, which bring about synthesis of ATP, instead of glycogen synthesis, which bring about storage of energy in the form of glycogen (Glick 2012). It’s found in one examination, a strain of *Rhizobium tropici* was developed with a deletion in the gene for glycogen synthase (Glick 2012).

1.9 Production of Indolic Compounds

It has been suggested that 80% of rhizosphere bacteria produce IAA (Spaepen et al. 2007). The impact of bacteria in the rhizosphere of plants is largely because of the production of auxin phytohormones (de Souza et al. 2015). Many bacterial species can produce indolic compounds (ICs), for example the auxin phytohormone indole-3-acetic acid (IAA), which exhibit awesome physiological relevance for bacteria-plant interactions, shifting from pathogenesis to phytostimulation (de Souza et al. 2015). IAA influences plant cell division, extension and differentiation; stimulates seed and tuber germination increases the rate of xylem and root improvement; control procedures of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light gravity and florescence; influence photosynthesis, pigment formation, biosynthesis of different metabolites, and resistance to distressing conditions (Glick 2012). The synthesis of ICs in bacteria relies upon the presence of precursor in root exudates (de Souza et al. 2015). Among the different exudate, L-tryptophan has been recognized as the main precursor of the route of IC biosynthesis in bacteria (de Souza et al.

2015). The characterization of intermediate compounds has prompted to the identification of different pathways that use L-tryptophan as the main precursor. The different pathways of IAA synthesis in bacteria demonstrate a high degree of comparability with the IAA biosynthesis pathways in plants. Helpful bacteria predominantly synthesize IAA through the indole-3-pyruvic acid pathway, an alternative pathway dependent on L-tryptophan. In phytopathogenic bacteria, IAA is produced from L-tryptophan means of the indole-acetoamide pathway (de Souza et al. 2015). In *A. brasilense* no less than three biosynthesis pathways have been depicted to produce IAA: two L-tryptophan-dependent (indole-3-pyruvic acid and indole-acetamide pathways) and one L-tryptophan-independent, with the indole-3-pyruvic (de Souza et al. 2015).

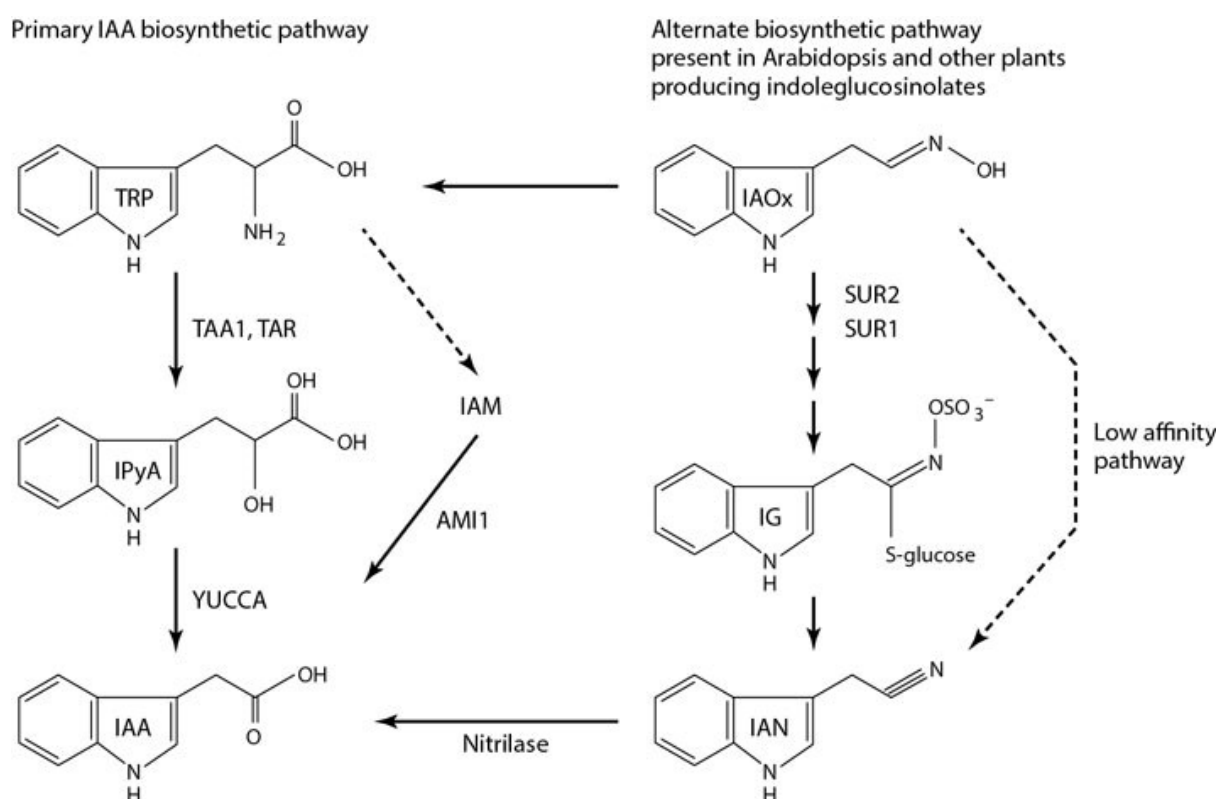


Figure 1-3: tryptophan-dependent pathways of IAA biosynthesis in Arabidopsis. Dashed arrows represent that neither a gene nor an enzyme activity has been identified in Arabidopsis. TRP, tryptophan; IAM, indole-3-acetamide; IPyA, indole-3-pyruvic acid; IAox, indole-3-acetaldoxime; IG, indole-3-methylglucosinolate; TRM, tryptamine; IAN, indole-3-acetonitrile (Normanly 2010).

1.10 Siderophore Productions

Siderophores can be referred to small peptidic molecules containing side chains and functional groups that can give a high-affinity set of ligands to facilitate uptake of ferric ions (Beneduzi et al. 2012). Bacteria siderophores have been classified into four main classes (carboxylate, hydroxamates, phenol catecholates and pyoverdines) based on their iron-coordinating functional group, structural factors and types of ligands (Beneduzi et al. 2012). Iron is rich in the Earth's crust yet most of it is in the highly insoluble form of ferric hydroxide, and thus unavailable to organisms in soil solution. Some bacteria have developed iron uptake systems (Timmusk 2003). In the aerobic condition, iron occurs principally as Fe^{3+} and is

probable to form insoluble hydroxides and oxy-hydroxides, hence making it for the most part inaccessible to both plants and microorganisms (Ahemad and Kibret 2014). Generally, bacteria acquire iron by the secretion of low-molecular mass iron chelators called siderophores which have high association constants or complexing iron. The clear majority of the siderophores are water-soluble and can be divided into extracellular siderophores and intracellular siderophores (Ahemad and Kibret 2014). Generally, rhizobacteria varies with respect to siderophore cross utilizing ability; some are capable in utilizing siderophores of the similar genus (homologous siderophores) while others could use those produced by other rhizobacteria of different genera (heterologous siderophores) (Ahemad and Kibret 2014). In both Gram-negative and Gram-positive rhizobacteria, iron (Fe^{3+}) in Fe^{3+} -siderophore complex on bacterial membrane is reduced to Fe^{2+} which is additionally released into the cell from the siderophore by means of a gating mechanism connecting the inner and outer membranes. During this reduction process, the siderophore might be destroyed or recycled (Ahemad and Kibret 2014). Therefore, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation (Ahemad and Kibret 2014). Not just iron, siderophores likewise form stable complexes with other heavy metals that are of environmental concern such as Al, Cd, Cu, Ga, In, Pb, Zn, and in addition with radionuclides including U and Np (Ahemad and Kibret 2014). Binding of the siderophore to a metal increases the soluble metal concentration (Ahemad and Kibret 2014). Hence, bacterial siderophores help to ease the stresses forced on plants by high soil levels of heavy metals (Ahemad and Kibret 2014).

1.11 ACC Deaminase Activity

The plant hormone ethylene is one of the simplest molecules with biological activity (Glick 2012). Generally, ethylene is an essential metabolite for the normal growth and development of plants (Ahemad and Kibret 2014). Ethylene is an endogenously produced gaseous phytohormone that acts at low concentrations occurring in the regulation of plant growth, development, senescence (de Souza et al. 2015). Under abiotic and biotic stresses such as pathogen damage, flooding, drought, salt, and organic and inorganic contaminants, endogenous ethylene production is significantly accelerated and unfavorably influences the growth of the roots and thus the growth of the plant (de Souza et al. 2015). Several mechanisms have been examined importantly to reduce the levels of ethylene in plants. One of these mechanisms includes the activity of the bacterial enzyme 1-aminoclopropane-1-carboxylate (ACC) deaminase. ACC deaminase controls the production of plant ethylene by metabolizing ACC (the immediate precursor of ethylene biosynthesis in higher plants) into α -ketobutyric acid and ammonia.

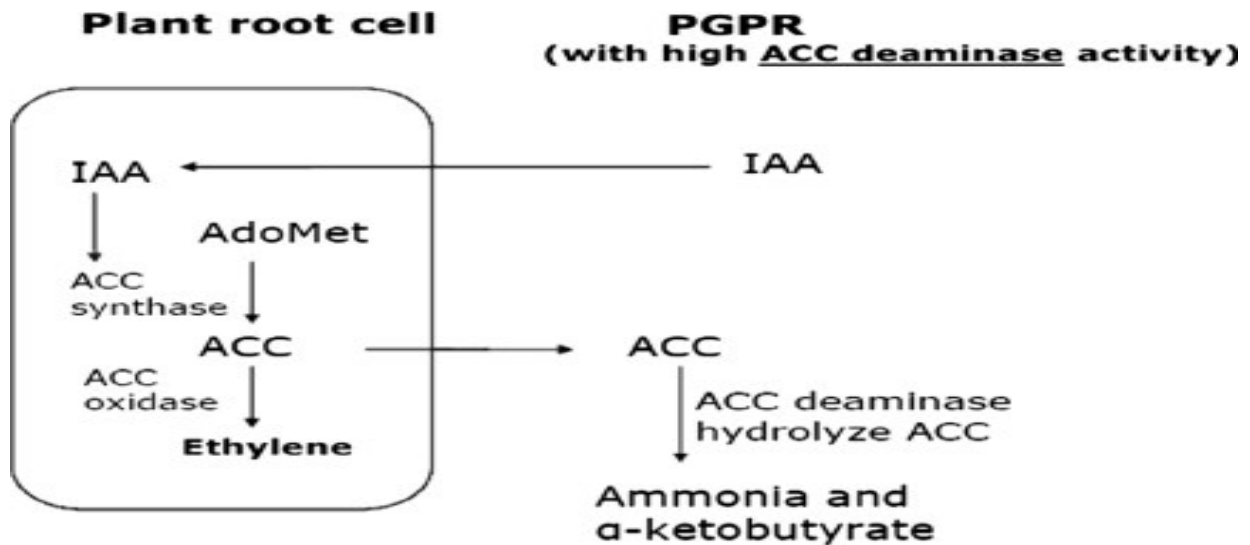


Figure 1-4: The ACC deaminase in PGPR degrades the ethylene precursor ACC. The ACC deaminase PGPR bring down ethylene level in plants by degrading ACC to ammonia and α -ketobutyrate (Saraf et al. 2010).

A lot of plant ACC may be excreted from the plant roots and therefore taken up by soil microorganisms and hydrolyzed by the enzyme ACC deaminase, consequently diminishing the amount of ACC in the environment. At the point when related with plant roots, soil microbial communities with ACC deaminase action may have a superior growth than other free microorganisms, as these organisms utilize ACC as a source of nitrogen (de Souza et al. 2015). Aside from being a plant growth regulator, ethylene has additionally been established as a stress hormone (Ahemad and Kibret 2014). Under stress conditions like these formed by salinity, drought, water logging, heavy metals and pathogenicity, the endogenous level of ethylene is significantly increased which negatively affects the overall plant growth. For examples, high concentration of ethylene induces defoliation and other cellular processes that may prompt to reduced crop performance (Ahemad and Kibret 2014). Plant growth promoting rhizobacteria which have the catalyst, ACC deaminase, facilitate plant growth and development by diminishing ethylene levels, stimulate salt tolerance and decreasing drought stress in plants (Ahemad and Kibret 2014). currently, bacteria strains displaying ACC deaminase action have been recognized in an extensive variety of genera for example, *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia* and *Rhizobium* etc. (Ahemad and Kibret 2014) such rhizobacteria take up the ethylene precursor ACC and convert it into 2-oxobutanoate and NH_3 (Ahemad and Kibret 2014).

1.12 Phosphate Solubilisation

Phosphorus (P) is an important nutrient for plants, partaking as a structural component of nucleic acids, phospholipids and ATP, as a key element of metabolic and biochemical pathways, important especially for BNF and photosynthesis (de Souza et al. 2015). Regardless of the way that the amount of Phosphorus in the soil is generally quite high (often between 400 and 1,200 mg/kg of soil) the majority of this phosphorous is insoluble and in this way not accessible to support plant growth (Glick 2012). The insoluble phosphorous is available as either an inorganic mineral, for example, apatite or as one of a few organic forms including

inositol phosphate (soil phytate), phosphomonesters, and phosphotriesters (Glick 2012). Low levels of P reflect the high reactivity of phosphate with other soluble components (de Souza et al. 2015). For example, aluminum in acid soil of pH <5 and calcium in alkaline soils of pH >7 (de Souza et al. 2015). Organic P incorporated into biomass or soil organic matter and inorganic compounds essentially as insoluble mineral complexes, are generally sources of available P in the soil (de Souza et al. 2015). In this manner, the availability of P relies on upon the solubility of this element, which could be affected by the action of plant roots and microorganisms in the soil. Phosphate-solubilizing bacteria (PSB) and fungi constitute around 1-50% and 0.1-0.5% separately, of the total population of cultivable microorganisms in the soil (de Souza et al. 2015). Among the diverse sources of P in the soil, (as already said), the solubilization of inorganic phosphates has been the principal focus of research studies (de Souza et al. 2015). Phosphate-solubilizing bacteria solubilize inorganic soil phosphates, such as FePO_4 , $\text{Ca}_3(\text{PO}_4)_2$, AlPO_4 , through the production of organic acids, siderophores, and hydroxyl ions (de Souza et al. 2015). A few bacteria just solubilize calcium phosphate, while different microorganism capable of solubilizing different types of inorganic phosphates at various intensities. Bacteria isolates belonging to genera *Enterobacter*, *Pantoea* and *Klebsiella* solubilize $\text{Ca}_3(\text{PO}_4)_2$ better than both FePO_4 and AlPO_4 (de Souza et al. 2015). The production of organic acids, especially gluconic and carboxylic, is one of the mechanisms well-study used by microorganisms to solubilize inorganic phosphates (de Souza et al. 2015). Bacterial genera like *Azobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Erwinia*, *Pseudomonas*, *Rhizobium* and *Serratia* are reported as the most significant phosphate solubilizing bacteria (Ahemad and Kibret 2014). Commonly, the solubilization of organic phosphorus occurs as an outcome of the activity of low molecular weight organic acids which are synthesized by different soil microbes (Ahemad and Kibret 2014). Then again, the mineralization of organic phosphorus occurs through the synthesis of a several of different phosphatases, catalyzing the hydrolysis of phosphoric esters (Ahemad and Kibret 2014). Significantly, phosphate solubilization and mineralization can exist together in the same bacterial strain (Ahemad and Kibret 2014).

1.13 Production of Volatile Organic Compounds

Microorganisms produce an extensive variety of VOCs when grown in pure culture and when effectively utilizing metabolizing organic matter in litter and soil (Ramirez et al. 2009). VOCs promote plant growth directly, through induced systemic resistance (ISR), or indirectly through suppression of phytopathogens (biocontrol) (Santoro et al. 2015). Probably the most widely recognized VOCs emitted from soils and litters include methanol, monoterpenes, alcohols, and ethers, yet the types and amount of VOCs released during microbial decomposition are highly variable and affected by both substrate type (e.g litter chemistry) and microbial community composition (Santoro et al. 2015). VOCs may act additionally as a carbon source for microorganisms, increasing soil CO_2 generation and diminishing nitrogen mineralization rates (Ramirez et al. 2009). VOCs can likewise also regulate bacterial growth rates (either stimulation or inhibition) by means of different mechanisms that remain undetermined (Ramirez et al. 2009). The discovery of rhizobacteria-produced VOCs are important mechanism for the elicitation of plant growth by rhizobacteria (Bhattacharyya and Jha 2012). It has now been established that the VOCs produced by the rhizobacterial strains can act as

signalling molecule to mediate plant-microbe interactions as volatiles produced by PGPR colonizing roots are produced at adequate concentration to trigger the plant responses (Ryu et al. 2003). Low-molecular weight of plant volatiles such as terpenes, jasmonates, and green leaf components have been distinguished as potential signal molecules for plants and organisms of other trophic level, the role volatile emissions from bacteria play in plant development is unknown (Ryu et al. 2003). Ryu et al. (2003) recorded some PGPR strains namely *Bacillus subtilis* GBO3, *B. Amylolique-faciens* IN937a and *Enterobacter cloacae* JM22 that releases a blend of volatile components, especially, 2, 3-butanediol and acetoin, which promoted growth of *Arabidopsis thaliana*, recommending that synthesis of bioactive VOCs is a strain-specific phenomenon (Bhattacharyya and Jha 2012).

1.14 Induced Systemic Resistance

Non-pathogenic bacteria have been identified to suppress disease by stimulating a resistance mechanism in the plant called 'Induced Systemic Resistance' (ISR) (Beneduzi et al. 2012). Induced resistance is the state of an improved protective capacity created by plants when suitably stimulated (Beneduzi et al. 2012). The defense mechanism of ISR is initiated only when there is an attack of pathogenic agent (Kundan et al. 2015). ISR is not particular against specific pathogen but rather helps the plant to control diseases. ISR involves jasmonate and ethylene signalling within the plant and these hormones induce the host plant's defence responses to a range of pathogens (Kundan et al. 2015). ISR has been demonstrated in the model plant *Arabidopsis* (*Arabidopsis thaliana*), and is effective against a wide range of plant pathogens, including fungi, bacteria, viruses, and even insect herbivores (Hua Guo and Hao Jiang 2015). The rhizobacteria strain *Pseudomonas fluorescens* WCS417r (WCS417r hereafter) has been appeared to trigger ISR in several plant species (Hua Guo and Hao Jiang 2015). It has been demonstrated by previous studies that PGPRs induced systemic resistance by activating the signalling pathways in plants, for example, SA, JA- or Ethylene-signalling pathwys. Diverse PGPR activated ISR relied upon various pathways. Reported that WCS417r-activated ISR was dependent on the JA/ET signalling pathway and NPR1 in *Arabidopsis* (Hua Guo and Hao Jiang 2015).

1.15 Protein Phosphatases

1.15.1 Protein Phosphorylation and Dephosphorylation

The phosphorylation and dephosphorylation of proteins are crucial for regulation of metabolism, cell division, development, growth and stress responses in all organisms (Lillo et al. 2014). Changing in phosphorylation status stand out amongst the most well-known methods for modifying the action of proteins and influence reaction rates, cellular localization, stability and interactions with other proteins (Lillo et al. 2014). Protein phosphorylation involves the enzyme-catalyzed transfer of the terminal phosphate group of an ATP molecule to the hydroxyl group of Ser, Thr, and Tyr side chain of the protein. This reaction is catalyzed by a protein kinase, and the reaction is basically unidirectional because of the extensive amount of free energy released when the phosphate-phosphate bond in ATP is broken to produce ADP. The reverse reaction of phosphate removal, or dephosphorylation, is rather catalyzed by a protein phosphatase (Alberts et al. 2002) as illustrated in figure 1-5.

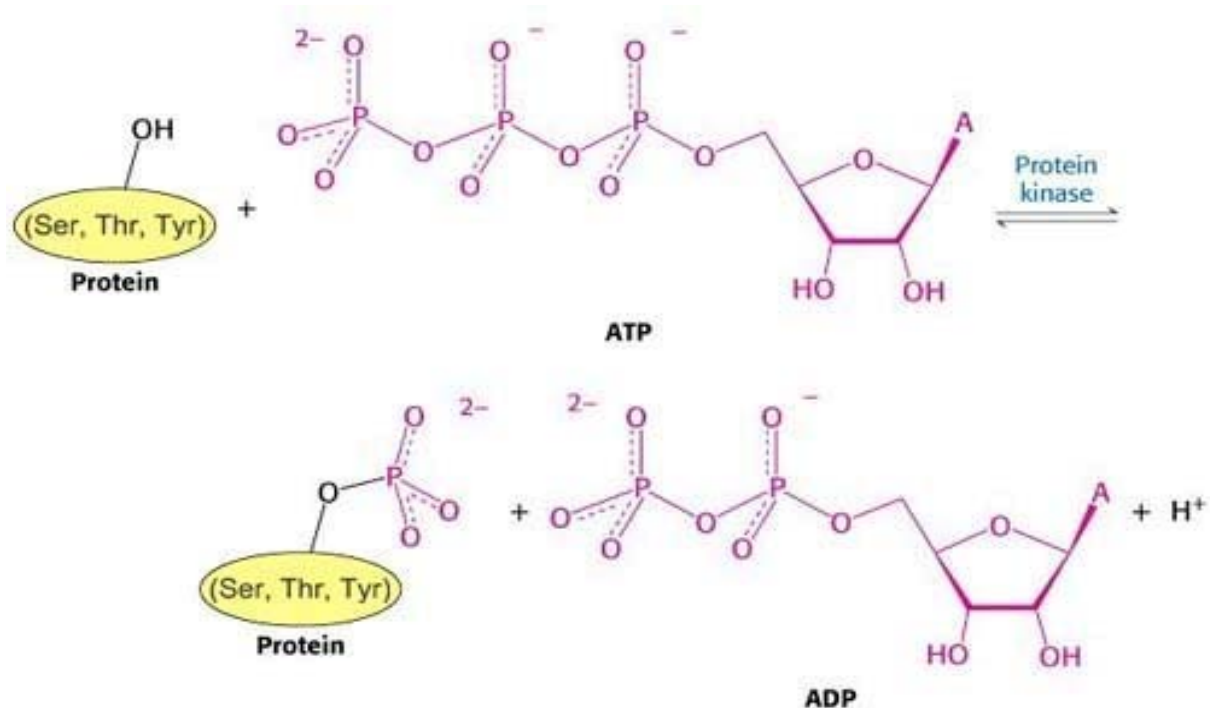


Figure1-5: Protein phosphorylation and dephosphorylation. Transfer of a phosphate group from ATP to an amino acid side chain of the target protein is catalyzed by a protein kinase. Removal of the phosphate group is catalyzed by a protein phosphatase. In this case, the phosphate is added to Ser, Thr or Tyr side chain. This diagram was copied from Stryer Biochemistry Textbook (5th Edition, page 397) (Berg et al. 2002).

Arabidopsis has around 1125 protein kinases and 150 protein phosphatases. The state of phosphorylation of a protein at any moment, and hence its activity, relies on upon the relative condition of the protein kinases and phosphatases that alter it (Alberts et al. 2002). For many reasons, the protein phosphatases have been much less studied than the protein kinase since

they are difficult to study and many of them are just functional in the cell as part of complexes with at least one regulatory subunits (Lillo et al. 2014).

1.15.2 The PPP Family of Protein Phosphatases

The PPP family of serine/threonine phosphatases is the most quantitatively huge source of protein phosphatase activity in eukaryotes (Shi et al. 1998). Eukaryotic protein phosphatases can be divided into four distinct gene families each with various active site signatures: (1) PPP (serine/threonine-specific phosphor-protein phosphatases) -GDxHG(x)₂₃GDxVDRG(x)₂₅GNHE-; (2) PPM/PP2C (Mg²⁺-dependent protein phosphatases) – (E/Q)D(x)_nDGH(A/G)(x)_nD(N/D)-; (3) Asp-based protein phosphatases -D_xD_x(T/V/I)L-; and (4) phosphor-tyrosine phosphatases (PTP)-CX₅R- (Lillo et al. 2014). The PPPs are most highly conserved proteins across eukaryotic species, and this family represents around 80% of protein phosphatases action in eukaryotic cells (Lillo et al., 2014). The PPP family can be additionally divided into subgroups: PP1, PP2/PP2A, PP3/PP2B (only in animals), PP4, PP5, PP6, PP7, PPKL/Kelch (only in plants and alveolates), and bacterial-like protein phosphatases (SLP, RLPH, ALPH). PP2A, PP4 and PP6 form a different cluster among the PPPs, suggestive of a common ancestor (Lillo et al. 2014). In vivo, plant catalytic subunits of PP2A, PP4 and PP6 are found in trimeric and also dimeric forms for instance with TAP46 (PP2A phosphatase associated protein of 46 KD) (Lillo et al. 2014).

1.15.3 Protein Phosphatase 2A (PP2A)

Protein phosphatase 2A (PP2A) is one of the most abundant types of serine/threonine phosphatase in all eukaryotic cells, showing a high level of conservation as far as both sequence and functional properties (Ballesteros et al. 2013). It is a holoenzyme comprising of catalytic (C) subunit, that occurs in relationship with regulatory A subunit and together with a third variable B subunit. The B subunits, which determine the substrate specificity and subcellular localization of PP2As are classified into B, B' B'' families (País et al. 2009). For instance, *Arabidopsis* has three scaffolding and 17 regulatory subunits that are part of trimeric complexes with the five catalytic subunits belonging to the PPP2A group, making a total of 255 possible combinations (Lillo et al. 2014). Some of the regulatory and catalytic subunits in *Arabidopsis* are given in figure 1-6. Five genes that encode PP2A catalytic subunits (PP2Ac) are found in *Arabidopsis*, and these five C subunits are assembled into two subfamilies: subfamily I (PP2A-C1, PP2A-C2, and PP2A-C5) and subfamily II (PP2A-C3 and PP2A-C4); Individuals from subfamily I are accepted to be required in plant stress and defence responses (Chen et al. 2014). Aside from reduced germination rate in the pp2a-c2 knockout line, *Arabidopsis* single mutants have no visible phenotype under standard conditions. Knocking out all individuals in both two subfamilies has serious effects (Lillo et al. 2014).

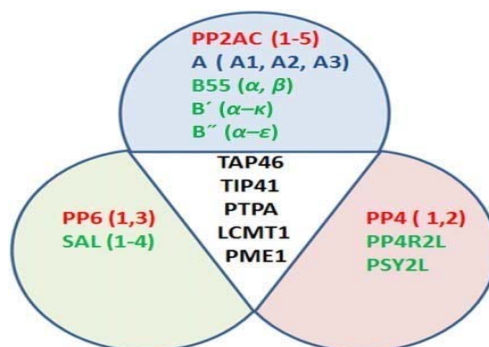


Figure 1-6: List of catalytic and regulatory subunits of PP2A, PP4, and PP6 in *Arabidopsis thaliana*. Catalytic subunits are represented in red, canonical scaffolding subunits in blue and regulatory subunits are in green. Putative interactors common for PP2A, PP4, and PP6 are shown in black in the central triangle (Lillo et al. 2014).

1.15.4 PP2A Catalytic Subunits in Physiological Processes in Arabidopsis

PPP2s have been involve in auxin transport, blue-light, abscisic acid signalling and photosynthetic active light (Lillo et al. 2014). The C2 mutant was originally isolated in the Wasslewskija ecotype (Ballesteros et al. 2013). PP2A-C2 is involved in blue-light-induced chloroplast movement and Arabidopsis PP2A C2 subunit has a function in ABA signalling. In the pp2a-c2 knockout, effect of ABA was strengthened, that is, ABA treatment inhibited root growth and germination more strongly in the mutant than in WT. Then again, in C2 overexpressor lines, ABA impacts were less prominent (Lillo et al. 2014). Additionally, photosynthetic active light promotes dephosphorylation of nitrate reductase, sucrose phosphate synthase and hydroxymethylglutaryl-CoA reductase. These three proteins are all cytosolic enzymes of primary metabolism being regulated by PP2A and are key enzymes in nitrogen assimilation, biogenesis of sucrose, and biogenesis of lipids, respectively (Lillo et al. 2014).

2. MATERIAL AND METHODS

2.1 Materials

2.1.1 Plant Materials

For this study, all work was done on plants species *Arabidopsis thaliana* (WT) ecotype Columbia and four types of *Arabidopsis* mutants; C2, C4, C2C4, C2C5 single and double mutants and tomato plants (Gemini original).

2.1.2 Hoagland Plant Nutrient Solution

The Hoagland solution was used as nutrient solution for plants during growth time in soil. The chemicals that utilized for making Hoagland plant supplement arrangement are listed in table 2-1.

Table 2-1: Hoagland plant Nutrient solution

Chemicals	Concentration of Stock (for 5 litre concentrated 10× solution) ml	Nutrient concentration in (1x) concentration solution (mM)
Potassium Nitrate, KNO ₃	250	5 mM
Potassium dihydrogen phosphate, KH ₂ PO ₄	50	1 mM
Calcium nitrate, Ca(NO ₃) ₂ ×4H ₂ O	250	5 mM
Magnesium sulphate heptahydrate, MgSO ₄ ×7H ₂ O	100	2 mM
1% Iron chelate, Fe-EDTA	50	1 mM
Micronutrient solution	Grams (g) dissolved in 1 litre of H₂O	
Boric Acid, H ₃ BO ₃	2.86	46.23 μM
Manganese chloride, MnCl ₂ ×4H ₂ O	1.81	9.2 μM
Copper Sulfate, CuSO ₄ ×5H ₂ O	0.089	0.36 μM
Zinc sulfate heptahydrate, ZnSO ₄ ×7H ₂ O	0.22	0.77 μM
Sodium molybdate, Na ₂ MoO ₄ ×2H ₂ O	0.029	1.12M

2.1.3 Preparation of Gamborg Medium for Sowing Seeds

Took 20 ml of 1/50 Gamborg/B5 and 0.78 g MES were added into 500 ml of distilled water. The pH was adjusted to 5.7 and 480 ml of distilled water was added to make 1 litre and medium was autoclaved.

2.1.4 Preparation of MS Medium for Sowing Seeds

Made 1 litre of 1× MS Medium from a prepared stock solutions shown in table 2-2. These were added to 500 ml of dH₂O. The pH was adjusted to 5.8 with 5 M of NaOH and 356 ml of distilled water was added to make 1 litre and 5 g of sucrose (0.5%) and 7g of Agar-agar were added and the medium was autoclaved.

Table 2-2: Murashige and skoog (MS) medium

Chemicals	Concentration of Stock (g/L)	Nutrient concentration in (1x) concentration solution
KNO₃	95	20 ml
NH₄PO₃	120	13 ml
MgSO₄×7H₂O	37	10 ml
KH₂PO₄	17	20 ml
CaCl₂×2H₂O	44	10 ml
Minor I		10ml
ZnSO ₄ ×H ₂ O	0.920	
H ₃ BO ₃	0.620	
MnSO ₄ ×4H ₂ O		
Minor II		10 ml
Na ₂ MoO ₄ ×2H ₂ O	0.025	
CuSO ₄ ·5H ₂ O	0.003	
CoCl ₂ ·6H ₂ O	0.003	
KI	0.083	
Fe/EDTA		50 ml

Na ₂ · EDTA	0.373	
FeSO ₄ ×7H ₂ O	0.278	
M7150 Vitamin (1000×)	mg/L	1 ml
Glycine	2.0	
Myo-inositol	100	
Nicotinic acid	0.5	
Pyridoxine · HCl	0.5	
Thiamine · HCl	0.1	

2.2 Methods

2.2.1 Soil Seed Sowing

The soil was filled into 20 pots and the soil was soaked in nutrient solution for 30 minutes. Two seeds of Arabidopsis (WT), C2, C4, and C2C5 mutants were sown into each pot and placed in the cold and dark room for 48 h then transferred to growth chamber (artificial light environment of 16 h light/ 8 h dark) at 20-23°C. Throughout the whole growth period the plants were regularly watered with Hoagland solution (fig. 2-1). After 2 months, the seeds were harvested.

2.2.2 Sterilizing of Seeds

Ca-hypochlorite of 0.25 g was dissolved into 25 ml of dH₂O and was stirred on a magnet stirrer, then a drop of tween was added, the solution was shake and left to settle. 1ml of the supernatant with pipette into 9 ml of 95% ethanol. The seeds Arabidopsis (WT) and mutants of C2, C4, C2C4 and C2C5 was placed in different tubes and 1 ml of ethanol/hypochlorite solution was added into each tube. The solution was kept for 3 min and the supernatant was removed. 1 ml of 95 % ethanol was added and removed and this step was repeated twice by washing. The seeds were left to dry overnight in the sterile hood to remove the ethanol.

2.2.3 Plant Growth Conditions

After sterilizing the seeds, 50 seeds of Arabidopsis (WT), C2, C4, C2C4 and C2C5 were sown on Petri dishes containing Gammberg/B5 (1×) or 1× MS medium, agar with sucrose (0.5%) and placed in temperature of 4⁰C in a dark room for 2 days and afterward put into growth chamber (artificial light environment of 16 h light/ 8 h dark) at 20-23°C for 5 days.

2.2.4 Isolation of Bacteria from Rhizosphere

The 0.9 g of hydroponic tomato root (*S. Pennelli*) was cut using scissors. Roots were washed five times with 25ml PBS buffer then the roots were sterilized as stated here. The root was soaked in 1% of 10 ml Ca-hypochlorite for 3 min and the supernatant was discarded and rinsed with autoclave dH₂O five times. The last rinsing was used to test sterilization and pipette 20 µl of crude extract and 80 µl of MgSO₄ into LB-agar plate as 5-fold dilution and 10 µl of extract was pipette into 90 µl MgSO₄ into LB-agar plate as 10-fold dilution and last washed was used as control. This was placed at 30 °C for seven days.

There was different colony with different colour in the LB-agar plates after seven days. Two different colonies were chosen and was streaked on LB-agar plate and placed at room temperature. Following day, took each bacterial colony into 10 ml of LB medium (low salt) and placed on a shaker for 24 h at 30 °C per 120 rpm. The optical density was read using spectrophotometer. 1.5 ml of each cultures in six Eppendorf tubes, three tubes for G-positive and three tubes for G-negative pre-treatment for bacteria. Two cultures with twelve Eppendorf tubes in total were centrifuged for 10 min at 5000 × g describe in 2.10 and 2.11.

2.2.5 Preparation of Enzymatic Lysis Buffer (Stock)

Tris (20 µl) was added into Eppendorf tube and 4 µl of EDTA and 60 µl of Triton was added to 716 µl of dH₂O mixed together excluding 200 µl lysozyme. Lysozyme was added before use.

2.2.6 Pre-treatment for Gram-Positive Bacteria

Two cultures with three Eppendorf tubes were centrifuged for 10 min at 5000 × g and supernatant was discarded. The bacteria pellet was resuspended in lysis buffer and incubated for 30 min at 37 °C. 25 µl of proteinase K and 200 µl Buffer AL (without ethanol) was added to the three Eppendorf tubes and mixed by vortex. The three Eppendorf tubes were incubated at 56 °C for 30 min. After incubation, 200 µl of 96 % ethanol was added to the samples and mixed thoroughly by vortexing. A white precipitate was observed and the mixtures were pipetted into the DNeasy Mini spin column placed in 2 ml collection tubes and centrifuged at 6000 × g for 1 min and supernatant was discarded. The DNeasy Mini spin column was placed into a new collection tubes and 500 µl of buffer AW1 was added and centrifuged for 1 min at 6000 × g and supernatant was discarded. The last step was repeated and 500 µl of buffer AW2 was added and centrifuged for 3 min at 20,000 × g to dry the DNeasy membrane and the supernatant was discarded. The DNeasy Mini spin column was placed in a clean 1.5 ml micro centrifuged tubes and 50 µl of sterilized dH₂O was added directly into the DNeasy membrane and was incubated at room temperature and centrifuged for 1 min at 6000 × g to elute and this step was repeated twice.

2.2.7 Pre-treatment for Gram-Negative Bacteria

Two cultures with three Eppendorf tubes were centrifuged for 10 min at $5000 \times g$ and supernatant was discarded. The bacteria pellet was resuspended in 180 μl buffer ATL and 20 μl of proteinase K was added and mixed thoroughly by vortex and incubated for 56°C for two hours and was vortexed every 15 min until it completely lysed. Vortex for 15 s and 200 μl buffer AL was added to the samples and mixed thoroughly by vortex. Then 200 μl of 96 % ethanol was added and mixed again thoroughly by vortex. The mixtures were pipetted into the DNeasy Mini spin column placed in 2 ml collection tubes and centrifuged at $6000 \times g$ for 1 min and supernatant was discarded. The DNeasy Mini spin column was placed into a new collection tubes and 500 μl of buffer AW1 was added and centrifuged for 1 min at $6000 \times g$ and supernatant was discarded. The last step was repeated and 500 μl of buffer AW2 was added and centrifuged for 3 min at $20,000 \times g$ to dry the DNeasy membrane and the supernatant was discarded. The DNeasy Mini spin column was placed in clean 1.5 ml micro centrifuged tubes and 50 μl of sterilized dH_2O was added directly into the DNeasy membrane and was incubated at room temperature and centrifuged for 1 min at $6000 \times g$ to elute and this step was repeated twice.

2.2.8 Concentration Measurement

The concentration of G-positive and G-negative bacterial was measured using Nanodrop as shown in table 2-3.

Table 2-3: Concentration of G-positive and G-negative bacterial after pre-treatment

Samples	Concentration (ng/ μl)	A260/A280	A260/A230
1p	25.7	1.81	0.88
C1p	37.2	1.96	1.16
1n	37.5	1.87	1.35
C1n	34.2	2.14	2.22

2.2.9 Polymerase Chain Reaction (PCR)

PCR was used by repeated cycles of strand separation, annealing of primers, and extension of the primed strands. Basically, the objective DNA is repeated in vitro, numerous, multiple occasions, to acquire a lot of the DNA that lies between the two primer regions.

2.2.10 Primer used for Genotyping

In this study, forward and reverse primers were used and can be seen in table 2-4.

Table 2-4: List of primers and nucleotide sequence used for genotyping

Primers Name	Nucleotide sequence (5' to 3')
26FBactSpecific 16S	AGA GTT TGA TCC TGG CTC AG
1520R	AAG GAG GTG ATC CAG CCG GA
1492R	GGT TAC CTT GTT ACG ACT T

2.2.11 PCR Mix and PCR Program used when Genotyping

For genotyping Thermo Scientific Dream Taq DNA polymerase kits was used with two samples per Gram-positive and Gram-negative of different colony per each. This can be seen in table 2-5.

Table 2-5: PCR mix used for genotyping of control and two samples per G-positive and G-negative bacterial.

Reagent	Control (µl)	1p (µl)	C1p (µl)	1N (µl)	C1N (µl)
10X Dream Taq Buffer	5	5	5	5	5
dNTP Mix, 2Mm each (#R0241)	5	5	5	5	5
Forward primer 26F (10 µM)	1	1	1	1	1
Reverse primer (1520R) (10 µM)	1	1	1	1	1
Template DNA (Samples)	-	1	1	1	1
Dream Taq DNA Polymerase	0.25	0.25	0.25	0.25	0.25
Water, nuclease-free (#R0581)	37.0	36.7	36.7	36.7	36.7

The samples were gently vortexed and spin down and PCR Machine was set in thermal cycling condition and this can be seen table 2-6.

Table 2-6: Thermal cycling condition of G-positive and G-negative bacterial for PCR

Steps	Temperature, °C	Time
Initial Denaturation	95	3 min
Denaturation	95	30s
Annealing	60	30s
Extension	72	1h 30min
Final Extension	72	10

Note: Denaturation, Annealing, and Extension were repeated 30 times.

2.2.12 Agarose Gel Electrophoresis

To make the gel, 1g of agarose was used in 100 ml 1x TAE buffer. The agarose-buffer mixture was heated for 2 min, then poured into the cast with comb for 20 min to solidify.

2.2.13 DNA Bands Visualization

The mixture of the samples with other reagents were used and visualized the DNA band by using VisiDoc-It (Imaging System Doc). Marker (HyperLadder™ 1kb and Quick-Load® 100 bp) were used for determination of DNA size. 10 µl of the samples were pipette from PCR products.

Table 2-7: Mixture of samples (PCR products) with loading buffer and gel red.

	Samples (µl)	HyperLadder™ (µl)	Loading Buffer (µl)	Gel Red (µl)
Control	-	5	-	1
1p	10	-	1.5	1.5
C1p	10	-	1.5	1.5
1n	10	-	1.5	1.5
C1n	10	-	1.5	1.5

The mixtures were mixed and 13 µl of the solution pipette into the gel-well and run for 40 min at 90 V and analyzed using UV light to visualize the DNA bands.

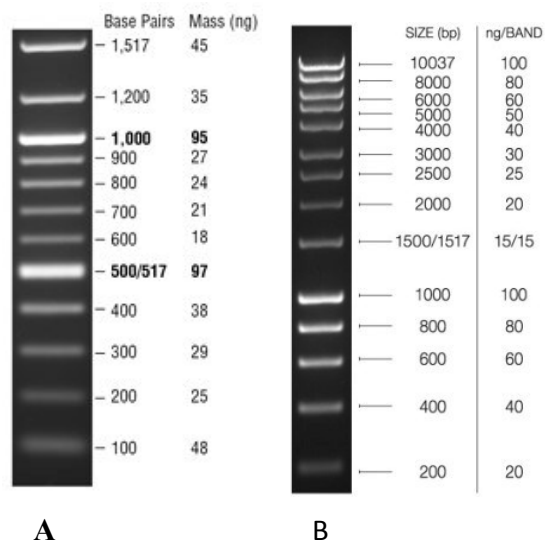


Figure 2-1. Overview of the Markers, (A) Quick-Load® 100 bp (B) HyperLadder™ 1kb and with size and concentration (per 5 µl).

2.2.14 Scaling up for DNA Extraction

The same procedure was repeated as shown in 2.16 except that the remaining 40 µl of the samples (PCR products) were divided into two and 24 µl of the mixture of the samples with reagents were pipette into well gels as shown in table 2-8.

Table 2-8: Mixture of loading buffer and gel red with the remaining samples

	Samples (µl)	HyperLadder™ (µl)	Loading Buffer (µl)	Gel Red (µl)
Control	-	4	-	4
1p	20	-	4	4
1.1p	20	-	4	4
C1p	20	-	4	4
C1.1P	20	-	4	4
1n	20	-	4	4
1.1n	20	-	4	4
C1n	20	-	4	4
C1.1n	20	-	4	4

The empty 1.5 µl Eppendorf tubes were weighed and the bands were carefully cut using scalpel into the tubes and this was used for DNA extraction.

2.2.15 DNA Extraction from Agarose Gels

The size of the gel with DNA of interest was calculated by subtracting the weight of Eppendorf tube with the gel and empty Eppendorf tube and this was used to know the amount of NTI to be used. For each 100 mg of agarose gel < 2% (1:2), 200 µl of buffer NTI was added to the samples and incubated for 5 min and the samples were vortexed every 2 min until the gel slice completely dissolved. After dissolving the samples, 700 µl of the samples were loaded into NucleoSpin® gel with PCR Clean-up column and was centrifuged at $11,000 \times g$ for 30 min to bind the DNA and the supernatant was discarded. The silica membrane was washed by adding 700 µl of buffer NT3 into NucleoSpin® gel with PCR Clean-up column and was centrifuged at $11,000 \times g$ for 30 s and supernatant was discarded. The silica membrane was dried by centrifuge the NucleoSpin® gel with PCR Clean-up column at $11,000 \times g$ for 1 min and finally incubate the columns for 2 min at 70 °C to remove buffer NT3. The DNA was eluted by placing NucleoSpin® gel and PCR Clean-up column into a new 1.5 ml micro centrifuge tube and 15 µl of autoclaved dH₂O was added to the tubes and was incubated for 1 min at room temperature and centrifuged at $11,000 \times g$ for 1 min. This was repeated twice.

2.2.16 Sequencing of G-positive and G-negative Bacterial

The 12 samples were sent to sequence Laboratory (Seqlab) Göttingen GmbH, address; Hannah-Vogt-Str.1, 37085 Göttingen, postfach 3343, 37023 Göttingen, Germany.

Premixed DNA sequencing was applied for all the 12 samples by mixing the plasmid (containing DNA template) and the primer. See the details in table 2-3, the list of primers and their nucleotides sequence. The sequence obtained from the Seqlab was translated using MEGA 6, the reverse nucleotides were pasted into reverse complement program and the nucleotides sequence of forward and reverse were pairwise using Emboss (local alignment) for both the forward and reverse complement. The result gotten from the alignment was BLASTed at NCBI with BLASTn program against 16S ribosomal RNA Sequence (Bacteria and Archaea) database. The query sequences were retrieved and was saved as FASTA format.

2.2.17 Procedure for Rhizosphere and Endospheric Bacteria

The bacterial stains 5, 6, 9, 15, 10, 15, 16, 18, and cl8 were inoculated into growth medium of 10 ml (LB Broth Lumina low salt) and placed on a shaker for 48 h at 30 °C. The optical density (O D₆₀₀) was read using spectrophotometer until the absorbance was 0.5. 1 ml of bacterial strains were pipette into Eppendorf tubes and was centrifuged at 4000 rpm for 20 min at room temperature and then the supernatant was discharged. The pellets were washed and centrifuge with 1 ml of MgSO₄ for 5 min and this procedure was repeated twice and the supernatant were discharged. 650 µl of MgSO₄ were added into each of the tubes and mixed with pipette.

2.2.18 Procedure for *Pseudomonas simiae* WCS417r Bacterial Inoculation

The *Pseudomonas simiae* WCS417r bacterial strain was grown overnight at 28 °C on King's medium B agar medium supplemented with 1 ml of rifampicin 50 mg/ml stock solution. Following day, 5 ml of 10 mM MgSO₄ × 7H₂O was added to WCS417r bacteria on the plate

and left for 5 min and pipette it into a tube. Addition of 5 ml of 10 mM $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ was added and mixed. 1 ml of the bacteria suspension was added to four Eppendorf tubes and centrifuged at $3200 \times g$ for 5 min. supernatant was discarded. The pellets were washed and centrifuge with 1 ml of 10 mM $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ for 5 min at $3200 \times g$ and this procedure was repeated twice and the supernatant were discharged. The optical density (OD_{600}) was read using spectrophotometer until the absorbance was adjusted to 2×10^6 colony-forming units (cfu) m/L of 10 mM $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ ($\text{OD}_{600} = 0.002$).

2.2.19 Procedure for *Sphingobium limneticum* and *Acidovorax delafieldii* strains

The *Sphingobium limneticum* strain was grown for 3 days at room temperature on King's medium B agar medium supplemented with 1 ml of rifampicin 50 mg/ml stock solution and *Acidovorax delafieldii* strain was grown on LB agar (low salt) for 3 days at room temperature. The bacterial stains of *Sphingobium limneticum* and *Acidovorax delafieldii* were inoculated into growth medium of 10 ml (LB Broth Lumina low salt) and placed on a shaker for 48h at 30 °C. After the following day, 1 ml of bacterial strains were pipette into Eppendorf tubes and was centrifuged at $3200 \times g$ for 5 min. supernatant was discarded. The pellets were washed and centrifuge with 1 ml of 10 mM $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ for 5 min at $3200 \times g$ and this procedure was repeated twice and the supernatant were discharged. The optical density (OD_{600}) was read using spectrophotometer until the absorbance was adjusted to 2×10^6 colony-forming units (cfu) m/L of 10 mM $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ ($\text{OD}_{600} = 0.002$).

2.2.20 Preparation for Tomato Plants (Gemini original)

The tomato plants (7) were planted into vermiculite containing pots, allowed to grow for two weeks and 5× superba was used to water the plants and the bacteria were used to treat the plants. The same procedure was used to prepare the bacterial (WCS417r and *Sphingobium limneticum*) except the amount required to treat the tomato plants was 120 ml per pots containing tomato plants and 120 ml of 10 mM $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ for the control. The primary shoot length, shoot fresh weight was determined.

2.2.21 Growth Media

Bacterial, 650 µl of bacterial strains 5, 6, 9, 15, 10, 15, 16, 18, cl8, 400 µl of WCS417r *Sphingobium limneticum* and *Acidovorax delafieldii* were spread over 2 cm from the top and downward the surface of the plates containing 1/50 Gammberg medium without sucrose or 1× MS medium with 0.5 % sucrose and allowed to dry for 45 min. As a control, five seeds were sown into separated four Petri dishes containing 1/50 Gammberg or 1× MS medium without bacterial, and then five seeds were sown into separated four Petri dishes containing bacteria and the Petri dishes were moved into growth chamber for 8 days to stimulate the growth. This was observed daily for 8 days. Following a sum of 8 days, the Petri dishes were examined for growth using image J to examine the primary root length and lateral roots length by counting each of the lateral roots and shoot fresh weight was weighed.

3. RESULTS

3.1 Phenotype of *Arabidopsis thaliana* and Mutants

The *Arabidopsis* (WT) and the C2, C2C4 mutants were sown into the soil. The C2C4 mutants displayed impaired growth, taking about 3 weeks longer to mature and produce seeds when compared to the wild type and this can be seen in figure 3-1. After three months, the seed were harvested and sterilized. Then the seeds were used to test plant growth promoting effect using bacteria.

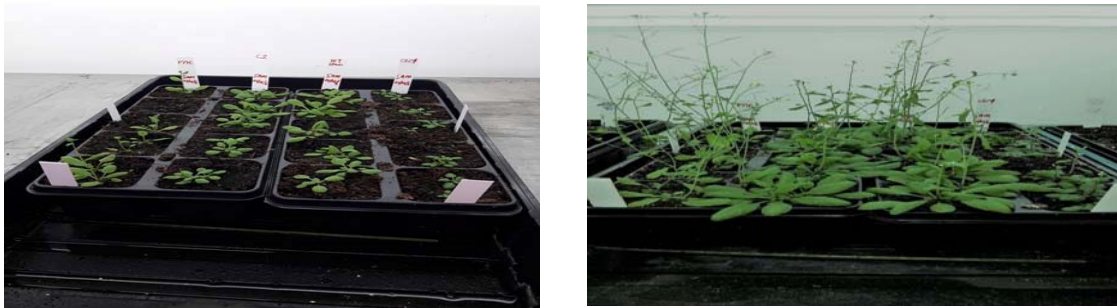


Figure 3-1: Seeds of rcn, C2, *Arabidopsis* (WT) and C2C4 mutants plated on soil.

Left figure: from left to right: seeds of rcn, C2, *Arabidopsis* (WT) and C2C4 mutants planted on soil, three weeks after sowing. Right figure: same tray two months after sowing. The C2C4 mutants displayed impaired growth when compared to the wild type showing in the last row.

3.2 DNA Bands Visualization by using PCR, Gel Electrophoresis, and DNA Extraction

The bacteria had been isolated from root of hydroponic tomato (*Solanum pennellii*). The samples were subjected to PCR as described in table 2-4 and electrophoreses on a 1% agarose-TAE gel. 13 μ l of each samples were pipette into gel-well, followed by separation at 90 V for 40 min. The gel was exposed to uv light and the picture was taken with a Imaging System Doc. Hyperladder is denoted H, negative control is denoted C- with forward primer (26F) and reverse primer (150R) and positive control is denoted C+ with forward primer (26F) and reverse primer (1492R). Also, 1n, c1n, 1p, c1p contains forward primer with 150R and 1.1n, c1.1n, 1.1p, c1.1p contains forward primer with 1492R.

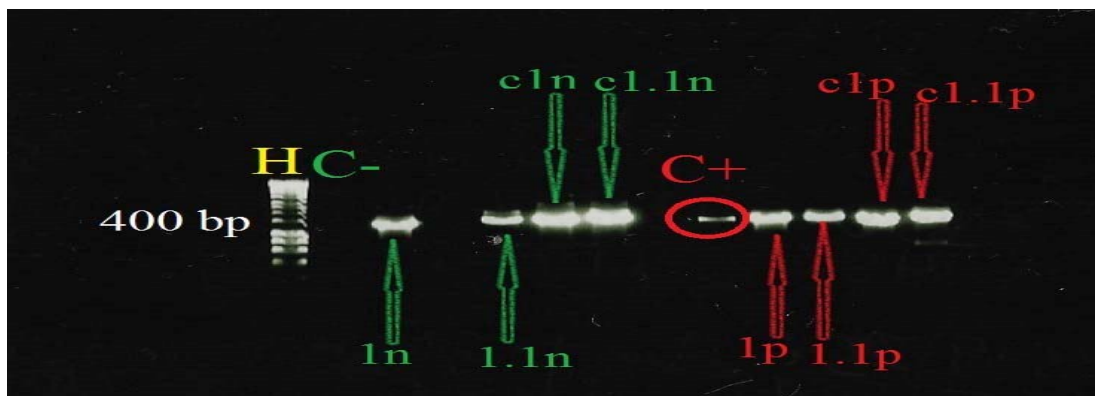


Figure 3-2: Agarose gel electrophoresis results of unidentified Gram-negative and Gram-positive bacteria.

Two colonies for Gram-negative and Gram-positive were sequenced and identified as *Sphingobium limneticum* and *Acidovorax delafieldii* by blasting the compliment sequence at NCBI with BLASTn program against 16S ribosomal RNA Sequence (Bacteria and Archaea). The query protein of *Sphingobium limneticum* and *Acidovorax delafieldii* shows 99 percent identity (see details in the appendix number 1 to 4).

```
NCTTCGGGTCTAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCTTGGGTTTCGGAATAACGTCGGGAAACTGACGCTAATACCGGATGATGACGAAAGTC
CAAAGATTATCGCCAGGGATGAGCCCGCTAGGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCTACGATCCTTAGCTGGTCTGAGAGGATGATCAGC
CACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATATTGGACAATGGGGCAACCCCTGATCCAGCAATGCCGCGTGAGTGATGAA
GGCCTTAGGGTTGTAAAGCTCTTTTACCGAGATGATAATGACAGTATCGGGAGAATAAGCTCCGGCTAACTCCGTGCCAGCAGCCGCGTAATACGGAGGGAG
CTAGCGTTGTTTCGGAATTAAGTGGGCGTAAAGCGCACGTCAGGCGCGAATTAAGTCAGAGGTGAAAGCCCGGGGCTCAACCCCGGAACTGCCTTTGAGACTGGAT
TGCTTGAATCCTGGAGAGGTGAGTGGAATCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGGCTCACTGGACAGGTATTG
ACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGATAACTAGCTGCCGGGGCACATGGTGTTCGGTGG
CGCAGCTAACGCATTAAGTTATCCGCTGGGGAGTACGGTCCGAAAGTAAAACTCAAAGGAATTGACGGGGGCTGCACAAGCGGTGGAGCATGTGGTTTAAT
TCGAAGCAACGCGCAGAACCTTACCAACGTTTACATCCCTATCGCGGATCGTGGAGACTTTCTTCAGTTCCGGCTGGATAGGTGACAGGTGCTGCATGGCT
GTCGTCAGCTCGTG
```

Figure 3-3: One of Sequence of unidentified colony for Gram-negative bacteria. See details in the appendix 1-4 to see the rest of the sequence with forward primer and reversed primer.

Sphingobium limneticum strain 301 16S ribosomal RNA gene, partial sequence
 Sequence ID: [NR_109484.1](#) Length: 1441 Number of Matches: 1

Range 1: 69 to 1017 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1748 bits(946)	0.0	948/949(99%)	0/949(0%)	Plus/Plus

Figure 3-4: Identification of *Sphingobium limneticum* after sequencing. The first hit showing 99% identity after blasting the nucleotides sequence with BLASTn program against 16S ribosomal RNA Sequence (Bacteria and Archaea).

```
GTCGAACGGTAACAGGTCTTCGGATGCTGACGAGTGGCGAACGGGTGAGTAATACATCGGAACGTGCCCGAGAGTGGGGATAACGAAGCGAAAGCTTTGCTAA
TACCGCATACGATCTCAGGATGAAAGCAGGGGACCGCAAGGCCTTGCCTCACGAGCGGCCGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGC
CGACGATCTGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTGGACAATGGGGC
CAAGCCTGATCMAGCCATGCCGCGTGCAGGATGAAGGCCTTCGGTTGTAAACTGCTTTTGTACGGAACGAAAAGACTCTGGTTAATACCTGGGGTCCATGACG
GTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTCCGAGGGCGG
TTATATAAGACAGATGTGAAATCCCGGGCTCAACCTGGGAACGCAATTTGTGACTGTATAGCTAGAGTACGGCAGAGGGGGATGGAATCCCGCTGTAGCAGT
G
```

Figure 3-5: One of Sequence of unidentified colony for Gram-positive bacteria. See detail in the appendix 1 to 4 to see the rest of the sequence with forward primers and reversed primers.

Acidovorax delafieldii strain 133 16S ribosomal RNA gene, partial sequence
 Sequence ID: [NR_028714.1](#) Length: 1515 Number of Matches: 1

Range 1: 54 to 678 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1134 bits(614)	0.0	621/625(99%)	0/625(0%)	Plus/Plus

Figure 3-6: Identification of *Acidovorax delafieldii* after sequencing. The first hit showing 99% identity after blasting the nucleotides sequence with BLASTn program against 16S ribosomal RNA Sequence (Bacteria and Archaea).

3.3 Effect of Plant Growth Promoting Bacteria

Bacteria CL8 was isolated from tomato, *Solanum lycopersicum*, cv. Heinz. Lillo laboratory (University of Stavanger), *Pseudomonas simiae* WCS417r obtained from Wintermans et al; 2016 and some bacteria from Belgium (Abbamondi et al. 2016) were used and these rhizosphere bacterial are represented as bacterial strains 5, 6, and endospheric bacterial represented as *Pseudomonas* sp. (number 9), 10, *Agrobacterium* sp. (number 15 and 18), *Rhizobium* sp. (number 16).

The effect of bacteria on plant growth and root system architecture of Arabidopsis (WT) and the mutants C2, C2C4, C2C5 and tomato plants (Gemini original) were investigated. To study the PGPR-mediated plant growth promotion, the seeds were sown on 1/50 Gamborg medium or 1× MS medium agar-solidified medium supplemented with 0.5% sucrose and placed in cold dark room for 2 days. The bacterial strains 5, 6, 9, 10, 15, 16, 18, cl8, and *Sphingobium limneticum* and *Acidovorax delafieldii* strains were inoculated into growth medium overnight and WCS417r was grown on King's medium B agar medium supplemented with 50 mg/ml rifampicin stock solution and the optical density was measured and Arabidopsis (WT) and the mutant seedlings growing vertically on 1/50 Gamborg medium, 1% Phyto agar or 1× MS medium (0.7% Agar-agar) to test the development of bacterial strains on seedlings of WT and the mutants. The bacterial suspensions of each strains (650 µl) of bacterial strain 5, 6, 9,10,15, 16, 18, cl8 and (400 µl) of WCS417r, *Sphingobium limneticum* and *Acidovorax delafieldii* strains were spread out lower of the agar plates and 650 µl or 400 µl of 10 mM MgSO₄ × 7H₂O were also spread out lower of agar plates as a control. After 6 days for experiment 2, 3, 4, expect 8 days for experiment 1, 5, 6, 7, 8, and 9 the primary root length, lateral root, the shoot fresh weight was measured and root hair was observed using electron microscope. The photo of seedling growing on control plates and plates containing bacterial was taken (see details in the appendix).

3.4 Observation made for Tomato Plants (Gemini original)

The tomato plants were planted into vermiculite containing pots, soaked with 5× superba nutrient solution. After two weeks, the plants were treated with 120 ml solution of WCS417r and *Sphingobium limneticum* bacterial per each pots and 120 ml of 10mM MgSO₄ × 7H₂O for the control placed separately in another tray.

BEFORE



Figure 3-7: showing the Tomato plants (Gemini original) before treatment with bacteria.

AFTER



Control

WCS417r

Sphingobium limneticum

Figure 3-8: showing the Tomato plants (Gemini original) with control (without bacteria) and separated pots with WCS417r and *Sphingobium limneticum* respectively. Number of the plants n= 7. Generally, the plants were stressed due to the lack of nutrients, the stems of tomato treated with *Sphingobium limneticum* are thicker and decreasing in primary shoot length compared with control.

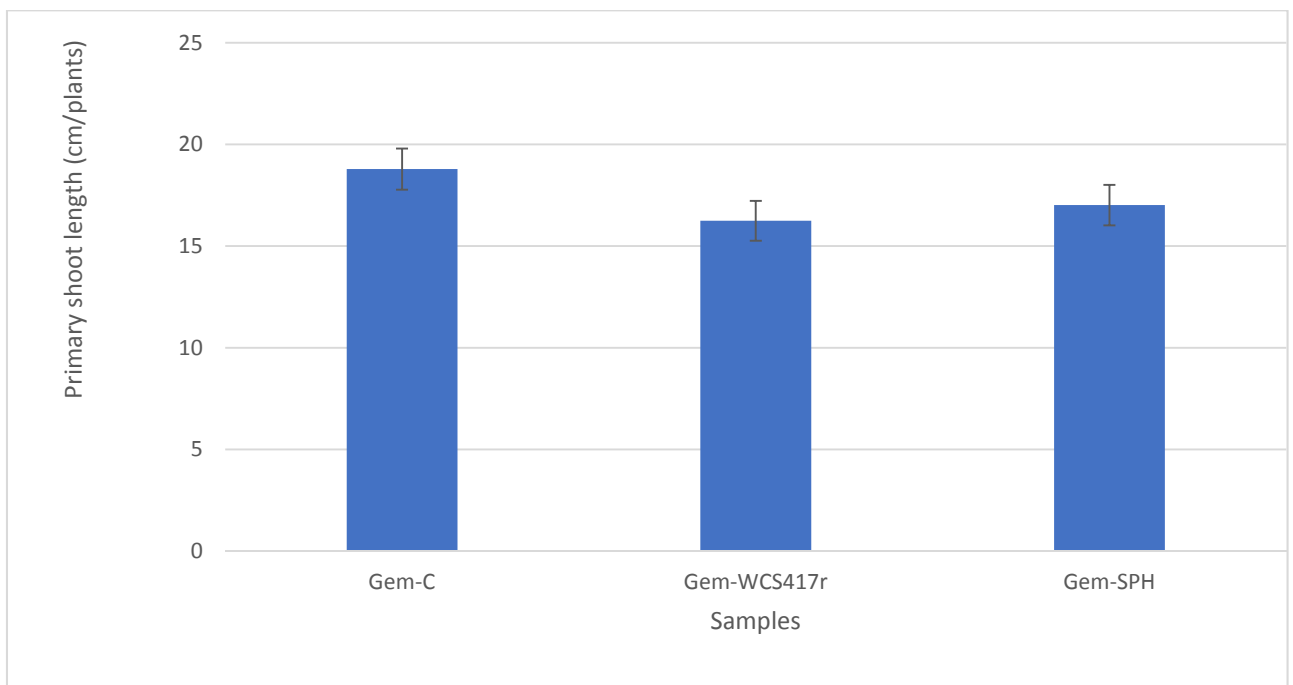


Figure 3-9: Primary shoot length of tomato plants (Gemini). The result showing the primary shoot length of tomato plants (Gemini original) with control (without bacteria) and bacterial strains WCS417r and *Sphingobium limneticum* after three weeks of treatment. n=7

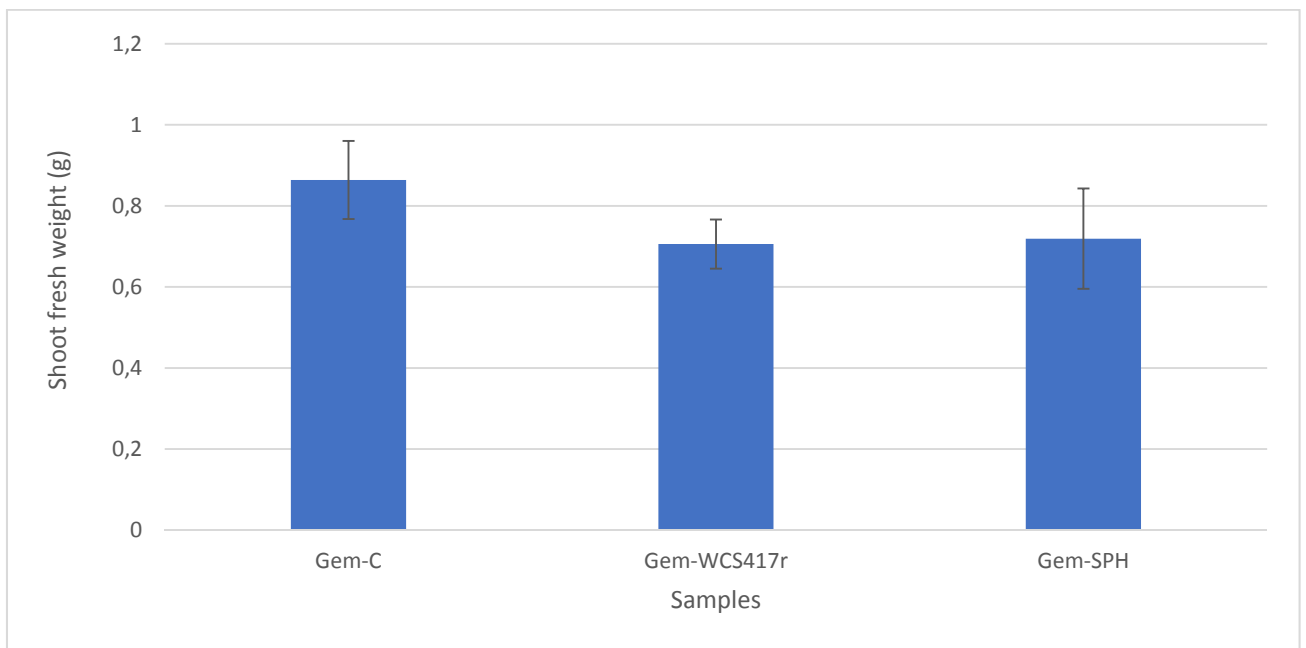


Figure 3-10: shoot fresh weight of tomato plants (Gemini). The result showing the shoot fresh weight of tomato plants (Gemini original) with control (without bacteria) and bacterial strains WCS417r and *Sphingobium limneticum* after three weeks of treatment. n=7

Note: Gem: Represent tomato plant Gemini original
 SPH: Represent *Sphingobium limneticum*
 WCS417r: Represent *Pseudomonas simiae* WCS417r bacterial

3.5 Measurement Parameters for Experiment 1, 2, 3, 4, and 5

3.5.1 Primary root length for WT

After the Arabidopsis WT seedlings, had been growing for 6 days on 1/50 Gamborg medium, 1% Phyto-agar (without sucrose) containing 650 μ l of bacterial strains and 650 μ l of MgSO₄ for the control. The image J. was used to measure the primary root length. Bacteria from (Abbamondi et al. 2016) and bacteria (CL8) from Lillo laboratory were used and some are rhizosphere and endospheric bacterial. The rhizosphere bacterial which are represented as bacterial strains 5, 6, and endospheric bacterial represented as *Pseudomonas sp.* (number 9), 10, *Agrobacterium sp.* (number 15), *Rhizobium sp.* (number 16), *Agrobacterium sp.* (number 18), and CL8 were used to test the effect of each bacterial strains on plants.

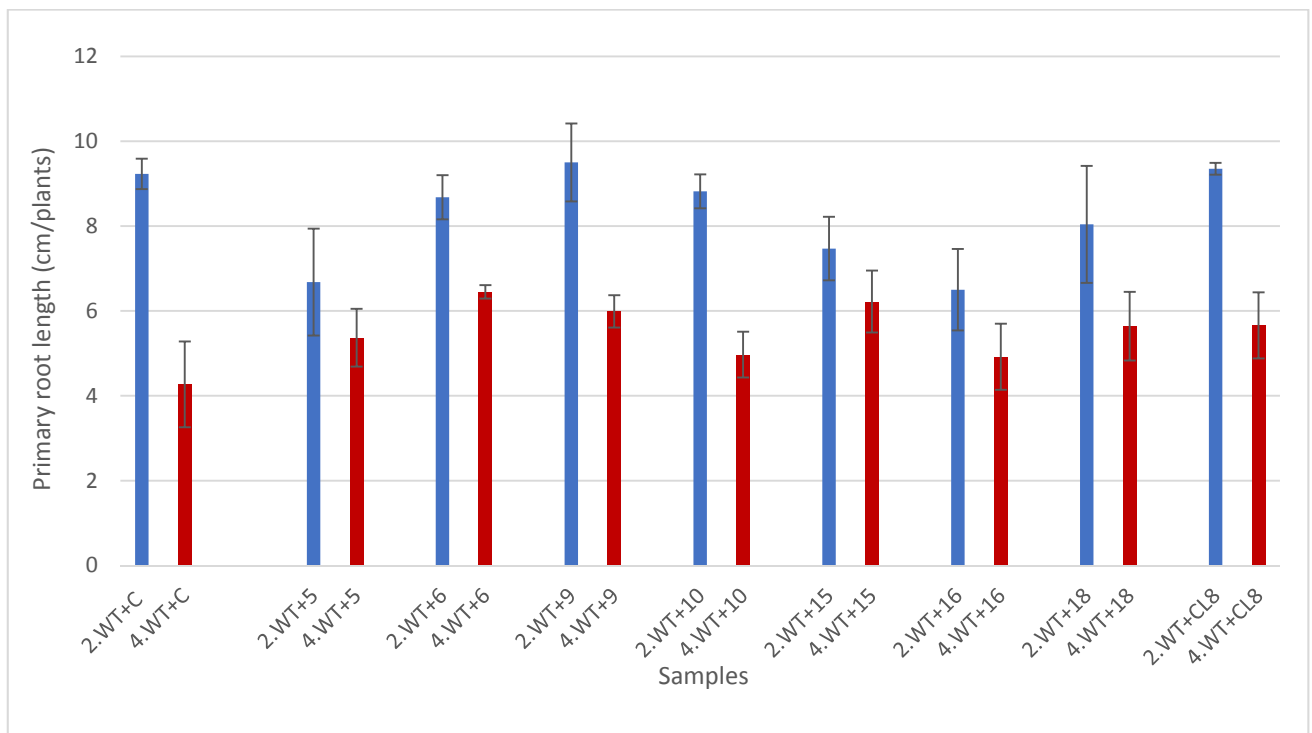


Figure 3-11: (Exp.2 and 4) Primary root length for WT. After six days, the primary root length (cm/plant) of wild type Arabidopsis (WT) with control without sucrose and with no bacterial and wild type with different bacterial strains were measured using image J. The average, standard deviation and standard error were calculated. The graph showing the primary root length (cm/plant) of wild type with control without sucrose and without bacterial. The bacterial strains 5, 6, 9, 10, 15, 16, 18 and CL8 were added to WT without sucrose. n=5

The first experiment (exp.2) indicating that some of the bacteria strains inhibit primary root growth but this was not affirmed in the second experiment (exp.4) (fig.3-11). In conclusion, the test did not demonstrate reproducible impacts of the bacterial strains on primary root length.

3.5.2 Lateral root length for WT

After the Arabidopsis WT seedlings, had been growing for 6 days on 1/50 Gamborg medium, 1% Phyto agar (without sucrose) containing 650 µl of bacterial strains and 650 µl of MgSO₄ for the control. The image J. was utilized to measure the lateral root length.

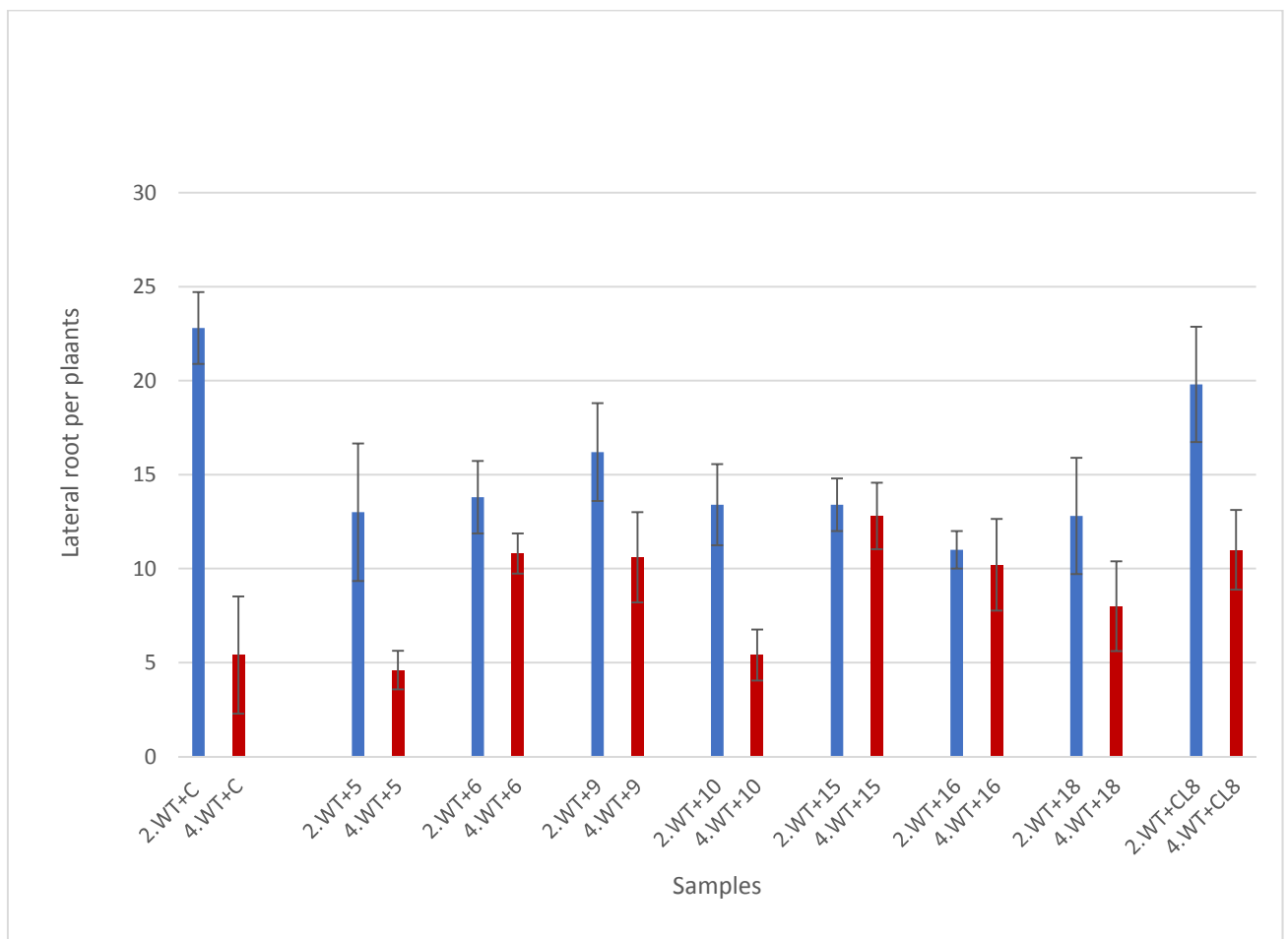


Figure 3-12: (Exp. 2 and 4) Lateral root length for WT. After six days, the lateral root per plants of wild type Arabidopsis with control without sucrose and with no bacterial and wild type with different bacterial strains were measured using image J. The lateral root was counted per plants, the average, standard deviation and standard error were calculated. The graph showing the lateral root /plants of wild type with control without sucrose and without bacterial. The bacterial strains 5, 6, 9, 10, 15, 16, 18 and CL8 were added to WT without sucrose. n=5

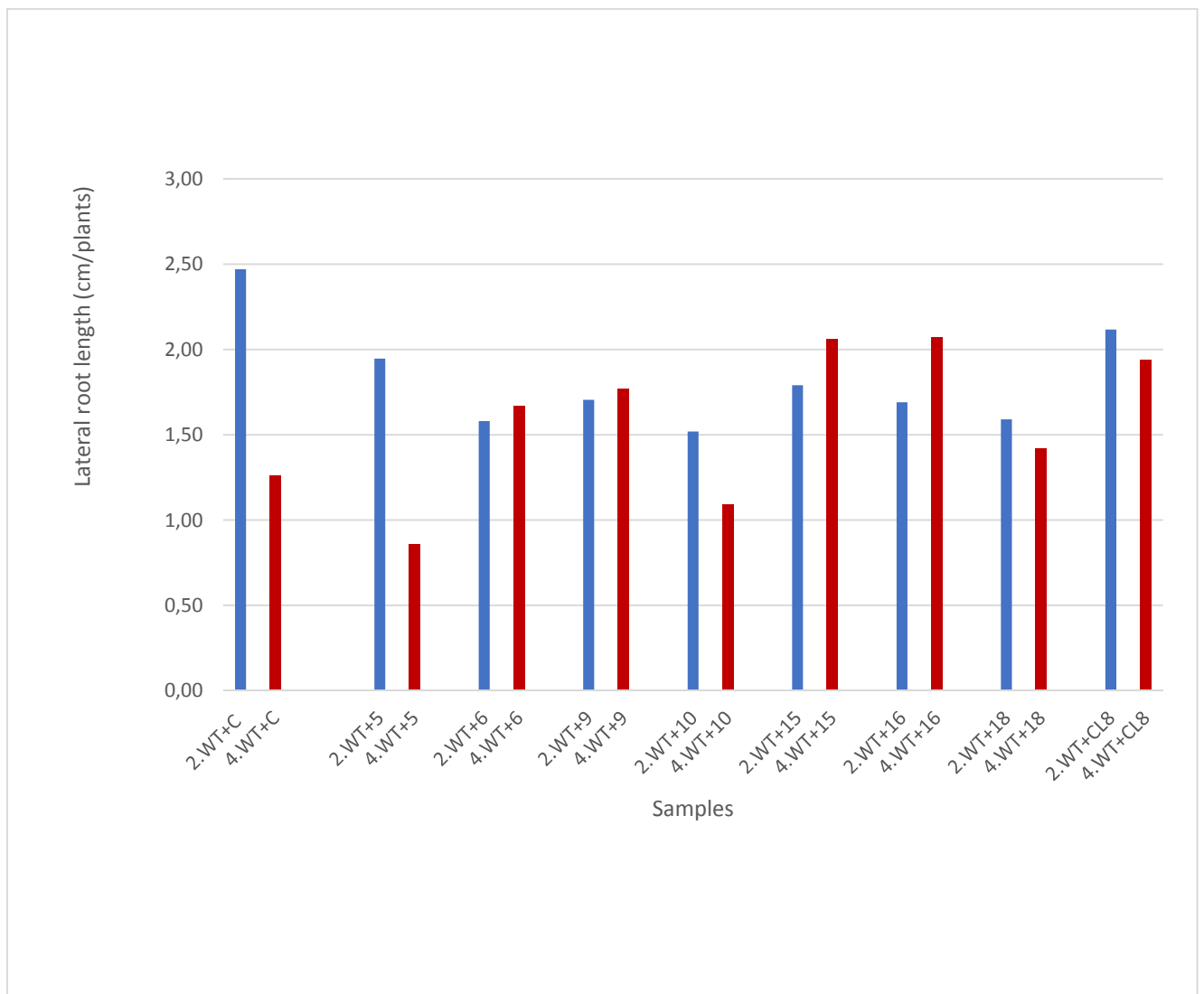


Figure 3-13. (Exp. 2 and 4) Lateral root length of WT in cm/plants. The experiment was repeated (fig. 3-8). The average of lateral root per cm were calculated by dividing the mean of lateral root with the average mean of the primary root length standard deviation and standard error were calculated. n=5

The first experiment (exp2) showing that some of the bacteria strains inhibit lateral root length and this was not confirmed in the second experiment (exp4) (fig.3-12 and 13). In conclusion, the test did not demonstrate reproducible impacts of the bacterial strains on lateral root.

3.5.3 Primary root length for the mutants

After the seedlings of mutants, had been growing for 6 or 8 days on 1/50 Gamborg medium, 1% Phyto agar (without sucrose) containing 650 μ l of bacterial strains and 650 μ l of MgSO₄ for the control. The image J. was used to measure the primary root length.

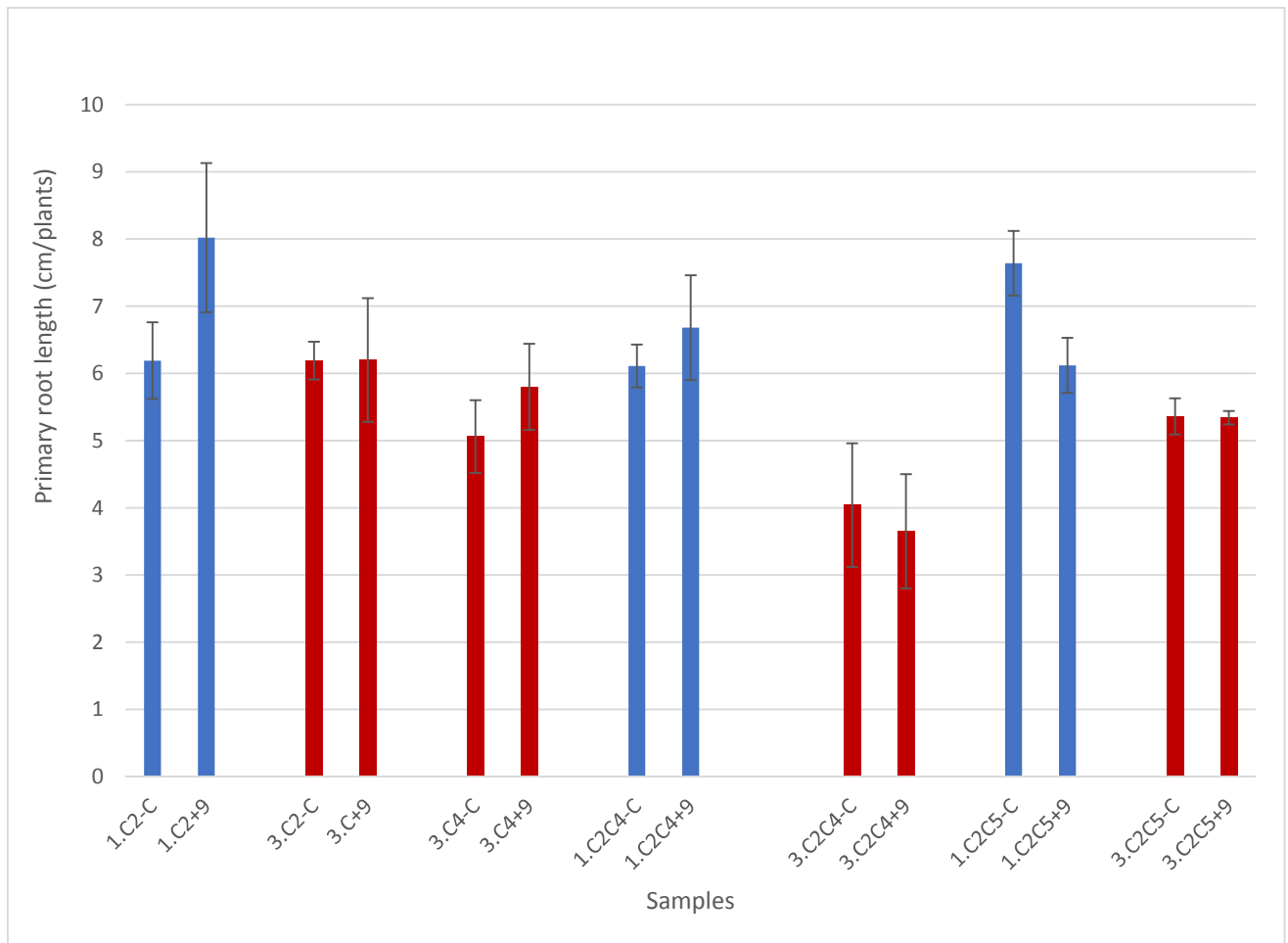


Figure 3-14: (Exp. 1, and 3) Primary root length cm/plant with bacteria strain 9. After 8 days for experiment 1, and 6 days for experiment 3, the primary root length (cm/plant) were measured using image J. The average, standard deviation and standard error were calculated. The graph showing the primary root length (cm/plants) of C2, C2C4, C2C4 and C2C5 with control without sucrose and without bacterial. The bacterial strain 9 was added to the C2, C4, C2C4, and C2C5 without sucrose. For experiment 1: n=6 and experiment 3: n=5

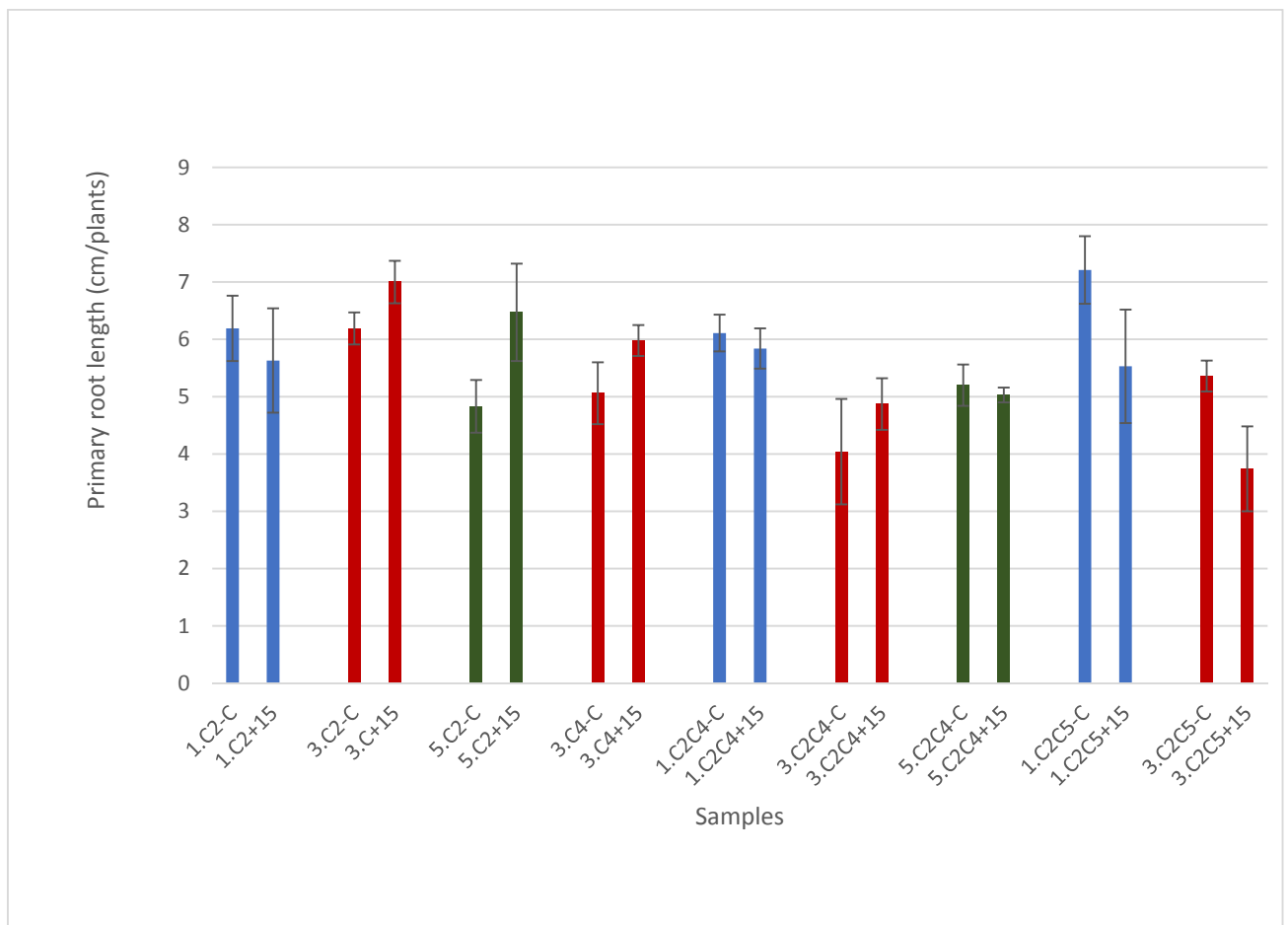


Figure 3-15: (Exp. 1, 3 and 5) Primary root length for the mutants with bacteria strain 15. The same procedure was repeated in fig 3-13 except the addition of bacteria strain 15. For experiment 1, 5: n=6 and experiment 3: n=5

It was observed there was increase in growth of primary root length of C2 (exp.1), C4 (exp.3), and C2C4 (exp.1) with bacterial strain 9 except the C2C5 (exp.3) and C2C4 (exp.1) that shows decrease in primary root length (fig.3-14). Also, there was increase in growth development of primary root length of C2 (exp.3 and 5), C4 (exp.3), and C2C4 (exp.3) with bacterial strain 15 aside from the C2, C2C4 (exp.1) C2C4 (exp.1, 5) and C2C5 (exp.1 and 3) that shows decrease in primary root length (fig. 15). Therefore, C2C5 (exp.5) with bacteria strain 15 and C2C5 (exp.1) with bacteria strain 9 inhibit primary root length in contrast with mutants.

3.5.4 Lateral root length for the mutants

After the seedlings of mutants, had been growing for 6 or 8 days on 1/50 Gamborg medium, 1% Phyto agar (without sucrose) containing 650 µl of bacterial strains and 650 µl of MgSO₄ for the control. The image J. was used to measure the lateral root.

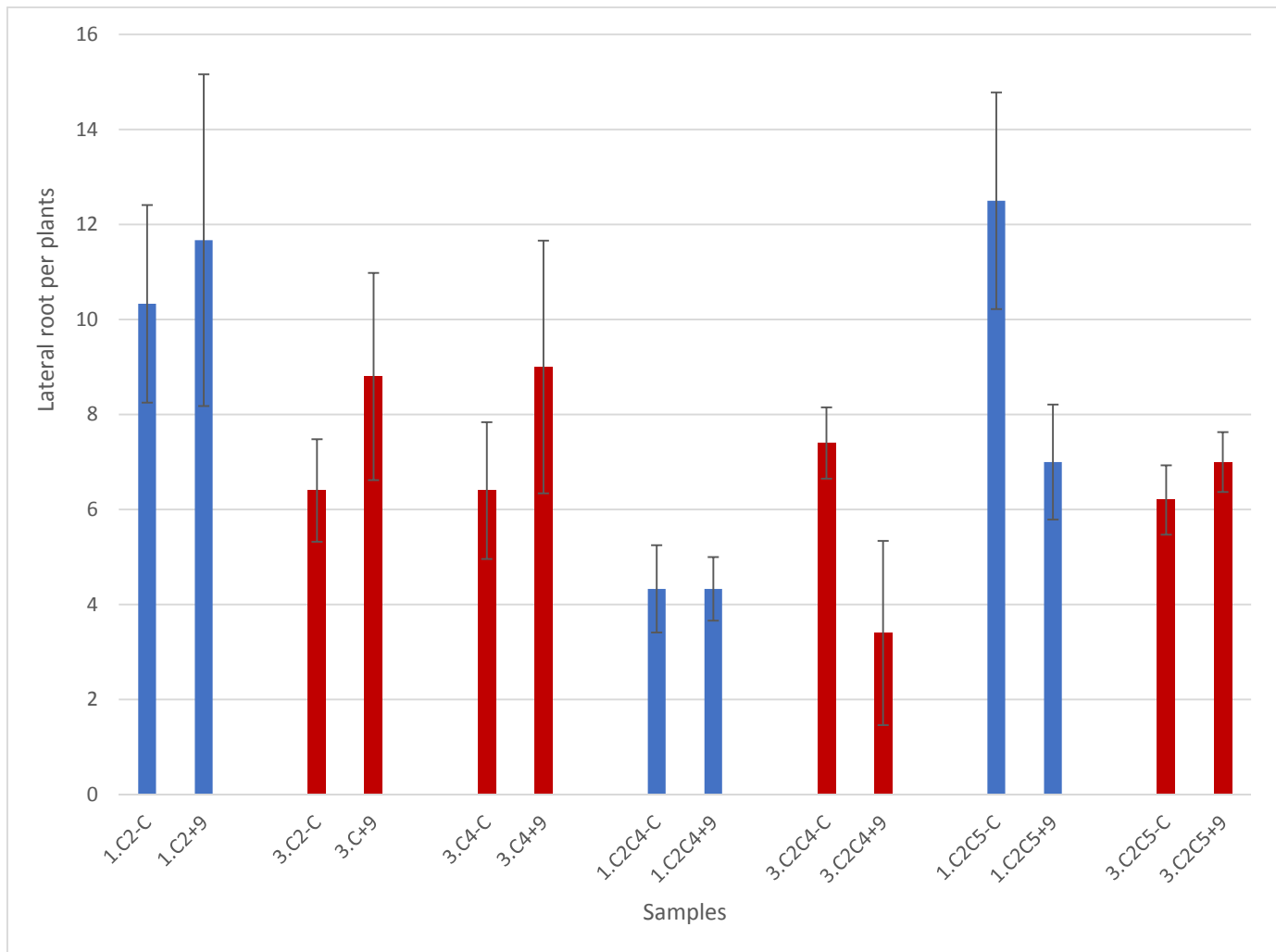


Figure 3-16: (Exp. 1 and 3) Lateral root length for the mutants with bacteria strain 9. After 8 days for experiment 1, and 6 days for experiment 3, the lateral root per plants were measured using image J. The average, standard deviation and standard error were calculated. The graph showing the lateral root/plants of C2, C4, C2C4 and C2C5 with control without sucrose and without bacterial. The bacterial strain 9 was added to C2, C4, C2C4 and C2C5 without sucrose. For experiment 1: n=6 and experiment 3: n=5

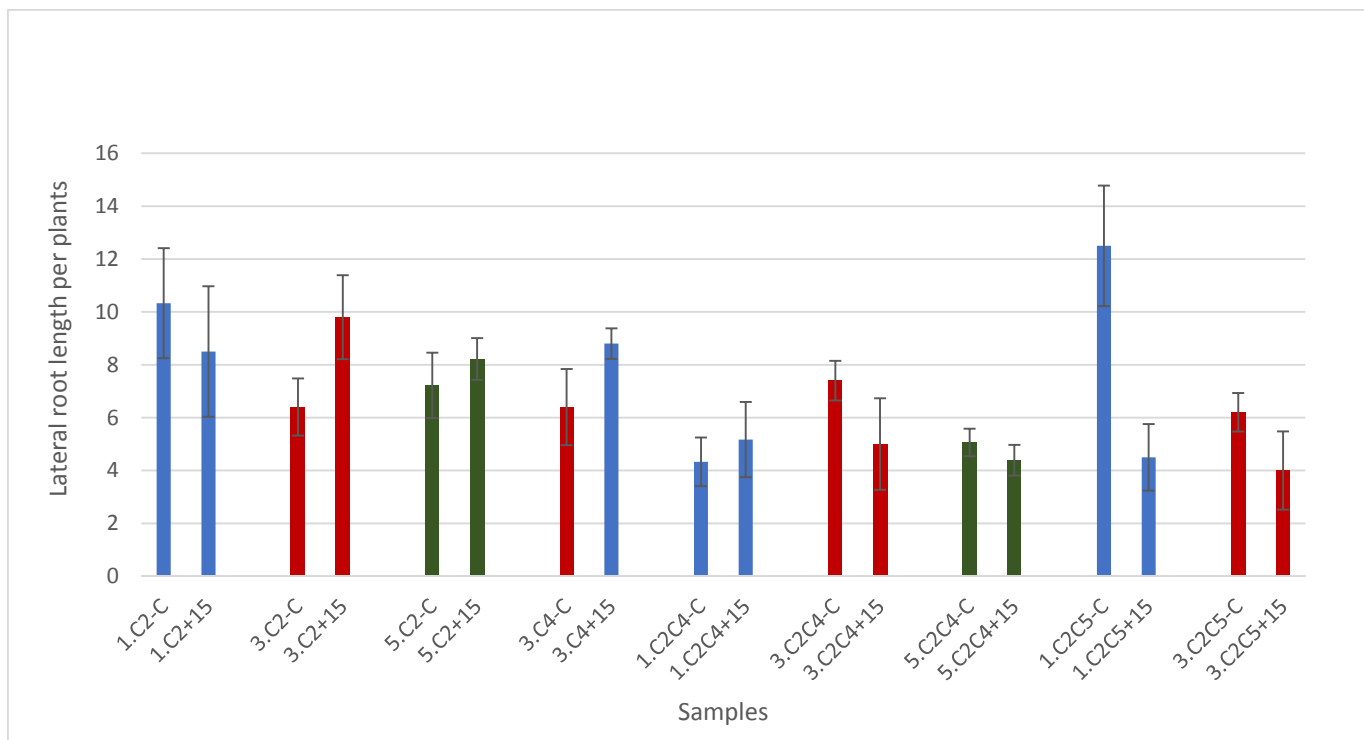


Figure 3-17: (Exp. 1, 3 and 5) Lateral root length for the mutants with bacteria strain 15. The same procedure was repeated in fig. 3-12 except addition of bacteria strain 15. For experiment 1, 5: n=6 and experiment 3: n=5

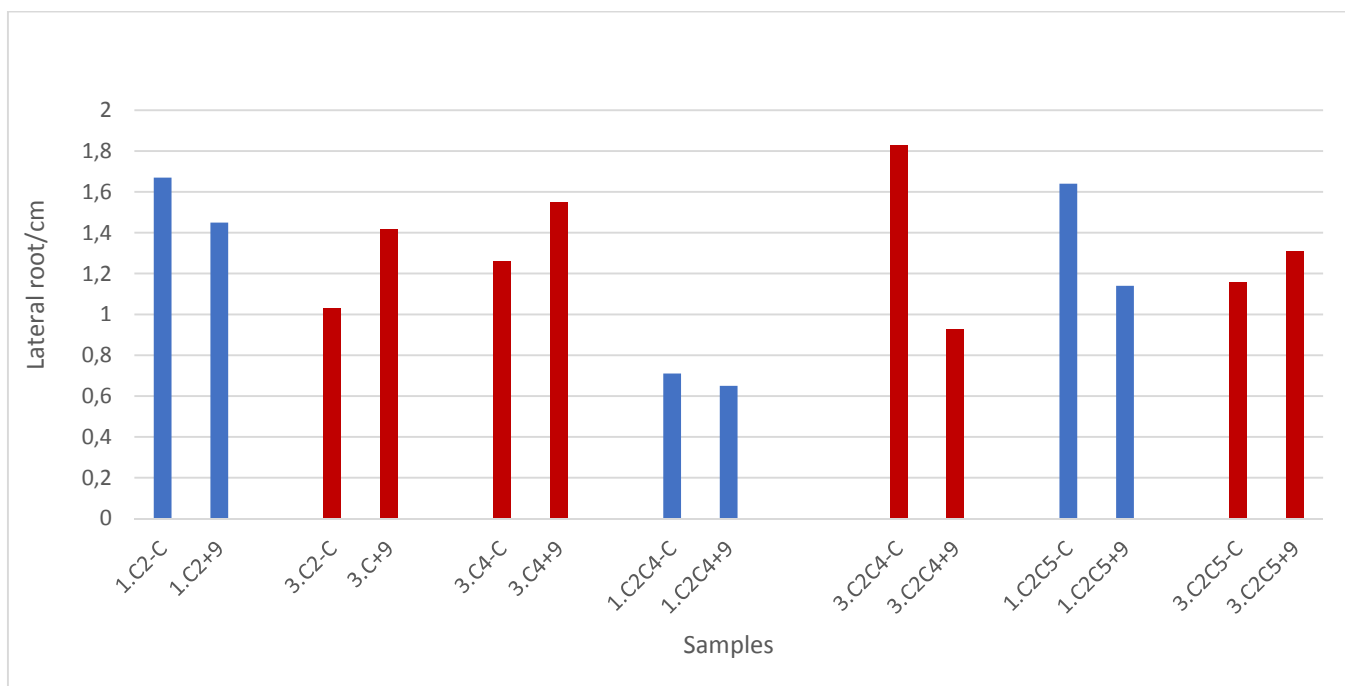


Figure 3-18: (Exp. 1, 3 and 5) Lateral root/cm for the mutants with bacteria strain 9. The average of lateral root per cm were calculated by dividing the mean of lateral root with the average mean of the primary root length. The graph showing the lateral roots/cm of C2, C4, C2C4 and C2C5 with control without sucrose and without bacterial. The bacterial strain 9 was added to C2, C4, C2C4 and C2C5 without sucrose. For experiment 1: n=6 and experiment 3: n=5

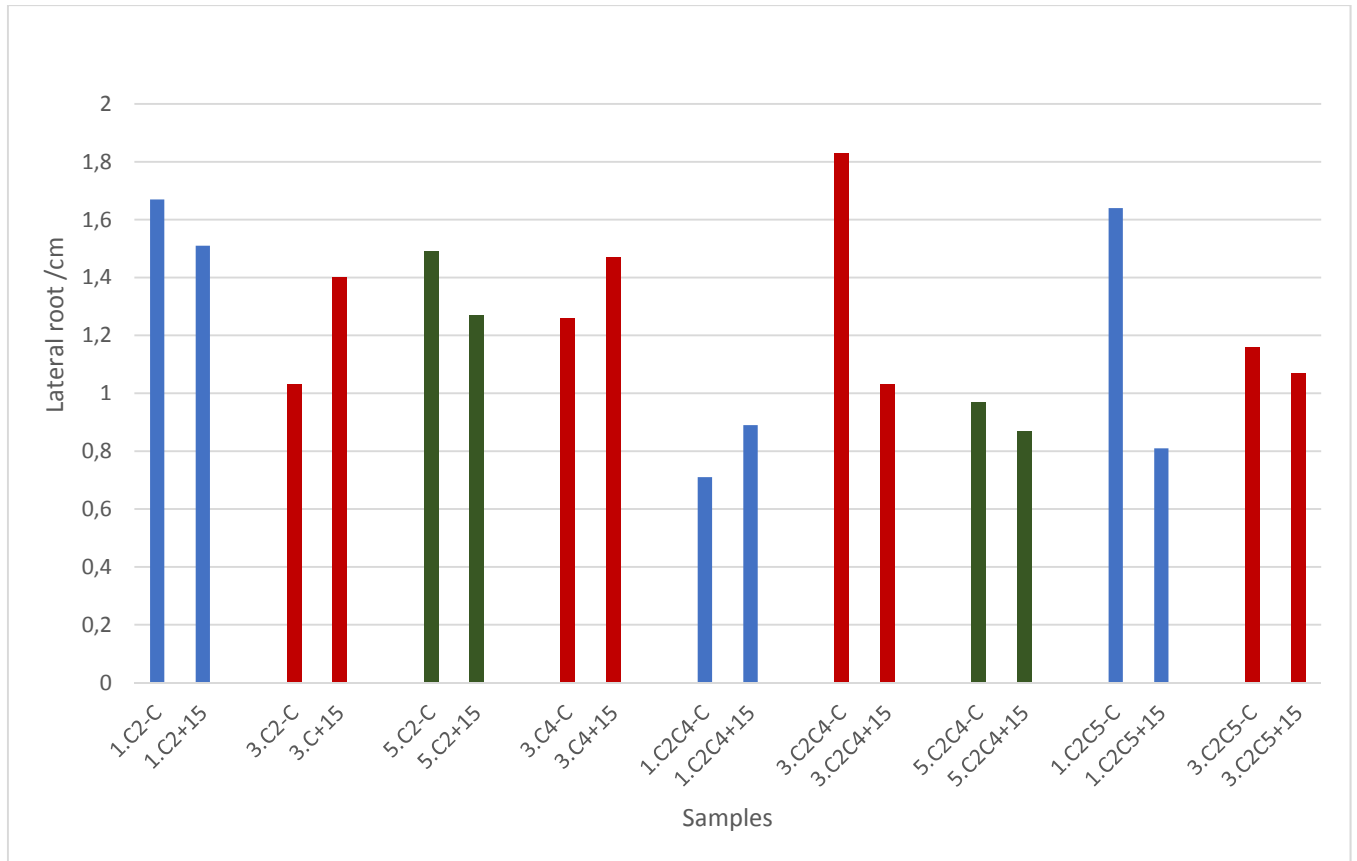


Figure 3-19: (Exp. 1, 3 and 5) Lateral root/cm for the mutants with bacteria strain 15. The same procedure was repeated in fig. 3-14. Except the addition of bacteria strain 15. For experiment 1, 5: n=6 and experiment 3: n=5

A diminishing pattern was observed in lateral root of C2C4, C2C5 (exp3), C2C5 (epx.1) with both bacteria strains 9 and 15 per plants and there was decrease in lateral root of C2, C2C4 (exp.1, 5) per plants. This was repeated after dividing the mean of the lateral root with the average mean of the primary root length. In conclusion, some mutants inhibit lateral root length. More specifically, the strains number 15.

3.6 Measurement Parameters for Experiment 6 and 7

3.6.1 Primary root length of WT, C2, C2C4 and C2C5

After the Arabidopsis (WT), C2, C2C4, and C2C5 mutant seedlings had been growing for 8 days on 1× MS medium without sucrose (exp.6) and (0.5 % sucrose (exp. 7) with WCS417r bacteria and 10 mM MgSO₄ × 7H₂O for the control. The image J. was used to measure the primary root length.

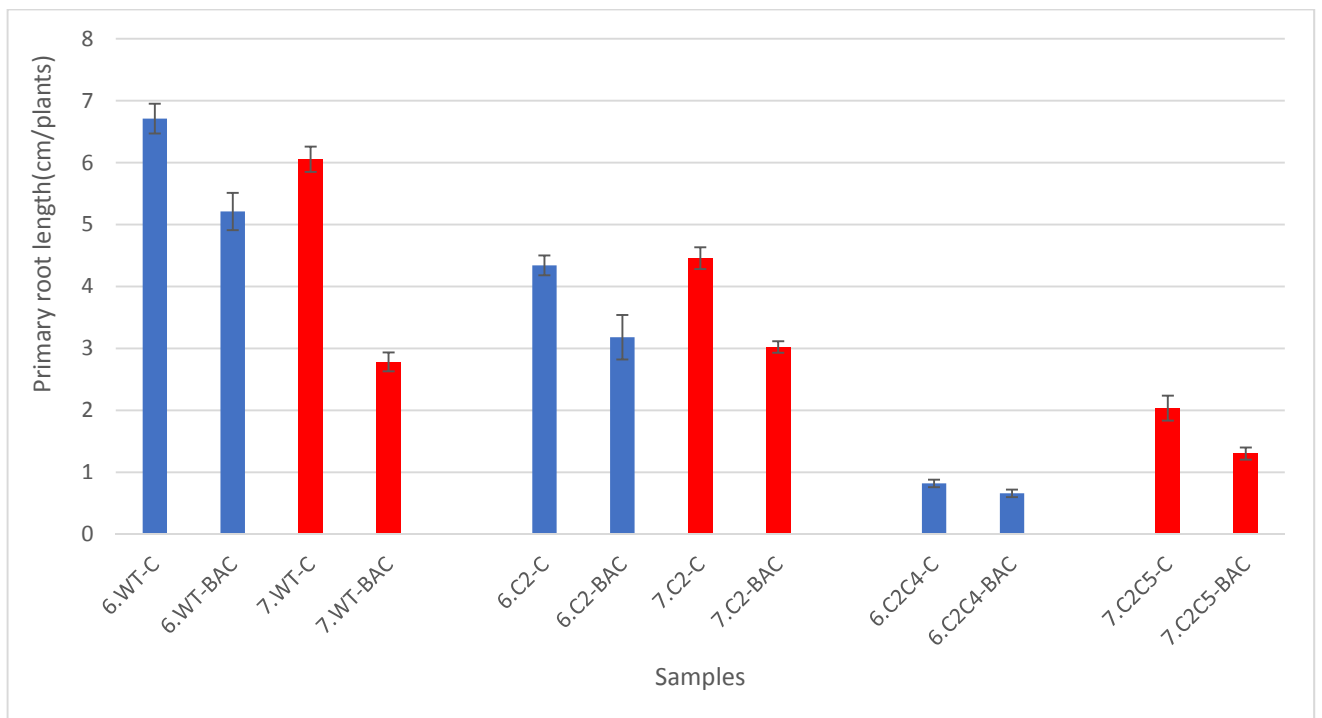


Figure 3-20: (Exp. 6 and 7) Primary root length of WT, C2 and C2C5 with WCS417r bacterial. After 8 days, the primary root length (cm/plants) of Wild Type Arabidopsis (WT), C2, C2C4, and C2C5 with control without sucrose and with no bacterial and WT, C2, C2C4 and C2C5 with WCS417r bacterial were determined by calculating the average, standard deviation and standard error. The graph showing the primary root length (cm/plant) of Arabidopsis (WT), C2, C2C4, C2C5 mutants with control and without sucrose for experiment 6, and 0.5% sucrose for experiment 7, and without bacterial. The WCS417r bacterial were added to WT, C2, C2C4, and C2C5 without sucrose (Exp. 6) and with sucrose 0.5% (Exp. 7). n=20

In experiment six, the Arabidopsis (WT) and C2, C2C4 mutants with WCS417r bacterial decrease in primary root length except WT-BAC4 that demonstrate increase in primary root length (see details in appendix table 33). This was repeated in experiment seven due to the sucrose supplement. Therefore, the effect of the WCS417r bacterial on WT and mutants inhibits the primary root elongation (fig. 3-20).

3.6.2 Lateral root length of WT, C2, C2C4, C2C5

The Arabidopsis (WT), C2, C2C4 and C2C5 seedlings had been growing for 8 days on 1× MS medium (without sucrose for exp. 6) and (0.5% sucrose for exp. 7) containing WCS417r bacterial and 10 mM MgSO₄ × 7H₂O for the control. The lateral roots were counted and recorded.

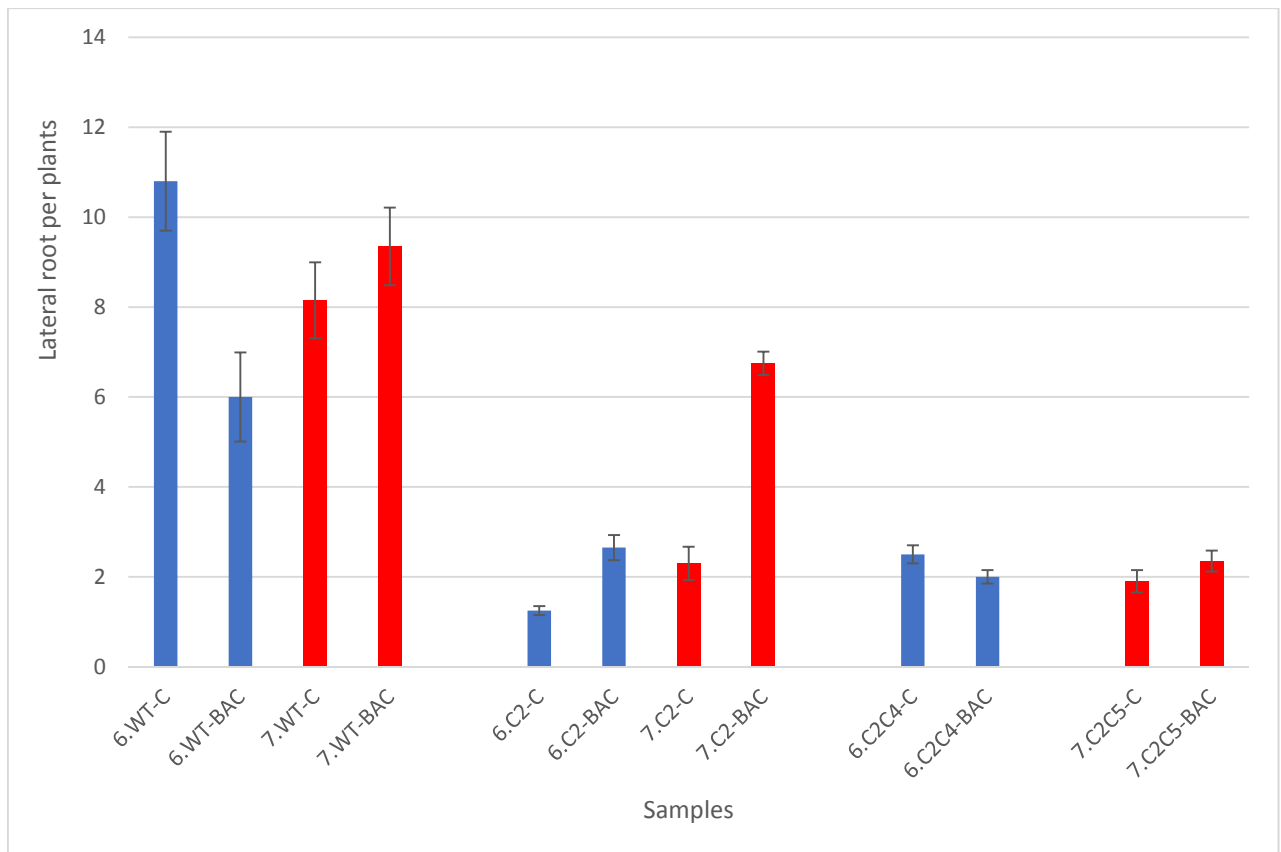


Figure 3-21: (Exp. 6 and 7) Lateral per plants of WT, C2, C2C4 and C2C5 with WCS417r bacterial. After 8 days, the lateral root of Arabidopsis (WT), C2, C2C4, and C2C5 with control without sucrose and with no bacterial and WT, C2, C2C4 and C2C5 with WCS417r bacterial were determined by counting the number of lateral root per plants and calculating the average, standard deviation and standard error. The graph showing the lateral root /plants of Arabidopsis (WT), C2, C2C4 and C2C5 with control without sucrose (Exp. 6) and 0.5% sucrose (Exp. 7) and without bacterial. The WCS417r bacterial were added to WT, C2, and C2C4 and C2C5 without sucrose (Exp. 6) and 0.5% sucrose (Exp. 7). Number of seedlings n=20

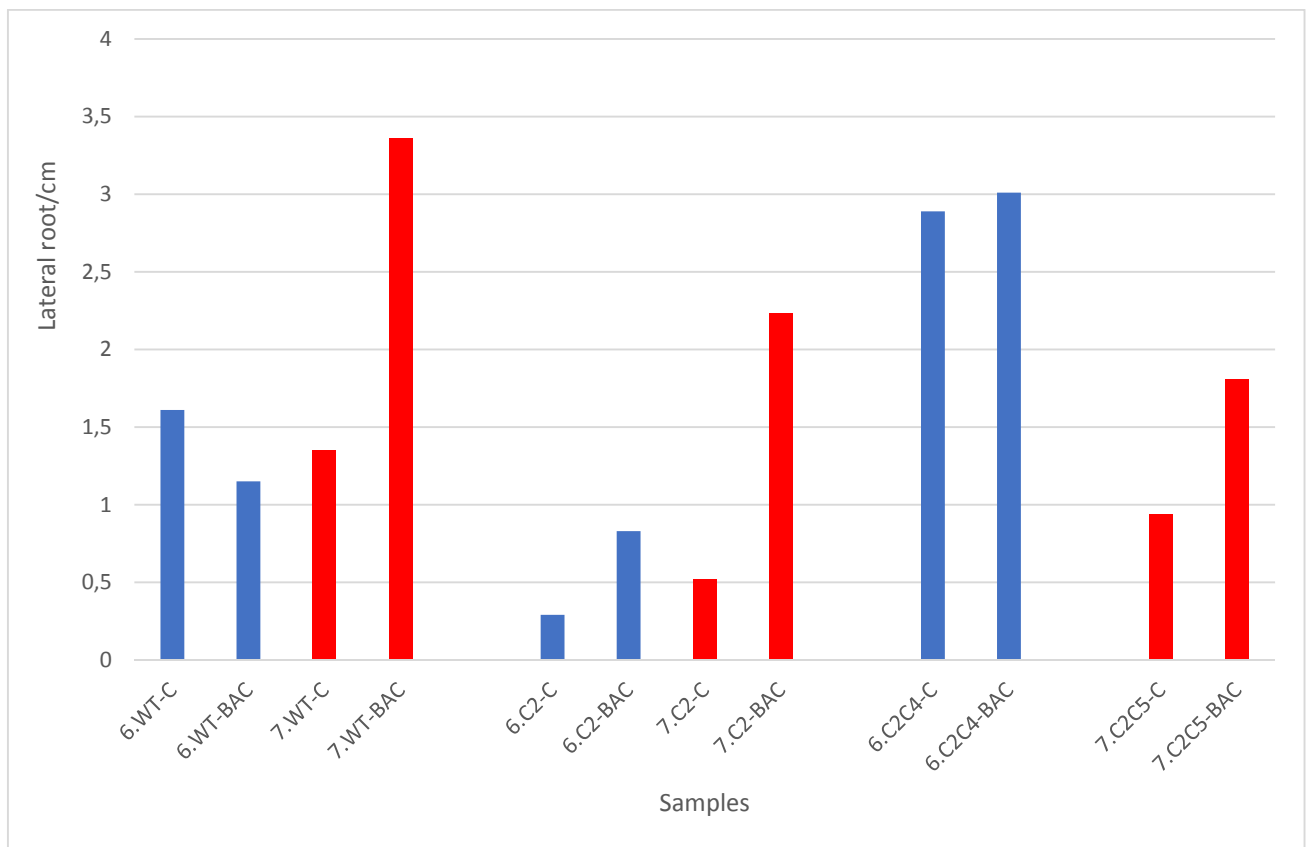


Figure 3-22: (Exp. 6 and 7) Lateral root/cm of WT, C2, C2C4, and C2C5 with WCS417r bacterial. The average of lateral root per cm were calculated by dividing the mean of lateral root with the average mean of the primary root length.

The Arabidopsis (WT) and C2C4 with WCS417r bacterial decrease in lateral root per plants except C2 mutant that demonstrate increase in lateral root compare with the control in experiment 6 (see detail in appendix table 39 and 41). There was increase in lateral root of WT and the mutants in experiment 7 because of addition of sucrose, which is contrary in experiment 6 without sucrose aside from C2 mutants that shows increase in lateral root (exp. 6). In conclusion, a trend of lateral root increase was observed with C2 mutant both in experiment 6 and 7 (see details in table 21). The WT inhibit primary root length and promote the growth development of plants with the bacterial without sucrose in experiment 6.

3.6.3 Shoot Fresh Weight

The Arabidopsis (WT) and C2, C2C4 and C2C5 seedlings was grown for 8 days on 1× MS without sucrose (exp.6) and 0.5% sucrose (exp.7) containing WCS417r bacterial and 10 mM MgSO₄ × 7H₂O for the control. The weighing scale was used to measure the shoot fresh weight.

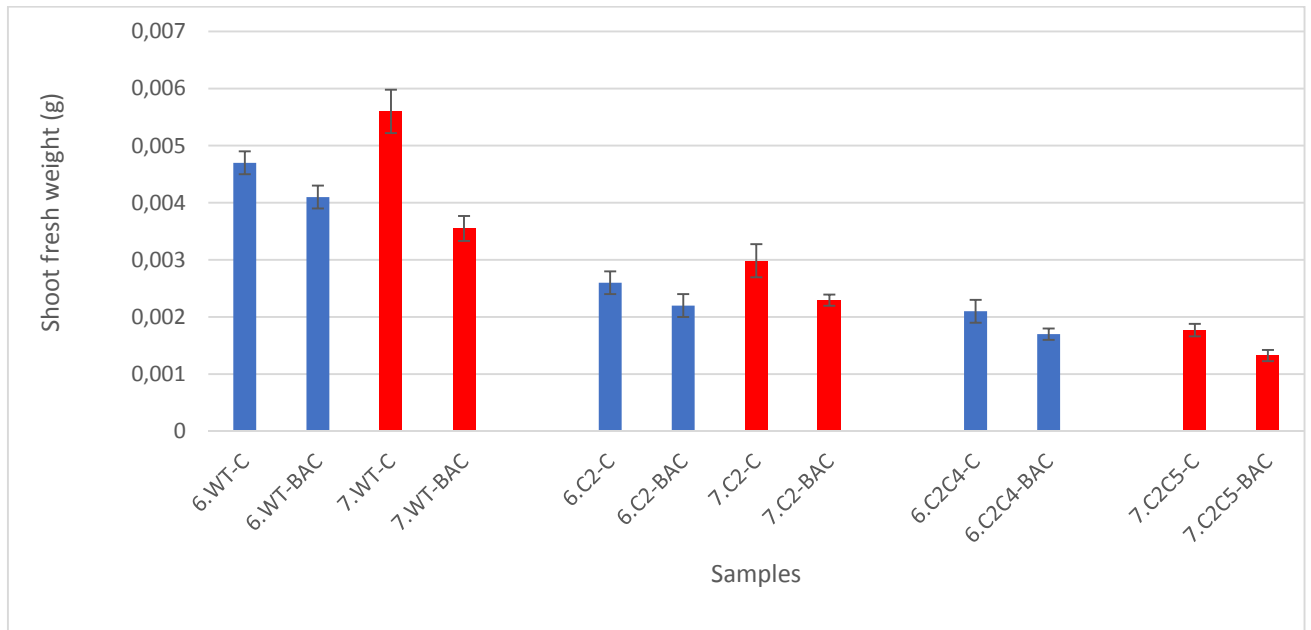


Figure 3-23: (Exp. 6 and 7) Shoot Fresh Weight of WT, C2, C2C4, and C2C5 with WCS417r bacterial. After 8 days, the shoot fresh weight of Arabidopsis (WT), C2, C2C4, and C2C5 with control without sucrose and with no bacterial and WT, C2, C2C4 and C2C5 with WCS417r bacterial were determined. The graph showing the shoot fresh weight (g) of WT, C2, C2C4 and C2C5 with control without sucrose (Exp. 6) and 0.5% sucrose (Exp. 7) without bacterial. The WCS417r bacterial were added to WT, C2, and C2C4 without sucrose (Exp. 6) and 0.5% sucrose (Exp. 7). Number of seedlings n=20

Upon exposure of WT, C2, C2C4 and C2C5 to WCS417r bacterial showed decrease in shoot fresh weight and increase with control (fig. 3-23) both the experiment 6 & 7. Therefore, the weight of the shoot reduces with bacteria.

From the photographs (see appendix for details table 51, 52, 53, 54, 55, & 56), the Arabidopsis (WT) and the mutants demonstrate the presence of anthocyanin properties identified by the colour and the mutant plants were stressed compare with WT. However, there is increase in number of lateral root of WT and they were longer while C2 mutants has less lateral roots.

Note: It was observed C2C4 and C2C5 demonstrate a poor growth on 1× MS medium compare with the 1/50 Gamborg medium.

3.7 Measurement Parameters for Experiment 8, 9 and 10

3.7.1 Primary root length of WT, C2, and C2C4

The Arabidopsis (WT), C2, and C2C4 mutant seedlings had been growing for 8 days on 1 × MS medium with 0.5% sucrose (exp.8 and 9) and without sucrose (exp. 10) with *Sphingobium limneticum* and *Acidovorax delafieldii* strains and 10 mM MgSO₄ × 7H₂O for the control. The image J. was used to measure the primary root length.

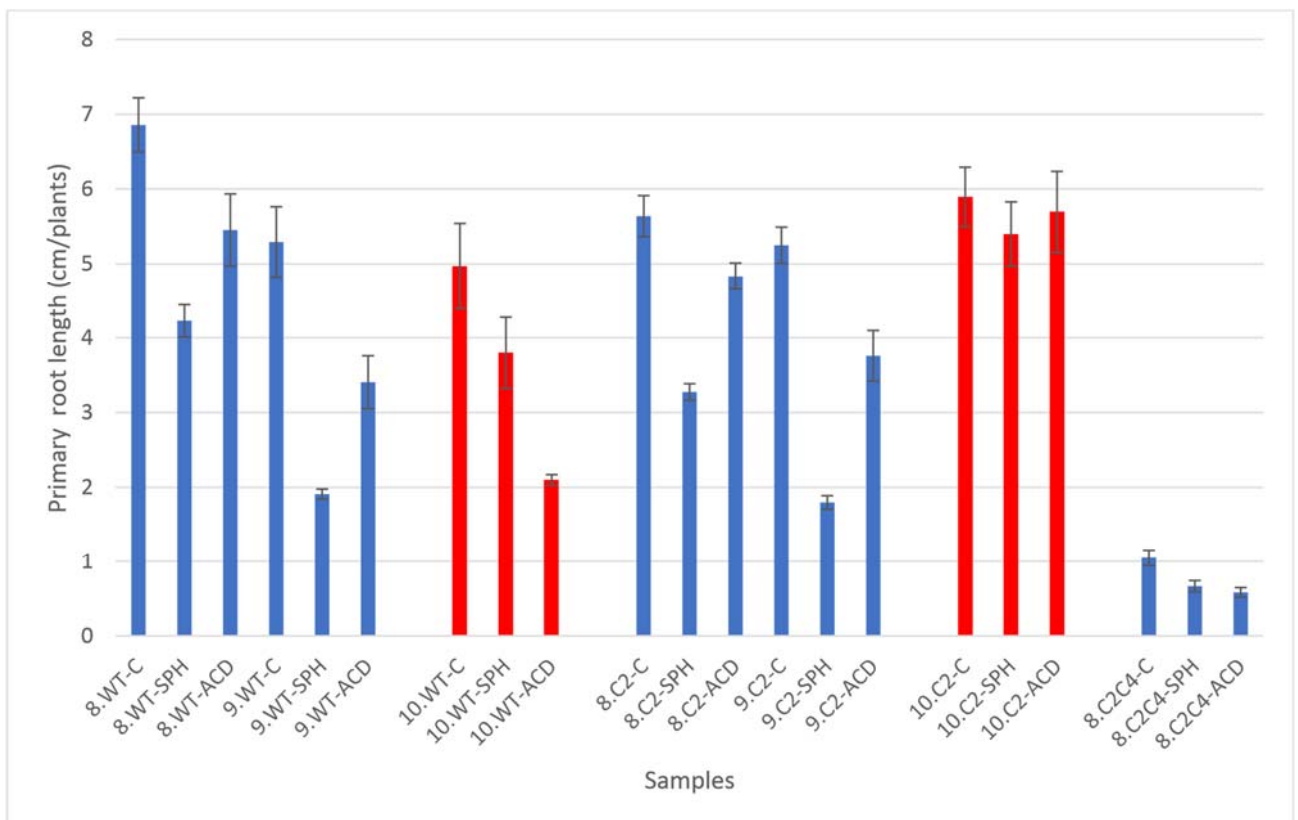


Figure 3-24: (Exp. 8, 9 and 10) Primary root length of WT, C2 and C2C4 with *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial. After 8 days, the primary root length (cm/plants) of Arabidopsis (WT), C2, and C2C4, with control 0.5% sucrose for experiment 8, 9 and without sucrose in experiment 10 and with no bacterial and WT, C2, and C2C4 with *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial were determined by calculating the average, standard deviation and standard error. The graph showing the primary root length (cm/plant) of Arabidopsis (WT), C2, and C2C4, mutants with control with 0.5 % sucrose for experiment 8 and 9 in blue colour, and without sucrose for experiment 10 in red colour, and without bacterial. The *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial were added to WT, C2, and C2C4 with 0.5% sucrose (Exp. 8 and 9 blue colour) and without sucrose (Exp. 10 red colour). n=15

In experiment eight and nine, the Arabidopsis (WT) and C2, C2C4 mutants with *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial decrease the primary root length except. This was repeated in experiment ten without sucrose and this is represented in red colour in the graph. Therefore, the effect of the *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial on WT and mutants inhibits the primary root elongation (fig. 3-24).

3.7.2 Lateral root of WT, C2, and C2C4

The Arabidopsis (WT), C2, and C2C4 seedlings had been growing for 8 days on 1× MS medium (with 0.5% sucrose for exp.8 and 9) and (without sucrose for exp.10) containing *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial and 10 mM MgSO₄ × 7H₂O for the control. The lateral root was counted and recorded.

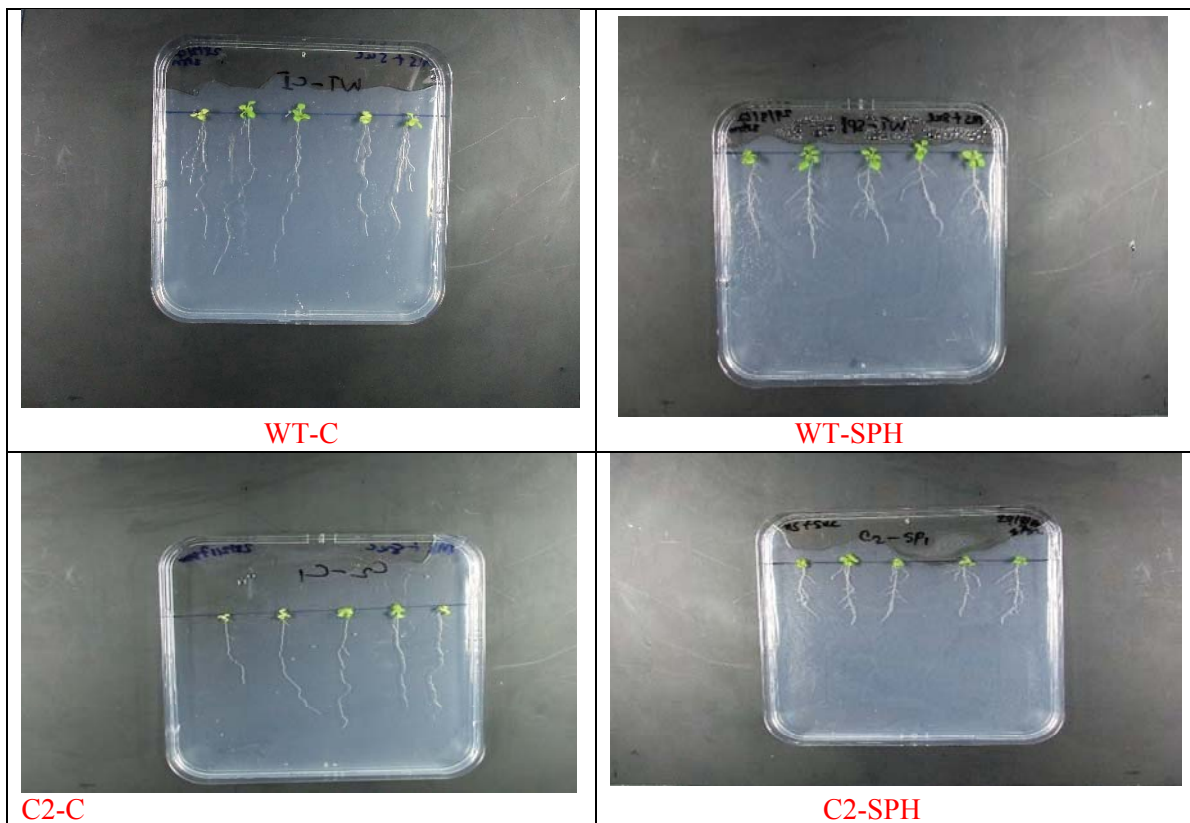


Figure 3-25: Responsiveness of Arabidopsis (WT) and C2 mutants to the plant growth-promoting effect with *Sphingobium limneticum*. Shown are photographs of 1× MS agar plates with WT and C2 mutant with 400 µl of 10 mM MgSO₄ × 7H₂O spread over the plates as control (WT-C) and WT and C2 with 400 µl *Sphingobium limneticum* bacterial suspension (2 × 10⁶ cfu mL) spread all over the plates. (See details in appendix number 11 table 83-94).

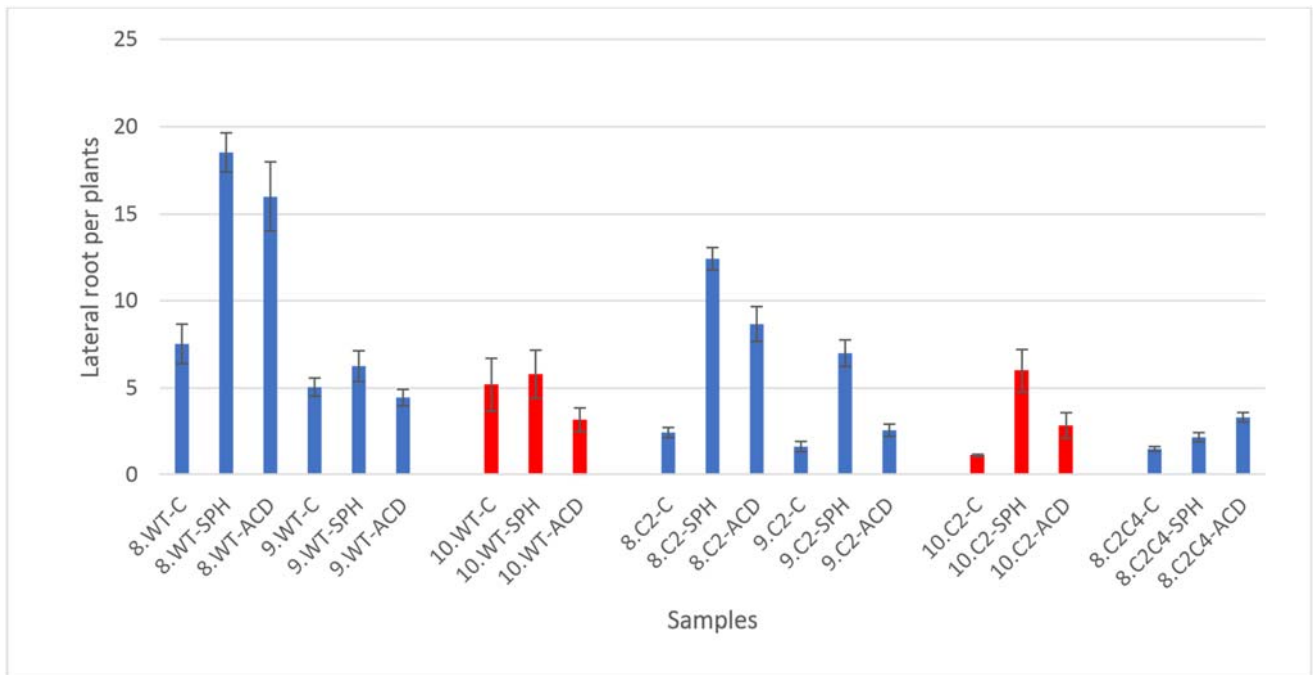


Figure 3-26: (Exp. 8, 9 and 10) Lateral root of WT, C2 and C2C4 with *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial. After 8 days, the lateral root /plants of Arabidopsis (WT), C2, and C2C4, with control 0.5% sucrose for experiment 8, 9 and without sucrose in experiment 10 and with no bacterial and WT, C2, and C2C4 with *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial were determined by counting the lateral root per plants and calculating the average, standard deviation and standard error. The graph showing the lateral root per plants of Arabidopsis (WT), C2, and C2C4, mutants with control with 0.5 % sucrose for experiment 8 and 9 in blue colour, and without sucrose for experiment 10 in red colour, and without bacterial. The *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial were added to WT, C2, and C2C4 with 0.5% sucrose (Exp. 8 and 9 blue colour) and without sucrose (Exp. 10 red colour). n=15

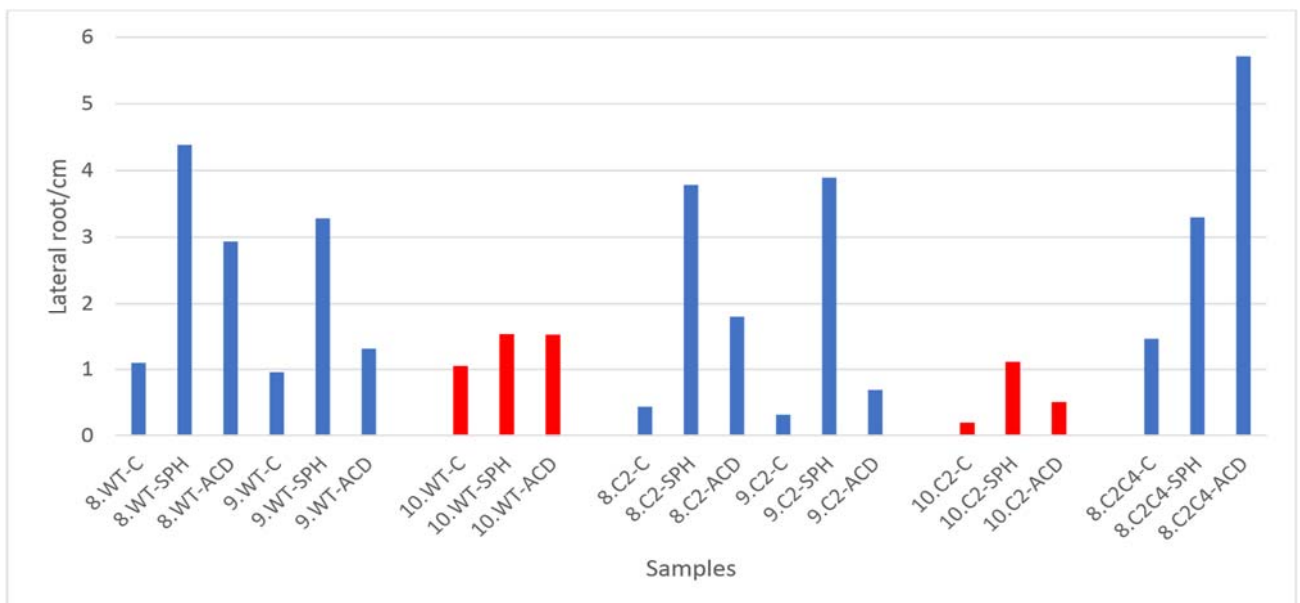


Figure 3-27: (Exp. 8, 9 and 10) Lateral root/cm of WT, C2, and C2C4, with *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial. The average of lateral root per cm were calculated by dividing the mean of lateral root with the average mean of the primary root length.

The Arabidopsis (WT), C2 and C2C4 with *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial increase lateral root compare with the control (fig. 3-26). There were 63 and 49 additional lateral roots for five plants of WT and C2 mutant (see details in fig. 3-25) compare with control. In conclusion, there is a significant growth of lateral roots with WT and the mutants with the bacterial and this promote the plant growth development.

3.7.3 Shoot Fresh Weight

The Arabidopsis (WT) and C2, and C2C4 seedlings was grown for 8 days on 1× MS with 0.5% sucrose (exp.8 and 9) and without sucrose (exp.10) containing *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial and 10 mM MgSO₄ × 7H₂O for the control. The weighing scale was used to measure the shoot fresh weight.

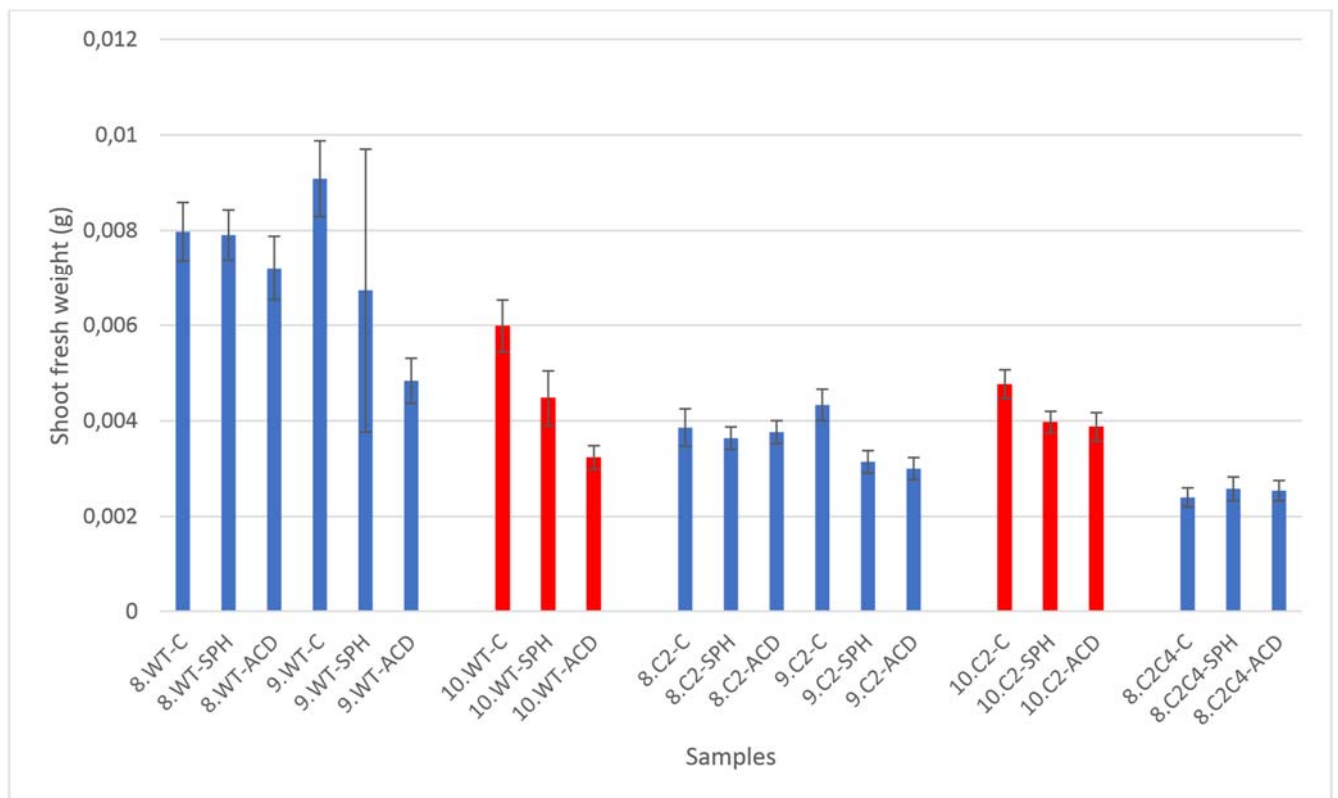


Figure 3-28: (Exp. 8, 9 and 10) Shoot fresh weight (g) of WT, C2 and C2C4 with *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial. After 8 days, the shoot fresh weight of Arabidopsis (WT), C2, and C2C4, with control 0.5% sucrose for experiment 8, 9 and without sucrose in experiment 10 and with no bacterial and WT, C2, and C2C4 with *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial were determined by weighing the shoot per plants and calculating the average, standard deviation and standard error. The graph showing the shoot fresh weight (g) of Arabidopsis (WT), C2, and C2C4, mutants with control with 0.5 % sucrose for experiment 8 and 9 in blue colour, and without sucrose for experiment 10 in red colour, and without bacterial. The *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial were added to WT, C2, and C2C4 with 0.5% sucrose (Exp. 8 and 9 blue colour) and without su-crose (Exp. 10 red colour). n=15

Upon exposure of WT, C2, and C2C4 to *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial decreases in shoot fresh weight compare with control (fig. 3-28) in experiment 8, 9, & 10. Therefore, the weight of the shoot reduces with bacteria.

4. DISCUSSION

This study was designed to use PGPR with the aim to improve plant health and crop productivity based on microbial inoculation. Bacteria CL8 was isolated from tomato, *Solanum lycopersicum*, cv. Heinz, and also, *Sphingobium limneticum* and *Acidovorax delafieldii* were isolated from *Solanum pennellii* in Lillo laboratory (University of Stavanger), and *Pseudomonas simiae* WCS417r obtained from Wintermans et al; 2016. Also, some bacteria were obtained from Belgium (Abbamondi et al. 2016). They were named as bacterial strains 5, 6 (rhizosphere) and endospheric bacterial represented as *Pseudomonas* sp. (number 9), 10, *Agrobacterium* sp. (number 15 and 18), *Rhizobium* sp. (number 16).

In this study, we made use of *Arabidopsis* (WT) and mutants (C2 C2C4 and C2C5) on 1/50 Gamborg and 1 × MS to investigate the influence of endospheric and rhizosphere bacterial from Abbamondi et al. 2016 on plant-growth and to gain insight on how *Arabidopsis* (WT) and the mutants react in exposure to *Pseudomonas simiae* WCS417r, *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial. The growth promotion parameters of shoot fresh weight, primary root length and lateral root formation in response to the bacteria treatment were used to examine the plants.

4.1 Inoculation of bacterial strains (*Pseudomonas* sp., *Agrobacterium* sp., *Rhizobium* sp.) obtained from (Abbamondi et al. 2016) on *Arabidopsis* WT and mutants

It was observed for the growth of *Arabidopsis thaliana* WT with primary root length in experiment two was not the same compare with the experiment four. In experiment two, WT with bacterial strains 5, 9, 15, 16 decreases in primary root length compare with control, and in experiment four, which shows a different result in which bacteria strains 6, 9, 15, 18, CL8 increases in primary root length compare with control (fig. 3-11). The plants in the media with bacterial strains 6, 10, CL8 and control were longer in primary root length compared with other bacterial strains 5 and 16 (fig. 3-11). In experiment one, C2C5 with bacterial strain 15 showing decrease in primary root length and this was repeated in experiment three (fig. 3-15) but in the case of C2C5, there was decrease in growth of the plants with bacterial strain 9. But C2C5 with bacterial strains 9 in experiment three shows no significant result with control (fig. 3-14).

The WT decreased in number of lateral root in experiment two with bacteria strains 5, 6, 9, 10, 15, 16, 18 and CL8 while in experiment four, promotes the plant growth by increasing the lateral root with help of bacterial strains 6, 9, 15, 16, 18, and CL8 (fig. 3-12) and it was repeated for C2, and C4 with bacterial strains 9 and 15 in experiment three (fig. 3-16). However, the numbers of inoculated plants planted in the experiment one was six and experiment three was five and this can bring about a considerable measure of progress in the information.

Therefore, the effect of the bacteria is not strong enough to display the growth and inhibitory effect of each plants. It has been discovered that the bacterial strains 9, increases the lateral root formation while bacterial strains 6, 15, 16, and 18 decreases lateral root. Endophytes is a source of bioactive compounds that can positively influence plant growth through a numerous mechanisms. Moreover, bacterial endophytes are competing with phytopathogens because they

colonize the ecological niches; therefore, they frequently create abilities that protect plants from infections (biocontrol agents) (Abbamondi et al. 2016).

4.2 Inoculation of *Pseudomonas simiae* WCS417r (from Wintermans et al; 2016), and *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial isolated from *Solanum pennellii* on Arabidopsis WT, mutants and Gemini tomato

The second part of this study, *Sphingobium limneticum* and *Acidovorax delafieldii* were isolated from hydroponic *Solanum pennellii*. They were identified using 16S rRNA gene sequencing and they were slow growing bacteria (takes 3 days to grow on LB agar).

It was observed that growth of *Arabidopsis thaliana* (WT) and mutants inhibit primary root length when exposure to *Pseudomonas simiae* WCS417r, *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial (fig. 3-24 and 3-25). Also, Gemini tomato inhibit the primary root length with exposure with *Pseudomonas simiae* WCS417r and *Sphingobium limneticum* (fig. 3-9). The similar outcome was repeated in (Zamioudis et al.), the primary root length of seedlings exposed to WCS417r bacteria was decreased by around 40% compared with the mock treated roots, showing a suppressive impact of WCS417r on primary root elongation (Zamioudis et al. 2013). And WCS417r, *Sphingobium limneticum* and *Acidovorax delafieldii* bacterial promotes the lateral root formation in both experiment 6, 7, 8, 9 and 10 except WT and C2C4 mutant in experiment 6 that demonstrate decrease in plants growth (fig. 3-20 and 3-24). Most often, there is strong positive effect of bacteria by increasing lateral root formation of C2 mutant. Hence, WT and the mutants gain more shoot fresh weight without bacteria (fig. 3-23 and 3-28) and this is contrary to Wintermans et al; 2016. The PGPR-mediated increase in shoot fresh weight is at least identified to PGPR-mediated changes in root architecture (Wintermans et al. 2016).

All things considered, these root architectural changes broaden the limit of the root system to take up water and nutrients supplements, which may add to the observed correlation between additional numbers of lateral roots formed and increased shoot fresh weight in WCS417r-related *Arabidopsis* seedlings. It was noticed that *Arabidopsis* seedling were developed on plates in which nutrient supplement accessibility was not constraining, subsequently it cannot be decide the possibility that the correlation between root architectural changes and increased shoot fresh weight is caused by another, so far unknown process (Wintermans et al. 2016). It was suggested that IAA produced by *Sphingomonas* sp. isolated from the leaves of *Tephrosia apollinea* should be linked to the increase surface area and root length, the loss of cell wall and the release of exudates in tomato (*Solanum lycopersicum* L) (Abbamondi et al. 2016).

It has additionally been discovered that the plant-promoting rhizobacterium PGPR *Pseudomonas simiae* WCS417r stimulates lateral root formation and increase shoot growth in *Arabidopsis thaliana*. These plants stimulating impacts are brought on by volatile organic compounds (VOCs) produced by the bacterium (Wintermans et al. 2016). Regardless of the way that WCS417r does not produce auxin, it might produce other molecules with auxin activity, such as diketopiperazines, quorum-sensing bacterial molecules recently demonstrated to functionally imitate the binding of IAA to its receptor. In addition to secreted molecules, the volatile blend of WCS417 likewise seems to have a key role in promoting LR formation in *Arabidopsis* (Zamioudis et al. 2013).

In this study, no definite conclusion can be drawn from inoculation of bacteria strains 5, 6 (rhizosphere) and endospheric bacterial represented as *Pseudomonas* sp. (number 9), 10, *Agrobacterium* sp. (number 15 and 18), *Rhizobium* sp. (number 16) obtained from (Abbamondi et al. 2016) on Arabidopsis WT and mutants. Therefore, more work is needed to replicate the same experiment performed by Abbamondi et al. 2016. Also, further work is needed to investigate the activities of different bacterial strains especially *Agrobacterium* sp., *Sphingobium limneticum* and *Pseudomonas simiae* WCS417r on Arabidopsis WT and protein phosphatase 2A catalytic subunit mutant (C2).

REFERENCES

- Abbamondi GR, Tommonaro G, Weyens N, Thijs S, Sillen W, Gkorezis P, Iodice C, de Melo Rangel W, Nicolaus B, Vangronsveld J (2016) Plant growth-promoting effects of rhizospheric and endophytic bacteria associated with different tomato cultivars and new tomato hybrids. *Chemical and Biological Technologies in Agriculture* 3 (1):1
- Abushita AA, Hebshi EA, Daood HG, Biacs PA (1997) Determination of antioxidant vitamins in tomatoes. *Food Chemistry* 60 (2):207-212. doi:10.1016/S0308-8146(96)00321-4
- Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King Saud University-Science* 26 (1):1-20
- Alberts B, Johnson A, Lewis J, Walter P, Raff M, Roberts K (2002) *Molecular Biology of the Cell* 4th Edition: International Student Edition. Routledge,
- Amils R (2011) Gram-Positive Bacteria. In: Gargaud M, Amils R, Quintanilla JC et al. (eds) *Encyclopedia of Astrobiology*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 685-685. doi:10.1007/978-3-642-11274-4_664
- Arumuganathan K, Earle E (1991) Estimation of nuclear DNA content of plants by flow cytometry. *Plant Molecular Biology Reporter* 9 (3):229-241. doi:10.1007/BF02672073
- Bai Y, Lindhout P (2007) Domestication and Breeding of Tomatoes: What have We Gained and What Can We Gain in the Future? *Annals of Botany* 100 (5):1085-1094. doi:10.1093/aob/mcm150
- Ballesteros I, Domínguez T, Sauer M, Paredes P, Duprat A, Rojo E, Sanmartín M, Sánchez-Serrano JJ (2013) Specialized functions of the PP2A subfamily II catalytic subunits PP2A-C3 and PP2A-C4 in the distribution of auxin fluxes and development in *Arabidopsis*. *The Plant Journal* 73 (5):862-872. doi:10.1111/tpj.12078
- Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology* 35 (4 Suppl):1044-1051
- Berg J, Tymoczko J, Stryer L (2002) *Biochemistry, Fifth Edition: International Version (hardcover)*. W. H. Freeman. doi:citeulike-article-id:166333
- Bergougnoux V (2014) The history of tomato: From domestication to biopharming. *Biotechnology Advances* 32 (1):170-189. doi:<http://doi.org/10.1016/j.biotechadv.2013.11.003>
- Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J Microbiol Biotechnol* 28 (4):1327-1350. doi:10.1007/s11274-011-0979-9
- Bolger A, Scossa F, Bolger ME, Lanz C, Maumus F, Tohge T, Quesneville H, Alseekh S, Sorensen I, Lichtenstein G, Fich EA, Conte M, Keller H, Schneeberger K, Schwacke R, Ofner I, Vrebalov J, Xu Y, Osorio S, Aflitos SA, Schijlen E, Jimenez-Gomez JM, Ryngajllo M, Kimura S, Kumar R, Koenig D, Headland LR, Maloof JN, Sinha N, van Ham RC, Lankhorst RK, Mao L, Vogel A, Arsova B, Panstruga R, Fei Z, Rose JK, Zamir D, Carrari F, Giovannoni JJ, Weigel D, Usadel B, Fernie AR (2014) The genome of the stress-tolerant wild tomato species *Solanum pennellii*. *Nat Genet* 46 (9):1034-1038. doi:10.1038/ng.3046
- Brunen M, Engelhardt H, Schmid A, Benz R (1991) The major outer membrane protein of *Acidovorax delafieldii* is an anion-selective porin. *Journal of Bacteriology* 173 (13):4182-4187
- Cheema DS, Dhaliwal MS (2005) Hybrid Tomato Breeding. *Journal of New Seeds* 6 (2-3):1-14. doi:10.1300/J153v06n02_01
- Chen H, Jogler M, Rohde M, Klenk HP, Busse HJ, Tindall BJ, Sproer C, Overmann J (2013) *Sphingobium limneticum* sp. nov. and *Sphingobium boeckii* sp. nov., two freshwater planktonic members of the family Sphingomonadaceae, and reclassification of *Sphingomonas suberifaciens* as *Sphingobium suberifaciens* comb. nov. *Int J Syst Evol Microbiol* 63 (Pt 2):735-743. doi:10.1099/ijs.0.040105-0

- Chen J, Hu R, Zhu Y, Shen G, Zhang H (2014) Arabidopsis PHOSPHOTYROSYL PHOSPHATASE ACTIVATOR Is Essential for PROTEIN PHOSPHATASE 2A Holoenzyme Assembly and Plays Important Roles in Hormone Signaling, Salt Stress Response, and Plant Development. *Plant Physiology* 166 (3):1519-1534. doi:10.1104/pp.114.250563
- Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry* 42 (5):669-678. doi:<http://doi.org/10.1016/j.soilbio.2009.11.024>
- de Souza R, Ambrosini A, Passaglia LMP (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and Molecular Biology* 38 (4):401-419. doi:10.1590/S1415-475738420150053
- Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. *Scientifica (Cairo)* 2012:963401. doi:10.6064/2012/963401
- Heldens JWG, Ykema M, Herlaar F, Stee VMP, Lambalk JJM (2009) Promotor sequence and gene construct for increasing crop yield in tomato. Google Patents,
- Hua Guo J, Hao Jiang C (2015) The Plant Healthy and Safety Guards Plant Growth Promoting Rhizo Bacteria (PGPR). *Transcriptomics: Open Access* 03 (02). doi:10.4172/2329-8936.1000109
- Innerebner G, Knief C, Vorholt JA (2011) Protection of Arabidopsis thaliana against Leaf-Pathogenic Pseudomonas syringae by Sphingomonas Strains in a Controlled Model System. *Applied and Environmental Microbiology* 77 (10):3202-3210. doi:10.1128/aem.00133-11
- Jones JB (2008) Tomato plant culture: in the field, greenhouse, and home garden. 2nd ed. edn. CRC Press, Boca Raton, Fla
- Kadam PD, Chuan HH (2016) Erratum to: Rectocutaneous fistula with transmigratio of the suture: a rare delayed complication of vault fixation with the sacrospinous ligament. *Int Urogynecol J* 27 (3):505. doi:10.1007/s00192-016-2952-5
- Kundan R, Pant G, Jadon N, Agrawal PK (2015) Plant Growth Promoting Rhizobacteria: Mechanism and Current Prospective. *Journal of Fertilizers & Pesticides* 06 (02). doi:10.4172/2471-2728.1000155
- Lillo C, Kataya AR, Heidari B, Creighton MT, NEMIE-FEYISSA D, Ginbot Z, Jonassen EM (2014) Protein phosphatases PP2A, PP4 and PP6: mediators and regulators in development and responses to environmental cues. *Plant, cell & environment* 37 (12):2631-2648
- Martínez-Viveros O, Jorquera MA, Crowley DE, Gajardo G, Mora ML (2010) MECHANISMS AND PRACTICAL CONSIDERATIONS INVOLVED IN PLANT GROWTH PROMOTION BY RHIZOBACTERIA. *Journal of soil science and plant nutrition* 10:293-319
- Mattern V (2013) Hybrid seeds vs. GMOs.(Ask Our Experts). *Mother Earth News* (256):76
- Normanly J (2010) Approaching cellular and molecular resolution of auxin biosynthesis and metabolism. *Cold Spring Harb Perspect Biol* 2 (1):a001594. doi:10.1101/cshperspect.a001594
- Opena R, Chen J, Kalb T, Hanson P (2001) Hybrid seed production in tomato. AVRDC International Cooperators Guide Publication No:01-527
- País SM, Téllez-Iñón MT, Capiati DA (2009) Serine/threonine protein phosphatases type 2A and their roles in stress signaling. *Plant signaling & behavior* 4 (11):1013-1015
- Persello-Cartieaux F, David P, Sarrobert C, Thibaud M-C, Achouak W, Robaglia C, Nussaume L (2001) Utilization of mutants to analyze the interaction between Arabidopsis thaliana and its naturally root-associated Pseudomonas. *Planta* 212 (2):190-198. doi:10.1007/s004250000384
- Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* 11 (11):789-799. doi:10.1038/nrmicro3109
- Ramirez KS, Lauber CL, Fierer N (2009) Microbial consumption and production of volatile organic compounds at the soil-litter interface. *Biogeochemistry* 99 (1-3):97-107. doi:10.1007/s10533-009-9393-x

- Razdan MK (2006) Genetic Improvement of Solanaceous Crops Volume 2. Tomato.
- Rick CM (1960) Hybridization between *Lycopersicon Esculentum* and *Solanum Pennellii*: Phylogenetic and Cytogenetic Significance. *Proceedings of the National Academy of Sciences of the United States of America* 46 (1):78-82
- Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Pare PW, Kloepper JW (2003) Bacterial volatiles promote growth in *Arabidopsis*. *Proc Natl Acad Sci U S A* 100 (8):4927-4932. doi:10.1073/pnas.0730845100
- Santi C, Bogusz D, Franche C (2013) Biological nitrogen fixation in non-legume plants. *Annals of Botany* 111 (5):743-767. doi:10.1093/aob/mct048
- Santoro M, Cappellari L, Giordano W, Banchio E (2015) Production of Volatile Organic Compounds in PGPR. In: Cassán FD, Okon Y, Creus CM (eds) *Handbook for Azospirillum: Technical Issues and Protocols*. Springer International Publishing, Cham, pp 307-317. doi:10.1007/978-3-319-06542-7_17
- Saraf M, Jha CK, Patel D (2010) The Role of ACC Deaminase Producing PGPR in Sustainable Agriculture. 18:365-385. doi:10.1007/978-3-642-13612-2_16
- Shi L, Potts M, Kennelly PJ (1998) The serine, threonine, and/or tyrosine-specific protein kinases and protein phosphatases of prokaryotic organisms: a family portrait. *FEMS Microbiology Reviews* 22 (4):229-253. doi:10.1111/j.1574-6976.1998.tb00369.x
- Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiology Reviews* 31 (4):425-448. doi:10.1111/j.1574-6976.2007.00072.x
- Timmusk S (2003) Mechanism of action of the plant growth promoting bacterium *Paenibacillus polymyxa*. *Acta Universitatis Upsaliensis*,
- Timmusk S, Paalme V, Pavlicek T, Bergquist J, Vangala A, Danilas T, Nevo E (2011) Bacterial Distribution in the Rhizosphere of Wild Barley under Contrasting Microclimates. *PLOS ONE* 6 (3):e17968. doi:10.1371/journal.pone.0017968
- Truyens S, Beckers B, Thijs S, Weyens N, Cuypers A, Vangronsveld J (2016) The effects of the growth substrate on cultivable and total endophytic assemblages of *Arabidopsis thaliana*. *Plant and Soil* 405 (1):325-336. doi:10.1007/s11104-015-2761-5
- Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moenne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dye F, Prigent-Combaret C (2013) Plant growth-promoting rhizobacteria and root system functioning. *Front Plant Sci* 4:356. doi:10.3389/fpls.2013.00356
- Velivelli SLS, Sessitsch A, Prestwich BD (2014) The Role of Microbial Inoculants in Integrated Crop Management Systems. *Potato Research* 57 (3):291-309. doi:10.1007/s11540-014-9278-9
- Wang ET, Martínez-Romero E (2000) *Sesbania herbacea*-*Rhizobium huautlense* Nodulation in Flooded Soils and Comparative Characterization of *S. herbacea*-Nodulating Rhizobia in Different Environments. *Microbial Ecology* 40 (1):25-32. doi:10.1007/s002480000010
- Willems A, Falsen E, Pot B, Jantzen E, Hoste B, Vandamme P, Gillis M, Kersters K, De Ley J (1990) *Acidovorax*, a new genus for *Pseudomonas facilis*, *Pseudomonas delafieldii*, E. Falsen (EF) group 13, EF group 16, and several clinical isolates, with the species *Acidovorax facilis* comb. nov., *Acidovorax delafieldii* comb. nov., and *Acidovorax temperans* sp. nov. *Int J Syst Bacteriol* 40 (4):384-398. doi:10.1099/00207713-40-4-384
- Wintermans PC, Bakker PA, Pieterse CM (2016) Natural genetic variation in *Arabidopsis* for responsiveness to plant growth-promoting rhizobacteria. *Plant Mol Biol* 90 (6):623-634. doi:10.1007/s11103-016-0442-2

Zamioudis C, Mastranesti P, Dhonukshe P, Blilou I, Pieterse CM (2013) Unraveling root developmental programs initiated by beneficial *Pseudomonas* spp. bacteria. *Plant Physiol* 162 (1):304-318. doi:10.1104/pp.112.212597

APPENDIX

1. The sequences were translated and blasted at NCBI with BLASTn program against 16S ribosomal RNA Sequence (Bacteria and Archaea). The name of query protein and the possible bacteria are given below with the sequence.

1p. 26f

```
NCTTCGGGTCTAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCTTGGGTTTCGGAATAACGTCGGGAACTGACGCTAATACCGGATG
ATGACGAAAAGTCCAAAGATTTATCGCCCAGGGATGAGCCCGCTAGGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCTACGATCCTT
AGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATATTGGACAATGGGG
GCAACCCTGATCCAGCAATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTTACCCGAGATGATAATGACAGTATCGGGAGAA
TAAGTCCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGAGCTAGCGTTGTTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGG
CGATTTAAGTCAGAGGTGAAAGCCCGGGCTCAACCCGGAACCTTTGAGACTGGATTGCTTGAATCCTGGAGAGGTGAGTGAATTC
GAGTGTAGAGGTGAAATTCGTAGATATTTCGGAAGAACCAGTGGCGAAGGCGGCTCACTGGACAGGTATTGACGCTGAGGTGCCAAAGCGT
GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCGTAAACGATGATAACTAGCTGCCGGGCACATGGTGTTCGGTGGCGCAGCTA
ACGCATTAAGTTATCCGCTGGGGAGTACGGTCCGAAGATTAACAAAGGAAATTGACGGGGCCGCACAAGCGGTGGAGCATGTGGTT
TAATTCGAAGCAACGCGCAGAACCTTACCAACGTTTGACATCCCTATCGCGGATCGTGAGACACTTTCCTTCAGTTCGGCTGGATAGGTGA
CAGGTGCTGCATGGCTGTCGTCAGCTCGTG
```

Spingobium limneticum strain 301 16S ribosomal RNA gene, partial sequence

Sequence ID: [NR_109484.1](#) Length: 1441 Number of Matches: 1

Range 1: 69 to 1017 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1748 bits(946)	0.0	948/949(99%)	0/949(0%)	Plus/Plus

<input checked="" type="checkbox"/>	Spingobium limneticum strain 301 16S ribosomal RNA gene, partial sequence	1748	1748	99%	0.0	99%	NR_109484.1
<input type="checkbox"/>	Spingobium amiense strain NBRC 102518 16S ribosomal RNA gene, partial sequence	1709	1709	99%	0.0	99%	NR_114136.1
<input type="checkbox"/>	Spingobium amiense strain Y 16S ribosomal RNA gene, partial sequence	1709	1709	99%	0.0	99%	NR_028622.1
<input checked="" type="checkbox"/>	Spingobium limneticum strain 301 16S ribosomal RNA gene, partial sequence	1663	1663	94%	0.0	100%	NR_118123.1
<input type="checkbox"/>	Spingobium vanoikuyae strain NBRC 15102 16S ribosomal RNA gene, partial sequence	1626	1626	99%	0.0	98%	NR_113730.1
<input type="checkbox"/>	Spingobium vermicomposti strain VC-230 16S ribosomal RNA gene, partial sequence	1626	1626	99%	0.0	98%	NR_115107.1
<input checked="" type="checkbox"/>	Spingobium mellinum strain WI4 16S ribosomal RNA, partial sequence	1615	1615	99%	0.0	97%	NR_133859.1

1p2. 1520R

```
AGTCGCTAAACCCACTGTGGTCGCTGCCTCCTTGCGGTTAGCTCAACGCCTTCGAGTGAATCCAACCTCCCATGGTGTGACGGGCGGTGTGT
ACAAGG
```

Reverse complement

```
CCTTGTACACACCCCGCTCACACCATGGGAGTTGGATTCACTCGAAGGCGTTGAGCTAACCGCAAGGAGGCAGGCGACCACAGTGGGTTTA
GCGACT
```

Blast result reversed

Sphingobium mellinum strain WI4 16S ribosomal RNA, partial sequence

Sequence ID: [NR_133859.1](#) Length: 1451 Number of Matches: 1

Range 1: 1326 to 1423 [GenBank](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
182 bits(98)	3e-46	98/98(100%)	0/98(0%)	Plus/Plus
Query 1	CCTTGTACACACCCGCCGTCACACCATGGGAGTTGGATTCACTCGAAGGCGTTGAGCTAA	60		
Sbjct 1326	CCTTGTACACACCCGCCGTCACACCATGGGAGTTGGATTCACTCGAAGGCGTTGAGCTAA	1385		
Query 61	CCGCAAGGAGGCGAGGCGACACAGTGGGTTTAGCGACT	98		
Sbjct 1386	CCGCAAGGAGGCGAGGCGACACAGTGGGTTTAGCGACT	1423		

1b). 1.1p-1492R

```
TCGCCCTCCTTGGCGTTAGGCTAACTACTTCTGGCAGAACCCGCTCCCATGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCAC
CGCGACATCTGATCCCGGATTACWAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGACTGGCTTTATGGGATT
GGCTCCCCCTCGCGGGTTGGCAACCCCTCTGTACCAGCCATTGTATGACGTGTGTAGCCCCACCTATAAAGGGCCATGAGGACTTGACGTATC
CCCACCTTCTCCGGTTTGTACCGGCAGTCTCATTAGAGTGCCCAACTGAATGTAGCAACTAATGACAAGGGTTGCGCTCGTTGCGGGACT
TAACCCAAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTTACGGTTCTCTTTTCGAGCACTCCCTCTATCTCTAAAGGATTC
CGTACATGTCAAAGGTGGGTAAGGTTTTTTCGCGTTGTCATCGAATTAACACATCATCCACCGCTTGTGCGGGTCCCCGTCGAATTCCTTTGA
GTTTCAACCTTGGCGCGTACTCCCCAGGCGGTCAACTTCACGCGTTAGCTTTCGTTACTGAGTCAGTGAAGACCCAAACACAGTTGACATC
GTTTAGGGCGTGGACTACMAGGTTATCTAATCTGTTTGTCTCCCCACGCTTTCGTCATGAGCGTCAGTACAGGCCNAGGGGATGACCTTCG
CCATCGGTGTTCTCCGCATATCTACGCATTTCACTGCTACACGCGGAATCCATCCCCCTCTGCCGTACTCNAGCTATACAGTCACAAATG
CAGTTCCNAGGTTGAGCCCGGGGATTTACATCTGTCTTATATAACCCGCTGCCACGCTTTACGCCAGTAATTCGGATTAACGCTTGAC
CCTACGTATTACCGCGGTGCTGGCACGTAGTTAGC
```

Reverse complement

```
GCTAATACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATATAAG
ACAGATGTGAAATCCCCGGGCTCAACCTNGGAAGTGCATTTGTGACTGTATAGCTNGAGTACGGCAGAGGGGGATGGAATTCGCGGTGTAGC
AGTGAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCTNGGCCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCTGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTTCTCACTGACTCAGTAACGAAGCTAACGCGTGAAGT
TGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGACCCGCACAAGCGGTGGATGATGTGGTTTAAATTCGATGC
AACCGCAAAAACCTTACCACCTTTGACATGTACGGAATCCTTTAGAGATAGAGGAGTGTCTCGAAAGAGAACCGTAACACAGGTGCTGCATG
GCTGTGCTGAGCTCGTGTGATGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTTGTCAATTAGTTGCTACATTCACTTGGGCACTCTA
ATGAGACTGCCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCTCATGGCCCTTATAGGTGGGGCTACACACGTCATACAATGGC
TGGTACAGAGGGTTGCCAACCCGCGAGGGGGAGCCAATCCCATAAAGCCAGTCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGT
CGGAATCGCTGTAATCGCGGATCAGAATGTGCGGTGAATACGTTCCCGGCTTGTACACACCGCCGTCACACCATGGGAGCGGGTTCTG
CCAGAAGTAGTTAGCCTAACCCGCAAGGAGGGCGA
```

EMBOSS (LOCAL ALIGNMENT)

```
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
# Length: 1361
# Identity: 457/1361 (33.6%)
# Similarity: 457/1361 (33.6%)
```

```

# Gaps:          818/1361 (60.1%)
# Score: 1833.5

EMBOSS_001      1 NCTTCGGGTCTAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCTT      50
EMBOSS_001      1 -----                                                    0
EMBOSS_001     51 GGGTTCGGAATAACGTCGGGAAACTGACGCTAATACCGGATGATGACGAA      100
EMBOSS_001      1 -----                                                    0
EMBOSS_001    101 AGTCCAAAGATTTATCGCCCAGGGATGAGCCC GCGTAGGATTAGCTAGTT      150
EMBOSS_001      1 -----                                                    0
EMBOSS_001    151 GGTGAGGTAAAGGCTCACCAAGGCTACGATCCTTAGCTGGTCTGAGAGGA      200
EMBOSS_001      1 -----                                                    0
EMBOSS_001    201 TGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA      250
EMBOSS_001      1 -----                                                    0
EMBOSS_001    251 GCAGTAGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCAATGCCGC      300
EMBOSS_001      1 -----                                                    0
EMBOSS_001    301 GTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTTACCCGAGATGATA      350
EMBOSS_001      1 -----                                                    0
EMBOSS_001    351 ATGACAGTATCGGGAGAATAAGCTCCGGCTAACTCCGTGCCAGCAGCCGC      400
                                     |||...|||
EMBOSS_001      1 -----GCTAACTACGTGCCAGCAGCCGC      23
EMBOSS_001    401 GGTAATACGGAGGGAGCTAGCGTTGTTCCGGAATTACTGGGCGTAAAGCGC      450
|||...|||...|||...|||...|||...|||...|||...|||...|||...
EMBOSS_001      24 GGTAATACGTAGGGTCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGT      73
EMBOSS_001    451 ACGTAGCGGCGATTTAAGTCAGAGGTGAAAGCCCGGGGCTCAACCCCGG      500
      .||...|||...||...|||...|||...|||...|||...|||...|||...
EMBOSS_001      74 GCGCAGGCGGTTATATAAGACAGATGTGAAATCCCCGGGCTCAACCTNGG      123
EMBOSS_001    501 AACTGCCTTTGAGACTGGATTGCTTGAATCCTGGAGAGGTGAGTGGAATT      550
      |||...|||...|||...||...|||...||...||...|||...||...|||
EMBOSS_001    124 AACTGCATTTGTGACTGTATAGCTNGAGTACGGCAGAGGGGGATGGAATT      173
EMBOSS_001    551 CCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGCGGA      600
      |||...|||...|||...|||...|||...|||...|||...|||...|||
EMBOSS_001    174 CCGCGTGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGCGA      223
EMBOSS_001    601 AGGCGGCTCACTGGACAGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAG      650
      |||...|||...|||...|||...|||...|||...|||...|||...|||
EMBOSS_001    224 AGGCAATCCCCTNGGCTGTACTGACGCTCATGCACGAAAGCGTGGGGAG      273
EMBOSS_001    651 CAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGATAACTA      700
|||...|||...|||...|||...|||...|||...|||...|||...|||...

```

EMBOSS_001	274	CAAACAGGATTAGATACCCT-GTAGTCCACGCCCTAAACGATGTCAACTG	322
EMBOSS_001	701	GCTGCCGGG--GCACATGGTGTTCGGTGGCGCAGCTAACGCATTAAGT	747
		. .	
EMBOSS_001	323	GTTGTTGGGTCTTCAC----TGACTCAGTAACGAAGCTAACGCGTGAAGT	368
EMBOSS_001	748	TATCCGCTGGGGAGTACGGTCGCAAGATTAAACTCAAAGGAATTGACG	797
		. .	
EMBOSS_001	369	TGACCGCTGGGGAGTACGGCCGCAAGGTTGAACTCAAAGGAATTGACG	418
EMBOSS_001	798	GGGCGCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCA	847
		. .	
EMBOSS_001	419	GGGACCCGCACAAGCGGTGGATGATGTGGTTTAATTCGATGCAACGCGAA	468
EMBOSS_001	848	GAACCTTACCAACGTTTGACATCCCTATCGCGGATCGTGGAGACACTTTC	897
		. .	
EMBOSS_001	469	AAACCTTACCCACCTTTGACAT--GTA---CGGA-----ATC	500
EMBOSS_001	898	CTTCAGTTCGGCTGGATAG----GTG-----ACAGG	924
		. .	
EMBOSS_001	501	CTTTAG-----AGATAGAGGAGTGCTCGAAAGAGAACCGTAACACAGG	543
EMBOSS_001	925	TGCTGCATGGCTGTCGTCAGCTCGTG-----	950
		
EMBOSS_001	544	TGCTGCATGGCTGTCGTCAGCTCGTGCTGAGATGTTGGGTTAAGTCCC	593
EMBOSS_001	951	-----	950
EMBOSS_001	594	GCAACGAGCGCAACCCTTGTATTAGTTGCTACATTCAGTTGGGCACTCT	643
EMBOSS_001	951	-----	950
EMBOSS_001	644	AATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTC	693
EMBOSS_001	951	-----	950
EMBOSS_001	694	CTCATGGCCCTTATAGGTGGGGCTACACACGTCATAAATGGCTGGTACA	743
EMBOSS_001	951	-----	950
EMBOSS_001	744	GAGGGTTGCCAACCCGCGAGGGGAGCCAATCCCATAAAGCCAGTCGTAG	793
EMBOSS_001	951	-----	950
EMBOSS_001	794	TCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTGTAAT	843
EMBOSS_001	951	-----	950
EMBOSS_001	844	CGCGGATCAGAATGTCGCGGTGAATACGTTCCCGGTCTTGTACACACCG	893
EMBOSS_001	951	-----	950
EMBOSS_001	894	CCCGTCACACCATGGGAGCGGGTCTGCCAGAAGTAGTTAGCCTAACCGC	943
EMBOSS_001	951	----- 950	
EMBOSS_001	944	AAGGAGGGCGA 954	

BLAST RESULT

Acidovorax delafieldii strain 133 16S ribosomal RNA gene, partial sequence

Sequence ID: [NR_028714.1](#) Length: 1515 Number of Matches: 1

Range 1: 491 to 1446 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1720 bits(931)	0.0	947/956(99%)	2/956(0%)	Plus/Plus
Query 1	GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACT	60		
Sbjct 491	GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTAATCGGAATTACT	550		
Query 61	GGGCGTAAAGCGTGCGCAGGCGGTTATATAAGACAGATGTGAAATCCCCGGGCTCAACCT	120		
Sbjct 551	GGGCGTAAAGCGTGCGCAGGCGGTTATATAAGACAGATGTGAAATCCCCGGGCTCAACCT	610		

2.

b. 1N. 26F

CTTCGGGTCTAGTGGCGCACGGGTGCGTAAACGCGTGGGAATCTGCCCTTGGGTTTCGGAATAACGTCGGGAAACTGACGCTAATACCGGATGATGACGAAAAGTCCAAAGATTTATCGCCCAGGGATGAGCCCGCGTAGGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCTACGATCCTTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATATTGGACAATGGGGCAACCCCTGATCCAGCAATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTTACCCGAGATGATAATGACAGTATCGGGAGAATAAGCTCCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGAGCTAGCGTTGTTTCGGAATTACTGGGCGTAAAGCGCACGTTAGGCGGC GATTTAAGTCAGAGGTGAAAGCCCGGGGCTCAACCCCGGAACGCCTTTGAGACTGGATTGCTTGAATCCTGGAGAGGTGAGTGAATTCAGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGGCTCACTGGACAGGTATTGACGCTGAGGTGCGAAAAGCGTG GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGATAACTAGCTGCCGGGCACATGGTGTTCGGTGGCCGACGTAA CGCATTAAAGTTATCCGCCTGGGAGTACGGTTCGCAAGATTAAGAACTCAAAGGAATTGACGGGGCCTGCACAAGCGGTGGAGCATGTGGTTT AATTCCAAGCAACCGCGAGAACCTTACCAACGTTTGACATCCCTATCGCGGATCGTGA

BLAST RESULT

Sphingobium limneticum strain 301 16S ribosomal RNA gene, partial sequence

Sequence ID: [NR_109484.1](#) Length: 1441 Number of Matches: 1

Range 1: 69 to 953 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1635 bits(885)	0.0	885/885(100%)	0/885(0%)	Plus/Plus

1N2. 1520R

TTACCCAGTCGCTAAACCCACTGTGGTGCCTGCCTCCTTGGGTTAGCTCAACGCCTTCGAGTGAATCCAATCCCATGGTGTGACGGG CCGTGTGTACAAGGCCCTGGGAACGTATTACCCGCGCATGCTGATCCGCGATTACTAGCGATTCCGCCTTCACGCTCTCGAGTTGCGAGAA CGATCCGAACTGAGACGACTTTTGGAGATTAGCTCCCTCTCGCGAGGTGGCTGCCACTGTAGTCGCCATTGTAGCACGTGTGTAGCCCAAC GCGTAAGGGCCATGAGGACTTGACGTCAATCCACCTTCTCCGGCTTATCACCGCGGTTCCCTTAGAGTACCCAATAAATGATGGCAAC TAAAGGCGAGGGTTGCGCTCGTTGCGGACTTAACCCAACATCTCACGACAGGCTGACGACAGCCATGCAGCACCTGTCACCTATCCAGC CGAACTGAAGGAAAGTGTCTCCACGATCCGCGATAGGGATGTCAAACGTTGGTAAGGTTCTGCGGTTGCTTCGAATTAACACCATGCTCC ACCGCTTGTGACAGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTGCACCGTACTCCCCAGGCGGATAACTTAATGCGTTAGCTGCGCCACC GAAACACCATGTGCCCCGGCAGCTAGTTATCATCGTTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGTCTCCCAACGCTTTCCGACC TCAGCGTCAATACCTGTCCAGTGAGCCGCTTCGCCACTGGTGTCTTCCGAATATCTACGAATTCACCTCTACACTCGGAATTCACCTCA CCTCTCCAGGATTCAAGCAATCCAGTCTCAAAGGCAGTTCGGGGTTGAGCCCGGGCTTTCACCTCTGACTTAATCGCCGCTACGTGCGC TT

REVERSE COMPLEMENT

AAGCGCACGTAGGCGCGGATTAAGTCAGAGGTGAAAGCCCGGGGCTCAACCCCGGAACGCCTTTGAGACTGGATTGCTTGAATCCTGGAGA GGTGAGTGAATTCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACCAGTGGCGAAGGCGGCTCACTGGACAGGTATTGACGCT GAGGTGCGAAAAGCGTGGGGAGCAAACAGGATTAGATACCTGGTAGTCCACGCGTAAACGATGATAACTAGCTGCCGGGGCACATGGTGT TCGGTGGCGCAGCTAACGCATTAAGTTATCCGCTGGGGAGTACGGTTCGCAAGATTAAGAACTCAAAGGAATTGACGGGGGCTGCACAAGCG GTGGAGCATGTGGTTAATTCGAAGCAACGCGAGAACCTTACCAACGTTTGACATCCCTATCGCGGATCGTGGAGACACTTTCCTTCAGTT CCGCTGGATAGGTGACAGGTGCTGCATGGCTGTCGTACCTCGTGTGAGATGTTGGTTAAGTCCCGCAACGAGCGCAACCTTCGCCTT

TAGTTGCCATCATTAGTTGGTACTCTAAAGGAACCGCCGGTGATAAGCCGGAGGAAGGTGGGGATGACGTCAAGTCCATGGCCCTTAC
 GCGTTGGGCTACACACGTGCTACAATGGCGACTACAGTGGGCAGCCACCTCGCGAGAGGGAGCTAATCTCCAAAAGTCTCAGTTCGGAT
 CGTTCTGCAACTCGAGAGCGTGAAGGCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGTGAATACGTTCCAGGCCTTGTACACAC
 CGCCCGTCACACCATGGGAGTTGGATTCACTCGAAGGCGTTGAGCTAACCGCAAGGAGGCAGGCGACCACAGTGGGTTTAGCGACTGGGGTG
 AA

EMBOSS (LOCAL ALIGNMENT)

```
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
# Length: 1366
# Identity:      441/1366 (32.3%)
# Similarity:   441/1366 (32.3%)
# Gaps:         924/1366 (67.6%)
# Score: 2191.0

EMBOSS_001      1  CTTCGGGTCTAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCTTG      50
EMBOSS_001      1  -----
EMBOSS_001     51  GGTTCCGAATAACGTCCGGAAACTGACGCTAATACCGGATGATGACGAAA     100
EMBOSS_001      1  -----
EMBOSS_001    101  GTCCAAAAGATTTATCGCCCAGGGATGAGCCCGCTAGGATTAGCTAGTTG     150
EMBOSS_001      1  -----
EMBOSS_001    151  GTGAGGTAAAGGCTCACCAAGGCTACGATCCTTAGCTGGTCTGAGAGGAT     200
EMBOSS_001      1  -----
EMBOSS_001    201  GATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG     250
EMBOSS_001      1  -----
EMBOSS_001    251  CAGTAGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCAATGCCGCG     300
EMBOSS_001      1  -----
EMBOSS_001    301  TGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTTACCCGAGATGATAA     350
EMBOSS_001      1  -----
EMBOSS_001    351  TGACAGTATCGGGAGAATAAGCTCCGGCTAACTCCGTGCCAGCAGCCGCG     400
EMBOSS_001      1  -----
EMBOSS_001    401  GTAATACGGAGGGAGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCA     450
EMBOSS_001      1  -----
EMBOSS_001      1  -----AAGCGCA      7
```

EMBOSS_001	451	CGTAGGCGGCGATTTAAGTCAGAGGTGAAAGCCCGGGGCTCAACCCCGGA	500
EMBOSS_001	8	CGTAGGCGGCGA-TTAAGTCAGAGGTGAAAGCCCGGGGCTCAACCCCGGA	56
EMBOSS_001	501	ACTGCCTTTGAGACTGGATTGCTTGAATCCTGGAGAGGTGAGTGGAATTC	550
EMBOSS_001	57	ACTGCCTTTGAGACTGGATTGCTTGAATCCTGGAGAGGTGAGTGGAATTC	106
EMBOSS_001	551	CGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGCGGAA	600
EMBOSS_001	107	CGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGCGGAA	156
EMBOSS_001	601	GGCGGCTCACTGGACAGGTATTGACGCTGAGGTGCGAAAAGCGTGGGGAGC	650
EMBOSS_001	157	GGCGGCTCACTGGACAGGTATTGACGCTGAGGTGCGAAAAGCGTGGGGAGC	206
EMBOSS_001	651	AAACAGGATTAGATACCTGGTAGTCCACGCCGTAAACGATGATAACTAG	700
EMBOSS_001	207	AAACAGGATTAGATACCTGGTAGTCCACGCCGTAAACGATGATAACTAG	256
EMBOSS_001	701	CTGCCGGGGCACATGGTGTTCGGTGGCGCAGCTAACGCATTAAGTTATC	750
EMBOSS_001	257	CTGCCGGGGCACATGGTGTTCGGTGGCGCAGCTAACGCATTAAGTTATC	306
EMBOSS_001	751	CGCCTGGGGAGTACGGTCGCAAGATTAAAACCTCAAAGGAATTGACGGGGG	800
EMBOSS_001	307	CGCCTGGGGAGTACGGTCGCAAGATTAAAACCTCAAAGGAATTGACGGGGG	356
EMBOSS_001	801	CCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAAC	850
EMBOSS_001	357	CCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAAC	406
EMBOSS_001	851	CTTACCAACGTTTGACATCCCTATCGCGGATCGTGA-----	886
EMBOSS_001	407	CTTACCAACGTTTGACATCCCTATCGCGGATCGTGGAGACTTTCCTTC	456
EMBOSS_001	887	-----	886
EMBOSS_001	457	AGTTCGGCTGGATAGGTGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT	506
EMBOSS_001	887	-----	886
EMBOSS_001	507	CGTGAGATGTTGGGTTAAGTCCCACGAGCGCAACCCTCGCCTTTAGT	556
EMBOSS_001	887	-----	886
EMBOSS_001	557	TGCCATCATTTAGTTGGTACTCTAAAGGAACCGCCGGTGATAAGCCGGA	606
EMBOSS_001	887	-----	886
EMBOSS_001	607	GGAAGTGGGGATGACGTCAAGTCTCATGGCCCTTACGCGTTGGGCTAC	656
EMBOSS_001	887	-----	886

EMBOSS_001	657	ACACGTGCTACAATGGCGACTACAGTGGGCAGCCACCTCGCGAGAGGGAG	706
EMBOSS_001	887	-----	886
EMBOSS_001	707	CTAATCTCCAAAAGTCGTCTCAGTTCGGATCGTTCTCTGCAACTCGAGAG	756
EMBOSS_001	887	-----	886
EMBOSS_001	757	CGTGAAGGCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATA	806
EMBOSS_001	887	-----	886
EMBOSS_001	807	CGTCCCAGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGATTC	856
EMBOSS_001	887	-----	886
EMBOSS_001	857	ACTCGAAGGCGTTGAGCTAACCAGGAGGCAGGCGACCACAGTGGGTT	906
EMBOSS_001	887	-----	886
EMBOSS_001	907	TAGCGACTGGGGTGAA	922

Blast reversed

Sphingobium limneticum strain 301 16S ribosomal RNA gene, partial sequence

Sequence ID: [NR_109484.1](#) Length: 1441 Number of Matches: 1

Range 1: 512 to 1434 [GenBank](#) [Graphics](#)

[Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1692 bits(916)	0.0	921/923(99%)	1/923(0%)	Plus/Plus

b).1.1N. 1492R

GCCTGCCCTCCTTGCGGTTAGCTCAACGCCTTCGAGTGAATCCAACCTCCATGGTGTGACGGGCGGTGTGTACAAGGCCTGGGAACGTATTCA
 CCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGCCTTCACGCTCTCGAGTTGCAGAGAACGATCCGAACCTGAGACGACTTTTGGAGATT
 AGCTCCCTCTCGCGAGGTGGCTGCCACTGTAGTCGCCATTGTAGCACGTGTGTAGCCCAACGCGTAAGGGCCATGAGGACTTGACGTATC
 CCCACCTTCCTCCGGCTTATCACCGGCGGTTCCCTTTAGAGTACCCAATAAATGATGGCAACTAAAGGGGAGGGTTGCGCTCGTTGCGGGAC
 TTAACCCAACATCTCAGACACGAGCTGACGACAGCCATGCAGCACCTGTACCTATCCAGCCGAACCTGAAGGAAAGTGTCTCCACGATCCG
 CGATAGGGATGTCAAACGTTGGTAAGGTTCTGCGCGTTGCTTCGAATTAACACATGCTCCACCGCTTGTGCAGGCCCGTCAATTCCTT
 TGAGTTTTAATCTTGCGACCGTACTCCCAGGCGGATAACTTAATGCGTTAGCTGCGCCACCAGAAACACCATGTGCCCGGCGAGCTAGTTAT
 CATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGTCTCCACGCTTTCGCACCTCAGCGTCAATACCTGTCCAGTGAGCCGCC
 TTCGCCACTGGTGTCTTCCGAATATCTACGAATTTCCACCTTACACTCGGAATTCACCTCACCTCTCCAGGATTCAGCAATCCAGTCTCA
 AAGGCAGTCCCGGGTTGAGCCCGGGCTTTCACCTCTGACTTAAATCGCCGCTACGTGCGCTTACGCCAGTAATTCGAACAACGCTA
 GCTCCCTCCGTATTACCGCGGCT

Complement

AGCCCGGTAATACGGAGGGAGCTAGCGTTGTTCCGAATTACTGGGCGTAAAGCGCACGTAGGCGGGGATTTAAGTCAGAGGTGAAAGCCCG
 GGGCTCAACCCCGAACTGCCTTTGAGACTGGATTGCTTGAATCCTGGAGAGGTGAGTGAATTCAGAGTGTAGAGGTGAAATTCGTAGATA
 TTCGGAAGAACCAGTGGCGAAGGCGGCTCACTGGACAGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCTT
 GGTAGTCCACGCGTAAACGATGATAACTAGCTGCCGGGCGACATGGTGTTCGGTGGCGCAGCTAACGCATTAAGTTATCCGCCTGGGGAG
 TACGGTCGCAAGATTAACCTCAAAGGAATTGACGGGGGCTGCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACCGCGAGAACCTT
 ACCAACGTTTGACATCCCTATCGCGGATCGTGGAGACTTTCCTTCAGTTCGGCTGGATAGGTGACAGGTGCTGCATGGCTGTCTGCTAGCT
 CGTGTCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCTCGCCTTTAGTTGCCATCATTTAGTTGGGTACTCTAAAGGAACCGCCG
 GTGATAAGCCGGAGGAAGGTGGGGATGACGTCAAGTCTCATGGCCCTTACGCGTTGGGCTACACACGTGCTACAATGGCGACTACAGTGGG
 CAGCCACCTCGCGAGAGGGAGCTAATCTCCAAAAGTCGTCTCAGTTCGGATCGTTCTCTGCAACTCGAGAGCGTGAAGGCGGAATCGCTAGT
 AATCGCGGATCAGCATGCCGCGGTGAATACGTTCCAGGCCTTGTACACACCGCCCGTACACCATGGGAGTTGGATTCACTCGAAGCGGTT
 GAGCTAACCGCAAGGAGGAGGC

EMBOSS (LOCAL ALIGNMENT)

Aligned_sequences: 2

1: EMBOSS_001

2: EMBOSS_001

Matrix: EDNAFULL

Gap_penalty: 10.0

Extend_penalty: 0.5

Length: 1336

Identity: 492/1336 (36.8%)

Similarity: 492/1336 (36.8%)

Gaps: 843/1336 (63.1%)

Score: 2456.0

```
EMBOSS_001      1 CTTCGGGTCTAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCTTG      50

EMBOSS_001      1 -----                                0
EMBOSS_001     51 GGTTTCGGAATAACGTCGGGAAACTGACGCTAATACCGGATGATGACGAAA     100
EMBOSS_001      1 -----                                0
EMBOSS_001    101 GTCCAAAGATTTATCGCCAGGGATGAGCCCGCTAGGATTAGCTAGTTG     150
EMBOSS_001      1 -----                                0
EMBOSS_001    151 GTGAGGTAAAGGCTCACCAAGGCTACGATCCTTAGCTGGTCTGAGAGGAT     200
EMBOSS_001      1 -----                                0
EMBOSS_001    201 GATCAGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCGAG     250
EMBOSS_001      1 -----                                0
EMBOSS_001    251 CAGTAGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCAATGCCGCG     300
EMBOSS_001      1 -----                                0
EMBOSS_001    301 TGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTTACCCGAGATGATAA     350
EMBOSS_001      1 -----                                0
EMBOSS_001    351 TGACAGTATCGGGAGAATAAGCTCCGGCTAACTCCGTGCCAGCAGCCGCG     400
                                     |||||
EMBOSS_001      1 -----AGCCGCG                                7
EMBOSS_001    401 GTAATACGGAGGGAGCTAGCGTTGTTTCGGAATTACTGGGCGTAAAGCGCA     450
      |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      8 GTAATACGGAGGGAGCTAGCGTTGTTTCGGAATTACTGGGCGTAAAGCGCA     57
EMBOSS_001    451 CGTAGGCGGCGATTTAAGTCAGAGGTGAAAGCCCGGGGCTCAACCCCGGA     500
      |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001    58 CGTAGGCGGCGATTTAAGTCAGAGGTGAAAGCCCGGGGCTCAACCCCGGA     107
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EMBOSS_001      501 ACTGCCTTTGAGACTGGATTGCTTGAATCCTGGAGAGGTGAGTGGAAATTC      550
      |||
EMBOSS_001      108 ACTGCCTTTGAGACTGGATTGCTTGAATCCTGGAGAGGTGAGTGGAAATTC      157
EMBOSS_001      551 CGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAA      600
      |||
EMBOSS_001      158 CGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAA      207
EMBOSS_001      601 GGCGGCTCACTGGACAGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGC      650
      |||
EMBOSS_001      208 GGCGGCTCACTGGACAGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGC      257
EMBOSS_001      651 AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGATAACTAG      700
      |||
EMBOSS_001      258 AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGATAACTAG      307
EMBOSS_001      701 CTGCCGGGGCACATGGTGTTCGGTGGCGCAGCTAACGCATTAAGTTATC      750
      |||
EMBOSS_001      308 CTGCCGGGGCACATGGTGTTCGGTGGCGCAGCTAACGCATTAAGTTATC      357
EMBOSS_001      751 CGCCTGGGGAGTACGGTCGCAAGATTA AAACTCAAAGGAATTGACGGGGG      800
      |||
EMBOSS_001      358 CGCCTGGGGAGTACGGTCGCAAGATTA AAACTCAAAGGAATTGACGGGGG      407
EMBOSS_001      801 CCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAAC      850
      |||
EMBOSS_001      408 CCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAAC      457
EMBOSS_001      851 CTTACCAACGTTTGACATCCCTATCGCGGATCGTGA-----      886
|||
EMBOSS_001      458 CTTACCAACGTTTGACATCCCTATCGCGGATCGTGGAGACACTTTCCTTC      507
EMBOSS_001      887 -----      886
EMBOSS_001      508 AGTTCGGCTGGATAGGTGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT      557
EMBOSS_001      887 -----      886
EMBOSS_001      558 CGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTTAGT      607
EMBOSS_001      887 -----      886
EMBOSS_001      608 TGCCATCATTTAGTTGGGTACTCTAAAGGAACCGCCGGTGATAAGCCGGA      657
EMBOSS_001      887 -----      886
EMBOSS_001      658 GGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGCGTTGGGCTAC      707
EMBOSS_001      887 -----      886
EMBOSS_001      708 ACACGTGCTACAATGGCGACTACAGTGGGCAGCCACCTCGCGAGAGGGAG      757
EMBOSS_001      887 -----      886
EMBOSS_001      758 CTAATCTCCAAAAGTCGTCTCAGTTCGGATCGTTCTCTGCAACTCGAGAG      807

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EMBOSS_001      887 ----- 886
EMBOSS_001      808 CGTGAAGGCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATA 857
EMBOSS_001      887 ----- 886
EMBOSS_001      858 CGTCCCAGGCCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGATTC 907
EMBOSS_001      887 ----- 886
EMBOSS_001      908 ACTCGAAGGCGTTGAGCTAACCGCAAGGAGGCAGGC 943

```

BLAST RESULT

Sphingobium limneticum strain 301 16S ribosomal RNA gene, partial sequence

Sequence ID: [NR_109484.1](#) Length: 1441 Number of Matches: 1

Range 1: 462 to 1404 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1736 bits(940)	0.0	942/943(99%)	0/943(0%)	Plus/Plus

3.

2. C1P. 26F

```

GTCGAACGGTAAACAGGCTTCGGATGCTGACGAGTGGCGAACGGGTGAGTAATACATCGGAACGTGCCCGAGAGTGGGGGATAACGAAGCGA
AAGCTTTGCTAATACCGCATACGATCTCAGGATGAAAGCAGGGGACCGCAAGGCCTTGCCTCACGGAGCGGCCGATGGCAGATTAGGTAGT
TGGTGGGATAAAAAGCTTACCAAGCCGACGATCTGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATTTGGACAATGGGCGCAAGCCTGATCMAGCCATGCCCGCTGCAGGATGAAGGCCTTCGGGTTGTAAACTGC
TTTTGTACGGAACGAAAAGACTCTGGTTAATACCTGGGGTCCATGACGGTACCCTAAGAATAAGCACGGGCTAACTACGTGCCAGCAGCCGC
GGTAATACGTAGGGTCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCCGAGGCGGTTATATAAGACAGATGTGAAATCCCCGGGCTC
AACCTGGGAACGCATTTGTGACTGTATAGCTAGAGTACGGCAGAGGGGGATGGAATTCGCCGTGTAGCAGTG

```

Acidovorax delafieldii strain 133 16S ribosomal RNA gene, partial sequence

Sequence ID: [NR_028714.1](#) Length: 1515 Number of Matches: 1

Range 1: 54 to 678 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1134 bits(614)	0.0	621/625(99%)	0/625(0%)	Plus/Plus

C1P2. 1520R

```

TCTCTATTCTTAAGATTGAAGAAAAGTTAACATCTGTTTGTCTAATTCAGATAATATTATCTGATTCTACATAGCCNACGTTCCGGATTATT
TCTTGGTGTGTTGTTAAGTTTTCCCTATGACGAGTCAGGCAGTAAGATTTGACATAGTGAATGTGTTCTGTGCAGGGGANATACGACATTA
TGAGAAACTACATGCCGAAAGTTGGTACCCTTGGTCTTGATATGATGCTCCNAACGTGTACTGTTTCAGGTTAGATTAATATTCTGACTTTGC
GCCTTTTCATA

```

REVERSE COMPLEMENT

```

TATGAAAAGGCGCAAAGTCAGAATATTAATCTAACCTGAACAGTACACGTTNGGAGCATCATATCAAGACCAAGGGTACCAACTTTCCGGCAT
GTAGTTTCTCATAATGTCGTATNTCCCTGCACAGAACACATTACACTATGTCAAATCTTACTGCCTGACTCGTCATAGGAAAACCTAACAC
AAACACCAAGAAATAATCCGAACGTNGGCTATGTAGAATCAGATAATATTATCTGAATTAGAAAACAAACAGATGTTAACTTTCTCAATCTT
AAGAATAGAGA

```

EMBOSS (LOCAL ALIGNMENT)

Aligned_sequences: 2

1: EMBOSS_001

2: EMBOSS_001

Matrix: EDNAFULL

Gap_penalty: 10.0

Extend_penalty: 0.5

Length: 685

Identity: 187/685 (27.3%)

Similarity: 187/685 (27.3%)

Gaps: 458/685 (66.9%)

Score: 248.0

```
EMBOSS_001      1  GTCGAACGGTAACAGGTCTTCGGATGCTGACGAGTGGCGAACGGGTGAGT      50
EMBOSS_001      1  -----
EMBOSS_001     51  AATACATCGGAACGTGCCCGAGAGTGGGGGATAACGAAGCGAAAGCTTTG     100
EMBOSS_001      1  -----
EMBOSS_001    101  CTAATACCGCATAACGATCTCAGGATGAAAAGCAGGGGACCGCAAGGCCTTG     150
                                     .||||| | | | |||||.
EMBOSS_001      1  -----TATGAAA--AGG----CGCAAAG-----      17
EMBOSS_001    151  CGCTCACGGAGCGGCCGATGGCAGATTA-----GGTA---GT      184
                                     ||| |.|.||| |.||| ||
EMBOSS_001     18  ---TCA-----GAATATTAATCTAACCTGAACAGTACACGT      50
EMBOSS_001    185  TGGTGGGATAA---AAGCTTACCAAG---CCGACGATC---TGTAGC      222
|.|.|.|.|.|. ||| ||||| ||.|||.|| |||||.
EMBOSS_001     51  TNGGAGCATCATATCAAG---ACCAAGGGTACCAACTTTCGGCATGTAGT      97
EMBOSS_001    223  TGGTCTGAGAGGACGACCAGCCACACTG-----GGACTGAGA      259
                                     | ||| ||.|.|| |.||.|| |
EMBOSS_001     98  T--TCT-----CATAATGTCGTATNTCCCTGCACAGA-A      129
EMBOSS_001    260  CACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACA--AT      307
                                     ||| ||| ||| ||
EMBOSS_001    130  CAC-----ATT----ACACTAT      142
EMBOSS_001    308  GGGCGCAA-----GCCTGA-TCMAGCCATGCCGCGTGCAGGATGAAG      348
                                     | .||| ||||| |||.||| ||||
EMBOSS_001    143  G---TCAAATCTTACTGCCTGACTC--GTCAT-----AGGA-----      173
EMBOSS_001    349  GCCTTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAGACTCTGGTTAA      398
                                     ||||| |.|||.|||.|
```

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EMBOSS_001      174 -----AAACT-----TAACACAAACA-----      189
EMBOSS_001      399 TACCTGGGGTCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGT      448
                               |||.||           |||||  .|||  .|||
EMBOSS_001      190 -----CCAAGA-----AATAA--TCCG-----AACGT      209
EMBOSS_001      449 --GCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGA----A      492
                               ||           ||.||||           |||.||  |
EMBOSS_001      210 NGGC-----TATGTAG-----AATCAGATAATA      232
EMBOSS_001      493 TTA-CTGGGCGTAAAGCGTGCGCAGGCGGTTATATA-AGACAGATGTGAA      540
                               ||| |||  ||           |||.|. | .||| |||.||
EMBOSS_001      233 TTATCTG-----AA-----TTAGAAACAAACAGATGTTAA      262
EMBOSS_001      541 ATCCCCGGGCTCAACCTGGGAACGCATTTGTGACTGTATAGCTAGAGTA      590
                               .|. |  .|||.||  .||           |.||||  |||
EMBOSS_001      263 CTTTC----TTCAATCT--TAA-----GAATAG--AGA---      287
EMBOSS_001      591 CGGCAGAGGGGATGGAATTCCGCGGTAGCAGTG      625
EMBOSS_001      288 -----      287

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b). C1P2. 1492R

```

TGCGGTTAGCTCAACGCCTTCGAGTGAATCCAACCTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCTGGGAACGTATTACCGCGGCATGC
TGATCCGCGATTACTAGCGATTCCGCTTCACGCTCTCGAGTTGCAGAGAACGATCCGAACTGAGACGACTTTTGGAGATTAGCTCCCTCTC
GCGAGGTGGCTGCCACTGTAGTCGCCATTGTAGCAGCTGTGTAGCCCAACGCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCT
CCGGCTTATCACCAGCGGTTCTTTAGAGTACCCAACCTAAATGATGGCAACTAAAGGCGAGGGTTGCGCTCGTTGCGGGACTTAAACCAACA
TCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCACCTATCCAGCCGAACTGAAGGAAAGTGTCTCCACGATCCGCGATAGGGATG
TCAAACGTTGGTAAGGTTCTGCGCGTTGCTTCGAATTAACCACATGCTCCACCGCTTGTGCAGGCCCCCGTCAATTCCTTTGAGTTTAAAT
CTGCGACCGTACTCCCAGGCGGATAACTTAATGCGTTAGCTGCGCCACCGAAACACCATGTGCCCGGCAGCAGNAGTTATCATCGTTTACG
GCGTGGACTACCAGGTTATCTAATCCTGTTTGTCTCCCACGCTTTCGCACCTCAGCGTCAATACCTGTCCAGTGAGCCGCTTCGCCACTGG
TGTTCTCCGAATATCTACGAATTCACCTCTACACTCGGAATTCACCTCACCTCTCCAGGA

```

REVERSE COMPLEMENT

```

TCCTGGAGAGGTGAGTGAATTCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGGCTCACTGGACAGGT
ATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATAACTNGCTGCCGGGCA
CATGGTGTTCGGTGGCGCAGCTAACGCATTAAGTTATCCGCTGGGGAGTACGGTCGCAAGATTAACAACTCAAAGGAATTGACGGGGCCCT
GCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACGCGCAGAACCTTACCAACGTTTGACATCCCTATCCGCGGATCGTGGAGACACTTT
CCTTCAGTTCCGGCTGGATAGGTGACAGGTGCTGCATGGCTGTCGTGAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC
CCTCGCTTTAGTTGCCATCATTAGTTGGGTACTCTAAAGGAACCGCGGTGATAAGCCGAGGAAGTGGGGATGACGTCAAGTCCCTCAT
GGCCCTTACGCGTTGGGTACACACGTGCTACAATGGCGACTACAGTGGGCGCCACCTCGCGAGAGGGAGCTAATCTCCAAAAGTCTGCTC
AGTTCCGATCGTTCTCTGCAACTCGAGAGCGTGAAGGCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCAGGCCT
TGTACACACCGCCCGTACACCATGGGAGTTGATTCACTCGAAGGCGTTGAGCTAACCGCA

```

EMBOSS (LOCAL ALIGNMENT)

```

# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EDNAFULL
# Gap_penalty: 10.0

```

```

# Extend_penalty: 0.5
# Length: 938
# Identity:      378/938 (40.3%)
# Similarity:   378/938 (40.3%)
# Gaps:         453/938 (48.3%)
# Score: 609.0

EMBOSS_001      1  -----GTCGAACGGTAA-----CAGG      16
|.|| | |||. | .||.
EMBOSS_001      1  TCCTGGAGAGGTGAGTGAATTCCGAGTGTAGA--GGTGAAATTCGTAGA      48
EMBOSS_001     17  TCTTCGGATGCTGACGAGTGGCGAA--CGGGTGA-----GTAATACA      56
      |.|||||.|. . . .|. ||||||| | |||. | |||. |
EMBOSS_001     49  TATTCGGAAGAACACCAGTGGCGAAGCGGCTCACTGGACAGGTATT---      95
EMBOSS_001     57  TCGGA-----ACGTGCCCGAGAGTGGGG-----GATA-----      83
      || |.|||||. . .|. ||||||| ||||
EMBOSS_001     96  ---GACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCT      142
EMBOSS_001     84  -----ACG-----AAGCGA-----AA---GCT-----T      98
      ||| ||.||| || |||
EMBOSS_001    143  GGTAGTCCACGCCGTAAACGATGATAACTNGCTGCCGGGGCACATGGTGT      192
EMBOSS_001     99  T-----GCTAATACCGCA-----TACGA      116
| ||||| |||| ||||.
EMBOSS_001    193  TTCGGTGGCGCAGCTAA---CGCATTAAGTTATCCGCCTGGGGAGTACGG      239
EMBOSS_001    117  TCTCAGGATGAAAGC----AGG---GGACCGCAAGGCCTTGCCTCACGG      159
      ||.|||.|||.|||. | ||| .|||. | .||| | ||. ||
EMBOSS_001    240  TCGCAAGATTAATACTCAAAGGAATTGACGG--GGGCCT---GCACA---      281
EMBOSS_001    160  AGCGGCCGATGGCAG-ATTAGGTAGTTGGTGGGATAA-----      195
      ||||| ||| || | ||||. . |||
EMBOSS_001    282  AGCGG----TGG-AGCAT-----GTGGTTTAATTCGAAGCAACGC      316
EMBOSS_001    196  ----AAGCTTACCAAGCC----GACGAT--CTGTAGCTGGTCTGAGAGGA      235
      ||.||||||| | ||| || |||.|||. |. |||
EMBOSS_001    317  GCAGAACCTTACCAA--CGTTTGAC-ATCCCTATCGCGGATC---GTGGA      360
EMBOSS_001    236  CGAC-----CAGCCACACTGG-----GAC---TGAGACACGGC--      265
      ||| |||. . . . ||| ||| ||. . . ||. |||
EMBOSS_001    361  -GACACTTTCCTTCAGTTCGGCTGGATAGGTGACAGGTGCTGCATGGCTG      409
EMBOSS_001    266  ---CCAGACTCCTACGGGAGGCAGCAGTGGGGA-ATTTTGG-----      302
      .||| |||. | |||. | |||. |
EMBOSS_001    410  TCGTCAG-CTCGT-----GTCGTGAGATGTTGGGTTAAGTCC      445

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EMBOSS_001      303 -ACAATGGGCGCAAGCCT-----GATCMAGCCAT----- 330
. |||.|.|||||.|||      |.|  |||||
EMBOSS_001      446 CGCAACGAGCGCAACCCTCGCCTTTAGTT---GCCATCATTAGTTGGGT 492
EMBOSS_001      331 -----GCCCGGT-----GCAGGATGAAGGCCTTCGGGTTGT 361
                        |||| |  ||.||||.|||||  |.||||.||
EMBOSS_001      493 ACTCTAAAGGAACCGCCG-GTGATAAGCCGGAGGAAG---TGGGGATG- 537
EMBOSS_001      362 AAAC TGCTTTTGTACGGAACGAAAAGACTCTGGTTAATACCTGGGGTCCA 411
|||.|||||.||  |.||||  ||. EMBOSS_001      538 -----
ACGTCAAGTC-CT-----CATGG---CCC 557
EMBOSS_001      412 TGACGGTACCGTAAGAATAAGCACCGGCTA-----ACTAC-GTGCCA 452
|.||||  |||.|.|.|||||.||||  ||||| ||| .
EMBOSS_001      558 TTACG----CGTTGGGCTACACACGTGCTACAATGGCGACTACAGTG--G 601
EMBOSS_001      453 GCAGCC-----GCGGTAAT-----ACGTAG-----GG 474
      |||||  |.|.||||  |.|||.  ||
EMBOSS_001      602 GCAGCCACCTCGCGAGAGGGAGCTAATCTCCAAAAGTCGTCTCAGTTCGG 651
EMBOSS_001      475 -----TGCA-----AGCGTTAA-TCGGAATTACTGG-----GCG 502
                        ||||  |||||.|| .|||||.|.|||.  |||
EMBOSS_001      652 ATCGTTCTCTGCAACTCGAGAGCGTGAAGGCGGAATCGCTAGTAATCGCG 701
EMBOSS_001      503 TA-AAGCGTGCGCAGCGGTTATATAAG---ACAG---ATGT-GAAATCC 544
.| .||||.||||  .|||||.|| |||.|  .|||  .|||  .|.|.|.
EMBOSS_001      702 GATCAGCATGC---CGCGGTGA-ATACGTTCCAGGCCTTGTACACACCG 747
EMBOSS_001      545 CCGGGCTCAACCTGGGAACTGCATTTGTGACT-----GTATAGCTAGA 587
      |||.|.|||||.|||||.|||.||  |.||||  ||.|||||
EMBOSS_001      748 CCCGTCACACCATGGGAGTTGGAT---TCACTCGAAGGCGTTGAGCTA-- 792
EMBOSS_001      588 GTACGGCAGAGGGGATGGAATCCGCGTGTAGCAGTG 625
      ||.||||
EMBOSS_001      793 --ACGCA----- 798

```

BLAST RESULT

Sphingobium limneticum strain 301 16S ribosomal RNA gene, partial sequence

Sequence ID: [NR_109484.1](#) Length: 1441 Number of Matches: 1

Range 1: 596 to 1393 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1465 bits(793)	0.0	796/798(99%)	0/798(0%)	Plus/Plus

4.

C1N. 26F

```
ATGCAGTCGAACGGTAACAGGTCTTCGGATGCTGACGAGTGGCGAACGGGTGAGTAATACATCGGAACGTGCCCGAGAGTGGGGGATAACGA
AGCGAAAAGCTTTGCTAATACCGCATAACGATCTCAGGATGAAAAGCAGGGGACCGCAAGGCCTTGCCTCACGGAGCGGCCGATGGCAGATTAG
GTAGTTGGTGGGATAAAAGCTTACCAAGCCGACGATCTGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCAGAC
TCCTACGGGAGGCAGCAGTGGGAATTTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCTGCAGGATGAAGGCCTTCGGTTGTAA
ACTGCTTTTGTACGGAACGAAAAGACTCTGGTTAATACCTGGGGTCCATGACGGTACCCTAAGAATAAGCACCGGCTAACTACGTGCCAGCA
GCCGCGTAATACGTAGGGTGAAGCGTTAATCGGAATTAAGCGTAAAGCGTGGCAGGCGGTTATATAAGACAGATGTGAAATCCCCG
GGCTCAACCTGGGAACCTGCATTGTGACTGTATAGCTAGAGTACGGCAGAGGGGGATGGAATTCGCGGTGAGCAGTGAATGCGGTAGATAT
GCGGAGGAACACCGATGGCGAAGGCAATCCCTGGGCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGCAAAACAGGATTAGATACCTTG
GTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCAGTAACGAAGCTAACGCGTGAAGTTGACCGCCTGGGAGTA
CGGCCGCAAGGTTGAACTCAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGTTAATTCGATGCAACCGGAAAAACCTTAC
CCACCTTTGACATGTACGGAAT
```

BLAST RESULT

Acidovorax delafieldii strain 133 16S ribosomal RNA gene, partial sequence

Sequence ID: [NR_028714.1](#) Length: 1515 Number of Matches: 1

Range 1: 48 to 990 [GenBank](#) [Graphics](#)

[Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1712 bits(927)	0.0	938/943(99%)	1/943(0%)	Plus/Plus

C1N2. 1520R

```
ACCCAGTCACGAACCTGCCGTGGTAATCGCCCTCCTTGCGGTTAGGCTAACTACTTCTGGCAGAACCCTCCATGGTGTGACGGGCGG
TGTGTACAAGACCCGGGAACGTATTACCGCGACATTCTGATCCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGA
TCCGGACTACGACTGGCTTTATGGGATTGGCTCCCCCTCGCGGGTTGGCAACCCTCTGTACCAGCCATTGTATGACGTGTGTAGCCCCACCT
ATAAGGGCCATGAGGACTTGACGTCAATCCACCTTCCCTCCGTTTGTACCGGCAGTCTCATTAGAGTGCCCACTGAATGTAGCAACTAA
TGACAAGGGTTGCGCTCGTTGCGGGACTTAACCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTTACGGTTCTCTTT
CGAGCACTCCTCTATCTCTAAAGGATTCGATCATGTCAAAGGTGGGTAAGGTTTTTCGCGTTGCATCGAATTAACACATCATCCACGC
TTGTGCGGGTCCCGTCAATTCCTTTGAGTTTCAACCTTGGCGCGTACTCCCGAGGCGTCAACTTCACGCGTTAGCTTCGTTACTGAGTC
AGTGAAGACCAACAACAGTTGACATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCATGAGCG
TCAGTACAGGCCAGGGGATTGCTTCCGATCGGTTTCCCTCCGATATCTACGCATTTCACTGCTACACGCGGAATTCATCCCCCTCTG
CCGTACTCTAGCTATACAGTCACAAATGCGATTCCAGGTTGAGCCCGGGGATTTACATCTGTCTTATATAACCGCTGCGCACGCTTTAC
GCCAGTAATTCGATTAACGCTTGACCCCTACGTA
```

REVERSE COMPLEMENT

```
TACGTAGGGTGCAAGCGTTAATCGGAATTAAGCGTGGCAGGCGGTTATATAAGACAGATGTGAAATCCCGGGCTCAACCT
GGGAACGTGCATTTGTGACTGTATAGCTAGAGTACGGCAGAGGGGATGGAATTCGCGGTGAGCAGTGAATGCGTAGATATGCGGAGGAAC
ACCGATGGCGAAGGCAATCCCTGGGCTGTACTGACGCTCATGCACGAAAGCGTGGGAGCAAAACAGGATTAGATACCTGGTAGTCCACG
CCCTAAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCAGTAACGAAGCTAACGCGTGAAGTTGACCGCTGGGGAGTACGGCCGCAAG
GTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGTTAATTCGATGCAACCGGAAAAACCTTACCCACCTTTGA
CATGTACGGAATCCTTTAGAGATAGAGGAGTGCCTCGAAAGAGAACCCTAACACAGGTGCTGCATGGCTGTCGTGAGTGTGAGATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCAATAGTTGGTACATTCAGTTGGGCACTAATGAGACTGCCGGTGACAAACCGGAGG
AAGGTGGGGATGACGTCAAGTCTCATGGCCCTTATAGGTGGGCTACACAGTCATAACAATGGCTGGTACAGAGGGTTGCCAACCCGCGAG
GGGGAGCAATCCATAAAGCCAGTCTGATCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAAATCGCTAGTAATCGCGGATCAGA
ATGTCGCGGTGAATACGTTCCCGGGTCTTGTACACACCCTGTCACACCATGGGAGCGGGTTCTGCCAGAAGTAGTTAGCCTAACCCGAAG
GAGGGCGATTACCACGGCAGGGTTCTGTGACTGGGGT
```

Aligned_sequences: 2

1: EMBOSS_001


```

# 2: EMBOSS_001
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
# Length: 1426
# Identity:      472/1426 (33.1%)
# Similarity:    472/1426 (33.1%)
# Gaps:          954/1426 (66.9%)
# Score: 2360.0

EMBOSS_001      1 ATGCAGTCGAACGGTAACAGGTCTTCGGATGCTGACGAGTGGCGAACGGG      50
EMBOSS_001      1 -----                                                    0
EMBOSS_001     51 TGAGTAATACATCGGAACGTGCCCGAGAGTGGGGGATAACGAAGCGAAAG    100
EMBOSS_001      1 -----                                                    0
EMBOSS_001    101 CTTTGCTAATACCGCATACGATCTCAGGATGAAAGCAGGGGACCGCAAGG    150
EMBOSS_001      1 -----                                                    0
EMBOSS_001    151 CCTTGCCTCACGGAGCGGCCGATGGCAGATTAGGTAGTTGGTGGGATAA    200
EMBOSS_001      1 -----                                                    0
EMBOSS_001    201 AAGCTTACCAAGCCGACGATCTGTAGCTGGTCTGAGAGGACGACCAGCCA    250
EMBOSS_001      1 -----                                                    0
EMBOSS_001    251 CACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGA    300
EMBOSS_001      1 -----                                                    0
EMBOSS_001    301 ATTTTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCCGGTGCAGGATG    350
EMBOSS_001      1 -----                                                    0
EMBOSS_001    351 AAGGCCTTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAGACTCTGGT    400
EMBOSS_001      1 -----                                                    0
EMBOSS_001    401 TAATACCTGGGGTCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTA    450
EMBOSS_001      1 -----                                                    0
EMBOSS_001    451 CGTGCCAGCAGCCCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTA    500
EMBOSS_001      1 -----TACGTAGGGTGCAAGCGTTAATCGGAATTA                    30
EMBOSS_001    501 CTGGGCGTAAAGCGTGCGCAGGCGGTTATATAAGACAGATGTGAAATCCC    550
EMBOSS_001      1 -----                                                    0
EMBOSS_001    31 CTGGGCGTAAAGCGTGCGCAGGCGGTTATATAAGACAGATGTGAAATCCC    80
EMBOSS_001    551 CGGGCTCAACCTGGGAACTGCATTTGTGACTGTATAGCTAGAGTACGGCA    600
EMBOSS_001      1 -----                                                    0
EMBOSS_001    81 CGGGCTCAACCTGGGAACTGCATTTGTGACTGTATAGCTAGAGTACGGCA    130

```

EMBOSS_001	601	GAGGGGGATGGAATTCGCGTGTAGCAGTGAAATGCGTAGATATGCGGAG	650
EMBOSS_001	131	GAGGGGGATGGAATTCGCGTGTAGCAGTGAAATGCGTAGATATGCGGAG	180
EMBOSS_001	651	GAACACCGATGGCGAAGGCAATCCCCTGGGCCTGTACTGACGCTCATGCA	700
EMBOSS_001	181	GAACACCGATGGCGAAGGCAATCCCCTGGGCCTGTACTGACGCTCATGCA	230
EMBOSS_001	701	CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCT	750
EMBOSS_001	231	CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCT	280
EMBOSS_001	751	AAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCAGTAACGAAGCTA	800
EMBOSS_001	281	AAACGATGTCAACTGGTTGTTGGGTCTTCACTGACTCAGTAACGAAGCTA	330
EMBOSS_001	801	ACGCGTGAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAA	850
EMBOSS_001	331	ACGCGTGAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTGAAACTCAA	380
EMBOSS_001	851	AGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGTTTAATTCGA	900
EMBOSS_001	381	AGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGTTTAATTCGA	430
EMBOSS_001	901	TGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAAT-----	942
EMBOSS_001	431	TGCAACGCGAAAAACCTTACCCACCTTTGACATGTACGGAATCCTTTAGA	480
EMBOSS_001	943	-----	942
EMBOSS_001	481	GATAGAGGAGTGCTCGAAAGAGAACCGTAACACAGGTGCTGCATGGCTGT	530
EMBOSS_001	943	-----	942
EMBOSS_001	531	CGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC	580
EMBOSS_001	943	-----	942
EMBOSS_001	581	CCTTGTCAATTAGTTGCTACATTAGTTGGGCACTCTAATGAGACTGCCGG	630
EMBOSS_001	943	-----	942
EMBOSS_001	631	TGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCTCATGGCCCTTAT	680
EMBOSS_001	943	-----	942
EMBOSS_001	681	AGGTGGGGCTACACACGTCATAACAATGGCTGGTACAGAGGGTTGCCAACC	730
EMBOSS_001	943	-----	942
EMBOSS_001	731	CGCGAGGGGGAGCCAATCCATAAAGCCAGTCGTAGTCCGGATCGCAGTC	780
EMBOSS_001	943	-----	942
EMBOSS_001	781	TGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGGGATCAGAAT	830

```

EMBOSS_001      943 ----- 942
EMBOSS_001      831 GTCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCAT 880
EMBOSS_001      943 ----- 942
EMBOSS_001      881 GGGAGCGGGTTCGCCAGAAGTAGTTAGCCTAACCGCAAGGAGGGCGATT 930
EMBOSS_001      943 ----- 942
EMBOSS_001      931 ACCACGGCAGGGTTCGTGACTGGGGT 956

```

BLAST RESULT

Acidovorax delafieldii strain 133 16S ribosomal RNA gene, partial sequence

Sequence ID: [NR_028714.1](#) Length: 1515 Number of Matches: 1

Range 1: 519 to 1474 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1744 bits(944)	0.0	952/956(99%)	0/956(0%)	Plus/Plus

C11N. 1492R

```

CCTGCCTCCTTGCGGTTAGCTCAACGCCTTCGAGTGAATCCAAC'TCCCATGGTGTGACGGGCGGTGT
GTACAAGGCCTGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGCC'TTCA
CGCTCTCGAGTTGCAGAGAACGATCCGAACTGAGACGACTTTTGGAGATTAGCTCCCTCTCGCGAG
GTGGCTGCCCACTGTAGTCGCCATTGTAGCACGTGTGTAGCCCAACGCGTAAGGGCCATGAGGACT
TGACGTCATCCCCACCTTCCTCCGGCTTATCACCGCGGTTCC'TTTAGAGTACCCAAC'TAAATGAT
GGCAACTAAAGGCGAGGGTTGCGCTCGTTGCGGGACTTAAACCAACATCTCACGACACGAGCTGAC
GACAGCCATGCAGCACCTGTACCTATCCAGCCGAACTGAAGGAAAGTGTCTCCACGATCCGCGAT
AGGGATGTCAAACGTTGGTAAGGTTCTGCGCGTTGCTT'CGAATTAACCACATGCTCCACCGCTTG
TGCAGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGATAACTTAA
TGCGTTAGCTGCGCCACCGAAACACCATGTGCCCGGCAGCTAGTTATCATCGTTTACGGCGTGGA
CTACCAGGGTATCTAATCCTGTTTGCTCCCCACGTTTTCGCACCTCAGCGTCAATACCTGTCCAGT
GAGCCGCCTTCGCCACTGGTGTCTTCCGAATATCTACGAATTTACCTCTACACTCGGAAT'TCCA
CTCACCTCTCCAGGATTCAAGCAATCCAGTCTCAAAGGCAGTTCGGGGTTGAGCCCCGGGCTTTC
ACCTCTGACTTAATCGCCGCCTACGTGCGCTTTACGCCAGTAATTC

```

REVERSE COMPLEMENT

EMBOSS (LOCAL ALIGNMENT)

```

# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
# Length: 1405
# Identity:      368/1405 (26.2%)
# Similarity:   368/1405 (26.2%)

```

Gaps: 962/1405 (68.5%)

Score: 1476.0

```
EMBOSS_001      1 ATGCAGTCGAACGGTAAACAGGTCTTCGGATGCTGACGAGTGGCGAACGGG      50
EMBOSS_001      1 -----                                                    0
EMBOSS_001     51 TGAGTAATACATCGGAACGTGCCCGAGAGTGGGGGATAACGAAGCGAAAG     100
EMBOSS_001      1 -----                                                    0
EMBOSS_001    101 CTTTGCTAATACCGCATAACGATCTCAGGATGAAAGCAGGGGACCGCAAGG     150
EMBOSS_001      1 -----                                                    0
EMBOSS_001    151 CCTTGCCTCACGGAGCGGCCGATGGCAGATTAGGTAGTTGGTGGGATAA     200
EMBOSS_001      1 -----                                                    0
EMBOSS_001    201 AAGCTTACCAAGCCGACGATCTGTAGCTGGTCTGAGAGGACGACCAGCCA     250
EMBOSS_001      1 -----                                                    0
EMBOSS_001    251 CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGA     300
EMBOSS_001      1 -----                                                    0
EMBOSS_001    301 ATTTTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGCAGGATG     350
EMBOSS_001      1 -----                                                    0
EMBOSS_001    351 AAGGCCTTCGGGTGTAAACTGCTTTTGTACGGAACGAAAAGACTCTGGT     400
EMBOSS_001      1 -----                                                    0
EMBOSS_001    401 TAATACCTGGGGTCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTA     450
EMBOSS_001      1 -----                                                    0
EMBOSS_001    451 CGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTA     500
                                                    |||||
EMBOSS_001      1 -----GAATTA                                           6
EMBOSS_001    501 CTGGGCGTAAAGCGTGCGCAGGCGGTTATATAAGACAGATGTGAAATCCC     550
      |||||.....|..|.....|..| ||||..|||..|.....|..|
EMBOSS_001      7 CTGGGCGTAAAGCGCACGTAGGCGCGAT-TAAGTCAGAGGTGAAAGCCC     55
EMBOSS_001    551 CGGGCTCAACCTGGGAAGTGCATTTGTGACTGTATAGCTAGAGTACGGCA     600
      .||.....|..|.....|..|..|.....|..|..|..|..|..|..|
EMBOSS_001     56 GGGGCTCAACCCCGGAAGTGCCTTTGAGACTGGATTGCTTGAATCCTGGA     105
EMBOSS_001    601 GAGGGGATGGAATTCGCGTGTAGCAGTGAAATGCCGTAGATATGCCGAG     650
|||..|..|.....|..|.....|..|.....|..|.....|..|.....|..|
EMBOSS_001    106 GAGGTGAGTGAATTCAGAGTGTAGAGGTGAAATTCGTAGATATTCGGAA     155
EMBOSS_001    651 GAACACCGATGGCGAAGGCAATCCCCTGGGCCTGTACTGACGCTCATGCA     700
||.....|..|.....|..|.....|..|.....|..|.....|..|..|..|
EMBOSS_001    156 GAACACCAGTGGCGAAGGCGGCTCACTGGACAGGTATTGACGCTGAGGTG     205
EMBOSS_001    701 CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCT     750
```


BLAST RESULT

Sphingobium limneticum strain 301 16S ribosomal RNA gene, partial sequence

Sequence ID: [NR_109484.1](#) Length: 1441 Number of Matches: 1

Range 1: 497 to 1403 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1663 bits(900)	0.0	905/907(99%)	1/907(0%)	Plus/Plus

5.

Table 1: After three weeks, the primary root length (cm/plant) of tomato plant (Gemini original) with control without bacteria and bacterial strains WCS417r and *Sphingobium limneticum* were measured using ruler. The average, standard deviation and standard error were calculated. n=7

	Gem-C	Ger-WCS417r	Gem-SPH
	21	19.5	16
	22	13	17.5
	16.8	18.5	17.6
	19.2	15.7	15
	19.5	16	19
	19	13	13
	14	18	21
Mean	18.79	16.24	17.01
Stdev.	2.67	2.59	2.63
SE	1.01	0.98	1.00

Table 2: After three weeks, the shoot fresh weight of tomato plant (Gemini original) with control without bacteria and bacterial strains WCS417r and *Sphingobium limneticum* were weighed. The average, standard deviation and standard error were calculated. n=7

	Gem-C	Gem-WCS417r	Gem-SPH
	0.7509	0.6782	0.6148
	1.0436	0.6855	0.9588

	1.3632	0.6273	1.0066
	0.8224	1.0263	0.0723
	0.7132	0.5775	0.9272
	0.6686	0.5608	0.6045
	0.685	0.7853	0.8501
Mean	0.864	0.706	0.719
Stdev.	0.255	0.160	0.327
SE	0.096	0.061	0.124

6.

Table 3: Different bacterial strains for experiment 2 with optical density measurements

Bacterial strains	Optical Density (O. D₆₀₀)
5	0.492
6	0.459
9	0.691
10	0.404
15	0.455
16	0.530
18	0.637
C18	0.266

Table 4: Different bacterial strains for experiment 4 with optical density measurements

Bacterial strains	Optical Density (O. D₆₀₀)
5	0.272
6	0.515
9	0.492
10	0.467
15	0.675
16	0.515
18	0.515
C18	0.125

7.

Table 5: (Exp2) After six days, the primary root length (cm/plant) of wild type Arabidopsis (WT) with control without sucrose and with no bacterial and wild type with different bacterial strains were measured using image J. The average, standard deviation and standard error were calculated then there 5 plants per treatment as shown below.

	Control	WT BACT 5	WT BACT 6	WT BACT 9	WT BACT 10	WT BACT 15	WT BACT 16	WT BACT 18	WT BACT cl8	
		9.59	7.48	11.28	8.66	9.03	7.66	7.15	9.12	
		9.99	9.49	11.29	8.22	7.25	6.66	10.53	9.86	
		9.86	7.55	8.88	8.07	4.7	5.01	10.58	9.41	
		8.85	10.12	6.34	8.85	8.25	3.87	8.8	9.22	
		8.02	8.74	9.71	10.32	8.1	9.3	3.14	9.16	
Mean		9.23	6.68	8.68	9.50	8.82	7.47	6.50	8.04	9.35
Stdev		0.82	2.81	1.17	2.05	0.89	1.67	2.14	3.08	0.30
SE		0.36	1.26	0.52	0.92	0.40	0.75	0.96	1.38	0.14

Table 6 : (Exp. 4) The experiment was repeated (table 5)

	Control WT	WT+5	WT+6	WT+9	WT+10	WT+15	WT+16	WT+18	WT+CL8
	3.56	6.03	6.55	4.64	5.49	5.51	5.41	4.66	6.30
	1.91	5.36	6.21	6.31	5.20	7.18	7.76	6.57	6.35
	7.50	6.34	6.85	6.24	6.27	6.43	3.77	2.92	7.22
	5.59	6.39	6.67	6.91	4.88	8.11	3.53	6.86	5.78
	2.79	2.74	5.98	5.83	3.03	3.86	4.10	7.22	2.67
Mean	4.27	5.37	6.45	5.99	4.97	6.22	4.92	5.64	5.66
Stdev.	2.26	1.53	0.35	0.85	1.20	1.63	1.75	1.82	1.75
SE	1.01	0.68	0.16	0.38	0.54	0.73	0.78	0.81	0.78

Table 7: (Exp. 2) After six days, the numbers of lateral root of wild type with control without sucrose and without bacterial and wild type with different bacterial strains were counted. The average, standard deviation and standard error were calculated then there were 5 plants per treatment as shown below.

	Control WT	WT BACT 5	WT BACT 6	WT BACT 9	WT BACT 10	WT BACT 15	WT BACT 16	WT BACT 18	WT BACT cl8
	22	18	12	22	13	14	10	11	14
	29	20	17	23	8	8	12	17	13
	25	17	8	12	11	16	11	20	18
	19	10	19	13	14	14	8	14	28
	19	0	13	11	21	15	14	2	26
Mean	22.80	13.00	13.80	16.20	13.40	13.40	11.00	12.80	19.80
Stdev	4.27	8.19	4.32	5.81	4.83	3.13	2.24	6.91	6.87
SE	1.91	3.66	1.93	2.60	2.16	1.40	1.00	3.09	3.07

Table 8: (Exp.4) The experiment was repeated (table 7)

	Control WT	WT+5	WT+6	WT+9	WT+10	WT+15	WT+16	WT+18	WT+CL8
	1	6	11	2	7	7	2	4	12
	1	4	8	14	4	12	17	10	11
	17	7	14	10	9	12	11	1	18
	7	5	12	16	6	17	9	11	9
	1	1	9	11	1	16	12	14	5
Mean	5.4	4.6	10.8	10.6	5.4	12.8	10.2	8	11
Stdev.	6.99	2.30	2.39	5.37	3.05	3.96	5.45	5.34	4.74
SE	3.12	1.03	1.07	2.40	1.36	1.77	2.44	2.39	2.12

Table 9: (Exp. 2) After six days, the numbers of lateral root of WT with control without sucrose and without bacterial and WT with different bacterial strains were counted. The average of lateral root per plants were calculated by dividing the mean of lateral root with the average mean of the primary root length, standard deviation and standard error were calculated then there were 5 plants per treatment as shown below.

	Control WT	WT BACT 5	WT BACT 6	WT BACT 9	WT BACT 10	WT BACT 15	WT BACT 16	WT BACT 18	WT BACT cl8
	22	18	12	22	13	14	10	11	14
	29	20	17	23	8	8	12	17	13
	25	17	8	12	11	16	11	20	18
	19	10	19	13	14	14	8	14	28
	19	0	13	11	21	15	14	2	26
Mean (lateral root/plants)	22.80	13.00	13.80	16.20	13.40	13.40	11.00	12.80	19.80
Mean (primary roo length cm/plants)	9.23	6.68	8.68	9.50	8.82	7.47	6.50	8.04	9.35
Mean (lateral root cm/plants)	2.47	1.95	1.58	1.71	1.52	1.79	1.69	1.59	2.12

Table 10: (Exp. 4) The experiment was repeated (table 9)

	Control WT	WT +5	WT +6	WT +9	WT +10	WT +15	WT +16	WT +18	WT +CL8
	1	6	11	2	7	7	2	4	12
	1	4	8	14	4	12	17	10	11
	17	7	14	10	9	12	11	1	18
	7	5	12	16	6	17	9	11	9
	1	1	9	11	1	16	12	14	5
Mean	5.4	4.6	10.8	10.6	5.4	12.8	10.2	8	11
(lateral root/plants)									
Mean (primary root length cm/plants)	4.27	5.37	6.45	5.99	4.97	6.22	4.92	5.64	5.66
Mean (lateral root cm/plants)	1.26	0.86	1.67	1.77	1.09	2.06	2.07	1.42	1.94

Table 11: Different bacterial strains for experiment 1 with optical density measurements

Bacterial strains	Optical Density (O. D₆₀₀)
9	0.449, 0.500
15	0.514

Table 12: Different bacterial strains for experiment 3 with optical density measurements

Bacterial strains	Optical Density (O. D₆₀₀)
9	0.570, 0.629
15	0.614, 0.617

Table 13: Different bacterial strains for experiment 5 with optical density measurements

Bacterial strains	Optical Density (O. D₆₀₀)
15	0.500, 0.514, 0.525

Table 14: (Exp. 1) After 8 days, the primary root length (cm/plant) of C2, C2C4, C2C5 with a control without sucrose and bacterial and C2, C2C4, C2C5 without sucrose and with bacterial strains 9 and 15 were measured using image J. The average, standard deviation and standard error were calculated then there were 6 plants per treatment as shown below.

	C2 +C (cm)	C2 +9 (cm)	C2 +15 (cm)	C2C4 +C (cm)	C2C4 +9 (cm)	C2C4 +15 (cm)	C2C 5+C (cm)	C2C5 +9 (cm)	C2C5 +15 (cm)
	9.50	9.03	5.14	3.84	2.90	4.39	5.93	4.72	6.00
	7.53	9.91	3.73	6.94	7.38	6.00	5.28	6.56	6.90
	6.38	10.12	9.20	7.03	7.46	6.50	7.13	7.49	2.64
	8.60	3.56	7.17	7.66	7.40	5.53	8.08	6.64	7.37
	6.00	9.78	5.34	7.36	8.21	6.87	9.26	5.20	2.37
	7.31	5.72	3.21	6.63	6.71	5.72	7.59	6.11	7.92
Mean	7.55	8.02	5.63	6.58	6.68	5.84	7.21	6.12	5.53
Stdev.	1.32	2.73	2.23	1.39	1.91	0.87	1.45	1.01	2.43
SE	0.54	1.11	0.91	0.57	0.78	0.35	0.59	0.41	0.99

Table 15: (Exp. 3) After six days, the primary root length (cm/plant) of C2 with control without sucrose and without bacterial and C2 with different bacterial strains were measured using image J. The average, standard deviation and standard error were calculated, then there were 5 plants per treatment as shown below.

	Control C2	C2 + 9	C2 + 15	C2 + CL8
	6.03	2.75	7.57	7.11
	5.16	6.74	5.67	7.28
	6.64	6.48	7.77	6.11
	6.56	8.24	6.92	6.58
	6.57	6.77	7.06	7.17
MEAN	6.19	6.20	7.00	6.85
Stdev.	0.63	2.05	0.82	0.49
SE	0.28	0.92	0.37	0.22

Table 16: (Exp. 3) The experiment was repeated for C4 (table 15)

	Control C4	C4 + 9	C4 + 15	C4 + CL8
	6.03	7.20	6.08	2.04
	4.44	6.62	5.79	3.33
	3.21	6.26	5.06	5.94
	5.89	5.38	6.61	7.18
	5.73	3.54	6.36	5.44
MEAN	5.06	5.80	5.98	4.79
Stdev.	1.21	1.43	0.60	2.07
SE	0.54	0.64	0.27	0.93

Table 17: (Exp. 3) The experiment was repeated for C2C4 (table 15)

Number of Samples	Control C2C4	C2C4 + 9	C2C4 +15	C2C4 + CL8
	2.33	3.62	5.50	5.60
	4.96	6.14	3.23	4.73
	5.89	1.77	4.53	1.67
	5.65	1.84	5.49	5.00
	1.38	4.85	5.59	4.05
MEAN	4.04	3.65	4.87	4.21
Stdev.	2.05	1.90	1.01	1.52
SE	0.92	0.85	0.45	0.68

Table 18: (Exp. 3) The experiment was repeated for C2C5 (table 15)

	Control C2C5	C2C5 + 9	C2C5 + 15	C2C5 + CL8
	5.94	5.31	4.41	1.75
	6.03	5.31	5.14	5.14
	4.61	5.19	5.22	4.30
	5.04	5.72	1.74	4.15
	5.15	5.19	2.21	5.16
MEAN	5.36	5.34	3.74	4.10
Stdev.	0.61	0.22	1.66	1.40
SE	0.27	0.10	0.74	0.62

Table 19: (Exp. 5) After 8 days, the primary root length (cm/plant) of selected eighteen out of twenty plants of C2, and C2C4 with control without sucrose and without bacterial and C2, and C2C4 with bacterial strain 15 were measured using image J. The average, standard deviation was calculated, then there were eighteen plants per treatments.

	Control C2	C2 +15	Control C2C4	C2C4 +15
	7.07	7.29	2.12	5.64
	7.2	6.68	5.31	5.13
	5.22	5	5.85	5.65
	3.98	5.51	4.38	5.74
	1.84	6.51	5.15	4.58
	3.69	5.71	6.71	5.88
	3.61	6.4	2.83	4.82
	3.85	6.28	5.96	5.84
	2.23	6.05	6.45	4.8

	2.73	6.64	2.06	5.16
	3.38	6.42	4.12	5.11
	6.2	5.15	6.36	4.83
	2.61	5.69	5.5	4.36
	5.88	7.84	6.33	5.07
	5.89	7.33	7.13	4.35
	7.17	7	6.49	4.16
	7.91	7.86	5.81	5.17
	6.54	7.11	4.97	4.22
MEAN	4.83	6.47	5.20	5.03
Stdev.	1.95	0.85	1.54	0.56
SE	0.46	0.20	0.36	0.13

Table 20: (Exp. 1) After 8 days, the numbers of lateral root of C2, C2C4, C2C5 with control without sucrose and without bacterial and with bacterial strains 9 and 15 were counted. The average of lateral root per plants, standard deviation and standard error were calculated as shown below.

Samples	Control C2 (-suc)	C2 +9 (-suc)	C2 +15 (-suc)	Control C2C4 (-suc)	C2C4 +9 (-suc)	C2C4 +15 (-suc)	Control C2C5 (-suc)	C2C5 +9 (-suc)	C2C5 +15 (-suc)
	20	8	10	3	2	2	16	6	5
	8	22	1	6	6	2	19	10	5
	12	22	19	8	4	9	11	11	1
	8	5	7	3	3	10	3	6	6
	7	11	5	4	6	4	11	3	1
	7	2	9	2	5	4	15	6	9
Mean	10.33	11.67	8.50	4.33	4.33	5.17	12.50	7.00	4.50
Stdev.	5.09	8.55	6.06	2.25	1.63	3.49	5.58	2.97	3.08
SE	2.08	3.49	2.47	0.92	0.67	1.42	2.28	1.21	1.26

Table 21: (Exp. 3) After six days, the numbers of lateral root of C2 with control without sucrose and without bacterial and C2 with different bacterial strains were counted. The average of lateral root per cm were calculated by dividing the mean of lateral root with the average mean of the primary root length and standard deviation and standard error were calculated as shown below.

Number of Samples	Control C2	C2 + 9	C2 + 15	C2 + CL8
1	8	1	10	14
2	3	8	6	15
3	5	10	15	9
4	7	14	11	11
5	9	11	7	13

Later root Mean	6.4	8.8	9.8	12.4
Mean (Primary root length)	6.19	6.20	7.00	6.85
Mean (Lateral root per cm)	1.03	1.42	1.40	1.81
Stdev.	2.41	4.87	3.56	2.41
SE	1.08	2.18	1.59	1.08

Table 22: (Exp. 3) After six days, the numbers of lateral root of C4 with control without sucrose and without bacterial and C4 with different bacterial strains were counted. The average of lateral root per cm were calculated by dividing the mean of lateral root with the average mean of the primary root length and standard deviation and standard error were calculated as shown below.

Number of Samples	Control C4	C4 + 9	C4 + 15	C4 + CL8
	9	16	10	1
	4	12	8	2
	2	11	7	7
	8	5	10	14
	9	1	9	6
Later root Mean	6.4	9	8.8	6
Mean (Primary root length)	5.06	5.80	5.98	4.79
Mean (Lateral root per cm)	1.26	1.55	1.47	1.25
Stdev.	3.21	5.96	1.30	5.15
SE	1.44	2.66	0.58	2.30

Table 23: (Exp. 3) After six days, the numbers of lateral root of C2C4 with control without sucrose and without bacterial and C2C4 with different bacterial strains were counted. The average of lateral root per cm were calculated by dividing the mean of lateral root with the average mean of the primary root length band standard deviation and standard error were calculated as shown below.

	Control C2C4	C2C4 + 9	C2C4 +15	C2C4 + CL8
	7	1	10	6
	5	11	1	9
	9	1	2	1
	7	1	8	9
	9	3	4	3
Later root Mean	7.4	3.4	5	5.6
Mean (Primary root length)	4.04	3.65	4.87	4.21
Mean (Lateral root per cm)	1.83	0.93	1.03	1.33
Stdev.	1.67	4.34	3.87	3.58
SE	0.75	1.94	1.73	1.60

Table 24: (Exp. 3) After six days, the numbers of lateral root of C2C5 with control without sucrose and without bacterial and C2C5 with different bacterial strains were counted. The average of lateral root per cm were calculated by dividing the mean of lateral root with the average mean of the primary root length standard deviation and standard error were calculated as shown below.

	Control C2C5	C2C5 + 9	C2C5 + 15	C2C5 + CL8
	8	9	3	1
	8	6	7	7
	5	8	8	3
	5	6	1	4
	5	6	1	7
Later root Mean	6.2	7	4	4.4

Mean (Primary root length)	5.36	5.34	3.74	4.10
Mean (Lateral root per cm)	1.16	1.31	1.07	1.07
Stdev.	1.64	1.41	3.32	2.61
SE	0.73	0.63	1.48	1.17

Table 25: (Exp. 5) After eight days, the numbers of lateral root of selected eighteen out of twenty plants of C2, C2C4 with control without sucrose and without bacterial and C2, C2C4 with bacterial strain 15 were counted. The average of lateral root per plants, standard deviation and standard error were calculated as shown below.

	Control C2	C2 +15	Control C2C4	C2C4 +15
	17	14	10	7
	7	8	5	5
	1	5	5	1
	15	3	7	3
	5	8	5	2
	2	6	6	2
	5	7	6	9
	5	10	4	3
	5	10	3	3
	4	13	1	5
	1	5	8	8
	5	1	5	2
	4	11	3	8
	13	11	1	6

	6	9	5	4
	11	11	5	3
	18	8	6	2
	6	8	6	6
Mean	7.22	8.22	5.06	4.39
Stdev.	5.28	3.37	2.21	2.45
SE	1.24	0.79	0.52	0.58

Table 26: (Exp. 5) After eight days, the numbers of lateral root of selected eighteen out of twenty plants of C2, C2C4 with control without sucrose and without bacterial and C2, C2C4 with different bacterial strains were counted. The average of lateral root per plants were calculated by dividing the mean of lateral root with the average mean of the primary root length, standard deviation and standard error were calculated as shown below.

NOS	Control C2	C2 +15	Control C2C4	C2C4 +15
	17	14	10	7
	7	8	5	5
	1	5	5	1
	15	3	7	3
	5	8	5	2
	2	6	6	2
	5	7	6	9
	5	10	4	3
	5	10	3	3
	4	13	1	5
	1	5	8	8

	5	1	5	2
	4	11	3	8
	13	11	1	6
	6	9	5	4
	11	11	5	3
	18	8	6	2
	6	8	6	6
Mean (lateral root/plants)	7.22	8.22	5.06	4.39
Mean (primary root length cm/plants)	4.83	6.47	5.20	5.03
Mean (lateral root cm/plants)	1.49	1.27	0.97	0.87

8.

Table 27 (Exp. 5): Photographs of four plates of WT on agar plates with five plants per each on the plates with control (CI-CIV), and bacterial strain 15 (BAC1-BAC4) were taken after 8 days on growth chambers.




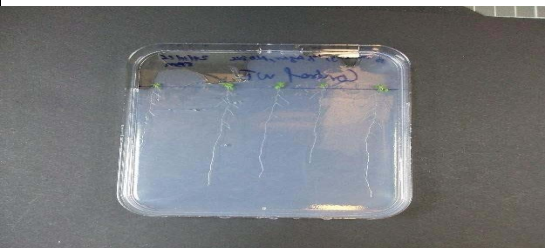
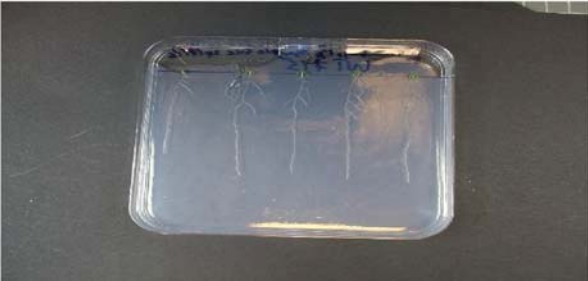

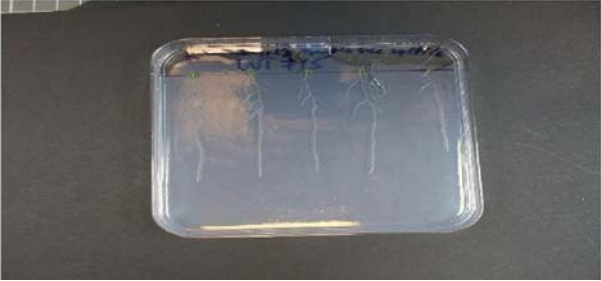

	
WT-CI	WT-CII
	
WT-CIII	WT-CIV
	
WT-BAC1	WT-BAC2
	
WT-BAC3	WT-BAC4

Table 28 (Exp. 5): Photographs of four plates of C2 on agar plates with five plants per each on the plates with control (CI-CIV), and bacterial strain 15 (BAC1-BAC4) were taken after 8 days on growth chambers.

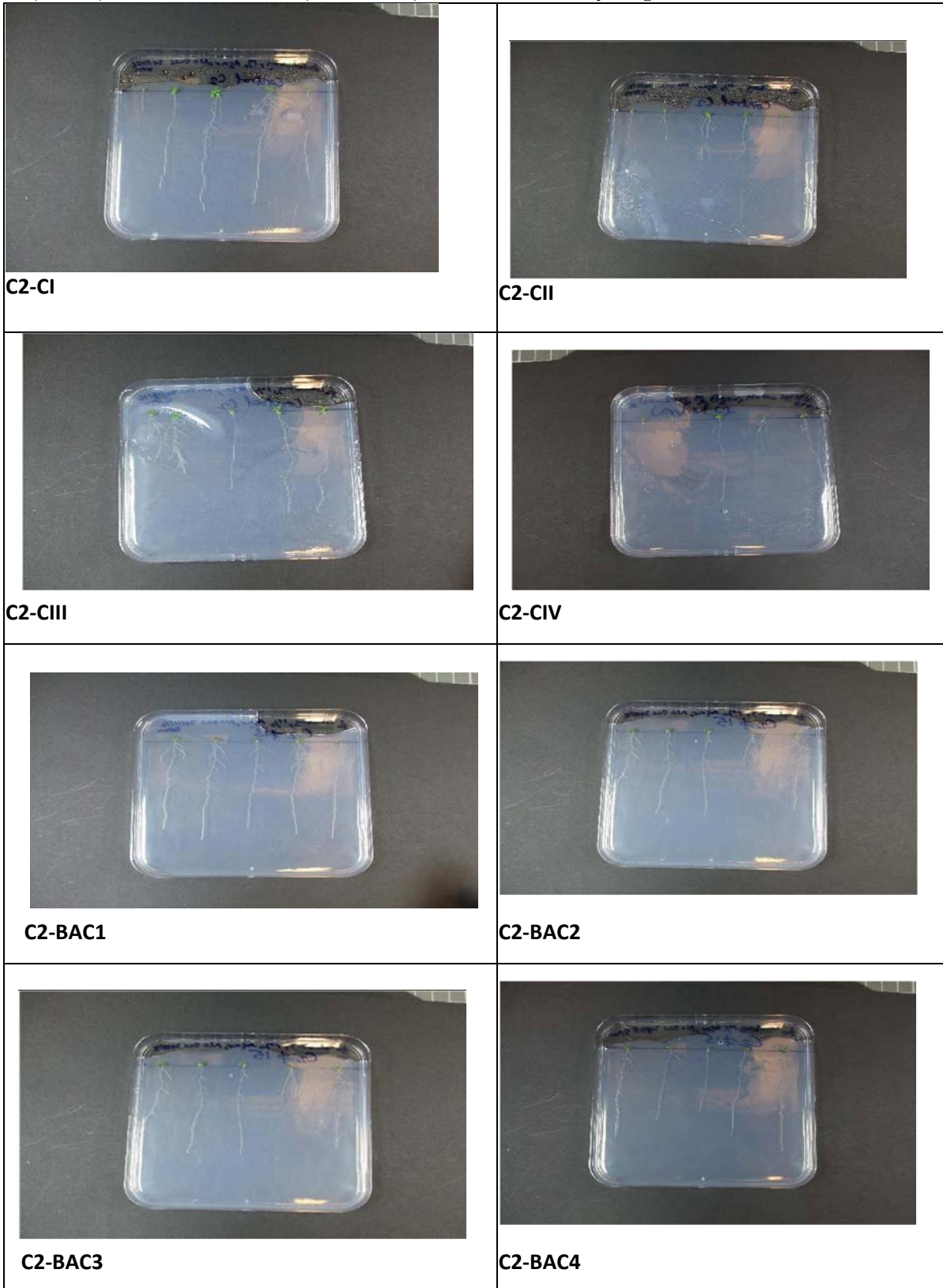
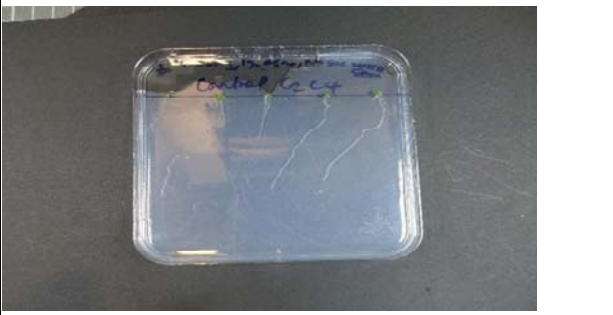






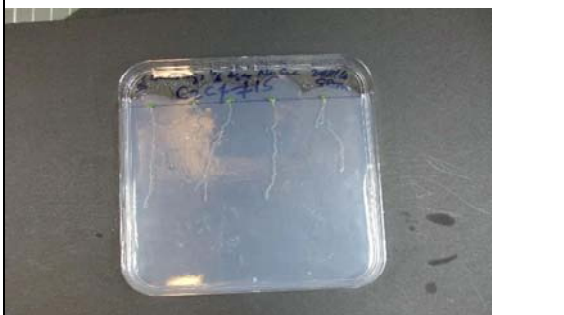


Table 29 (Exp. 5): Photographs of four plates of C2C4 on agar plates with five plants per each on the plates with control (CI-CIV), and bacterial strain 15 (BAC1-BAC4) were taken after 8 days on growth chambers.

 <p>C2C4-CI</p>	 <p>C2C4-CII</p>
 <p>C2C4-CIII</p>	 <p>C2C4-CIV</p>
 <p>C2C4-BAC1</p>	 <p>C2C4-BAC2</p>
 <p>C2C4-BAC3</p>	 <p>C2C4-BAC4</p>

9.

Table 30 (Exp. 6): After 8 days, the primary root length (cm/plants) of Wild Type Arabidopsis (WT) with four controls (CI-CIV) without sucrose and with no bacterial and wild type with WCS417r bacterial (BAC1-BAC4) were measured using image J.

WT-CI	WT-CII	WT-CIII	WT-CIV	WT-BAC1	WT-BAC2	WT-BAC3	WT-BAC4
6.63	6.75	6.64	3.25	6.74	4.82	4.14	5.71
6.92	5.76	6.77	5.54	6.64	4.53	1.25	6.33
7.7	6.69	7.5	7.44	3.63	5.75	5.23	5.71
5.35	6.91	8.16	7.42	5.12	5.02	5.18	6.58
7.11	6.79	7.99	6.89	5.36	6.19	3.63	6.66

Table 31 (Exp. 6): The same experiment was done for C2 mutants (see table 2 for details)

C2-CI	C2-CII	C2-CIII	C2-CIV	C2-BAC1	C2-BAC2	C2-BAC3	C2-BAC4
3.43	3.87	2.54	4.92	2.2	4.5	4.36	1.19
4.29	4.25	4.32	5.27	5.29	1.82	4.15	3.7
5.01	4.74	4.29	5.18	1.08	4.81	1.71	0.98
3.74	5.31	4.16	5.2	1.79	5.37	4.88	1.23
3.26	4.34	3.99	4.66	1.82	4.72	4.56	3.42

Table 32 (Exp. 6): The same experiment was done for C2C4 mutants (table 2)

C2C4-CI	C2C4-CII	C2C4-CIII	C2C4CIV	C2C4-BAC1	C2C4-BAC2	C2C4-BAC3	C2C4-BAC4
1.39	1.21	0.79	0.83	1.01	1.16	0.26	0.58
0.78	0.95	0.54	0.96	0.59	0.78	0.97	0.32
0.62	0.86	0.83	1.03	0.3	0.72	0.32	0.98
1.21	1.19	0.75	1.02	0.38	0.92	0.61	0.79
0.46	0.69	0.51	0.71	0.77	0.32	0.69	0.81

Table 33 (Exp. 6): The average, standard deviation and standard error were calculated then there were 20 plants per treatment as shown below.

	(WT-CI) - (WTCIV)	(WT-BAC1) - (WTBAC4)	(C2-CI) - (C2CIV)	(C2BAC1)- (C2-BAC4)	(C2C4-CI)- (C2C4CIV)	(C2C4BAC1) - (C2C4BAC4)
	6.63	6.74	3.43	2.2	1.39	1.01
	6.92	6.64	4.29	5.29	0.78	0.59
	7.7	3.63	5.01	1.08	0.62	0.3
	5.35	5.12	3.74	1.79	1.21	0.38
	7.11	5.36	3.26	1.82	0.46	0.77
	6.75	4.82	3.87	4.5	1.21	1.16
	5.76	4.53	4.25	1.82	0.95	0.78
	6.69	5.75	4.74	4.81	0.86	0.72
	6.91	5.02	5.31	5.37	1.19	0.92
	6.79	6.19	4.34	4.72	0.69	0.32
	6.64	4.14	2.54	4.36	0.79	0.26
	6.77	1.25	4.32	4.15	0.54	0.97
	7.5	5.23	4.29	1.71	0.83	0.32
	8.16	5.18	4.16	4.88	0.75	0.61
	7.99	3.63	3.99	4.56	0.51	0.69
	3.25	5.71	4.92	1.19	0.83	0.58
	5.54	6.33	5.27	3.7	0.96	0.32
	7.44	5.71	5.18	0.98	1.03	0.98
	7.42	6.58	5.2	1.23	1.02	0.79
	6.89	6.66	4.66	3.42	0.71	0.81
MEAN	6.71	5.21	4.34	3.18	0.87	0.66
Stdev.	1.09	1.34	0.74	1.61	0.25	0.28
SE	0.24	0.30	0.16	0.36	0.06	0.06

Table 34 (Exp. 7): After 8 days, the primary root length (cm/plants) of Wild Type Arabidopsis (WT) with four controls (CI-CIV) with 0.5% sucrose and with no bacterial and WT with WCS417r bacterial (BAC1-BAC4) were measured using image J.

WT-CI	WT-CII	WT-CIII	WT-CIV	WT-BAC1	WT-BAC2	WT-BAC3	WT-BAC4
6.98	5.21	6.71	6.71	2.29	3.66	2.08	2.08
6.76	5.04	6.02	6.02	2.30	1.68	1.63	1.63
5.82	6.32	6.50	6.50	3.51	3.09	3.30	3.30
6.39	6.30	7.30	7.30	3.60	3.21	2.95	2.95
4.64	5.94	4.30	4.30	2.82	3.43	3.06	3.06

Table 35 (Exp. 7): The same experiment was done for C2 mutants (see table 6 for details)

C2-CI	C2-CII	C2-CIII	C2-CIV	C2-BAC1	C2-BAC2	C2-BAC3	C2-BAC4
3.31	4.27	5.73	4.88	2.90	2.57	2.10	3.18
4.44	4.42	2.22	5.46	3.06	2.81	3.89	3.21
4.52	4.96	4.52	3.87	3.01	3.21	2.95	3.10
4.73	4.62	5.19	3.60	2.59	2.26	3.25	3.12
4.08	4.84	4.98	4.52	3.50	3.26	3.46	3.01

Table 36 (Exp. 7): The same experiment was done for C2C5 mutants (see table 2 for details)

C2C5-CI	C2C5-CII	C2C5-CIII	C2C5-CIV	C2C5-BAC1	C2C5-BAC2	C2C5-BAC3	C2C5-BAC4
1.73	2.04	1.50	1.29	1.16	1.77	1.57	0.92
2.93	2.33	2.82	3.45	1.77	1.56	1.02	0.60
2.45	1.88	1.12	3.70	2.18	1.68	1.40	0.70
0.63	1.28	1.26	3.11	1.18	1.24	1.56	0.85
0.73	2.44	1.31	2.69	1.22	1.79	1.33	0.55

Table 37 (Exp. 7): The average, standard deviation and standard error were calculated then there were 20 plants per treatment as shown below.

	(WT-CI) - (WT-CIV)	(WT-BAC1) - (WT-BAC4)	(C2-CI) - (C2-CIV)	(C2-BAC1) (C2-BAC4)	(C2C5-CI) - (C2C5-CIV)	(C2C5-BAC1) (C2C5-BAC4)
	6.98	2.29	3.31	2.90	1.73	1.16
	6.76	2.30	4.44	3.06	2.93	1.77
	5.82	3.51	4.52	3.01	2.45	2.18
	6.39	3.60	4.73	2.59	0.63	1.18
	4.64	2.82	4.08	3.50	0.73	1.22
	5.21	3.66	4.27	2.57	2.04	1.77
	5.04	1.68	4.42	2.81	2.33	1.56
	6.32	3.09	4.96	3.21	1.88	1.68
	6.30	3.21	4.62	2.26	1.28	1.24
	5.94	3.43	4.84	3.26	2.44	1.79
	6.71	2.08	5.73	2.10	1.50	1.57
	6.02	1.63	2.22	3.89	2.82	1.02
	6.50	3.30	4.52	2.95	1.12	1.40
	7.30	2.95	5.19	3.25	1.26	1.56
	4.30	3.06	4.98	3.46	1.31	1.33
	6.71	2.08	4.88	3.18	1.29	0.92
	6.02	1.63	5.46	3.21	3.45	0.60
	6.50	3.30	3.87	3.10	3.70	0.70
	7.30	2.95	3.60	3.12	3.11	0.85
	4.30	3.06	4.52	3.01	2.69	0.55
MEAN	6.05	2.78	4.46	3.02	2.03	1.30
Stdev.	0.92	0.68	0.79	0.42	0.90	0.44
SE	0.20	0.15	0.18	0.09	0.20	0.10

Table 38 (Exp. 6): After 8 days, the numbers of lateral root of Arabidopsis (WT), C2, C2C5 with control (CICIV) without sucrose and without bacterial) and wild type with WCS417r bacterial (BAC1-BAC4) were counted.

WT-CI	WT-CII	WT-CIII	WT-CIV	WT-BAC1	WT-BAC2	WT-BAC3	WT-BAC4
10	5	12	14	12	12	5	7
9	1	4	12	6	8	5	7
4	10	11	6	17	11	15	8
11	7	8	6	10	12	13	5
14	5	3	11	6	15	9	4

Table 39 (Exp. 6): The same experiment was done for C2 mutants (see table 10 for details)

C2-CI	C2-CII	C2-CIII	C2-CIV	C2-BAC1	C2-BAC2	C2-BAC3	C2-BAC4
3	2	1	1	7	8	6	6
2	4	4	1	6	7	7	8
2	2	6	1	8	4	7	8
4	1	1	6	6	8	6	6
1	2	1	1	8	8	6	5

Table 40 (Exp. 6): The same experiment was done for C2C4 mutants (see table 10 for details)

C2C5-CI	C2C5-CII	C2C5-CIII	C2C5-CIV	C2C5-BAC1	C2C5-BAC2	C2C5-BAC3	C2C5-BAC4
1	4	2	1	4	2	2	1
1	1	4	1	2	3	2	2
4	2	3	1	3	4	2	2
3	2	2	1	4	4	2	1
2	1	1	1	2	3	1	1

Table 41 (Exp. 6): The average, standard deviation and standard error were calculated then there were 20 plants per treatment without sucrose as shown below.

	(WT-CI) - (WT-CIV)	(WT-BAC1) (WT-BAC4)	(C2-CI) - (C2-CIV)	(C2-BAC1) -(C2-BAC4)	(C2C5-CI) (C2C5CIV)	(C2C5-BAC1) (C2C5-BAC4)
	10	12	3	7	1	4
	9	6	2	6	1	2
	4	17	2	8	4	3
	11	10	4	6	3	4
	14	6	1	8	2	2
	5	12	2	8	4	2

	1	8	4	7	1	3
	10	11	2	4	2	4
	7	12	1	8	2	4
	5	15	2	8	1	3
	12	5	1	6	2	2
	4	5	4	7	4	2
	11	15	6	7	3	2
	8	13	1	6	2	2
	3	9	1	6	1	1
	14	7	1	6	1	1
	12	7	1	8	1	2
	6	8	1	8	1	2
	6	5	6	6	1	1
	11	4	1	5	1	1
MEAN	8.15	9.35	2.3	6.75	1.9	2.35
Stdev.	3.79	3.86	1.66	1.16	1.12	1.04
SE	0.85	0.86	0.37	0.26	0.25	0.23

Table 42 (Exp. 7): After 8 days, the numbers of lateral root of Arabidopsis (WT), C2, C2C5 with control (CICIV) with 0.5% sucrose and without bacterial and WT with WCS417r bacterial (BAC1-BAC4) were counted.

WT-CI	WT-CII	WT-CIII	WT-CIV	WT-BAC1	WT-BAC2	WT-BAC3	WT-BAC4
10	5	12	14	12	12	5	7
9	1	4	12	6	8	5	7
4	10	11	6	17	11	15	8
11	7	8	6	10	12	13	5
14	5	3	11	6	15	9	4

Table 43 (Exp. 7): The same experiment was done for C2 mutants (see table 14 for details)

C2-CI	C2-CII	C2-CIII	C2-CIV	C2-BAC1	C2-BAC2	C2-BAC3	C2-BAC4
3	2	1	1	7	8	6	6
2	4	4	1	6	7	7	8
2	2	6	1	8	4	7	8
4	1	1	6	6	8	6	6
1	2	1	1	8	8	6	5

Table 44 (Exp. 7): The same experiment was done for C2C5 mutants (see table 14 for details)

C2C5-CI	C2C5-CII	C2C5-CIII	C2C5-CIV	C2C5-BAC1	C2C5-BAC2	C2C5-BAC3	C2C5-BAC4
1	4	2	1	4	2	2	1
1	1	4	1	2	3	2	2
4	2	3	1	3	4	2	2
3	2	2	1	4	4	2	1
2	1	1	1	2	3	1	1

Table 45 (Exp. 6): The average, standard deviation and standard error were calculated then there were 20 plants per treatment with 0.5% sucrose as shown below.

	(WT-CI) - (WT-CIV)	(WT-BAC1) (WT-BAC4)	(C2-CI) - (C2-CIV)	(C2-BAC1) (C2-BAC4)	(C2C5-CI) - (C2C5-CIV)	(C2C5-BAC1) (C2C5-BAC4)
	10	12	3	7	1	4
	9	6	2	6	1	2
	4	17	2	8	4	3
	11	10	4	6	3	4
	14	6	1	8	2	2
	5	12	2	8	4	2
	1	8	4	7	1	3
	10	11	2	4	2	4
	7	12	1	8	2	4
	5	15	2	8	1	3
	12	5	1	6	2	2
	4	5	4	7	4	2
	11	15	6	7	3	2
	8	13	1	6	2	2
	3	9	1	6	1	1
	14	7	1	6	1	1
	12	7	1	8	1	2
	6	8	1	8	1	2
	6	5	6	6	1	1
	11	4	1	5	1	1
MEAN	8.15	9.35	2.3	6.75	1.9	2.35
Stdev.	3.79	3.86	1.66	1.16	1.12	1.04
SE	0.85	0.86	0.37	0.26	0.25	0.23

Table 46 (Exp. 6): The average of lateral root per plants without sucrose were calculated by dividing the mean of lateral root with the average mean of the primary root length then there were 20 plants per treatment as shown below.

	(WT-CI) - (WT-CIV)	(WT-BAC1) - (WT-BAC4)	(C2-CI) - (C2-CIV)	(C2-BAC1) - (C2-BAC4)	(C2C5-CI) - (C2C5-CIV)	(C2C5-BAC1) - (C2C5-BAC4)
	10	12	3	7	1	4
	9	6	2	6	1	2
	4	17	2	8	4	3
	11	10	4	6	3	4
	14	6	1	8	2	2
	5	12	2	8	4	2
	1	8	4	7	1	3
	10	11	2	4	2	4
	7	12	1	8	2	4
	5	15	2	8	1	3
	12	5	1	6	2	2
	4	5	4	7	4	2
	11	15	6	7	3	2
	8	13	1	6	2	2
	3	9	1	6	1	1
	14	7	1	6	1	1
	12	7	1	8	1	2
	6	8	1	8	1	2
	6	5	6	6	1	1
	11	4	1	5	1	1
Mean (Lateral root per plants)	8.15	9.35	2.3	6.75	1.9	2.35
Mean (Primary root length cm/plants)	6.05	2.78	4.46	3.02	2.03	1.30
Lateral root length cm/plants	1.35	3.36	0.52	2.23	0.93	1.80

Table 47 (Exp. 7): The average of lateral root per plants with sucrose were calculated by dividing the mean of lateral root with the average mean of the primary root length then there were 20 plants per treatment as shown below

	(WT-CI)- (WT-CIV)	(WT-BAC1) (WT-BAC4)	(C2-CI)- (C2-CIV)	(C2-BAC1)- (C2-BAC4)	(C2C5-CI) - (C2C5-CIV)	(C2C5-BAC1) (C2C5-BAC4)
	10	12	3	7	1	4
	9	6	2	6	1	2
	4	17	2	8	4	3
	11	10	4	6	3	4
	14	6	1	8	2	2
	5	12	2	8	4	2
	1	8	4	7	1	3
	10	11	2	4	2	4
	7	12	1	8	2	4
	5	15	2	8	1	3
	12	5	1	6	2	2
	4	5	4	7	4	2
	11	15	6	7	3	2
	8	13	1	6	2	2
	3	9	1	6	1	1
	14	7	1	6	1	1
	12	7	1	8	1	2
	6	8	1	8	1	2
	6	5	6	6	1	1
	11	4	1	5	1	1
Mean (Lateral root per plants)	8.15	9.35	2.3	6.75	1.9	2.35
Mean (Primary root length cm/plants)	6.05	2.78	4.46	3.02	2.03	1.30
Lateral root length cm/plan	1.35	3.36	0.52	2.23	0.93	1.80

Table 48 (Exp. 6): After 8 days, the shoot fresh of Arabidopsis (WT), C2, C2C4 with control without sucrose and without bacterial (CI-CIV) and WT with WCS417r bacterial (BAC1-BAC4) were weighed (g) per plants then there were 20 plants per treatment as shown below.

	(WT-CI)- (WTCIV)	(WTBAC1)- (WTBAC4)	(C2-CI) -(C2CIV)	(C2-BAC1) - (C2-BAC4)	(C2C5-CI) - (C2C5-CIV)	(C2C5-BAC1) (C2C5- BAC4)
	0.0035	0.0020	0.0012	0.0017	0.0022	0.0011
	0.0039	0.0051	0.0022	0.0018	0.0015	0.0012
	0.0043	0.0048	0.0020	0.0028	0.0016	0.0018
	0.0061	0.0025	0.0028	0.0021	0.0016	0.0012
	0.0039	0.0053	0.0038	0.0023	0.0007	0.0020
	0.0039	0.0019	0.0013	0.0022	0.0020	0.0011
	0.0083	0.0039	0.0034	0.0027	0.0013	0.0009
	0.0047	0.0035	0.0024	0.0020	0.0016	0.0015
	0.0048	0.0026	0.0025	0.0022	0.0018	0.0022
	0.0067	0.0050	0.0021	0.0022	0.0027	0.0019
	0.0034	0.0040	0.0032	0.0018	0.0015	0.0009
	0.0069	0.0039	0.0051	0.0029	0.0021	0.0012
	0.0070	0.0031	0.0056	0.0019	0.0017	0.0011
	0.0073	0.0033	0.0022	0.0031	0.0021	0.0020
	0.0080	0.0035	0.0056	0.0019	0.0028	0.0011
	0.0048	0.0036	0.0024	0.0018	0.0012	0.0011
	0.0080	0.0032	0.0029	0.0029	0.0021	0.0011
	0.0044	0.0026	0.0029	0.0026	0.0017	0.0012
	0.0046	0.0033	0.0043	0.0027	0.0016	0.0014
	0.0075	0.0039	0.0018	0.0023	0.0016	0.0005
MEAN	0.0056	0.0036	0.0030	0.0023	0.0018	0.0013
Stdev.	0.0017	0.0010	0.0013	0.0004	0.0005	0.0004
SE	0.0004	0.0002	0.0003	0.0001	0.0001	0.0001

Table 49 (Exp. 7): After 8 days, the shoot fresh of Arabidopsis (WT), C2, C2C4 with control with 0.5% sucrose and without bacterial (CI-CIV) and WT with WCS417r bacterial (BAC1-BAC4) were weighed (g) per plants then there were 20 plants per treatment as shown below.

	(WT-CI) - (WT-CIV)	(WT-BAC1) - (WT-BAC4)	(C2-CI) - (C2- CIV)	(C2-BAC1) - (C2-BAC4)	(C2C5-CI) - (C2C5-CIV)	(C2C5-BAC1) - (C2C5-BAC4)
	0.0035	0.0020	0.0012	0.0017	0.0022	0.0011
	0.0039	0.0051	0.0022	0.0018	0.0015	0.0012
	0.0043	0.0048	0.0020	0.0028	0.0016	0.0018
	0.0061	0.0025	0.0028	0.0021	0.0016	0.0012
	0.0039	0.0053	0.0038	0.0023	0.0007	0.0020
	0.0039	0.0019	0.0013	0.0022	0.0020	0.0011
	0.0083	0.0039	0.0034	0.0027	0.0013	0.0009
	0.0047	0.0035	0.0024	0.0020	0.0016	0.0015
	0.0048	0.0026	0.0025	0.0022	0.0018	0.0022
	0.0067	0.0050	0.0021	0.0022	0.0027	0.0019
	0.0034	0.0040	0.0032	0.0018	0.0015	0.0009
	0.0069	0.0039	0.0051	0.0029	0.0021	0.0012
	0.0070	0.0031	0.0056	0.0019	0.0017	0.0011
	0.0073	0.0033	0.0022	0.0031	0.0021	0.0020
	0.0080	0.0035	0.0056	0.0019	0.0028	0.0011
	0.0048	0.0036	0.0024	0.0018	0.0012	0.0011
	0.0080	0.0032	0.0029	0.0029	0.0021	0.0011
	0.0044	0.0026	0.0029	0.0026	0.0017	0.0012
	0.0046	0.0033	0.0043	0.0027	0.0016	0.0014
	0.0075	0.0039	0.0018	0.0023	0.0016	0.0005
MEAN	0.0056	0.0036	0.0030	0.0023	0.0018	0.0013
Stdev.	0.0017	0.0010	0.0013	0.0004	0.0005	0.0004
SE	0.0004	0.0002	0.0003	0.0001	0.0001	0.0001

10.

Table 51 (Exp.6): Photographs of five WT plants on agar plates without sucrose as control (CI-CIV), and WCS417r bacterial (BAC1-BAC4) were taken after 8 days on growth chambers

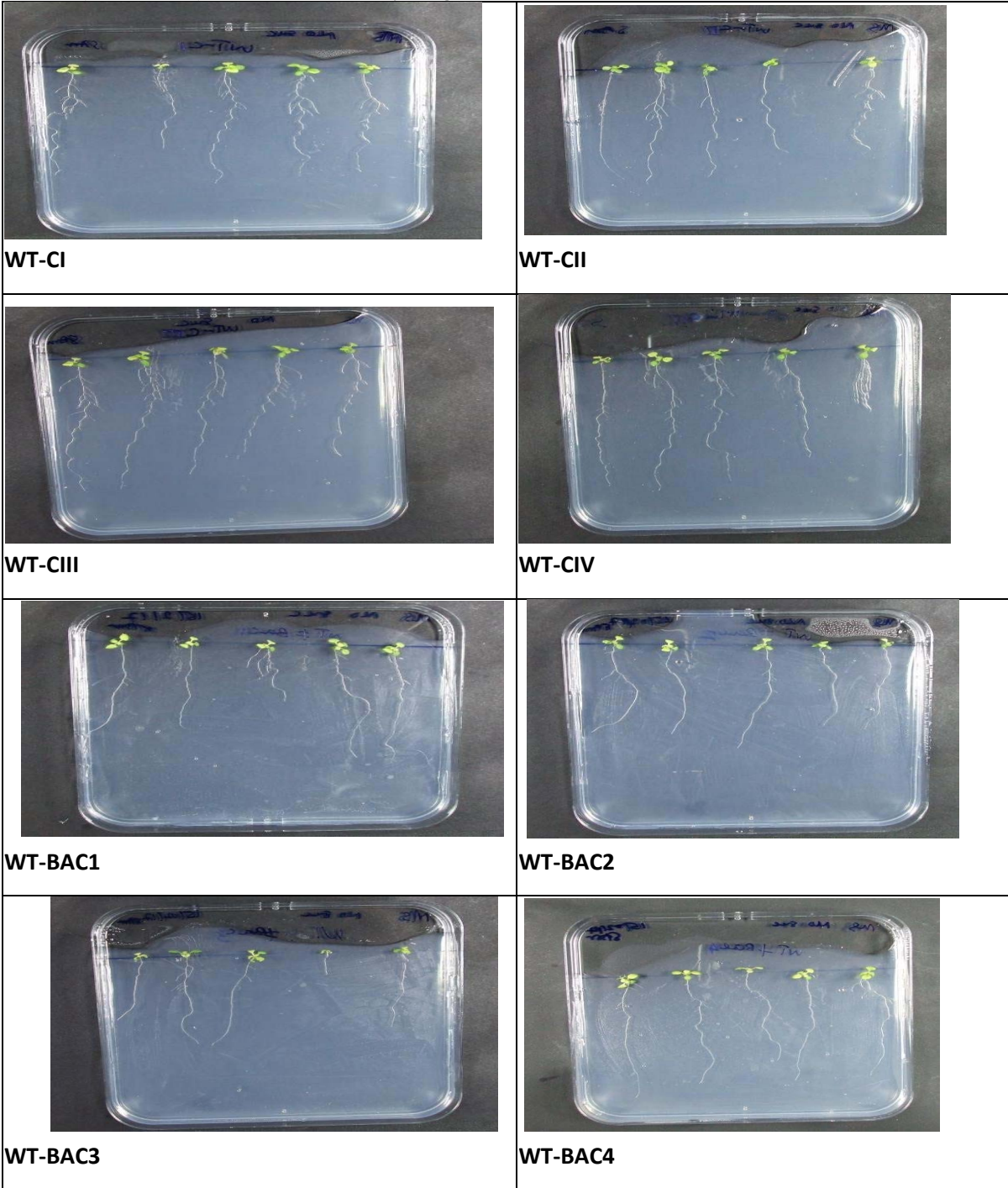


Table 52 (Exp.6): The same procedure was carried out for C2 (see table 51 for details)

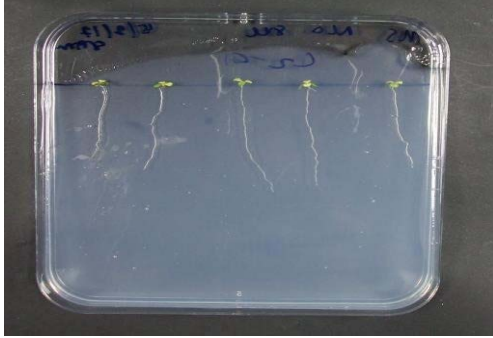
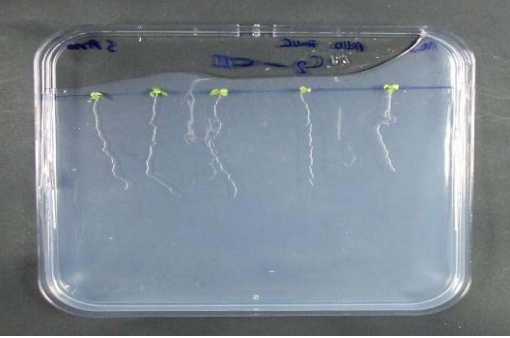






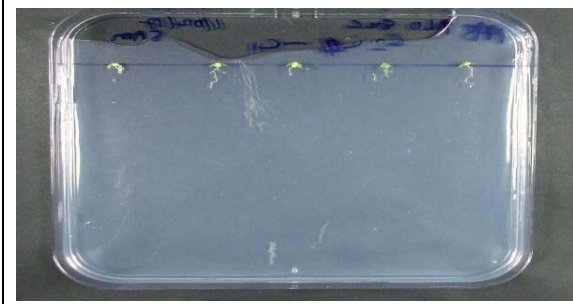
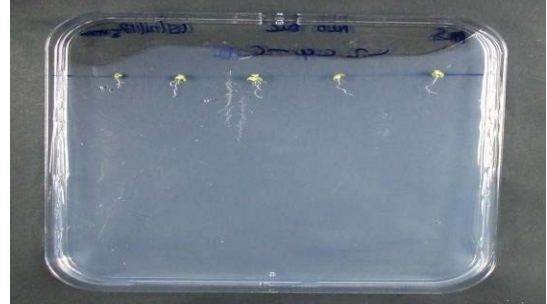
 <p>C2-CI</p>	 <p>C2-CII</p>
 <p>C2-CIII</p>	 <p>C2-CIV</p>
 <p>C2-BAC1</p>	 <p>C2-BAC2</p>
 <p>C2-BAC3</p>	 <p>C2-BAC4</p>

Table 53 (Exp.6): The same procedure was carried out for C2C4 (see table 51 for details)



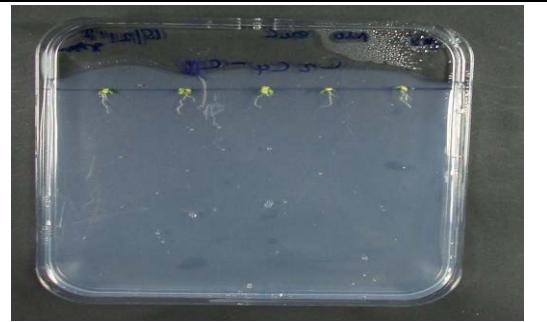
C2C4-CI



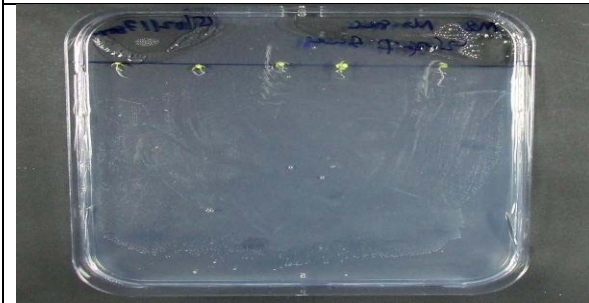
C2C4-CII



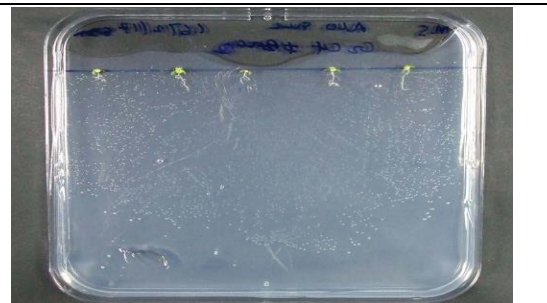
C2C4-CIII



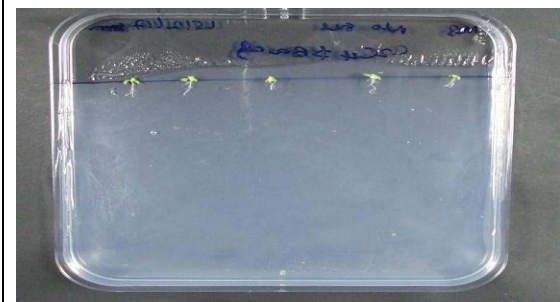
C2C4-CIV



C2C4-BAC1



C2C4-BAC2

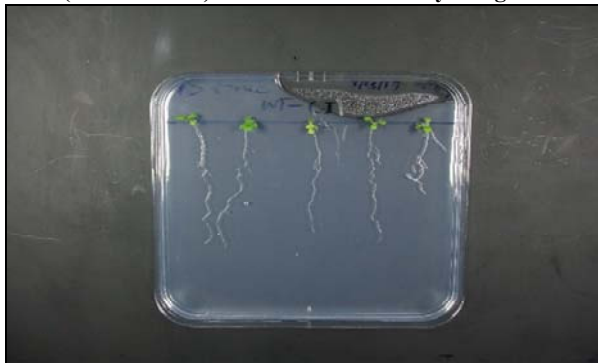


C2C4-BAC3



C2C4-BAC4

Table 54 (Exp. 7): Photographs of five WT plants on agar plates with 0.5% sucrose as control (CI-CIV), and WCS417r bacterial (BAC1-BAC4) were taken after 8 days on growth chambers



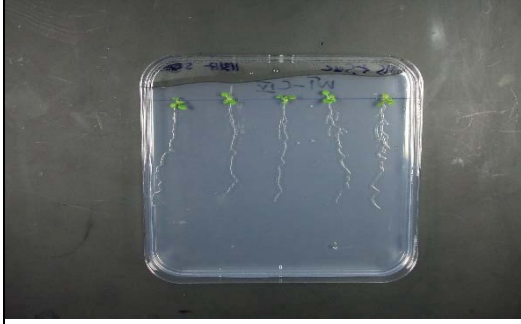
WT-CI



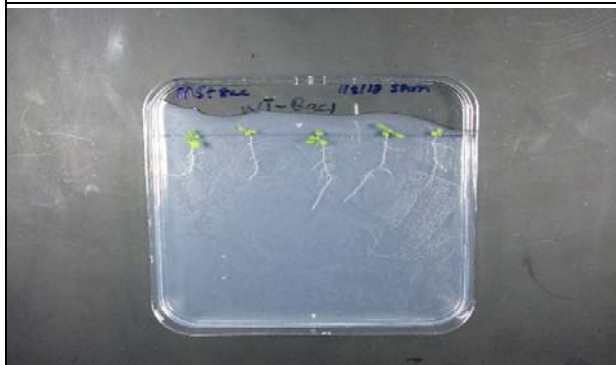
WT-CII



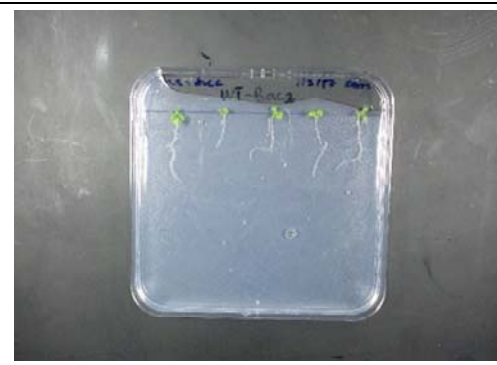
WT-CIII



WT-CIV



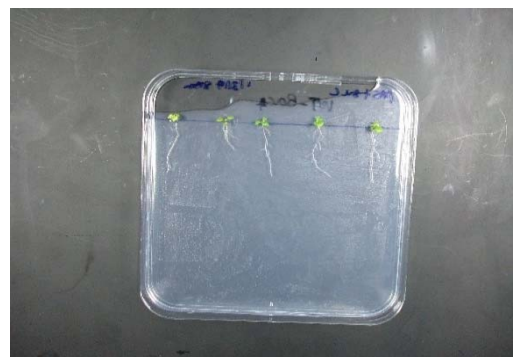
WT-BAC1



WT-BAC2



WT-BAC3



WT-BAC4

Table 55 (Exp.7): The same procedure was carried out for C2 (see table 54 for details)

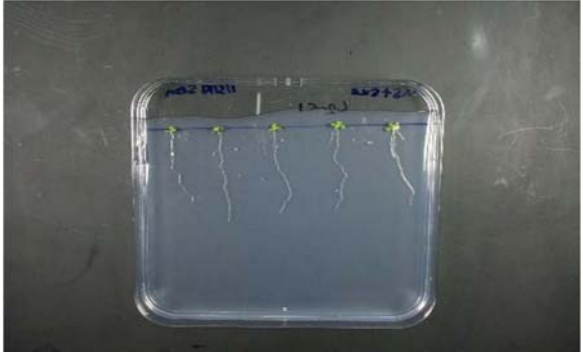
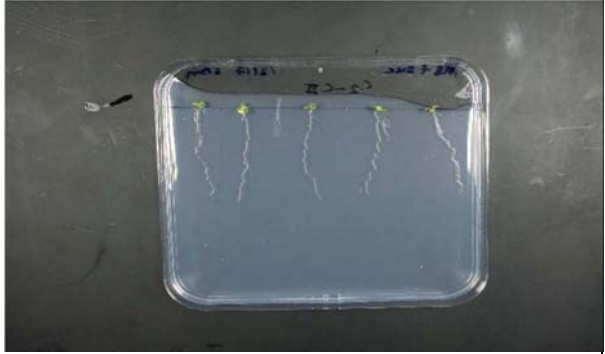
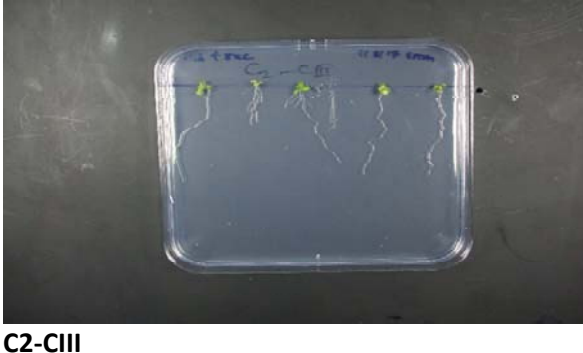
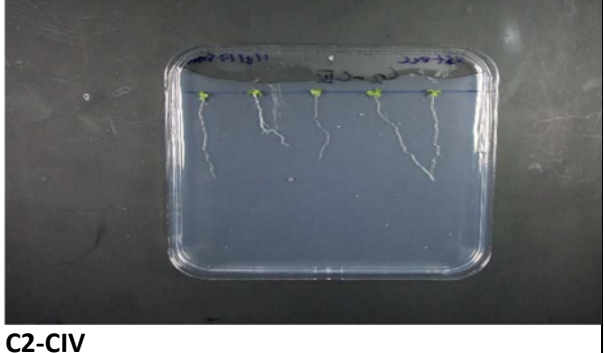
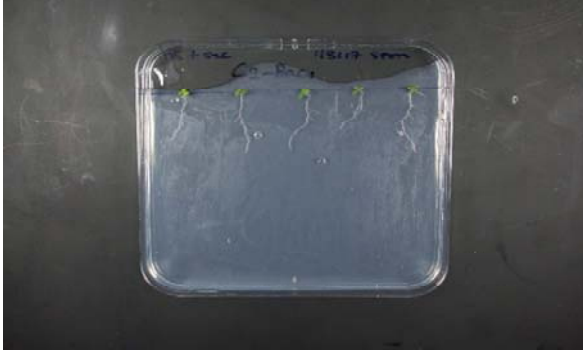
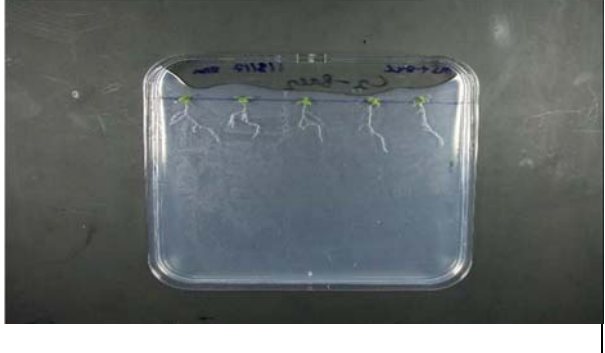

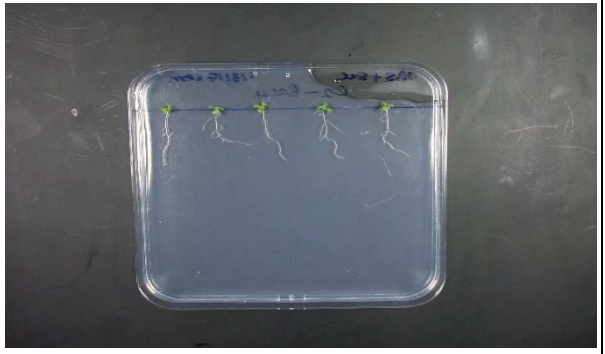
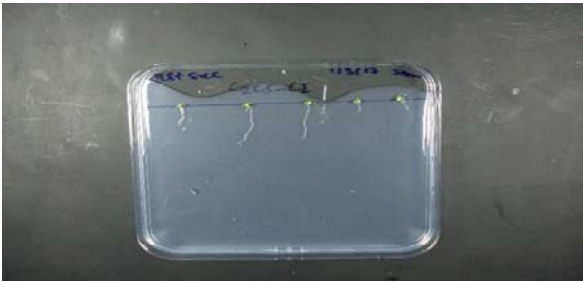






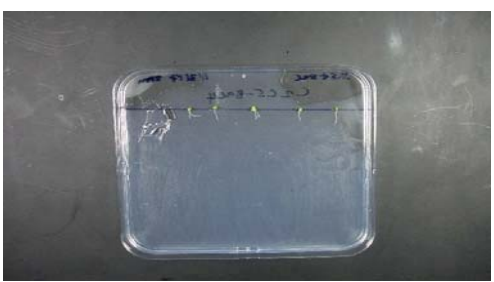
	
C2-CI	C2-CII
	
C2-CIII	C2-CIV
	
C2-BAC1	C2-BAC2
	
C2-BAC3	C2-BAC4

Table 56 (Exp.7): The same procedure was carried out for C2C4 (see table 54 for details)

 <p>C2C5-CI</p>	 <p>C2C5-CII</p>
 <p>C2C5-CIII</p>	 <p>C2C5-CIV</p>
 <p>C2C5-BAC1</p>	 <p>C2C5-BAC2</p>
 <p>C2C5-BAC3</p>	 <p>C2C5-BAC4</p>

11.

Table 57 (Exp. 8): After 8 days, the primary root length (cm/plants) of Wild Type Arabidopsis (WT) with four controls (CI-CIII) with 0.5% sucrose and with no bacterial and wild type with *Sphingobium limneticum* and *Acidovorax delafieldii* strains (SPH1-SPH3) and (ACD1-ACD3) were measured using image J.

WT-CI	WT-CII	WT-CIII	WT-SPH1	WT-SPH2	WT-SPH3	WT-ACD1	WT-ACD2	WT-ACD3
5.78	6.02	7.41	2.56	4.11	4.76	7.92	7.27	5.72
5.71	7.86	6.46	3.85	5.00	5.09	2.08	6.73	5.37
7.25	8.25	8.08	3.79	5.00	5.03	7.59	3.06	5.65
7.85	3.35	8.43	4.57	4.85	2.65	5.58	7.80	5.38
5.80	6.30	8.29	3.57	3.87	4.73	3.20	3.08	5.33

Table 58 (Exp. 8): The same experiment was done for C2 mutants (see table 57 for details)

C2-CI	C2-CII	C2-CIII	C2-SPH1	C2-SPH2	C2-SPH3	C2-ACD1	C2-ACD2	C2-ACD3
6.94	6.04	5.44	3.18	3.46	3.78	4.00	5.48	4.98
5.83	5.65	5.09	3.28	3.34	3.92	4.44	4.53	5.27
7.28	6.36	6.11	3.29	3.15	3.85	3.71	5.86	5.27
6.53	5.87	5.23	2.88	3.03	3.32	4.03	5.43	5.75
4.08	5.00	3.13	3.45	2.99	2.21	4.05	4.54	5.13

Table 59 (Exp. 8): The same experiment was done for C2C4 mutants (see table 57 for details)

C2C4-CI	C2C4-CII	C2C4-CIII	C2C4-SPH1	C2C4-SPH2	C2C4-SPH3	C2C4-ACD1	C2C4-ACD2	C2C4-ACD3
0.83	1.18	1.05	0.63	0.31	0.62	0.55	0.80	0.33
1.19	0.75	0.43	0.48	0.43	0.43	0.50	1.17	0.28
1.50	0.97	1.37	0.99	0.23	0.93	0.60	0.62	0.35
0.74	1.83	1.55	1.18	0.48	1.05	0.66	0.39	0.93
1.25	0.46	0.65	0.91	0.55	0.81	0.42	0.40	0.75

Table 60 (Exp. 8): The average, standard deviation and standard error were calculated then there were 15 plants per treatment.

	(WT-CI)- (WT-CIII)	(WT-SPH1) - (WT-SPH3)	(WT-ACD1) - (WT-ACD3)	(C2-CI) - (C2-CIII)	(C2-SPH1) -(C2-SPH3)	(C2-ACD1) -(C2-ACD3)	(C2C4-CI) -(C2C4-CIII)	(C2C4-SPH1) - (C2C4-SPH3)	(C2C4-ACD1) -(C2C4-ACD3)
	5.78	2.56	7.92	6.94	3.18	4.00	0.83	0.63	0.55
	5.71	3.85	2.08	5.83	3.28	4.44	1.19	0.48	0.50
	7.25	3.79	7.59	7.28	3.29	3.71	1.50	0.99	0.60
	7.85	4.57	5.58	6.53	2.88	4.03	0.74	1.18	0.66
	5.80	3.57	3.20	4.08	3.45	4.05	1.25	0.91	0.42
	6.02	4.11	7.27	6.04	3.46	5.48	1.18	0.31	0.80
	7.86	5.00	6.73	5.65	3.34	4.53	0.75	0.43	1.17
	8.25	5.00	3.06	6.36	3.15	5.86	0.97	0.23	0.62
	3.35	4.85	7.80	5.87	3.03	5.43	1.83	0.48	0.39
	6.30	3.87	3.08	5.00	2.99	4.54	0.46	0.55	0.40
	7.41	4.76	5.72	5.44	3.78	4.98	1.05	0.62	0.33
	6.46	5.09	5.37	5.09	3.92	5.27	0.43	0.43	0.28
	8.08	5.03	5.65	6.11	3.85	5.27	1.37	0.93	0.35
	8.43	2.65	5.38	5.23	3.32	5.75	1.55	1.05	0.93
	8.29	4.73	5.33	3.13	2.21	5.13	0.65	0.81	0.75
Mean	6.86	4.23	5.45	5.64	3.28	4.83	1.05	0.67	0.58
Stdev.	1.40	0.84	1.87	1.06	0.42	0.69	0.41	0.29	0.25
SE	0.36	0.22	0.48	0.27	0.11	0.18	0.11	0.07	0.06

Table 61 (Exp. 9): The same experiment was repeated with 0.5 % sucrose (see details in table 57).

WT-CI	WT-CII	WT-CIII	WT-SPH1	WT-SPH2	WT-SPH3	WT-ACD1	WT-ACD2	WT-ACD3
4.21	3.69	6.77	1.65	2.02	1.61	5.56	3.47	4.08
5.84	4.60	3.06	2.18	1.53	2.44	2.18	3.56	5.05
4.24	6.59	8.65	1.72	2.08	2.32	3.20	1.58	6.42
4.46	2.89	7.33	1.85	1.80	1.99	2.46	2.25	2.78
8.11	3.69	5.24	1.94	1.69	1.82	2.98	2.14	3.37

Table 62 (Exp. 9): The same experiment was done for C2 mutants (see table 57 for details).

C2-CI	C2-CII	C2-CIII	C2-SPH1	C2-SPH2	C2-SPH3	C2-ACD1	C2-ACD2	C2-ACD3
3.15	5.60	5.68	1.53	1.39	2.04	3.76	5.05	4.91
6.90	5.41	6.26	2.18	2.00	2.03	1.49	5.07	4.17
4.13	5.55	5.64	1.62	1.80	2.19	2.45	6.32	2.79
3.98	5.56	5.36	1.56	1.32	1.68	1.92	4.44	3.93
5.68	4.57	5.26	2.45	1.32	1.85	3.28	2.91	3.91

Table 63 (Exp. 9): The average, standard deviation and standard error were calculated then there were 15 plants per treatment.

	(WT-CI) -(WT-CIII)	(WT-SPH1) -(WT-SPH3)	(WT-ACD1) -(WTACD3)	(C2-CI) -(C2-CIII)	(C2-SPH1) -(C2-SPH3)	(C2-ACD1) -(C2-ACD3)
	4.21	1.65	5.56	3.15	1.53	3.76
	5.84	2.18	2.18	6.90	2.18	1.49
	4.24	1.72	3.20	4.13	1.62	2.45
	4.46	1.85	2.46	3.98	1.56	1.92
	8.11	1.94	2.98	5.68	2.45	3.28
	3.69	2.02	3.47	5.60	1.39	5.05
	4.60	1.53	3.56	5.41	2.00	5.07
	6.59	2.08	1.58	5.55	1.80	6.32
	2.89	1.80	2.25	5.56	1.32	4.44
	3.69	1.69	2.14	4.57	1.32	2.91
	6.77	1.61	4.08	5.68	2.04	4.91
	3.06	2.44	5.05	6.26	2.03	4.17
	8.65	2.32	6.42	5.64	2.19	2.79
	7.33	1.99	2.78	5.36	1.68	3.93
	5.24	1.82	3.37	5.26	1.85	3.91
Mean	5.29	1.91	3.41	5.25	1.80	3.76
Stdev.	1.83	0.26	1.37	0.94	0.35	1.31
SE	0.47	0.07	0.35	0.24	0.09	0.34

Table 64 (Exp. 10): The same experiment was repeated except without sucrose (see details in table 57).

WT-CI	WT-CII	WT-CIII	WT-SPH1	WT-SPH2	WT-SPH3	WT-ACD1	WT-ACD2	WT-ACD3
6.04	5.76	5.64	2.54	1.89	4.82	2.00	2.11	2.42
6.85	8.34	2.01	2.40	5.33	6.52	2.30	1.68	2.13
3.52	2.51	2.71	2.12	4.49	2.78	1.73	2.02	1.90
5.36	7.91	3.30	4.96	2.07	1.84	1.81	1.85	2.33
8.27	3.84	2.46	6.55	2.24	6.45	2.43	2.27	2.52

Table 65 (Exp. 10): The same experiment was done for C2 mutants (see table 64 for details)

C2-CI	C2-CII	C2-CIII	C2-SPH1	C2-SPH2	C2-SPH3	C2-ACD1	C2-ACD2	C2-ACD3
3.00	4.60	5.84	3.92	6.90	4.38	4.60	4.84	7.13
7.48	6.72	6.43	5.77	6.66	1.32	6.08	8.06	2.65
6.55	5.99	5.58	6.48	6.91	6.36	2.03	7.18	1.87
6.11	7.36	2.32	4.07	6.81	4.35	6.14	7.61	7.63
7.61	6.99	5.84	7.38	4.03	5.62	6.08	5.64	7.87

Table 66 (Exp. 10): The average, standard deviation and standard error were calculated then there were 15 plants per treatment.

	(WT-CI)- (WT-CIII)	(WT-SPH1)- (WT-SPH3)	(WT-ACD1)- (WT-ACD3)	(C2-CI)- (C2-CIII)	(C2-SPH1)- (C2-SPH3)	(C2-ACD1)- (C2-ACD3)
	6.04	2.54	2.00	3.00	3.92	4.60
	6.85	2.40	2.30	7.48	5.77	6.08
	3.52	2.12	1.73	6.55	6.48	2.03
	5.36	4.96	1.81	6.11	4.07	6.14
	8.27	6.55	2.43	7.61	7.38	6.08
	5.76	1.89	2.11	4.60	6.90	4.84
	8.34	5.33	1.68	6.72	6.66	8.06
	2.51	4.49	2.02	5.99	6.91	7.18
	7.91	2.07	1.85	7.36	6.81	7.61
	3.84	2.24	2.27	6.99	4.03	5.64
	5.64	4.82	2.42	5.84	4.38	7.13
	2.01	6.52	2.13	6.43	1.32	2.65
	2.71	2.78	1.90	5.58	6.36	1.87
	3.30	1.84	2.33	2.32	4.35	7.63
	2.46	6.45	2.52	5.84	5.62	7.87
Mean	4.97	3.80	2.10	5.89	5.40	5.69
Stdev.	2.22	1.84	0.27	1.54	1.67	2.10
SE	0.57	0.48	0.07	0.40	0.43	0.54

Table 67 (Exp. 8): After 8 days, the numbers of lateral root of Arabidopsis (WT) with control (CI-CIII) with 0.5% sucrose and without bacterial and (WT) with *Sphingobium limneticum* and *Acidovorax delafieldii* strains (SPH1- SPH3) and (ACD1-ACD3) were counted.

WT-CI	WT-CII	WT-CIII	WT-SPH1	WT-SPH2	WT-SPH3	WT-ACD1	WT-ACD2	WT-ACD3
5	3	8	15	14	24	19	14	20
3	10	7	14	24	21	4	21	21
6	15	5	21	16	21	14	3	25
5	2	10	22	26	16	8	17	24
5	15	14	15	13	16	5	19	26

Table 68 (Exp. 8): The same experiment was done for C2 mutants (see table 67 for details)

C2-CI	C2-CII	C2-CIII	C2-SPH1	C2-SPH2	C2-SPH3	C2-ACD1	C2-ACD2	C2-ACD3
5	3	3	13	8	15	12	8	3
2	3	1	15	14	15	15	8	5
2	4	2	11	13	17	12	5	11
1	1	2	10	10	12	13	4	7
2	3	3	12	11	10	12	4	11

Table 69 (Exp. 8): The same experiment was done for C2C4 mutants (see table 67 for details)

C2C4-CI	C2C4-CII	C2C4-CIII	C2C4-SPH1	C2C4-SPH2	C2C4-SPH3	C2C4-ACD1	C2C4-ACD2	C2C4-ACD3
1	2	2	1	2	3	2	4	2
1	2	2	2	2	4	4	2	2
1	1	2	1	3	4	4	4	4
1	2	2	2	2	2	2	3	5
1	1	2	1	1	3	4	4	4

Table 70 (Exp. 8): The average, standard deviation and standard error were calculated then there were 15 plants per treatment.

	(WT-CI)- (WT-CIII)	(WT- SPH1)- (WTSPH3)	(WT- ACD1)- (WT-ACD3)	(C2- CI) -(C2- CIII)	(C2- SPH1)- (C2-SPH3)	(C2- ACD1)- (C2-ACD3)	(C2C4- CI)- (C2C4- CIII)	(C2C4- SPH1)- (C2C4- SPH3)	(C2C4- ACD1)- (C2C4- ACD3)
5	15	19	5	13	12	1	1	2	
3	14	4	2	15	15	1	2	4	
6	21	14	2	11	12	1	1	4	
5	22	8	1	10	13	1	2	2	
5	15	5	2	12	12	1	1	4	
3	14	14	3	8	8	2	2	4	
10	24	21	3	14	8	2	2	2	
15	16	3	4	13	5	1	3	4	
2	26	17	1	10	4	2	2	3	
15	13	19	3	11	4	1	1	4	
8	24	20	3	15	3	2	3	2	
7	21	21	1	15	5	2	4	2	
5	21	25	2	17	11	2	4	4	
10	16	24	2	12	7	2	2	5	
14	16	26	3	10	11	2	3	4	
Mean	7.53	18.53	16.00	2.47	12.40	8.67	1.53	2.20	3.33
Stdev.	4.36	4.32	7.73	1.13	2.47	3.87	0.52	1.01	1.05
SE	1.12	1.12	2.00	0.29	0.64	1.00	0.13	0.26	0.27

Table 71 (Exp. 9): The same experiment was repeated with 0.5% sucrose (see details in table 67).

WT-CI	WT-CII	WT-CIII	WT-SPH1	WT-SPH2	WT-SPH3	WT-ACD1	WT-ACD2	WT-ACD3
3	5	6	3	8	3	2	4	6
3	6	6	12	8	3	3	7	6
3	5	8	4	11	2	3	4	6
4	3	4	4	6	5	4	8	3
5	5	10	5	12	8	5	4	2

Table 72 (Exp. 9): The same experiment was done for C2 mutants (see table 67 for details)

C2-CI	C2-CII	C2-CIII	C2-SPH1	C2-SPH2	C2-SPH3	C2-ACD1	C2-ACD2	C2-ACD3
1	2	2	6	5	12	3	3	1
5	1	1	5	12	9	2	2	2
1	1	2	6	9	7	4	4	1
2	1	1	9	5	4	6	2	2
3	1	1	1	7	8	3	3	1

Table 73 (Exp. 9): The average, standard deviation and standard error were calculated then there were 15 plants per treatment.

	(WT-CI)- (WT-CIII)	(WT-SPH1)- (WT-SPH3)	(WT-ACD1)- (WT-ACD3)	(C2-CI)- (C2-CIII)	(C2-SPH1)-(C2- SPH3)	(C2-ACD1)- (C2-ACD3)
	3	3	2	1	6	3
	3	12	3	5	5	2
	3	4	3	1	6	4
	4	4	4	2	9	6
	5	5	5	3	1	3
	5	8	4	2	5	3
	6	8	7	1	12	2
	5	11	4	1	9	4
	3	6	8	1	5	2
	5	12	4	1	7	3
	6	3	6	2	12	1
	6	3	6	1	9	2
	8	2	6	2	7	1
	4	5	3	1	4	2
	10	8	2	1	8	1
Mean	5.07	6.27	4.47	1.67	7.00	2.60
Stdev.	1.98	3.39	1.81	1.11	2.95	1.35
SE	0.51	0.88	0.47	0.29	0.76	0.35

Table 74 (Exp. 10): The same experiment was repeated except without sucrose (see details on table 67).

WT-CI	WT-CII	WT-CIII	WT-SPH1	WT-SPH2	WT-SPH3	WT-ACD1	WT-ACD2	WT-ACD3
16	1	1	6	1	17	1	3	4
3	16	1	2	2	9	1	5	6
1	4	5	1	4	3	1	1	3
3	9	1	7	2	1	1	1	6
15	1	1	13	4	15	3	10	2

Table 75 (Exp. 10): The same experiment was done for C2 mutants (see details in table 74).

C2-CI	C2-CII	C2-CIII	C2-SPH1	C2-SPH2	C2-SPH3	C2-ACD1	C2-ACD2	C2-ACD3
1	1	2	1	13	2	10	1	2
1	1	1	3	10	1	4	8	1
1	1	1	11	10	5	1	1	1
1	2	1	1	7	1	3	5	3
1	1	1	8	14	3	1	1	1

Table 76 (Exp. 10): The average, standard deviation and standard error were calculated then there were 15 plants per treatment.

	(WT-CI)- (WT-CIII)	(WT-SPH1)- (WT-SPH3)	(WT-ACD1)- (WT-ACD3)	(C2-CI)- (C2-CIII)	(C2-SPH1)- (C2-SPH3)	(C2-ACD1)- (C2-ACD3)
	16	6	1	1	1	10
	3	2	1	1	3	4
	1	1	1	1	11	1
	3	7	1	1	1	3
	15	13	3	1	8	1
	1	1	3	1	13	1
	16	2	5	1	10	8
	4	4	1	1	10	1
	9	2	1	2	7	5
	1	4	10	1	14	1
	1	17	4	2	2	2
	1	9	6	1	1	1
	5	3	3	1	5	1
	1	1	6	1	1	3
	1	15	2	1	3	1
Mean	5.20	5.80	3.20	1.13	6.00	2.87
Stdev.	5.85	5.35	2.62	0.35	4.71	2.83
SE	1.51	1.38	0.68	0.09	1.21	0.73

Table 77 (Exp. 8): The average of lateral root per plants with sucrose were calculated by dividing the mean of lateral root with the average mean of the primary root length then there were 15 plants per treatment.

	(WT-CI) (WT-CIII)	(WT-SPH1)- (WT-SPH3)	(WT-ACD1)- (WT-ACD3)	(C2-CI)- (C2CIII)	(C2-SPH1)- (C2-SPH3)	(C2-ACD1)- (C2-ACD3)	(C2C4-CI)- (C2C4-CIII)	(C2C4-SPH1)- (C2C4-SPH3)	(C2C4-ACD1)- (C2C4-ACD3)
	5	15	19	5	13	12	1	1	2
	3	14	4	2	15	15	1	2	4
	6	21	14	2	11	12	1	1	4
	5	22	8	1	10	13	1	2	2
	5	15	5	2	12	12	1	1	4
	3	14	14	3	8	8	2	2	4
	10	24	21	3	14	8	2	2	2
	15	16	3	4	13	5	1	3	4
	2	26	17	1	10	4	2	2	3
	15	13	19	3	11	4	1	1	4
	8	24	20	3	15	3	2	3	2
	7	21	21	1	15	5	2	4	2
	5	21	25	2	17	11	2	4	4
	10	16	24	2	12	7	2	2	5
	14	16	26	3	10	11	2	3	4
Mean (Lateral root per plants)	7.5	18.5	16.0	2.5	12.4	8.7	1.5	2.2	3.3
Mean (Primary root length cm/plants)	6.9	4.2	5.5	5.6	3.3	4.8	1.1	0.7	0.6
Lateral root length cm/plants	1.1	4.4	2.9	0.4	3.8	1.8	1.5	3.3	5.7

Table 78 (Exp. 9): The same procedure was follow (see details in table 77). n=15

	(WT-CI) - (WT-CIII)	(WT-SPH1) (WT-SPH3)	(WT-ACD1) (WT-ACD3)	(C2-CI) - (C2-CIII)	(C2-SPH1) (C2-SPH3)	(C2-ACD1) (C2-ACD3)
	3	3	2	1	6	3
	3	12	3	5	5	2
	3	4	3	1	6	4
	4	4	4	2	9	6
	5	5	5	3	1	3
	5	8	4	2	5	3
	6	8	7	1	12	2
	5	11	4	1	9	4

	3	6	8	1	5	2
	5	12	4	1	7	3
	6	3	6	2	12	1
	6	3	6	1	9	2
	8	2	6	2	7	1
	4	5	3	1	4	2
	10	8	2	1	8	1
Mean (Lateral root per plants)	5.07	6.27	4.47	1.67	7.00	2.60
Mean (Primary root length cm/plants)	5.29	1.91	3.41	5.25	1.80	3.76
Lateral root length cm/plants	0.96	3.28	1.31	0.32	3.89	0.69

Table 79 (Exp. 10): The same procedure was follow (see details in table 77). n=15

	(WT-CI) - (WT-CIII)	(WT-SPH1) (WT-SPH3)	(WT-ACD1) (WT-ACD3)	(C2-CI) - (C2-CIII)	(C2-SPH1) (C2-SPH3)	(C2-ACD1) (C2-ACD3)
	16	6	1	1	1	10
	3	2	1	1	3	4
	1	1	1	1	11	1
	3	7	1	1	1	3
	15	13	3	1	8	1
	1	1	3	1	13	1
	16	2	5	1	10	8
	4	4	1	1	10	1
	9	2	1	2	7	5
	1	4	10	1	14	1
	1	17	4	2	2	2
	1	9	6	1	1	1
	5	3	3	1	5	1
	1	1	6	1	1	3
	1	15	2	1	3	1
Mean (Lateral root per plants)	5.20	5.80	3.20	1.13	6.00	2.87
Mean (Primary root length cm/plants)	4.97	3.80	2.10	5.89	5.40	5.69
Lateral root length cm/plants	1.05	1.53	1.52	0.19	1.11	0.50

Table 80 (Exp. 8): After 8 days, the shoot fresh of Arabidopsis (WT), C2, C2C4 with control with 0.5% sucrose and without bacterial (CI-CIII) and with bacterial (SPH1-SPH3) and (ACD1-ACD3) were weighed (g) per plants then there were 15 plants per treatment.

	(WT-CI)- (WT-CIII)	(WT-SPH1)- (WT-SPH3)	(WT-ACD1)- (WT-ACD3)	(C2-CI)- (C2-CIII)	(C2-SPH1)- (C2-SPH3)	(C2-ACD1)- (C2-ACD3)	(C2C4-CI)- (C2C4-CIII)	(C2C4-SPH1)- (C2C4-SPH3)	(C2C4-ACD1)- (C2C4-ACD3)
	0.0048	0.0057	0.0094	0.0018	0.0033	0.0025	0.0020	0.0029	0.0025
	0.0084	0.0120	0.0099	0.0035	0.0028	0.0049	0.0027	0.0022	0.0024
	0.0062	0.0114	0.0089	0.0068	0.0036	0.0042	0.0022	0.0012	0.0032
	0.0056	0.0062	0.0054	0.0066	0.0045	0.0046	0.0019	0.0022	0.0022
	0.0063	0.0067	0.0067	0.0039	0.0030	0.0050	0.0024	0.0021	0.0019
	0.0070	0.0061	0.0052	0.0057	0.0039	0.0044	0.0025	0.0056	0.0023
	0.0051	0.0097	0.0090	0.0047	0.0025	0.0030	0.0037	0.0029	0.0023
	0.0104	0.0089	0.0030	0.0039	0.0033	0.0052	0.0040	0.0026	0.0031
	0.0072	0.0088	0.0125	0.0024	0.0042	0.0038	0.0033	0.0019	0.0022
	0.0071	0.0072	0.0080	0.0040	0.0019	0.0034	0.0018	0.0022	0.0011
	0.0092	0.0057	0.0057	0.0022	0.0036	0.0039	0.0011	0.0025	0.0040
	0.0129	0.0067	0.0071	0.0035	0.0036	0.0024	0.0019	0.0021	0.0033
	0.0103	0.0086	0.0027	0.0029	0.0053	0.0032	0.0020	0.0030	0.0032
	0.0080	0.0062	0.0067	0.0034	0.0049	0.0031	0.0025	0.0027	0.0033
	0.0111	0.0087	0.0079	0.0026	0.0042	0.0029	0.0020	0.0026	0.0011
Mean	0.0080	0.0079	0.0072	0.0039	0.0036	0.0038	0.0024	0.0026	0.0025
Stdev.	0.0024	0.0020	0.0026	0.0015	0.0009	0.0009	0.0008	0.0010	0.0008
SE	0.0006	0.0005	0.0007	0.0004	0.0002	0.0002	0.0002	0.0002	0.0002

Table 81 (Exp. 9): The same procedure was follow (see details in table 80).

	(WT-CI)- (WT-CIII)	(WT-SPH1)- (WT-SPH3)	(WT-ACD1)- (WT-ACD3)	(C2-CI)- (C2-CIII)	(C2-SPH1)- (C2-SPH3)	(C2-ACD1)- (C2-ACD3)
	0.0077	0.0033	0.0033	0.0034	0.0019	0.0022
	0.0083	0.0038	0.0044	0.0028	0.0026	0.0025
	0.0077	0.0024	0.0085	0.0048	0.0044	0.0018
	0.0067	0.0070	0.0040	0.0053	0.0022	0.0021
	0.0045	0.0017	0.0040	0.0059	0.0037	0.0019
	0.0180	0.0480	0.0033	0.0032	0.0031	0.0025
	0.0094	0.0036	0.0039	0.0076	0.0027	0.0040
	0.0072	0.0049	0.0020	0.0045	0.0029	0.0045
	0.0115	0.0046	0.0064	0.0038	0.0052	0.0042
	0.0117	0.0046	0.0065	0.0036	0.0041	0.0036
	0.0094	0.0014	0.0041	0.0045	0.0037	0.0024

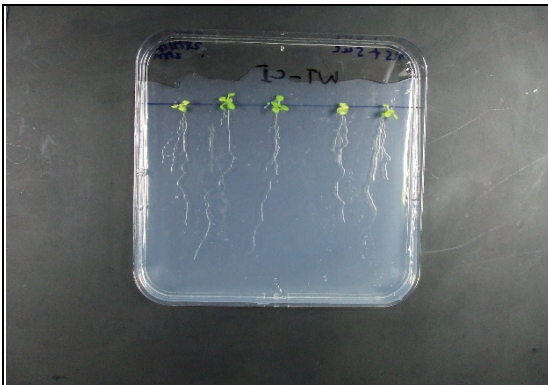
	0.0087	0.0039	0.0048	0.0035	0.0026	0.0034
	0.0087	0.0040	0.0042	0.0032	0.0026	0.0041
	0.0072	0.0038	0.0082	0.0052	0.0028	0.0028
	0.0096	0.0040	0.0050	0.0037	0.0027	0.0030
Mean	0.0091	0.0067	0.0048	0.0043	0.0031	0.0030
Stdev.	0.0031	0.0115	0.0018	0.0013	0.0009	0.0009
SE	0.0008	0.0030	0.0005	0.0003	0.0002	0.0002

Table 82 (Exp. 10): The same procedure was follow except without sucrose (see details in table 80).

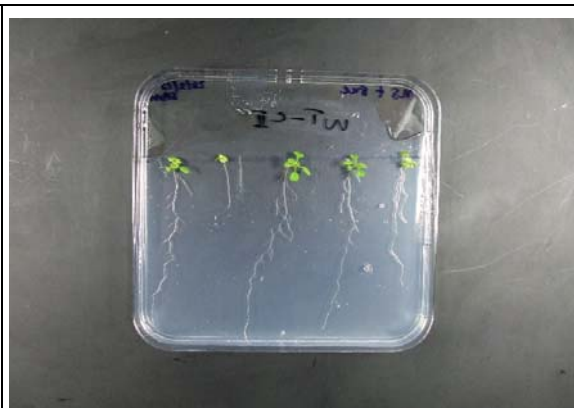
	(WT-CI)- (WT-CII)	(WT-SPH1)- (WT-SPH3)	(WT-ACD1)- (WT-ACD3)	(C2-CI)- (C2-CII)	(C2-SPH1)- (C2-SPH3)	(C2-ACD1)- (C2-ACD3)
	0.0086	0.0052	0.0034	0.0056	0.0053	0.0039
	0.0048	0.0029	0.0016	0.0060	0.0030	0.0041
	0.0044	0.0016	0.0027	0.0064	0.0043	0.0017
	0.0074	0.0050	0.0030	0.0053	0.0033	0.0050
	0.0082	0.0045	0.0034	0.0020	0.0027	0.0052
	0.0052	0.0036	0.0024	0.0037	0.0042	0.0015
	0.0091	0.0031	0.0029	0.0049	0.0044	0.0047
	0.0065	0.0030	0.0016	0.0055	0.0052	0.0050
	0.0092	0.0033	0.0038	0.0052	0.0047	0.0041
	0.0060	0.0032	0.0045	0.0052	0.0050	0.0045
	0.0047	0.0078	0.0048	0.0052	0.0034	0.0046
	0.0039	0.0026	0.0028	0.0045	0.0034	0.0043
	0.0053	0.0044	0.0043	0.0040	0.0044	0.0025
	0.0042	0.0072	0.0040	0.0050	0.0027	0.0032
	0.0023	0.0097	0.0033	0.0030	0.0036	0.0038
Mean	0.0060	0.0045	0.0032	0.0048	0.0040	0.0039
Stdev.	0.0021	0.0022	0.0010	0.0012	0.0009	0.0012
SE	0.0005	0.0006	0.0002	0.0003	0.0002	0.0003

12.

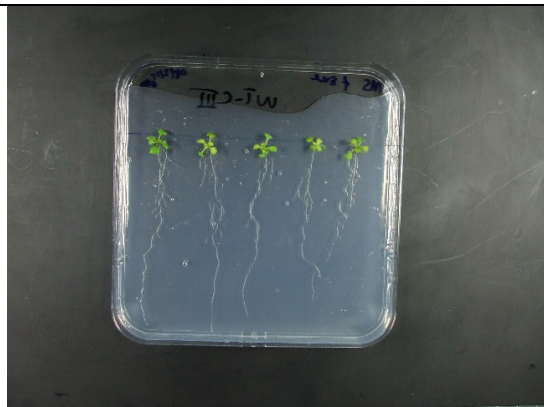
Table 83 (Exp. 8): Photographs of five WT plants on agar plates with 0.5% sucrose as control (CI-CIII), and bacterial (SPH1-SPH3) were taken after 8 days on growth chambers.



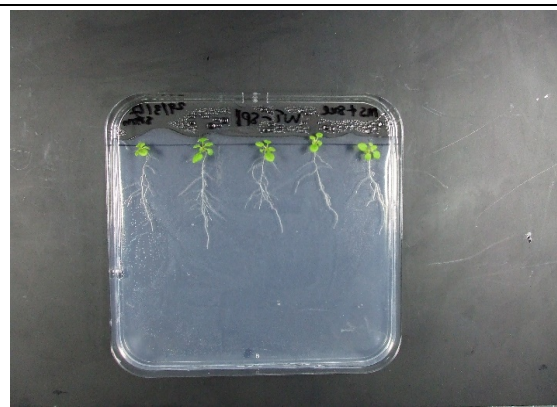
WT-CI



WT-CII



WT-CIII



WT-SPH1

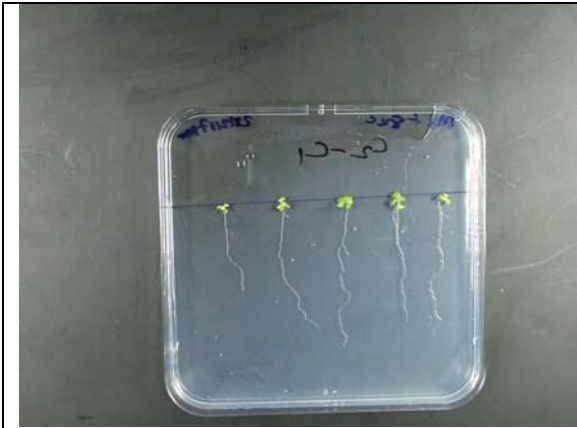


WT-SPH2

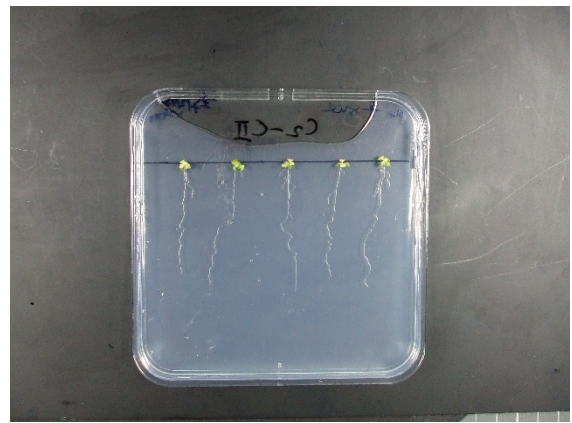


WT-SPH3

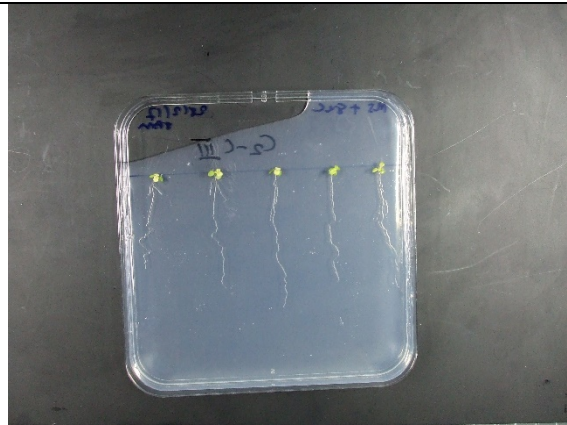
Table 84 (Exp.8): The same procedure was carried out for C2 (see table 83 for details).



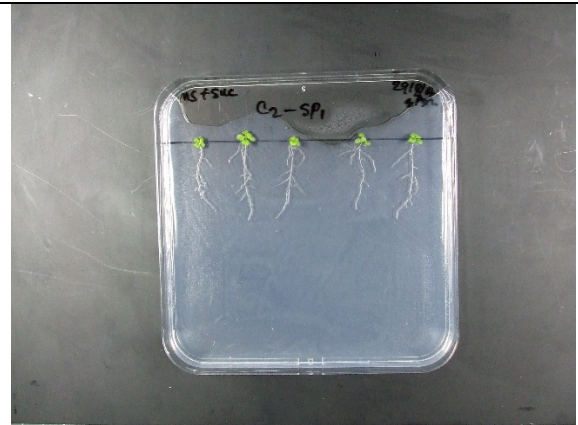
C2-CI



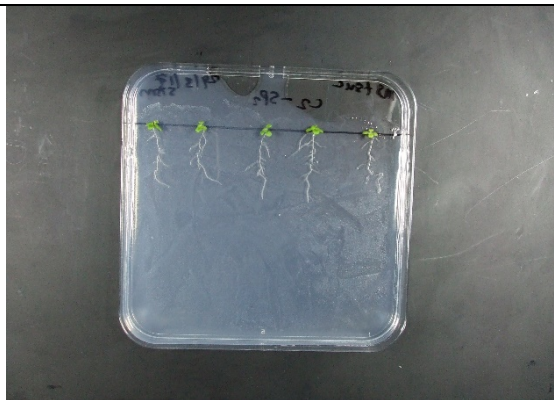
C2-CII



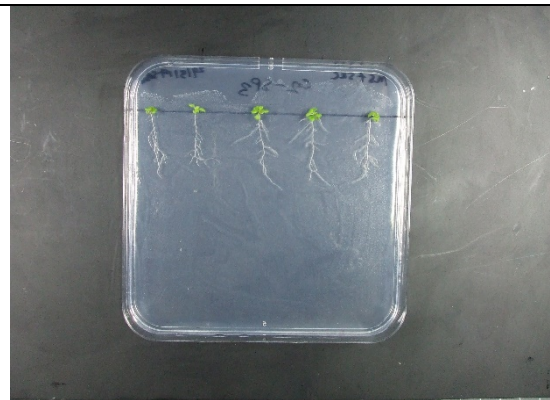
C2-CIII



C2-SPH1



C2-SPH2



C2-SPH3

Table 85 (Exp. 8): The same procedure was carried out for C2C4 (see table 83 for details)

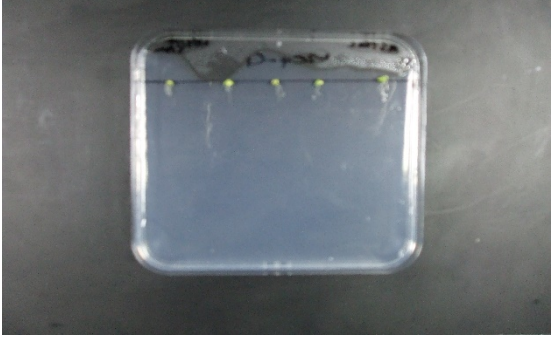
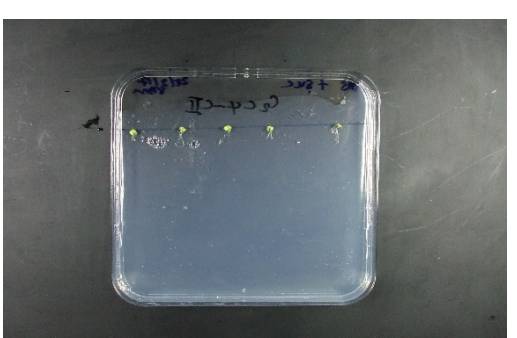
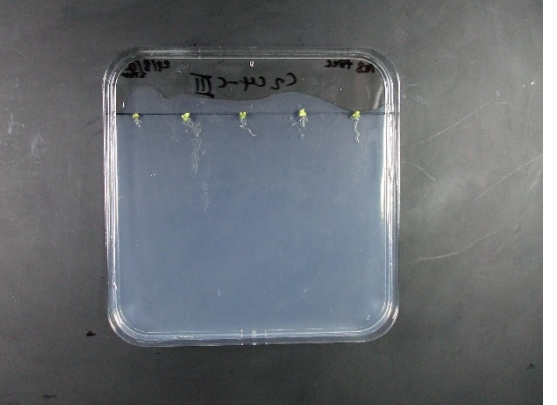
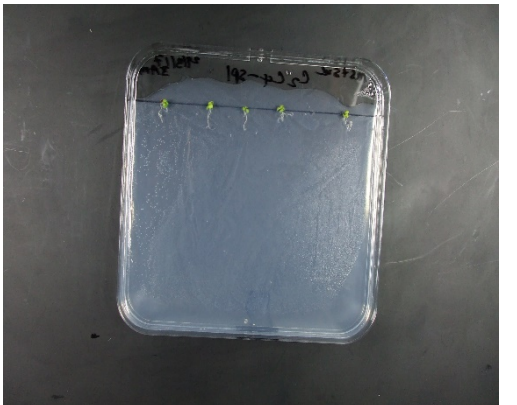
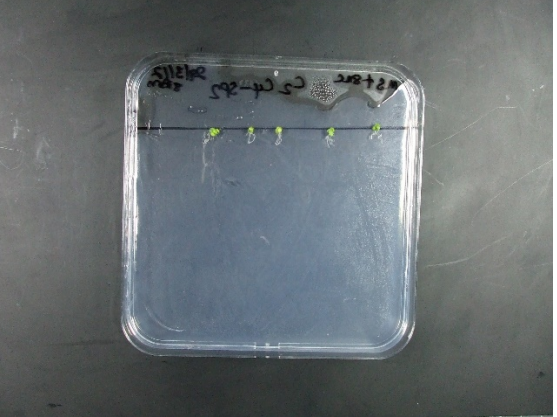

	
<p>C2C4-CI</p>	<p>C2C4-CII</p>
	
<p>C2C4-CIII</p>	<p>C2C4-SPH1</p>
	
<p>C2C4-SPH2</p>	<p>C2C4-SPH3</p>

Table 86 (Exp. 8): Photographs of five WT plants on agar plates with 0.5% sucrose as control (C1-C11), and bacterial (ACD1-ACD3) were taken after 8 days on growth chambers


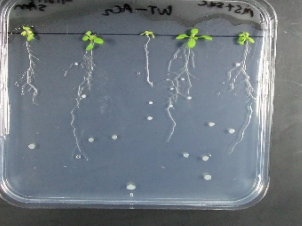

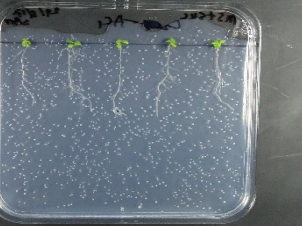






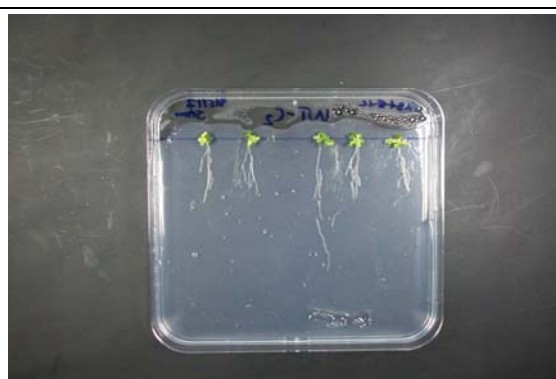
	
<p>WT-ACD1</p>	<p>WT-ACD2</p>
	
<p>WT-ACD3</p>	<p>C2-ACD1</p>
	
<p>C2-ACD2</p>	<p>C2-ACD3</p>
	
<p>C2C4-ACD1</p>	<p>C2C4-ACD2</p>
	
<p>C2C4-ACD3</p>	

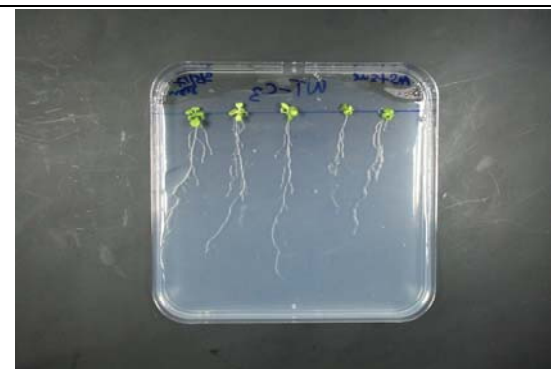
Table 87 (Exp. 9): Photographs of five WT plants on agar plates with 0.5% sucrose as control (CI-CIII), and bacterial (SPH1-SPH3) were taken after 8 days on growth chambers.



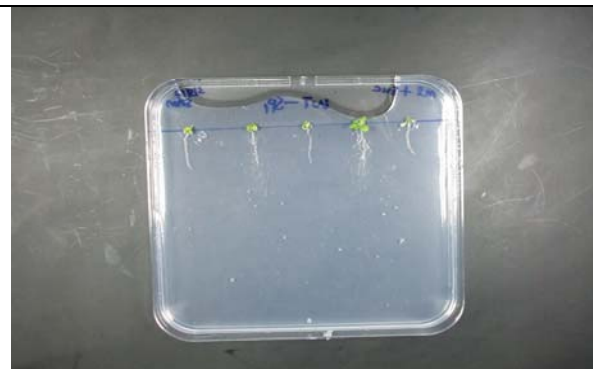
WT-CI



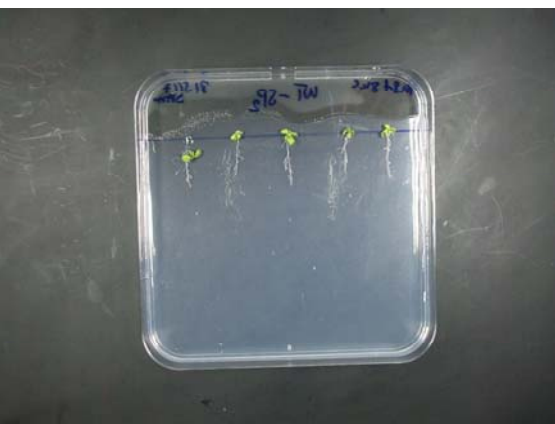
WT-CII



WT-CIII



WT-SPH1



WT-SPH2



WT-SPH3

Table 88 (Exp.9): The same procedure was carried out for C2 (see table 83 for details).


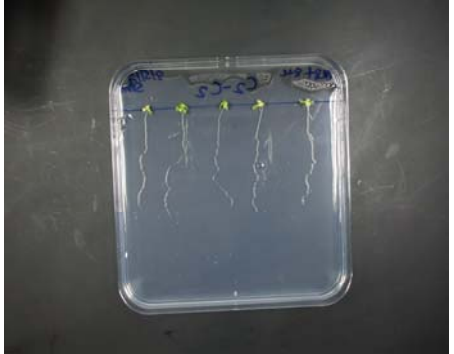
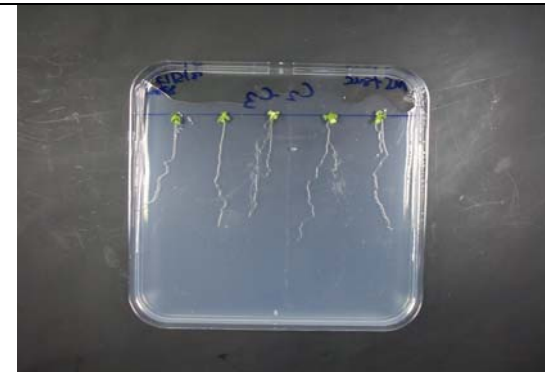

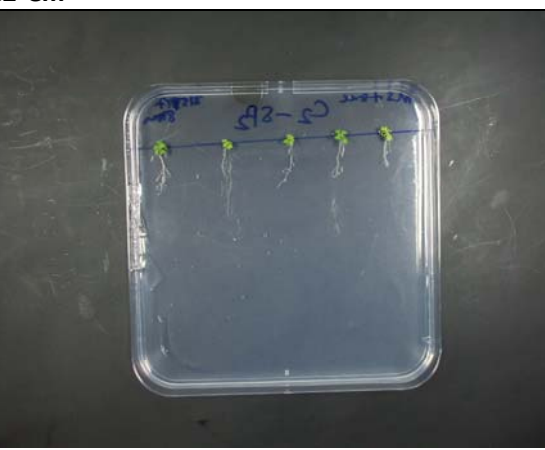
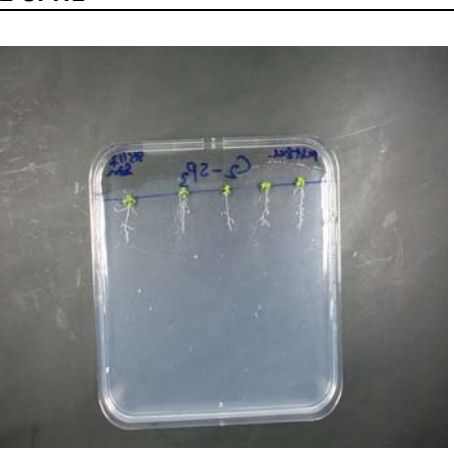
	
<p>C2-CI</p>	<p>C2-CII</p>
	
<p>C2-CIII</p>	<p>C2-SPH1</p>
	
<p>C2-SPH2</p>	<p>C2-SPH3</p>

Table 89 (Exp. 9): Photographs of five WT plants on agar plates with 0.5% sucrose as control, and bacterial (ACD1-ACD3) were taken after 8 days on growth chambers

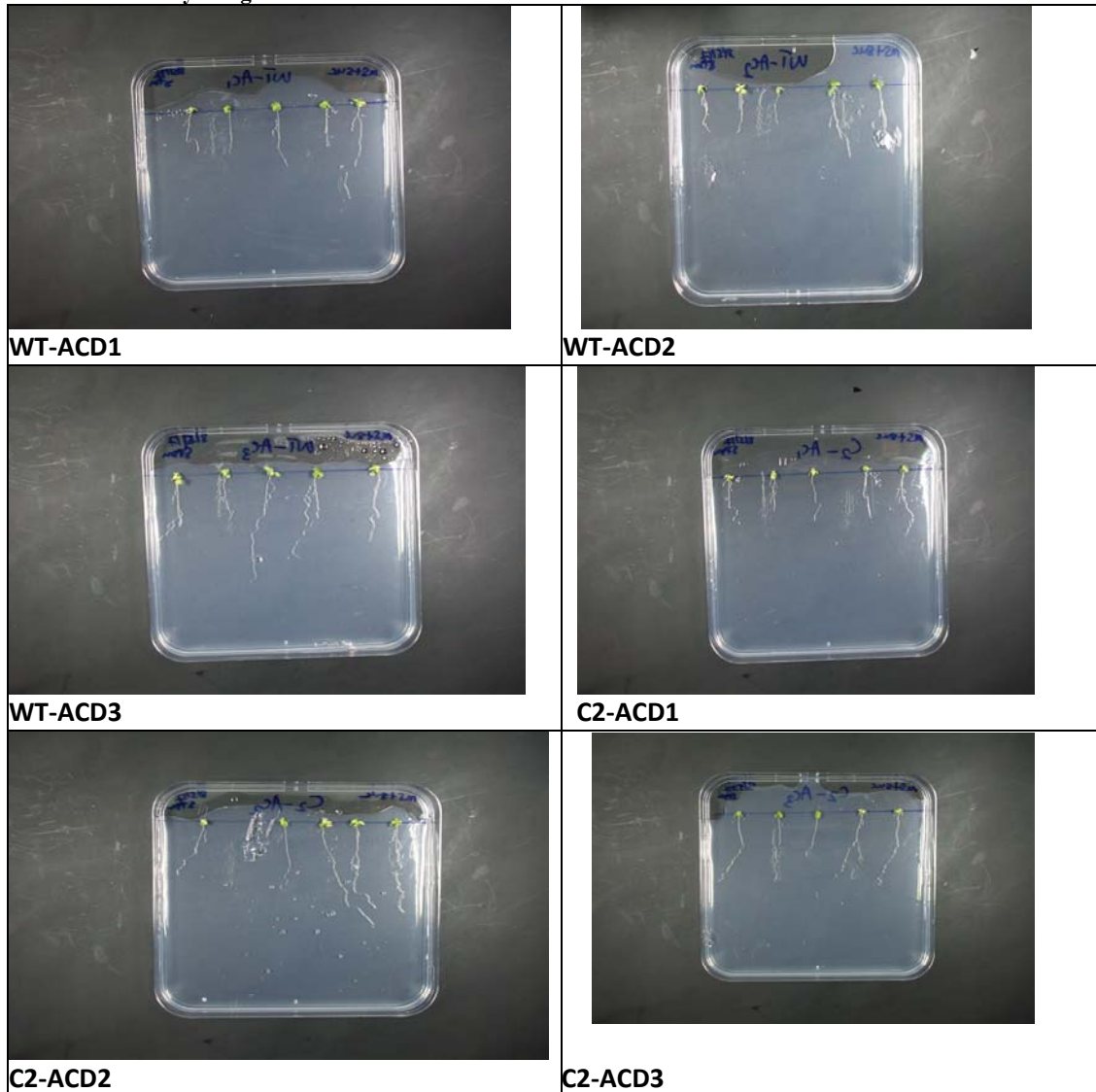
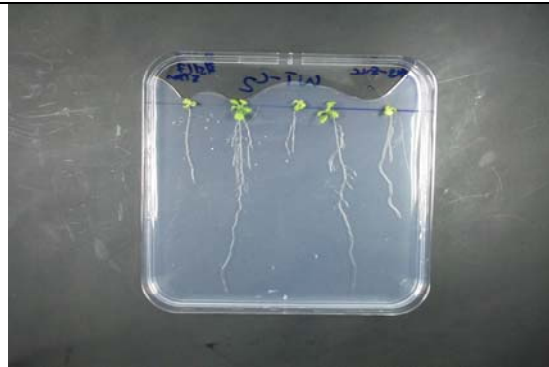


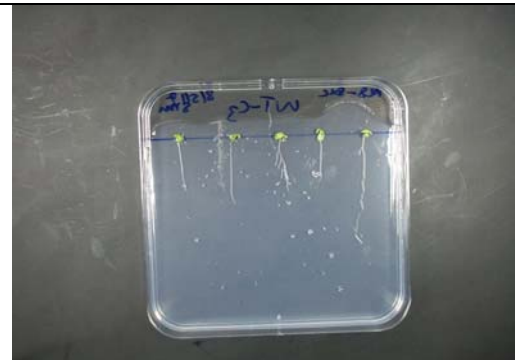
Table 90 (Exp. 10): Photographs of five WT plants on agar plates without sucrose as control (CI-CIII), and bacterial (SPH1-SPH3) were taken after 8 days on growth chambers.



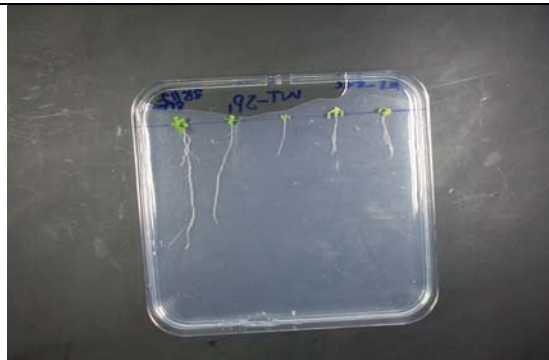
WT-CI



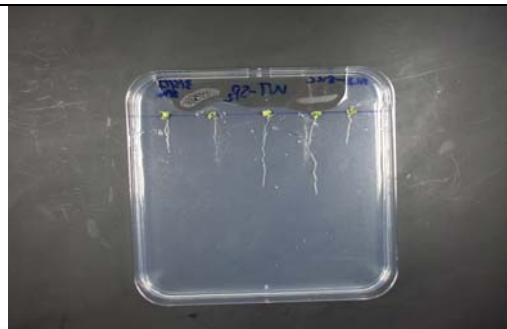
WT-CII



WT-CIII



WT-SPH1



WT-SPH2



WT-SPH3

Table 91 (Exp.10): The same procedure was carried out for C2 (see table 83 for details).

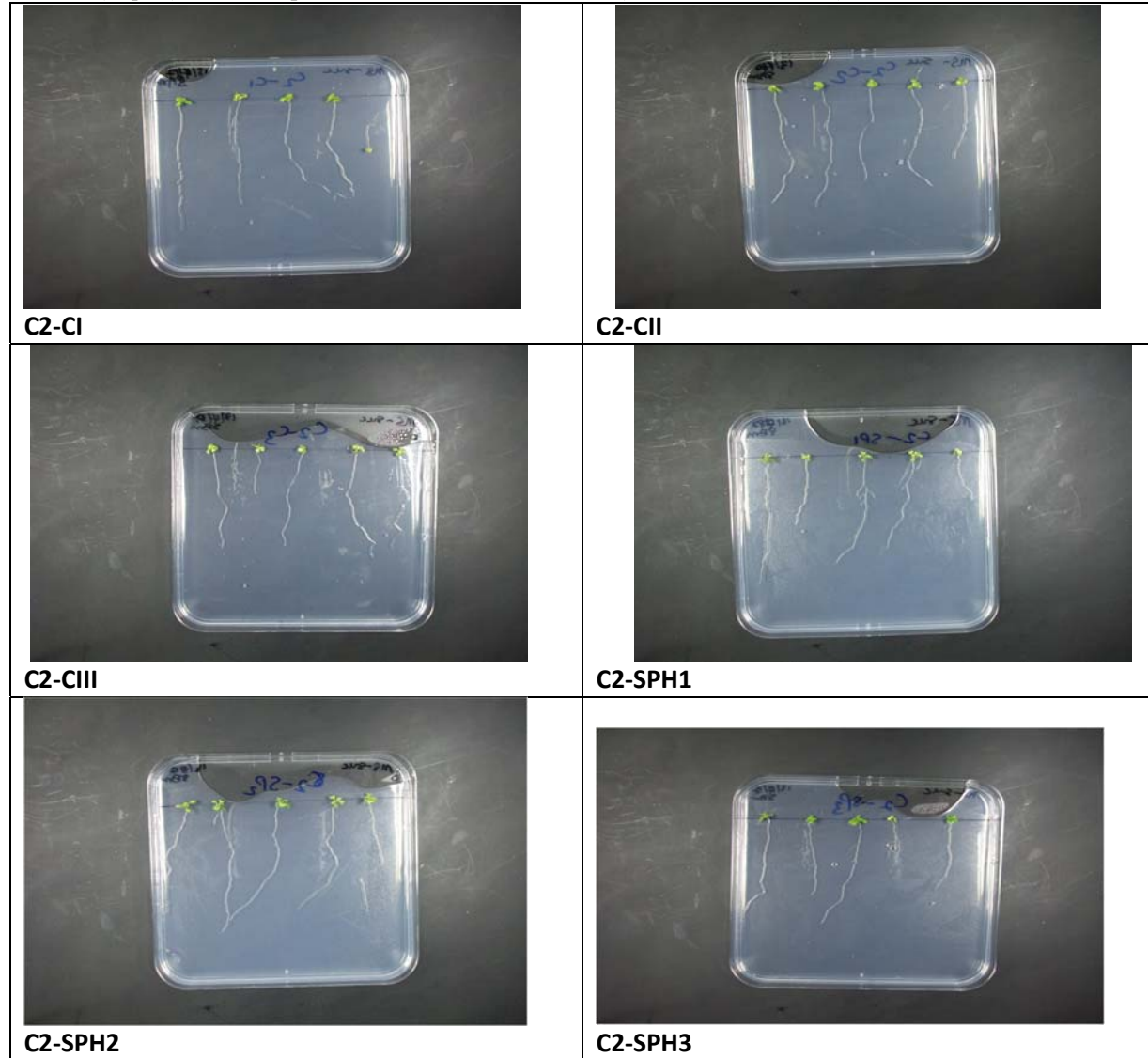
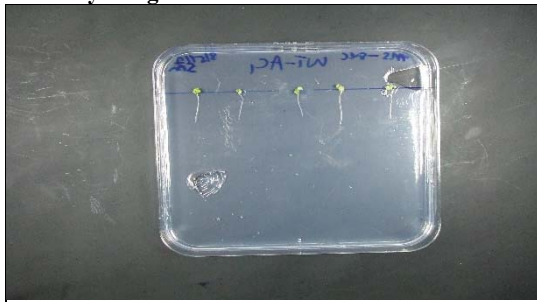
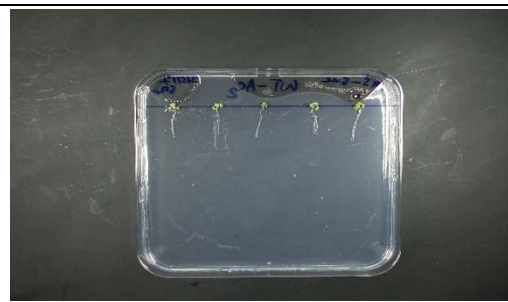


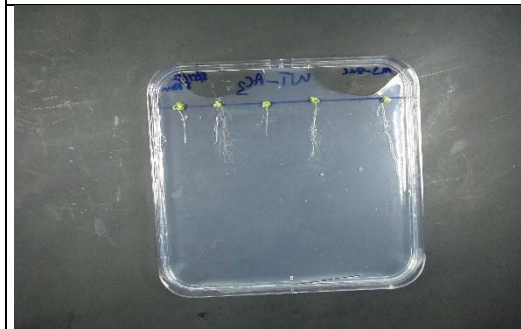
Table 94 (Exp. 10): Photographs of five WT plants on agar plates without sucrose as control, and bacterial (ACD) were taken after 8 days on growth chambers



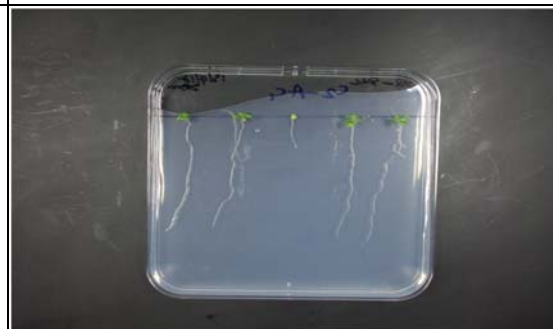
WT-ACD1



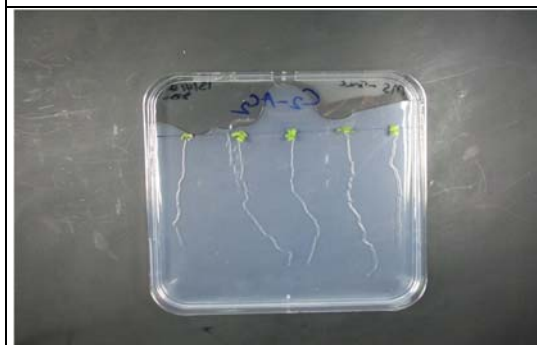
WT-ACD2



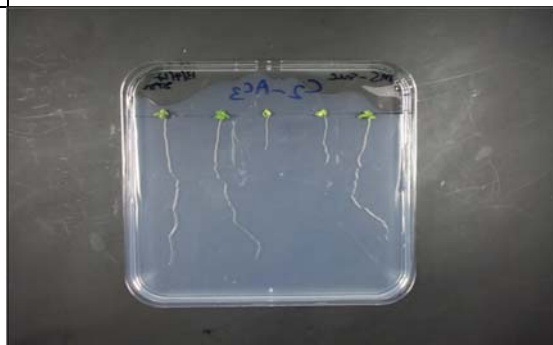
WT-ACD3



C2-ACD1



C2-ACD2



C2-ACD3