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BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
Department of Chemistry

IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION OF NOVEL BACTERIA ASSOCIATED WITH TRITICUM MONOCOCCUM SSP. MONOCOCCUM

DOCTOR OF PHILOSOPHY

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ACADEMIC SUPERVISOR
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BOLU, AUGUST - 2021

APPROVAL OF THE THESIS

IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION OF NOVEL BACTERIA ASSOCIATED WITH TRITICUM MONOCOCCUM SSP. MONOCOCCUM submitted by **Ömer Can ÜNÜVAR** and defended before the Examining Committee Members listed below in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in **Department of Chemistry, Institute of Graduate Studies of Bolu Abant Izzet Baysal University in 9.08.2021** by

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ABSTRACT

IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION OF NOVEL BACTERIA ASSOCIATED WITH TRITICUM MONOCOCCUM SSP. MONOCOCCUM
PHD THESIS
ÖMER CAN ÜNÜVAR
BOLU ABANT IZZET BAYSAL UNIVERSITY
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Triticum monococcum ssp. *monococcum* (einkorn) is an ancestral wheat species originated from Karacadağ Mountain, Turkey about 10-12,000 years ago. Wheat is one of the major food sources for growing world population. Since the food requirements have been increasing, fertilization is a necessity to increase the product yield. Uncontrolled and excessive usage of chemical fertilizers have been threatening the environment and food safety. In recent years, the demand for developing sustainable approaches to increase the yield is escalated. Usage of plant growth promoting bacteria is a promising alternative by increasing crop yield and increasing the plant resistance against environmental stresses. The major challenge for using biofertilizers in agriculture rise from the growth-incompetency of isolated bacteria in various environments. In this study, we hypothesize that growth of einkorn wheat can be promoted by using the bacteria isolated from rhizosphere of local wheat species (Iza wheat). In this thesis study we aimed to isolate bacteria that can promote yield increase in wheat. We collected root samples of *T. monococcum* ssp. *monococcum* and isolated ten different bacteria. According to interpretation of identification three methods (MALDI-TOF, 16S rDNA, and 16S-23S rDNA intergenic region) the bacteria isolated in this study are *Arthrobacter* spp. ESU164, *Arthrobacter* spp. ESU193, *Pseudomonas* spp. ESU131, *Pseudomonas* spp. ESU141, *Pseudomonas poae* strain ESU182, *Pseudomonas thivervalensis* strain ESU192, *Pseudomonas* spp. ESU1531, *Bacillus subtilis* strain ESU181, *Microbacterium foliorum* strain ESU172, *Microbacterium* spp. ESU121. For each isolate we investigated its biochemical properties along with their impact on *T. monococcum* ssp. *monococcum* development. The results show that all isolates have the ability of fixing nitrogen. The highest phosphate solubilization capacity has been reported as 2.805±0.439 for *Pseudomonas* spp. ESU131 while all the species show phosphate solubilization capacities except *Microbacterium foliorum* strain ESU172. All isolated species are IAA productors. In addition to these results, nine different bacteria positively affected the plant growth parameters. These species have important properties that can be used as biofertilizers in agricultural applications.

KEYWORDS: Agricultural biotechnology, Biofertilizer, PGPB, Sustainable agriculture, Iza wheat

ÖZET

TRITICUM MONOCOCCUM SSP. MONOCOCCUM KÖKLERİ İLE İLİŞKİLİ YENİ BAKTERİLERİN TANIMLANMASI VE BİYOKİMYASAL KARAKTERİZASYONU
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Triticum monococcum ssp. *monococcum* (einkorn) yaklaşık 10-12,000 yıl öncesine Karacadağ, Türkiye bölgesinde orjinlenen atasal bir buğday türüdür. Buğday, artan dünya nüfusu için başlıca besin kaynaklarından biridir. Gıda ihtiyacı arttıkça ürün verimini arttırmak amacıyla gübreleme zorunlu bir hal almıştır. Fakat kimyasal gübrelerin kontrolsüz ve aşırı kullanımı çevreyi ve gıda güvenliğini tehdit etmektedir. Son yıllarda verimi arttırmak için tarımda sürdürülebilir yaklaşımların geliştirilmesine ihtiyaç artmaktadır. Bitki büyümesini teşvik eden bakterilerin kullanımı, mahsul verimini ve bitkinin çevresel strese karşı direncini arttıran bir yaklaşım sağlamaktadır. Biyogübrelerin tarımda kullanımında en büyük zorluk çeşitli ortamlardan izole edilen bakterilerin uygulama alanlarına adaptasyon problemlerinden kaynaklanmaktadır. Bu çalışmada, yerel buğday türü olan Iza buğdayı rizosferinden izole edilen bakteriler kullanılarak bitki büyümesinin destekleneceği varsayılmıştır. Bu amaçla buğdayda verim artışını sağlayacak bakterilerin izolasyonu yapılmış olup, bakterilerin tanımlanması üç yöntemin (MALDI-TOF, 16S rDNA ve 16S-23S rDNA intergenik bölge) yorumlanmasına göre yapılmıştır. Bu kapsamda tanımlaması yapılan bakterilerin tür adları *Arthrobacter* spp. ESU164, *Arthrobacter* spp. ESU193, *Pseudomonas* spp. ESU131, *Pseudomonas* spp. ESU141, *Pseudomonas* poae strain ESU182, *Pseudomonas thivervalensis* strain ESU192, *Pseudomonas* spp. ESU1531, *Bacillus subtilis* strain ESU181, *Microbacterium foliorum* strain ESU172, *Microbacterium* spp. ESU121 olarak belirlenmiştir. Ayrıca çalışma kapsamında her izolatın biyokimyasal özellikleri ve *Triticum monococcum* ssp. *monococcum* gelişimi üzerine etkileri belirlenmiştir. Sonuçlar tüm bakterilerin azot fiksleme kabiliyetlerinin olduğunu göstermektedir. Fosfat çözebilme kapasitesi en yüksek tür 2.805±0.439 değeri ile *Pseudomonas* spp. ESU131 iken *Microbacterium foliorum* strain ESU172 izolatı hariç tüm izolatların fosfat çözebilme kapasitesi göstermektedir. Tüm izolatlar IAA üreticisidirler. Bu sonuçlara ek olarak, dokuz farklı izolat bitki büyüme parametreleri üzerinde olumlu etki göstermiştir. Bu türler tarımsal uygulamalara yönelik biyogübre olarak kullanılabilecek önemli özelliklere sahiptirler.
ANAHTAR KELİMELER: Tarımsal biyoteknoloji, Mikrobiyal gübre, PGPB, Sürdürülebilir tarım, Iza buğdayı

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LIST OF ABBREVIATIONS AND SYMBOLS

µ	: Micro
^{0}C	: Celsius degree
ACC	: 1-aminocyclopropane-1-carboxylate deaminase
Al	: Aluminium
As	: Arsenic
ATP	: Adenosine triphosphate
BNF	: Biological nitrogen fixation
Ca	: Calcium
Cd	: Cadmium
cfu.	: Colony-forming Unit
Cu	: Cupper
df	: Degrees of Freedom
Fe	: Iron
g	: Gram
GA	: Gibberellic acid
H	: Hydrogen
Hg	: Mercury
IAA	: Indole-3-acetic acid
K	: Potassium
L	: Litre
LB	: Luria-Bertani Medium
M	: Molar
m	: Milli
MALDI-TOF	: Matrix Assisted Laser Desorption Ionization-Time of Flight
Mg	: Magnesium
N	: The Total Number of Individuals
N	: Nitrogen
Ni	: Nickel
nm	: Nanometre

O	: Oxygen
OD	: Optical Density
P	: Phosphorus
Pb	: Lead
PBST	: Phosphate Buffered Saline Tween 20
PGPB	: Plant Growth Promoting Bacteria
Po	: Polonium
rDNA	: Ribosomal Deoxyribonucleic acid
SD	: Standard deviation
Sid	: Siderophore Production
spp.	: Species
Trp	: Tryptophan
U	: Uranium
v	: Volume
w	: Weight
Zn	: Zinc

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"…to the Bread of Life…"

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1. INTRODUCTION

1.1 Agriculture and Wheat

In *The Epic of Gilgamesh,* existence of the bread has come to represent the difference between barbarous humans and truly civilized humans. The dependence of the Enkidu on wilderness for survival has been eliminated by bread. Bread was a door which opened to the agrarian civilization (1).

The domestication of plants and animals developed separately in numerous regions of the earth. Archaeologists have realized that the domestication of plants and animals developed independently in several regions of the world. The Fertile Crescent, some parts of the Yangzi and Yellow River basins of China, and Mesoamerica have the strong arguments for archaeological priority. The Fertile Crescent which covered in southern Iraq, Syria, Lebanon, Jordan, Israel, Egypt, and parts of Turkey has known as the beginning point of the agriculture and food production dated back nearly ten thousand years. Figure 1.1 shows the borders of the Fertile Crescent (2).

Wheat that was the one of the crops domesticated more than 10 thousand years ago in the Fertile Crescent, from the day to today it has been providing fundamental food for more than 1/3 population of the world (3,4). For about 35% of the population of world, it is a basic nutrient, and it provides 20% of the energy source all over the world (5). The representation of the history of evolution of wheat species is given in Figure 1.2.

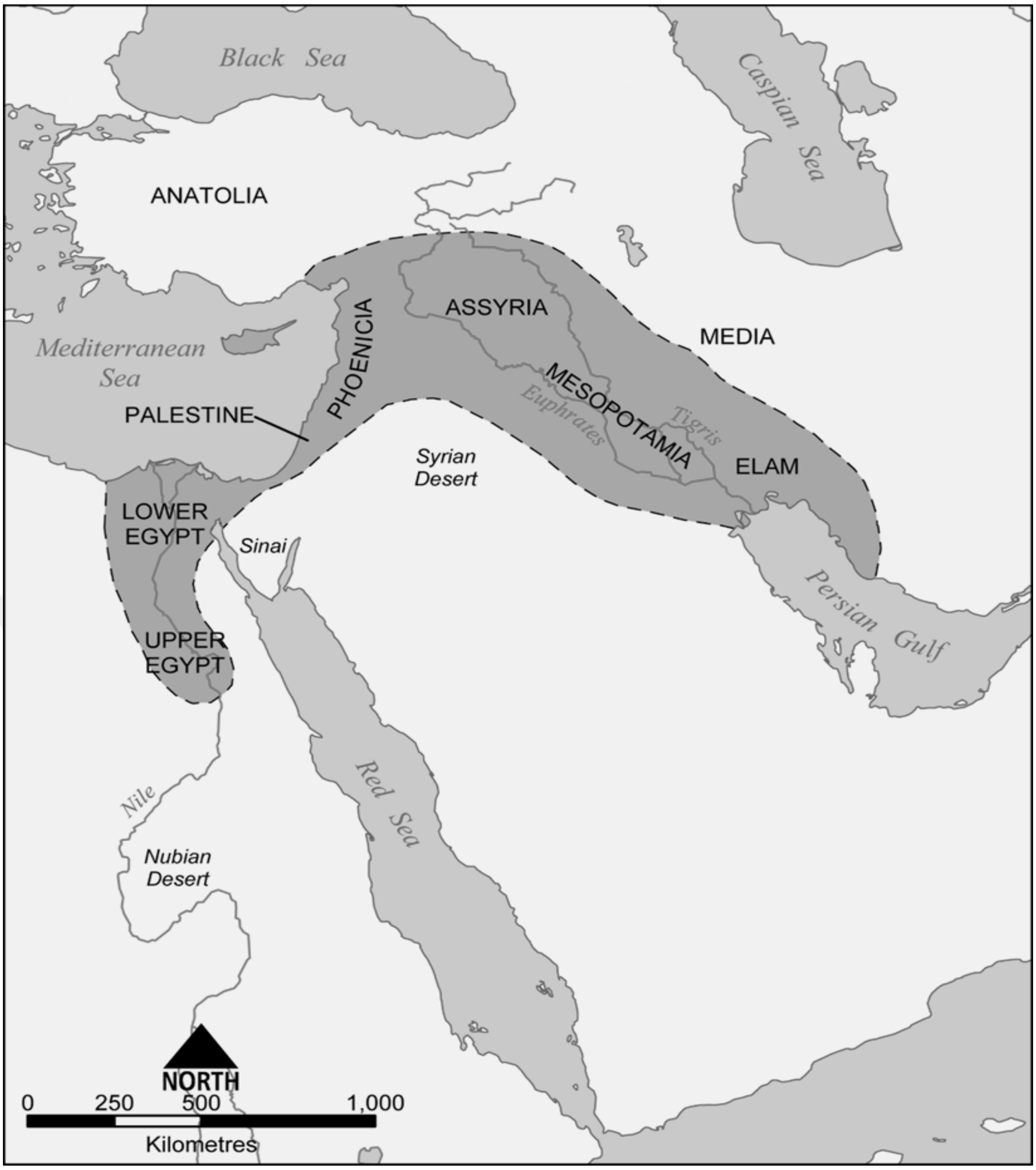

Figure 1.1 The Fertile Crescent

The demand for food is expected to rise approximately 40% in 2030 because of the growing world population. To meet this demand agricultural areas would stabilize at 1.5 billion hectares and the yield would rise of 2% for every year on the other hand, yield increasing has been reported as 0.9% over the last decade. It is worse in Europe because of climate change and its effects (6,7).

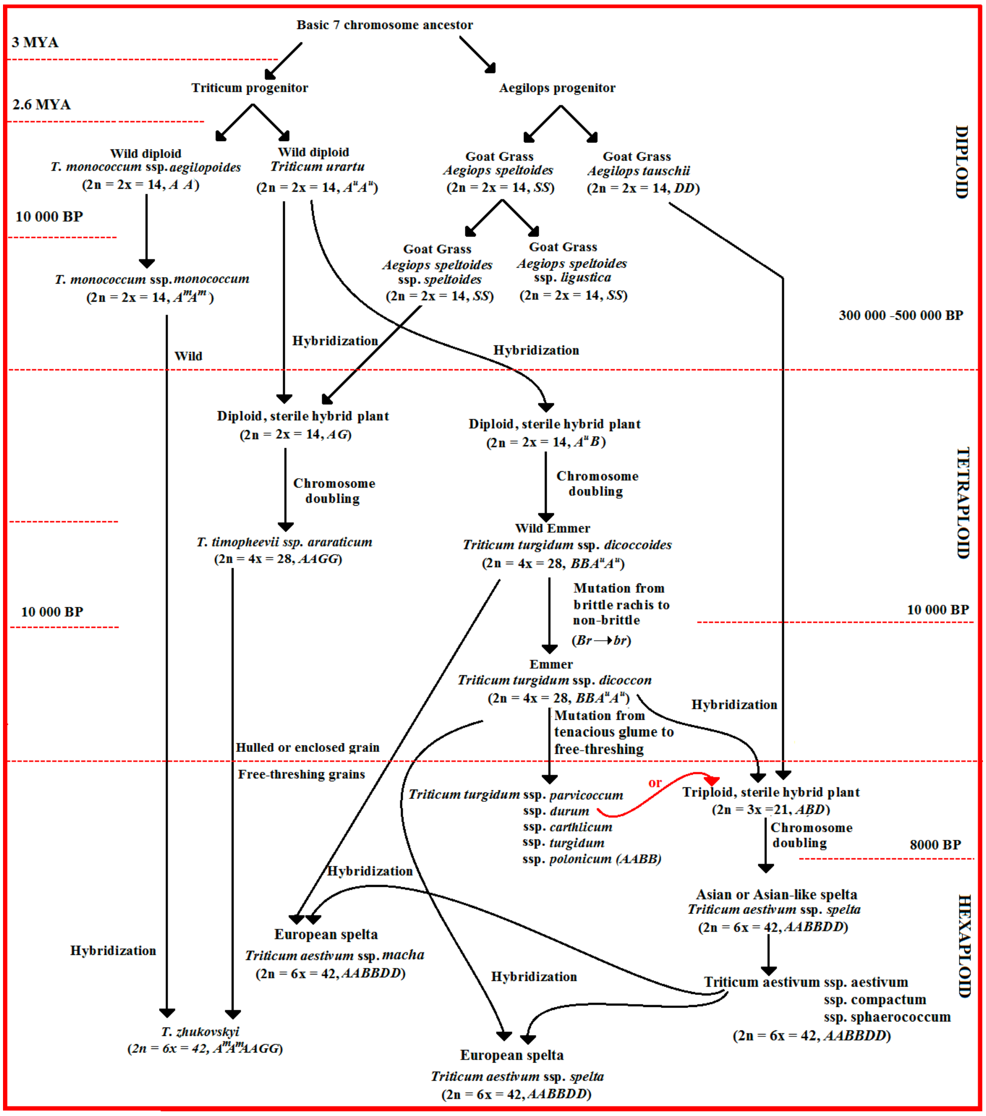

Figure 1.2 The history of evolution of wheat species (8,9).

There are both wild and domesticated species in *Triticum L.* genus. Wild types are able to spread their seeds, but the domestic types cannot. The endurance of the domesticated wheats cannot be continued without farming (planting, providing necessary inputs), the domesticated wheat species have hard rachis that does not allow spreading of the seeds unlike wild ones (10).

It is likely that *T. monococcum* ssp. *monococcum* is evoluated from its wild diploid progenitor, *T. boeoticum* that along with *T. urartu* originated from common ancestor, *Triticum* progenitor. The grains of ancient wheat as *Triticum monococcum* (AA), *Triticum dicoccum* (AABB) and *Triticum spelta* (AABBDD) has an enclosed cover, thus they are called hulled wheat (11).

There are diploid, tetraploid, and hexaploid types in *Triticum* species. *Triticum monococcum* (2n=14) is diploid, while *Triticum durum, Triticum turanicum, Triticum carthlicum, Triticum polonicum, Triticum dicoccum* and *Triticum timophevii* are tetraploid (2n=28), and *Triticum aestivum* and *Triticum spelta* are commonly known hexaploid (2n=42) species (12).

1.1.1 Einkorn Wheat

The diploid (A^mA^m) cultured *Triticum monococcum* ssp. *monococcum* is one of the first cultivated wheat known as einkorn that is derived from *T. monococcum* ssp. *boeoticum* in the region of Karacadağ Mountain, Turkey about 10-12,000 years ago (9). It is important in the daily diet because of its high carotenoid, antioxidants, vitamin B and E, and minerals (Zinc, iron, and copper) contents (13–15). The protein content of einkorn wheat ranges from 16.0% to 28.5%, and einkorn wheat has powerful genetic background to biotic and abiotic stresses (16,17).

1.1.2 Important Plant Growth Promoting Materials

1.1.2.1 Nitrogen

Nitrogen which is the essential part of proteins is the major element for plants. It takes roles in the building of amino acids which are vital for cells by catalysation, transportation, and photosynthesis with chlorophylls. Nitrogen promotes the vegetative parts of plants for growth and development. It also triggers growth of roots. Producing rapid growth, increasing the quality of fruits and leaves, and taking high protein content in crops are the main effects of Nitrogen on agricultural products. Additional uptake and utilization of other elements such as phosphorus and potassium are stimulated by nitrogen (18,19). On the other hand, the shortage of nitrogen causes commonly unhealthy growth, colour change of leaves from green to yellow named as chlorosis, and red-purple spots on leaves, lateral bud growth (20). All forms of nitrogen are not useful for uptake by plants, and most of the plants uptake nitrogen in nitrate form, while ammonium ion is the most useful and stable for rice (21). An optimum supply of nitrogen is important for the healthy plant growth. The deficiency causes reduction in crop yield, and excess amount of nitrogen causes adverse effects on plant.

The stable atmospheric dinitrogen gas is converted into biosynthetically available forms by nitrogen fixation process. The process can be briefly showed by the reaction below:

$$2N_{2(g)} + 8H^+ + 8e- \rightarrow 2NH_3 + H_2$$

The process occurs either by non-biological (lightning or industrial fixation) or biological. In geochemical fixation, lighting plays a key role by breaking of the triple bonds between N_2 molecules and recombination with oxygen to form NOx (22). In industrial fixation, nitrogen gas is converted to ammonia by the Haber-Bosch process under suitable conditions. The reaction briefly is given below:

$$N_2 + 3H_2 \rightarrow 2NH_3$$

The last one is the symbiotic fixation with the nitrogenase enzymes. Some rhizobium bacteria which are colonizing and living in the nodules of the plant roots, convert atmospheric nitrogen to available forms for plants (23). The detailed scheme of the nitrogen cycle in agricultural contexts is given Figure 1.3.

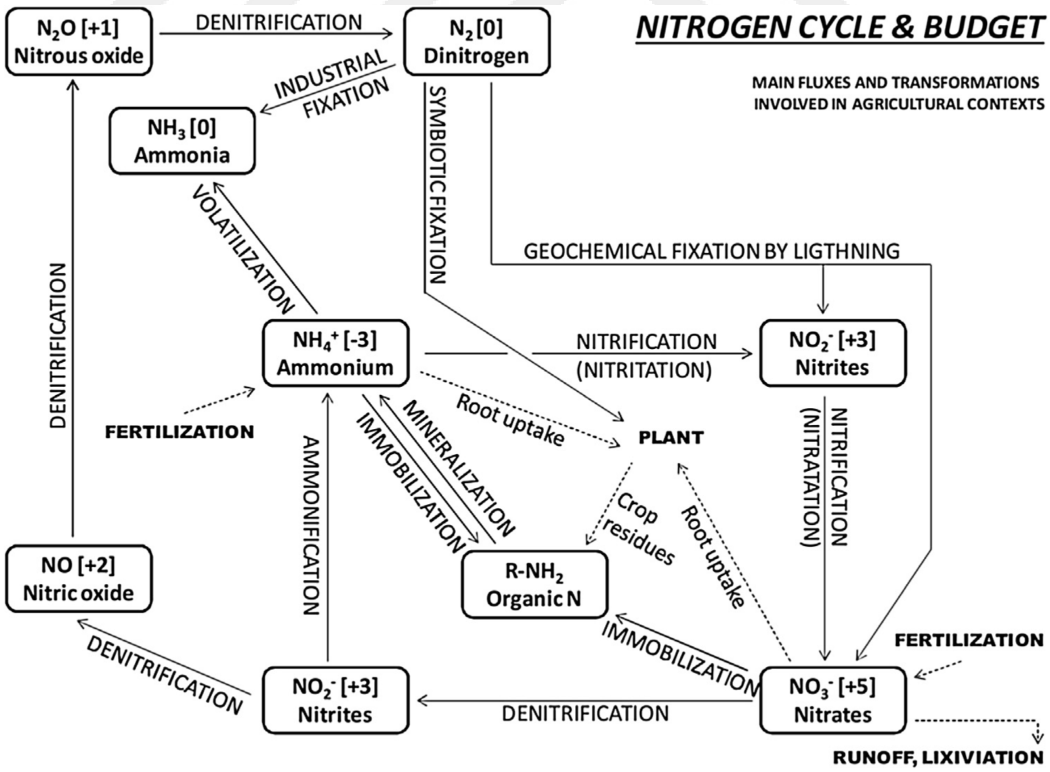

Figure 1.3 The nitrogen cycle in the agricultural contexts (23).

1.1.2.2 Phosphorus

Phosphorus (P) has known as the second most limiting element for plant growth after nitrogen (24). It takes a role in the building blocks of key molecules as nucleic acids, phospholipids, and ATP. In short, plant growth is not suitable without a reliable supply of P. Pi is also included in enzymatic reactions and in the metabolic pathways (25). The concentration of P is 2000-fold higher than plants (P concentration in total dry weight ranges from 0.05% to 0.5%) but the fixation in the form of Al/Fe or Ca/Mg phosphates turns it unavailable for plant uptake. Therefore, P deficiency problems occur in agricultural areas. At early stage of the growing, plants do not give visual symptoms under the deficiency of P. The deficiency reduces plant growth by both decreasing in photosynthesis and energy investment. Crop yield and quality are affected by P limitation. The prediction about that issue is that deficiency of P decreases the crop yields on 30-40% of the agricultural regions but the usage of excessive amount of P to correct the situation causes negatives impacts on ground and surface water (eutrophication) (26,27).

P exists many forms in the soil as plant available and unavailable inorganic forms, organic form, adsorbed P, primary mineral form. A simplified representation of phosphorus cycle is given in Figure 1.4. In this cycle, weathering, mineralization, and desorption enhance available forms of P for plants, on the other hand immobilization, precipitation and adsorption reduce available forms (28).

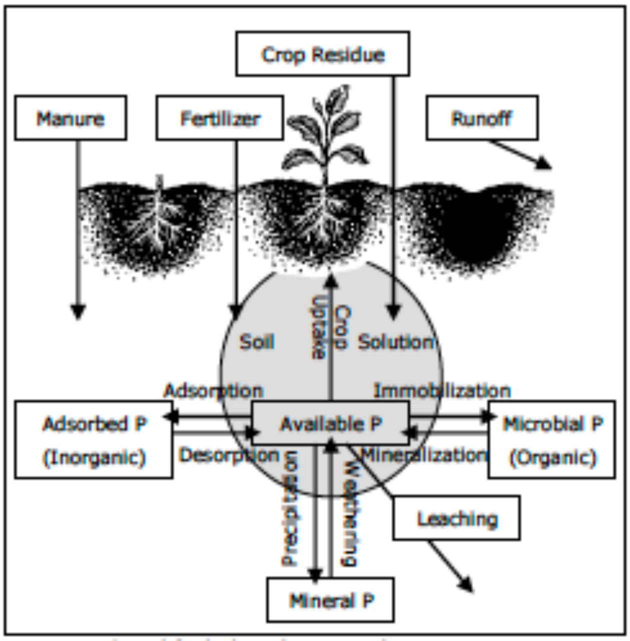

Figure 1.4 Phosphorus cycle (28).

1.1.2.3 Phytohormones

Plant hormones regulate plant growth and take part in developing a response to physical conditions. Auxin, gibberellin, cytokinin, abscisic acid, ethylene, brassinosteroids, jasmonic acid, salicylic acid, and strigolactones have been known as plant hormones to date (29).

Especially auxins that are small molecules trigger plant growth responses. They were the firstly studied phytohormones (30). The term “auxin” came from the Greek “auxein” which means “to grow”. Indole-3-acetic acid (IAA) has discovered by Salkowski in 1885 (31). IAA is the most abundant plant hormone of auxin class. IAA plays a key role in plant development as cell division, elongation, differentiation, development of fruits and photo-response (30,32). Other than the plants, there are several organisms that are able to produce IAA including certain bacterial species, actinomycetes, filamentous fungi, and yeast (33–35). The chemical structure of IAA is given in Figure 1.5. It is colourless solid and soluble in polar organic solvents.

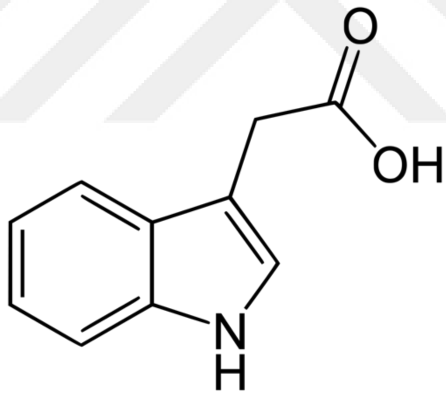

Figure 1.5 Chemical representation of IAA molecule

Cell elongation is stimulated by IAA with modifying cell conditions by enhancing the osmotic properties of the cell, increasing water permeability into cell and wall pressure decreasing etc. It increases the plant’s ability for flowering and fruiting (36). In addition, it induces longer root formation along with the root hairs and lateral roots that are necessary for nutrient uptake for plants (37).

1.2 Fertilizers

1.2.1 Chemical Fertilizers

With the growing population, the food requirement is increasing globally. To supply enough food, the usage of fertilizers is a necessity. Nevertheless, the unattended usage of fertilizers and lack of knowledge about the contents and possible side-effects threatens the environment and animal health. Chemical fertilizers are industrially produced compositions and contain potassium, phosphorus, and nitrogen that are major nutrients for plants (38). On the other hand, they also contain heavy metals such as Hg, Cd, As, Pb, Cu, Ni, and natural radionuclides like ^{238}U, ^{232}Th, and ^{210}Po (39).

Fertilizer usage has been increased with the growing population. This uncontrolled consumption of fertilizers affects the aggregation of heavy metals in soil. The absorption of these heavy metals by plants may lead them to enter the food chain (39). High usage of nitrogenous-rich fertilizers concentrates the soil with an extensive amount of nitrogen. This may cause plants to produce cancerogenic compound nitrosamine (40).

Another result of the excessive application of chemical fertilizers is eutrophication and water pollution. Eutrophication is the descension of water quality and aquatic living organisms' environment through the formation of algae as a result of the increasing amount of nitrogen and phosphate compounds in the water. Algal blooms in the water causes reduction of dissolved oxygen and production of the cytotoxic chemicals that are detrimental to other aquatic organisms (40). Fertilizers are considered as one of the main reasons for groundwater pollution caused by nitrates and salts (41). Nitrate can reach the depth of the soil. Plants can use 50% of the nitrogenous that is applied to the soil, 2-20% lost by evaporation, 15-25% reach with organic compounds in the clay soil and the remaining 2-10% reaches the ground and surface water (42).

1.2.2 Biofertilizers

To introduce plant growth promoting bacterial species, the term of “biofertilizer” must be defined. In the dictionaries, there is not a definition about it, if searched on the internet, numerous definitions such as seaweed extracts, urban waste composting, mixes of unidentified microbial constituents, or chemical fertilizers supplemented with organic substances can be found. On the other hand, the scientific literature is very clear about the definition. Scientifically, “biofertilizer” is defined as the liquid or solid mix that involves living microorganism(s) that colonizes the rhizosphere or some parts of the plant and promotes growth and development. There is a clear difference between organic fertilizer (fertilizers that contain organic compounds), and biofertilizers (43).

1.2.2.1 Common Species Used as Biofertilizer

Plant growth promoting bacteria (PGPB), that colonize the rhizosphere of the plant and help to increase the quantity and the quality of yields, they also increase the resistance of the plants against biotic and abiotic stresses (44). There are several beneficial species such as *Acinetobacter, Acetobacter, Alcaligenes, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Beijerinckia, Enterobacter, Flavobacterium, Methylobacterium, Pseudomonas, Rhizobium, Paenibacillus*, and *Pantoea* (43,45–48). In Table 1.1, the microorganisms with their functions are represented, and in Table 1.2, the roles of the various microorganisms in the literature under abiotic stress conditions are given.

Table 1.1 Plant and soil microorganisms with the functions (49)

Species	N	P	K	Sid	ACC	IAA	GA	References
Achromobacter xylosoxidans		+				+		(50)
Acinetobacter baumannii		+		+				(51)
Acinetobacter calcoaceticus		+		+		+		(52)
Aeromonas hydrophila		+				+	+	(53)
Arthroderma cuniculi		+		+				(54)
Aspergillus fumigatus				+		+	+	(55)
Aspergillus niger		+	+					(56)
Bacillus aerius	+	+		+		+		(57)
Bacillus altitudinis		+			+	+		(58)
Bacillus amyloliquefaciens	+	+		+		+		(57)
Bacillus licheniformis	+	+		+		+		(59)
Bacillus megaterium		+		+		+		(51)

Bacillus mucilaginosus	+	+		+		+		(57)
Bacillus subtilis	+	+		+		+		(57)
Bacillus thuringenesis		+		+		+		(60)
Burkholderia cepacia		+				+	+	(53)
Burkholderia gladioli		+				+	+	(53)
Enterococcus casseliflavus		+				+		(61)
Enterococcus gallinarum		+				+		(61)
Fusarium proliferatum		+				+	+	(55)
Lecanicillium psalliotae		+		+				(62)
Paenibacillus favisporus		+				+		(60)
Paenibacillus taichungensis		+		+		+		(51)
Pseudomonas entomophila		+				+		(63)
Pseudomonas koreensis		+				+		(64)
Pseudomonas luteola		+				+	+	(53)
Pseudomonas simiae		+			+	+		(64)
Pseudomonas stutzeri		+				+		(63)
Serratia marcescens		+				+		(65)
Serratia nematodiphila		+		+	+	+		(66)
Sphingomonas paucimobilis		+				+	+	(53)

N, Nitrogen fixation; P, Phosphate solubilization; K, Potassium solubilization; Sid, Siderophore Production; ACC, 1-aminocyclopropane-1-carboxylate deaminase; IAA, Indole-3-acetic acid; GA, Gibberellic acid.

Table 1.2 Role of microorganisms in plant growth affected on several abiotic stress conditions (49)

Cold Stress	**Crop**	**Response**	**References**
Azospirillum brasilense sp 245	Wheat	Dry weight increasing	(67)
Bacillus megaterium M3	Wheat	Dry weight increasing	(67)
Bacillus subtilis OSU142	Wheat	Dry weight increasing	(67)
Cellulomonas turbata AS1	Wheat	Growth and yield	(68)
Enterobacter cloacae AS6	Wheat	Growth and yield	(68)
Mycoplana bullata MpB46	Wheat	Growth (root and shoot)	(69)
Paenibacillus polymyxa BNH18	Wheat	Growth, Alleviating cold stress	(70)
Pantoea agglomerans 050309	Wheat	higher N, P, and K contents	(69)
Pseudomonas fluorescens PPRs4	Wheat	Alleviating cold stress	(71)
Pseudomonas jessani PGRs1	Wheat	Alleviating cold stress	(71)
Pseudomonas koreensis PBRs7	Wheat	Alleviating cold stress	(71)
Pseudomonas putida PGRs4	Wheat	Alleviating cold stress	(71)
Pseudomonas sp. NARs9	Wheat	Germination, root lengths	(71)

Drought and heat stress	**Crop**	**Response**	**References**
Azospirillum brasilense NO40	Wheat	Improved homeostatic	(72)
Bacillus amyloliquefaciens 5113	Wheat	Improved homeostatic	(72)
Piriformospora indica Pi	Wheat	Drought resistance, growth	(73)
Pseudomonas putida AKMP7	Wheat	Growth, Alleviating heat stress	(74)

Pantoea theicola NBRC 110557T	Wheat	Alleviating drought stress	(75)
Alcaligenes faecalis AF3	Maize	Increased root weight and protein content	(76)
Azospirillum brasilense SP-7	Maize	Increased biomass, N uptake	(77)
Herbaspirillum seropedicae Z-152	Maize	Increased biomass, N uptake	(77)
Pseudomonas putida GAP-P45	Sunflower	Increased survival, biomass	(78)

Salinity stress	**Crop**	**Response**	**References**
Achromobacter xylosoxidans 249	Wheat	Growth and SOD activity	(79)
Aeromonas hydrophila MAS-765	Wheat	Alleviate salinity, growth	(80)
Aeromonas vaga BAM-77	Wheat	Growth and Yield	(81)
Bacillus insolitus MAS17	Wheat	Alleviate salinity, growth	(80)
Pseudomonas fluorescens 153	Wheat	Salinity stress, growth	(82)
Pseudomonas putida 108	Wheat	Salinity stress, growth	(82)
Pseudomonas fluorescens	Maize	Enhanced biomass, uptake of Na^+ and K^+ ions	(83)
Pseudomonas fluorescens strain TDK1	Groundnut	Enhanced biomass	(84)
Achromobacter piechaudii	Tomato	Increased the fresh and dry weights of seedlings	(85)

Azospirillum sp.	Lettuce	Higher biomass and improved quality	(86)
Staphylococcus kloosii EY37	Radish	Increased biomass and chlorophyll content	(87)

Acidic and Alkalinity stress	**Crop**	**Response**	**References**
Aeromonas vaga BAM-77	Wheat	Growth and Yield	(81)
Micrococcus roseus SW1	Wheat	Growth and Yield	(88)
Bacillus licheniformis SA03	*Chrysanthemum*	Increased survival rates, photosynthesis, and biomass	(89)

Heavy metal-stressed stress	**Crop**	**Response**	**References**
Pseudomonas aeruginosa KP717554	Brassica	Seed germination	(90)
Pseudomonas fluorescens 002	Maize	Alleviate Al stress	(91)
Pseudomonas koreensis AGB-1	*Miscanthus sinensis*	Increased biomass, chlorophyll content, protein content	(92)
Kluyvera ascorbata SUD165	Canola	Root elongation	(93)
Enterobacter asburiae KE17	Soyabean	Increased growth rates	(94)
Pseudomonas fluorescens	*Mirabilis jalapa*	Enhanced biomass, bioaccumulation of metals	(95)
Pseudomonas fluorescens G10	*Brassica napus*	Increased root elongation, biomass, Pb uptake	(96)

1.2.2.2 Plant Growth Promoting Properties of Biofertilizers

PGPB increase growth via several mechanisms such as biological nitrogen fixation, phosphate solubilization, production of several hormones (auxins, cytokinin, gibberellins, and ethylene, enhancing the uptake of some important elements (Fe, Zn, K, and organic matters), induction of resistance, ACC deaminase production, representing antagonistic effect against pathogenic microorganisms by siderophores, b-1,3-glucanase, chitinases, and antibiotics (44,97–101).

1.2.2.2.1 Nitrogen Fixation

In biological nitrogen fixation (BNF), molecular nitrogen (N_2) is reduced in electron transfer reactions, the reaction gives ammonia and hydrogen gas (102). This reduction of N_2 to ammonium is catalysed by ATP-dependent nitrogenase enzyme complex, and the reaction is ahigh energy consuming process. Figure 1.6 represents the general reaction of nitrogen fixation and schematic structure of nitrogenase enzyme in action (103).

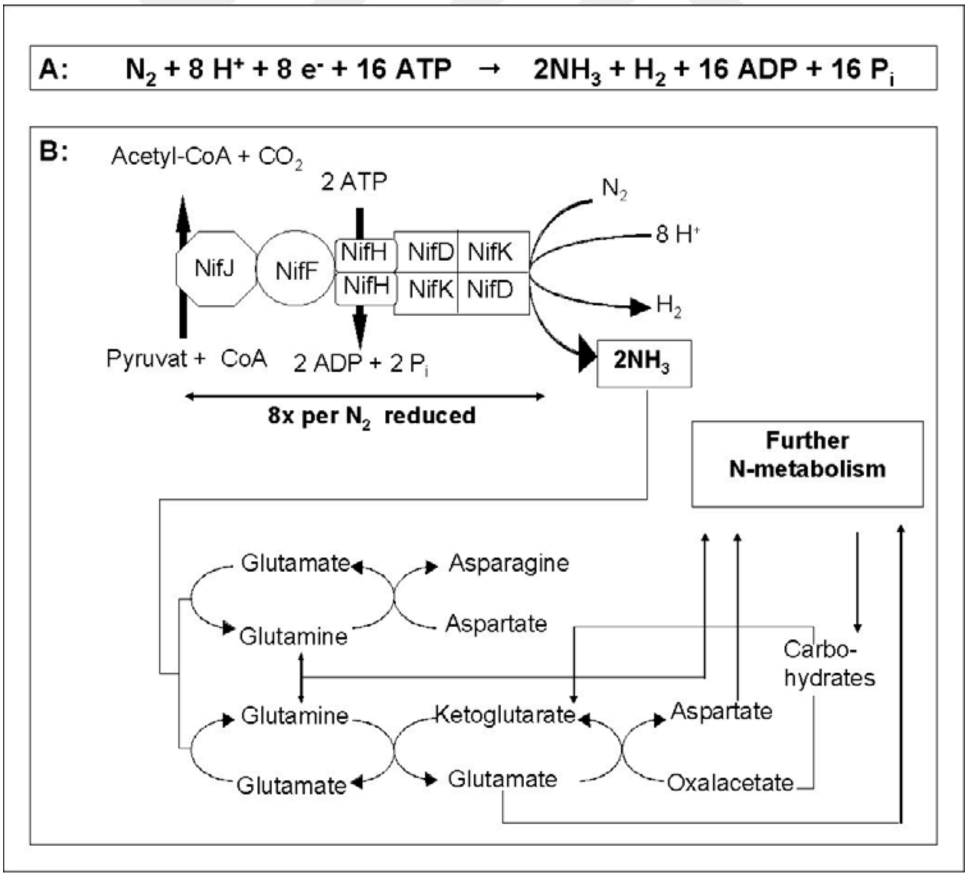

Figure 1.6 General reaction of nitrogen fixation (A); The structure and operation of nitrogenase enzyme complex(B)

Electrons are transferred from reduced ferredoxin (or flavodoxin) by azoferredoxin to molybdoferredoxin. Each mol of fixed nitrogen requires 16 mol ATP hydrolysed by the NifH protein. The produced NH_3 can be utilized for the synthesis of glutamine or glutamate, for N-metabolism. NifJ: pyruvate flavodoxin/ferredoxin oxidoreductase, NifF: Flavodoxin/Ferredoxin) (103).

1.2.2.2.2 Phosphate Solubilization

Several bacteria have the ability of mineralization and solubilization of inorganic and organic phosphorus (104,105). This process occurs with some metabolites as organic acids via their hydroxyl and carboxyl groups chelate the cation side of phosphate, and converted to soluble forms (106). The solubilization of phosphate happens through many microbial processes consisting of organic acid production and proton subtraction (107–109). These organic acids have been reported as mainly gluconic and keto gluconic acid (110,111), also lowering the pH of the rhizosphere by biotic production of proton/bicarbonate release and oxygen/carbon dioxide exchanges. The organic acids which are released by phosphate solubilizing bacteria solubilize phosphate compounds by lowering pH, cation chelation, and competing with phosphate for adsorption parts in soil (109).

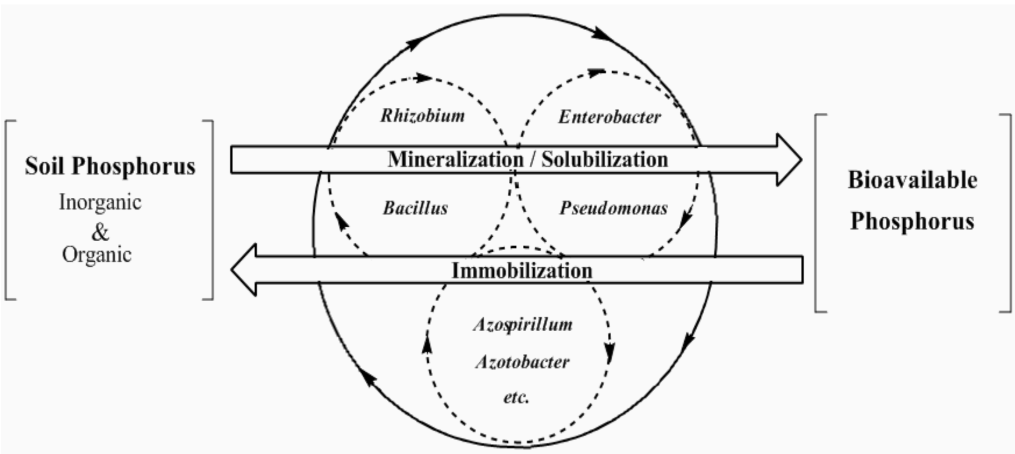

Figure 1.7 Diagram of soil phosphorus mobilization by bacterial species (112).

1.2.2.2.3 Indole-3-acetic acid (IAA) Production

Bacterial IAA production occur through two paths as the trp-independent and the trp-dependent by conversion of tryptophan (trp) to several intermediates.

Trp-independent synthesis of IAA has been reported in the study of Wright et al., 1991 firstly, this pathway may take place via either indole or indole-3-glycerol phosphate (113). In 2018, the existence of an unclear Trp-independent pathway for IAA synthesis in the lack of exogenous trp supply from am endogenous precursor in *Arthrobacter pascens* ZZ21 has been reported (114).

In Figure 1.8, the biosynthetic pathways of IAA in gram-negative bacterial species are given. Five different pathways include indole-3-acetamide (IAM) pathway, Indole-3-pyruvic acid (IPyA) pathway, tryptophan side-chain oxidase (TSO) pathway, tryptamine (TAM) pathway, and indole-3-acetonitrile (IAN) pathway (115).

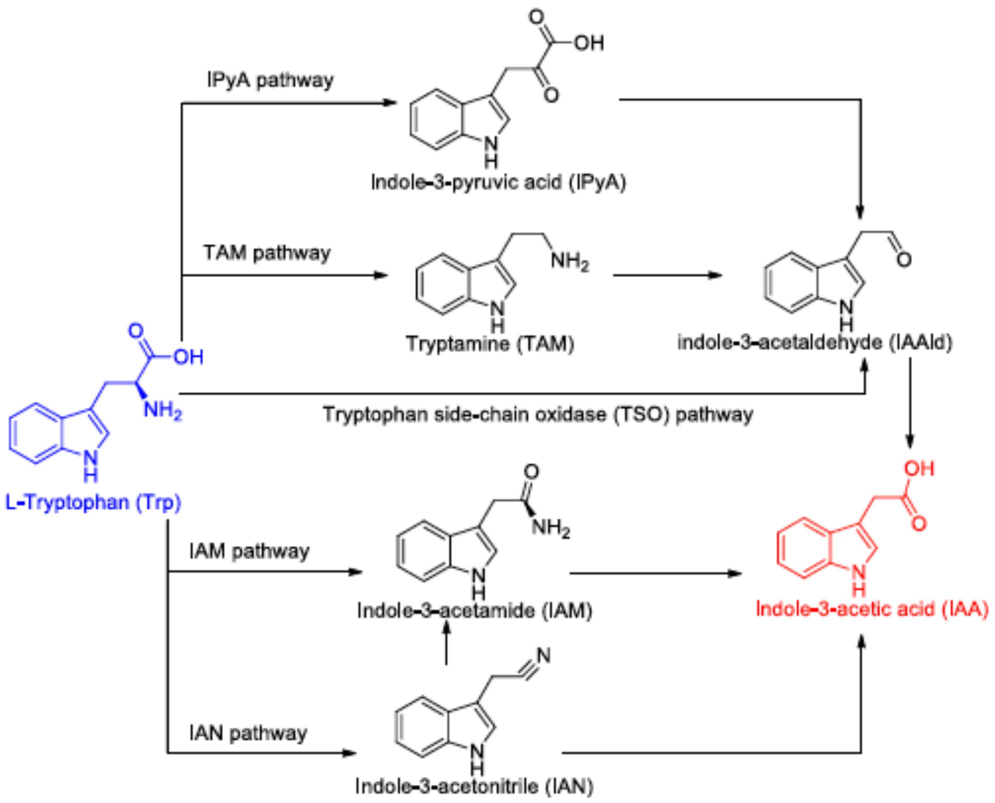

Figure 1.8 IAA synthesis pathways in gram-negative bacterial species (115).

1.2.2.2.4 Catalase

Catalase is a well-known haem containing enzyme that belongs to the oxido-reductase class. It is an important part of the cell defence mechanism against oxidative stress. Hydrogen peroxide is turned to oxygen and water by catalase enzyme, the two stages mechanism is given in the Figure 1.9 (116). The damaging effect of the active oxygen is prevented with it (117). The activity of catalase is essential for bacteria to protect itself against H_2O_2 (hazardous to bacteria and roots of plants). Thus, PGPB with higher catalase activity can survive longer than others and promote plant growing (118).

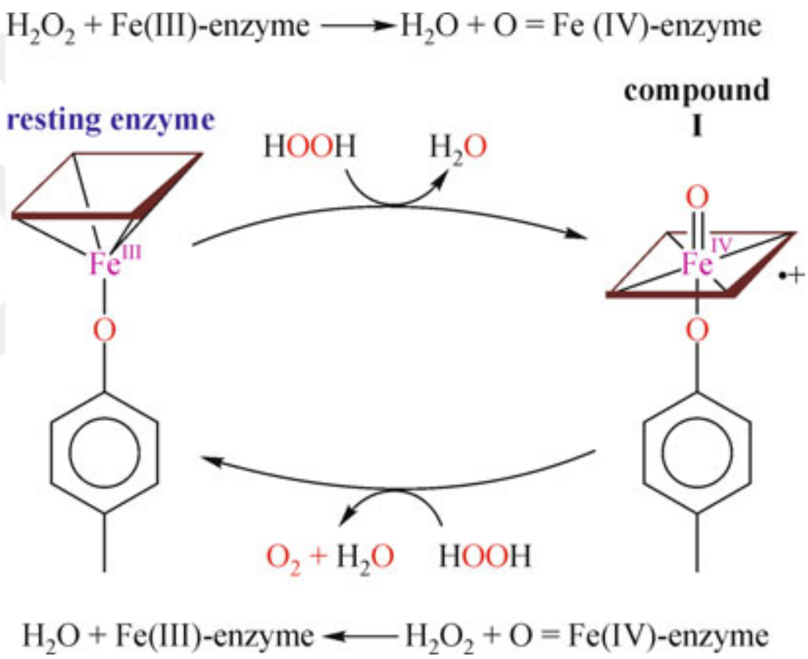

Figure 1.9 Mechanism of catalase (119).

2. AIM AND SCOPE OF THE STUDY

The major challenge for using biofertilizers in agriculture rise from the growth-incompetency of isolated bacteria in various environments. In this study, we hypothesize that the growth of einkorn wheat can be promoted by using the bacteria isolated from the rhizosphere of local wheat species (Iza wheat).

Our goal is to isolate the most effective bacteria that promote yield increase in wheat development. To achieve this goal, we aim to identify bacterial species and carry out biochemical characterization studies.

With this thesis research, we plan to isolate and characterize several bacterial species related to Iza wheat. We also plan to have at least one bacterial species that can statistically change the yield of Iza wheat growth by increasing nitrogen intake, phosphate solubilizing capacity, IAA production. In addition, catalase activities of the isolates were measured.

3. MATERIALS AND METHODS

3.1 Materials

The materials which were used in this study is alphabetically given in Table 3.1.

Table 3.1 Materials

No.	Chemicals	Firm	Cat No.
1	10X Taq Buffer	New England Biolabs	B9015S
2	25 mM Magnesium chloride ($MgCl_2$)	New England Biolabs	B9021S
3	Agar	Liofilchem	611001
4	Bacterial Genomic DNA Isolation Kit	Norgen Biotek Corp.	17900
5	Bovine Serum Albumin	Sigma-Aldrich	A7511
6	Copper (II) sulfate pentahydrate ($CuSO4.5H_2O$)	Sigma-Aldrich	C7631
7	Dipotassium hydrogen phosphate (K_2HPO_4)	Merck	1.05099.0250
8	Folin-Ciocalteu’s phenol reagent	Sigma-Aldrich	F9252
9	Gram Staining Kit	Norateks	106092
10	Hydrogen peroxide (H_2O_2)	Sigma-Aldrich	18304-1L
11	IAA Standard	Duchefa Biochemie	10901.0005
12	Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$)	Merck	1.03943.0250
13	Jensen’s Medium	Himedia	M710-500G
14	L-tryptophan	Merck	1.08374.0010
15	Nuclease-free water	Sigma	W4502
16	Perchloric acid ($HClO_4$)	Merck	1.09065.1000
17	Pikovskaya’s Agar	Himedia	M520-500G

18	Potassium dihydrogen phosphate (KH_2PO_4)	Merck	104871
19	Potassium sodium tartrate tetrahydrate ($C_4H_4KNaO_6.4H_2O$)	Merck	108087
20	Sodium carbonate (Na_2CO_3)	Merck	106392
21	Sodium chloride (NaCl)	Sigma-Aldrich	31434-1KG-R
22	Sodium hydroxide (NaOH)	Merck	106462
23	Taq DNA Polymerase	New England Biolabs	M0320S
24	Tetramethtl-p-phenlylenediamine dihydrochloride	A.D.R. Group	29010640020
25	Triton X-100 ($C_{14}H_{21}(C_2H_4O)_nOH$)	Merck	112298
26	Tryptone	Merck	1.07213.1000
27	Tween20	Sigma-Aldrich	P1379
28	Yeast Extract	Bioshop	YEX401.1

3.2 Methods

3.2.1 Sampling

The root samples of the *Triticum monococcum* spp. *monococcum* were collected from Seben region of Bolu, Turkey on February 19, 2018, and on July 5, 2018, by random sampling. The coordinates of the sampling area are 40°23’39.2’’North, 31°33’02.6’’East. After collection, the samples were immediately transferred to the laboratory.

3.2.2 Isolation of Rhizospheric Bacterial Communities

The isolation procedure was applied according to White et al., 2015. Briefly, the root samples were cleaned gently and carefully from the excessive amount of the soil particles with dH_20 to avoid any damage to the roots and risks of losing of sample content. After the cleaning the larger soil particles, roots were cut near the plant stem and placed into the 50 mL centrifuge tubes vertically and the tubes were filled with 45 mL of freshly prepared (Phosphate buffered saline Tween 20) PBST solution. The tubes were subjected to sonication for 1 minute. Then, the roots were transferred to the second tube which was filled with PBST, and the second sonication step was applied for 1 minute. After the second sonication step, final step was applied the same as step 1 and step 2, but the third sonication was applied for 10 minutes. The final solutions were diluted as 1000x. 100 µL of the diluted samples were dispensed onto the petri dish and spread onto the LB medium in the sterilized cabinet. The isolates were incubated at 30^0C for 18 hours. The growing bacterial species were selected and isolated to achieve one type of colony in a petri dish (120).

Phosphate buffered saline Tween 20 was prepared as 500 mL, pH 7.2. 0.605 g of K_2HPO_4 was completely dissolved in 300 mL of dH_2O. 0.17 g of KH_2PO_4 was added to the mixture, and 4.1 g of NaCl was dissolved into the solution. The pH of the solution was adjusted to 7.2, after the pH adjustment the solution was completed to 500 mL with dH_2O, stirred, and autoclaved with liquid cycle, 121^0C for 30 minutes. Finally, 250 µL of Tween20 was added to the sterilized solution carefully to avoid bubble formation (120).

Luria-Bertani (LB) medium was prepared as 1000 mL, pH 7.0. 10 g of tryptone, 5 g of yeast extract and 5 g of NaCl were dissolved in dH_2O, pH was adjusted to 7.0 and autoclaved. For the preparation of the solid medium 15 g of agar was added before the sterilization step (121).

3.2.3 Identification of the Isolates

The identification of the isolates was carried out by using three types of techniques as MALDI-TOF, 16S rDNA, and 16S-23S rDNA intergenic region amplifications.

The bacterial species' names were firstly identified by MALDI-TOF in the Scientific, Industrial and Technological Application and Research Center, Bolu Abant İzzet Baysal University, BOLU.

Genomic DNA isolations of the bacterial species were carried out by using Bacterial Genomic DNA Isolation Kit, Norgen Biotek Corp., 17900. For the amplification of the 16S rDNA and 16S-23S rDNA intergenic region universal primers were used. The primers are given in Table 3.2. The PCR mixture is given in Table 3.3. The PCR conditions are given in Table 3.4. The appropriate annealing temperatures were determined by gradient PCR as 57 ^{0}C for 16S rDNA amplification and 65 ^{0}C for 16S-23S rDNA intergenic region. Biometra T Professional Thermocycler was used for PCR studies.

Table 3.2 The primers for the genetical identification of the isolates

16S rDNA – P1 (forward)	5-AGAGTTTGATCCTGGTCAGAACGAACGCT-3
16S rDNA – P6 (reverse)	5-TACGGCTACCTTGTTACGACTTCACCCC-3
16S-23S rDNA – FGPS 1490 (forward)	5- TGGGGCTGGATCACCTCCTT-3
16S-23S rDNA – FGPS 132 (reverse)	5-CCGGGTTTCCCCATTCGG-3

Table 3.3 The PCR reaction components

10X Taq Buffer	2.5 µL
dNTP Mix (10 mM)	0.5 µL
25 mM $MgCl_2$	1.5 µL
Forward primer (10 µM)	0.5 µL
Reverse primer (10 µM)	0.5 µL
Taq DNA Polymerase	0.2 µL
Nuclease-free water	18.3 µL
Template DNA	1.0 µL

Table 3.4 The PCR conditions

Step	Temperature, ^0C	Time	Number of cycles
Initial denaturation	95	00:30	-
Denaturation	68	02:00	-
Annealing	95 57 and 65 68	00:30 00:45 01:00	35
Final extension	68	05:00	-

3.2.4 Gram Staining of the Isolates

Gram Staining Kit (Norateks, 106092) was used in this part of the study. After the incubation of the isolates for 16 hours, cells were stained with crystal violet dye for 1-minute, next iodine solution was added (1 minute) to form a complex between crystal violet and iodine. The decolourizer was used to decolorize samples, and samples were washed with water. Safranin (counterstain) was added to the samples and stained for 30 seconds. Finally, the samples were washed with water, dried, and visualized by 100x objective.

3.2.5 Qualitative Catalase and Cytochrome *c* Oxidase Tests

Hydrogen peroxide (3%) was dropped onto the colonies, formation of the bubbles was the sign for the catalase activities (122).

The strips of sterilized filter papers were soaked into the freshly prepared 1% solution of tetramethyl-p-phenylenediamine dihydrochloride. The colonies were smeared over the filter papers and deep-purple colour formation was the sign to the positive oxidase activity (123).

The tests in this part were repeated three times.

3.2.6 Nitrogen Fixation Test

For the characterization of the nitrogen-fixing bacteria, nitrogen-free Jensen's medium was used and survival abilities of the isolates in the medium were reported (124). Firstly, the isolates were incubated in LB liquid medium at 30^0C for 18 hours. Then, OD_{600} values were taken with VWR UV-6300PC double beam spectrophotometer, the OD_{600} values of the isolates were adjusted to 1.0 and they were spotted to the Jensen's agar medium as 10 μL. The isolates were incubated onto the Jensen's agar medium at 30^0C for 8 days. The Jensen's medium (1L, pH 7.0) composition is given in Table 3.5. After preparation of the medium, the mixture was autoclaved for sterilization.

The tests in this part were repeated as three times.

Table 3.5 The composition of the Jensen's medium (1.0 L, pH 7.0)

Sucrose	20.0 g/L
Dipotassium phosphate	1.0 g/L
Magnesium sulphate	0.5 g/L
Sodium chloride	0.5 g/L
Ferrous sulphate	0.1 g/L
Sodium molybdate	0.005 g/L
Calcium carbonate	2.0 g/L
Agar	15.0 g/L

3.2.7 Phosphate Solubilization Test

The phosphate solubilization test works by plating the isolates to the sterile Pikovskaya's agar medium (125). The medium is supplemented with insoluble tricalcium phosphate. The isolates were incubated in LB liquid medium at 30^0C for 18 hours. After the incubation step, the OD_{600} values of the isolates were adjusted to 1.0, and they were spotted on the Pikovskaya's agar medium as 10 μL. The isolates were incubated at 37^0C for 48 hours. The clear halo zone diameter around each colony and the diameter of the colonies were taken, and the solubilization indices (SI) were calculated as (Clearance zone) / (Colony diameter).

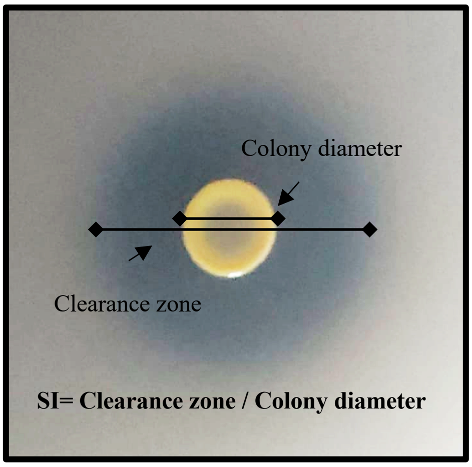

Figure 3.1 Calculation of phosphate solubilizing indices

The composition of Pikovskaya's agar medium (1L, pH 7.0) is given in Table 3.6. After preparation of the medium, the mixture was autoclaved for sterilization.

The tests in this part were repeated three times.

Table 3.6 The composition of the Pikovskaya's medium (1.0 L, pH 7.0)

Yeast extract	0.5 g/L
Dextrose	10.0 g/L
Calcium phosphate	5.0 g/L
Ammonium sulphate	0.5 g/L
Potassium chloride	0.2 g/L
Magnesium sulphate	0.1 g/L
Manganese sulphate	0.0001 g/L
Ferrous sulphate	0.0001 g/L
Agar	15.0 g/L

3.2.8 Characterization of Indole-3-acetic acid (IAA) Production

The determination of the amounts of the IAA produced by the isolates was performed by using Salkowski reagent (126). The isolates were grown in LB medium, and 0.1% L-tryptophan (w/v) supplemented LB medium for 11 days at 30^0C. 1 mL of supernatants were taken from each sample in 24-hour periods, and they were combined with 2 mL freshly prepared Salkowski's reagent (2 mL 0.5M $FeCl_3$ and 49 mL water and 49 mL 70% perchloric acid). The mixtures were incubated for 30 minutes at room temperature. After the incubation, colorimetric measurements were taken at 535 nm. The standard curve was plotted by using the IAA standard to calculate the production quantity of each isolate as 5, 10, 15, 20, 25, and 30 μg/mL (127).

The tests in this part were repeated three times for each sample.

3.2.9 Determination of Catalase Activities

3.2.9.1 Protein Extraction from the Isolates

The isolates were incubated in LB medium at 30^0C. 4.5 mL of stationary cultures were centrifuged at 1200xg, 4^0C, 3 minutes and the pellets were washed with 500 μL of ice-cold 50 mM phosphate buffer. After the washing step, the mixtures were centrifuged again under the same conditions, supernatants were removed. 1000 μL of ice-cold 50 mM phosphate buffer was added to each pellet and was sonicated in an ice-water bath by 10 times 10 seconds, between each 10 seconds sonication was paused and the mixtures were left on ice-bath for 10 seconds. Finally, the sonicated mixtures were centrifuged at 1200xg, 4^0C, 10 minutes. The supernatants were used in both protein determination and total catalase activity assay.

3.2.9.2 Protein Determination

The concentrations of the proteins extracted from the isolates were determined by the method of Lowry et al. (1951) (128). Bovine serum albumin (BSA) was used to plot the standard curve with five concentrations (0.020, 0.050, 0.100, 0.150, and 0.200 mg/mL). For the determination of the protein of the extracts, 250 μL of the extract diluted with 250 μL dH_2O. There was no extract in the blank solution. 2.5 mL of alkaline copper reagent (1:1:100) (freshly prepared by 2% copper sulphate, 2% sodium potassium tartrate, and 20% sodium carbonate in 0.1 N sodium hydroxide) was added to each tube. Reaction mixtures were incubated for 10 minutes at room temperature. After that, 1 N 0.25 M Folin-Ciocalteu's phenol reagent was poured to each tube and vortexed immediately. After 30 minutes of incubation, the results were taken by the spectrophotometer at

660 nm. The calibration curve was plotted to determine the protein contents of the isolates.

The experiments in this part were repeated five times for each isolate.

3.2.9.3 Quantitative Catalase Activities

Catalase activities of the isolates were determined according to the method described by Aebi (1984) (129). The decomposition of the hydrogen peroxide (H_2O_2) to water and oxygen with the presence of catalase enzyme is the principle of the procedure.

The optimizations of the assay were carried out with different enzyme-substrate concentrations and pH. Each measurement was taken by using 1% Triton x-100 in 10 mM phosphate buffer, pH 7.0 as 1:10 dilution and the mixtures were incubated on an ice-bath for 10 minutes. Then, 9 mL of 50 mM phosphate buffer, pH 7.0 was added to each 1 mL of the incubated Triton X-100 and sample mixture. 2 mL of sample and 1 mL of phosphate buffer was used as blank and 2 mL of the sample with 1 mL of 120 mM H_20_2 was used for the determination of the activities of each isolate at 240 nm for 3 minutes.

The catalase activities of the isolates were calculated as μmol H_20_2 consumed per mg of protein in 1 minute at 25^0C. (Extinction coefficient was taken as 0.0364 $mM^{-1}.cm^{-1}$.)

3.2.10 Visualizing of the Effects of the Isolates on the Germination of *Triticum monococcum* ssp. *monococcum*

3.2.10.1 Sterilization of the Seeds

The seeds of *Triticum monococcum* ssp. *monococcum* were sterilized according to the study of Örgeç et al. (2018). 5 drops of Tween20 were added to 100 mL of dH_20. The seeds were immersed in the mixture and were stirred for 1 minute properly followed by adding sodium hypochlorite 40% with stirring for 15 minutes. The procedure was suitable for the sterilization of the all seeds in the experiments without decreasing the germination capacities (14).

3.2.10.2 Germination on Filter Paper

Five seeds in all triplicates were germinated on Whatman No.1 filter paper at 23±1^0C for 8 days in a controlled climate chamber (130). Bacterial applications were carried out by immersing the seeds into 10^9 cfu/mL isolates for 15 minutes, and they were dried for 15 minutes in the sterile cabinet. There was no bacterial application to the control seeds.

Root numbers, root lengths, coleoptile lengths and germination powers were measured after 8 days.

3.2.10.3 Germination in Soil

Five seeds in all triplicates were germinated in soil at 23±1^0C for 14 days in a controlled climate chamber (130). Bacterial applications were carried out by immersing the seeds into 10^9 cfu/mL isolates for 15 minutes, and they were dried for 15 minutes in the sterile cabinet. No bacteria isolate was mixed with the control seeds.

The germination powers were counted after 8 days. Root numbers, root lengths, root fresh weights, root dry weights, and coleoptile lengths were measured after 14 days.

3.2.10.4 Statistical Analysis

Statistical analysis of the results was carried out with SPSS 22.0 as independent samples t-test against control.

4. RESULTS

4.1 Identification of the Species

4.1.1 Genomic DNA (gDNA) Isolation

The concentrations of the isolated gDNA are given in Table 4.1. All calculations were carried out by using the formula.

"Concentration (µg/ml) = (A_{260} reading – A_{320} reading) × dilution factor × 50µg/ml"

Table 4.1 Genomic DNA concentrations of the isolates

ESU_LAB Codes	Identities	gDNA concentrations, ng/mL
ESU_164	*Arthrobacter* spp. ESU164	201
ESU_193	*Arthrobacter* spp. ESU193	134
ESU_131	*Pseudomonas* spp. ESU131	280
ESU_141	*Pseudomonas* spp. ESU141	192
ESU_182	*Pseudomonas poae* strain ESU182	224
ESU_192	*Pseudomonas thivervalensis* strain ESU192	316
ESU_1531	*Pseudomonas* spp. ESU1531	348
ESU_181	*Bacillus subtilis* strain ESU181	34
ESU_172	*Microbacterium foliorum* strain ESU172	300
ESU_121	*Microbacterium* spp. ESU121	158

4.1.2 MALDI-TOF Results

The results of MALDI-TOF are given in Table 4.2 as logarithmic scores equal or greater than 2.0.

Table 4.2 MALDI-TOF results

ESU_LAB Codes	Detected species	Log Score
ESU_164	*Arthrobacter aurescens* DSM 20116T DSM *Arthrobacter aurescens* CIP 102364T CIP *Arthrobacter aurescens* HKI 11247 HKJ	2.327 2.149 2.111
ESU_193	*Arthrobacter oxydans* IMET 10684T HKJ *Arthrobacter oxydans* DSM 20119T DSM	2.072 2.060
ESU_131	*Pseudomonas brassicacearum* DSM 13227T HAM *Pseudomonas thivervalensis* DSM 13194T HAM *Pseudomonas kiloensis* DSM 13647T HAM	2.169 2.092 2.042
ESU_141	*Pseudomonas chlororaphis* ssp. *aurantica* CIP 106718T HAM *Pseudomonas chlororaphis* ssp. *chlororaphis* DSM 50083T HAM	2.052 2.037
ESU_182	*Pseudomonas poae* DSM 14936T HAM *Pseudomonas antarctica* DSM 15318T HAM *Pseudomonas trivialis* DSM 14937T HAM	2.158 2.091 2.033
ESU_192	*Pseudomonas kiloensis* DSM 13647T HAM *Pseudomonas brassicacearum* DSM 13227T HAM *Pseudomonas thivervalensis* DSM 13194T HAM *Pseudomonas chlororaphis* ssp. *aurantica* CIP 106718T HAM	2.153 2.126 2.118 2.041
ESU_1531	*Pseudomonas corrugata* DSM 7228T HAM	2.021

ESU_181	*Bacillus subtilis* DSM 5552 DSM	1.956
ESU_172	*Microbacterium foliorum* DSM 12966T DSM	2.271
ESU_121	*Microbacterium luteolum* DSM 20143T DSM	1.803

4.1.3 16S rDNA

Amplified 16S rDNA results were analysed by BLAST comparing the sequence data against the NCBI 16S database and Standard Databases-Nucleotide collection (nr/nt).

The results are shown in Table 4.3 and Table 4.4, respectively.

Table 4.3 16S rRNA results from 16S database

Code No.	Species	Max score	Total score	Query Cover	E value	Percent Ident
ESU_164	*Pseudomonas trivialis* strain P 513/19	1679	1679	98%	0.0	100.00%
	Pseudomonas poae strain P 527/13	1679	1679	98%	0.0	100.00%
	Pseudomonas extremorientalis strain KMM 3447	1668	1668	98%	0.0	99.78%
ESU_193	-	-	-	-	-	-
ESU_131	*Pseudomonas brassicacearum* subsp. *neoaurantiaca* strain CIP 109457	1197	1197	7%	0.0	89.55%
	Pseudomonas corrugata strain Slade 939/1	1190	1190	97%	0.0	89.44%
	Pseudomonas migulae strain CIP 105470	1190	1190	97%	0.0	89.44%

ESU_141	*Pseudomonas kilonensis* strain 520-20	1657	1657	99%	0.0	99.34%
	Pseudomonas thivervalensis strain SBK26	1655	1655	99%	0.0	99.34%
	Pseudomonas brassicacearum subsp. *neoaurantiaca* strain CIP 109457	1650	1650	99%	0.0	99.13%
	Pseudomonas corrugata	1648	1648	99%	0.0	99.23%
	Pseudomonas brassicacearum strain DBK11	1639	1639	99%	0.0	99.02%
ESU_182	*Pseudomonas trivialis* strain P 513/19	1672	1672	99%	0.0	99.78%
	Pseudomonas poae strain P 527/13	1672	1672	99%	0.0	99.78%
	Pseudomonas extremorientalis strain KMM 3447	1661	1661	99%	0.0	99.56%
ESU_192	*Pseudomonas chlororaphis* strain DSM 50083	1205	1205	96%	0.0	90.41%
	Pseudomonas thivervalensis strain SBK26	1205	1205	95%	0.0	90.66%
	Pseudomonas frederiksbergensis strain DSM 13022	1205	1205	96%	0.0	90.42%
ESU_1531	*Pseudomonas brassicacearum* subsp. *neoaurantiaca* strain CIP 109457	1179	1179	98%	0.0	89.44%
	Pseudomonas migulae strain CIP 105470	1168	1168	98%	0.0	89.22%
	Pseudomonas migulae strain NBRC 103157	1168	1168	98%	0.0	89.22%

ESU_181	*Bacillus nakamurai* strain NRRL B-41091 *Bacillus amyloliquefaciens* strain NBRC 15535 *Bacillus subtilis* strain BCRC 10255	1149 1149 1147	1149 1149 1147	98% 98% 98%	0.0 0.0 0.0	89.05% 89.05% 89.06%
ESU_172	-	-	-	-	-	-
ESU_121	-	-	-	-	-	-

Table 4.4 16S rRNA results (Standard Databases-Nucleotide collection (nr/nt))

Code No.	**Species**	**Max score**	**Total score**	**Query Cover**	**E value**	**Percent Ident**
ESU_164	*Pseudomonas* sp. PG-3-10 *Pseudomonas poae* strain T23 *Pseudomonas* sp. strain S2WE	1683 1683 1681	1683 1683 1681	99% 99% 100%	0.0 0.0 0.0	99.78% 99.78% 99.67%
ESU_193	*Bacterium* DEN D7 Uncultured bacterium partial 16S rRNA gene, clone t0_12H85 *Arthrobacter* sp. strain AC147_JC338	861 815 782	861 815 782	85% 88% 84%	0.0 0.0 0.0	85.64% 83.83% 84.43%
ESU_131	*Pseudomonas chlororaphis* subsp. *piscium* strain IHBB 9882 *Pseudomonas* sp. G-229-22 *Pseudomonas fluorescens* strain F41	1240 1236 1214	1240 1236 1214	97% 97% 97%	0.0 0.0 0.0	90.41% 90.31% 89.88%

ESU_141	*Pseudomonas brassicacearum* strain JZY4-62	1676	1676	100%	0.0	99.67%
	Pseudomonas brassicacearum strain EH2	1676	1676	100%	0.0	99.67%
	Pseudomonas sp. strain H10R3	1674	1674	100%	0.0	99.67%
ESU_182	*Pseudomonas poae* strain T23	1676	1676	99%	0.0	99.67%
	Pseudomonas sp. strain Y171003	1674	1674	99%	0.0	99.56%
	Pseudomonas sp. strain Y171002	1674	1674	99%	0.0	99.56%
ESU_192	Pseudomonas chlororaphis subsp. piscium strain IHBB 9882	1260	1260	96%	0.0	91.45%
	Pseudomonas sp. G-229-22	1243	1243	96%	0.0	91.20%
	Pseudomonas putida strain F36	1238	1238	96%	0.0	91.09%
ESU_1531	*Pseudomonas chlororaphis* subsp. *piscium* strain IHBB 9882	1212	1212	98%	0.0	90.11%
	Pseudomonas sp. G-229-22	1212	1212	98%	0.0	90.11%
	Pseudomonas fluorescens strain F41	1201	1201	98%	0.0	89.89%
ESU_181	*Bacillus* sp. hb63	1232	1232	98%	0.0	90.71%
	Bacillus subtilis strain L1	1201	1201	97%	0.0	90.39%
	Bacillus sp. KT74	1201	1201	98%	0.0	90.13%

ESU_172	-	-	-	-	-	-
ESU_121	-	-	-	-	-	-

4.1.4 16S-23S rDNA Intergenic Region

Amplified 16S-23S rDNA results were again analysed using BLAST tool. The results are shown in Table 4.5.

Table 4.5 16S – 23S intergenic region rDNA results

Code No.	Species	Max score	Total score	Query Cover	E value	Percent Ident
ESU_164	*Arthrobacter* sp. YN, complete genome	869	5018	97%	0.0	90.94%
	Arthrobacter sp. Rue61a, complete genome	767	4405	97%	0.0	87.86%
	Paenarthrobacter aurescens TC1, complete genome	767	4480	97%	0.0	87.86%
ESU_193	*Pseudarthrobacter phenanthrenivorans* Sphe3 chromosome, complete genome	680	2528	97%	0.0	86.38%
	Arthrobacter sp. 24S4-2 chromosome, complete genome	590	2949	97%	4e-164	83.60%
	Arthrobacter sp. PAMC25564 chromosome, complete genome	573	2867	97%	4e-159	82.92%
ESU_131	*Pseudomonas fluorescens* strain CREA-C16 genome	1009	1009	95%	0.0	95.84%
	Pseudomonas fluorescens 16S-23S ribosomal RNA intergenic spacer and 23S ribosomal RNA gene, partial sequence	1003	1003	95%	0.0	95.69%

	Pseudomonas sp. UW4, complete genome	1003	6335	95%	0.0	95.69%
ESU_141	*Pseudomonas fluorescens* Pf29Arp contig024, whole genome shotgun sequence	1116	1116	98%	0.0	98.13%
	Pseudomonas thivervalensis strain SC5 genome	961	961	97%	0.0	93.92%
	Pseudomonas fluorescens strain FW300-N2E2 genome	961	3919	98%	0.0	93.92%
ESU_182	*Pseudomonas poae* RE*1-1-14, complete genome	1147	5717	97%	0.0	99.84%
	Pseudomonas trivialis strain BS3111 genome assembly, chromosome: I	1142	5700	97%	0.0	99.68%
	Pseudomonas poae strain BS2776 genome assembly, chromosome: I	1136	5684	97%	0.0	99.52%
ESU_192	*Pseudomonas fluorescens* Pf29Arp contig024, whole genome shotgun sequence	1129	1129	99%	0.0	98.01%
	Pseudomonas thivervalensis strain BS3779 genome assembly, chromosome: I	1099	5234	99%	0.0	97.39%
	Pseudomonas brassicacearum strain DF41, complete genome	976	4412	98%	0.0	93.87%
ESU_1531	*Pseudomonas brassicacearum* subsp. brassicacearum	1046	4737	99%	0.0	93.39%

	NFM421, complete genome					
	Pseudomonas brassicacearum strain S-1 chromosome, complete genome	1044	4946	99%	0.0	93.39%
	Pseudomonas thivervalensis strain BS3779 genome assembly, chromosome: I	1044	4314	99%	0.0	93.28%
ESU_181	*Bacillus amyloliquefaciens* strain R8-25 chromosome, complete genome	501	6274	99%	1e-137	98.26%
	Bacillus amyloliquefaciens strain R8-25 plasmid unnamed1, complete sequence	501	681	83%	1e-137	98.26%
	Bacillus sp. AM1(2019) chromosome, complete genome	501	6165	99%	1e-137	97.93%
ESU_172	*Microbacterium foliorum* strain NRRL B-24224 chromosome, complete genome	941	1671	97%	0.0	99.05%
	Microbacterium sp. 1.5R, complete genome	785	1571	97%	0.0	93.77%
	Microbacterium sp. CGR1, complete genome	785	1571	97%	0.0	93.76%
ESU_121	*Microbacterium* sp. 1.5R, complete genome	582	1165	97%	5e-162	86.80%
	Microbacterium sp. CGR1, complete genome	582	1165	97%	5e-162	86.80%
		573	1147	97%		

	Microbacterium foliorum strain 122 genome				3e-159	86.48%

The interpretation of the identification methods was carried out by comparing the results from MALDI-TOF, 16S rRNA, and 16S-23S intergenic region analysis with BLAST. In Table 4.6, putative species are given. The results show that two *Arthrobacter* species, five different *Pseudomonas* species, one *Bacillus subtilis* strain and two *Microbacterium* species have been identified.

Table 4.6 Interpretation of the identification methods

Code No.	MALDI-TOF	16S rRNA (16S database)	16S rRNA (Standard databases- Nucleotide collection (nr/nt)	16S – 23S intergenic region (Standard databases- Nucleotide collection (nr/nt)	Putative Species
164	*Arthrobacter aurescens* DSM 20116T DSM *Arthrobacter aurescens* CIP 102364T CIP *Arthrobacter aurescens* HKI 11247 HKJ	*Pseudomonas trivialis* strain P 513/19 *Pseudomonas poae* strain P 527/13 *Pseudomonas extremorientalis* strain KMM 3447	*Pseudomonas* sp. PG-3-10 *Pseudomonas poae* strain T23 *Pseudomonas* sp. strain S2WE	*Arthrobacter* sp. YN *Arthrobacter* sp. Rue61a *Paenarthrobacter aurescens* TC1	*Arthrobacter* spp. ESU164
193	Arthrobacter oxydans IMET 10684T HKJ Arthrobacter oxydans DSM 20119T DSM	-	*Bacterium* DEN D7 Uncultured bacterium clone t0_12H85 *Arthrobacter* sp. strain AC147_JC338	*Pseudarthrobacter phenanthrenivorans* Sphe3 chromosome Arthrobacter sp. 24S4-2 chromosome Arthrobacter sp. PAMC25564 chromosome	*Arthrobacter* spp. ESU193
131	*Pseudomonas brassicacearum* DSM 13227T HAM *Pseudomonas thivervalensis* DSM 13194T HAM Pseudomonas kiloensis DSM 13647T HAM	*Pseudomonas brassicacearum* subsp. *neoaurantiaca* strain CIP 109457 *Pseudomonas corrugata* strain Slade 939/1 *Pseudomonas migulae* strain CIP 105470	*Pseudomonas chlororaphis* subsp. *piscium* strain IHBB 9882 *Pseudomonas* sp. G-229-22 *Pseudomonas fluorescens* strain	*Pseudomonas fluorescens* strain CREA-C16 genome *Pseudomonas fluorescens* *Pseudomonas* sp. UW4	*Pseudomonas* spp. ESU131
141	*Pseudomonas chlororaphis* ssp. *aurantica* CIP 106718T HAM *Pseudomonas chlororaphis* ssp. *chlororaphis* DSM 50083T HAM	*Pseudomonas kilonensis* strain 520-20 *Pseudomonas thivervalensis* strain SBK26 *Pseudomonas brassicacearum* subsp. *neoaurantiaca* strain CIP 109457 *Pseudomonas corrugata*	*Pseudomonas brassicacearum* strain JZY4-62 *Pseudomonas brassicacearum* strain EH2 *Pseudomonas* sp. strain H10R3	*Pseudomonas fluorescens* Pf29Arp contig024, whole genome shotgun sequence *Pseudomonas thivervalensis* strain SC5 *Pseudomonas fluorescens* strain FW300-N2E2	*Pseudomonas* spp. ESU141

		Pseudomonas brassicacearum strain DBK11			
182	*Pseudomonas poae* DSM 14936T HAM *Pseudomonas antarctica* DSM 15318T HAM *Pseudomonas trivialis* DSM 14937T	*Pseudomonas trivialis* strain P 513/19 *Pseudomonas poae* strain P 527/13 *Pseudomonas extremorientalis* strain KMM 3447	*Pseudomonas poae* strain T23 *Pseudomonas* sp. strain Y171003 *Pseudomonas* sp. strain Y171002	*Pseudomonas poae* RE*1-1-14, complete genome *Pseudomonas poae* RE*1-1-14, complete genome *Pseudomonas poae* strain BS2776 genome assembly, chromosome: I	*Pseudomonas poae* strain ESU182
192	*Pseudomonas kiloensis* DSM 13647T HAM *Pseudomonas brassicacearum* DSM 13227T HAM *Pseudomonas thivervalensis* DSM 13194T HAM *Pseudomonas chlororaphis* ssp. *aurantica* CIP 106718T HAM	*Pseudomonas chlororaphis* strain DSM 50083 *Pseudomonas thivervalensis* strain SBK26 *Pseudomonas frederiksbergensis* strain DSM 13022	*Pseudomonas chlororaphis* subsp. *piscium* strain IHBB 9882 *Pseudomonas* sp. G-229-22 *Pseudomonas putida* strain F36	*Pseudomonas fluorescens* Pf29Arp contig024, whole genome shotgun sequence *Pseudomonas thivervalensis* strain BS3779 genome assembly, chromosome: I *Pseudomonas brassicacearum* strain DF41, complete genome	*Pseudomonas thivervalensis* strain ESU192
1531	*Pseudomonas corrugata* DSM 7228T HAM	*Pseudomonas brassicacearum* subsp. *neoaurantiaca* strain CIP 109457 *Pseudomonas migulae* strain CIP 105470 *Pseudomonas migulae* strain NBRC 103157	*Pseudomonas chlororaphis* subsp. *piscium* strain IHBB 9882 *Pseudomonas* sp. G-229-22 *Pseudomonas fluorescens* strain F41	*Pseudomonas brassicacearum* subsp. *brassicacearum* NFM421, complete genome Pseudomonas *brassicacearum* strain S-1 chromosome, complete genome *Pseudomonas thivervalensis* strain BS3779 genome assembly, chromosome: I	*Pseudomonas* spp. ESU1531
181	*Bacillus subtilis* DSM 5552 DSM	*Bacillus nakamurai* strain NRRL B-41091 *Bacillus amyloliquefaciens* strain NBRC 15535 *Bacillus subtilis* strain BCRC 10255	*Bacillus sp.* hb63 *Bacillus subtilis* strain L1 *Bacillus* sp. KT74	*Bacillus amyloliquefaciens* strain R8-25 chromosome *Bacillus amyloliquefaciens* strain R8-25 plasmid unnamed1 *Bacillus* sp. AM1(2019) chromosome	*Bacillus subtilis* strain ESU181
172	*Microbacterium foliorum* DSM 12966T DSM	-	-	*Microbacterium foliorum* strain NRRL B-24224 chromosome Microbacterium sp. 1.5R Microbacterium sp. CGR1	**Microbacterium foliorum* strain ESU172
121	*Microbacterium luteolum* DSM 20143T DSM	-	-	*Microbacterium sp.* 1.5R *Microbacterium* sp. CGR1 *Microbacterium foliorum* strain 122 g	**Microbacterium* spp. ESU121

*: Likely new species

4.1.5 Gram staining, Qualitative Catalase Test, Cytochrome *c* Oxidase Test Results

The results of gram staining, qualitative catalase and cytochrome oxidase test are represented in Table 4.7 as positive or negative for each identified isolate.

Table 4.7 Qualitative catalase and cytochrome *c* oxidase test results

Name	Gram staining results	Catalase test results	Cytochrome oxidase test results
Arthrobacter spp. ESU164	Negative	Positive	Positive
Arthrobacter spp. ESU193	Negative	Positive	Negative
Pseudomonas spp. ESU131	Negative	Positive	Positive
Pseudomonas spp. ESU141	Negative	Positive	Positive
Pseudomonas poae strain ESU182	Negative	Positive	Positive
Pseudomonas thivervalensis strain ESU192	Negative	Positive	Positive
Pseudomonas spp. ESU1531	Negative	Positive	Positive
Bacillus subtilis strain ESU181	Positive	Positive	Positive
Microbacterium foliorum strain ESU172	Negative	Positive	Negative
Microbacterium spp. ESU121	Negative	Positive	Negative

4.1.6 Nitrogen Fixation Abilities of the Species

Nitrogen fixation abilities of isolated species with nitrogen free Jensen's agar medium are given in Table 4.8. The results show that all the isolated species have the ability of fixing nitrogen.

Table 4.8 Nitrogen fixation abilities of isolated species

Name	Nitrogen fixation abilities
Arthrobacter spp. ESU164	Positive
Arthrobacter spp. ESU193	Positive
Pseudomonas spp. ESU131	Positive
Pseudomonas spp. ESU141	Positive
Pseudomonas poae strain ESU182	Positive
Pseudomonas thivervalensis strain ESU192	Positive
Pseudomonas spp. ESU1531	Positive
Bacillus subtilis strain ESU181	Positive
Microbacterium foliorum strain ESU172	Positive
Microbacterium spp. ESU121	Positive

4.1.7 Phosphate Solubilizing Capacities

Phosphate solubilizing capacities of isolated species are given in the Table 4.9. The calculated indices are shown in Figure 4.1. *Pseudomonas* spp. ESU131 has the maximum ability to phosphate solubilization as calcium phosphate form. On the other hand, only *Microbacterium foliorum* strain ESU172 was not able to solubilize phosphate.

Table 4.9 Phosphate solubilizing indices

Name	**Phosphate solubilizing results**
Arthrobacter spp. ESU164	2.144±0.269
Arthrobacter spp. ESU193	2.088±0.389
Pseudomonas spp. ESU131	2.805±0.439
Pseudomonas spp. ESU141	2.574±0.475
Pseudomonas *poae* strain ESU182	2.694±0.334
Pseudomonas thivervalensis strain ESU192	2.713±0.685
Pseudomonas spp. ESU1531	2.048±0.254
Bacillus subtilis strain ESU181	1.260±0.042
Microbacterium foliorum strain ESU17*2*	0.000
Microbacterium spp. ESU121	2.678±0.575

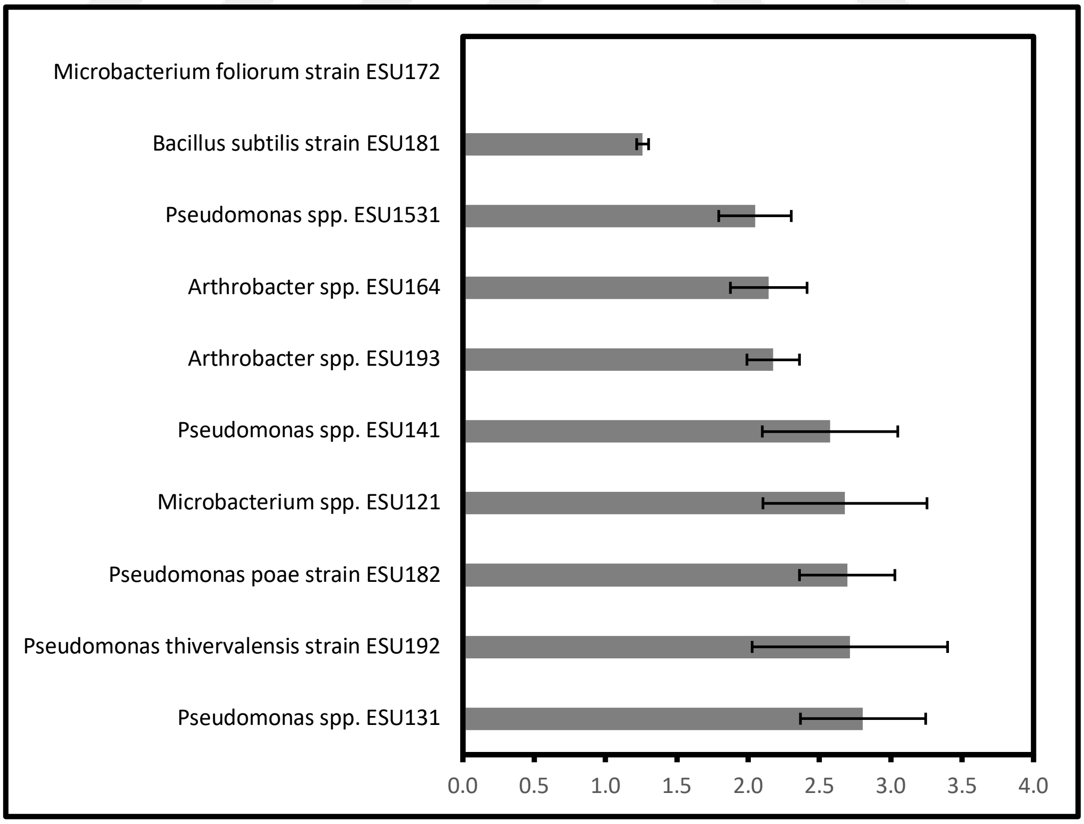

Figure 4.1 Graphical representation of phosphate solubilizing indices of the species

4.1.8 Quantitative Analysis of IAA Production Capacities of Isolated Species

IAA production abilities of the species in LB medium and LB medium supplemented with 0.1% trp are given in Figure 4.2 and 4.3 respectively. Although all the isolates can produce IAA in both LB and trp-supplemented LB media, *Arthrobacter* spp. ESU164 has been showed the maximum IAA production in LB medium and *Pseudomonas* spp. ESU131 has been showed the maximum IAA production in trp-supplemented LB medium. According to the results, in the high concentration of trp, higher IAA production capacities of the isolates have been recorded.

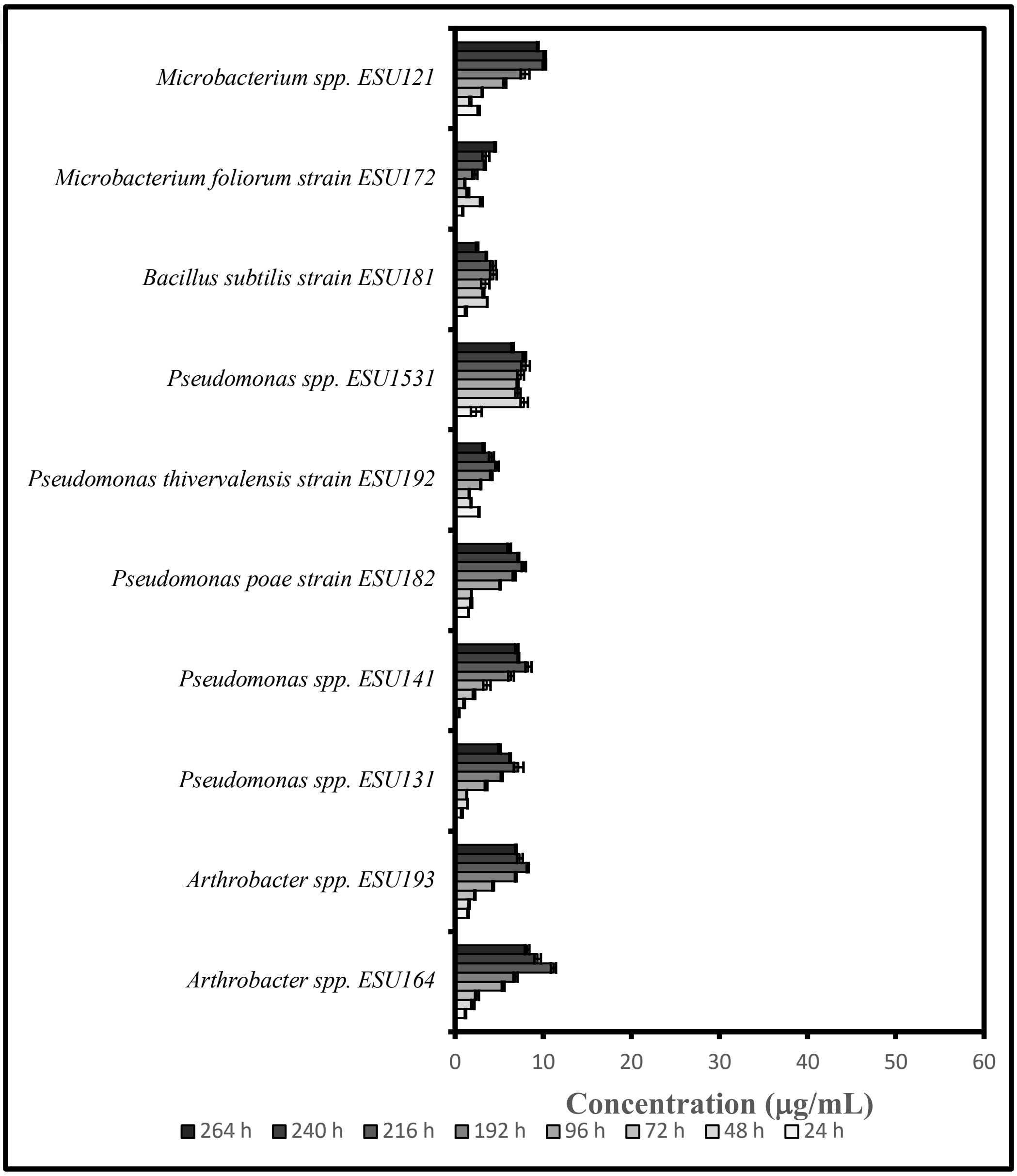

Figure 4.2 IAA production of the species in LB medium

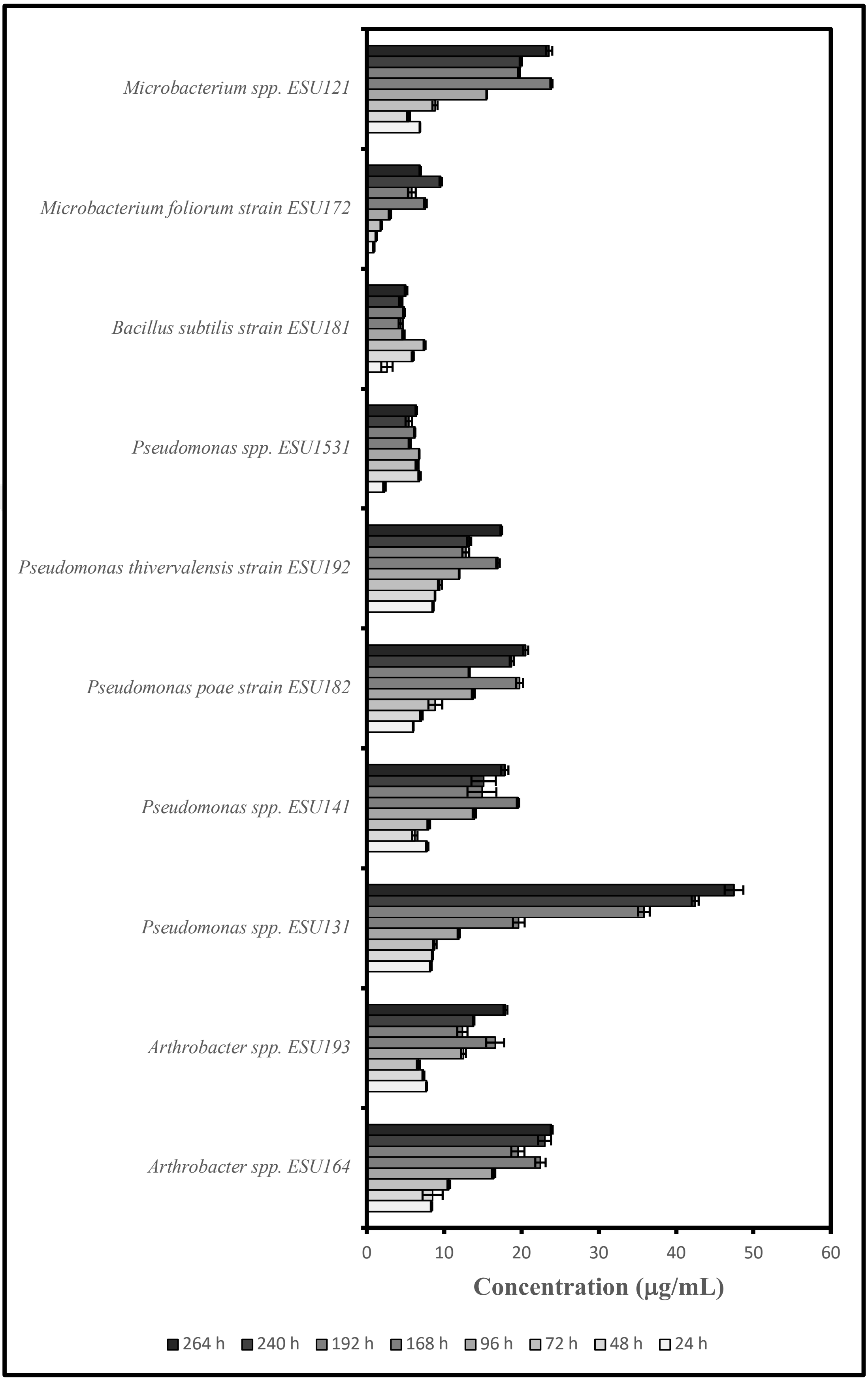

Figure 4.3 IAA production of the species in LB medium supplemented with 0.1% trp

4.1.9 Quantitative Catalase Activities of the Species

Quantitative analysis of the catalase activities of isolated species was carried out by Aebi method, and the results are represented in Figure 4.4 for each isolate. In this study, the highest catalase activities have been reported for *Pseudomonas thivervalensis* strain ESU192 and *Bacillus subtilis* strain ESU181. But the lowest catalase activity has been reported for *Microbacterium* spp. ESU121.

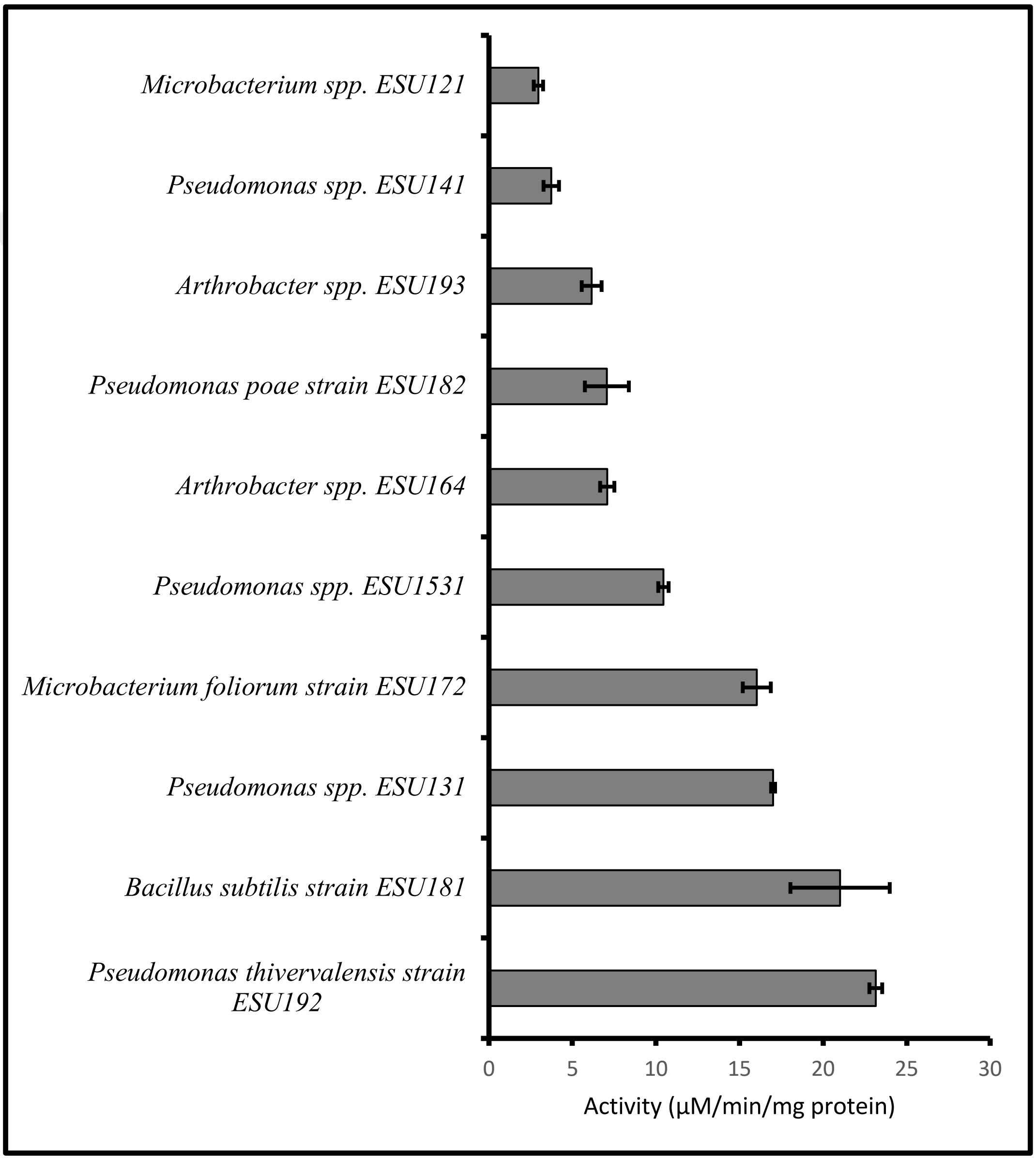

Figure 4.4 Catalase activities of the species

4.1.10 Germination on Filter Paper

The statistical results (independent t-test) of the plant variables after the application of the bacterial isolates against control are given in Tables 4.10-19 and Figures 4.5-14 in this section.

According to the statistical analysis results of the plant parameters on filter paper, *Arthrobacter* spp. ESU164 showed 8.89% increasing in root number ($p<0.05$). *Pseudomonas* spp. 131 increased the root number as 22.22%, the root length as 70.39%, and the coleoptile length as 63.01% ($p<0.05$). *Pseudomonas* spp ESU1531 increased the root length as 44.34% and the coleoptile length as 30.88% ($p<0.05$). *Bacillus subtilis* strain ESU181 increased the root length as 40.00% ($p<0.05$). *Microbacterium foliorum* strain ESU172 increased the root number as 31.11%, the root length as 296.12%, and coleoptile length as 187.51% ($p<0.05$).

Table 4.10 Independent samples t-test results for *Arthrobacter* spp. ESU164 on filter paper

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.000	0.000	8.89[a]	-2.177	27	.038
	Arthrobacter spp. ESU164	15	3.267	0.458				
Root Length (mm)	Control	14	6.143	1.562	12.87	-.960	27	.346
	Arthrobacter spp. ESU164	15	6.933	2.685				
Coleoptile Length (mm)	Control	14	6.214	1.847	16.93	-1.304	27	.203
	Arthrobacter spp. ESU164	15	7.267	2.434				
Germination Power	Control	14	-	-	7.14	-	-	-
	Arthrobacter spp. ESU164	15	-	-				

a: p < .05; b: p < .01; c: p < .001

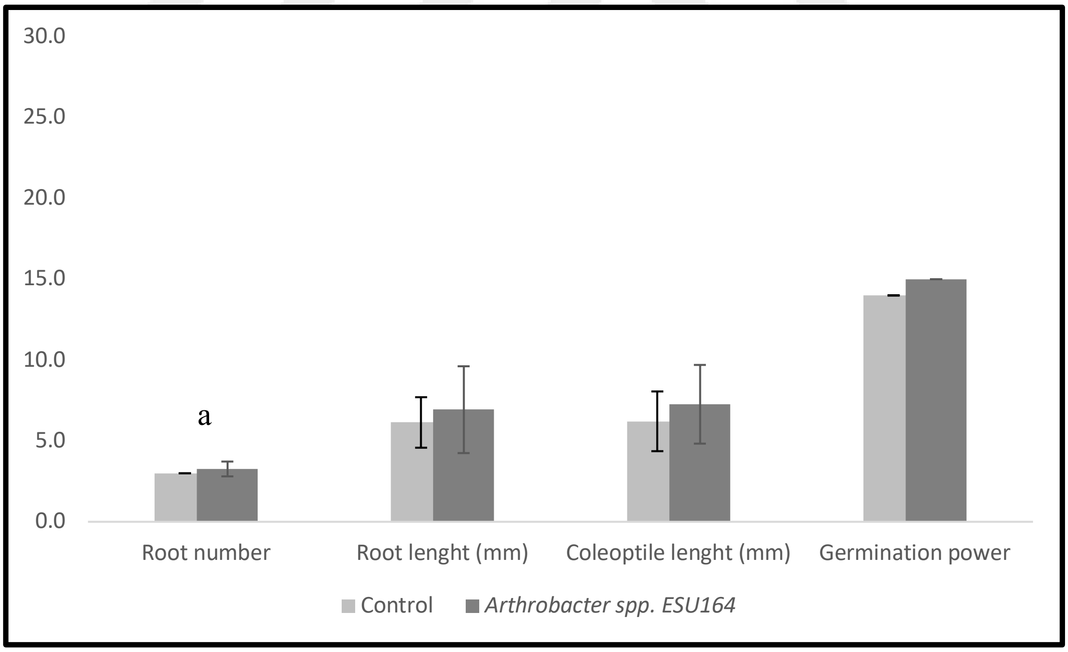

Figure 4.5 Changing in plant variables with *Arthrobacter* spp. ESU164 on filter paper.

Table 4.11 Independent samples t-test results for *Arthrobacter* spp. ESU193 on filter paper

Variables	**Species**	***N***	**Mean**	***SD***	**Percent Change**	***t***	***df***	***p***
Root Number	Control	14	3.000	.0000	0.00	-	-	-
	Arthrobacter spp. ESU193	15	3.000	.0000				
Root Length (mm)	Control	14	6.143	1.562	-1.24	.163	27	.872
	Arthrobacter spp. ESU193	15	6.067	0.884				
Coleoptile Length (mm)	Control	14	6.214	1.847	7.28	-.647	27	.523
	Arthrobacter spp. ESU193	15	6.667	1.915				
Germination Power	Control	14	-	-	7.14	-	-	-
	Arthrobacter spp. ESU193	15	-	-				

a: p < .05; b: p < .01; c: p < .001

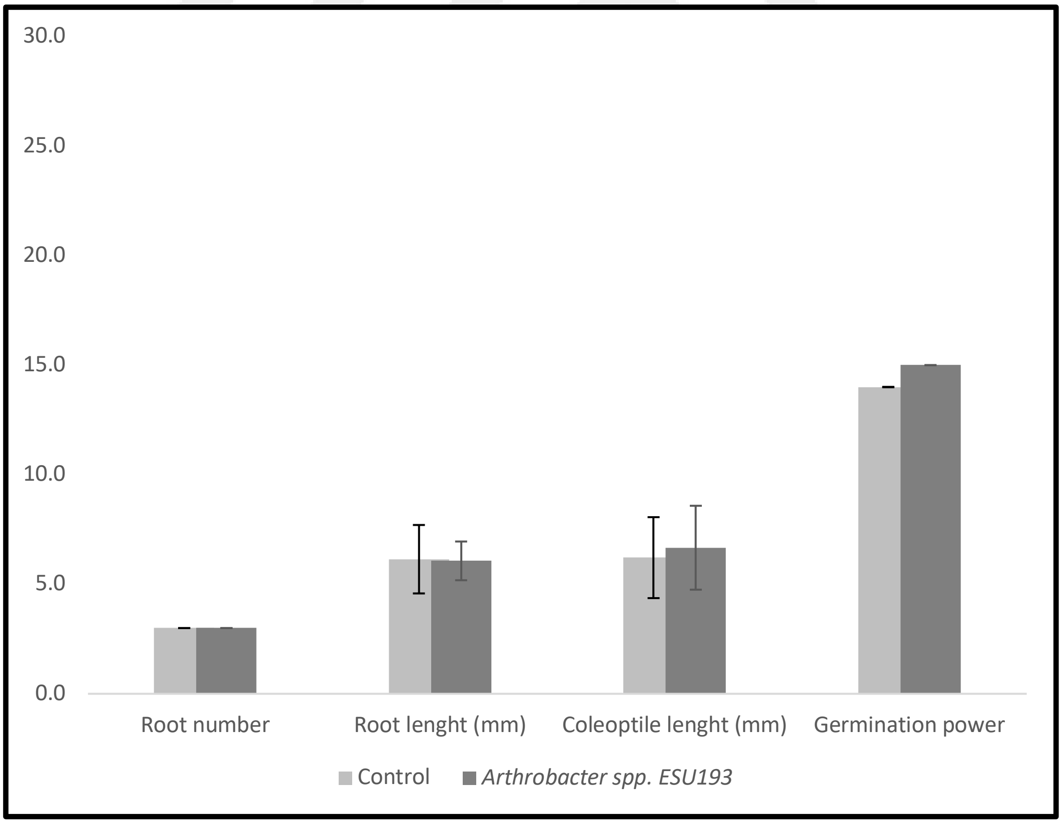

Figure 4.6 Changing in plant variables with *Arthrobacter* spp. ESU193 on filter paper

Table 4.12 Independent samples t-test results for *Pseudomonas* spp. ESU131 on filter paper

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.000	0.000	22.22[c]	-5.106	27	,000
	Pseudomonas spp. ESU131	15	3.667	0.488				
Root Length (mm)	Control	14	6.143	1.562	70.39[c]	-5.355	27	,000
	Pseudomonas spp. ESU131	15	10.467	2.615				
Coleoptile Length (mm)	Control	14	6.214	1.847	63.06[c]	-3.694	27	,001
	Pseudomonas spp. ESU131	15	10.133	3.543				
Germination Power	Control	14	3.000	-	7.14	-	-	-
	Pseudomonas spp. ESU131	15	3.667	-				

a: $p < .05$; b: $p < .01$; c: $p < .001$

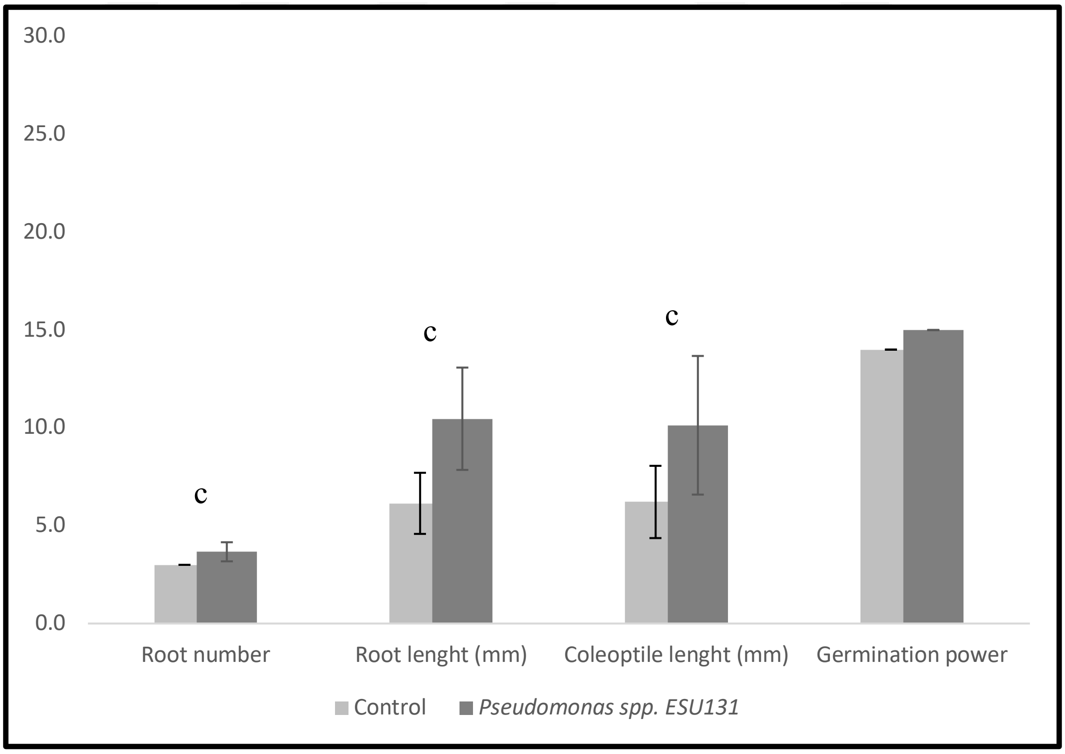

Figure 4.7 Changing in plant variables with *Pseudomonas* spp. ESU131 on filter paper

Table 4.13 Independent samples t-test results for *Pseudomonas* spp. ESU141 on filter paper

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.000	0.000	0.00	-	-	-
	Pseudomonas spp. ESU141	15	3.000	0.000				
Root Length (mm)	Control	14	6.143	1.562	6.36	-.685	27	.499
	Pseudomonas spp. ESU141	15	6.533	1.506				
Coleoptile Length (mm)	Control	14	6.214	1.847	-24.90	2.521	27	.018
	Pseudomonas spp. ESU141	15	4.667	1.447				
Germination Power	Control	14	-	-	7.14	-	-	-
	Pseudomonas spp. ESU141	15	-	-				

a: p < .05; b: p < .01; c: p < .001

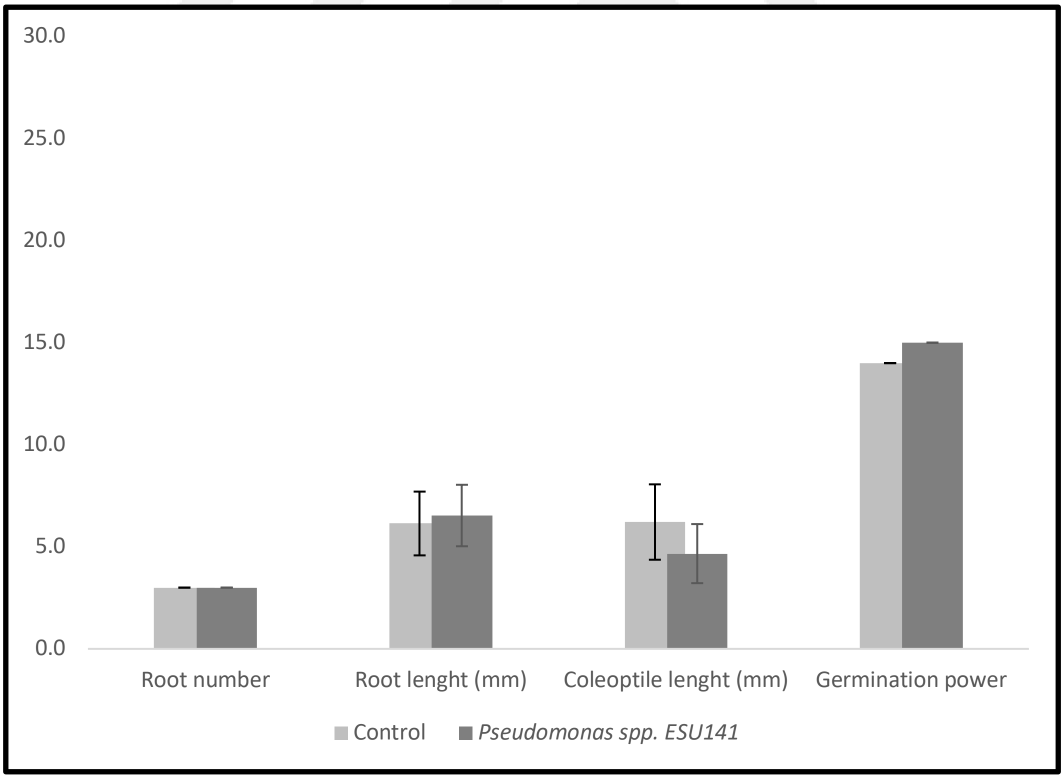

Figure 4.8 Changing in plant variables with *Pseudomonas* spp. ESU141 on filter paper.

Table 4.14 Independent samples t-test results for *Pseudomonas poae* strain ESU182 on filter paper

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.000	0.000	-7.14	1.385	26	.178
	Pseudomonas poae strain ESU182	14	2.786	0.579				
Root Length (mm)	Control	14	6.143	1.562	-9.30	.955	26	.348
	Pseudomonas poae strain ESU182	14	5.571	1.604				
Coleoptile Length (mm)	Control	14	6.214	1.847	-34.48	3.693	26	.001
	Pseudomonas poae strain ESU182	14	4.071	1.141				
Germination Power	Control	14	-	-	0.000	-	-	-
	Pseudomonas poae strain ESU182	14	-	-				

a: p < .05; b: p < .01; c: p < .001

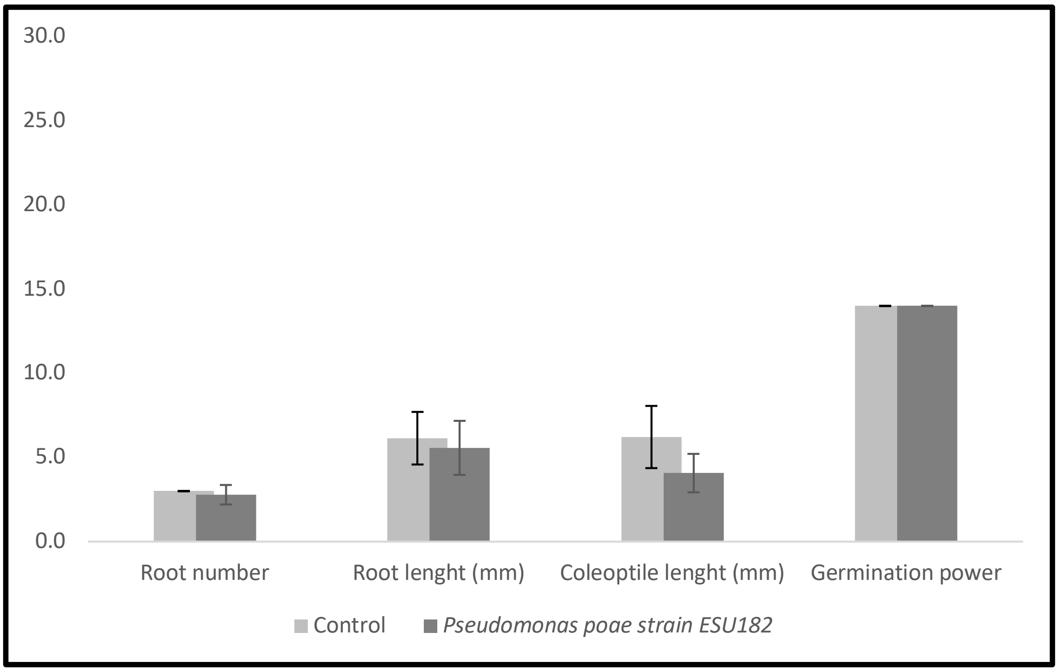

Figure 4.9 Changing in plant variables with *Pseudomonas poae* strain ESU182 on filter paper

Table 4.15 Independent samples t-test results for *Pseudomonas thivervalensis* strain ESU192 on filter paper

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.000	0.000	4.76	-.806	26	.427
	Pseudomonas thivervalensis strain ESU192	14	3.143	0.663				
Root Length (mm)	Control	14	6.143	1.562	33.72	-3.204	26	.004
	Pseudomonas thivervalensis strain ESU192	14	8.214	1.847				
Coleoptile Length (mm)	Control	14	6.214	1.847	24.14	-1.992	26	.057
	Pseudomonas thivervalensis strain ESU192	14	7.714	2.128				
Germination Power	Control	14	-	-	0.00	-	-	-
	Pseudomonas thivervalensis strain ESU192	14	-	-				

a: p < .05; b: p < .01; c: p < .001

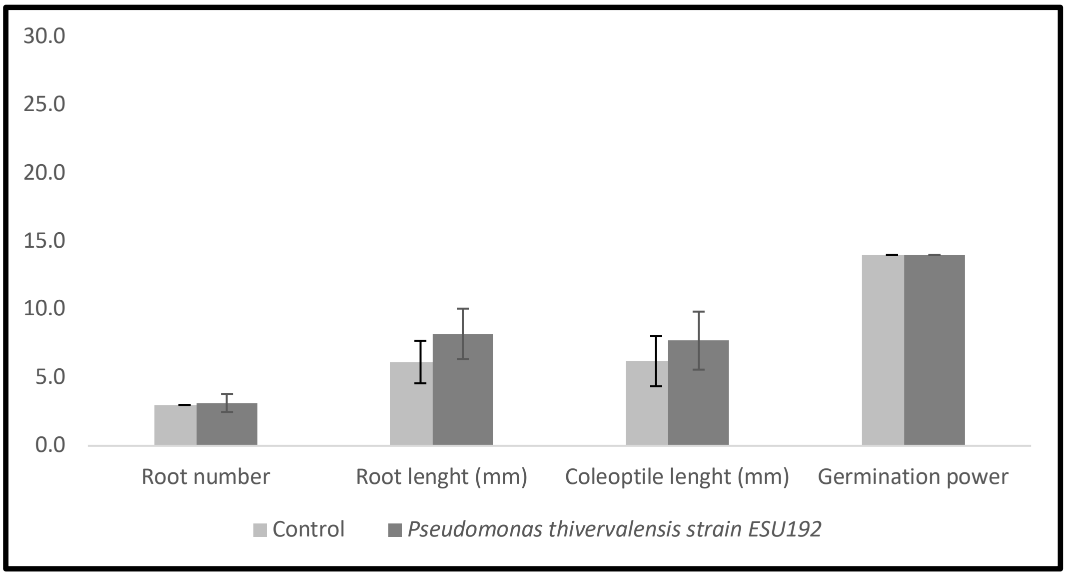

Figure 4.10 Changing in plant variables with *Pseudomonas thivervalensis* strain ESU192 on filter paper

Table 4.16 Independent samples t-test results for *Pseudomonas* spp. ESU1531 on filter paper

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.000	.00000	0.00	-	-	-
	Pseudomonas spp. ESU1531	15	3.000	.00000				
Root Length (mm)	Control	14	6.143	1.562	44.34[c]	-4.444	27	.000
	Pseudomonas spp. ESU1531	15	8.867	1.727				
Coleoptile Length (mm)	Control	14	6.214	1.847	30.88[a]	-2.251	27	.033
	Pseudomonas spp. ESU1531	15	8.133	2.642				
Germination Power	Control	14	-	-	7.14	-	-	-
	Pseudomonas spp. ESU1531	15	-	-				

a: $p < .05$; b: $p < .01$; c: $p < .001$

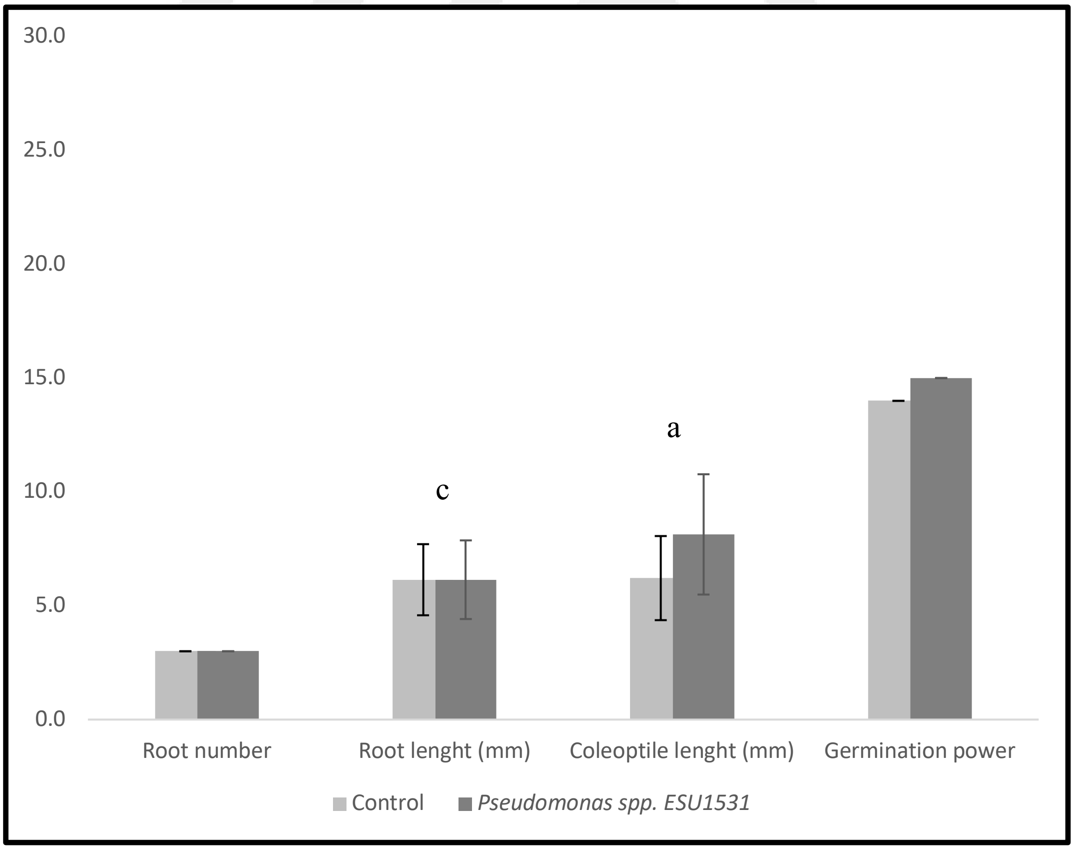

Figure 4.11 Changing in plant variables with *Pseudomonas spp.* ESU1531 on filter paper

Table 4.17 Independent samples t-test results for *Bacillus subtilis* strain ESU181 on filter paper

Variables	*Species*	*N*	*Mean*	*SD*	*Percent Change*	*t*	*df*	*p*
Root Number	Control	14	3.000	0.000	4.44	-.965	27	.343
	Bacillus subtilis strain ESU181	15	3.133	0.516				
Root Length (mm)	Control	14	6.143	1.562	40.00^{c}	-4.127	27	.000
	Bacillus subtilis strain ESU181	15	8.600	1.639				
Coleoptile Length (mm)	Control	14	6.214	1.847	-9.88	.937	27	.357
	Bacillus subtilis strain ESU181	15	5.600	1.682				
Germination Power	Control	14	-	-	7.14	-	-	-
	Bacillus subtilis strain ESU181	15	-	-				

a: p < .05; b: p < .01; c: p < .001

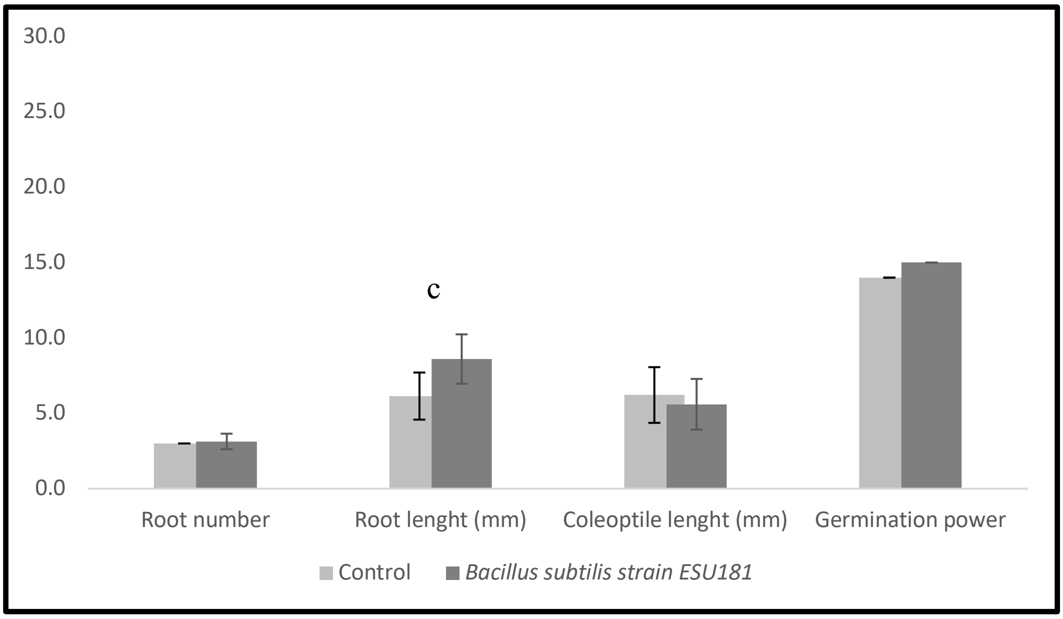

Figure 4.12 Changing in plant variables with *Bacillus subtilis* strain ESU181on filter paper

Table 4.18 Independent samples t-test results for *Microbacterium foliorum* strain ESU172 on filter paper

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.000	0.000	31.11^{c}	-5.876	27	.000
	Microbacterium foliorum strain ESU172	15	3.933	0.594				
Root Length (mm)	Control	14	6.143	1.562	296.12^{c}	-17.426	27	.000
	Microbacterium foliorum strain ESU172	15	24.333	3.599				
Coleoptile Length (mm)	Control	14	6.214	1.847	187.51^{c}	-12.009	27	.000
	Microbacterium foliorum strain ESU172	15	17.867	3.159				
Germination Power	Control	14	-	-	7.14	-	-	-
	Microbacterium foliorum strain ESU172	15	-	-				

a: p < .05; b: p < .01; c: p < .001

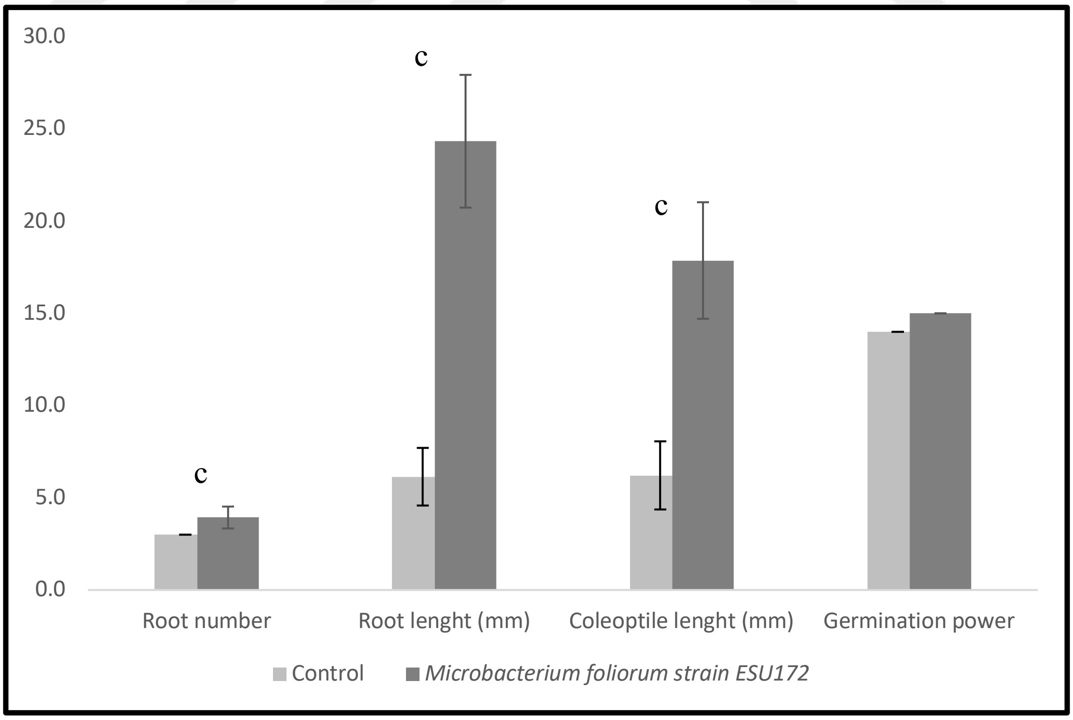

Figure 4.13 Changing in plant variables with *Microbacterium foliorum* strain ESU172 on filter paper

Table 4.19 Independent samples t-test results for *Microbacterium* spp. ESU121on filter paper

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.000	0.000	-11.90	1.794	26	.084
	Microbacterium spp. ESU121	14	2.643	0.745				
Root Length (mm)	Control	14	6.143	1.562	-24.42	2.370	26	.026
	Microbacterium spp. ESU121	14	4.643	1.781				
Coleoptile Length (mm)	Control	14	6.214	1.847	-55.17	5.580	26	.000
	Microbacterium spp. ESU121	14	2.786	1.369				
Germination Power	Control	14	-	-	0,00	-	-	-
	Microbacterium spp. ESU121	14	-	-				

a: p < .05; b: p < .01; c: p < .001

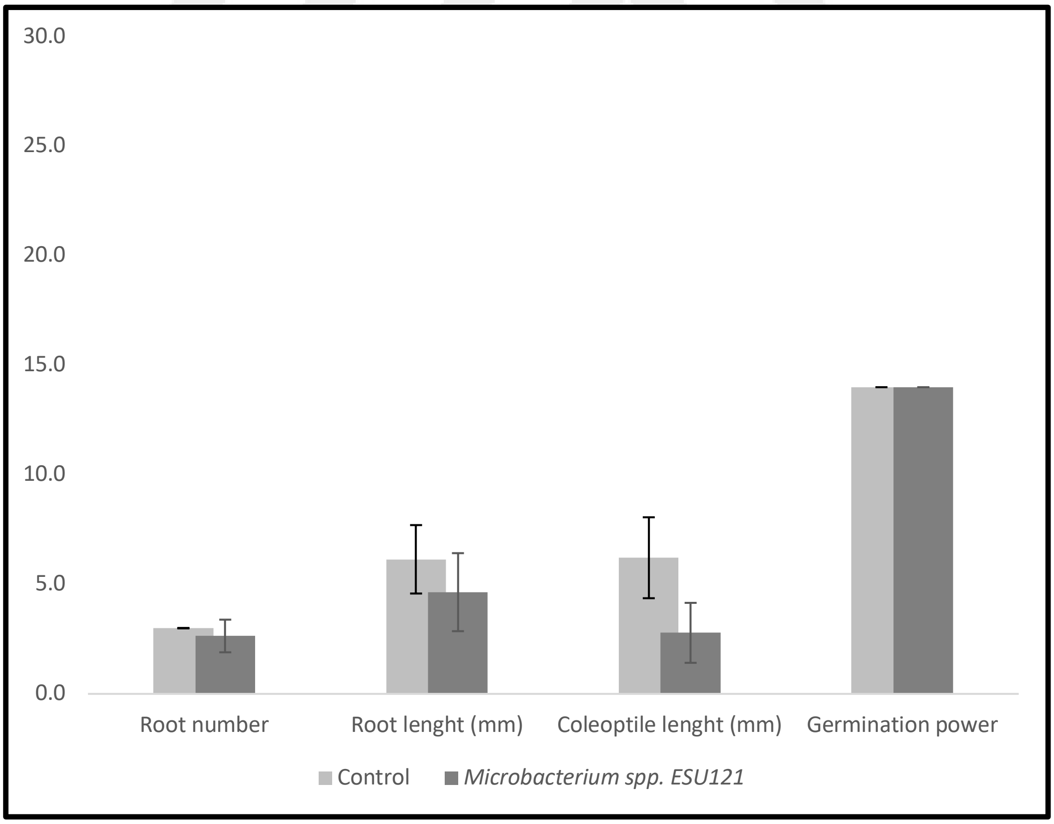

Figure 4.14 Changing in plant variables with *Microbacterium* spp. ESU121 on filter paper

4.1.11 Germination in Soil

The statistical results (independent t-test) of the plant variables after the application of the bacterial isolates against control are given in Tables 4.20-29 and Figures 4.15-24 in this section.

According to the statistical analysis results of the plant parameters in soil *Arthrobacter* spp. ESU164 showed 164.20% increase in the root fresh weight ($p<0.05$). *Arthrobacter* spp. ESU193 increased the root number as 24.00% ($p<0.05$). *Pseudomonas* spp. ESU131 increased the root number as 15.73%, the root length as 36.93%, and the root fresh weight as 89.38% ($p<0.05$). *Pseudomonas* spp. ESU141 increased the root number as 20.00%, the root length as 40.15%, and the root fresh weight as 117.72% ($p<0.05$). *Pseudomonas poae* strain ESU182 increased the root number as 22.00%, and the root fresh weight as 189.24% ($p<0.05$). *Pseudomonas thivervalensis* strain ESU182 increased the root length as 23.50% ($p<0.05$). *Pseudomonas* spp. ESU1531 increased the root number as 27.08% and the root length as 35.36% ($p<0.05$). *Bacillus subtilis* strain ESU181 increased the root length as 32.44% ($p<0.05$). *Microbacterium foliorum* strain ESU172 decreased the root fresh weight as 56.00% and the root dry weight as 47.15% ($p<0.05$).

Table 4.20 Independent samples t-test results for *Arthrobacter* spp. ESU164 in soil

Variables	**Species**	***N***	**Mean**	***SD***	**Percent Change**	***t***	***df***	***p***
Root Number	Control	14	3.571	0.646	7.33	-1.081	24	.290
	Arthrobacter spp. ESU164	12	3.833	0.577				
Root Length (cm)	Control	14	4.893	1.228	25.18	-1.865	24	.074
	Arthrobacter spp. ESU164	12	6.125	2.090				
Root Fresh Weight (mg)	Control	14	8.100	5.683	164.20[c]	-4.093	24	.000
	Arthrobacter spp. ESU164	12	21.400	10.521				
Root Dry Weight (mg) (mg)	Control	14	4.514	2.492	-11.58	.629	24	.536
	Arthrobacter spp. ESU164	12	3.992	1.552				
Germination Power	Control	14	-	-	-14.29	-	-	-
	Arthrobacter spp. ESU164	12	-	-				

a: p < .05; b: p < .01; c: p < .001

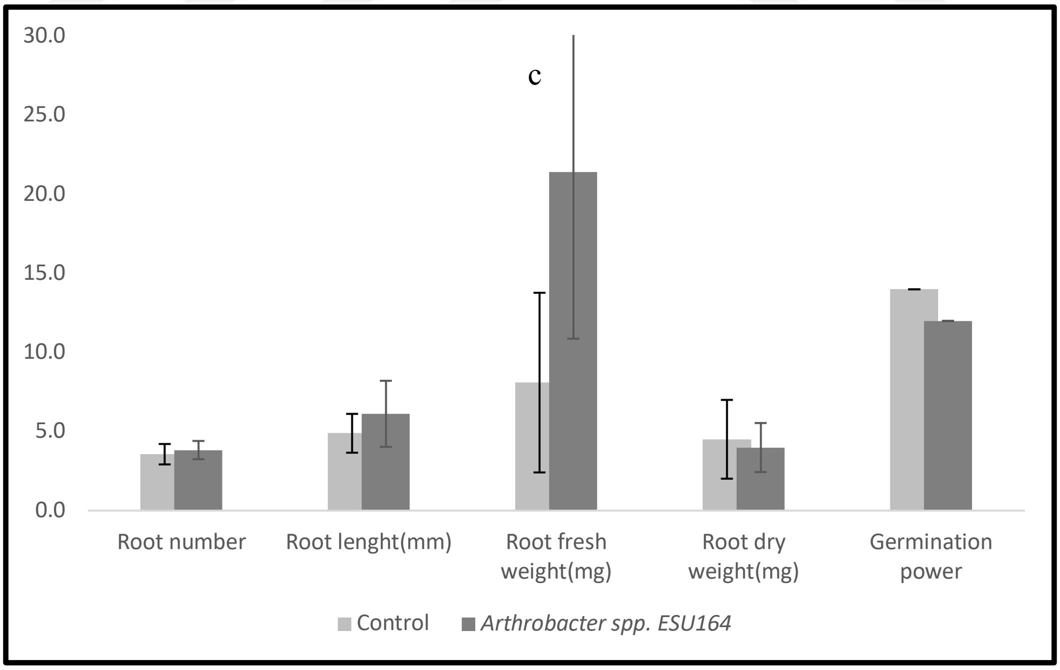

Figure 4.15 Changing in plant variables with *Arthrobacter* spp. ESU164 in soil

Table 4.21 Independent samples t-test results for *Arthrobacter* spp. ESU193 in soil

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.571	0.646	16.67[a]	-2.225	24	.036
	Arthrobacter spp. ESU193	12	4.167	0.718				
Root Length (cm)	Control	14	4.893	1.228	24.16	-1.596	24	.124
	Arthrobacter spp. ESU193	12	6.075	2.441				
Root Fresh Weight (mg)	Control	14	8.100	5.683	-32.10	1.468	24	.155
	Arthrobacter spp. ESU193	12	5.500	2.459				
Root Dry Weight (mg)	Control	14	4.514	2.492	-18.22	.947	24	.353
	Arthrobacter spp. ESU193	12	3.692	1.815				
Germination Power	Control	14	-	-	-14.29	-	-	-
	Arthrobacter spp. ESU193	12	-	-				

a: p < .05; b: p < .01; c: p < .001

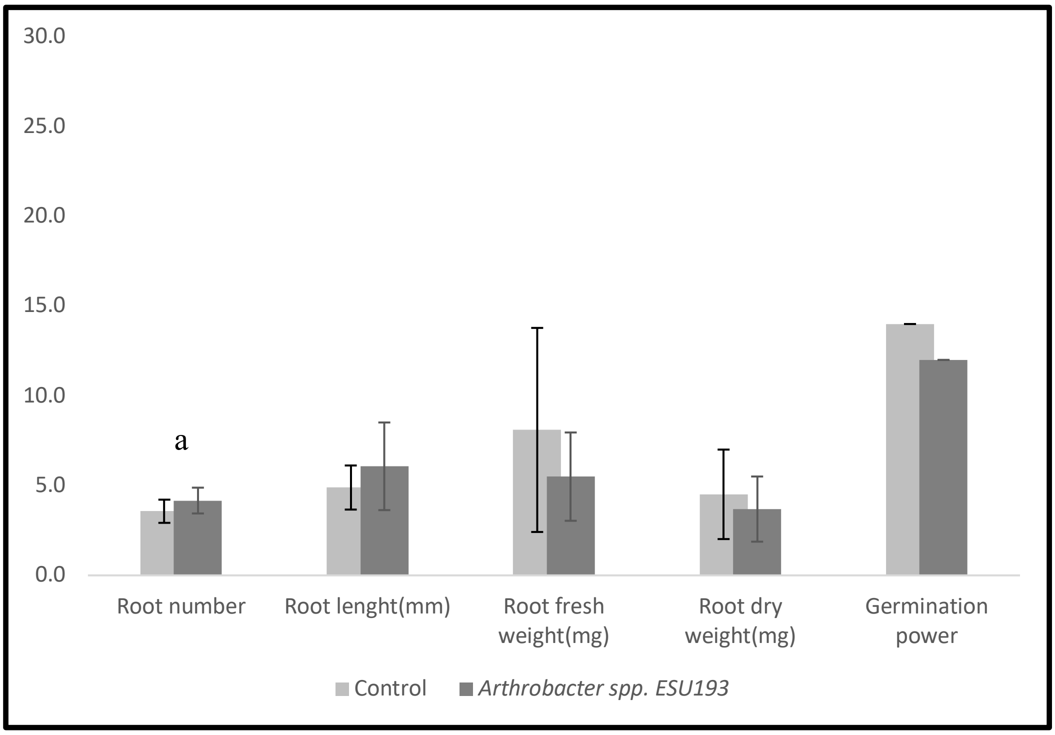

Figure 4.16 Changing in plant variables with *Arthrobacter* spp. ESU193 in soil

Table 4.22 Independent samples t-test results for *Pseudomonas* spp. ESU131 in soil

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.571	0.646	15.73[a]	-1.708	27	.099
	Pseudomonas spp. ESU131	15	4.133	1.060				
Root Length (cm)	Control	14	4.893	1.228	36.93[a]	-2.527	27	.018
	Pseudomonas spp. ESU131	15	6.700	2.396				
Root Fresh Weight (mg)	Control	14	8.100	5.683	89.38[a]	-2.301	27	.029
	Pseudomonas spp. ESU131	15	15.340	10.406				
Root Dry Weight (mg)	Control	14	4.514	2.492	3.82	-.202	27	.841
	Pseudomonas spp. ESU131	15	4.687	2.090				
Germination Power	Control	14	-	-	7.14	-	-	-
	Pseudomonas spp. ESU131	15	-	-				

a: $p < .05$; b: $p < .01$; c: $p < .001$

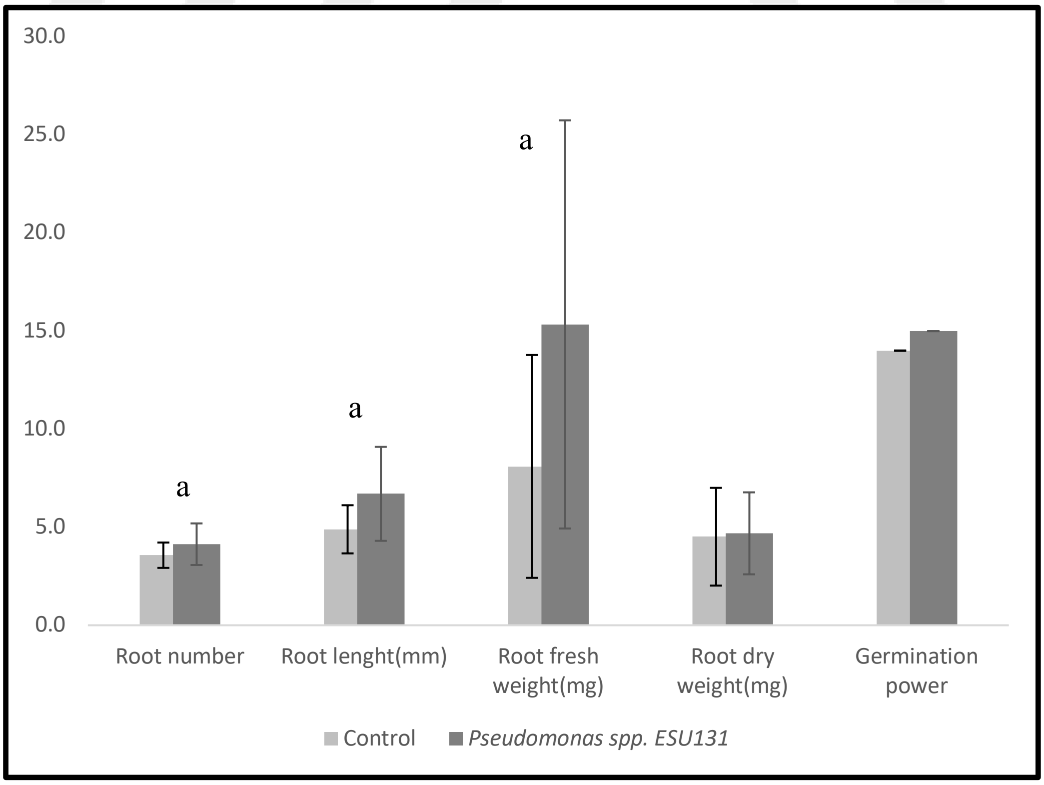

Figure 4.17 Changing in plant variables with *Pseudomonas* spp. ESU131 in soil

Table 4.23 Independent samples t-test results for *Pseudomonas* spp. ESU141 in soil

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.571	0.646	20.00[a]	-2.749	26	.011
	Pseudomonas spp. ESU141	14	4.286	0.726				
Root Length (cm)	Control	14	4.893	1.228	40.15[b]	-3.456	26	.002
	Pseudomonas spp. ESU141	14	6.857	1.737				
Root Fresh Weight (mg)	Control	14	8.100	5.683	117.72[b]	-2.944	26	.007
	Pseudomonas spp. ESU141	14	17.636	10.704				
Root Dry Weight (mg)	Control	14	4.514	2.492	-16.93	1.030	26	.313
	Pseudomonas spp. ESU141	14	3.750	1.225				
Germination Power	Control	14	-	-	0.00	-	-	-
	Pseudomonas spp. ESU141	14	-	-				

a: p < .05; b: p < .01; c: p < .001

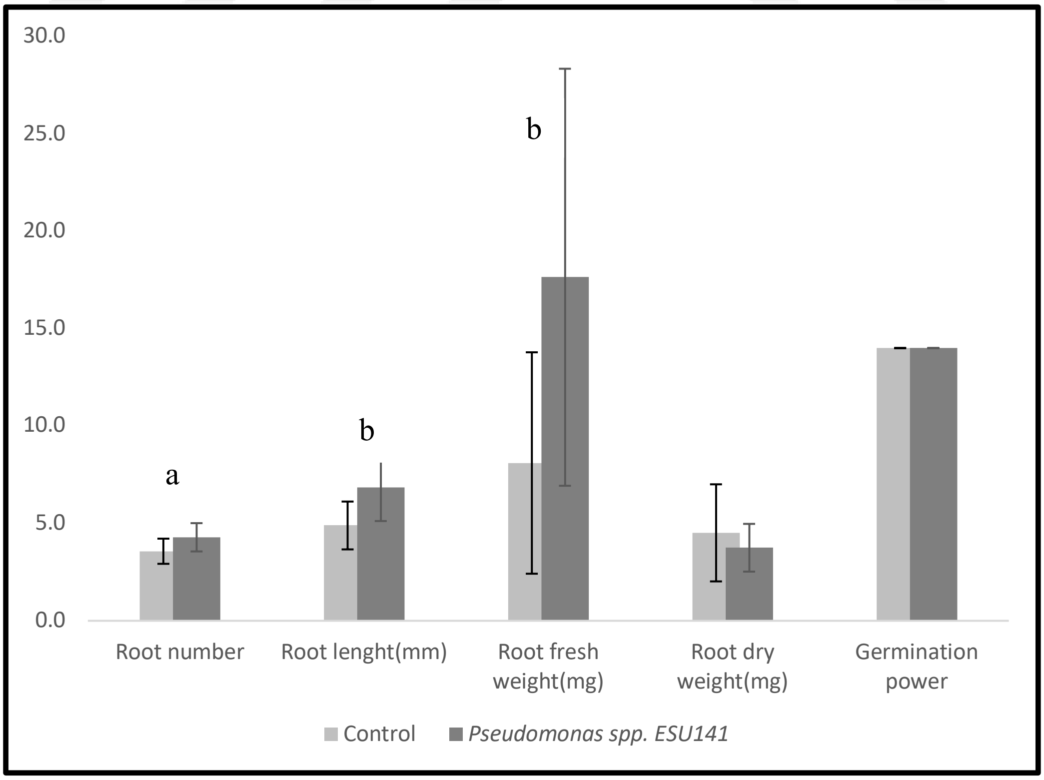

Figure 4.18 Changing in plant variables with *Pseudomonas* spp. ESU141 in soil

Table 4.24 Independent samples t-test results for *Pseudomonas poae* strain ESU182 in soil

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.571	0.646	22.00[a]	-2.598	26	.15
	Pseudomonas poae strain ESU182	14	4.357	0.929				
Root Length (cm)	Control	14	4.893	1.228	17.52	-1.546	26	.134
	Pseudomonas poae strain ESU182	14	5.750	1.673				
Root Fresh Weight (mg)	Control	14	8.100	5.683	189.24[b]	-3.933	26	.001
	Pseudomonas poae strain ESU182	14	23,429	13.430				
Root Dry Weight (mg)	Control	14	4.514	2.492	-17.25	.991	26	.331
	Pseudomonas poae strain ESU182	14	3.736	1.556				
Germination Power	Control	14	-	-				
	Pseudomonas poae strain ESU182	14	-	-	0.00	-	-	-

a: $p < .05$; b: $p < .01$; c: $p < .001$

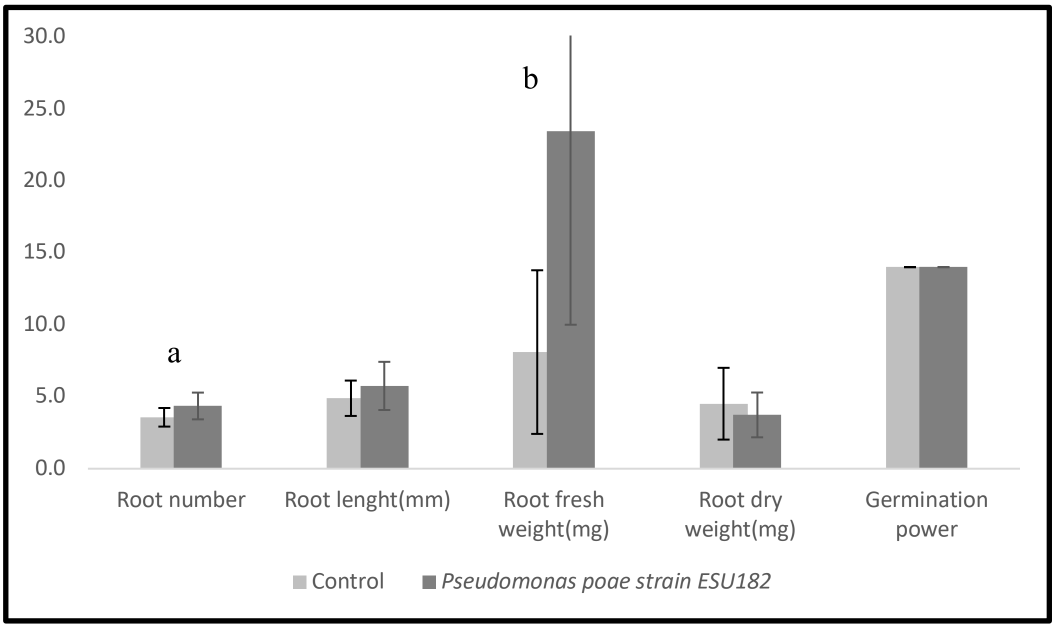

Figure 4.19 Changing in plant variables with *Pseudomonas poae* strain ESU182 in soil

Table 4.25 Independent samples t-test results for *Pseudomonas thivervalensis* strain ESU192 in soil

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.571	0.646	-6.00	.709	26	.485
	Pseudomonas thivervalensis strain ESU192	14	3.357	0.929				
Root Length (cm)	Control	14	4.893	1.228	23.50[a]	-2.166	26	.040
	Pseudomonas thivervalensis strain ESU192	14	6.043	1.561				
Root Fresh Weight (mg)	Control	14	8.100	5.683	0.09	-.004	26	.997
	Pseudomonas thivervalensis strain ESU192	14	8.107	4.930				
Root Dry Weight (mg)	Control	14	4.514	2.492	-34.81[a]	2.128	26	.043
	Pseudomonas thivervalensis strain ESU192	14	2.943	1.195				
Germination Power	Control	14	-	-	0.00	-	-	-
	Pseudomonas thivervalensis strain ESU192	14	-	-				

a: p < .05; b: p < .01; c: p < .001

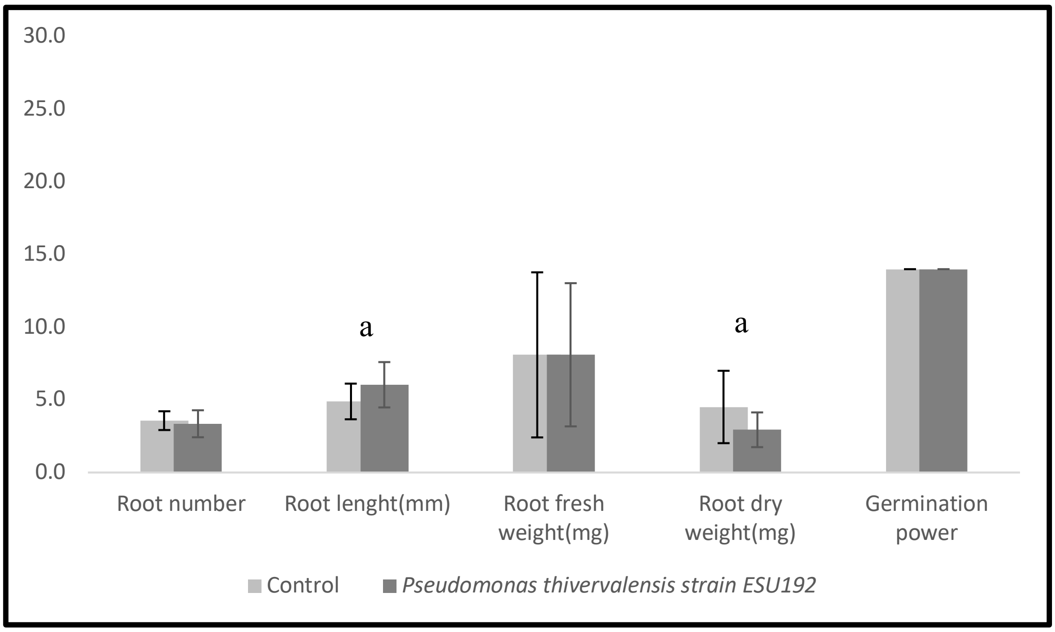

Figure 4.20 Changing in plant variables with *Pseudomonas thivervalensis* strain ESU192 in soil

Table 4.26 Independent samples t-test results for *Pseudomonas* spp. ESU1531 in soil

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*df*	*p*
Root Number	Control	14	3.571	0.646	27.08[b]	-3.279	25	.003
	Pseudomonas spp. ESU1531	13	4.538	0.877				
Root Length (cm)	Control	14	4.893	1.228	35.36[b]	-3.698	25	.001
	Pseudomonas spp. ESU1531	13	6.623	1.201				
Root Fresh Weight (mg)	Control	14	8.100	5.683	43.78	-1.622	25	.117
	Pseudomonas spp. ESU1531	13	11.646	5.668				
Root Dry Weight (mg)	Control	14	4.514	2.492	7.52	-.420	25	.678
	Pseudomonas spp. ESU1531	13	4.854	1.570				
Germination Power	Control	14	-	-	-7.14	-	-	-
	Pseudomonas spp. ESU1531	13	-	-				

a: p < .05; b: p < .01; c: p < .001

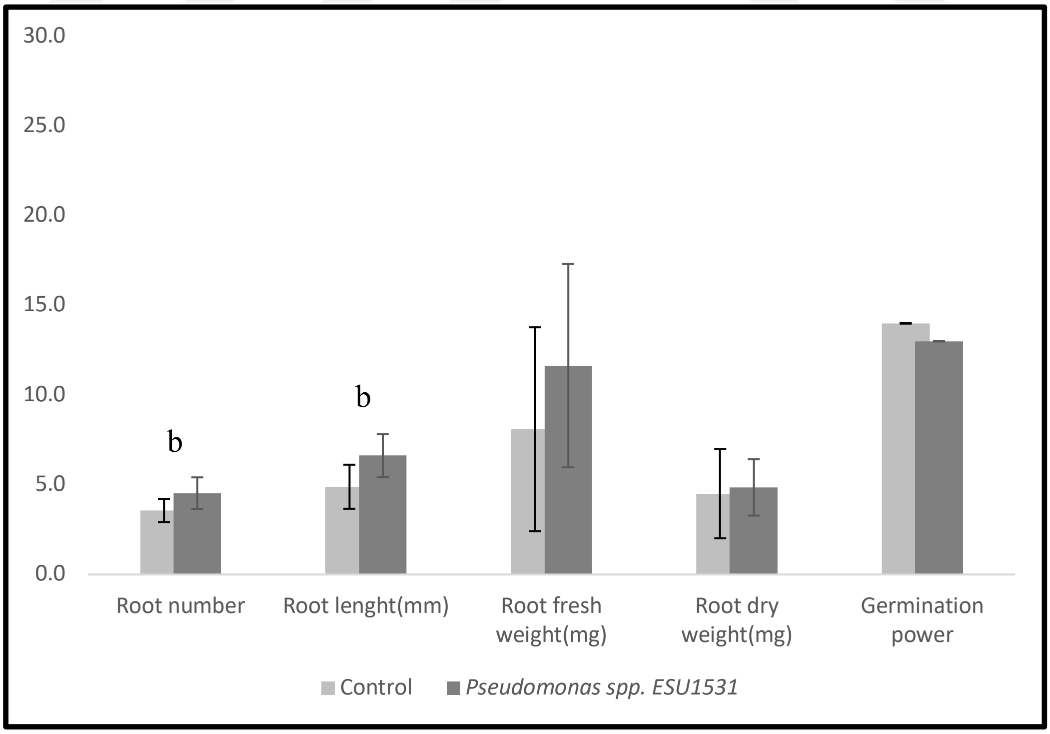

Figure 4.21 Changing in plant variables with *Pseudomonas* spp. ESU1531 in soil

Table 4.27 Independent samples t-test results for *Bacillus subtilis* strain ESU181in soil

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*Df*	*p*
Root Number	Control	14	3.571	0.646	8.27	-1.060	27	.298
	Bacillus subtilis strain ESU181	15	3.867	0.834				
Root Length (cm)	Control	14	4.893	1.228	32.44[a]	-2.121	27	.043
	Bacillus subtilis strain ESU181	15	6.480	2.534				
Root Fresh Weight (mg)	Control	14	8.100	5.683	9.30	-.343	27	.734
	Bacillus subtilis strain ESU181	15	8.853	6.124				
Root Dry Weight (mg)	Control	14	4.514	2.492	-12.13	.690	27	.496
	Bacillus subtilis strain ESU181	15	3.967	1.741				
Germination Power	Control	14	-	-	7.14	-	-	-
	Bacillus subtilis strain ESU181	15	-	-				

a: p < .05; b: p < .01; c: p < .001

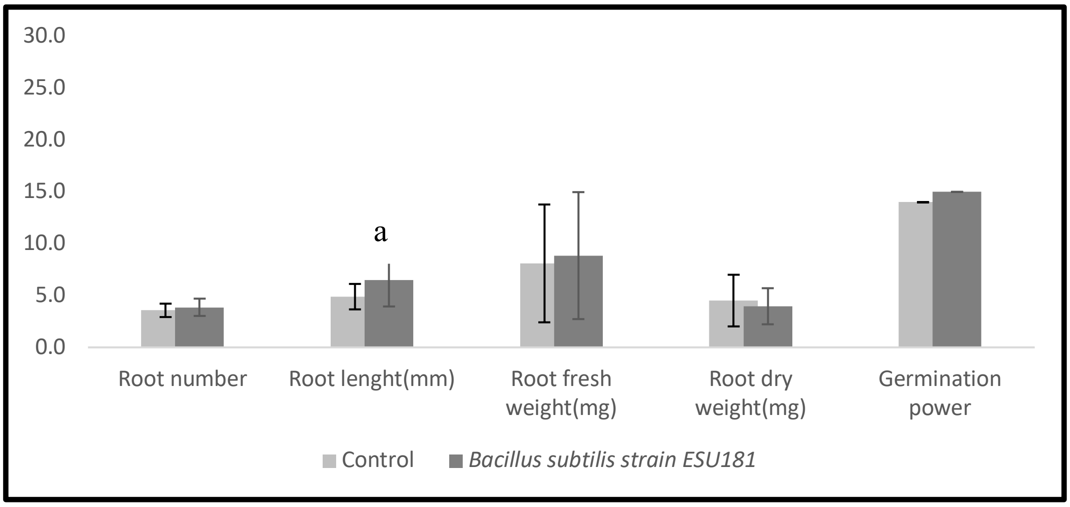

Figure 4.22 Changing in plant variables with *Bacillus subtilis* strain ESU181 in soil

Table 4.28 Independent samples t-test results for *Microbacterium foliorum* strain ESU172 in soil

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*Df*	*p*
Root Number	Control	14	3.571	0.646	-4.00	.585	26	.564
	Microbacterium foliorum strain ESU172	14	3.429	0.646				
Root Length (cm)	Control	14	4.893	1.228	-8.91	.973	26	.340
	Microbacterium foliorum strain ESU172	14	4.457	1.141				
Root Fresh Weight (mg)	Control	14	8.100	5.683	-56.00[b]	2.806	26	.009
	Microbacterium foliorum strain ESU172	14	3.564	2.067				
Root Dry Weight (mg)	Control	14	4.514	2.492	-47.15[b]	2.844	26	.009
	Microbacterium foliorum strain ESU172	14	2.386	1.278				
Germination Power	Control	14	-	-	0.00	-	-	-
	Microbacterium foliorum strain ESU172	14	-	-				

a: p < .05; b: p < .01; c: p < .001

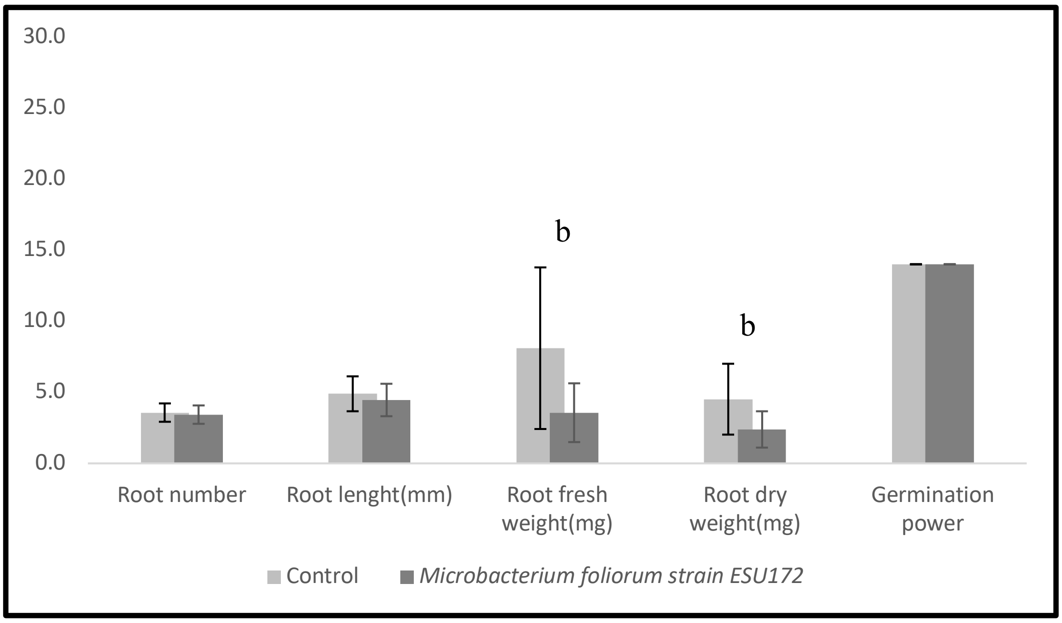

Figure 4.23 Changing in plant variables with *Microbacterium foliorum* strain ESU172 in soil

Table 4.29 Independent samples t-test results for *Microbacterium* spp. ESU121 in soil

Variables	Species	*N*	Mean	*SD*	Percent Change	*t*	*Df*	*p*
Root Number	Control	14	3.571	0.646	-5.23	.564	25	.578
	Microbacterium spp. ESU121	13	3.385	1.044				
Root Length (cm)	Control	14	4.893	1.228	5.33	-.531	25	.600
	Microbacterium spp. ESU121	13	5.154	1.329				
Root Fresh Weight (mg)	Control	14	8.100	5.683	-40.08	1.589	25	.125
	Microbacterium spp. ESU121	13	4.854	4.863				
Root Dry Weight (mg)	Control	14	4.514	2.492	-27.75	1.540	25	.136
	Microbacterium spp. ESU121	13	3.262	1.601				
Germination Power	Control	14	-	-	-7.14	-	-	-
	Microbacterium spp. ESU121	13	-	-				

a: $p < .05$; b: $p < .01$; c: $p < .001$

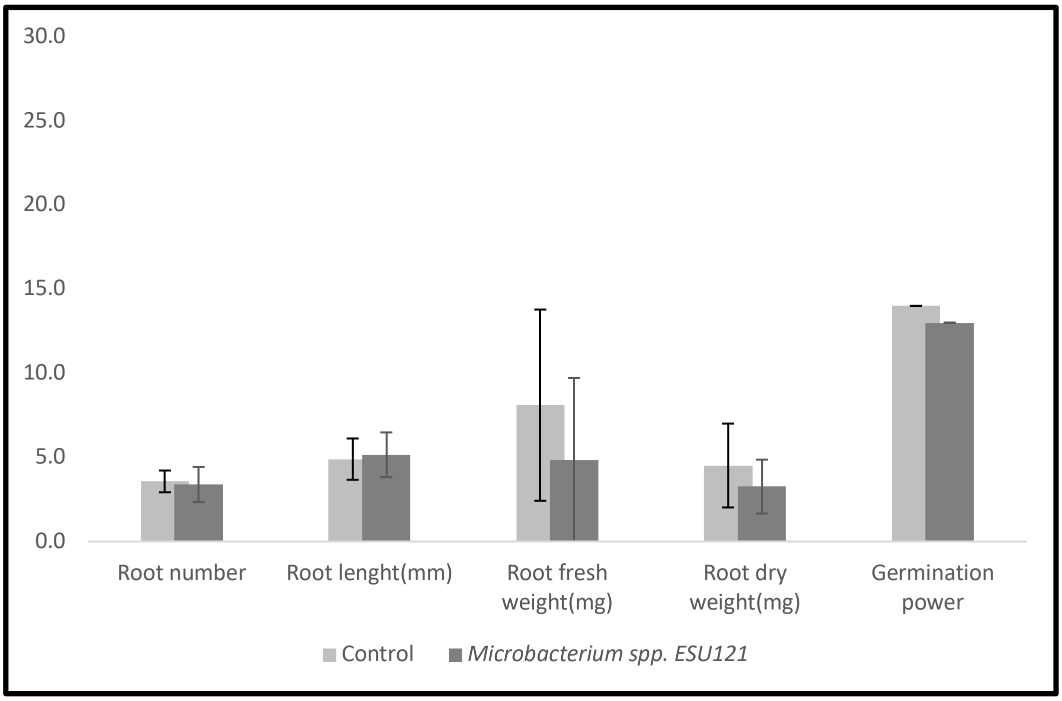

Figure 4.24 Changing in plant variables with *Microbacterium* spp. ESU121 in soil

5. DISCUSSION

The benefit of PGPB to plant growth and yield is a well-known phenomenon. The genotype of isolate not only represents its plant growth promoting functions, but also its interaction with the plant genotype (131–133). Furthermore, native biotic and abiotic factors such as localized bacterial species, soil properties, and climate impact the plant growth promoting effects of isolates. Commonly, biofertilizer development focuses on genetic and PGP properties. There is limited number of ecological studies to determine success under changing field conditions (131,132). In the literature, two approaches have been reported. One of them is the selection of strains that adapted to specific conditions such as osmotic tolerance (136). Another method is the isolation of native species which have biofertilization performance (137–139).

In this study, the isolation, the selection, the investigation of PGP effects and analysis of the bacterial species associated with locally named Iza wheat (Triticum monococcum ssp. monococcum) from Seben region of Bolu/Turkey were aimed. Three types of identification methods (MALDI-TOF, 16S rDNA, and 16S-23S rDNA intergenic region) and quadruple interpretation methods were applied to identify the putative species. Most of the studies in the literature identify the species by selecting only one method. Table 4.6 represents the approaches followed, in the right columns of the table identities of the isolates are given. According to the results, deciding the species' identity is not applicable by using only one technique.

Verifications of the identities of the putative species were carried out by using three variables as gram staining, catalase, and oxidase activities. All species except *Microbacterium foliorum* strain ESU172 and *Microbacterium* spp. ESU121 were verified. According to literature *Microbacterium* genus is gram-positive, on the other hand our results show that our isolate has a gram-negative cell wall.

In plant growth promoting bacteria, the property of binding nitrogen from the air is one of the most important features because nitrogen is the element which is used to increase the yield as fertilizer in most of the agricultural areas. Because of the cost and negative effects of the chemical fertilizers on environment, studies on nitrogen-fixing bacteria have gained momentum in recent years. In this study, nitrogen fixing abilities of the isolates were observed by using Jensen's medium, and the results show that all isolates are able to fix nitrogen from air.

Other important characteristics of the bacteria that are putative for using as biofertilizers is phosphate solubilization capacity. Because it is one of the limiting elements, and agricultural areas are poor in terms of available forms of phosphate compounds for plants. Although there are many microorganisms in soil, their densities and competition strengths are not sufficient. The necessity of interaction between more competitive phosphate solubilizing microorganisms and plants to improve the quality and quantity of crops has risen day by day. In the study phosphate solubilization capacities of the isolates were determined by using Pikovskaya's agar medium, and the results represent that *Pseudomonas* spp. ESU131 has the highest capacity to solubilize phosphate. On the other hand *Microbacterium foliorum* strain ESU172 is not able to solubilize phosphate in calcium phosphate form.

The activity of catalase is essential for bacteria which to protect itself against H_2O_2. So, PGPB with higher catalase activity can survive longer than others and promote plant growth (118). In this study *Pseudomonas thivervalensis* strain ESU192, *Bacillus subtilis* strain ESU181, and *Pseudomonas* spp. ESU131 give the highest three catalase activities.

L-tryptophan is known as an IAA precursor, addition of trp in incubation medium increases IAA biosynthesis (140). All 10 isolates have the ability for IAA production and the concentrations clearly accelerated with the addition of trp. For many bacterial species, the conversion of trp to IAA is the conserved pathway to produce IAA. Trp-depended production of IAA has been reported for *Streptomyces* spp. and *Bacillus subtilis* spp., and also other species (141,142). The colour change of the reaction between IAA and Salkowski reagent in the tubes are given under each graph. The reaction gives pink colour yield because of formation of IAA complex with Fe^{3+} reduction (143). *Arthrobacter* spp. ESU164 has the highest IAA production without trp in medium after 216 hours, and *Pseudomonas* spp. ESU131 is the best IAA producer with trp in medium after 264 hours.

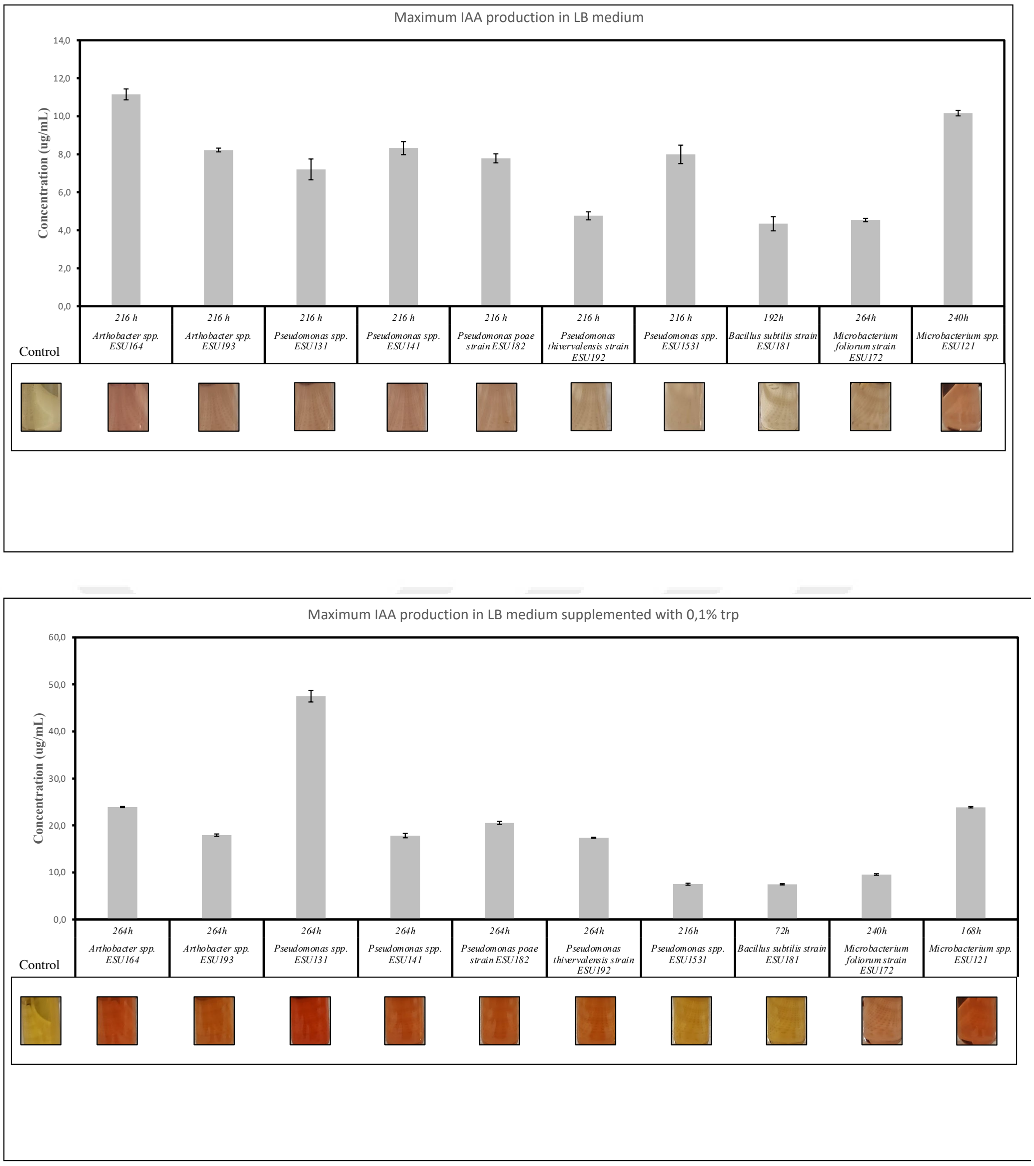

Figure 5.1 Maximum IAA productions of the species in LB medium and LB medium supplemented with 0.1% trp

Our aim was to isolate the most effective bacteria that promote yield increase in wheat development and at the end of this thesis research, also planned to have at least one bacterial species that can statistically change the yield of *Triticum monococcum* ssp. *monococcum*.

In reported studies, rhizobacteria can play a significant role in the plant growth and increasing the resistance of the host plant to different abiotic stresses especially drought and salinity. Bacterial species which are resistant to salinity and high temperatures can lead to an increase in yield amount and quality (144,145).

Arthrobacter species helps plants growth by ACC, IAA production and siderophore and the ability to solubilize phosphate ($Ca_3(PO_4)_2$) (146). In this study identified *Arthrobacter* spp. ESU164 and *Arthrobacter* spp. ESU193 give positive N_2-fixation, phosphate solubilizing capacities and IAA production, besides represents positive effects on plant growth parameters.

Pseudomonas genus that is diverse and ecologically versatile can colonize terrestrial, freshwater and marine habitats (147–149). This species shows diverging roles after the colonizing to host plant rhizosphere such as growth promoting, as biocontrol agents, and as pathogens(150,151). Another important property of *Pseudomonas* species is that they can withstand different kinds of stress. The isolated *Pseudomonas* species in this research represents remarkable results for plant growth parameters which are parallel with the literature.

The growth promoting effect of *B. subtilis* has shown in numerous studies. *B. subtilis* promotes plant growth and improves the host plants against a wide range of biotic (pathogens and pests) and abiotic (salinity, drought, temperature, heavy metals, etc.) stresses(152,153). The isolated *Bacillus subtilis* strain ESU181 increased the root length as over 30.00% significantly.

In the literature, the inoculation of a strain of *Microbacterium* sp. in durum wheat resulted positive results on height and fresh weight (154), the stem diameter and leave numbers increased by *Microbacterium foliorum* (155), and *Microbacterium arborescens* promoted plant elongation and Nitrogen, Phosphorus, Copper, Zinc, Manganese, and Iron assimilation (156). In this study isolated *Microbacterium foliorum* strain ESU172 increased the root parameters on the filter paper germination experiments, on the other hand it decreased the root fresh weight and the root dry weight in soil germination experiments.

Our aim was to isolate most effective bacteria that promote yield increase in wheat development, also planned to have at least one bacterial species that can statistically change the yield of *Triticum monococcum* ssp. *monococcum*.

The results from the plant growth experiments more than one species promote the plant parameters; they can be used as plant growth promoting bacteria for the agricultural applications. In Figure 5.1 represents the statistically significant effects of the isolated bacterial species on the growth parameters of *Triticum monococcum* ssp. *monococcum*. They can be used in the agricultural applications for specific properties as increasing root number, root length, root fresh weight, and coleoptile length.

Table 5.1 The effects of the isolated bacteria on the growth parameters of *Triticum monococcum* ssp. *monococcum*

	Root number	**Root length**	**Root fresh weight**	**Coleoptile length**
Pseudomonas spp. ESU131	✓	✓	✓	✓
Pseudomonas spp. ESU141	✓	✓	✓	
Pseudomonas poae strain ESU182	✓	✓	✓	
Pseudomonas spp. ESU1531	✓	✓		✓
Arthrobacter spp. ESU164	✓		✓	
Pseudomonas thivervalensis strain ESU192	✓		✓	
Arthrobacter spp. ESU193	✓			
Bacillus subtilis strain ESU181		✓		
Microbacterium foliorum strain ESU172				✓

6. CONCLUSIONS AND RECOMMENDATIONS

In this thesis study we aimed to isolate bacteria that can promote yield increase in *Triticum monococcum* ssp. *monococcum*. We identified 10 different species (*Arthrobacter* spp. ESU164, *Arthrobacter* spp. ESU193, *Pseudomonas* spp. ESU131, *Pseudomonas* spp. ESU141, *Pseudomonas poae* strain ESU182, *Pseudomonas thivervalensis* strain ESU192, *Pseudomonas* spp. ESU1531, *Bacillus subtilis* strain ESU181, *Microbacterium foliorum* strain ESU172, *Microbacterium* spp. ESU121). The evaluation of the biochemical properties and the impact of the isolates on *T. monococcum* ssp. *monococcum* development suggest that the isolates can be used to promote yield in wheat farming.

We were able to identify the bacteria at genus level for most of the isolates. Our data suggest that those isolates are likely new species. Thus, further investigation such as whole genome mapping is necessary to define exact species names.

Future studies such as testing the bacteria in agricultural areas can be beneficial to assess the impact of the bacteria on crop yield in under natural conditions. In addition, cumulative studies based on combination of different species during application can provide good insights in terms of product development.

To increase the commercial value of the isolates, optimization of the growth parameters at large scales is necessary.

Furthermore, to reduce to edaphic and environmental conditions such as interactions between local microbiome (competition, predation, antagonism), physicochemical properties of soil (nutrient levels, moisture, pH, salinity, temperature, organic content), and agricultural practises (fertilizers, pesticides, crop diversification strategies), local biofertilizers for specific areas and plants should be applied. This study represents the first steps of the isolation, identification and enlightening of the plant growth promoting properties of bacteria associated with local *Triticum monococcum* ssp. *monococcum* for Bolu region of Turkey.

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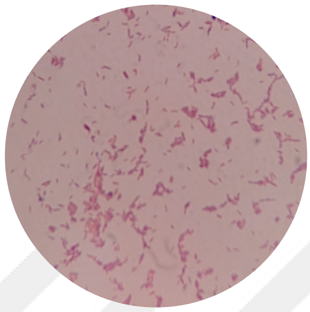
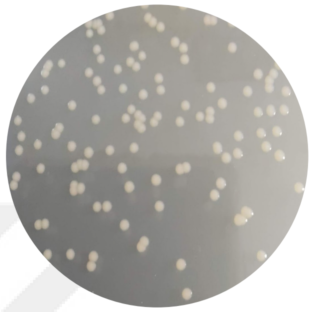
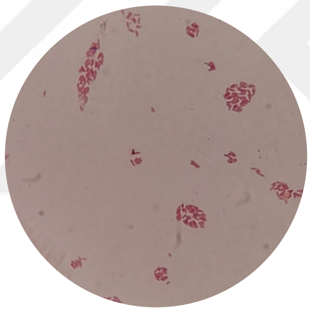
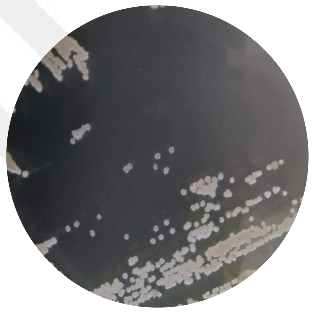
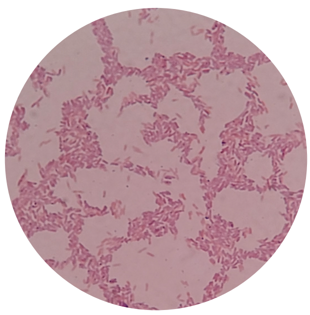
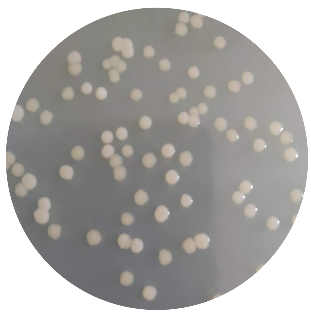
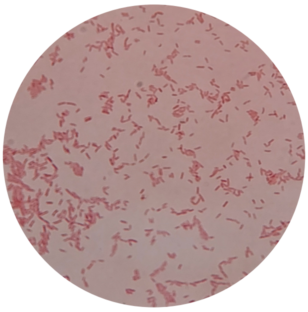
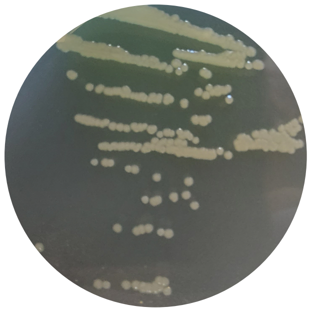
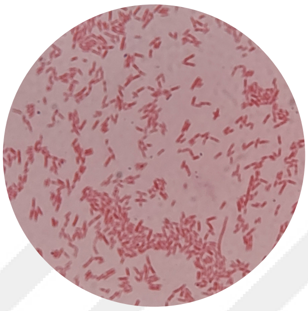
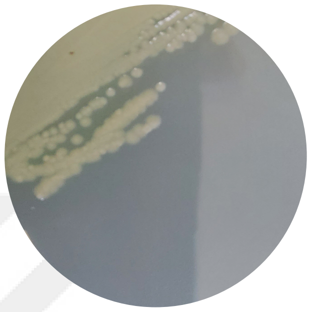
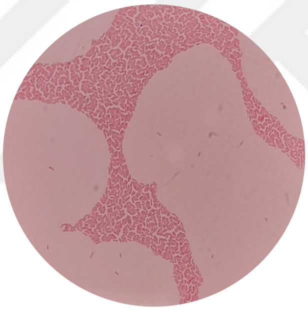
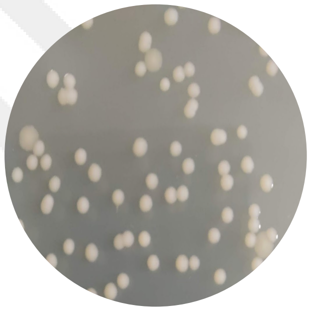
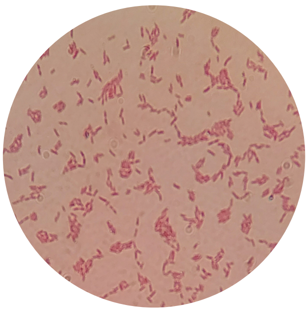
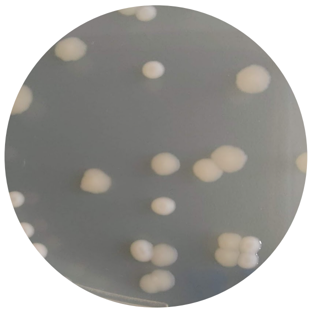
8. APPENDICES

In Figure 8.1, the photos of the gram-stained cells under 100X and the colony forms of the isolated species are given.

Species names	Gram staining of the cells	Colony forms onto LB plates
Arthrobacter spp. ESU164		
Arthrobacter spp. ESU193		
Pseudomonas spp. ESU131		

Pseudomonas spp. ESU141		
Pseudomonas poae strain ESU182		
Pseudomonas thivervalensis strain ESU192		
Pseudomonas spp. ESU1531		

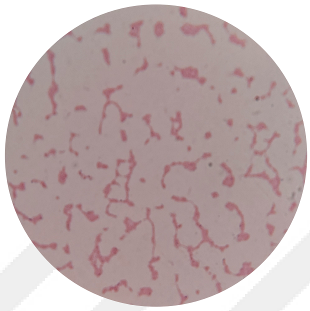
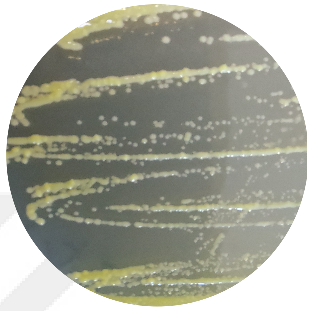
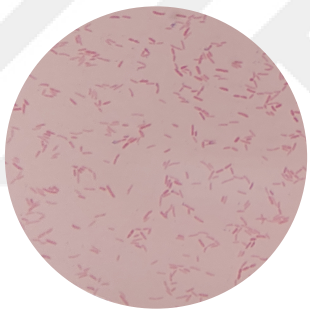
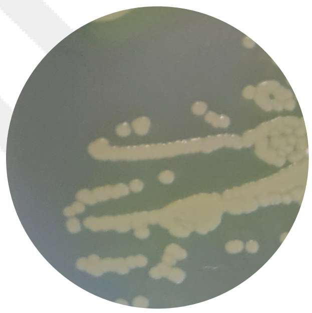
Bacillus subtilis strain ESU181		
Microbacterium foliorum strain ESU172		
Microbacterium spp. ESU121		

Figure 8.1 The photos of the species after gram staining and colony forms

In Figure 8.2, the photos of the germinated *T. monococcum* ssp. *monococcum* seeds both on the filter paper and in soil are shown as control group. The photos of the germinated *T. monococcum* ssp. *monococcum* seeds with the isolated species are represented in Figures 8.3-12.

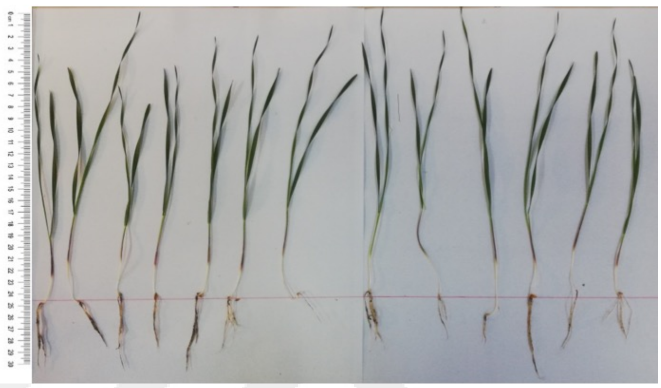
Germination on the filter paper (*Control*)	**Germination in soil** (*Control*)
	

Figure 8.2 The photos of the germinated *T. monococcum* ssp. *monococcum* seeds on filter paper and in soil as control

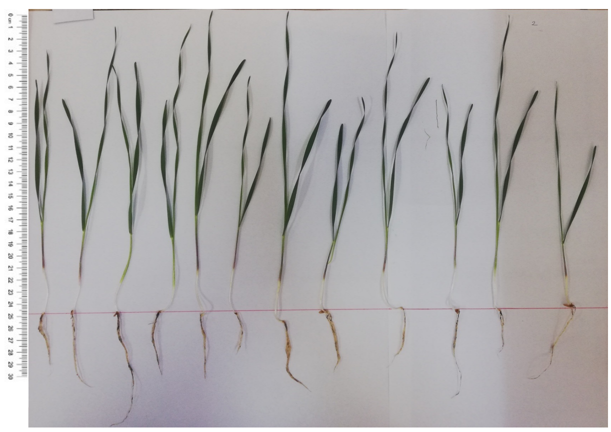
Germination on the filter paper (*Arthrobacter* spp. ESU164)	**Germination in soil** (*Arthrobacter* spp. ESU164)
	

Figure 8.3 The photos of the germinated *T. monococcum* ssp. *monococcum* seeds on filter paper and in soil with *Arthobacter* spp. ESU164

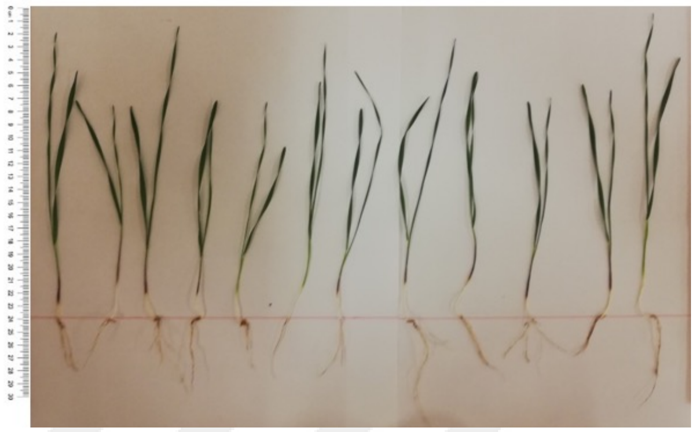
Germination on the filter paper (*Arthrobacter* spp. ESU193)	**Germination in soil** (*Arthrobacter* spp. ESU193)
	

Figure 8.4 The photos of the germinated *T. monococcum* ssp. *monococcum* seeds on filter paper and in soil with *Arthobacter* spp. ESU193

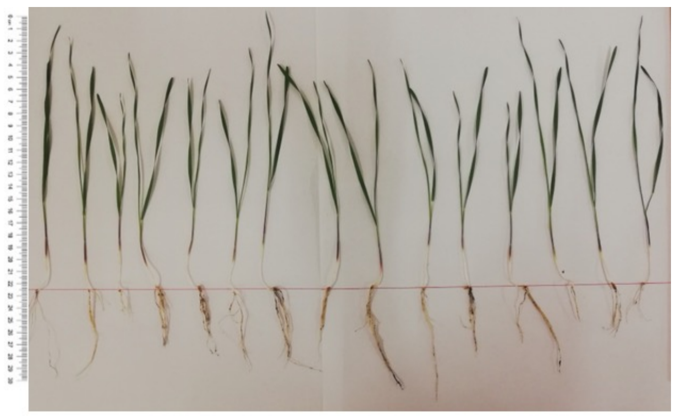
Germination on the filter paper (*Pseudomonas* spp. ESU131)	**Germination in soil** (*Pseudomonas* spp. ESU131)
	

Figure 8.5 The photos of the germinated *T. monococcum* ssp. *monococcum* seeds on filter paper and in soil with *Pseudomonas* spp. ESU131

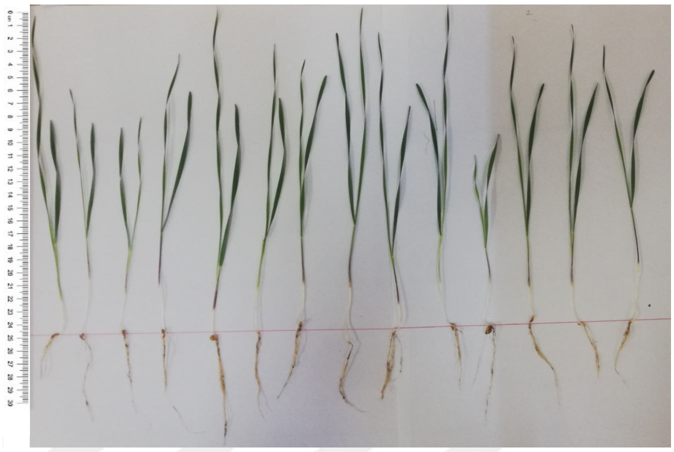
Germination on the filter paper (*Pseudomonas* spp. ESU141)	**Germination in soil** (*Pseudomonas* spp. ESU141)
	

Figure 8.6 The photos of the germinated *T. monococcum* ssp. *monococcum* seeds on filter paper and in soil with *Pseudomonas* spp. ESU141

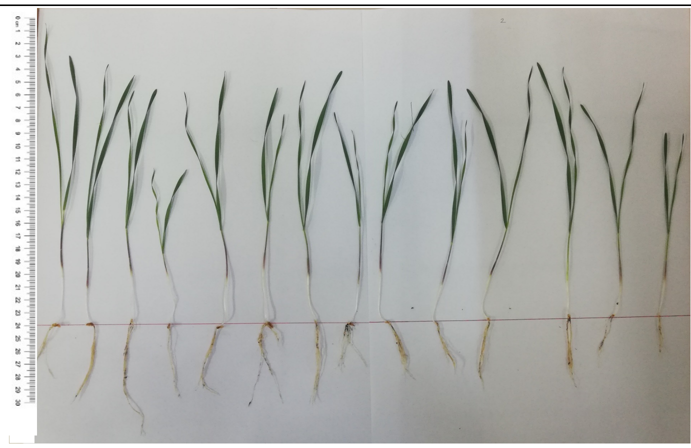
Germination on the filter paper (*Pseudomonas poae* strain ESU182)	**Germination in soil** (*Pseudomonas poae* strain ESU182)
	

Figure 8.7 The photos of the germinated *T. monococcum* ssp. *monococcum* seeds on filter paper and in soil with *Pseudomonas poae* strain ESU182

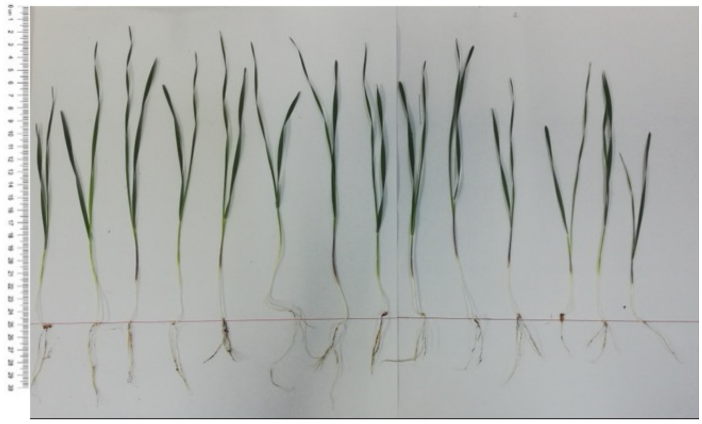
Germination on the filter paper (*Pseudomonas thivervalensis* strain ESU192**)**	**Germination in soil** (*Pseudomonas thivervalensis* strain ESU192**)**
	

Figure 8.8 The photos of the germinated *T. monococcum* ssp. *monococcum* seeds on filter paper and in soil with *Pseudomonas thivervalensis* strain ESU192

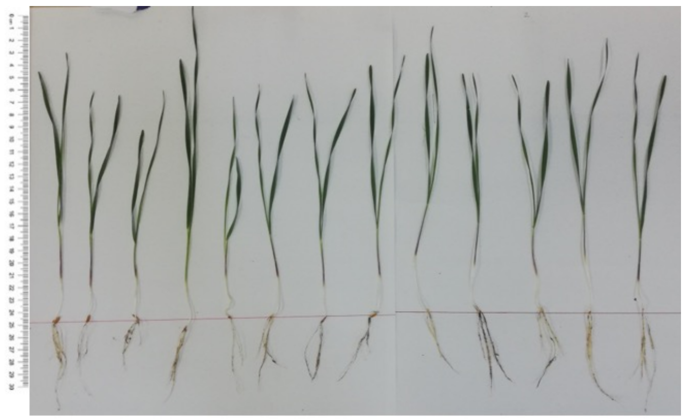
Germination on the filter paper (*Pseudomonas* spp. ESU1531**)**	**Germination in soil** (*Pseudomonas* spp. ESU1531**)**
	

Figure 8.9 The photos of the germinated *T. monococcum* ssp. *monococcum* seeds on filter paper and in soil with *Pseudomonas* spp. ESU1531

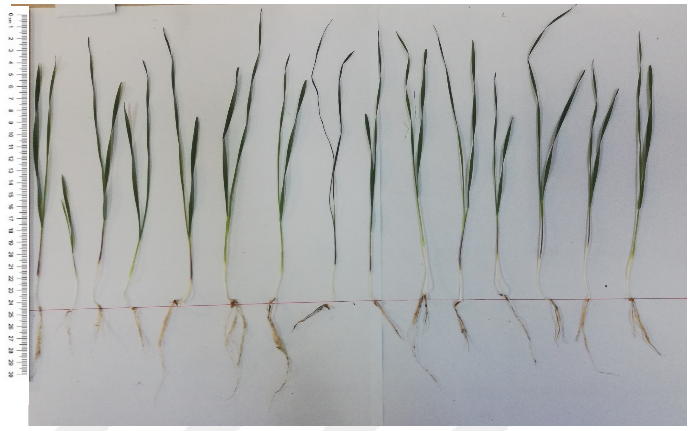
Germination on the filter paper (*Bacillus subtilis* strain ESU181)	**Germination in soil** (*Bacillus subtilis* strain ESU181)
	

Figure 8.10 The photos of the germinated *T. monococcum* ssp. *monococcum* seeds on filter paper and in soil with *Bacillus subtilis* strain ESU181

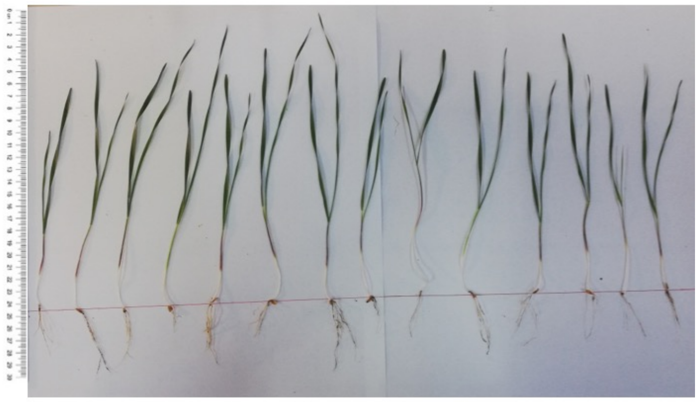
Germination on the filter paper (*Microbacterium foliorum* strain ESU172)	**Germination in soil** (*Microbacterium foliorum* strain ESU172)
	

Figure 8.11 The photos of the germinated *T. monococcum* ssp. *monococcum* seeds on filter paper and in soil with *Microbacterium foliorum* strain ESU172

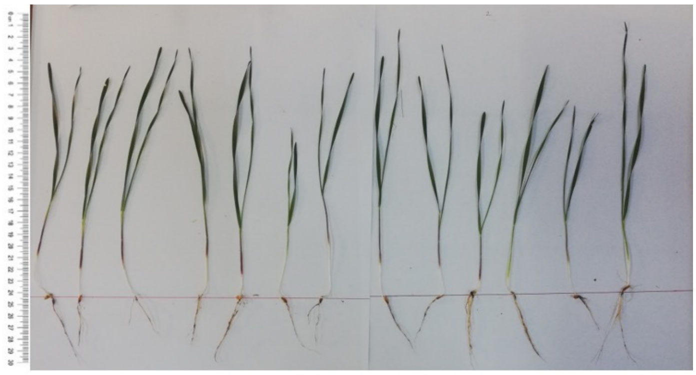
Germination on the filter paper (*Microbacterium* spp. ESU121)	**Germination in soil** (*Microbacterium* spp. ESU121)
	

Figure 8.12 The photos of the germinated *T. monococcum* ssp. *monococcum* seeds on filter paper and in soil with *Microbacterium* spp. ESU121