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ISOLATION AND CHARACTERIZATION OF NOVEL SILICATE-SOLUBILIZING BACTERIA FROM NATURAL HABITATS

MASTER OF SCIENCE

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APPROVAL OF THE THESIS

ISOLATION AND CHARACTERIZATION OF NOVEL SILICATE-SOLUBILIZING BACTERIA FROM NATURAL HABITATS

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Mercan YÜKSEK

ABSTRACT

ISOLATION AND CHARACTERIZATION OF NOVEL SILICATE-SOLUBILIZING BACTERIA FROM NATURAL HABITATS
MSC THESIS
MERCAN YÜKSEK
BOLU ABANT IZZET BAYSAL UNIVERSITY
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It is known that silica (Si) is a crucial element that is abundant in soil and helps plant development. However, it is not available in a form that plants can directly absorb. Silicate-dissolving bacteria convert the silicate minerals in the soil into a form that can be absorbed. They are helpful bacteria that enable the plant to absorb it as nutrients. Rice plant, which is in the high silica accumulator group, was used in this study. Root bacteria of plant samples collected from the Bolu/Kıbrıscık region were isolated, and biochemical properties characterized by Silica Solubility, Nitrogen Fixation, Indole acetic acid production, Catalase Test, and Phosphate Solubility tests were performed. The results showed that the isolated bacteria demonstrated a high capacity for solubilizing silicate minerals. This ability is a significant factor in plant nutrient availability and has significant potential for agricultural applications. The findings of this study offer insight into the mechanisms of silicate solubilization by bacteria and their potential for sustainable agriculture. This study contributes to the growing body of knowledge on the use of bacteria to promote plant growth. It offers a novel approach to the development of biofertilizers for sustainable agriculture.

KEYWORDS: Silicate-solubilizing bacteria, Silicate minerals, Silicon, Plant Evaluation, Rhizobacteria, Rice

ÖZET

DOĞAL HABİTATLARDAN YENİ SİLİKAT ÇÖZÜCÜ BAKTERİLERİN İZOLASYONU VE KARAKTERİZASYONU
YÜKSEK LİSANS TEZİ
MERCAN YÜKSEK
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
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Silkon elementinin toprakta çokça bulunduğu ve bitki büyüme ve gelişmine olan katkıları bilinmektedir. Ancak, toprakta bitkilerin direkt olarak emilim yapabileceği formda bulunmaz. Silikat çözebilen bakteriler, toprakta bulunan silikat minerallerini; besin olarak bitkilerin emilimini yapabileceği bir froma dönüştürür. Bu çalışmada yüksek silikat akümülatörü olarak bilinen pirinç bitkisi kullanılmıştır. Bu bitki Bolu'nun Kıbrıscık bölgesinden toplanıp kök bakterileri izole edilmiş ve biyokimyal özellikleri; silikat çözebilirliği, Azot fiksasyonu, Indol-3-asetik asit üretimi, Katalaz Testi, Sitokrom-c-oksidaz ve Fosfat çözünürlüğü testleri ile karakterize edilmiştir. Yapılan test sonuçlarında izole edilen bakterilerin yüksek silikat çözünürlüğüne sahip olduğu belirlenmiştir. İzole edilen bakterilerin gösterdiği bu özellik, bitkilere besin sağlanmasında önemli bir faktördür ve tarımsal uygulamalar için önemli bir potansiyele sahiptir. Bu çalışmanın bulguları, bakteriler tarafından silikat çözündürme mekanizmaları ve bunların sürdürülebilir tarım potansiyeli hakkında fikir vermektedir. Bu çalışma, bitki büyümesini teşvik etmek için bakterilerin kullanımına ilişkin giderek artan bilgi birikimine katkıda bulunmaktadır ve sürdürülebilir tarım için biyogübrelerin geliştirilmesine yeni bir yaklaşım sunmaktadır.

ANAHTAR KELİMELER: Silikat çözücü bakteriler, Silikat mineralleri, Silikon, Bitki Gelişimi, Rizobakteriler, Pirinç-bitki

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LIST OF ABBREVIATIONS AND SYMBOLS

µ	Micro
ºC	Celsius degree
ABA	Abscisic Acid
ACC	(1-Aminocyclopropane-1-Carboxylate) Deaminase
AU	Auxin
BR	Brassinosteroid
Cd	Cadmium
CK	Cytokinin
Co	Cobalt
Cr	Chrome
Cu	Copper
ET	Ethylene
Fe	Iron
g	Grams
GA	Gibberellin
HCl	Hydrochloric Acid
IAA	Indole-3-Acetic Acid
IAM	Indole-3-Acetamide
IAN	Indole-3-Acetonitrile

IAOX	Indole-3- Acetaldoxime
ID	Identity
IPA	Indole-3-Pyruvic Acid
IPNI	International Plant Nutrition Institute
ISR	Induced Systemic Resistance
JA	Jasmonate
K_2HPO_4	Di-Potassium Phosphate
KH_2PO_4	Mono-Potassium Phosphate
L	Liter
LB	Lysogeny Broth Medium
m	Milli
mL	Milliliter
Mn	Manganese
N	Nitrogen
Na	Sodium
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
nm	Nanometer
OD	Optical Density
P	Phosphorus

Pb	Lead
PBST	Phosphate Buffered Saline Tween-20
PGPB	Plant Growth Promoting Bacteria
PGPR	Plant Growth-Promoting Rhizobacteria
SA	Salicylic Acid
Se	Selenium
Si	Silicone
SSB	Silicate-Solubilizing Bacteria
Zn	Zinc

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1. INTRODUCTION

1.1 Silicon

The word "Silicon" originated from the Latin words "silicis" and "silex", which means stone or flint. (1) Silicon (Si) is the eighth most abundant element in the universe and the second most abundant element on Earth. The Earth's crust consists of 90% silicate minerals. The amount of silicon in soil ranges from 50 to 400 grams per kilogram, but it is not present in its pure form. Instead, it exists as various insoluble silicate minerals and silicic acid. (2)

In soil, while the solid phase of the silicon compound is insoluble and inactive, the liquid phase is known to be biogeochemically active. These liquids are in the form of mono- and ortho-silicic acid. Plants can absorb monomers of these acids [$Si(OH)_4$] through their roots or with the help of plant growth promoter microorganisms present in their roots. This transportation of Silicic acid can be an active, passive, or rejective passive pathway. International Plant Nutrition Institute (IPNI) categorized Silicon as a beneficial element for plants since its accumulation in plants decreases the risk of fungal diseases and abiotic and biotic stress levels in accumulator plants. Many researchers have shown that increasing silicic acid levels in plants benefit crop production in agriculture. (3) (4) (5) Silicic acid is mobilized as polysilicic acid during the dissolution of Si-rich solids. This polysilicic acid is depolymerizing to mono silicic acid until equilibrium is reached. (5)

As the world population grows, it is becoming increasingly difficult to access food sources high in nutritional value. In response, farmers often use chemical fertilizers and pesticides to boost crop production. However, these chemicals can cause irreversible soil and water pollution, leading to a variety of health risks. (6) Instead of using chemical fertilizers and pesticides, plant growth promoter bacteria known as biofertilizers can be utilized.

1.2 Plant Growth-Promoting Bacteria (PGPB)

Soil is a moist ecosystem and very rich in reduced carbon which promotes rhizomicrobiome communities living. Microbes of this community play a vital role in agriculture in the way nutrients and increasing stress tolerances likely to become more frequent because of run-on climate change. (7)

Plant growth-promoting bacteria are free-living bacteria that belong to the soil ecosystem. Rhizobacteria are the colonized PGPB in the root rhizosphere of the plants. Usage of PGPB as a biofertilizer increased in past decades since its benefits in crop production.

Plant growth-promoting bacteria (PGPB) have numerous benefits for crop production in agriculture, including nutritional support and protection against abiotic and biotic stress factors. Due to the increasing population, there is a higher demand for crop production leading to a rise in the use of chemical fertilizers and pesticides to improve yield. The use of chemical fertilizers leads to irreversible water and soil contamination. Therefore, instead of using chemical fertilizers, soil microorganisms that already exist in the soil can and should be utilized in agriculture to promote sustainable yields. (8) The mechanisms by which plant growth-promoting bacteria (PGPB) stimulate plant growth involve enhancing the availability of nutrients through genetic processes, such as phosphate solubilization, nitrogen fixation, and stress alleviation by modulating ACC deaminase expression. (9)

Table 1 Examples of Plant Growth-Promoter Rhizobacteria (10)

PGPR	Plant growth-promoting traits	References
Pseudomonas putida	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization	(11)(12)
Pseudomonas aeruginosa	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization	(13)(14)(15)
Klebsiella	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization	(16)(17)(18)
Enterobacter asburiae	IAA, siderophores, HCN, ammonia, exo-polysaccharides, phosphate solubilization	(19)(20)
Rhizobium (pea)	IAA, siderophores, HCN, ammonia, exo-polysaccharides	(21)(22,23)(23)
Mesorhizobium	IAA, siderophores, HCN, ammonia, exo-polysaccharides	(24)(25–27)
Acinetobacter p.	IAA, phosphate solubilization, siderophores	(28)
Rhizobium (lentil)	IAA, siderophores, HCN, ammonia, exo-polysaccharides	(29)(30)
Pseudomonas sp. *A3R3*	IAA, siderophores	(31)
Psychrobacter sp. *SRS8*	Heavy metal mobilization	(32)
Bradyrhizobium sp.	IAA, siderophores, HCN, ammonia, exo-polysaccharides	(33)(34)(35,36)
Pseudomonas aeruginosa 4EA	Siderophores	(36)
Bradyrhizobium sp. *750,*	Heavy metal mobilization	(37)

1.2.1 Mechanism of Plant Growth-Promoting Bacteria

PGPB directly enhances plant growth by aiding resource acquisition (N, P, and essential minerals), hormone level modulation, or induces the effect of pathogens. They have a positive impact on the growth of plants by creating different variants of plant growth regulators, enhancing the chemical characteristics of the soil, protecting the soil from different phytopathogens, they can also neutralize the soil from toxic heavy metals.

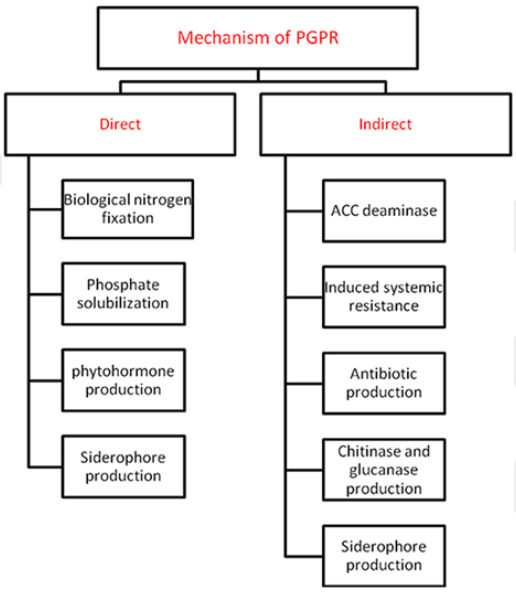


Figure 1. Simple presentation of Mechanism of PGPB.

1.2.1.1 Direct Mechanisms Of PGPB

1.2.1.1.1 Biological Nitrogen Fixation

Nitrogen is an abundant element in the atmosphere with almost 78% in air and it is a very important nutrient for plant growth. Although the high percentage, plants can not take this nitrogen directly from the air, thus nitrogen-fixing microorganisms in the soil convert atmospheric N_2 to plant-utilizable form which is ammonia. This is a complex enzymatic process called “Nitrogenase”. The reaction requires a significant amount of energy in the form of ATP.

Maize, rice, and wheat account for 80% of all grain production worldwide according to The Food and Agriculture Organization. In this manner, chemical nitrogen fertilizers are used very widely to gain high yields in cereal crops because

legumes have symbiotic root nodules with rhizobia to save the necessary nitrogen amount for their growth. The use of chemical nitrogen fertilizers releases most of its nitrogen into the environment and it causes environmental problems such as soil acidification, free heavy metal ions in the rhizosphere, and increasing greenhouse emissions. This is the reason cereal crops nitrogen emissions dropped by approximately 34% according to The Food and Agricultural Organization.

The use of plant growth-promoting bacteria (PGPB) for biological nitrogen fixation is crucial for producing healthy and sustainable, high-yield cereal crops. Biological nitrogen fixation is also an important process that belongs to nitrogen cycling. (38)

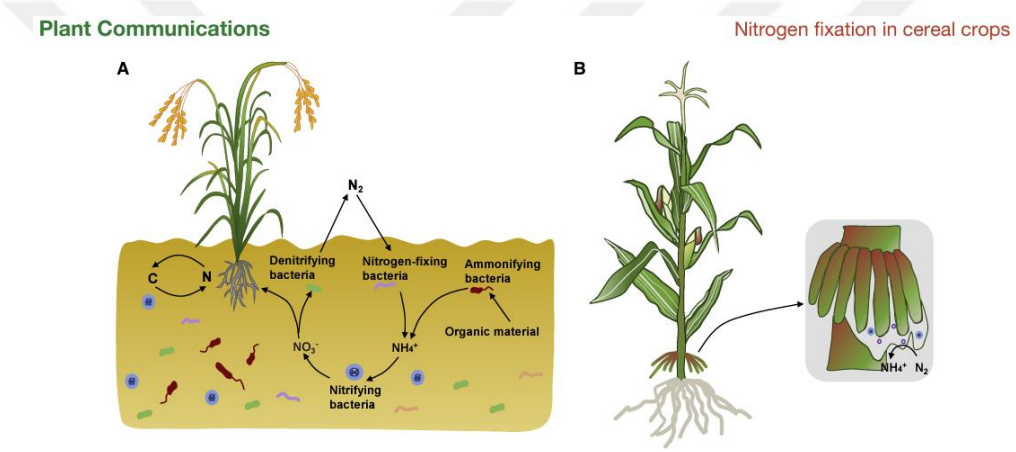


Figure 2. Nitrogen cycling and biological nitrogen fixation from microbiomes associated with cereal crops. (38)

1.2.1.1.2 Phosphate Solubilization

Phosphorus plays a vital role in plant growth as it is the second most essential macronutrient after nitrogen. It is involved in crucial cellular functions like cell division, energy generation, biosynthesis of macromolecules, signal transduction, membrane integrity, and photosynthesis. While soil is a rich source of phosphorus, it often gets complexed with metal ions, rendering it unavailable and insoluble for plant uptake. The use of phosphate fertilizers in agriculture to enhance available phosphorus in soil for plant uptake leads to the precipitation of this chemical fertilizer in soil with metal ions, causing health issues and posing a challenge to healthy food production.

Using phosphate-solubilizing microorganisms as fertilizers is a healthy solution to increase the yield of soluble phosphorus in soil. These microorganisms produce organic acids and enzymes and eliminate siderophores, which chelate metal ions and form complexes. This leads to improved soil quality and crop productivity. (39)

Enterobacter asburiae, *Bacillus*, *Pseudomonas*, and *Burkholderia* are involved in phosphate solubilization bacteria. Table 1 shows that many bacteria can solubilize phosphate. These microorganisms produce enzymes such as phytase and phosphatase to solubilize phosphate. (40)

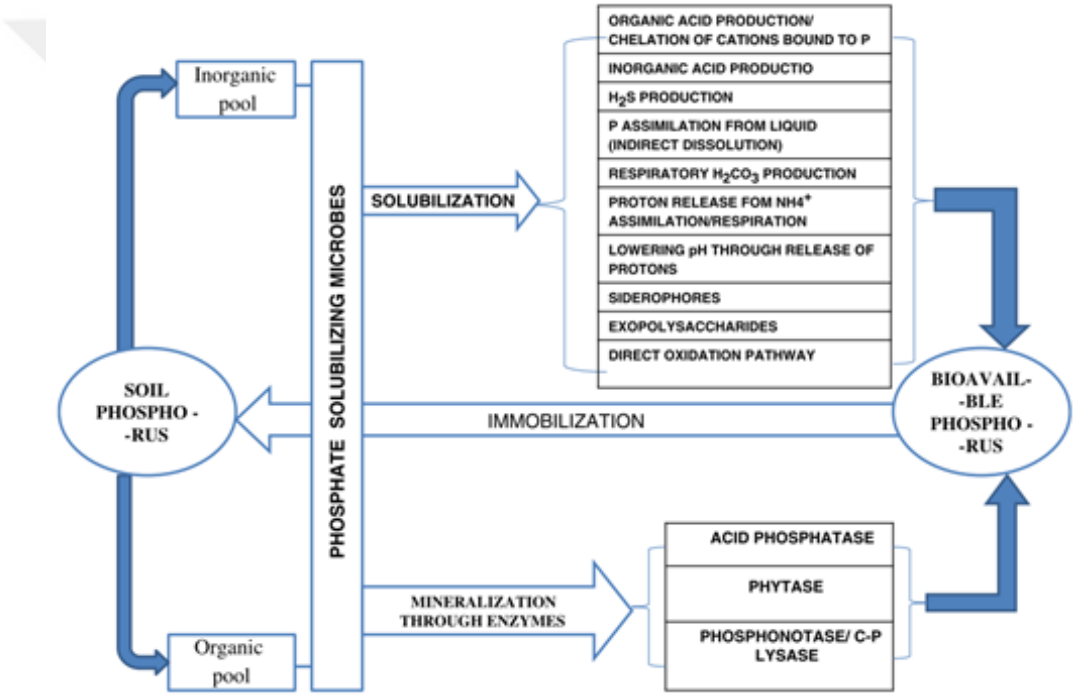


Figure 3 Simple presentation of soil P-solubilization. (41)

1.2.1.1.3 Silicon Impact on Phytohormones

Plants produce different phytohormones to defend themselves against different biotic and abiotic stress factors and environmental conditions. Auxins (AU), gibberellins (GA), ethylene (ET), cytokinins (CK), jasmonates (JA), abscisic acid (ABA), brassinosteroids (BR), salicylic acid (SA), and strigolactones are examples of phytohormones plant produces. Co, Na, Se, and Si are beneficial elements that have good impact on phytohormone stress resistance.

Heavy metals present in soil can create an abiotic stress situation for plants. Si prevents the transport of heavy metals. Si creates a Si-hemicellulose-Cd complex in the cell wall of roots and this way Si controls apoplast transport of Cd around the endodermis and Cd translocation from roots to shoots. Under Salinity stress, increasing the Si concentration has showed that increase in the amount of endogenous plant hormones such as GA, Indole-3-Acetic Acid, and ABA. (42)

A study has shown that, under cold stress, Silicon balances IAA, GA, and zeatin production by enhancing the homeostasis of cold stress-protective micronutrients, such as Zn and Mn. (43)

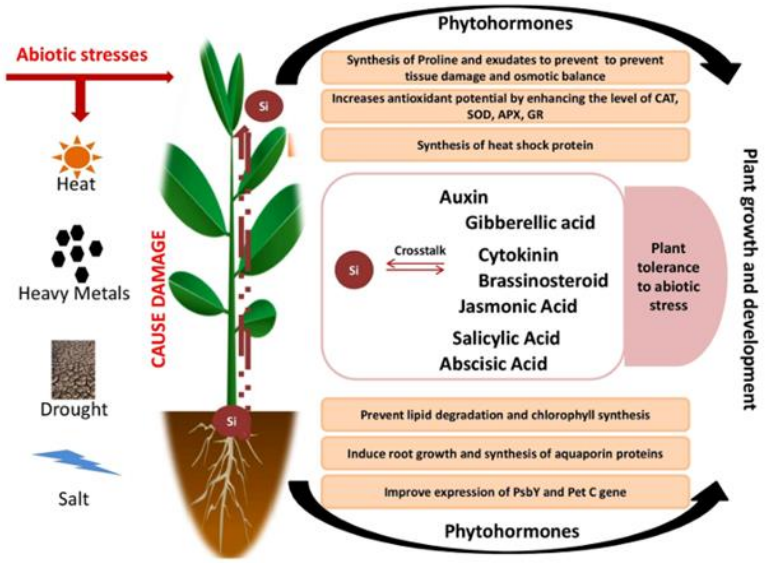


Figure 4. The role of silicon in triggering the production of phytohormones during abiotic stress. (44)

In the 19th century, Charles Darwin proposed the existence of a substance that transmits growth effects between different parts of a plant. Further years, this matter was discovered and defined as auxin in 1937. Auxin is a plant growth regulator and Indole-3-acetic acid (IIA) is a very physiologically active type of auxin hormone that promotes plant growth. (45)(46)

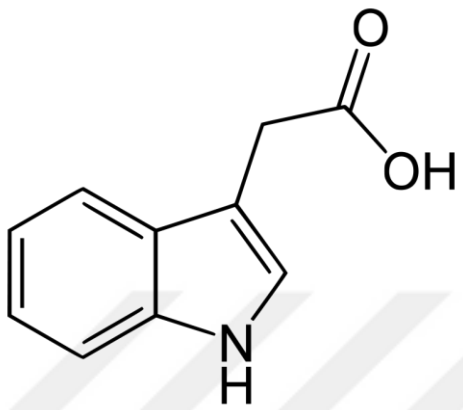


Figure 5. Chemical structure of Indole-3-Acetic acid.

IAA is a metabolite produced by microorganisms during L-tryptophan metabolism, including plant growth-promoting rhizobacteria. (PGPR). Bacterial production of IAA has two pathways Tryptophan dependent and Tryptophan independent in plants and bacteria. (46)

In the Trp-dependent pathway, tryptophan is converted to indole-3-acetamide (IAM) by tryptophan-2-monooxygenase and IAM is metabolized to IAA by IAM-hydrolase. The important well-studied IAA biosynthesis pathways include the indole-3- acetamide (IAM) pathway, the indole-3-pyruvic acid (IPA) pathway, and the indole-3-acetonitrile (IAN)/indole-3- acetaldoxime (IAOx) pathway. (47) (48) Figure 6. is a simple representation of these pathways.

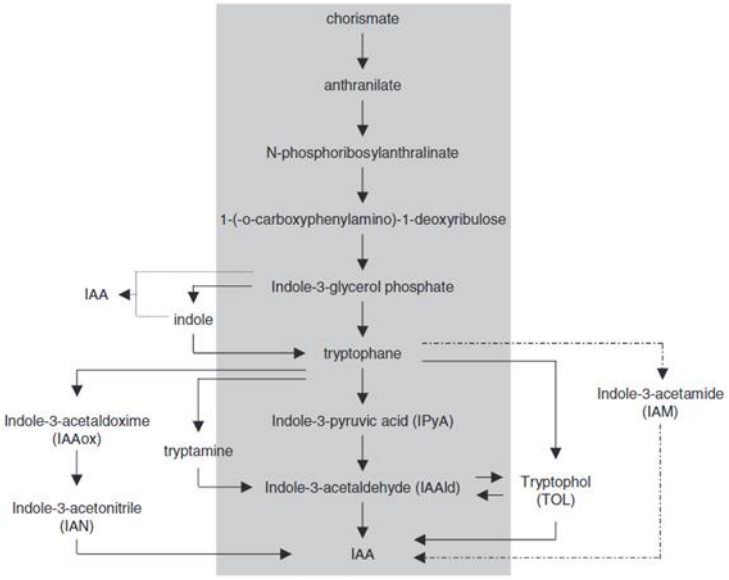


Figure 6. The important well-studied IAA biosynthesis pathways. (47)

1.2.1.1.4 Siderophore Production

Iron (Fe) is an important element for microorganisms since it plays a vital role such as a catalyst in enzymatic reactions, electron transfer, oxygen metabolism, and DNA, and RNA synthesis. Also, Fe increases the hydrophobicity of microbial surface and in Fe deficient situations limits biofilm formation. In this manner, microorganisms have produced siderophores which are low molecular weighted (200-2000 Da) metal chelating agents produced by plants and microorganisms against Fe defficient environments. The mechanism behind siderophore action involves the formation of a complex with free iron, which is then transported into the cell using membrane receptors. The operon contains five genes that encode receptor molecules. When the needed amount of iron has been taken into the cell, the operon is turned off.(49)

1.2.1.2 Indirect Mechanism of PGPB

1.2.1.2.1 ACC Deaminase

ACC (1-aminocyclopropane-1-carboxylate) deaminase is an enzyme that plays a crucial role in the regulation of ethylene levels in plants. Ethylene is a plant hormone involved in various aspects of plant growth and development, including seed germination, root development, fruit ripening, flower wilting, and leaf senescence. and response to stress. Plants produce ethylene using a chemical

process that involves the formation of ammonia and α-ketobutyrate as immediate precursors. (50)

1.2.1.2.2 Induced Systemic Resistance

Induced systemic resistance (ISR) is the plant's defensive system against any biotic stress factor such as pathogens or parasites in any form. It is the mechanism where the plant protects its nonexposed parts against future attack by threats such as graminivorous insects and pathogenic microorganisms. ISR is reinforced by PGPR, the most well-known strains belong to Pseudomonas. ISR mechanism relies on pathways that are regulated by jasmonate and ethylene. (43,51)

1.3 Silicic Acid and Stress Factors

Silicic acid, a form of soluble silicon, has been recognized for its significant impact on both biotic and abiotic stress factors in crop production. Its role in enhancing plant resilience and overall productivity has garnered attention from researchers and agricultural practitioners.

Drought is a threat to all living beings in the world. Climate change exacerbates the situation, and the increasing depletion of water resources jeopardizes food security. This is an important stress factor for plants and the threat of food security which is created with is an important issue for all living. Thus, every day, scientists search for and create new ways to combat this threat. Some scientists suggest that instead of developing drought-tolerant cultivars; better nutrition management can help drought management in plants. Silicon is a quasi-essential element and the accumulation of amorphous silica (SiO_2-nH_2O) in the cell wall can enhance the strength of stem and leaves. (52)

Rapid and irregular growth of industries can cause pressure on the global environment as chemical fertilizer, especially in water, soil, and air pollution. Thus, agricultural harm is inevitable. Past studies have shown that Silicon application in plants can reduce Cd and several heavy metal uptakes in plants and improve the nutritional quality of species. (53) Si-fertilizer can be used in this manner and it is also very accessible since Si-fertilizer is a side product in many industries. Silicon

in the soil has a high number of exchangeable cations and a small amount of H^+. This results in strong hydrolysis of the exchangeable cations, leading to a high proportion of NaOH in the soil solution, which further increases the soil pH. Si-fertilizer can help increase the pH in the soil and the soluble Silicon concentration. However, if the soil pH increases, it could reduce the availability of cations such as Cu, Cd, Cr, Pb, Fe, Mn, and Zn. (54)

Changing climate is a major stress factor in agriculture. The cold weather in spring accounts for cold soil and this negatively affects root growth. In a study done at University of Hohenheim the effects of Si which is a cold protective supplement were investigated with using Mazie –cold sensitive model plant-. They measured the phytohormones in maize seedlings with different temperatures (12°C and 18°C) in with/out Si-supplemented environments. They found that in cold temperatures; maize seedlings in Si-supplemented environments has produced more indole-3-acetic acid which is a vital plant growth hormone belonging to auxin family. (43)

Another abiotic stress factor is salinity. It is one of the major ones since it affects soil quality, and accounts for different stress factors such as oxidative stress with accumulation of reactive oxygen species like –OH radicals, and superoxide – O_2 and causes nutritional imbalance. In a study in 2019 at King Abdulaziz University, the combination of PGPB and Si-fertilizer reduced the salinity stress in coriander. Also, the combination of PGPB and Si fertilizer increased indole-3-acetic acid production as well as photosynthetic pigments, peroxidase activity, and salt tolerance index. (55)

Photosynthesis is a crucial physiological process that drives biomass production and plant growth and is highly sensitive to various stress factors. Silicon protects photosynthesis from stress by safeguarding machinery and function. Known benefits are protection of photosynthetic structure, improvement in water use efficiency, improvement in the electron transport during photosynthesis, from reactive oxygen species (ROS), gene regulations, and interacting with other physiological processes such as absorption of macro and micronutrients, and phytohormones which also influence photosynthetic activity (56).

1.4 Silicate-Solubilizing Bacteria (SSB)

Silicate minerals are not found in soil as plants can absorb, thus plants have symbiotic relations with these microorganisms in their roots. Silicate-solubilizing bacteria solubilize insoluble silicate minerals in the soil and turn into a form that plants can absorb as silicic acid. Silicon accumulator plants use SSB to accumulate silicon in their vital growing parts and accumulation of silicic acid enhances the strength of cell walls. Silicon accumulator plants can be divided into 3 parts; low-silicon accumulators, medium-silicon accumulator, and high-silicon accumulators. In this study, *Oryza Sativa* which is a rice species and a high silicon accumulator is used as a host plant.

Accumulation of Si enhances plants' defense mechanism, reduces stress and heavy metal uptake, and increases indole-3-acetic acid production. Thus, using SSB as a biofertilizer has many benefits and has no bad side effects like greenhouse emissions in comparison with chemical biofertilizers. There is limited research in the literature about SSB and needs more focus on agricultural applications. A study in the literature showed that the usage of SSB improved the growth of *Oryza Sativa.* (57)

The mechanism behind the silicate-solubilizing bacteria is not understood yet. One of the hypotheses in the literature is the "Silicase" enzyme which helps decompose the silicate compounds by catalyzing Si-O and Si-C bonds and forms silicic acid. (58)

1.5 Biosilicification

Biomineralization is an ancient phenomenon in which living organisms produce minerals with multifunctional properties. Biosilicification is a type of biomineralization also called silica biomineralization.

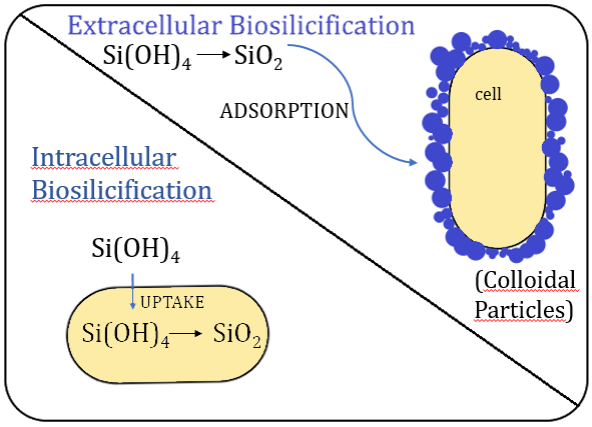


Figure 7 Intra- and Extracellular Biosilicification

Biosilicification is the process of consuming soluble monomeric silicic (orthosilicic acid; Si[OH]4) acid by organisms and polymerized and deposited as insoluble silica. The role of bacteria in this process is recently recognized, biosilicification by bacteria can be divided into two parts; Extracellular biosilicification and intracellular biosilicification.(59)

In 2010, Hirotaetal *et al*. (2010) reported that the silica layer acts as a coating agent around the bacterium protects the bacterium from acid, and helps the spore survive under acidic conditions.

2. AIM AND SCOPE OF THE STUDY

In this study, we aimed to isolate a novel silicate-solubilizing bacteria to improve plant growth. The isolation is made from a 1st class rice type *Oryza sativa* roots which is known as a high Si-accumulator. *Oryza sativa* is collected from the Kıbrıscık/Bolu region since it has a geographic signature. The *Oryza sativa* in this region has special properties such as up to 23-25% amylose. Amylose is an enzyme that helps starch hydrolysis through digestion. (60)

The biochemical characterization is made as; Nitrogen Fixation, Phosphate Solubilization, Silicate-Solubilization, Catalase Test, IAA Test, and Cytochrome Oxidase Test. For identification studies, 16S rRNA PCR is made.

There is limited information and work in this field so we hope that this work will fill a gap in the literature, and help further investigations of silicate-solubilizing bacteria and the mechanism behind also its benefits for crop production.

3. MATERIALS AND METHODS

3.1. Materials

The materials used in this thesis are given in Table 2.

Table 2 Materials

No	Chemical	Brand	Catalog Number
1	10X Standard Reaction Buffer	BioLabs	#B9015S
2	Agar	Liofilchem	611001
3	D(+)-Glucose monohydrate	Merck	1.08342.1000
4	Deoxynucleotide (dNTP)	BioLabs	NO447S
5	di-Magnesium trisilicate hydrate	HIMEDIA	RM-1809
6	Dipotassium hydrogen phosphate (K_2HPO4)	Merck	1.05099.0250
7	Hydrogen Peroxide	Sigma-Aldrich	183034-1L
8	Iron (III) chloride	Merck	1.03943.0250
9	Jensen Medium	HIMEDIA	M710-500G
10	L-tryptophan	Merck	1.03943.0250
11	Magnesium Chloride	BioLabs	#B9021S
12	Peptone	BioShop	PEP403.1
13	Pikovskaya's Agar	HIMEDIA	M520-500G
14	Potassium dihydrogen phosphate	Merck	104871
15	Sodium Chloride	Sigma-Aldrich	1.06404.1000
16	Sodium Hydroxide (NaOH)	Merck	106392
17	TAQ DNA Polymerase	BioLabs	#M0320S
18	Tween-20	Sigma-Aldrich	P1379
19	Yeast Extract	Bioshop	YEX401.1

3.2. Methods

3.2.1. Sampling

The root samples of *Oryza Sativa* were collected from Kıbrıscık, Bolu, Turkey on October 12th, 2022. The coordinates are 40°19'22.3"North, 31°40'49.8"East. 3 different host plants were selected randomly from the field. The collected samples are immediately taken into the Cold Room of the university until the isolation process has started.

Picture 1 The sampling location is 40°19'22.3"North, 31°40'49.8"East. Kıbrıscık/BOLU/TÜRKİYE

3.2.2. Isolation of Rhizobacteria

The isolation of rhizobacteria from *Oryza Sativa* roots was made according to White et al, 2015. (61) The rhizobacteria from host plants' roots are isolated by using phosphate-buffered saline Tween-20 (PBST). The ingredients are dissolved in 500 mL distilled water at pH 7.2 which is adjusted by using 1M NaOH and 1M HCl.

Table 3 Ingredients of 300 mL PBST solution.

Ingredients	Amount
K_2HPO_4	**0,605 grams**
KH_2PO_4	**0,17 grams**
NaCl	**4,1 grams**
Tween 20	**250 µL**

The root pieces are taken from 5 different randomly selected host plants in the field. They are washed with d-water and placed into the 15 ml tubes filled with 10 ml of PBST solutions as shown in Picture 2. Then, test tubes are placed into the sonicator which is filled with distilled water. Sonification is made 3 times for each root; firstly, roots are placed into Tube 1 for 60 seconds for the first sonification. The same roots are transferred into the second centrifuge tube filled with fresh 10 mL of PBST solution for second sonification for 60 seconds. Finally, roots are transferred into the third centrifuge tube filled with PBST solution, and sonification is made for 10 minutes.

Picture 2 Root samples in the PBST solution.

The isolated microorganisms floated into the PBST solution. Thus, tubes are centrifuged at 5000 RPM for 3-4 minutes. Centrifugates are separated and diluted to 100 times and 1000 times with distilled water. Diluted bacteria are cultivated to Lysogeny Broth / Agar (LB) medium in petri dishes, which are freshly prepared. 1 L LB medium is prepared and autoclaved for sterilization.

3.2.3. Cultivation

Cultivation is made on LB Agar medium and incubated for 3 days at 37 ºC. Grown colonies are counted and separated by different colony colors and shapes. Separated colonies are cultivated on LB Agar in petri dishes for every different host plant and incubated at 37 ºC in an incubator. After 3 days of

incubation, by using streaking technique the colonies are purificated to a single colony and characterization has started.

3.2.4 Biochemical Characterizations of Bacteria

3.2.4.1 Silicate-Solubilization Ability

Isolated and purified rhizosphere bacteria are checked for their Silicate-solubilizing activity by cultivating on glucose agar medium and 0,25% di-magnesium trisilicate ($2MgO.3SiO_2$) added glucose agar medium without silicate mineral (control medium).

Table 4 Ingredients of 0,25% di-magnesium Trisilicate added Glucose Agar Medium (1 L/7.0 pH)

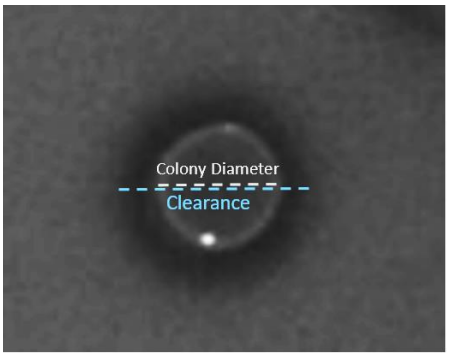
Ingredients	Amount
di-Magnesium Trisilicate	**2.0g/L**
Glucose monohydrate	**10.0g/L**
Agar	**20.g/L**

For easy observation of silicate solubilization by bacteria; the medium was poured as thin layers into petri dishes.

Table 5 Ingredients of Control Medium (1L/7.0 pH)

Ingredients	Amount
Glucose monohydrate	**10.0g/L**
Agar	**20.g/L**

Colonies are cultivated on petri dishes for both mediums for 3 days and incubated at 37ºC in the incubator. After 3 days some of the bacteria showed silicate solubilization as clear circles around colonies. This test is repeated 3 times for reliability. The non-silicate solubilizing bacteria are eliminated, and characterization is continued with silicate-solubilizing bacteria. Quantitative analysis is carried out with the ImageJ program. The clearance zone and colony diameters are measured in cm units. The SI indices are calculated as; clearance zone/colony diameter.



Picture 3 Silicate-solubilizing activity of ESU_85 is shown.

3.2.4.2 Nitrogen Fixation Test

To characterize nitrogen-fixing bacteria, Jensen Agar Medium is prepared. The ingredients are given in Table 5. First of all, bacteria are cultivated into tubes that contain LB liquid medium at 30ºC for 18 hours in an incubator. Then OD_{600} measurements are done by using a double-beam spectrophotometer. Obtained values are arranged to 1 OD by a simple calculation and 1 mL of bacteria are cultivated onto nitrogen-free Jensen agar medium for 8 days at 30ºC. This test is repeated 3 times for reliability. The growth of bacteria is considered proof of nitrogen-fixing.

Table 6 Ingredients of HIMEDIA's Jensen Agar Medium (1L/7.0 pH)

Ingredients	Amount (g/L)
Sucrose	20.0g/L
Dipotassium Phosphate	1.0g/L
Magnesium Sulphate	0.5g/L
Sodium Chloride	0.5g/L
Ferrous Sulphate	0.1g/L
Sodium Molybdate	0.005g/L
Calcium Carbonate	2.0g/L
Agar	15g/L

3.2.4.3 Phosphate Solubilization Test

The isolated bacteria samples are cultivated in liquid LB medium for 24 hours at 30 ºC. The OD_{600} measurements are done with a double-beam spectrophotometer. OD_{600} values are adjusted to 1 OD. Pikovskaya's Agar is prepared by using HIMEDIA's Pikovskaya's Agar. The composition of Pikovskaya's Agar is shown in Table 6.

Table 7 Ingredient of HIMEDIA's Pikovsykaya's Agar Medium.

Ingredients	Amount (g/L)
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

The species are cultivated on Pikovskaya's Agar medium for phosphate solubilization test for 48 hours at 37ºC. After 2 days of incubation clearance zone around colonies was seen. The UV-lighted photographs are taken. Clearance zone and colony diameters are measured with the ImageJ program. The calculation of solubility indices is done with a simple calculation; clearance zone/colony diameter.

3.2.4.4 Quantitative Analysis of Indole-3-Acetic Acid Production

The characterization for Indole-3-acetic acid production was performed with the Salkowski reagent. The isolated bacteria colonies are cultivated on LB medium and 0.1% L-tryptophan (w/v) supplemented LB medium incubated for 11 days at 30ºC in test tubes. After incubation 1 mL of supernatant is taken from tubes and directly added to freshly prepared 2 mL of Salkowski reagent. The mixtıre is incubated for 30 minutes at room temperature. Quantitative analysis is carried out by measurements of the species and control sample by double-beamed spectrophotometer.

Table 8 Ingredients of Salkowski Reagent.

Chemical	Amount
$FeCl_3$ (0.5 M)	2 mL
Distilled Water	49 mL
70% $HClO_4$	49 mL

3.2.4.5 Cytochrome *c* Oxidase Test

Filter papers are soaked into a 1% solution of tetramethyl-p-phenylenediamine dihydrochloride solution and colonies are transmitted onto the filter papers and purple color is observed in cytochrome *c* oxidase-positive ones.

3.2.4.6. Catalase Test

Colonies are transmitted onto parafilm and 3% H_2O_2 is dropped onto the colonies. Bubbles were seen in the catalase-positive bacteria. The mechanism depends on the conversion of hydrogen peroxide by catalase enzyme activity to water and oxygen. (62)

$$2H_2O_2 \xrightarrow{\text{CATALASE}} 2H_2O + O_2$$

3.2.4.7 Polymerase Chain Reaction (PCR) and Gel Electrophoresis

3.2.4.7.1 PCR

Genomic DNA of the 7 species is isolated by using the Bacterial Genomic DNA Isolation Kit, Norgen Biotek Corp., 17900. Amplification of the 16S rRNA is made by using primers which are given in Table 8. PCR components are given in Table 9 and reaction conditions in Table 10.

Table 9 Forward and Revers Primers.

Primer	Sequence
16S rRNA – P1 (forward)	5-AGAGTTTGATCCTGGTCAGAACGAACGCT-3
16S rRNA – P6 (reverse)	5-TACGGCTACCTTGTTACGACTTCACCCC-3

Table 10 Materials used in the PCR reaction.

Materials	Amount
10X Taq Buffer	2.5 µL
dNTP Mix (10 mM)	0.5 µL
25 mM MgCl2	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 11 PCR conditions.

Reaction Steps	Temperature (°C)	Time (sec)	Amount of Cycles
Inıtial Denaturation	95	30	-
Denaturation	95	30	35
Annealing	65	45	
Extension	72	90	
Final Extension	72	300	-

3.2.4.7.2 Gel Electrophoresis

After the polymerase chain reaction, gel electrophoresis was made to visualize the results. Gel was prepared from 1% Agarose concentration. 0.5 grams of Agarose was dissolved into the 50 mL of TAE buffer solution in an erlenmeyer flask and heated in a microwave until the agarose was completely dissolved. 16S rRNA PCR products were sequenced and the sequence data was analysed through BLAST using NCBI 16S ribosomal RNA sequences (Bacteria/Archaea) database.

4. RESULTS

4.1 Biochemical Characterization Results

4.1.1 Silicate-Solubilization Test Results

Silicate solubilization abilities are given graphically in Figure 8 and calculated indices in Table 12.

As a result of observations and calculations, it was determined that the ESU_85 bacterium was the best silicate-solubilizing bacterium among those isolated and ESU_80 was the least silicate-solubilizing bacterium among them.

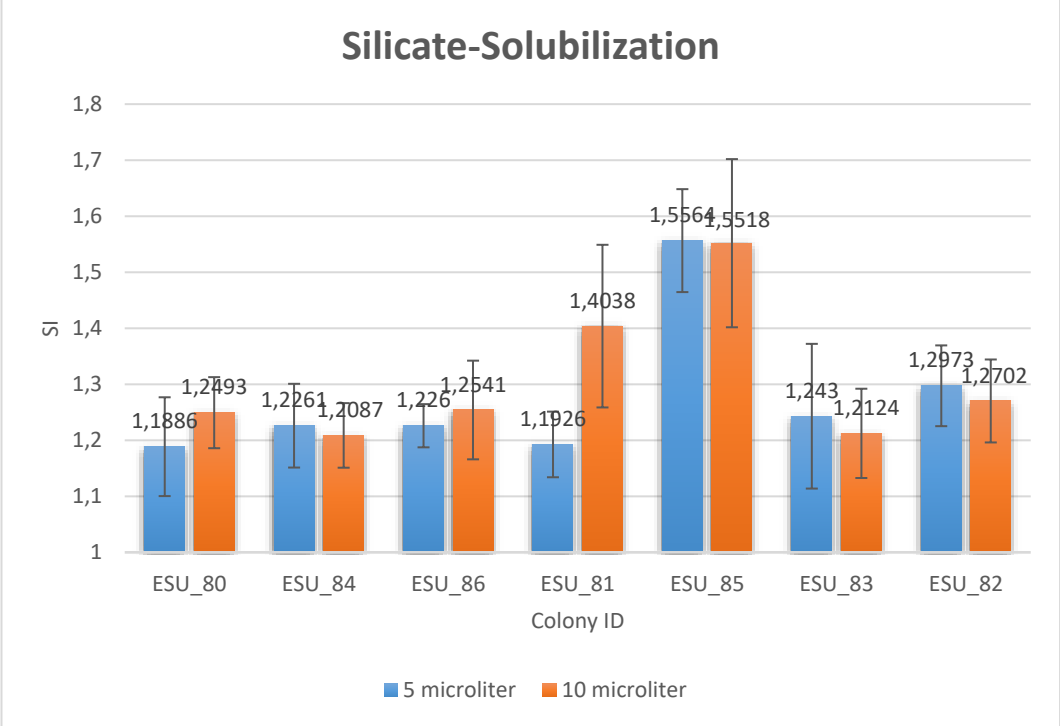


Figure 8 Graphical Representation of Silicate Solubilization Measurements of the Isolated Rhizobacteria

Table 12 Silicate-solubilization results for 5 µL and 10 µL cultivation.

Colony	5 microliter	10 microliter
ESU_80	**1,1886**±0,0881	**1,2493**±0,0634
ESU_84	**1,2261**±0,0747	**1,2087**±0,0576
ESU_86	**1,226**±0,0385	**1,2541**±0,0880
ESU_81	**1,1926**±0,0585	**1,4038**±0,1451
ESU_85	**1,5564**±0,0918	**1,5518**±0,1500
ESU_83	**1,243**±0,1291	**1,2124**±0,0796
ESU_82	**1,2973**±0,0720	**1,2702**±0,0740

4.1.2 Phosphate Solubilization Test Results

It is known that silicate-solubilizing bacteria can also solubilize phosphate from the soil and here we showed the phosphate solubilization activities of isolated SSB. (63) They all have similar results but the best phosphate solubilizer bacterium is ESU_81 and ESU_82 is the least. Graphical representation is given in Figure 9 and calculated indices in Table 13.

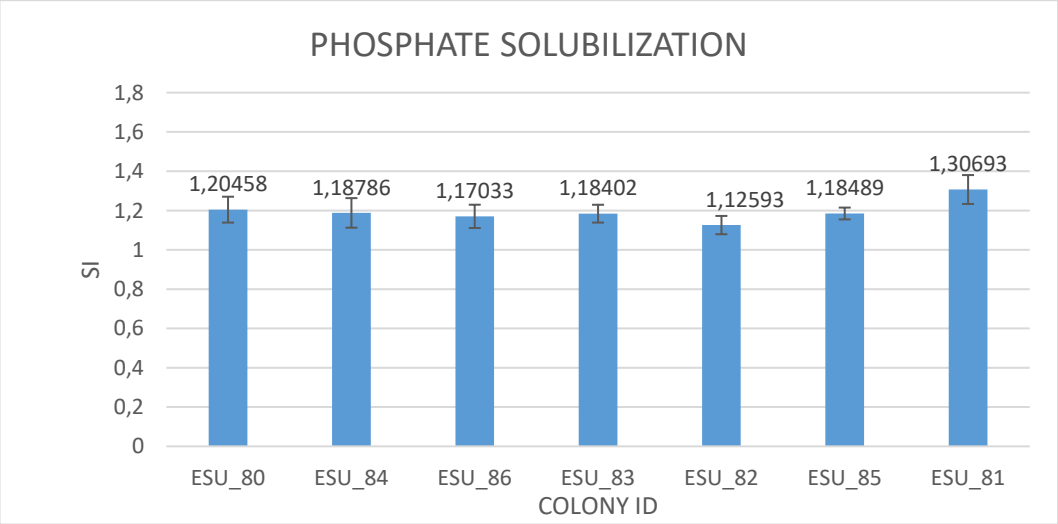


Figure 9 Graphical representation of phosphate solubilization measurements.

Table 13 Phosphate Solubilization Values.

ID	AVERAGE
ESU_80	**1,20458**±0,0658
ESU_84	**1,18786**±0,0752
ESU_86	**1,17033**±0,0592
ESU_83	**1,18402**±0,0453
ESU_82	**1,12593**±0,0465
ESU_85	**1,18489**±0,0300
ESU_81	**1,30693**±0,0734

4.1.3 Nitrogen Fixation Ability Test Results

All of the isolated bacteria are grown in an N-free environment which indicates that they are nitrogen-fixing bacteria. Table 14 shows the result of his test.

Table 14 Nitrogen Fixation of the isolated bacteria

ID	NITROGEN FIXATION ABILITY
ESU_86	+
ESU_85	+
ESU_84	+
ESU_81	+
ESU_80	+
ESU_83	+
ESU_82	+

4.1.4 Indole-3-Acetic Acid Test

All the silicate-solubilizing bacteria showed Indole-3-Acetic Acid production in the LB medium and also in the LB Medium with 0,1% Trp. ESU_84 showed the maximum IAA production in both mediums. ESU_85 bacterium showed the minimum IAA production with compassion with other bacteria in both mediums. Figure 11 is the graphical representation of Indole-3-Acetic Acid production of the isolated bacteria in both mediums. In Pictures 4 and 5 Salkowski reagent can be seen as colored, due to indole-3-acetic acid production by bacteria.

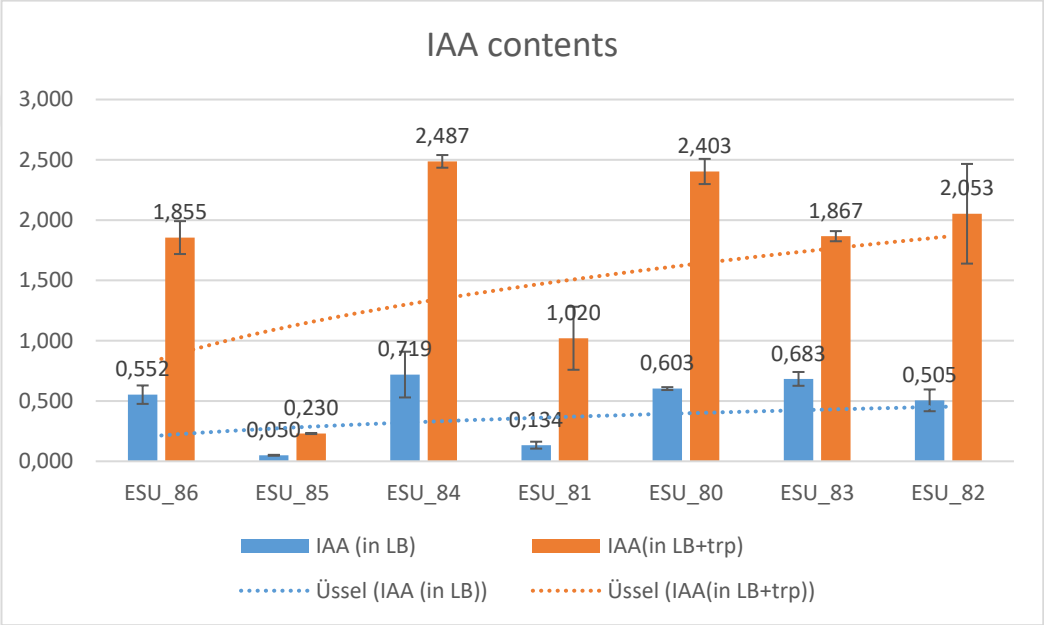
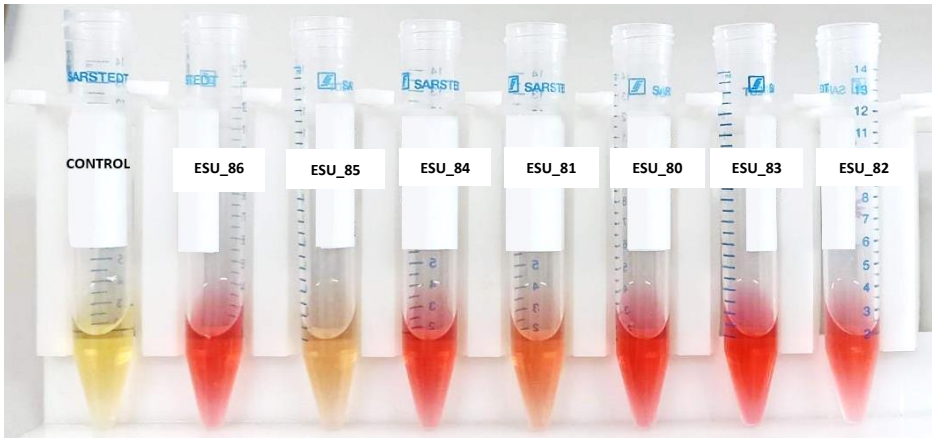


Figure 10 Graphical Representation of IAA Measurements on LB Medium and LB with Trp Measurements.



Picture 4 Control LB Medium and species are in the Salkowski Reagent without Tryptophan

Picture 8 Control LB Medium and species are in the Salkowski Reagent with Tryptophan

4.1.5 Catalase Test Results

All species were catalase-positive according to test results.

Table 15 Catalase Activity Test Result

ID	CATALASE TEST
ESU_86	+
ESU_85	+
ESU_84	+
ESU_81	+
ESU_80	+
ESU_83	+
ESU_82	+

4.1.6 Cytochrome *c* Oxidase Test Results

Except ESU_85 all bacteria showed negative results for this test. Table 16 shows cytochrome *c* oxidase activity.

Table 16 Cytochrome *c* oxidase test results.

ID	Cytochrome-*c*-Oxidase Test
ESU_86	-
ESU_85	+
ESU_84	-
ESU_81	-
ESU_80	-
ESU_83	-
ESU_82	-

4.1.7 Gel Electrophoresis of PCR

16S rRNA PCR is done for further identification processes. Loaded samples are mitigated under an electrical field and separated by their molecular charge, molecular weight.

Picture 9 PCR results of the isolated bacteria. From left to right; ESU_86, ESU_85, ESU_84, ESU_81, ESU_80, ESU_83, ESU_82.

4.1.8 16S rRNA Identification Analysis

Identification studies are done by using sequence analysis of 16 rRNA. Based on BLAST analysis, the information for possible species is given for ESU_80, ESU_81, ESU_82, ESU_83, ESU_84, ESU_85, and ESU_86 from Table 17 to Table 23. Table 24 shows suggested names for the species based on the best hit results obtained from the BLAST analysis.

Table 17 16S rRNA Identification Results of ESU_80

CODE NUMBER	SPECIES	MAX SCORE	TOTAL SCORE	QUERY COVER	E VALUE	PERCENT IDENT
ESU_80	*Enterobacter chengduensis* strain WCHECl-C4 16S ribosomal RNA,...	863	863	88%	0.0	82.43
	Enterobacter sichuanensis strain WCHECL1597 16S ribosomal RNA,...	861	861	89%	0.0	82.34
	Enterobacter oligotrophicus strain CCA6 16S ribosomal RNA,...	857	857	89%	0.0	82.22
	Enterobacter wuhouensis strain WCHEs120002 16S ribosomal RNA,...	856	856	89%	0.0	82.22
	Enterobacter chuandaensis strain 090028 16S ribosomal RNA,...	856	856	89%	0.0	82.22

Table 18 16S rRNA Identification Results of ESU_81

CODE NUMBER	SPECIES	MAX SCORE	TOTAL SCORE	QUERY COVER	E VALUE	PERCENT IDENT
ESU_81	*Enterobacter chengduensis* strain WCHECl-C4 16S ribosomal RNA,...	983	983	94%	0.0	83.08
	Enterobacter oligotrophicus strain CCA6 16S ribosomal RNA,...	977	977	94%	0.0	82.94
	Enterobacter wuhouensis strain WCHEs120002 16S ribosomal RNA,...	976	976	94%	0.0	82.94
	Enterobacter sichuanensis strain WCHECL1597 16S ribosomal RNA,...	976	976	94%	0.0	82.94
	Enterobacter chuandaensis strain 090028 16S ribosomal RNA,...	970	970	94%	0.0	82.83

Table 19 16S rRNA Identification Results of ESU_82

CODE NUMBER	SPECIES	MAX SCORE	TOTAL SCORE	QUERY COVER	E VALUE	PERCENT IDENT
ESU_82	*Enterobacter sichuanensis strain* WCHECL1597 16S ribosomal RNA,...	1158	1158	97%	0.0	86.24
	Enterobacter chengduensis strain WCHECl-C4 16S ribosomal RNA,...	1157	1157	97%	0.0	86.16
	Enterobacter wuhouensis strain WCHEs120002 16S ribosomal RNA,...	1155	1155	97%	0.0	86.13
	Enterobacter chuandaensis strain 090028 16S ribosomal RNA,...	1153	1153	97%	0.0	86.13
	Enterobacter kobei strain JCM 8580 16S ribosomal RNA, partial...	1149	1149	97%	0.0	86.03

Table 20 16S rRNA Identification Results of ESU_83

CODE NUMBER	SPECIES	MAX SCORE	TOTAL SCORE	QUERY COVER	E VALUE	PERCENT IDENT
ESU_83	*Bacillus wiedmannii* strain FSL W8-0169 16S ribosomal RNA,...	1380	1380	97%	0.0	91.65
	Bacillus proteolyticus strain MCCC 1A00365 16S ribosomal RNA,...	1380	1380	97%	0.0	91.65
	Bacillus sanguinis strain BML-BC004 16S ribosomal RNA, partial...	1380	1380	97%	0.0	91.65
	Bacillus fungorum strain 17-SMS-01 16S ribosomal RNA, partial...	1380	1380	97%	0.0	91.65
	Bacillus cereus strain JCM 2152 16S ribosomal RNA, partial...	1375	1375	97%	0.0	91.55

Table 21 16S rRNA Identification Results of ESU_84

CODE NUMBER	SPECIES	MAX SCORE	TOTAL SCORE	QUERY COVER	E VALUE	PERCENT IDENT
ESU_84	*Enterobacter cloacae* subsp. dissolvens strain ATCC 23373 16S...	987	987	74%	0.0	88.16
	Pantoea agglomerans strain JCM1236 16S ribosomal RNA, partial...	987	987	74%	0.0	88.16
	Enterobacter ludwigii strain EN-119 16S ribosomal RNA, partial...	987	987	74%	0.0	88.16
	Leclercia adecarboxylata ATCC 23216 = NBRC 102595 strain LMG...	985	985	74%	0.0	88.16
	Leclercia adecarboxylata strain CIP 82.92 16S ribosomal RNA,...	985	985	74%	0.0	88.16

Table 22 16S rRNA Identification Results of ESU_85

CODE NUMBER	SPECIES	MAX SCORE	TOTAL SCORE	QUERY COVER	E VALUE	PERCENT IDENT
ESU_85	*Pseudomonas urethralis* strain BML-PP042 16S ribosomal RNA,...	1352	1352	98%	0.0	91.72
	Pseudomonas laurentiana strain GSL-010 16S ribosomal RNA,...	1352	1352	98%	0.0	91.72
	Pseudomonas promysalinigenes strain RW10S1 16S ribosomal RNA,...	1352	1352	98%	0.0	91.72
	Pseudomonas kurunegalensis strain RW1P2 16S ribosomal RNA,...	1352	1352	98%	0.0	91.72
	Pseudomonas ceruminis strain BML-PP028 16S ribosomal RNA,...	1347	1347	98%	0.0	91.61

Table 23 16S rRNA Identification Results of ESU_86

CODE NUMBER	SPECIES	MAX SCORE	TOTAL SCORE	QUERY COVER	E VALUE	PERCENT IDENT
ESU_86	*Enterobacter cloacae* strain DSM 30054 16S ribosomal RNA, parti…	597	597	54%	5E-170	85.58
	Enterobacter cloacae subsp. dissolvens strain ATCC 23373 16S...	597	597	54%	5E-170	85.58
	Enterobacter cloacae strain NBRC 13535 16S ribosomal RNA,...	597	597	54%	5E-170	85.58
	Pantoea agglomerans strain JCM1236 16S ribosomal RNA, partial...	597	597	54%	5E-170	85.58
	Enterobacter cloacae strain 279-56 16S ribosomal RNA, partial...	597	597	54%	5E-170	85.58

Table 24 Suggested Names for the Species

CODE NUMBER	SPECIES NAME
ESU80	*Enterobacter* sp. strain ESU80
ESU81	*Enterobacter* sp. strain ESU81
ESU82	*Enterobacter* sp. strain ESU82
ESU83	*Bacillus* sp strain ESU83
ESU84	*Enterobacter* sp. strain ESU84
ESU85	*Pseudomonas* sp strain ESU85
ESU86	*Enterobacter* sp. strain ESU86

5. DISCUSSION

Silicon is an abundant element in the earth's crust and is used in many industries for many different reasons. Silicon is not considered an essential plant nutrient but has many benefits for crop production such as reducing biotic and abiotic stress factors. (64)(44) However, in the soil there is no plant-absorbable form of silicate minerals. They are found in nature as polymerized forms such as magnesium trisilicate. Thus, Si-accumulator plants can only absorb silicon via silicate-solubilizing bacteria in the monomeric form of silicic acids. SSB is a type of plant growth-promoter bacteria that enhances plant growth, especially in crop production. There is limited research in the literature on silicate-solubilization by bacteria. SSB can be found in soil, water, and marine sediments but their number is not as much overall bacteria, which is the reason for their uniqueness.

In this work rice *(Oryza Sativa),* known as a high Si-accumulator plant is used for the isolation and characterization of SSB. *Oryza Sativa* is collected from Kıbrıcık/Bolu region. Kıbrıscık is known for having the 1st class of *Oryza Sativa*.(60)

102 bacteria are isolated from the roots of 3 randomly collected plants. However, seven bacteria had the best ability of silicate-solubilization. They transform polymerized silicate minerals –magnesium trisilicate is used- into silicic acid. Absorbed silicic acid accumulates at the growing points of the plant enhances plant strength, and reduces heavy metal uptake according to the literature.(54) Further investigation is needed for plant applications of these isolated bacteria.

According to indole-3-acetic acid test results all isolates can produce indole-3-acetic acid with and without tryptophan in LB medium. Indole-3-acetic acid is an auxin which is the most important type of plant growth regulator that plays a vital role in root formation. Thus, it can be understood that all the isolated bacteria have benefits and may be used in the plant industry and increase the crop production yield in agriculture. Indole-3-acetic acid production has 2 pathways tryptophan dependent and typtophan independent. Both pathways are tried on all bacteria and results showed that tryptophan-dependent pathways are more actively used in all isolated silicate-solubilizer bacteria. In the literature, there are no studies about why

the bacteria synthesize the indole-3-acetic acid hormone and further investigations and studies can be focused on this question.

Phosphorus is a vital element and plays an active role in metabolic pathways such as energy transfer through plants, photosynthesis, macromolecular biosynthesis, and also nitrogen fixation in legumes. Soil is a rich source of phosphorus in organic and inorganic forms so roots can not uptake directly. (41) The results of this study showed that all the isolated silicate-solubilizing bacteria can also solubilize phosphate. This is an important finding for agriculture and applications can be done in the future.

Nitrogen is the most abundant element in the atmosphere but it is also the most limited nutrient for plants. All isolated silicate-solubilizing bacteria are also nitrogen-fixing bacteria. Nitrogen-fixing bacteria are an important part of nitrogen cycling. They take atmospheric dinitrogen gas and transform it to ammonia by using an enzyme dinitrogen synthase reductase, dinitrogen synthase, and cofactors (38). Since N is the most limited and efficient element for plants, especially important in crop production. In the soil ecosystem, the decomposition of organic matter can create alkalinity by forming ammonia and amines that can also solubilize silicates. (65)

According to 16S rRNA identification analysis shown that ESU_80, ESU_81, ESU_82, ESU_84, and ESU_86 are *Enterobacter* sp. strains which are known as plant growth-promoting bacteria. They have the ability of biological nitrogen fixation, inorganic phosphate solubilization, production of phytohormones and siderophores, and silicate solubilization. In past studies, it has also proven that they can enhance the abiotic and biotic stress tolerances.

Enterobacter spp. is also known for their ability for bioremediation. Bioremediation is a process used to treat contaminated water and soil. They can trap heavy metals within the cell, without releasing them back to the environment by using an enzymatic system. It is an efficient and eco-friendly process and can be used in agricultural applications. (66)

ESU_83 and ESU_85 are identified as *Bacillus* sp. and *Pseudomonas* sp. Past studies in the literature had proven that both has the ability of silicate solubilization. The mechanism of silicate-solubilization varies by the silicate type and between different organisms. (65)

Different types of bacteria that dissolve silicate can live together in the same plant. They produce different organic acids through various mechanisms that bind with different silicate minerals and release nutrients like magnesium, iron, potassium, and more. Besides to being able to dissolve silicate minerals, biochemical characterizations showed that these bacteria are very beneficial by providing the release and biomineralization of different macro and micro-nutrients and this is the reason their uniqueness and being beneficial plant growth rhizobacteria.

6. CONCLUSIONS AND RECOMMENDATIONS

In this study, we aimed to isolate a new silicate-solubilization bacteria from nature. We have successfully isolated 102 bacteria and 7 bacteria of these groups has the best ability for silicate solubilization. Our test results demonstrated the potential for these bacteria to enhance plant growth by increasing the availability of silicon, an essential nutrient for many plant species, and producing indole-3-acetic acid which is a vital hormone for plant growth. This could have important implications for sustainable agriculture and the development of new biofertilizers. It would be useful to further explore the mechanisms by which these bacteria solubilize silicate, as well as their potential for use in large-scale agricultural applications. This could involve additional laboratory studies, as well as field trials to assess the effectiveness of these bacteria under real-world conditions, especially in stressful conditions such as drought, heavy metal stress, etc.

7. REFERENCES

Vancouver citation system was used in this thesis.

1. Ball DW. Elemental Etymology: What's in a Name? 1985.

2. Cruz JA. Silicate-Solubilizing Bacteria in Louisiana Soils: Identification, Profiling, and Functions in Crop Production [Internet]. 2021. Available from: https://repository.lsu.edu/gradschool_dissertations/5473

3. Prabhu AS, Barbosa Filho MP, Filippi MC, Datnoff LE, Snyder GH. Chapter 18 Silicon from rice disease control perspective in Brazil 293.

4. Pozza EA, Pozza AAA, Dos Santos Botelho DM. Silicon in plant disease control. Vol. 62, Revista Ceres. Universidade Federal de Vicosa; 2015. p. 323–31.

5. Understanding_biosilification_Biomaterials. Materials Today. 2008 Jun;11(6).

6. Dhankhar N, Kumar J. Impact of increasing pesticides and fertilizers on human health: A review. Mater Today Proc. 2023 Apr 26;

7. Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, et al. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Vol. 871, Frontiers in Plant Science. Frontiers Media S.A.; 2018.

8. Ramakrishna W, Yadav R, Li K. Plant growth promoting bacteria in agriculture: Two sides of a coin. Vol. 138, Applied Soil Ecology. Elsevier B.V.; 2019. p. 10–8.

9. de Souza R, Ambrosini A, Passaglia LMP. Plant growth-promoting bacteria as inoculants in agricultural soils. Vol. 38, Genetics and Molecular Biology. Brazilian Journal of Genetics; 2015. p. 401–19.

10. Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. Vol. 26, Journal of King Saud University - Science. Elsevier B.V.; 2014. p. 1–20.

11. Ahemad M, Khan MS. Pseudomonasaeruginosa strain PS1 enhances growth parameters of greengram [Vignaradiata (L.) Wilczek] in insecticide-stressed soils. J Pest Sci (2004) [Internet]. 2011;84(1):123–31. Available from: https://doi.org/10.1007/s10340-010-0335-0

12. Ahemad M, Khan MS. Evaluation of plant-growth-promoting activities of rhizobacterium Pseudomonas putida under herbicide stress. Ann Microbiol [Internet]. 2012;62(4):1531–40. Available from: https://doi.org/10.1007/s13213-011-0407-2

13. Ahemad M, Saghir Kha M. Assessment of Plant Growth Promoting Activities of Rhizobacterium Pseudomonas putida under Insecticide-Stress. Microbiology Journal. 2011 Feb 15;1(2):54–64.

14. Ahemad M, Khan MS. Alleviation of fungicide-induced phytotoxicity in greengram [Vigna radiata (L.) Wilczek] using fungicide-tolerant and plant growth promoting Pseudomonas strain. Saudi J Biol Sci. 2012 Oct;19(4):451–9.

15. Ahemad M, Khan MS. Phosphate-Solubilizing and Plant-Growth-Promoting Pseudomonas aeruginosa PS1 Improves Greengram Performance in Quizalafop-p-ethyl and Clodinafop Amended Soil. Arch Environ Contam Toxicol [Internet]. 2010;58(2):361–72. Available from: https://doi.org/10.1007/s00244-009-9382-z

16. Ahemad M, Khan MS. Effects of insecticides on plant-growth-promoting activities of phosphate solubilizing rhizobacterium Klebsiella sp. strain PS19. Pestic Biochem Physiol [Internet]. 2011;100(1):51–6. Available from: https://www.sciencedirect.com/science/article/pii/S0048357511000332

17. Ahemad M, Khan MS. Biotoxic impact of fungicides on plant growth promoting activities of phosphate-solubilizing Klebsiella sp. isolated from mustard (Brassica campestris) rhizosphere. J Pest Sci (2004) [Internet]. 2012;85(1):29–36. Available from: https://doi.org/10.1007/s10340-011-0402-1

18. Ahemad M, Saghir Khan Md. Toxicological Effects of Selective Herbicides on Plant Growth Promoting Activities of Phosphate Solubilizing Klebsiella sp. Strain PS19. Curr Microbiol [Internet]. 2011;62(2):532–8. Available from: https://doi.org/10.1007/s00284-010-9740-0

19. Ahemad M, MS K. Influence of Selective Herbicides on Plant Growth Promoting Traits of Phosphate Solubilizing Enterobacter asburiae Strain PS2. Res J Microbiol. 2010 Sep 1;5:849–57.

20. Ahemad M, Khan M. Plant Growth Promoting Activities of Phosphate Solubilizing Enterobacter as- buriae as Influenced by Fungicides. Eurasian J Biosci. 2010 Jan 1;4:88–95.

21. Ahemad M, MS K. Ecological assessment of biotoxicity of pesticides towards plant growth promoting activities of pea (Pisum sativum)-specific Rhizobium sp. Strain MRP1. J Sci Food Agric. 2012 Aug 1;24:334–43.

22. Ahemad M, Khan M. Effect of tebuconazole-tolerant and plant growth promoting Rhizobium isolate MRP1 on pea-Rhizobium symbiosis. Sci Hortic. 2011 Jun 1;129:266–72.

23. Ahemad M, Khan M. Comparative toxicity of selected insecticides to pea plants and growth promotion in response to insecticide-tolerant and plant growth promoting Rhizobium leguminosarum. Crop Protection. 2010 Apr 1;29:325–9.

24. Ahemad M, Khan M. Effects of pesticides on plant growth promoting traits of Mesorhizobium strain MRC4. Journal of the Saudi Society of Agricultural Sciences. 2012 Jan 31;11:63–71.

25. Ahemad M, Khan M. Ameliorative effects of Mesorhizobium sp. MRC4 on chickpea yield and yield components under different doses of herbicide stress. Pestic Biochem Physiol. 2010 Oct 1;98:183–90.

26. Ahemad M, MS K. Improvement in the growth and symbiotic attributes of fungicide-stressed chickpea plants following plant growth promoting fungicide-tolerant Mesorhizobium inoculation. African Journal of Basic and Applied Sciences. 2010 Jan 1;2:111–6.

27. Ahemad M, Khan M. Effect of insecticide-tolerant and plant growth-promoting Mesorhizobium on the performance of chickpea grown in insecticide stressed alluvial soils. J Crop Sci Biotechnol. 2010 Dec 1;12:217–26.

28. Rokhbakhsh Zamin F, Sachdev D, Kazemi-Pour N, Engineer A, Pardesi K, Zinjarde S, et al. Characterization of Plant-Growth-Promoting Traits of Acinetobacter Species Isolated from Rhizosphere of Pennisetum glaucum. J Microbiol Biotechnol. 2011 Jun 1;21:556–66.

29. Ahemad M, Khan M. Ecotoxicological assessment of pesticides towards the plant growth promoting activities of Lentil (Lens esculentus)-specific Rhizobium sp. strain MRL3. Ecotoxicology. 2011 Feb 1;20:661–9.

30. Ahemad M, Khan M. Plant-Growth-Promoting Fungicide-Tolerant Rhizobium Improves Growth and Symbiotic Characteristics of Lentil (Lensesculentus) in Fungicide-Applied Soil. J Plant Growth Regul. 2011 Sep 1;30:334–42.

31. Ma Y, Rajkumar M, Luo Y, Freitas H. Inoculation of endophytic bacteria on host and non-host plants—Effects on plant growth and Ni uptake. J Hazard Mater [Internet]. 2011;195:230–7. Available from: https://www.sciencedirect.com/science/article/pii/S0304389411010399

32. Ma Y, Rajkumar M, Vicente JAF, Freitas H. Inoculation of Ni-Resistant Plant Growth Promoting Bacterium Psychrobacter sp. Strain SRS8 for the Improvement of Nickel Phytoextraction by Energy Crops. Int J Phytoremediation [Internet]. 2010 Dec 28;13(2):126–39. Available from: https://doi.org/10.1080/15226511003671403

33. Ahemad M, Khan MS. Productivity of greengram in tebuconazole-stressed soil, by using a tolerant and plant growth-promoting Bradyrhizobium sp. MRM6 strain. Acta Physiol Plant [Internet]. 2012;34(1):245–54. Available from: https://doi.org/10.1007/s11738-011-0823-8

34. Ahemad M, Khan M. Effect of Pesticides on Plant Growth Promoting Traits of Greengram-Symbiont, Bradyrhizobium sp. strain MRM6. Bull Environ Contam Toxicol. 2011 Feb 1;86:384–8.

35. Ahemad M, Khan M. Insecticide-tolerant and plant growth promoting Bradyrhizobium sp. (vigna) improves the growth and yield of greengram [Vigna radiata (L.) Wilczek] in insecticide-stressed soils. Symbiosis. 2011 Sep 1;54:17–27.

36. Naik M, Dubey S. Lead-Enhanced Siderophore Production and Alteration in Cell Morphology in a Pb-Resistant Pseudomonas aeruginosa Strain 4EA. Curr Microbiol. 2011 Feb 1;62:409–14.

37. Dary M, Chamber M, Palomares AJ, Pajuelo E. “In situ” phytostabilisation of heavy metal polluted soils using Lupinus luteus inoculated with metal resistant plant-growth promoting rhizobacteria. J Hazard Mater. 2010 May 15;177:323–30.

38. Guo K, Yang J, Yu N, Luo L, Wang E. Biological nitrogen fixation in cereal crops: Progress, strategies, and perspectives. Vol. 4, Plant Communications. Cell Press; 2023.

39. Rawat P, Das S, Shankhdhar D, Shankhdhar SC. Phosphate-Solubilizing Microorganisms: Mechanism and Their Role in Phosphate Solubilization and Uptake. Vol. 21, Journal of Soil Science and Plant Nutrition. Springer Science and Business Media Deutschland GmbH; 2021. p. 49–68.

40. Lee KE, Adhikari A, Kang SM, You YH, Joo GJ, Kim JH, et al. Isolation and characterization of the high silicate and phosphate solubilizing novel strain enterobacter ludwigii GAK2 that promotes growth in rice plants. Agronomy. 2019 Mar 20;9(3).

41. Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA. Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. Vol. 2, SpringerPlus. SpringerOpen; 2013.

42. Lang DY, Fei PX, Cao GY, Jia XX, Li YT, Zhang XH. Silicon promotes seedling growth and alters endogenous IAA, GA3 and ABA concentrations in Glycyrrhiza uralensis under 100 mM NaCl stress. J Hortic Sci Biotechnol [Internet]. 2019 Jan 2;94(1):87–93. Available from: https://doi.org/10.1080/14620316.2018.1450097

43. Moradtalab N, Weinmann M, Walker F, Höglinger B, Ludewig U, Neumann G. Silicon improves chilling tolerance during early growth of maize by effects on micronutrient homeostasis and hormonal balances. Front Plant Sci. 2018 Apr 26;9.

44. Sarraf M, Janeeshma E, Arif N, Qudrat Ullah Farooqi M, Kumar V, Ansari NA, et al. Understanding the role of beneficial elements in developing plant stress resilience: Signalling and crosstalk with phytohormones and microbes. Plant Stress. 2023 Dec;10:100224.

45. Spaepen S, Vanderleyden J, Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling. Vol. 31, FEMS Microbiology Reviews. 2007. p. 425–48.

46. Duca DR, Glick BR. Indole-3-acetic acid biosynthesis and its regulation in plant-associated bacteria. Appl Microbiol Biotechnol [Internet]. 2020;104(20):8607–19. Available from: https://doi.org/10.1007/s00253-020-10869-5

47. Persello-Cartieaux F, Nussaume L, Robaglia C. Tales from the underground: Molecular plant-rhizobacteria interactions. Vol. 26, Plant, Cell and Environment. 2003. p. 189–99.

48. Ünüvar ÖC, Ünlü ES. From Hologenomes to Biofertilizers in Wheat Production. In: Zencirci N, Ulukan H, Baloch FS, Mansoor S, Rasheed A, editors. Ancient Wheats [Internet]. Cham: Springer International Publishing; 2022. p. 181–96. Available from: https://doi.org/10.1007/978-3-031-07285-7_8

49. Durve-Gupta A, Birla BK, Kalyan C, Chaudhary Y, Gosavi P. Impact Factor: 5.2 IJAR. 2017;3(4):246–50. Available from: www.allresearchjournal.com

50. Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B. Promotion of Plant Growth by Bacterial ACC Deaminase. CRC Crit Rev Plant Sci [Internet]. 2007 Oct 23;26(5–6):227–42. Available from: https://doi.org/10.1080/07352680701572966

51. Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM. Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol. 2014;52:347–75.

52. Sharma A, Gupta A, Ramakrishnan M, Ha C Van, Zheng B, Bhardwaj M, et al. Roles of abscisic acid and auxin in plants during drought: A molecular point of view. Plant Physiology and Biochemistry [Internet]. 2023;204:108129. Available from: https://www.sciencedirect.com/science/article/pii/S098194282300640X

53. Li N, Feng A, Liu N, Jiang Z, Wei S. Silicon application improved the yield and nutritional quality while reduced cadmium concentration in rice. Environmental Science and Pollution Research. 2020 Jun 1;27(16):20370–9.

54. Khan I, Awan SA, Rizwan M, Ali S, Hassan MJ, Brestic M, et al. Effects of silicon on heavy metal uptake at the soil-plant interphase: A review. Vol. 222, Ecotoxicology and Environmental Safety. Academic Press; 2021.

55. Al-Garni SMS, Khan MMA, Bahieldin A. Plant growth-promoting bacteria and silicon fertilizer enhance plant growth and salinity tolerance in Coriandrum sativum. J Plant Interact. 2019 Jan 1;14(1):386–96.

56. Rastogi A, Yadav S, Hussain S, Kataria S, Hajihashemi S, Kumari P, et al. Does silicon really matter for the photosynthetic machinery in plants…? Plant Physiology and Biochemistry. 2021 Dec 1;169:40–8.

57. Kang SM, Waqas M, Shahzad R, You YH, Asaf S, Khan MA, et al. Isolation and characterization of a novel silicate-solubilizing bacterial strain Burkholderia eburnea CS4-2 that promotes growth of japonica rice (Oryza sativa L. cv. Dongjin). Soil Sci Plant Nutr. 2017 May 4;63(3):233–41.

58. Kaur P, Sharma A, Bhardwaj NK, Singh A, Dalal S, Sharma J. A novel, simple, and quick plate assay to screen silicolytic bacteria and silicase production using different substrates. Bioresour Technol Rep [Internet]. 2022;17:100971. Available from: https://www.sciencedirect.com/science/article/pii/S2589014X22000287

59. Ikeda T. Bacterial biosilicification: A new insight into the global silicon cycle. Vol. 85, Bioscience, Biotechnology and Biochemistry. Oxford University Press; 2021. p. 1324–31.

60. Ettiren T. No: 513-Menşe Adı BOLU KIBRISCIK PİRİNCİ.

61. White L, Bruzel V, Subramanian S. Isolation of Rhizosphere Bacterial Communities from Soil. Bio Protoc. 2015;5(16).

62. Catalase Test Protocol [Internet]. 2010. Available from: www.asmscience.org

63. Naureen Z, Aqeel M, Hassan MN, Gilani SA, Bouqellah N, Mabood F, et al. Isolation and Screening of Silicate Bacteria from Various Habitats for Biological Control of Phytopathogenic Fungi. Am J Plant Sci. 2015;06(18):2850–9.

64. Ahmad HM, Fiaz S, Hafeez S, Zahra S, Shah AN, Gul B, et al. Plant Growth-Promoting Rhizobacteria Eliminate the Effect of Drought Stress in Plants: A Review. Vol. 13, Frontiers in Plant Science. Frontiers Media S.A.; 2022.

65. Vasanthi N, Saleena LM, Raj SA. Silica Solubilization Potential of Certain Bacterial Species in the Presence of Different Silicate Minerals. Silicon. 2018 Mar 1;10(2):267–75.

66. Khalifa A. Enterobacter. In: Beneficial Microbes in Agro-Ecology: Bacteria and Fungi. Elsevier; 2020. p. 259–70.

8. APPENDICES

ANNEX 1 Research permit for field work.

T.C.
TARIM VE ORMAN BAKANLIĞI
Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü

Sayı : E-50411936-605.01-7165125
Konu : Araştırma İzni (Doç. Dr. Ercan Selçuk ÜNLÜ)

BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ REKTÖRLÜĞÜ
(Fen Edebiyat Fakültesi)

İlgi : 26.09.2022 tarihli ve E-43776244-605.01-2200133883 sayılı yazınız.

Bolu Abant İzzet Baysal Üniversitesi Rektörlüğü Fen Edebiyat Fakültesi Kimya Bölümü öğretim üyesi Doç. Dr. Ercan Selçuk ÜNLÜ' nün danışmanlığında yüksek lisans çalışmalarına başlayacak olan Kimya Anabilim Dalı Yüksek Lisans öğrencisi Mercan YÜKSEK'in Üniversiteniz Lisansüstü Eğitim Müdürlüğü tarafından başlığı onaylanmış *"Doğal Habitatlardan Yeni Silikat Çözücü Bakterilerin İzolasyonu ve Karakterizasyonu"* başlıklı tez çalışması için Bolu-Kıbrıscık bölgesinde bulunan bir pirinç tarlasındaki toprakta mikrobiyolojik yük çalışması yapılacağı belirtilmektedir. 25.09.2022-20.10.2022 tarihleri projede toplanacak materyaller için öngörülen zamandır. Toplanacak materyallerin henüz başvurusu hazırlık aşaması olan TÜBİTAK projesi öncesi ön çalışma niteliğinde kullanılacağı belirtilerek Genel Müdürlüğümüzden izin talep edilmektedir.

Söz konusu proje ile ilgili taahhütnamede belirtildiği gibi kurumumuzun görüşleri aşağıda açıklanmıştır;

-Toplanacak materyalin birer örneğinin pasaport/teşhis ve tanı bilgileri ile birlikte Ankara Zirai Mücadele Merkez Araştırma Enstitüsü Müdürlüğü'nde muhafaza edilmek üzere Bakanlığımıza teslim edilmesi,

-Toplanan materyalle moleküler düzeyde yapılacak laboratuvar çalışmalarının, toplanan materyal ülke dışına çıkarılmadan, Türkiye'deki bir kuruluşta yapılması,

-Toplanan materyalin kullanılması ile elde edilecek bilgiler ve bunlara dayalı olarak yapılan her yayının Bakanlığımıza da gönderilmesi,

-Toplanan materyal üzerinde hiçbir sahiplik ya da ıslahçı hakkı iddia edilmemesi,

-Toplanan ve/veya bundan elde edilecek herhangi bir materyalin ticari amaçla kullanılmaması, üçüncü kişilere verilmemesi koşuluyla söz konusu araştırmada materyallerin toplanması amacıyla çalışmaların Doç. Dr. Ercan Selçuk ÜNLÜ tarafından yürütülmesinde bir sakınca görülmemektedir.

Bilgilerinizi ve gereğini rica ederim.

Dr. Şerafettin ÇAKAL
Bakan a.
Genel Müdür Yardımcısı V.