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Entry to the Stockholm Junior Water Prize 2024

Enhancing Plant Salinity Stress Tolerance Through Rhizobacteria-Mediated Salinity Mitigation.

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The research was conducted in the Zuckerberg Institute for Water Research laboratory at the Blaustain Institute for Desert Research Of Ben Gurion University under the guidance of Prof. Zeev Ronen and Damiana Diez Reck.

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Abstract

Worldwide, agriculture accounts for 70% of all water consumption, compared to 20% for industry and 10% for domestic use. Climate changes lead to an increase of seven percent per year in the arid land area, and an increase use of irrigation water, specifically the brackish and treated effluent water that increases the extent of salt-stressed soils and hurts plants. Salinity stress impacts the whole world's food security and quality. Food security comes second in the UN 17 goals list, indicating its importance. Regrettably, we are not heading to a better place, with the world population increasing irrespective of the amount of food we produce. While the population is expected to reach 10 billion by 2050, the a-biotic stress only increases and already leads to a 50% loss in yield. Climate changes lead to an increase of seven percent per year in the arid land area. The standard solution to the food security problem is based on artificial or non-sustainable fertilizers and additives. Still, their effect on the soil and the microbiome in the rhizosphere is ignored. Salt stress has become a world problem. As of 2020, this stress affects about 3 billion hectares worldwide out of 5.2 billion hectares of agricultural land. If this situation does not change, we will lose more than half of our agricultural fields.

The microbiome in the rhizosphere plays a significant role in plant health and offers potential solutions for mitigating stress and improving crop yield. Biological solutions such as inducing plant growth-promoting bacteria (PGPR) are promising. However, even these bacteria require artificial carbon additives, which may affect the overall sustainability of this solution. The goal of this research is to find PGPR originating from the plant rhizosphere of natural halophytic plants and check the ability to sustain itself and reduce the ethylene produced by plants under stress. Decreasing the ethylene rate will lower the damage to the plants, therefore saving water and making freshwater available for domestic use.

This study shows that plant growth-promoting bacteria (PGPR) from halophytic plants can degrade and use aminocyclopropane-1-carboxylic acid (ACC) as sole carbon and nitrogen. By doing so, these bacteria reduce the salinity effect on plants. The novelty of the findings is the ability of the PGPR to sustain itself in the rhizosphere during brackish water and treated effluent irrigation. Therefore, harnessing these alternative water resources in agriculture has the potential to enhance the availability of fresh water for fulfilling domestic demand.

The future research whould contain finding a way to measure the change in biomass when growing on ACC as sole carbon and nitrogen; measuring change in the 16S rRNA gene using qPCR; screening the isolates to express other salinity-improving traits and antioxidant

enzymes; and finally growing tomatoes in a greenhouse using brackish water irrigation with and without the bacteria and observing changes in root length and overall biomass yield of the plants including comparison of different application times for adding the bacteria, whether during germination or after the formation of the first leaves.

Abbreviations and Acronyms

ACC - aminocyclopropane-1-carboxylic acid

α-Keto - α-Ketobutyric acid

PGPB – plant growth-promoting bacteria

PGPR – plant growth promoting rhizosphere

IAA - Indole-3-acetic acid

ACCD - ACC deaminase

SAM - S-adenosyl L- methionine

MMDF – minimal media

PAF - Pseudomonas Agar F

L-methionine - L-aspartic acid

Acknowledgments

I am grateful for the opportunity to research and explore in the "Alpha" program, which allows high school students to integrate into academic scientific research while in high school. Due to this experience, I've realized I love doing research and that I'd like to continue it in water treatment and microbiology further in the future.

While writing this report, many people helped me. I couldn't have done it without them, I'd like to express my gratitude to them.

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Introduction

Global water crisis and climate changes lead to an increase of seven percent per year in arid land areas and desertification [Zahedi 2021]. Besides, the high salt concentration in the irrigation water, salinizes soils and damages plants [Oleńska et al. 2020]. Salinity stress on plants impacts the whole world's food security and quality. Food security comes second in the UN 17 goals list, indicating its importance. Regrettably, we are not heading to a better place, with the world population increasing beyond the the increase of food we produce.

The current solution to the food stress problem is based on artificial, non-sustainable fertilizers and additives, which have a detrimental effect on soils. As of 2020, this stress affects about 30 of 52 million square kilometers of agricultural land. If this situation doesn't change, we will lose more than half of our agricultural fields.

The microbiome, the overall microbial community in the rhizosphere (plant roots environment), affects plant growth and can ease stress and improve the crop. Inducing plant growth-promoting bacteria (PGPR) is a possible biological solution for the mitigation of salt stress. PGPR needs artificial carbon additives for application in the field, which diminishes the benefits of this solution as a sustainable option.

As an alternative, the goal of this research is to find PGPR originating from the halophytic rhizosphere of natural plants that can sustain itself and prevent salinity stress from irrigation via control of the stress hormone ethylene. Decreasing the ethylene level in the rhizosphere lowers the damage to plant function, increase yield, and consequently improve food security. Because the bacteria can sustain themselves, we could reduce plant damage while irrigating with brackish water and treated effluents. Using these water resources allows us to save fresh water for ever-increasing domestic demand.

Salinity Stress

Worldwide, 40% of the agricultural land is arid land. Arid lands are exposed to ongoing water deficits due to climate change. Therefore, farmers can't produce their crops on rainwater only. In the past, most of the water used for irrigation in arid land was freshwater. As the demand for freshwater rose and with the ongoing droughts, farmers began irrigating their crops with alternative water sources such as treated wastewater and brackish water. These types of water contain up to thousands of milligrams of salt per liter, unlike freshwater, which typically contains only tens of milligrams. High concentrations of salts for irrigation result in soil

salinization. As a result, today, most irrigated agricultural land suffers from levels of salinity stress [Fiorela et al. 2021].

Salinity stress has a detrimental impact on the plant, from the germination quality and water intake to damage to the eco-balance and plant structure. Researchers observed such plants damage in the plant's ability to take nitrogen, resulting in reduced yield potential. The salinity stress causes osmotic imbalance, which decreases the plant's ability to uptake water and nutrients from the soil. Furthermore, this imbalance harms the photosynthesis process that nourishes the plant [Otlewska et al. 2020],

Besides these changes, there are also structural changes to adapt to the salinized environment. Different Auxins, which are plant hormones, especially Indole-3-acetic acid (IAA), are produced and transported to the root tips. In high concentrations, the IAA delays the root's growth, damaging the whole plant. Unlike higher auxin levels that occur in every stress, ethylene levels rise in response to salt. The decrease in root growth led to direct and indirect increases in ethylene synthesis. High ethylene level causes poor oxidation-reduction and plant withering. A compendium of salinity stress effects is displayed in Fig. 1 [Middleton et al. 1997].

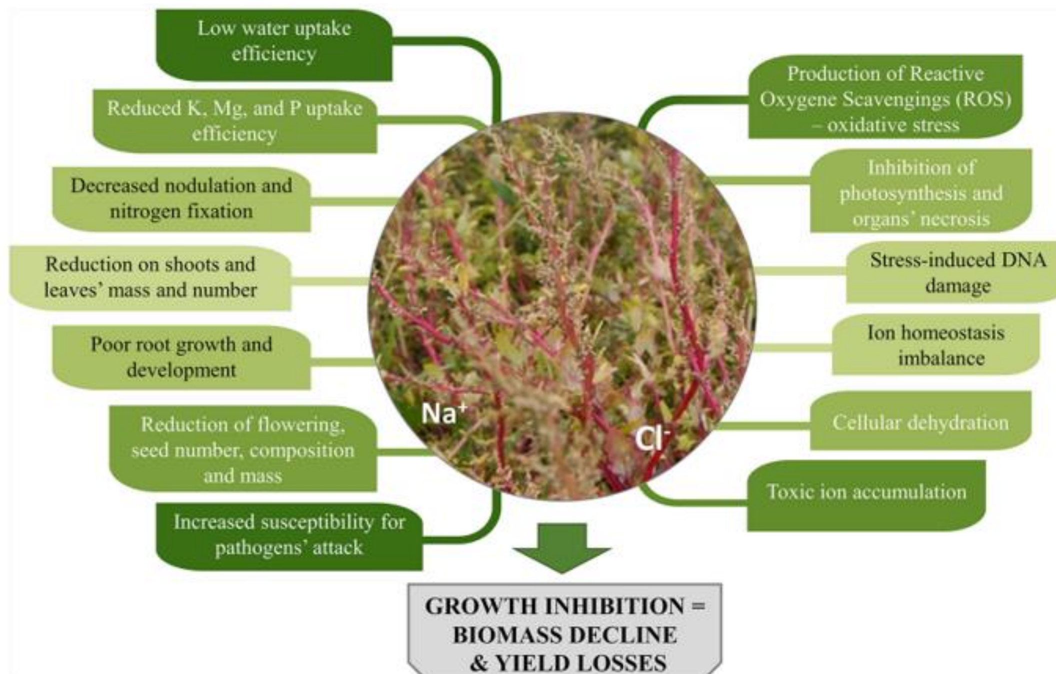

Figure 1: salinity stress effacts [Middleton et al. 1997]

Ethylene's Role in Stress

Ethylene is a gaseous hydrocarbon molecule present in the entire plant life circle. It is a plant hormone responsible for growth and development and is affected by high saltconcentration in

the plants environment. Ethylene affects several processes in the plant, including germination, root development, blooming, flower gender, and leaves or flowers aging. (Hayat et. al. 2010). Its most known role is ripening fruits like bananas and apples. Even though these roles are essential, high ethylene concentrations cause the opposite effect. Too much ethylene causes early rot, faster aging of the whole plant, oxidation-reduction problems, slower root growth, and nutrient intake issues. The average ethylene level in the roots is 0.1-1 ppm [Pathania et al. 2020]. During salinity stress, these levels rise and become detrimental to plant and crop quality. This rise is due to the high rates of IAA during stress that increased the availability of aminocyclopropane-1-carboxylic acid (ACC), the precursor of ethylene synthesis.

Rhizobacteria in the Rhizosphere

Rhizosphere is the volume surrounding the root. It contains water, nutrients, and minerals mutually transferring to the roots and back. In the rhizosphere, many microorganisms affect the plant's ability to grow and thrive [Figure 2]. The rhizobacteria live around the root, but in some cases, they enter the root tissue. In the root tissue, there are endobacteria and exobacteria. Many rhizosphere contain endobacteria which have a better connection to the plant and they are called endophytes. The endophytes are also separated as extracellular and intracellular [Dimkpa et al. 2009]. The extracellular bacteria are on the root surface. We separate them into three groups: next to the root without touching it, by the root surfaces, and in the space between the root hair. Due to the immediacy of the plant rhizobacteria, plants that survive under stress depend on their rhizobacteria. The rhizobacteria can balance the plant hormones and nutrients and protect them from different pathogens. Furthermore, nitrogen fixation, exclusively performed by symbiotic or free-leaving rhizobacteria, and phosphorus solubilization produces rhizobitauxin, which promotes plant development under stress.

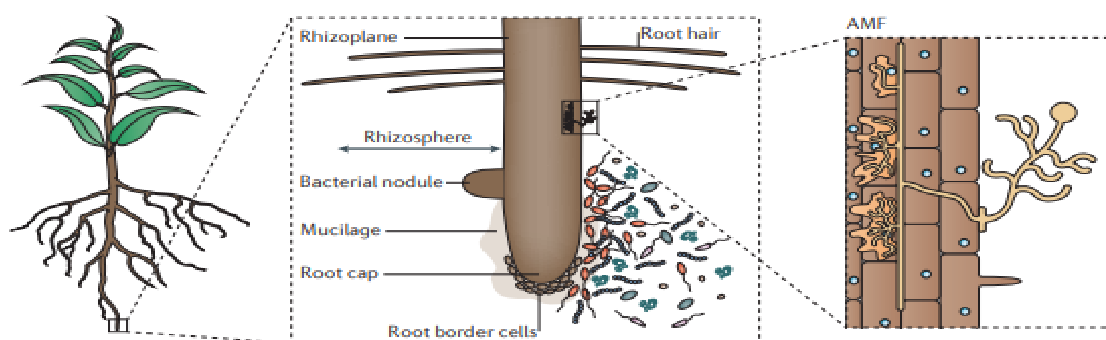

Figure 2: rhizosphere and root peel (to the right) [Laurent et al. 2013]

Plant Growth-Promoting Bacteria (PGPB)

Plant Growth Promoting Bacteria (PGPB) are bacteria that colonize the plant rhizosphere and affect it in various ecological and biological ways. PGPB affects plant nutrient intake and are responsible for the hormonal balance by allocating the growth regulator and the signalling molecules. Above all, PGPB induces resistance to diverse pathogens and creates metabolites such as biosurfactants that can exterminate the pathogens.

PGPBs are known mainly for their ability to produce growth stimulants, e. g., exopolysaccharides, rhizobitoxine, and signaling molecules. Rhizobitoxines help the plant by limiting the ethylene produced. Similarly, the signal molecule helps recognize stress and regulate the microbiome, which enhances plant development. In addition, these molecules interact with the plant, thus protecting it from the stress negative effect [Pathania et al 2020]. ACCD enzyme improves the plant's health by degrading ACC (aminocyclopropane-1-carboxylic acid) before it is transformed to ethylene. Plants with PGPR containing ACCD showed longer roots and higher disease resistance. PGPR even improved regular growth, although it was under stress, by reducing pressure and the negative impact of ethylene [Ha-Tran et al. 2021].

ACC and ACCD

Plants under stress produce more IAA (Indole-3-acetic acid) as it elongates the roots. However, IAA accumulation increases the transcription of ACC synthesis, which in turn increases ethylene production above normal. Ethylene is a plant hormone that acts as a regulator. It has roles in plants germination, development, blooming, and aging. Ethylene encourages rooting and sprouting, reduces dormancy, and signals hazards in low concentrations. Under stress, the ethylene causes apoptosis and wilt acceleration.

Ethylene production starts from the activity of the enzyme ACC synthase after IAA accumulation. ACC is synesized from L-methionine precursor transformed to SAM (S-adenosyl L-methionine), by SAM ligase. The SAM becomes ACC with synthases. Then, methionine turns into ethylene via ACC. There are two options to reduce ethylene production.

In this research, the second option was examined. Thus, the ACCD enzyme hydrolyses the ACC into ammonium and α-Keto butyrate [Oleńska et al. 2021], [Figure 3].

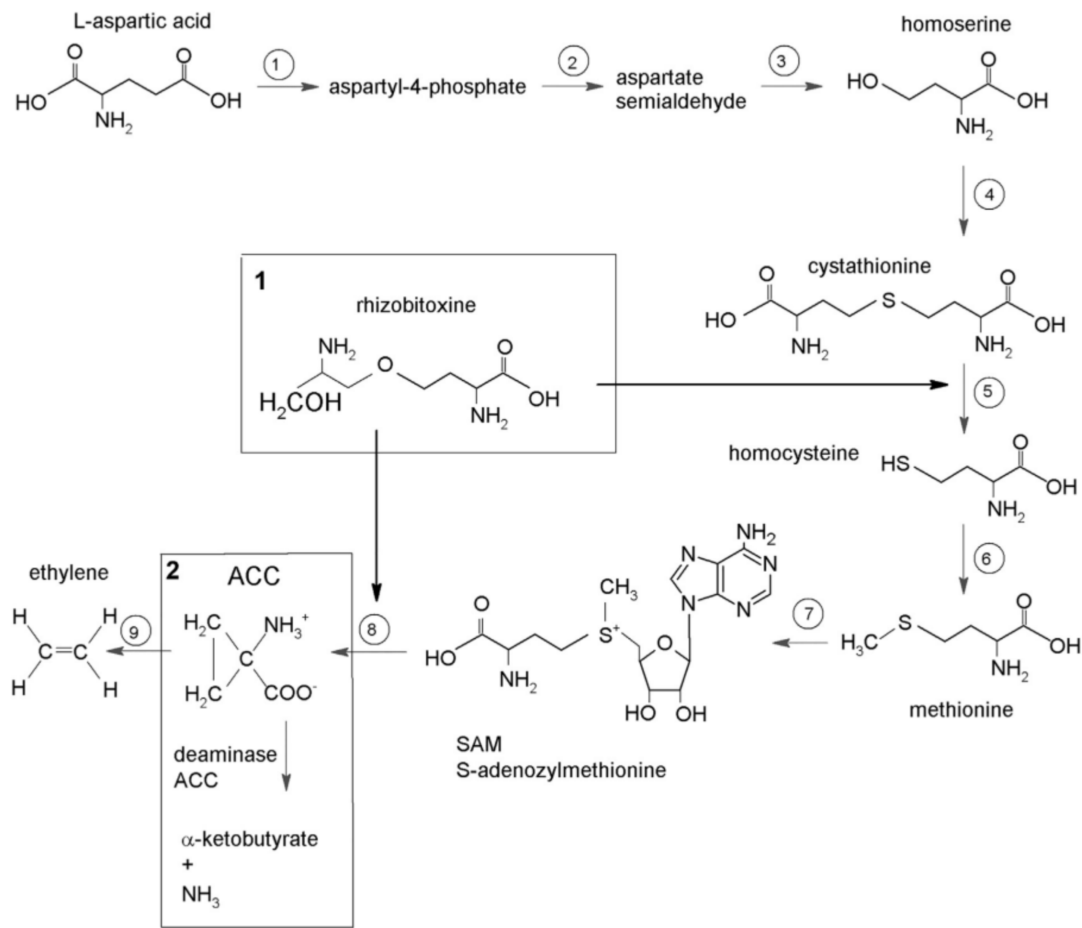

Figure 3: the ethylene synthesis. The bacteria intervene in stages 5 and 8 [Oleńska et al 2020]

Plants inoculation with ACCD PGPB

Numerous researchers showed that rhizobacteria producing ACCD improved plant growth under stress. For example, researchers in 2013 isolated PGPR from tomatoes that grew in high salt concentrations environment, which reduces ACCD and reduces the plant stress [Otlewska et al 2009]. In 2014, *Acinetobacter* spp. and *Pseudomonas* sp showed improved growth in barley due to their ACCD and IAA production.

In 2015, *Hartmannibacter diazotrophicus*, isolated from Plantago Winter, was used to inoculate the wild barely and improve its growth [Table 1]. In 2013, peppers suffering from salinity stress were supplanted with *Bacillus licheniformis* and then showed increased biomass and longer roots [Deka&Goswami 2020], [Table 2]. In 2009, lettuces inoculated with *Pseudomonas mendocina* showed improved nutrient intake because of the ACCD produced

by the bacteria. In 2004, researchers observed higher resistance in tomatoes and peppers suffering from salinity stress after they grafted with *Achromobacter piechaudii*, an ACCD-producing PGPR.

Table 1: List of plants inoculated with PGPRr [Otlewska et al 2009]

Plants	Microbes	Effect/Mechanism	References
Groundnut (*Arachis hypogaea* L.)	*Brachybacterium saurashtrense* (JG-06), *Brevibacterium casei* (JG-08), and *Haererohalobacter* (JG-11)	Higher K^+/Na^+ ratio and higher Ca^{2+}, phosphorus, and nitrogen content. Shoot and root has higher concentra-tion of auxin	Shukla et al. (2012a, 2012b)
Mung bea (Vigna radiate)	*Rhizobium* and *Pseudomonas*	ACC-deaminase for improving growth, nodulation and yield of mung bean under natural salt-affected conditions	Ahmad et al. (2011)
Barley and oats	*Acinetobacter spp. and Pseudomonas* Sp.	Production of enzyme ACC deaminase lower ethylene and IAA promote plant growth	Chang et al. (2014)
Wheat	*Azospirillum* Sp.	Increased shoot dry weight and grain yield. Plants accumulate some organic solutes (e.g. proline and soluble sugars)and inorganic ions to maintain osmotic adjustment	
	Pseudomonas Sp. Serratia Sp.	Have ACC deaminase activity, reduce ethylene level and enhance plant height, root length and yield	Zahir et al. (2009)
Maize (Zeya Mays)	*Pseudomonas* and *Enterobacter*	Reduce triple response and more N, P, and K uptake and high K^+–Na^+ ratios	Nadeem et al. (2009)
Rice GJ-17	*Pseudomonas pseudoalcaligenes and Bacillus pumilus*	Reduced the toxicity of reactive oxygen species(ROS) and reduce lipid peroxidation and superoxide dismutase activity. Reduce lipid peroxidation and superoxide dismutase activity	Jha and Subramanian, (2014)
Rice	*Bacillus amyloliquefaciens* NBRISN13 (SN13)	Modulating differential transcription in a set of at least 14 genes	Nautiyal et al. (2013)
Barley (*Hordeum vulgare* L.)	*Hartmannibacter diazotrophicus* E19	Increased root and shoot dry weight. ACC-deaminase activity of and lower ethylene content	Suarez et al. (2015)
lettuce seeds	*Azospirillum*	Promoted higher biomass, ascorbic acid content antiox- idant capacity, and a lower browning intensity	Fasciglione et al. (2015)
Brassica napus (canola) and Maize	*Pseudomonas putida* UW4	Modulation of plant protein differential expression and ACC deaminase activity	Cheng et al. (2011)

Table 2 Effects of PGPR on tomatoes growing under stress[Goswami & Deka 2020]

Plant growth-promoting rhizobacteria (PGPR)-induced changes in plant morphological, physiological, and molecular traits under environmental stresses

PGPR	Plant	Changes in plant morphological, physiological, or molecular traits	Reference
Azospirillum brasilense Sp245	Wheat (*Triticum aestivum*)	Increased relative and absolute water content, water potential, and apoplastic water fraction	Creus *et al.*, 2004
Pseudomonas putida H-2-3	Soybean (*Glycine max* L.)	Increased production of gibberellins	Kang *et al.*, 2014b
Phyllobacterium brassicacearum STM196	Oilseed rape (*Arabidopsis thaliana*)	Improved osmotic tolerance as a result of increased abscisic acid (ABA) content which decreases leaf transpiration	Bresson *et al.*, 2013
Achromobacter piechaudii ARV8	Tomato (*Lycopersicum esculentum* cv. F144) and pepper (*Capsicum annuum* L. cv. Maor)	Increased fresh and dry weights of tomato and pepper plants with significantly decreased ethylene production	Mayak *et al.*, 2004b
Paenibacillus polymyxa	Oilseed rape	Expression of drought responsive gene (*ERD15*) and ABA responsive gene in *Arabidopsis* plants	Timmusk and Wagner, 1999
Bacillus sp. KB129	Sorghum (*Sorghum bicolor* var. CSV-15)	Significantly increased relative water content	Grover *et al.*, 2014
Bacillus licheniformis K11	Pepper	Decreased plant ethylene concentration due to sequestration and cleavage of plant produced 1-aminocyclopropane-1-carboxylate (ACC) deaminase	Lim and Kim, 2013

α-Keto as a Carbon Source

Until today, every research on ACCD plant growth promoting bacteria assumed that the ACC is a nitrogen source, while carbon was an external part of the medium. Accumulation of α-Keto was the indicator of ACCD presence. The prospect that PGPR can use ACC as a nitrogen and carbon source, was no tinvestigated. The underlying assumption of this research is that bacteria with such ability would have an advantage over other bacteria as they do not depend on outside carbon sources to survive.

Materials and Methods

Herein a description of our experimental setup aimed at observing the impact of bacteria in soltion simulating the liquids in the rizosphere.

Concentrations

Ammonium concentrations were evaluated in the cultures that degraded AAC with a spectrophotometer. The culture samples were filtered with 0.2-micron filters and mixed with the solution described in the addendum. Using known standards of ammonium, a calibration curve was made, allowing to calculate the unknown concentration.

The α-Keto level in the cultures was measured with an HPLC machine. The samples were filtered with 0.2-micron filters and transferred into a glass vial. Using known standards, a calibration curve was made, allowing to calculate the unknown concentration. Using this data, figures for the different experiments were created combining the change in ammonium and α-Keto over time.

In the current study, the change in biomass wasn't evaluated, as the bacteria grew in clusters. Therefore, conventional methods like OD couldn’t be used.

Bacteria Extraction

Rhizobacteria A14: one gram of soil from the Nahal Zin dry stream bed was added to 50 ml of PAF medium (addendum). This suspension was incubated for 24 hours on a shaker at 150 rotations per minute (rpm) at 25°C. Then, one ml from the sample was transferred to a fresh 50 ml MMDF medium. The MMDF had 300 microliters of 3 mM ACC as a nitrogen source. The sample was subjected to 150 rpm at 25°C was incubated, and then one ml was transferred to a new MMDF medium with ACC. The process repeats three more times. The culture on LB agar plates was then spread and incubated in a 35°C incubator [Figure 1].

Rhizobacteria A14a: From the LB plate, one specific colony was chosen and purified on an LB agar plate. To be sure it was a pure bacterium, a single colony was transferred to a new plate three times daily. [Figure 4].

Zugan rhizobacteria: the ground The Jacob Blaustein Institutes for Desert Research (BIDR) Sede Boker campus provided *Zygophyllum dumosum* with their roots. Soil from the roots area was crushed to powder, and 1 gram was added to the PAF medium. The suspension was incubated on a shaker for a week (as above). After one week, one ml from the old medium was

transferred to the new MMDF (addendum) medium with 3 Mm ACC. In the new medium, the ACC was a carbon and nitrogen source. One ml from the medium was transformed into a new fresh medium once a week over six weeks.

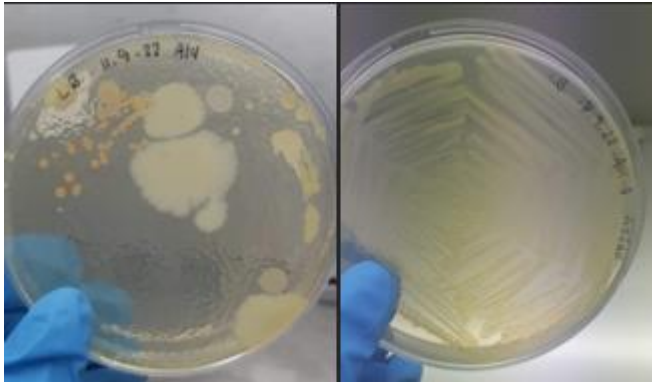

Figure 4: A picture of LB agar with A14 and A14a bacteria. From left to right

Experiment Prep

Fifty ml falcon tubes were filled with 40 ml MMDF with the respective rhizobacteria. All samples were incubated on a shaker in a dark 25°C. and sampled according to a predetermined timetable [Table 1]. At each sampling day, a set of tubs was directly frozen at -20°C to stop additional growth.

Before analysis, the falcon tube was defrosted in warm water and centrifuged at 4000 rpm for 5 minutes. The medium was divided into 2 Eppendorf with 1.5 ml each. The rest of the medium was frozen at -20°C.

Table 1: experiment samples timetable

Time/ Semple	13.3	15.3	17.3	19.3	21.3	23.3	25.3	27.3	29.3	31.3
A14+ACC	V	V	V	V	V	V	V	V	V	V
A14+ammonium+ α-Keto	V	V	V	V	V	V	V	V	V	V
Zugan+ACC	V	V	V	V	V	V	V	V	V	V
Zugan+ ammonium+ α-Keto	V	V	V	V	V	V	V	V	V	V
A14a+ACC	V	V	V	V	V	V	V	V	V	V
MMDF +ACC	V									V
MMDF+ ammonium+ α-Keto	V									V

Results

First Experiment: ACC as Carbon and Nitrogen Source

In the A14 culture incubated on ACC [Figure 5], a minimal increase in ammonium level was observed, with a peak after five days and then a graduate decrease. The α-Keto level also remained minimal during the experiment. The final ammonium concentration of about 0.7mM at the end represents the recovery of 23% of the N from the ACC added.

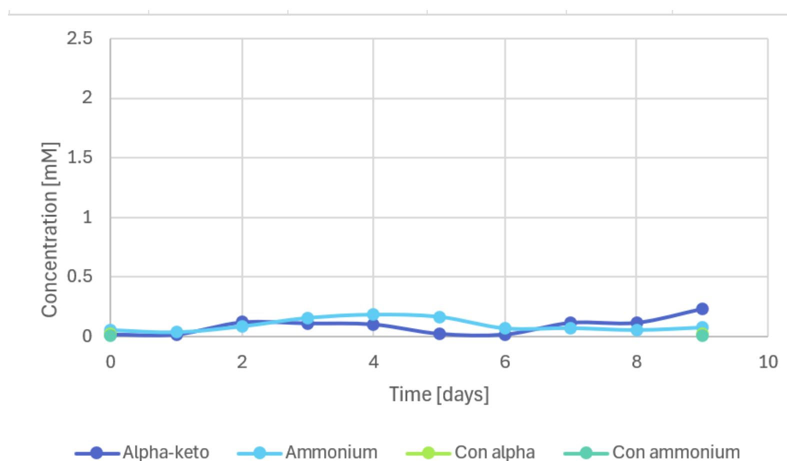

Figure 5: change in ammonium and α-Keto level over time in ACC fed a14 culture.

In the culture enriched from the Zugan rhizosphere [Figure 6], an increase in ammonium level was observed after two days, then decreased, rose to a peak of 1.4 mM after six days, and graduated decreased. On the opposite, the α-Keto level almost 'doesn't change over time. The final ammonium remaining is 47% of the initial ACC added.

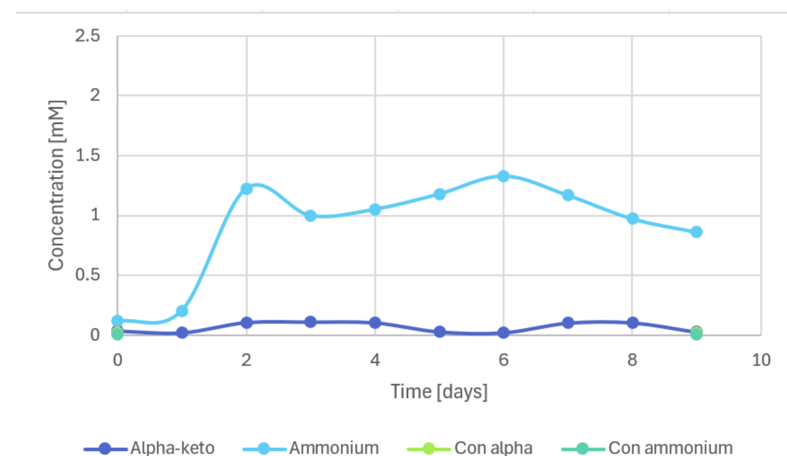

Figure 6: change in ammonium and α-Keto level over time in ACC fed Zugan culture

Likewise, in the A14a culture cultivated on ACC as sole carbon and nitrogen [Figure 7], a late (after six days) increase in ammonium level was observed then decrease and further increase. The final ammonium remained is about 50% of the initial ACC added. On the other hand, the α-Keto level was not accumulating.

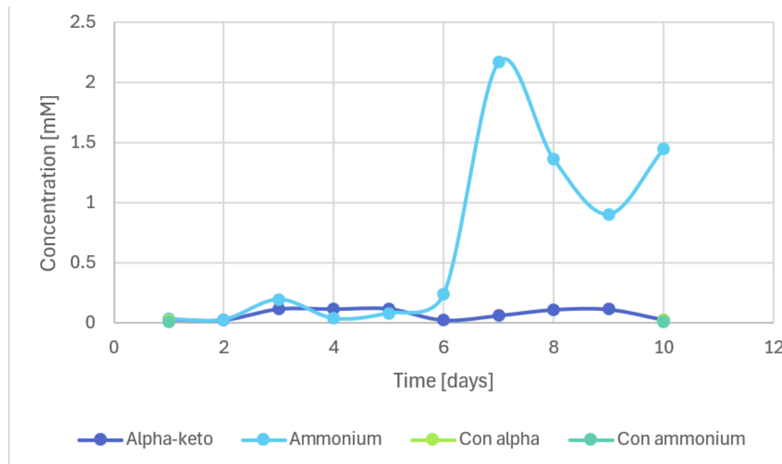

Figure 7: change in ammonium and α-Keto level over time in ACC fed 14a culture

Second Experiment: α-Keto and Ammonium as Carbon and Nitrogen Sources

In the A14 culture cultivated on ammonium and α-Keto [Figure 9], a concurrent graduate decrease in ammonium level was observed with α-Keto. There is 90% utilization of initial ammonium added. Moreover, 94% of the initial amount of a-Keto was utilized as well after ten days of incubation.

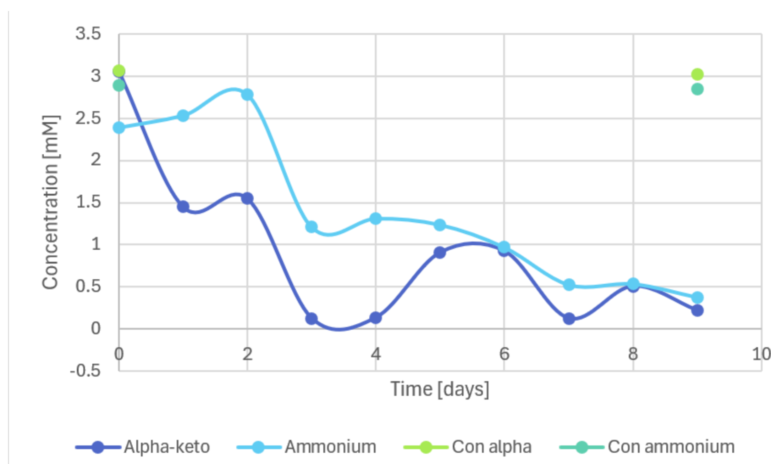

Figure 8: change in ammonium and α-Keto levels over time

With the Zugan culture that was grown on ammonium and α-Keto [Figure 9], a slow decrease in ammonium level was observed. The rate of a-Keto consumption is faster, and after two

days most of it was degraded. After ten days, there is 87% use of initial ammonium and 98% use of the initial amount of the added a-Keto.

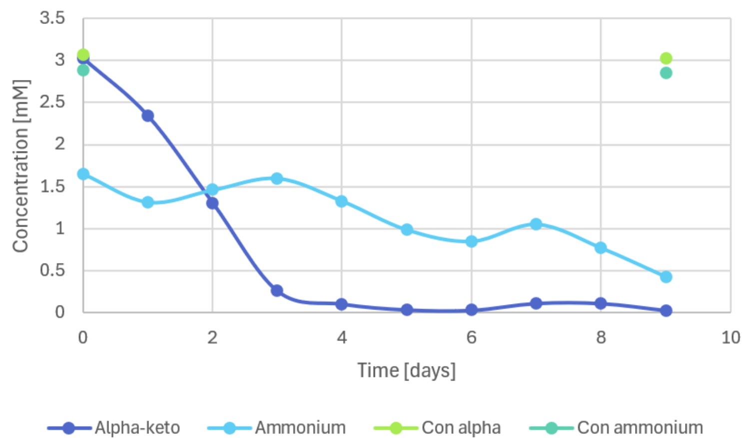

Figure 9: shows the change in ammonium and α-Keto levels over time.

Discussion

The results strongly suggest that the bacteria cultures grew and degraded ACC (aminocyclopropane-1-carboxylic acid) to ammonium and α-Keto (Figs. 8 and 9). In the ACC-fed bacteria, the consumption of ACC can be seen, but we can't be sure the bacteria used in α-Keto as the carbon source. The lack of α-Keto accumulation suggests that it may be further utilized. Observing the experiments of the α-Keto and ammonium-fed bacteria complete the picture and point out that almost 100% of the α-Keto added was consumed. The observation indicates that the disappearance of a-Keto is biological because, in the control treatments, there was no change in the concentrations.

Bacteria that degrade ACC and use it as carbon and nitrogen sources will benefit the plants under salinity stress. The assumption is that this ability provides the bacteria with a competitive advantage in the rhizosphere. Application of these PGPBs will allow irrigation with salty water and treated effluents without harming the plants. The bacteria sustained themself and didn't rely on additional chemical additions, so their land damage would be minimal. That way, we can use brackish water and effluents without concern about damaging plants and crop yield. Using alternative irrigation water types with concurrent application of PGPR will allow for saving fresh water for domestic use in arid environments.

In the first experiments (Figs. 5-7) an increase in ammonium level and a decrease through the end was observed. That observation fits the hypothesis that the bacteria decay the ACC as this

is the only source of nitrogen. There is no need to add more ACC over time. Therefore, it's reasonable to assume that the ammonium level will decrease near the end of the incubation period as the bacteria consume the residual carbon added to the medium or the degradation products of ACC like α-Keto.

Moreover, in the second experiment (Figs. 8,9), there is a decrease in the ammonium lever throughout the incubation period. Mass balance calculation shows 92% use of the initial amount of nitrogen in the ACC consumed. There is no change in the control group. Thus, it can be assumed that the consumption happens because of microbial growth.

In the first experiment (Figs. 5-7), α-Keto changes are barely observed, which contradicts the observation of many other studies. But, due to the change in the ammonium on the same medium, the ACC certainly degraded. So, the accumulation of α-Keto, like the ammonium, is expected, but this is not the case. A change in α-Keto is barely seen. Therefore, it can be suggested that α-Keto is also a carbon source in our cultures.

In the second experiment (Figs 8,9), there is a continuous decrease in α-Keto levels and no decrease in the control. By calculating the change from the initial amount, there is a 95% utilization for the α-Keto by the bacteria. As was hypothesized, it can be concluded that the α-Keto is consumed as a carbon source for microbial growth. The results are novel because so far all ACCD containing PGPB were accumulating α-Keto and thus required external carbon for growth.

Significant of the Research

The examined bacteria, isolated from saline soil and halophytic plant rhizosphere, are capable of degrading ACC, the precursor for ethylene production in the plant, utilizing it as carbon and nitrogen sources. This ability helps disrupt the ethylene signal and, by doing so, allows the plant to thrive under drought and salinity stress. Unlike previous plant growth-promoting bacteria, this bacteria wouldn't need external additives to proliferate in the rhizosphere. Those properties allow better application of PGPB to plants that are irrigated with brackish water and treated effluents. Consequently, shifting saline water-treated effluents to irrigation will increase the availability of freshwater for domestic use.

Future Research

In the future, it is planned to find a way to measure the change in biomass when growing on ACC as sole carbon and nitrogen. The bacteria grow in clusters, so OD isn't possible, and measuring change in protein is not efficient. The plan is to measure the change in the 16S rRNA gene using qPCR. In addition, isolating other salinity-improving traits and antioxidant enzymes such as superoxide dismutase, glutathione oxidase, and catalase will be screened. The next step would be to try growing tomatoes in a greenhouse using brackish water irrigation with and without the bacteria and observe changes in root length and overall biomass yield of the plants. Lastly, different application times for adding the bacteria, whether during germination or after the formation of the first leaves, will be compared.

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Addendum-

Medium

MMDF medium: [1 Liter]

KH_2PO_4 – 4 grams

Na_2HPO_4 – 6 grams

$MgSO_4*7H_2O$ – 0.2 grams

$FeSO_4*7H_2O$ – 100 microliters (sterile)

Trace elements – 100 microliters

PAF medium: [1 Liter]

protease peptone – 10 grams

casein hydrolysate – 10 grams

anhydrous$MgSO_4$ – 1.5 grams

K_2HPO_4 – 1.5 grams

Glycerol – 10 ml

Lb agar: [1 Liter]

Broth - 25 grams

Agar – 15 grams