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RHIZOSPHERE-COMPETENT ACTINOBACTERIAL ISOLATES WITH ACC DEAMINASE ACTIVITY ALLEVIATE SALT STRESS IN TOMATO PLANTS IN THE UAE

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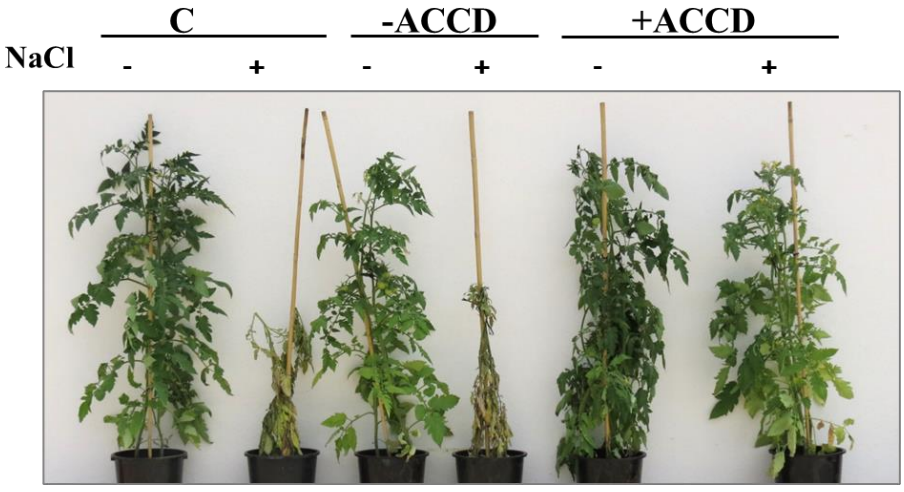

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Cover: Effect of salinity on tomato plants inoculated with ACC deaminase-producing actinobacterial isolate

(Photo: By Alaa Ahmed Abbas Elbadawi)

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Declaration of Original Work

I, Alaa Ahmed Abbas Elbadawi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Rhizosphere-Competent Actinobacterial Isolates with ACC Deaminase Activity Alleviate Salt Stress in Tomato Plants in the UAE*", hereby, solemnly declare that this is the original research work done by me under the supervision of Prof. Synan AbuQamar, in the College of Science at UAEU. This work has not previously formed the basis for the award of any academic degree, diploma, or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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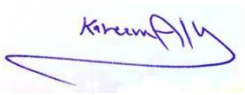
   Signature  Date 8/6/2022

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Abstract

Tomato (*Solanum lycopersicum*) is one of the most popular vegetables in the world, including the United Arab Emirates (UAE). Salinity is a global menace to plant growth and development, causing significant economic losses to tomato and other crop plants. It is well-known that 1-aminocyclopropane-1-carboxylic acid (ACC**)** deaminase (ACCD) can increase plant tolerance to environmental stresses**.** This can be attributed to the increased activity of the enzyme ACCD, which breaks down ACC [the immediate precursor of ethylene (ET)] to ammonia and α-ketobutyrate, to lower the overproduction of ET levels *in planta* and to reduce the damages caused by salt stress. Consequently, the application of beneficial actinobacteria was assessed to alleviate the destructive effects of salt stress on tomato plants. The main objective of this project was to isolate and apply ACCD-producing actinobacteria with increased salt tolerance to relieve any stress on tomato plants cultivated under high salt conditions. Four hundred ninety-one actinobacteria were isolated from the rhizosphere soils of Sweihan area in Abu Dhabi-UAE. *In vitro* screening demonstrated that three actinobacterial isolates produced ACCD, while tolerating up to 8% NaCl. In the greenhouse, the most promising ACCD-producing isolate (referred to as +ACCD isolate) significantly ($P<0.05$) enhanced the growth of tomato seedlings in response to salt stress. This was evident in the increase in the length, fresh and dry weight of the shoots and roots as well as the total chlorophyll content in leaves of tomato plants treated with the +ACCD isolate. The results also showed that the +ACCD isolate reduced the endogenous ACC levels by three- and four-fold in both root and shoot tissues, respectively, compared to those of control and non-ACCD-producing isolate treatments. This study has shed light on the identification of a potential ACCD-producing isolate that can reduce the negative effects of salt stress and enhance salinity tolerance of crop plants, such as tomato, in the UAE and elsewhere.

Keywords: ACC deaminase, actinobacteria, ethylene, plant growth promoting rhizobacteria, soil salinity, *Streptomyces*, tomato.

Title and Abstract (in Arabic)

استخدام وعزل الأكتينوبكتيريا التي تعيش حول الجذور والمفرزة لإنزيم ACC Deaminase للحد من ارتفاع الملوحة على نبات الطماطم في دولة الإمارات العربية المتحدة

الملخص

يعد نبات الطماطم (*Solanum lycopersicum*) أحد أكثر أنواع الخضروات استهلاكاً عالمياً وفي دولة الإمارات العربية المتحدة. تشكل الملوحة، بشكل عام، تهديداً لنمو النباتات، والتي تتسبب في خسائر اقتصادية كبيرة للعديد من المحاصيل بما في ذلك محصول الطماطم. يزيد إنزيم 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (ACCD) من قدرة النباتات على تحمل الإجهاد الفيسيولوجي الناتج عن الظروف البيئية الصعبة. وذلك لأن هذا الإنزيم يقوم بتكسير ACC -المادة الأولية للإيثلين- إلى α-ketobutyrate و ammonia، وعليه تنخفض مستويات هرمون الإيثيلين العالية داخل أنسجة النبات، وبالتالي يتقلص الضرر الناتج عن الإجهاد الملحي على النباتات. ووفقًا لذلك، تم تقييم استخدام الأكتينوبكتيريا النافعة للتقليل من أضرار الإجهاد الملحي على نباتات الطماطم. يهدف هذا المشروع البحثي إلى عزل وتقييم الأكتينوبكتيريا المنتجة لإنزيم ACCD والتي تعتبر ذات تحمل عال للملوحة، ليتم استخدامها في تثبيط الاجهاد الحيوي لمحصول الطماطم المزروعة في ظروف ذات ملوحة عالية. تم عزل 491 نوع مختلف من الأكتينوبكتيريا من ترب ملحية مختلفة من منطقة سويحان في امارة أبو ظبي في دولة الإمارات العربية المتحدة. لقد أثبتت الاختبارات المخبرية أن ثلاثة عزلات من الأكتينوبكتيريا قادرة على إنتاج ACCD، مع تحمل تركيزات ملحية عالية جدا قد تصل إلى 8٪ من كلوريد الصوديوم. أظهرت تجارب البيوت البلاستيكية، أن الأكتينوبكتيريا المنتجة لإنزيم ACCD عملت على تحسين نمو نباتات الطماطم بوجود تركيزات ملوحة مختلفة وبنتيجة ذات دلالة إحصائية معنوية ($P<0.05$) من زيادة في الطول، والوزن لكل من الجذور والسيقان، وفي محتوى الكلوروفيل الكلي في أوراق نباتات الطماطم أيضا. كما أظهرت النتائج أن الأكتينوبكتيريا المنتجة لإنزيم ACCD، عملت على تخفيض مستوى ACC إلى الثلث داخل الجذور، وإلى الربع داخل السيقان، بالمقارنة مع المعاملات التي لم يتم اضافة الأكتينوبكتيريا أو تلك التي تم اضافة الأكتينوبكتيريا غير المنتجة لإنزيم ACCD. تلقي هذه الدراسة الضوء على التعريف بإحدى العزلات المنتجة لإنزيم ACCD، والتي لها القدرة على التقليل من الآثار السلبية للإجهاد الملحي، وزيادة قدرة تحمل المحاصيل (مثل الطماطم) لملوحة التربة في الإمارات العربية المتحدة أو أي منطقة أخرى.

مفاهيم البحث الرئيسية: ACC deaminase، أكتينوبكتيريا، الإيثيلين، البكتيريا المحفزة لنمو النبات، ملوحة التربة، *Streptomyces*، الطماطم.

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Finally, I would like to express my deep sense of gratitude to my beloved parents, for always surrounding me with love, encouragement, and blessings. I owe special gratitude to my siblings and family for always being by my side, for their love, support, and prayers.

Dedication

To my beloved parents and family

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List of Abbreviations

ACC	1-Aminocyclopropane-1-Carboxylate
ACCD	ACC Deaminase
CAT	Catalases
CKs	Cytokinins
CWDEs	Cell-Wall-Degrading Enzymes
EC	Electrical Conductivity
ET	Ethylene
IAA	Indole Acetic Acid
OMYEA	Oatmeal Yeast Extract Agar
PGPR	Plant Growth Promoting Rhizobacteria
PGPA	Plant Growth Promoting Actinobacteria
ROS	Reactive Oxygen Species
SAM	S-Adenosylmethionine
SNA	Starch Nitrate Agar
SOD	Superoxide Dismutase

Chapter 1: Introduction

1.1 Overview

Plant Growth Promoting Rhizobacteria (PGPR) are free-living beneficial bacteria within the soil with great potential in agriculture. As a biotechnological tool, PGPR can be utilized to alleviate abiotic stresses in crop plants, such as red pepper and cucumber (Siddikee et al., 2011; Cho et al., 2015). In general, PGPR comprise of a wide range of bacterial species with various mechanisms of plant growth promotion. This study evaluated the potential of actinobacterial isolates to ameliorate salt-induced stress on tomato seedlings in the UAE. Soil salinity causes severe damage to plants leading to reduced crop growth and yield; thus, threatening food security in the UAE and elsewhere. The outcomes of this study report the findings of novel actinobacterial isolates with the potential to be applied as biofertilizers for salinized areas in the UAE.

1.2 Statement of the Problem

Today, there is an urgent need to explore alternative approaches to feed the growing world's population, which is estimated to reach 9.5 billion in 2050 (Santoyo et al., 2021), creating a threat to global food security in the presence of climate change and other biotic and abiotic stresses. Soil salinity is one of the significant environmental issues that affect over a billion hectares worldwide (Machado & Serralheiro, 2017). Salt stress limits the ability of plants to absorb water and nutrients from the soil. Salt stress further induces the production of the stress ethylene (ET) hormone in plants, which accelerates the rate of damage in tissues, and ultimately results in a huge drop in crop yield. PGPR have a significant role in sustainable agriculture and presents an effective, eco-friendly method to enhance salt tolerance in plants.

1.3 Research Objectives

The overall objective of this research is to evaluate the application of beneficial rhizosphere actinobacteria isolated from the UAE cultivated soils for their role in salinity stress tolerance in tomato plants.

The specific aims are to:

1. Isolate rhizosphere-competent actinobacteria capable of producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase (ACCD) from UAE soils.
2. *In vitro* evaluate the most promising ACCD-producing isolates with high salt tolerance.
3. Determine the response of tomato seedlings grown under normal and saline conditions to inoculation with the rhizosphere-competent ACCD-producing isolate(s) under greenhouse conditions.

1.4 Relevant Literature

1.4.1 Soil Salinity

Plants are subjected to various abiotic and biotic stresses during their life cycle. While abiotic stress refers to flooding, salinity, drought, and heavy metal pollution, biotic stress includes “living” pathogens such as bacteria, fungi, viruses, insects, and weeds and causes plant diseases (Ghosh et al., 2018).

Soil salinity is considered one of the most serious environmental factors limiting the productivity of crop plants, due to their sensitivity to salinity caused by the lowest concentrations of salts in the soil (Ventura et al., 2015). In general, salinity affects crop production in 20% of the total agricultural land around the world and 33% of the arid and semi-arid environments that are grown under irrigation (Mayak et al., 2004). The area of salt affected soils is increasing by rate of 1% annually, covering 833 million hectares in 118 countries (FAO, 2021). According to (Wang et al., 2021) Crop yield losses in saline lands resulted in annual economic losses worth a total US$ 27.3 billion globally.

Soil salinity is measured in terms of electrical conductivity (EC) of the saturation extract (ECe). When the EC of the root zone exceeds 4 deci Siemens per meter (dS/m; ~40 mM NaCl at 25°C), the soil is considered saline (Jamil et al., 2011). At this level of EC, most legume plants are affected by salt stress and exhibit retardation in their overall growth, leading to a reduction in crop productivity. However, vegetable plants are sensitive to salinity at a much lower rate of 1–2.5 dS/m (Vaishnav et al., 2019).

1.4.2 Soil Salinity in the UAE

In the arid and semi-arid areas, including the United Arab Emirates (UAE), the levels of salinity in the soil vary. In the desert areas, where there is no irrigation with saline water, the EC levels are low (2-4 dS/m). However, the salinity level is moderate in the irrigation water coming from wells or groundwater; thus, ranging between 4-8 dS/m. It has also been reported that soil salinity in some groundwaters may reach extreme levels from over 16 up to 50 dS/m, similar to the salinity level of seawater. As a result, irrigation with saline groundwater affects over 90% of the farms in Al Ain city (EAD, 2017). The area of saline lands in the UAE is increasing each year, covering around 39% of the soils in the Emirate of Abu Dhabi (Wiede, 2010; EAD, 2017).

1.4.3 The Causes of Soil Salinity

Salinization can be caused by anthropogenic activities, such as poor agricultural practices, and the recurrent use of poor drainage facilities. It also results from using brackish groundwater for irrigation and from the elongated use of irrigation water (FAO, 2021). Saline soils can be also developed from natural processes, including climate change, rock weathering, and the disposition of either seawater or marine sediments into the land by wind or rain (Paul & Lade, 2014; FAO, 2021).

1.4.4 Effect of Salt Stress on Plants

With the continuation of the natural and anthropogenic causes of salinity, large quantities of ions accumulate in the soil, including sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), carbonate (CO_3^{2-}), sulfate ($SO4^{2-}$) and bicarbonate (HCO^{3-}). All these water-soluble ions are toxic to plants, especially Na^+ and chloride (Cl^-) ions. Their presence in the soil in high quantities may lead to lowering the osmotic potential of the roots; thus, preventing roots from absorbing water from the soil, which eventually inhibits plant growth (Numan et al., 2018).

The exposure of plants to salt stress immediately generates osmotic stress. After a few days, Na^+ ions accumulate in the shoots, creating toxic ionic stress and resulting in a nutrient imbalance in plants (Vaishnav et al., 2019). Salinity stress can have a direct impact on major cell processes, such as protein synthesis, photosynthesis, and energy metabolism

(Gamalero & Glick, 2012). The morphological symptoms of the damage are apparent in the reduction of leaves surface, defoliation, chlorophyll content, and the dry weight of the plant. It also affects seed germination, flowering, and fruiting patterns leading to a drastic decrease in crop yield (Ghanem et al., 2009).

Most importantly, the primary effect of salinity leads to the overproduction of reactive oxygen species (ROS), specifically H_2O_2, O^{-2}, and O^{-}. The accumulation of ROS compounds is toxic to plant cells due to the damage they cause to DNA, RNA, and proteins (Habib et al., 2016). To overcome the effect of ROS compounds, plant cells produce multiple antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD). These enzymes can scavenge the ROS compounds and keeping them at very low levels; thus, their production is often used as an indicator of oxidative stress in plants (Paul & Lade, 2014).

1.4.5 Plant Response to Abiotic Stress

Plant response to changes in their environment or internal stimuli is often controlled by plant hormones. Phytohormones are crucial in the adaptation ability of plants, as they control multiple functions. For example, plants tend to overproduce many hormones such as auxins, cytokinins (CKs), abscisic acid and ET in response to abiotic stresses (Forni et al., 2017).

Auxins play a major role in cell proliferation, differentiation, and elongation of root tissues (Spaepen et al., 2007). Although the process of their synthesis in plants is unknown, auxin can be synthesized from tryptophan and transported throughout the plant via the phloem where they create concentration gradients and accumulate in various tissues (Duca et al., 2014). CKs play a major role in facilitating cell division and stimulating protein synthesis, especially in plant shoot tissues. CKs are produced in the tips of the roots and then transferred to the shoot system through the xylem, where they promote plant growth (Forni et al., 2017). In addition to the negative effect of salt stress on many organs in plants, the levels of auxins and CKs are substantially reduced leading to decreased branching of the roots and the overall plant growth (Trifunović-Momčilov et al., 2021).

ET is a gaseous phytohormone that plays a role in the transduction of the signal from the recognition of salt stress to the initiation of physiological responses (Mayak et al., 2004). ET is also crucial for plant growth and development when it is produced in low concentrations (~10 µg/L). It regulates abscission, seed germination, flowering, fruit ripening, and elongation of shoots and roots (Nascimento et al., 2016). In higher plants, ET is synthesized from S-adenosylmethionine (SAM). SAM gets converted to ACC by the enzyme ACC synthase. Afterward, ACC is oxidized by the enzyme ACC oxidase to form ET along with H_2O, HCN and CO_2 (Swarnalakshmi & Ramakrishnan, 2016; Ghosh et al., 2018). Another compound resulting from the reaction of ACC synthase is methylthioadenosine, which is then used to produce methionine in the following steps in the so-called "Yang cycle" (Figure 1).

Upon the exposure of the plant to salt stress, ET is synthesized in two peaks. The first smaller peak (10 µg/L) triggers the transcription of genes required for defense against stress. The second larger peak (~25 µg/L) is referred to as "stress ET", causes retardation affecting seed germination and root elongation, eventually leading to senescence and death of the plant (Forni et al., 2017). Mayak et al. (2004) have demonstrated that the increase in ET levels is proportionally related to the increase in salt stress. ET levels can be significantly elevated when tomato seedlings were exposed to 207 mM NaCl, which leads to an overall deterioration in growth. Therefore, the approaches to combat the effect of salinity stress on plants must focus on lowering the levels of the endogenous ET in plants.

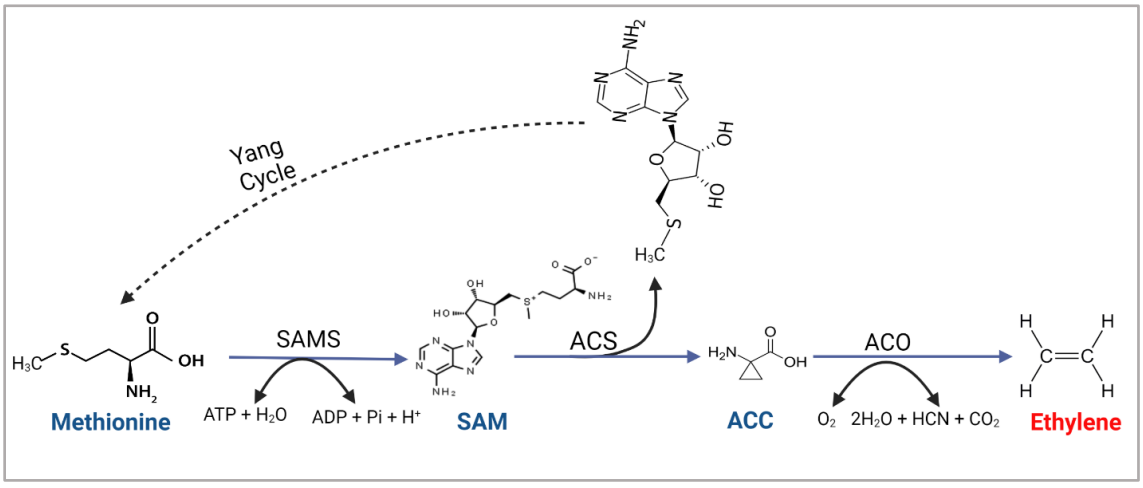

Figure 1: Yang cycle of ethylene synthesis. Adapted from (Houben & Van de Poel, 2019)

1.4.6 Plant Growth Promoting Rhizobacteria (PGPR)

The use of PGPR may represent an effective, environmentally friendly, and safe management option to alleviate salt stress. PGPR is a group of plant beneficial bacteria that exist in the rhizosphere (the narrow region of the soil) that is rich in roots exudates and is utilized by bacteria to support their growth. Many rhizobacteria may colonize the outer root surfaces as rhizosphere-competent PGPR (Beneduzi et al., 2012). In addition, bacteria inhabiting the internal plant tissues, called endophytic PGPR (Glick, 2013). Each plant secretes a unique mixture of compounds through their root exudates that attract different PGPR. Thus, it leads to the presence of different PGPR within and surrounding the roots of the same plant (Glick & Gamalero, 2021).

PGPB isolates from the endosphere and rhizosphere have been found to stimulate growth of plants directly by regulating phytohormones and increasing nutrient uptake or indirectly via eliminating plant pathogens (Gamalero & Glick, 2012). However, endophytic PGPR have a better effect on plant growth and yield; which is probably because endophytic PGPR are not in direct interaction with harsh conditions in soils (Carvalho et al., 2017; Khare et al, 2018).

Many PGPR strains of the genera *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Bacillus* and *Streptomyces* have been identified to be effective in growth promotion and salinity tolerance in various plant species (Vinayarani & Prakash, 2018; Mathew et al., 2020; El-Tarabily et al., 2019; 2020; 2021a). As reported by Gupta & Pandey (2019), *Aneurinibacillus aneurinilyticus* and *Paenibacillus* sp., were isolated from the rhizosphere of garlic (*Allium sativum*) plants. The two strains inoculated into the rhizosphere of the French bean (*Phaseolus vulgaris*) successfully reduced the levels of endogenous ET to 40% and promoted the growth of seedlings under drought and salt stress conditions. In the present study, however, the focus was on isolating candidates of plant growth promoting actinobacteria (PGPA) to determine their effect on growth promotion and high salinity tolerance in tomato, like those found in the arid environments and the UAE (Goodfellow & Williams, 1983).

1.4.7 Actinobacteria

Actinobacteria are a unique group of Gram-positive bacteria. They are unicellular and have peptidoglycan cell walls like bacteria, but they possess fungus-like substrates and aerial mycelia (Sharma et al., 2014). Like other soil bacteria, actinobacteria grow at temperatures of 25-30°C. However, some actinobacteria can survive in temperatures as high as 60°C. Actinobacteria are characterized with a high guanine and cytosine (G+C) content in their genomes, which has been linked to adaptation to higher growth temperatures (Bohlin et al., 2017). Ecologically, actinobacteria exist as aerobic microorganisms, but others are found in extreme environments *i.e.*, chemoautotrophic capable of utilizing inorganic compounds as a source of carbon and energy (Barka et al., 2016). Actinobacteria are mostly abundant in the soil in densities of 10^6 - 10^9 cells/g of soil. The genus *Streptomyces* comprises 95% of the actinobacteria isolated from the soil. *Streptomyces* are naturally active in the dry, arid soil microflora and can be easily isolated. Actinobacteria can also be found in other habitats, including fresh water and marine environments; hence, they vary in their environmental requirements for optimal growth (Sharma et al., 2014).

1.4.7.1 Identification and Classification of Actinobacteria

Actinobacteria can be identified to the genus level according to the presence/absence of the morphology of substrate and aerial mycelia. The aerial mycelia have various morphological differences that range from simple coccoid (*e.g. Nocardia* spp.), to branched hyphae (*e.g. Streptomyces* spp.). Both substrate and aerial mycelia can be found in a variety of colors: black, brown, blue, and yellow among others. The substrate mycelium can be differentiated into aerial hyphae that develop into asexual reproducing spores (Figure 2) (Li et al., 2016a). The shape of spores is another characteristic that is used in the classification of actinobacterial species. Following their production from aerial hyphae, the germination spores develop into substrate mycelium. In addition, spores allow actinobacteria to survive in harsh environments in dormant states (Sharma et al., 2014). Those are molecular structures formed through the oxidative polymerization of phenolic compounds and are found in different colors. Today, the most significant method for the

identification of actinobacteria is based on the genomic sequencing and/or the 16s rRNA gene sequencing (Li et al., 2016b).

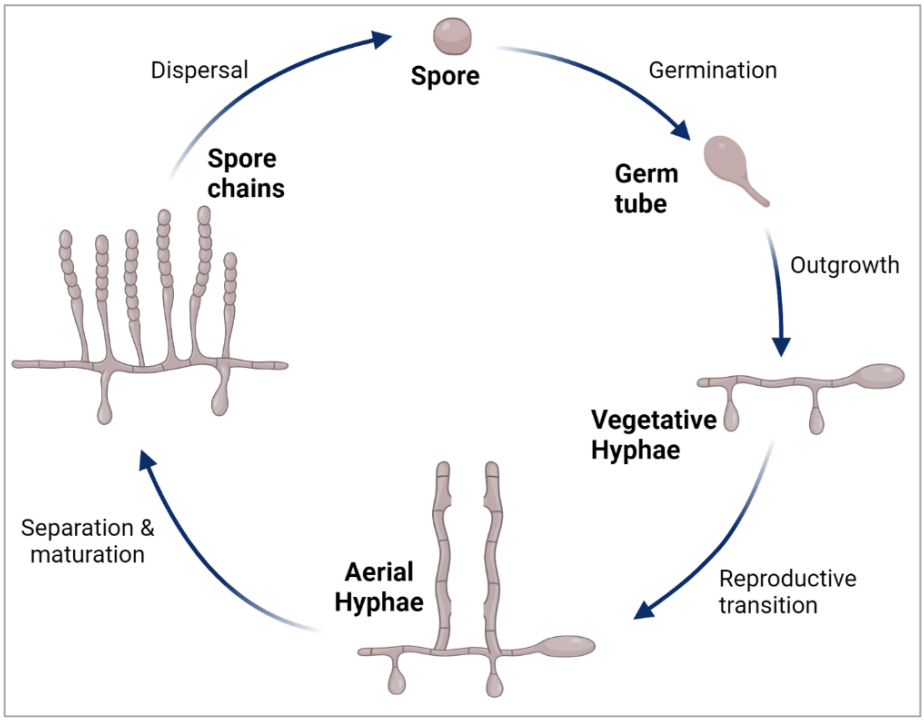

Figure 2: The life cycle of sporulating mycelia. Adapted from (Prudence et al., 2020)

1.4.7.2 Isolation of Actinobacteria from Soil Rhizosphere

The soil environment contains different (actino) bacterial species and fungi species (Sapkota et al., 2020). The soil samples (5-30 cm depth) should be collected from areas rich with actinobacteria and are suitable for the specificity of the research *i.e.*, salt lakes and alkaline soils and stored in sterile bags (Jiang et al., 2016). Proper soil pretreatment should take place to minimize the growth of Gram-negative bacteria. Such methods of air-drying for 5 days or drying/heating at 120^{o}C can be done on these samples (Nonomura & Ohara, 1969). For the isolation of non-*Streptomycete* actinobacterial colonies, other methods are used to lower the dominance of the *Streptomyces* spp. Approaches, like using *Streptomyces* phages, can be included (Kurtböke et al., 1992). In addition, the composition of the isolation media should favor the growth of actinobacteria over other microbes. Antifungal compounds such as nystatin and cycloheximide are usually added to the

isolation media to inhibit the growth of yeast and fungi. To isolate actinobacteria with increased tolerance to high salt, resistance to alkalinity, heat, antibiotics and other unique physiological properties, the isolation media can be adjusted to support their growth (Jiang et al., 2016).

1.4.7.3 Applications of Actinobacteria

Actinobacteria are of great biotechnological, medical, and economical importance, due to their ability to produce secondary metabolites (Arasu et al., 2016). Over 7000 bioactive compounds (mostly antibiotics) have been isolated from *Streptomycetes* in particular. This has classified them as the primary antibiotic-producing organism. Some of the structural antibiotic classes produced by actinobacteria include tetracycline, lactam (cephalosporins) and aminoglycosides (streptomycin and kanamycin) (Anandan et al., 2016).

Actinobacteria are also a source of enzyme inhibitors used in treating cancer and enhancing the immune response (Barka et al., 2016). Moreover, they can produce a wide range of enzymes such as lipases that are used in diagnostics and pharmaceutical research. Amylase, which is used in the food industry and paper manufacturing, is an enzyme produced by actinobacteria (Abdulkhair & Alghuthaymi, 2016).

1.4.8 Actinobacteria Acting as PGPR

PGPA can stimulate plant growth directly through several mechanisms: Production of auxins such as indole acetic acid (IAA), solubilization of inorganic phosphorus (P), nitrogen (N) fixation, providing roots with iron (Fe) by producing Fe chelators such as siderophores and production of the enzyme ACCD (Mayak et al., 2004).

1.4.8.1 Production of IAA

Auxin is a phytohormone essential for many metabolic processes during plant growth and development. IAA is one of the major auxins that act on regulating plant growth (Numan et al., 2018). IAA helps in elongating the lateral roots of plants by enhancing root cell proliferation; hence, increasing the nutrient uptake. This type of auxin can function in leaves and fruit production (Ghosh et al., 2018).

IAA is also produced in microorganisms, mainly actinobacteria, in which it plays a crucial role in spore germination and cell differentiation (Numan et al., 2018). IAA-producing actinobacteria can either biosynthesize IAA through the tryptophan-dependent pathway or the tryptophan-independent pathway (Duca et al., 2014).

The endogenous IAA synthesized by plants along with the IAA produced by actinobacteria stimulates root elongation, ET synthesis and ACC production, making the IAA-producing actinobacteria a significant plant growth promoter (Wahyudi et al., 2019). Anwar et al. (2016) isolated 98 actinobacteria from tomato and wheat plants, and three isolates produced the highest concentrations of IAA. At 500 µg/ mL L-tryptophan, *Streptomyces nobilis* WA-3 produced 79.5 µg/ mL IAA, whereas *Streptomyces kunmingenesis* WC-3 and *Streptomyces enissocaesilis* TA-3 synthesized 79.23 and 69.26 µg/mL IAA, respectively. *S. nobilis* WA-3 along with the other IAA producers were applied as a PGPA on wheat seeds. It has been found that *S. nobilis* WA-3 can enhance shoot length by 65%, root length by 81% and the fresh and dry weights of the plant by 85% compared to control plants.

1.4.8.2 Production of ACCD

PGPR may promote plant growth directly through the production of ACCD. This enzyme reduces the elevated levels of endogenous ET in plants that are overexpressed as a response to salt-induced stress (Glick et al., 1998; Mayak et al., 2004; El-Tarabily, 2008). ACCD hydrolyzes ACC, the direct precursor of ET into ammonia and α-ketobutyrate, which can be used by the bacteria as nitrogen (N) and carbon (C) sources. In microorganisms, the ACCD enzyme was first reported in yeast (*Hansenula saturnus*; Honma & Shimomura, 1978). The production of ACCD has also been reported in PGPR, *Pseudomonas* (Li et al., 2011) and *Bacillus* (Kumar et al., 2014) as well as actinobacteria (El-Tarabily, 2008). ACCD is localized in the cytoplasm of the microorganisms; therefore, it requires the secretion of its substrate ACC in plant tissues to be hydrolyzed (Glick et al., 2007).

The potential ability of ACCD-producing PGPR to lower Et levels *in planta* was first suggested in the model reported by Glick et al. (1998). In this model, the secreted nutrients and tryptophan from plant roots recruit PGPR to bind to the surfaces of the tissue.

The bacteria then produce IAA that along with the endogenous IAA, either stimulate root elongation or synthesize ACC. A portion of this ACC is exuded and taken up by the ACCD-producing bacteria to be cleaved into ammonia and α-ketobutyrate. This directly reduces the level of endogenous ET; thus, enhancing plant growth (Figure 3).

Several studies have reported the use of salt-tolerant actinobacteria to not only limit the negative effect of salt stress on plants but also to promote growth. The *Streptomyces* sp. strain PGPA39 that exhibited ACCD enzyme activity with high IAA levels, P-solubilization and tolerance to 1 mol of NaCl enhanced tolerance of salinity in cherry tomato (*Solanum lycopersicum*) under greenhouse conditions (Palaniyandi et al., 2014). The same strain enhanced the overall growth of seedlings up to 60 days. Inoculated-tomato seedlings with *Streptomyces* sp. PGPA39 further increased the total dry weight, chlorophyll content and proline levels compared to control plants. In another study by Albdaiwi et al. (2019), nine halotolerant bacterial isolates possessing ACCD activity also enhanced the salinity tolerance of wheat (*Triticum turgidum*). The bacterial isolates belonging to *Firmicutes*, *Proteobacteria* and actinobacteria phyla, showed plant growth-promoting traits including auxin and siderophore production and inorganic P-solubilization. The PGPR strains also increased seed germination and root length in the wheat seedlings.

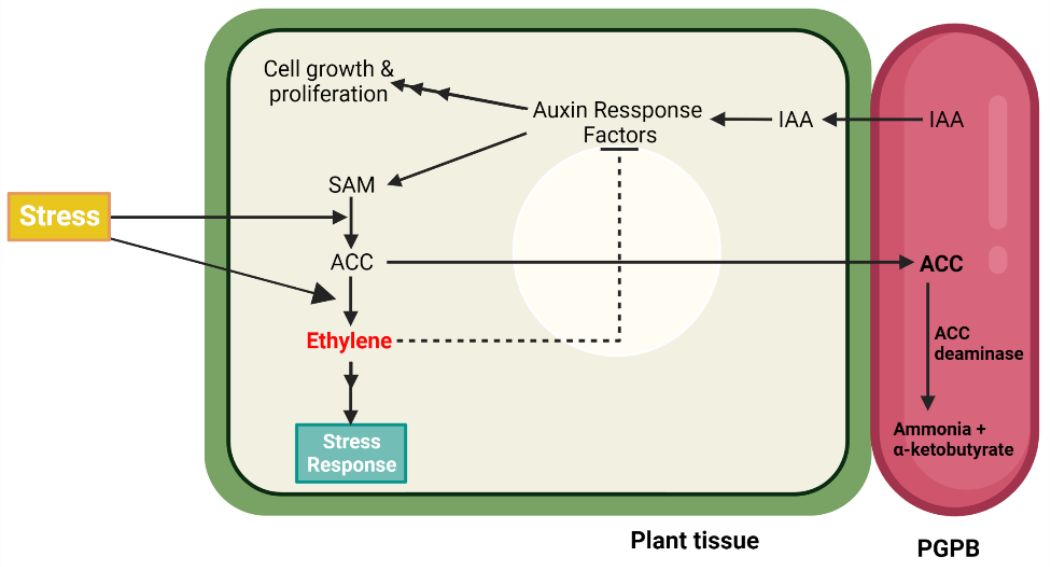

Figure 3: Glick model of plant growth promoting rhizobacteria (PGPR). Adapted from (Orozco-Mosqueda et al., 2020)

1.4.8.3 Phosphate Solubilization

P is one of the essential macronutrients, that is required for plant growth and development. In the soil environment, it is mostly found in its inorganic form, where it forms insoluble mineral compounds with other ions. It is also present in the soil in an organic form that is degradable by microorganisms. However, the soluble, free P in soil is insufficient for supporting the growth of plants (Numan et al., 2018). PGPR with P-solubilizing abilities can provide soluble phosphate to plants, which can easily be taken through their root systems. The process by which PGPBs solubilize P involves the synthesis of some organic acids such as oxalic acid and citric acid (Nimaichand et al., 2016). These acids chelate the cations bound to the phosphate through their hydroxyl groups, converting P into the soluble form (Santoyo et al., 2021). Soumare et al. (2021) have isolated four P solubilizing actinobacteria belonging to *Streptomyces* and *Nocardiopsis* genera. Under low-P conditions all the isolates demonstrated high efficiency in P-solubilization. Under greenhouse conditions, the inoculation with these strains also resulted in a significant increase in acid and alkaline phosphatase production in the maize rhizosphere. The *in vivo* results showed that these PGPA strains improved the growth and yield of maize (*Zea mays*) plants, particularly, *Streptomyces griseorubens* and *Norcardiopsis alba* which enhanced the yield by 126.91% and 135.33% over the positive controls.

1.4.8.4 PGPA as Biological Control Agents

Actinobacteria can support plant growth indirectly by reducing the growth inhibitory effects of pathogenic attacks by utilizing their antibacterial activity (Celador-Lera et al., 2018). Streptomycetes have been widely applied as active biocontrol agents, effective against many pathogenic fungi (Saeed et al., 2017; Kaur et al., 2019; Al Raish et al., 2021; Al Hamad et al., 2021; Alblooshi et al., 2022; Kaur & Manhas, 2022). Kamil et al. (2018) isolated three actinobacterial isolates from the soils of the UAE that successfully inhibited the pathogenic fungus *Lasiodiplodia theobromae*, the causative agent of mango dieback disease. The three isolates: *Micromonospora tulbaghiae* UAE1, *Streptomyces cavourensis* UAE1, and *Streptomyces samsunensis* UAE produced extracellular cell-wall-degrading enzymes (CWDEs) and diffusible antifungal

metabolites. The antagonistic effect of all isolates was assessed *in vivo* under greenhouse conditions. Although all isolates significantly suppressed the fungal growth and reduced the disease symptoms on mango seedlings, *S. samsunensis* exhibited the greatest effect among the three isolates. Kaari et al. (2022) have also reported a *Streptomyces* strain UT4A49, that significantly inhibited phytopathogen *Ralstonia solanacearum* under greenhouse conditions. The antagonistic effect of *S.* UT4A49 was attributed to the production of the bioactive compound, 2,4-Di-tert-butylphenol, along with ammonia, protease, cellulase, and amylase.

1.4.9 The Importance of Tomato Crop Worldwide

In the present study, tomato was used as a model plant for investigating the growth promotion and salinity tolerance by actinobacteria isolated from the UAE rhizosphere soils. Tomato, which belongs to the Solanaceae family, is the second most-consumed vegetable worldwide and is considered a highly valuable agricultural and economic product (Rao et al., 2020). It is mainly cultivated in tropical and temperate climates, specifically in China, India, Turkey, Spain, and the USA, as the top five countries in tomato production (FAO, 2019). Globally, the production of tomatoes has reached 182 million tonnes in 2019, in a surface area of almost 5 million hectares. Whereas in the UAE, 63042 kg of tomato were produced in 2020 in an area of 684 hectares (FAOSTAT, 2020). Tomato has high nutritional values as it contains vitamins A, C and B complex as well as Fe and carotenoids (Domínguez et al., 2020). It is also considered a low-calorie vegetable with 20 calories per fruit. Tomato is a fast-growing, brittle tree, reaching between 3.0-5 ft in height (Fanasca et al., 2006). The salinity of the irrigation water negatively affects seed germination and flowering and inhibits fruit development in tomato, causing significant economic losses (Ghanem et al., 2009).

Chapter 2: Methods

2.1 Soil Sample Collection and Treatment

Forty-three soil samples were collected from the rhizospheres of different halotolerant plants including *Atriplex leucoclada*, *Sesuvium verrucosum*, *Portulaca oleracea*, *Zygophyllum mandaveli*, *Amaranthus graecizans*. The soil samples were collected from Sweihan area in Abu Dhabi-UAE (24°24'09.2"N 55°05'42.3"E). Sweihan is an agricultural area, rich in private farms irrigated with ground water. However, the groundwater in this area is characterized by high salinity (Al-Alawi, 2014).

To lower the numbers of the bacteria other than actinobacteria, soil samples were air-dried for 4 days at 25°C (Williams et al., 1972). Samples were then sieved to eliminate plant debris and pebbles and stored in sterile containers for further experiments. In order to minimize the growth of Gram-negative bacteria and to isolate non-*Streptomycete* actinobacteria, heat treatment technique was applied to soil samples, of which 10 g were dry-heated in the oven at 120°C for 1 hour (Nonomura & Ohara, 1969).

2.2 Isolation and Culture of Actinobacteria

The soil dilution method was used to isolate actinobacteria from the soil samples. Ten grams of each soil sample were mixed with 90 mL of deionized water and placed in the shaker for 30 minutes. The soil solutions were serially diluted to 10^{-2}-10^{-5}. From each dilution, 0.2 mL aliquots were spread into inorganic Starch Nitrate Agar (SNA; Küster, 1959). Three replicates were made for each dilution and the plates were kept in dark at 28°C for 7-10 days. For the dry-heated soil samples, the same technique was applied and serial dilutions to 10^{-1}-10^{-3} were prepared. Aliquots (0.3 mL) were spread on SNA medium amended with 50 µg/mL cycloheximide (Sigma–Aldrich).

After the completion of the incubation period, colonies of actinobacteria were selected and isolated based on their morphology and were streaked on oatmeal yeast extract agar (OMYEA) plates (Küster, 1959). Actinobacterial cultures were repeatedly streaked on OMYEA to obtain pure bacterial colonies; and were maintained for 5 months with serial subculture and were preserved at 4^{o}C.

2.3 Assessment of Tolerance of the Isolates to Different NaCl Concentrations

All isolated actinobacterial cultures were tested for their ability to tolerate salt on SNA media prepared with 40 g/L (4%) and 80 g/L (8%) of NaCl in triplicates and incubated in dark at 28°C for 7 days (Williams et al., 1972). The cultures that presented strong growth on SNA containing 4% NaCl were considered halotolerant as suggested by (Sharma et al., 2016); thus, they were applied to SNA containing 8% NaCl.

2.4 Qualitative Determination of ACCD Production

All the salt-tolerant actinobacteria were screened qualitatively for their PGP characteristics. The production of ACCD was tested using Dworkin and Foster's (DF) salts minimal agar medium (Dworkin & Foster, 1958). Five-day-old actinobacterial isolates were streaked on the DF medium amended with either 2 g of $(NH_4)_2SO_4$ (control) or 3 mM ACC (Sigma) l^{-1}, as a sole nitrogen (N) source. The salt medium was autoclaved prior to adding the heat-labile ACC-filter sterilized through Millipore membranes (pore size 0.22 µm, Millipore Corporation, MA, USA). The plates were incubated in dark at 28°C for 7 days. The isolates that grew and sporulated on the DF-ACC medium were considered as ACCD producers, due to their utilization of ACC in the medium.

2.5 Qualitative Evaluation of P-solubilization

The efficiency of the isolates to solubilize the inorganic and insoluble phosphate is evaluated using Pikovskaya's agar medium (Pikovskaya, 1948). The medium was amended with bromophenol blue as an indicator of P-solubilization. The appearance of white clear zones around the actinobacterial cultures indicates the solubilization of P.

2.6 Identification of the Actinobacterial Isolates

The most promising ACCD-producing actinobacterial isolate (+ACCD) and the non-ACCD-producing isolate (-ACCD isolate) were identified based on cultural, morphological, and physiological properties outlined by Locci (1989), which include the presence or absence of aerial mycelia, the formation of spores and substrate mycelia.

2.7 Preparation of Actinobacterial Inoculum for *In Vivo* Experiments

Liquid inocula of the +ACCD and -ACCD isolates were prepared for soil application in the greenhouse experiment. Isolates were cultured in ISSB and shaken for 8 days at 250 rpm at 28°C in the dark in an orbital shaker incubator. The concentration of actinobacteria in each sample was adjusted to 10^8 CFU/mL. A volume of 100 mL inoculum of each of the two actinobacterial isolate was applied to the seedlings as a soil drench. Noncolonized ISSB broth that had been autoclaved twice served as the control.

2.8 Effect of the Most Promising Isolates Under Greenhouse Conditions

The effectiveness of the +ACCD isolate in enhancing the salinity tolerance of tomato plants (*Solanum lycopersicum*) cultivar Castlemart II grown under saline conditions was further evaluated *in vivo* in the greenhouse. In this study, seedlings were subjected to no salt stress (0 mM NaCl), moderate (60 mM NaCl), and extreme (120 mM NaCl). Salt treatments were applied by pouring 100 mL NaCl to each pot.

The treatments used for this experiment were as follow:

1. Control: Tomato seedlings without (0 mM NaCl), or with salt stress (60 and 120 mM NaCl).
2. Tomato seedlings with the -ACCD isolate without (0 mM NaCl), or with salt stress (60 and 120 mM NaCl).
3. Tomato seedlings with the +ACCD isolate without (0 mM NaCl), or with salt stress (60 and 120 mM NaCl).

For each treatment/group, three pots (each containing two seedlings), arranged in a completely randomized design, were used. The treatments were administered over 12 weeks in two phases. In the first phase, the actinobacterial isolates suspensions were applied once a week for 4 weeks in total. While in the second phase, the salinity treatment (60 and 120 mM NaCl) was applied to the seedlings two times a week for 2 weeks. All the control and inoculated seedlings were maintained under controlled greenhouse conditions of at 27°C±3°C.

2.8.1 Morphological and Physiological Analysis of the Tomato Plants

By the end of the experiment (After 12 weeks), the plants and fruits were harvested. The following growth parameters of shoots and roots were determined: length (cm), fresh weights (g), Shoots and roots were oven-dried at 70°C for three days before recording the dry weights (g). Measurements of number of leaves, number of fruits, weight of fruits (g) were also recorded.

2.8.2 Extraction of Photosynthetic Pigments and ACC From the Tissues of the Tomato Plants

Endogenous ACC was extracted from the tissues of the shoots and roots using the method described by Lizada and Yang (1979). ACC was derivatized by adding phenylisothiocyanate (Sigma). As described by Lanneluc-Sanson et al. (1986), HPLC chromatograms were created by injecting 10 µL of the resulting phenylthiocarbamyl-ACC samples dissolved in acetonitrile onto a 10- µL reverse phase column (Waters Associates Bondapak C18, 4 mm × 30 cm) in a Waters Associates liquid chromatograph equipped with a differential UV detector set at 254 nm. A total of eight duplicate samples were examined.

Total chlorophyll content was determined spectrophotometrically based on the standard method of Holden (1965). To measure chlorophyll concentrations as mg/g fresh weight, 500 mg of fresh samples were coarsely chopped and maintained in a 50 mL conical flask containing 25 mL of 80% acetone. For 24 hours, these flasks were sealed with cork and kept in the dark, then the mixture was centrifuged for 15 minutes at 5000g. After decantation, the supernatant volume was increased to 40 mL using 80% acetone. The optical densities of chl a and chl b at 663 and 645 nm wave lengths were measured using a Shimadzu UV-2101/3101 PC scanning spectrophotometer (Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan). The following equations were then used to calculate the total chlorophyll content:

Total chlorophyll (mg/g) = 20.2 × OD645 + 18.2 × OD663 × V/[1000 × W] (Arnon, 1949).

2.9 Statistical Analysis

All the results from the *in vivo* study were analyzed using one-way ANOVA followed by Tukey's test at a significance level of 5%. The SPSS software was used to conduct all statistical analyses. The experiments were carried out three times, with the mean and standard deviation determined using Microsoft Excel 2016.

Chapter 3: Results

3.1 Isolation of Actinobacteria from Rhizosphere Soils

A total of 597 actinobacteria were isolated from all soil samples tested with serial dilutions on SNA media (Figure 4). All isolated actinobacteria were further cultured on OMYEA to determine their morphological characterization. The isolated actinobacteria were selected based on their unique morphology mostly as chalky and powdery colonies.

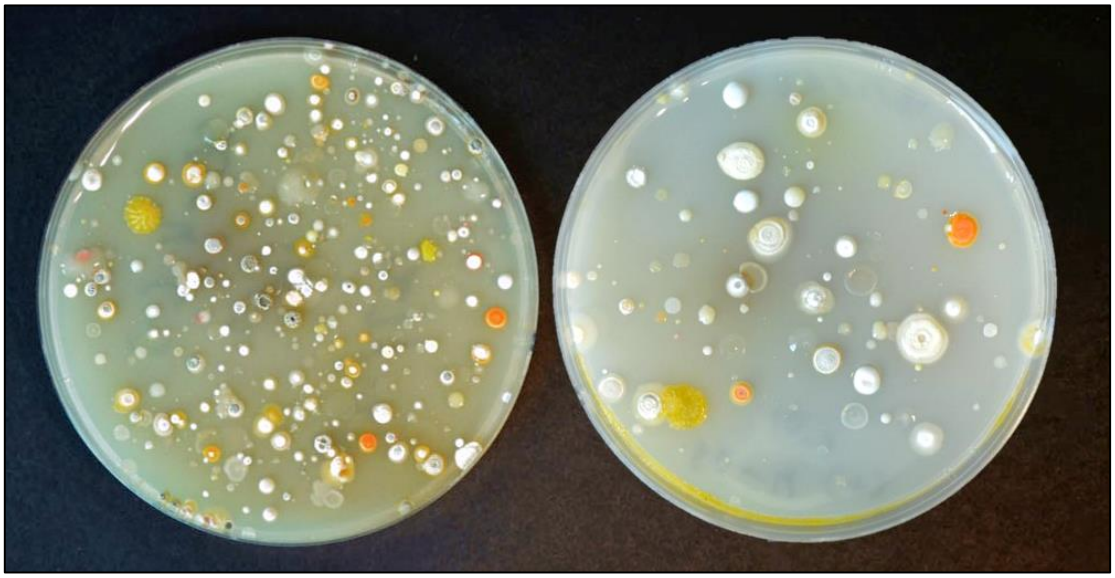

Figure 4: Colonies of actinobacteria isolated from the soil rhizosphere on (SNA) medium. Actinobacteria isolated from soil samples treated with (Right) and without (Left) heat (120°C)

3.2 Assessment of the Salt-Tolerance Ability in the Actinobacterial Isolates

All actinobacterial isolates were tested for their ability to tolerate 4% and 8% NaCl on SNA media (Figure 5). A total of 243 isolates showed tolerance to 4% NaCl, among which 47 isolates were able to tolerate up to 8% NaCl. For example, isolates (Sa-4, and Sa-6) tolerated up to 8% NaCl, while isolate (Sa-2) tolerated a salinity level of 4% only (Figure 5).

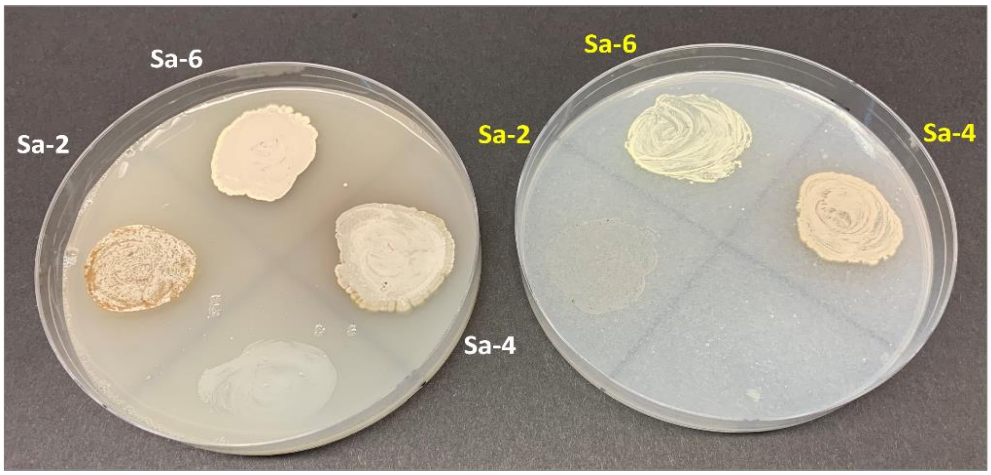

Figure 5: Salinity tolerance of actinobacterial isolates. Three isolates (Sa-2, Sa-4, and Sa-6) exhibited growth on SNA media supplemented with 40 g/L of NaCl (Left). Isolates Sa-4, and Sa-6 only were tolerant to 80 g/L of NaCl (Right)

3.3 *In vitro* Evaluation of P-solubilizing Actinobacteria

The salt-tolerant actinobacterial isolates were cultured on Pikovskaya's medium (Pikovskaya, 1948) to identify their ability to solubilize P. The development of the white halo zone around the isolates indicated their ability to solubilize P. Of all the halotolerant actinobacterial isolates, 14 isolates were P-solubilizers (Figure 6).

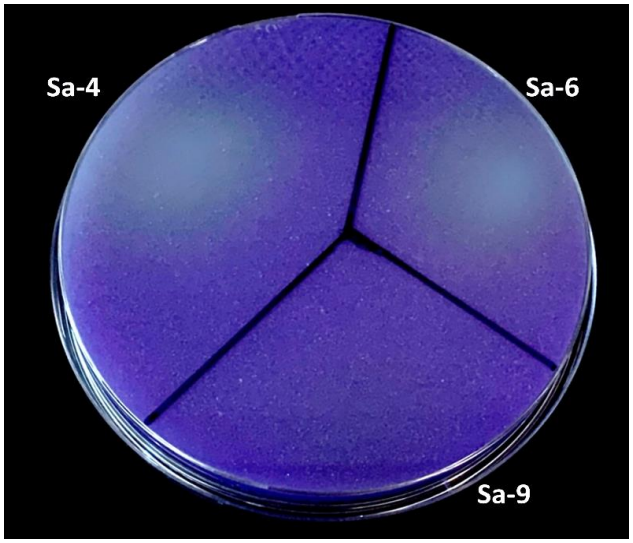

Figure 6: Qualitative assessment of P-solubilization on Pikovskaya's medium. Isolates Sa-2, Sa-4 exhibiting white halo zones on Pikovskaya's media, indicating P-solubilizing activity

3.4 *In vitro* Assessment of ACCD Production by Actinobacterial Isolates

The salt-tolerant isolates were tested for their ability to produce the enzyme ACCD. The test was conducted using the DF-$(NH_4)_2SO_4$ (control) or DF-ACC. Isolates were considered ACCD producers when they could grow on DF-ACC medium due to their ability to break down ACC. However, isolate Sa-4 exhibited better growth and sporulation on the DF-ACC medium, indicating higher ACCD production (Figure 7).

A summary of the 3 promising actinobacterial isolates (Sa-2, Sa-4, and Sa-6) were found to be halotolerant, P-solubilizers and ACC-producers (Table 1). For comparison purposes, the ACCD-producing actinobacterial isolate (Sa-4) and the non-ACCD-producing isolate (Sa-9) were selected for further greenhouse experiments. Sa-4 was considered as the +ACCD isolate; whereas isolate (Sa-9) was denoted as the -ACCD isolate.

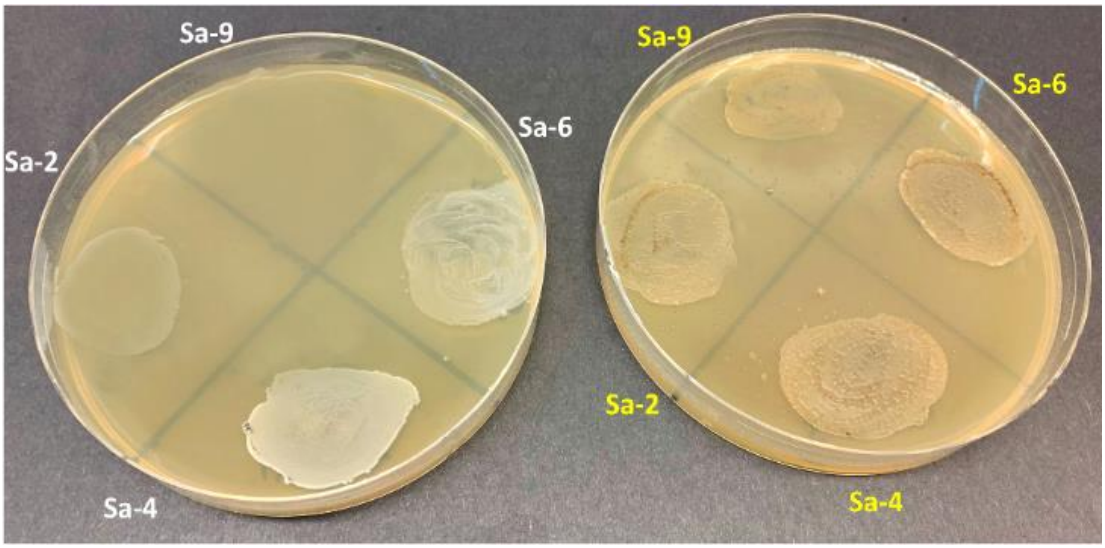

Figure 7: Qualitative assessment of ACCD production on DF medium. Isolates Sa-2, Sa-4, and Sa-6 showing growth on ACC-DF medium (Left) and the control DF medium containing $(NH_4)_2SO_4$ (Right)

Table 1: A summary of the qualitative screening of actinobacterial isolates. Evaluation of tolerance to salt stress (4% and 8% NaCl), production of ACCD, and solubilization of P. NaCl, sodium chloride; ACCD; P, phosphorus; -/+, absence/presence

Isolate #	4% NaCl	8% NaCl	ACCD	P-solubilization
Sa-2	+++	-	++	+
Sa-4	+++	+++	+++	+
Sa-6	+++	++	++	-
Sa-9	++	-	-	-

3.5 Identification of the Actinobacterial Isolates

Identification of the ACCD-producing and non-ACCD-producing actinobacterial isolates, Sa-4 and Sa-9, respectively, was confirmed based on their cultural, morphological, and physiological characteristics as described by Locci (1989). Isolate Sa-4 produced grayish brown aerial mycelium with dark gray substrate mycelial growth on ISP medium after 7 days of incubation (Figure 8). The morphology and presence of aerial and substrate mycelia are consistent with the morphology of the genus *Streptomyces*.

Figure 8: Identification of the most promising ACCD-producing isolate. Aerial (left) and substrate mycelia (right) of the ACCD-producing isolate (*Streptomyces* sp. Sa-4) growing on ISP3 medium

3.6 Effect of the ACCD-producing Isolate on the Growth Parameters of Tomato in Response to Salt Stress

To assess the effect of the ACCD-producing isolate in the growth promotion of tomato under saline conditions, the seedlings were inoculated with either +ACCD or -ACCD actinobacterial isolates. In this study, seedlings were subjected to no salt stress (0 mM NaCl), 60 mM and 120 NaCl. Tomato seedlings without any isolate inoculation were used as a control treatment.

In general, there were detrimental impacts of salt stress which resulted in a reduction in growth parameters, such as root length, shoot length, fresh and dry biomass of the roots and shoots of the tomato plants, in the non-stressed/non-inoculated seedlings.

Under 120 mM salt stress, there was a significant ($P < 0.05$) increase in the length of shoots (Figure 9A, 9B) when seedlings were treated with +ACCD isolate in comparison to the control or -ACCD isolate treatments. Furthermore, the +ACCD isolate significantly ($P < 0.05$) increased the fresh (Figure 9C) and dry (Figure 9D) weights of the shoots of tomato seedlings by about 2-fold in comparison to those non-inoculated (control) or inoculated with -ACCD isolate.

The application of +ACCD isolate to the soil increased the length of the roots by almost 50% compared to the other two treatments (Figure 10A, 10B). Moreover, fresh (Figure 10C) and dry (Figure 10D) weights of roots were significantly increased by 3-fold when compared to the control/-ACCD isolate treatments. There were no significant ($P > 0.05$), differences; however, in growth parameters of the roots or shoots of the tomato seedlings treated with the -ACCD isolate or control under any of the salinity levels tested (Figures 9,10).

Upon harvesting, the number of fruits per plant also tripled in seedlings inoculated with +ACCD compared to those of the control seedlings after 12 weeks of inoculation (Figure 11A). The fresh weight of fruits (Figure 11B) was significantly ($P < 0.05$) higher in the +ACCD treatments by more than 50% than in the other two treatments.

Moreover, the number of leaves of plants was found to be higher by 2-fold in the +ACCD treatment than in the control (Figure 12A). The +ACCD isolate was also found to be effective in enhancing the total chlorophyll content in leaves of tomato seedlings

when exposed to 60 mM NaCl treatment, compared to the control or the -ACCD isolate treatments (Figure 12B).

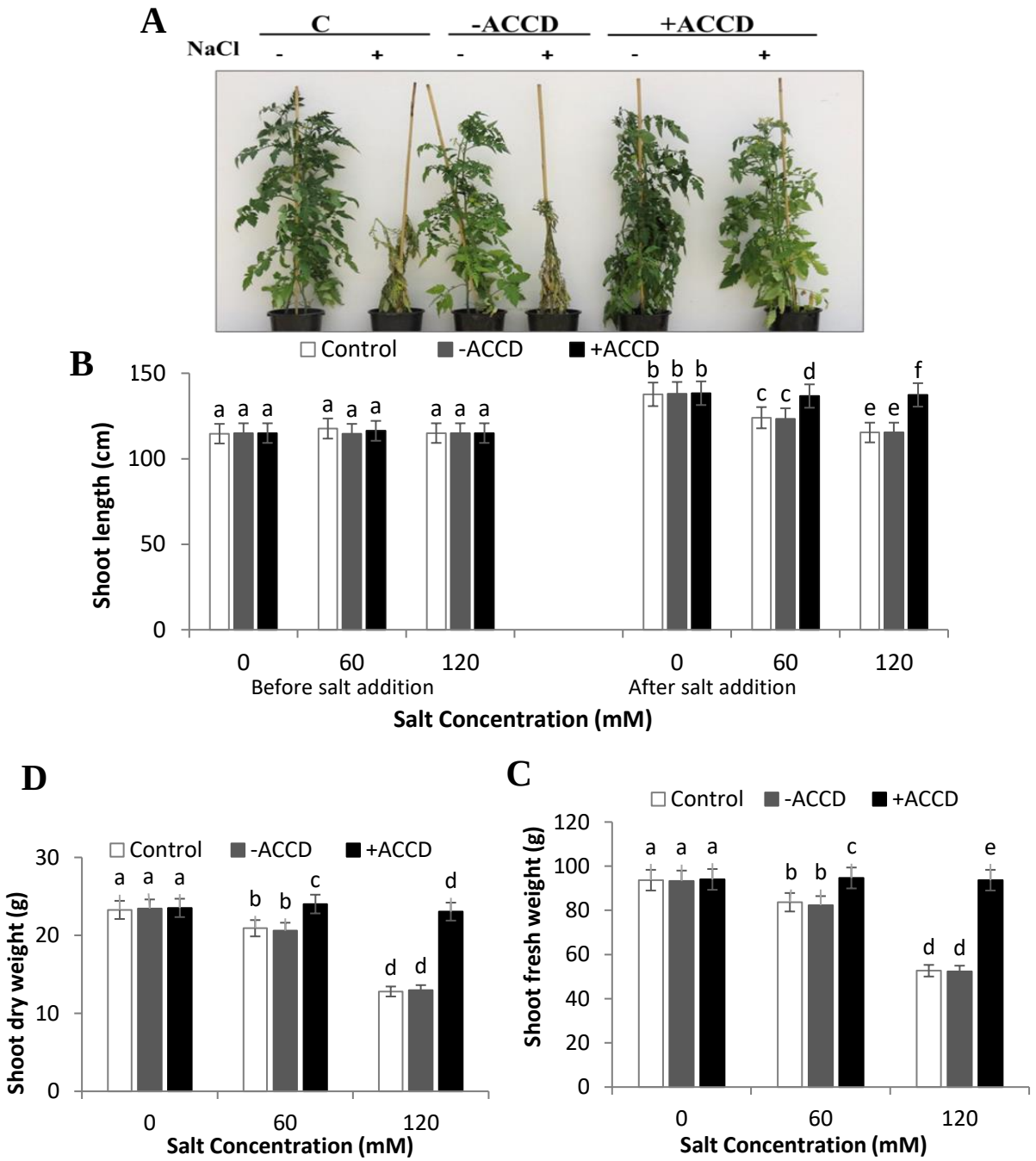

Figure 9: Effect of the application of +ACCD isolate and the –ACCD isolate or the control on the shoot parameters of the tomato seedlings under saline conditions. Measurements of the length (A, B), the fresh (C) and the dry (D) weights of the shoots. Columns represent the mean values of three replicates, while bars represent standard deviation. Significantly different values ($P<0.05$) between treatments are represented by letters as evaluated by Tukey's test. Control, seedling without isolate inoculation; +ACCD, seedlings inoculated with ACCD-producing isolate; -ACCD, seedlings inoculated with non-ACCD-producing isolate

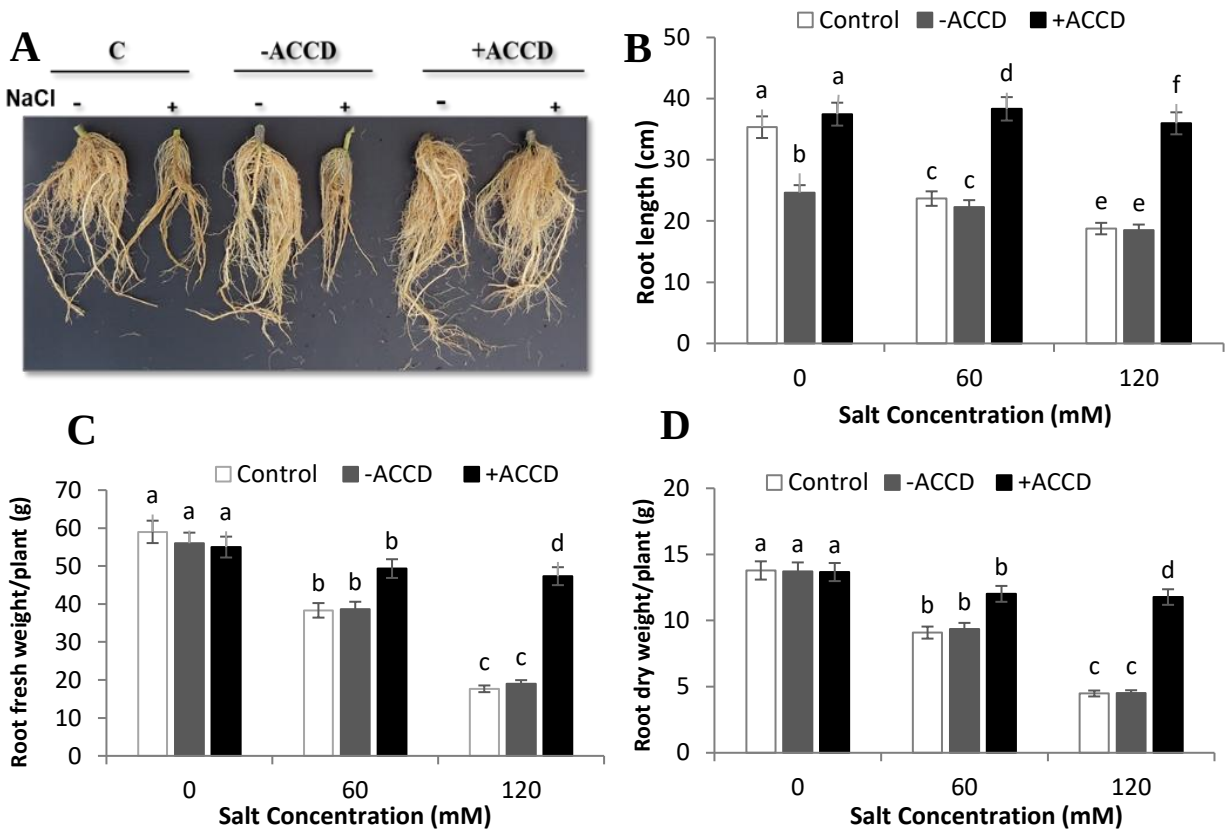

Figure 10: Effect of the application of +ACCD isolate and the –ACCD isolate or the control on the root parameters of the tomato seedlings under saline conditions. Measurements of the length (A, B), the fresh (C) and the dry (D) weights of the roots. Columns represent the mean values of three replicates, while bars represent standard deviation. Significantly different values ($P<0.05$) between treatments are represented by letters as evaluated by Tukey's test. Control, seedling without isolate inoculation; +ACCD, seedlings inoculated with ACCD-producing isolate; -ACCD, seedlings inoculated with non-ACCD-producing isolate

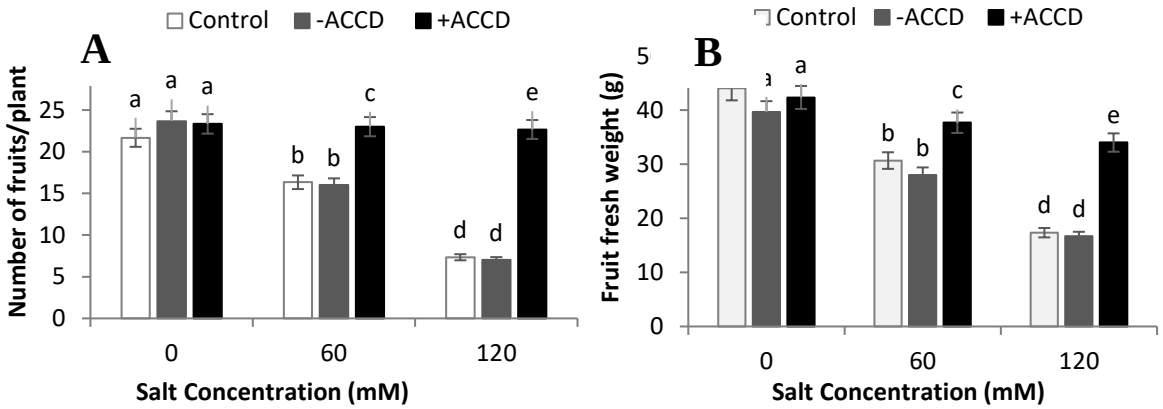

Figure 11: Effect of the application of +ACCD isolate and the –ACCD isolate or the control on the development of the tomato fruits under saline conditions. Measurements of the number of fruits (A) and fruit weight (B). Columns represent the mean values of three replicates, while bars represent Standard deviation. Significantly different values ($P<0.05$) between treatments are represented by letters as evaluated by Tukey's test. Control, seedling without isolate inoculation; +ACCD, seedlings inoculated with ACCD-producing isolate; -ACCD, seedlings inoculated with non-ACCD-producing isolate

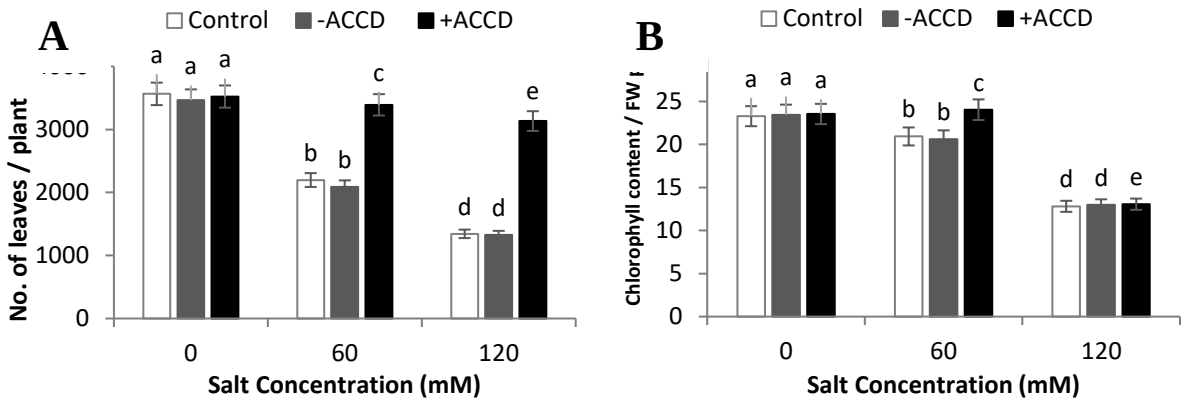

Figure 12: Effect of the application of +ACCD isolate and the –ACCD isolate or the control on the tomato leaves development under saline conditions. Measurements of the number of leaves (A) and the total chlorophyll content (B) of the tomato seedlings. Columns represent the mean values of three replicates, while bars represent Standard deviation. Significantly different values ($P<0.05$) between treatments are represented by letters as evaluated by Tukey's test. Control, seedling without isolate inoculation; +ACCD, seedlings inoculated with ACCD-producing isolate; -ACCD, seedlings inoculated with non-ACCD-producing isolate

3.7 Assessment of Endogenous ACC Levels in the Tomato Plants

The influence of the +ACCD isolate on the growth promotion of tomato seedlings under no stress and salinity stress conditions was assessed by measuring the endogenous levels of ACC *in planta* (Figure 13). The inoculation of tomato seedlings with the +ACCD isolate significantly (P<0.05) reduced the ACC content in shoots (A) and roots (B). For example, the ACC concentrations were 60% less in both shoots and roots of the +ACCD treatment than in the control group of seedlings in response to 60 mM NaCl. The endogenous ACC contents were reduced by 24% and 32%, in roots and shoots, respectively, compared to those of control and -ACCD isolate treatments under the extreme salt stress conditions when 120 mM NaCl was used.

These results suggest that the ACCD-producing isolate (+ACCD) has the potential to relieve the negative effects of salt stress and promote the growth of tomato plants under greenhouse conditions. However, no significant differences were observed in ACC levels of shoots or roots of the control tomato seedlings, or the seedlings treated with the -ACCD isolate under both salinity levels (Figure 13A, 13B).

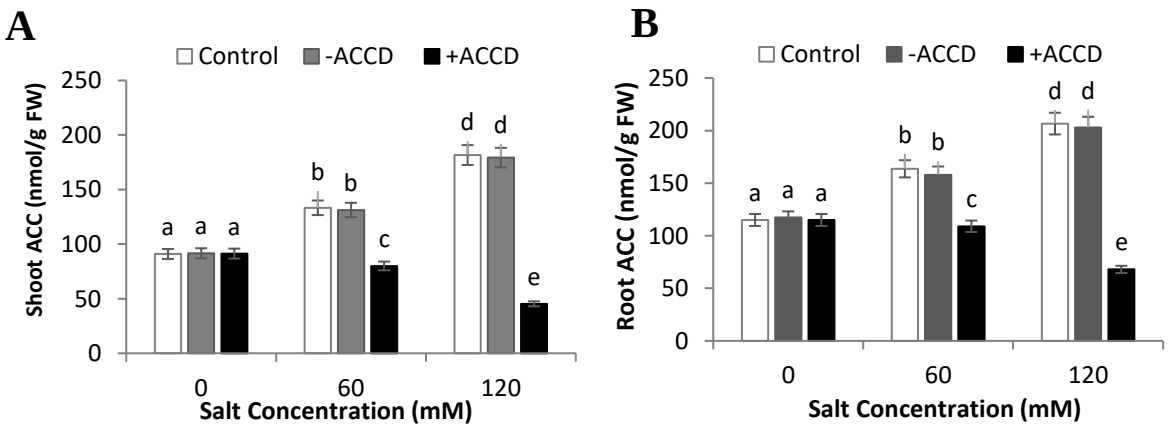

Figure 13: Effect of the application of +ACCD isolate and the –ACCD isolate or the control on the endogenous ACC content in the tomato seedlings under saline conditions. Measurements of the ACC content in the shoots (A) and the roots (B) of the tomato seedlings. Columns represent the mean values of three replicates, while bars represent Standard deviation. Significantly different values (P<0.05) between treatments are represented by letters as evaluated by Tukey's test. Control, seedling without isolate inoculation; +ACCD, seedlings inoculated with ACCD-producing isolate; -ACCD, seedlings inoculated with non-ACCD-producing isolate

Chapter 4: Discussion

Abiotic stress, such as drought and salt, lowers crop yield (Numan et al., 2018), posing a serious threat to global food security. In response to salt stress, an abrupt rise in ET concentration occurs and damages the plant morphology, physiology, molecular, and metabolism (Glick et al., 1998; El-Tarabily, 2008; Numan et al., 2018). Therefore, reducing ET contents in stressed plants is critical for enhancing crop yield.

PGPR play an essential role in sustainable agriculture, due to their ability to support plant growth under various stresses by lowering elevated ET levels and increasing nutrient uptake (El-Tarabily et al., 2019, Tolba et al., 2019; Mathew et al., 2020). In addition, PGPA have high resilience to arid and dry environments. They can also adapt to extreme environmental conditions that arise from their ability to form spores and survive for prolonged periods in the soil (Goodfellow & Williams, 1983; El-Tarabily et al., 2020). PGPA are well known for their ability to improve the growth of tomato plants under salt stress. For example, *Streptomyces* sp. KLBMP 5084 promoted the growth of tomato seedlings under salt-stress (Gong et al., 2020). Similarly, Rangseekaew et al. (2022), isolated *Dermacoccus abyssi* MT1.1T, a salt-tolerant actinobacterium, that enhanced the shoot length, dry weight, total chlorophyll content, and sugars content in tomato seedlings under 150 mM NaCl conditions. Therefore, I hypothesized that ACCD-producing rhizosphere-competent actinobacteria can increase salinity tolerance and stimulate the growth of tomato plants in the UAE.

Production of ACCD by some PGPR/PGPA is one of the direct mechanisms of plant growth promotion. This enzyme breaks down ACC (the immediate precursor of ET) into α-ketobutyrate and ammonia (Glick et al., 1998; Mayak et al., 2004). Singh et al. (2022) has demonstrated that inoculating wheat plant with the ACCD-producing soil bacterium *Enterobacter cloacae* (ZNP-4), significantly improved various growth parameters under salt-stress (150 mM and 200 mM NaCl). In addition, two ACCD-producing, actinobacterial isolates: *Streptomyces* sp. strain RA04 and *Nocardiopsis* sp. strain RA07 exhibited multiple PGP traits and promoted the growth of *Sorghum bicolor* by lowering the ET levels *in planta* (Silambarasan et al., 2022a). Hence, the enzymatic activity of ACCD determines the levels of endogenous ET in stressed plants.

Chemical inhibitors of ET, such as aminooxyacetic acid (AOA), copper sulphate (CuSO4), and silver ions (Ag+), can be applied as plant growth promoters; however, they are not favored due to their accumulation and toxicity in plant tissues (Ghosh et al., 2018). Unlike these chemical compounds, ACCD from microorganisms (e.g., PGPA) has the option of offering an eco-friendly and safer plant growth promotion. In the present study, we aimed at isolating salt-tolerant, ACCD-producing PGPA from various rhizospheres in the UAE. According to Ruppel et al. (2013), the application of microbes isolated from the rhizosphere of halophytes has led to increased plant growth and improved soil fertility under stress conditions. This could be attributed to their capabilities to withstand more than 30% NaCl concentrations.

P is the second most essential element for plant growth. In general, all the soluble forms of P are present as 0.1% of the total soil phosphate only (Nimaichand et al., 2016). In addition, P is linked directly to increased biomass production of plants (Hamdali et al., 2008; El-Tarabily et al., 2021a). Here, the process of the isolation of actinobacteria from the rhizosphere resulted in 491 isolates able to tolerate up to 8% NaCl, of which 14 were found to be strong P-solubilizers on Pikovskaya's media. According to the presence or absence of aerial mycelia, and the formation of spores and substrate mycelia, the isolates were morphologically identified to the genus level. All identified actinobacterial isolates belonged to *Streptomyces* spp. These findings were similar to other studies reporting P-solubilization by actinobacteria. According to Silambarasan et al. (2022b), the application of *Streptomyces corchorusii* strain CASL5 significantly increased the uptake of P from the soil under salt-stresses conditions. The P-solubilization capability of this isolate led to increased chlorophyll content in the leaves of the tomato seedlings and induced the overall growth of the plant. Moreover, *Streptomyces* sp. RHS33 was reported to promote the growth of tomato plants by enhancing fresh and dry weights and P uptake from the soil (El-Badan et al., 2019). Therefore, the ability of PGPR to solubilize inorganic P is one of the major characteristics that affect plant growth.

The halotolerant actinobacterial isolates were also *in vitro* evaluated for ACCD production. Three high salt-tolerant isolates were found to produce ACCD at varying levels. The isolate that showed excellent growth and heavy sporulation on the DF medium was considered as the ACCD-producing isolate (+ACCD) and was selected for *in vivo*

study in the greenhouse. One of the key characteristics shared by all plant growth-promoting bacteria is the production of ACC deaminase and the use of ACC as a sole source of nitrogen. As the level of ACC in plant tissues decreases, the amount of ethylene decreases, and plants are protected from the inhibitory effects of stress ethylene (Palaniyandi et al., 2014; Moon & Ali 2022).

In the *in vivo* study, the effect of the +ACCD isolate on the growth promotion of tomato plants was investigated in the greenhouse in response to different salt concentrations (0, 60, and 120 mM NaCl). In general, the +ACCD isolate enhanced the lengths as well as the fresh and dry biomass of shoots and roots of tomato plants in comparison with the control (no isolate) and the non-ACCD-producing isolate (-ACCD). Here, the ACCD-producing isolate enhanced the salinity tolerance of tomato. This was evident from the increment in the weight and number of fruits, number of leaves, and total chlorophyll contents compared to the other two treatments. Similarly, Djebaili et al. (2021) reported the isolation of fourteen rhizosphere-competent actinobacterial isolates that induced the salinity tolerance of *Triticum durum* up to 1 M NaCl. Most of those isolates produced ACCD, IAA, and solubilized inorganic phosphate. They increased total chlorophyll content and enhanced the plant length, yield, and biomass, in comparison to uninoculated plants, both with and without salt. Likewise, *Streptomyces hydrogenans* DH16, rhizosphere-competent, ACCD-producing isolate with plant growth-promoting characteristics was applied to tomato seedlings. *S. hydrogenans* DH16, enhanced the shoot and root length, lateral roots, and fresh and dry weights (Kaur & Manhas, 2022).

The outcomes of the current study revealed that there was a significant reduction in the endogenous ACC content in the root and shoot tissues by four- and three-fold, respectively. Our results are in agreement with another study that reported the inoculation of gray mangrove (*Avicennia marina*), with the ACCD-producing isolate *Streptomyces ferrugineus* under greenhouse conditions. *S. ferrugineus* significantly reduced the ACC levels in the shoots and roots of the mangrove seedlings *in planta* while increasing the dry biomass of the shoots and roots (El-Tarabily et al., 2021a).

The findings of the greenhouse experiments were consistent with recent studies reporting halotolerant, ACCD-producing actinobacteria that promoted plant growth

through lowering endogenous ACC levels. El-Tarabily et al. (2019) assessed the application of salt-tolerant actinobacterial isolate *Micromonospora chalcea* UAE1 to promote the growth of *Salicornia bigelovii* plants under greenhouse conditions. *M. chalcea* UAE1 significantly induced the growth of the seedlings, increases the concentration of photosynthetic pigments while reducing the endogenous ACC levels in the shoots and roots of *S. bigelovii*.

As suggested by Glick et al. (2007), the growth promotion of tomato observed, in the current study, could be attributed to the enzymatic activity of ACCD possessed by the Sa-4 isolate, which resulted in the reduction of ET in plant tissue. Moreover, the increase in the dry biomass of the seedlings could be attributed to the induction of the photosynthetic pigments. These findings support earlier studies linking the improvement in growth of tomato inoculated with PGPA to the induction chlorophyll content. For example, *Streptomyces* sp. strain PGPA39 (Palaniyandi et al., 2014), and *Dermacoccus abyssi* MT1.1T (Rangseekaew et al., 2022). However, other growth-promoting mechanisms, that were not tested in this study could have attributed to the growth promotion of the tomato seedlings.

The present study reported the isolation of actinobacteria from the UAE rhizosphere based on their tolerance to NaCl, P-solubilization, and ACCD-production. The findings of the *in vivo* study suggest that the +ACCD isolate has the ability of to enhance the salinity tolerance of the tomato seedlings under greenhouse conditions. This is because the +ACCD isolate increased the length, fresh and dry biomass of the roots and shoots, while reducing the endogenous ACC levels that is associated with stress ET.

Although the current research focused on relieving the damage caused by salt stress using ACCD, combining more actinobacterial isolates with other plant growth promoting traits would in higher yield of the tomato plants. Therefore, future experiments should focus on screening the all the ACCD-producing isolates for plant growth regulators such as IAA, siderophores and polyamines, to possibly apply them in consortia with the +ACCD isolate. This approach could also be applied to other crops to eventually promote their growth and enhance yield and productivity in the field under salt-stress conditions.

Chapter 5: Conclusion

This research reports the isolation of a *Streptomyces* isolate, namely Sa-4, from the UAE rhizosphere soils with promising high salt tolerance and ACCD synthesis. The *in vivo* results demonstrated the ability of this isolate to support the growth of tomato plants in conditions with high salt stress. The greenhouse studies revealed that the ACCD-producing isolate could be applied as a biological fertilizer to alleviate salinity stress in soils and water associated with irrigation systems in the UAE. Thus, the greenhouse experiment is the first step to apply such a "green" approach to uncover the ability of the ACCD-producing isolate under salt stress conditions on a larger scale *i.e.*, open field. Future field experiments are required to assess the effect of this actinobacterial candidate along with other PGPA to enhance the growth and increase the productivity of tomato under normal and salt-stressed field conditions.

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List of Publications

El- Tarabily, K.A., Ramadan, G.A., Elbadawi, A.A., Hassan, A.H., Tariq, S., Ghazal, E.W., Abo Gamar, M.I., AbuQamar, S.F. (2021). The marine endophytic polyamine-producing *Streptomyces mutabilis* UAE1 isolated from extreme niches in the Arabian Gulf promotes the performance of mangrove (*Avicennia marina*) seedlings under greenhouse conditions. *Frontiers in Marine Science*, 8, 710200. doi: https://doi.org/10.3389/fmars.2021.710200

El- Tarabily, K.A., Sham, A., Elbadawi, A.A., Hassan, A.H., Alhosani, B.K.K., El-Esawi, M.A., AlKhajeh, A.S., AbuQamar, S.F. (2021). A consortium of rhizosphere-competent actinobacteria exhibiting multiple plant growth-promoting traits improves the growth of *Avicennia marina* in the United Arab Emirates. *Frontiers in Marine Science*, 8, 715123. doi: https://doi.org/10.3389/fmars.2021.715123

Appendix

List of The Media Used in This Research

1. Inorganic Starch Nitrate Agar (SNA) (Küster, 1959).
2. Dworkin and Foster's Salts Minimal Agar Medium (DF) (Dworkin & Foster, 1958).
3. Oatmeal Yeast Extract Agar (OMYEA) (Küster, 1959).
4. Pikovskaya's Agar Medium (Pikovskaya, 1948).

Composition of Media:

1- Inorganic Starch Nitrate Agar (SNA)

Soluble starch	10 g
Potassium nitrate	2 g
Di-potassium hydrogen phosphate	1 g
Magnesium sulfate	0.5 g
Sodium chloride	0.5 g
Calcium carbonate	3 g
Ferrous sulfate	0.01 g
*Trace salt solution	1 mL
Cycloheximide (Sigma)	50 µg
Nystatin (Sigma)	50 µg
Distilled water	1 L
Agar	20 g

*Trace salt solution composed of 0.1 mg per liter of each of the following salts: ferrous sulfate, magnesium chloride, copper sulfate, and zinc sulfate.

2- Dworkin and Foster's Salts Minimal Agar Medium (DF) (Dworkin and Foster, 1958)

Di- hydrogen potassium phosphate	4.0 g
Di-sodium hydrogen phosphate	6.0 g
Magnesium sulfate	0.2 g
Ferrous sulfate	1.0 g
Boric acid	10 µg
Manganese sulfate	10 µg
Zinc sulfate	70 µg
Copper sulfate	50 µg
Molybdenum oxide	10 µg
Glucose	2.0 g
Gluconic acid	2.0 g
Citric acid	2.0 g
Agar	20 g
Distilled water	1 L

3- Oatmeal Yeast Extract Agar (OMYEA)

Twenty grams of oatmeal were steamed for 20 minutes in 1 liter of distilled water, and it was filtered through a cheesecloth. Distilled water was added to proceed the filtrate to 1 liter. 1 gram of yeast extract (Sigma) and 20 grams of agar (Sigma) were added, and the final medium pH has been adjusted to 7.2.

4- Pikovskaya's Agar Medium (Pikovskaya, 1948):

Glucose	10.0 g
Calcium Phosphate $Ca_3(PO_4)_2$	5.0 g
Ammonium sulfate $(NH_4)_2SO_4$	0.5 g
Sodium Chloride	0.2 g
Magnesium sulfate	0.1 g
Potassium chloride	0.2 g
Yeast Extract	0.5 g
Manganese sulfate	0.002 g
Iron sulfate	0.002 g
Distilled water	1000 mL
0.5% Bromophenol Blue	5 mL
Agar	20.0 g

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Soil salinity is a global issue that is increasing each year, leading to economical and agricultural losses. Plant growth promoting (actino)bacteria are an ecofriendly approach that can be applied to relieve plants from the stress hormone ethylene associated with high salt concentration. This study evaluated the application of a salt-tolerant actinobacterial isolate with the ability to produce the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase to alleviate the effects of salt stress in tomato plants in the UAE. The outcomes of this study present a potential PGPA isolate that could be applied to support the growth of crops (e.g., tomato) under salt stress conditions in the UAE.

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