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MANGROVE GROWTH PROMOTION BY ENDOPHYTIC ACTINOBACTERIA AND SEAWEED EXTRACT

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UAEU
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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Environmental Sciences

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Cover: Effect of the application of seaweed extract and *Streptomyces tubercidicus* UAE1 on mangrove growth.

(Photo: By Ameera Khalfan Salem Alkaabi)

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Declaration of Original Work

I, Ameera Khalfan Salem Alkaabi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Mangrove Growth Promotion by Endophytic Actinobacteria and Seaweed Extract"*, hereby, solemnly declare that this is the original research work done by me under the supervision of Prof. Khaled A. El-Tarabily, 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 Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions.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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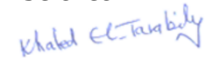
   Signature  Date 21/6/2022

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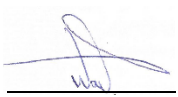
   Signature  Date 21/6/2022

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Abstract

In this study, I aimed to determine the impact of the application of a commercial seaweed extract (SWE) bio-stimulant and endophytic actinobacterial isolates on growth performance and endogenous hormonal levels of mangroves. Therefore, I isolated endophytic plant growth promoting (PGP) actinobacteria (PGPA) from mangrove roots; and evaluated their potential as biological inoculants on mangrove seedlings under greenhouse and open-field nursery conditions. Seven salt tolerant isolates had the ability to produce different levels of *in vitro* plant growth regulators (PGRs) and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (ACCD), and to solubilize phosphorus. Accordingly, only one isolate, *Streptomyces tubercidicus* UAE1 (*St*), was selected based on its relative superiority in displaying multiple mode of actions and in successfully colonizing mangrove tissues for 15 weeks. In the greenhouse experiments, plants treated with either *St* or SWE significantly ($P<0.05$) improved dry biomass by 40.2 and 55.1% in roots and 42.2 and 55.4% in shoots, respectively compared to seawater-irrigated non-treated mangrove plants (control). However, *St*+SWE caused greater significant ($P<0.05$) increase in dry weight of roots (67.6%) and shoots (65.7%) than control plants. Following the combined treatment of *St*+SWE, *in planta* PGR levels were found to be greatly enhanced over the non-treated control or treated plants grown in sediments inoculated with *St* or supplied with SWE only. This was evident from the significant ($P<0.05$) increases in the photosynthetic pigments and production of PGRs, as well as the reduction in the endogenous ACC levels of plant tissues compared to those in other treatments. Tissue nutrient contents of seedlings also increased by at least two-fold in *St*+SWE treatment compared to control. Similar effects were observed on all growth parameters under natural open-field nursery

conditions. This report is the first in the field of marine agriculture that uses SWE as a nutrient base for actinobacteria capable of producing PGRs and ACCD. By combining *St* with SWE, this does not only stimulate plant growth but also potentially has additive effects on mangrove ecosystem productivity in nutrient-impoverished soils in the Arabian coastal areas.

Keywords: Arabian Gulf, marine agriculture, mangrove, nutrient-base, plant growth promotion, seaweed extract.

Title and Abstract (in Arabic)

تعزيز نمو القرم عن طريق الأكتينوباكتريا الداخلية ومستخلص الأعشاب البحرية

الملخص

هدفي في هذه الدراسة هو تحديد تأثير تطبيق مستخلص الطحالب البحرية التجاري (SWE) والعزلات الجرثومية الشعاعية على أداء النمو والمستويات الهرمونية الذاتية لأشجار القرم(Mangroves). لذلك، قمت بعزل البكتيريا الشعاعية المعززة لنمو النبات (PGP) من جذور القرم ؛ وقمت بتقييم إمكاناتهم كملقحات بيولوجية على شتلات القرم في ظروف البيوت المحمية والمشاتل في الحقول المفتوحة. سبعة عزلات متحملة للملوحة لديها القدرة على إنتاج مستويات مختلفة من منظمات نمو النبات في المختبر (PGRs) وحمض 1-أمينوسيكلوبروبان-1-كربوكسيليك (ACC) ديميناز (ACCD)، وعلى إذابة الفوسفور. وفقًا لذلك، تم اختيار عزلة واحدة فقط، (*Streptomyces tubercidicus* UAE1 (*St*))، بناءً على تفوقها النسبي في عرض أنماط متعددة من الإجراءات واستعمار أنسجة المنغروف بنجاح لمدة 15 أسبوعًا في تجارب البيوت المحمية، النباتات التي تمت معالجتها باستخدام *St* أو SWE معنوياً ($P < 0.05$) حسنت الكتلة الحيوية الجافة بنسبة 40.2 و 55.1٪ في الجذور و 42.2 و 55.4٪ في البراعم، على التوالي مقارنة بنباتات القرم المروية بمياه البحر غير المعالجة. ومع ذلك، تسبب *St* + SWE في زيادة معنوية ($P < 0.05$) في الوزن الجاف للجذور (67.6٪) والبراعم (65.7٪) مقارنة بالنباتات الضابطة. بعد المعالجة المشتركة لـ *St* + SWE، وجد أن مستويات PGR في نبات بلانتا قد تحسنت بشكل كبير مقارنة بالنباتات غير المعالجة أو النباتات المعالجة المزروعة في الرواسب الملقحة بـ *St* أو المزودة بـ SWE فقط. كان هذا واضحًا من الزيادات الكبيرة ($P < 0.05$) في أصباغ التمثيل الضوئي وإنتاج PGRs، بالإضافة إلى انخفاض مستويات ACC الذاتية للأنسجة النباتية مقارنة بتلك الموجودة في المعالجات الأخرى. كما زادت محتويات الأنسجة المغذية للشتلات بمقدار الضعفين على الأقل في معاملة *St* + SWE مقارنةً بمجموعة التحكم. وقد لوحظت تأثيرات مماثلة على جميع مؤشرات النمو تحت ظروف الحضانة الطبيعية في الحقول المفتوحة.هذا التقرير هو الأول في مجال الزراعة البحرية الذي يستخدم SWE كقاعدة مغذية للبكتيريا الشعاعية القادرة على إنتاج PGRs و ACCD. من خلال الجمع بين *St* مع SWE، فإن هذا لا يحفز نمو النبات فحسب،بل يحتمل أيضًا أن يكون له تأثيرات إضافية على إنتاجية النظام الإيكولوجي للقرم في التربة الفقيرة بالمغذيات في المناطق الساحلية العربية.

مفاهيم البحث الرئيسية: الخليج العربي، الزراعة البحرية، القرم، المغذيات، تعزيز نمو النبات، مستخلص الأعشاب البحرية.

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Dedication

To my beloved parents and family

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List of Abbreviations

ACC	1-Aminocyclopropane-1-Carboxylate
AM	Arbuscular Mycorrhiza Fungi
CKs	Cytokinins
GAs	Gibberellins
GPB	Glucose Peptone Broth
OMYEA	Oat-Meal Yeast Extract Agar
DF	Dworkin and Foster's Salts Minimal Agar Medium
MDAM	Moeller's Decarboxylase Agar Medium
CSA	Modified Chrome Azurol Agar Plat
PGPB	Plant Growth Promoting Bacteria
SNA	Starch Nitrate Agar
SWE	Seaweed Extracts
UAE	United Arab Emirates

Chapter 1: Introduction

1.1 Overview

Mangrove cultivation in the UAE is generally limited by the plantation season, extremely poor plantation substrates, and high-water salinity due to less precipitation (El-Tarabily & Youssef, 2011), So the PGPB may benefit. The study of PGPB in mangroves ecosystems is in its infancy; however, several studies demonstrate the potential for using endophytes bacteria isolated from mangrove roots as PGPB.

1.2 Statement of the Problem

Mangrove forests in the Arabian Gulf are under continuous threat. In addition, the growth performance of mangroves in this region is generally limited by excessively high levels of salinity, wide seasonal temperature variation, poor nutrient availability, and adverse soil conditions (Habshi *et al.,* 2007). Therefore, finding an alternative eco-friendly way to enhance mangrove plants growth. Thus, this study was conducted to determine the mechanism of plant growth promotion by these beneficial endophytic bacteria.

1.3 Research Objectives

The objectives of the current study were to:

1. To isolate endophytic bacteria from mangrove roots.
2. To investigate mangrove growth promotion capabilities of the isolates.
3. To assess SWE as a bio- stimulant of mangrove growth.
4. To elucidate the potential mechanisms of mangrove growth promotion with a combination of SWE and endophytic bacteria.

1.4 Relevant Literature

Mangroves are unique tree species that help reduce the impact of climate change, act as a nursery to fish stocks, improve coastal water and protect coastlines (Hutchison *et al.,* 2014; Spalding & Parrett, 2019). Despite these advantages, mangrove habitats are globally in decline (Polidoro *et al.,* 2010). This can be attributed to the coastal development and land reclamation, aquaculture, oil spills, and coastal pollution as well as climate change effects (Ellison & Farnsworth, 1996). According to the Food and Agriculture Organization (FAO), mangrove areas in the United Arab Emirates (UAE) have, however, increased over the last 30 years (FAO, 2020). This is partly due to localized planting activities, alteration of shorelines, water-flow patterns, and increased public awareness and conservation efforts (Moore *et al.*, 2015; Elmahdy *et al.*, 2020). The highly salt tolerant gray mangrove (*Avicenna marina* (Forsk.) Vierh.) is the most common mangrove species in the UAE (Dodd *et al.,* 1999; Moore *et al.*, 2015). Within the UAE, mangrove plantations appear to be widely abundant in the Emirate of Abu Dhabi. Even though the mangrove ecosystem is very rich in microbial diversity, less than 5% of species have been described (Thatoi *et al.*, 2012).

Many attempts have focused on the microbial diversity in mangrove ecosystems to explore their potential applications in agricultural, environmental, industrial, and medical fields (Bashan *et al.*, 2000; Holguin *et al.*, 2001; Bashan & Holguin, 2002; Hong *et al.*, 2009; Allard *et al.,* 2020). Yet, little is known about the bacterial community living in mangrove, particularly actinobacteria, with potential to stimulate plant growth. Microbial endophytes are found in almost all plant species; thus, colonizing their internal tissues. Plant growth promoting (PGP) rhizobacteria (PGPR), including PGP actinobacteria (PGPA), are also known to affect plant fitness and soil quality, thereby increasing the productivity of agriculture and

stability of soils (El-Tarabily *et al.,* 2019; 2020; Mathew *et al.,* 2020). Endophytic PGPR stimulate plant growth directly by facilitating resource acquisition needed by plants or modulating the levels of plant growth regulators (Santoyo *et al.,* 2016), or indirectly by decreasing the inhibitory effects of pathogens on plants (Khare *et al.,* 2018). PGPR possessing the 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (ACCD) enzyme can also facilitate growth and induce tolerance to environmental stresses in plants by hydrolyzing ACC into α-ketobutyrate and ammonia (NH3); thus, decreasing ethylene (ET) levels in plant tissues (Glick, 2014; Olanrewaju *et al.,* 2017). Previously, endophytic actinobacteria have been isolated from mangrove to study their antimicrobial activities (Gayathri & Muralikrishnan, 2013; Jiang *et al.,* 2018; Chen *et al.,* 2021). Except of El-Tarabily, Ramadan *et al.* (2021), there are no reports about endophytic actinobacterial strains isolated from mangrove tissues to study their potential in plant growth activities of mangrove.

Biostimulants are natural or synthetic substances that can be applied to seeds, plants, and soils. In addition to their role in reducing the need for chemical fertilizers, bio-stimulants have been recognized for their efficiency in enhancing growth, improving stress tolerance and increasing productivity of plants (Shukla *et al.,* 2019; Ashour *et al.,* 2021; Hassan *et al.,* 2021). Seaweeds, also known as marine macroalgae or kelp, are sessile multicellular photosynthetic eukaryotes that can be differentiated from plants by their lack of specialized tissues such as root system and vascular structures (Graham & Wilcox, 2000). Seaweeds play a key role in marine ecosystems, mainly on rocky shores in coastal temperate marine environments. They provide food and space for marine microorganisms and higher organisms, act as nurseries and shelters for many invertebrate species and maintain the overall biodiversity structure (Schiel & Lilley, 2007; Egan *et al.,* 2013). Commercially, the growth of seaweed aquaculture has recently

increased, particularly in food markets, feedstocks and biofuel production (Borines *et al.,* 2011; Jacob *et al.*, 2016). In agriculture, seaweed extracts (SWE) are currently used as bio-stimulants in organic farming (Mukherjee & Patel, 2019; El Chami & Galli 2020; Ali *et al.,* 2021). Despite the extensive literature reports about using SWE in crop management, no study to date has deployed SWE in marine agriculture to promote growth of mangrove or Salicornia under greenhouse and/or open-field conditions.

It has been reported that a combination of natural SWE and PGPR can improve plant growth, increase crop production and quality, and ameliorate stress effects. For example, the combined Bacillus licheniformis and Pseudomonas fluorescens with Kelpak® (a seaweed-derived extract) improved the production and mineral contents of leafy vegetables (Ngoroyemoto *et al.,* 2019). In addition, supplying plants with SWE, PGPR (*Bacillus amyloliquefaciens* subsp. plantarum, Bacillus simplex and Pseudomonas sp.) and micronutrients improved tolerance to cold stress during early growth of maize (Bradáčová *et al.,* 2016). To date, however, there are no data in the literature to determine the impact of combining rhizosphere or endophytic PGPA with SWE treatments on growth of halophytic plants (e.g., mangrove) under greenhouse or field conditions.

1.5 An Overview of Mangrove Tree

Mangroves are halophytic salt-tolerant woody plants that occupy inter-tidal wetland ecosystems in the tropical and subtropical coastal areas withstand harsh coastal environments, high salinity, extreme tides, strong winds, high temperatures, and anaerobic soils. Consequently, mangrove wetlands are characterized as a humid climate, saline environment, and muddy soil. So, mangrove plants grow in muddy saline soil ranging from 2% to 90% (Veettil *et al.*, 2019; Barraclough *et al.*, 2020).

They are varied in size between 5 and 25 m, depending on the age and regional locations maritime climate on the coastal biosphere has a direct effect on the vegetation and is influenced by tides, wave action, salt spray, saline water, and the nature of substratum (Veettil *et al.,* 2019; Barraclough *et al.,* 2020).

Mangroves are one of the world's rich and productive ecosystems that occupy the land-sea interface. They consist of flowering trees, shrubs, a wide range of organisms including birds, mammals, and microorganisms (Gomes *et al.*, 2011).

Mangroves were grown in thick organic soil mixed with sediments in shallow coastal lagoons (Holguin *et al.*, 2001). Even though these ecosystems are highly productive and rich in organic matter, they are deficient in several nutrients. The major limiting factors found in the mangroves are nitrogen and phosphorous (Reef *et al.,* 2010). Studies have shown that mangrove soils have extremely low nutrient availability due to several factors such as tidal inundation, elevation in the tidal frame, soil type, and microbial activities. The mangrove soils are the major nutrient pools, and these are typically saline, anoxic, and waterlogged. The mangrove soils are rich in carbon content due to low rates of decomposition imposed by anoxic soils (Reef *et al.,* 2010).

1.6 Mangrove and its Distribution

Mangroves occupy the intertidal region between the sea and the land in the tropical and subtropical regions of the world between approximately 30°N and 30°S latitude (Figure 1) (Giri *et al.,* 2010; Li *et al.*, 2015). Which include nearly 27 genera, and more than 70 species of mangroves (Alongi, 2002) distribute in 105 countries across the world (Hamilton & Casey, 2016). Therefore, they cover around 60–75% of the world's estuarine coastlines. The majority are in India, Brazil, Australia, Mexico, and Nigeria (Ceccon *et al.,* 2019). The grey mangrove *Avicennia*

marina is one of the most common species and has the widest latitudinal range (Barraclough *et al.*, 2020).

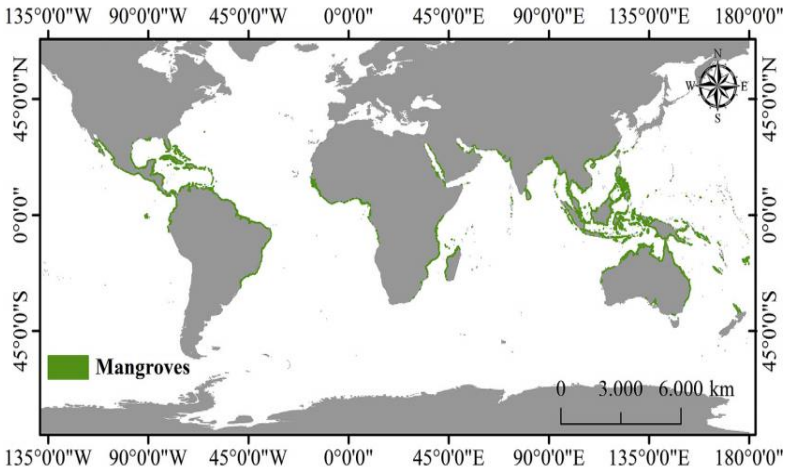

Figure 1: Global distribution of mangrove vegetation (Veettil *et al.*, 2019).

1.7 Importance of Mangrove

Mangroves are a highly productive wetlands ecosystem along with rainforest and coral reefs. This is due to the abundance of a variety of food items and turbid water (Sasekumar *et al.,* 1992). It possesses its value as it offers a wide range of products and services. They provide a wide range of services to the coastal communities (Tanner *et al.,* 2019; Rasquinha & Mishra, 2021).

1.7.1 Ecological Values of Mangroves

Mangroves provide a nursery habitat for many wildlife species for numerous marine and intertidal species including mammals, reptiles, and water birds. By providing, breeding, growing, and refuging zones for many marine organisms (Holguin *et al.*, 2001). It was reported that fishes, crabs, and shrimps are found in greater numbers in mangrove habitats compared

to shore habitats (Sasekumar *et al.,* 1992). Moreover, the aerial roots of mangroves which are called pneumatophores are a distinguishing feature that helps the plant to cope in oxygen-deprived sediments, thereby physically stabilizing the sediments. Mangroves can accumulate and excrete salt in their roots and leaves to exist in marine environments (Martin *et al.*, 2019).

Environment protection is another important fact about mangroves since it helps to stabilize shorelines and reduce the impact of natural disasters such as tsunamis and hurricanes (Sandilyan, 2015). Mangrove forests extend into the adjacent ocean holding down the coastal land where the ocean and shore meet. Thus, the strategic positioning of the mangroves on coastlines helps hold down the coastal land where the ocean and land meet thus stabilizing the shorelines. Their unique location in the intertidal areas prevents erosion of coastline caused by wave action and ocean currents (Bibi *et al.,* 2019).

Mangrove ecosystems are considered as global carbon sequestration and storage on the planet and are reported to store three to five times C per unit area higher than other forest ecosystems (Tue *et al.,* 2020). They sequester up to 25.5 million tons of carbon per year and provide more than 10% of essential organic carbon to oceans (Polidoro *et al*., 2010; Tanner *et al.,* 2019).

1.7.2 Mangroves in Medicine

It is known that mangrove possesses much essential medicinal importance which has supported humans for ages. Mangrove extracts are found to have medicinal values and are a source of proven activities against human, animal, and plant pathogens. They have been used traditionally by local medical practitioners worldwide. Moreover, it has been used as a treatment for diarrhea, dysentery, blood in urine, fever, angina, and diabetes (Chakraborty &

Raola, 2017). Also, mangrove actinobacteria produced a variety of antibiotics, anticancer compounds, and enzyme inhibitors (Arumugam *et al.,* 2017).

1.7.3 Economic Values of Mangroves

Mangrove forests are used as fuelwood. Many coastal communities are widely using mangrove wood for the construction of houses, fencing, and boats, to make furniture and utensils, as firewood and in charcoal production, as fish traps, and for timber (Rasquinha & Mishra, 2021).

Also, mangrove forests proved commercial fisheries. 80% of all commercial marine species in Florida, USA, have been estimated to depend upon mangrove estuarine areas (Jerath *et al.,* 2016). Due to the essential ecological support function that mangroves provide for commercial, recreational, and subsistence fisheries, by serving as a breeding ground and nursery habitat for marine life. Most studies estimate mangroves' contribution to fisheries in the range of 10–32 percent (Anneboina & Kumar, 2017).

1.7.4 Mangroves in Tourism

Mangrove forests have a lot of potentials to be developed as a tourist area. The recreational use of mangroves is widespread. The most popular mangrove sites attract hundreds of thousands of visitors per year and generate millions of dollars in a visitor. In addition, they can also provide socioeconomic benefits to local communities through the indirect value of natural resources and support the activities of the fishing industry (Spalding & Parrett, 2019; Fisu *et al.,* 2020). In the instance of that, Belize mangrove mangrove-related was estimated at US$60–78 million (Tanner *et al.,* 2019).

1.8 Mangroves in the UAE

Mangrove forests occur throughout the coastlines of Arabia (Figure 2), which are one of the most environmentally extreme regions for mangrove global distribution, which are reasons of the least biodiverse mangrove

ecosystems in the world. Also, the low limited growth of mangroves in this region is due to excessively high levels of salinity, wide seasonal temperature variation, low nutrient content, and adverse soil condition. The gray mangrove *Avicennia marina* is the only species is seen in the Arabia gulf side regions (El-Tarabily & Youssef, 2010; Friis & Burt, 2020).

In the United Arab Emirates (UAE), Mangroves cover approximately 3000 hectares of mangrove forest. The majority are founding in Abu Dhabi which cover 2300 hectares of the Abu Dhabi shoreline. The mangrove areas in UAE are steadily expanding in area and size due to the special interest of the rulers and the community. In instance of that, The Eastern Mangrove Lagoon National Park in Abu Dhabi is considered the most popular site for sea paddlers. It will be the first of five national parks identified in plan Abu Dhabi 2030. Other areas include Abu Abyadh, Al Aryam, and the Al Dhabeia islands of Abu Dhabi (Friis & Burt, 2020).

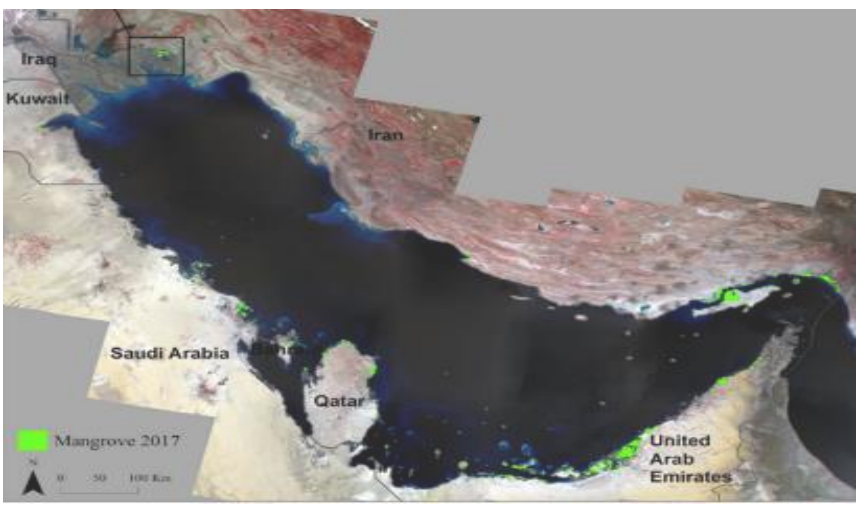

Figure 3: Mangrove distribution (green areas) along the Arabian Gulf shores (Almahasheer, 2018).

Avicennia marina or grey mangrove is the dominant species of mangrove. They play an integral part in preserving the coastlines of the UAE and reducing carbon emissions. The mangroves act as a green lunge for cities like Abu Dhabi and Dubai and provide a habitat for several marine animals, wildlife as well as creating an entertainment ground for humans Mangrove's ability to grow in the harsh condition is also an added advantage, especially in environments such as that in the UAE (Habshi *et al.,* 2007; Ghazal *et al.,* 2019).

1.9 Threats to Mangrove

Unfortunately, in the UAE and many other countries mangroves are under threat because of variety of natural and anthropogenic stresses. In addition, they are one of the highly endangered ecosystems around the world (Agoramoorthy *et al.,* 2008; Onyena & Sam, 2020). Furthermore, from 35 to 50% of the world's mangrove forests have become degraded over the past three decades especially in Asia (Mafi-Gholami *et al.,* 2020). Due to man-made disturbances ranging from deforestation and pollution (Agoramoorthy *et al.,* 2008; Rasquinha & Mishra, 2021).

The emission of heavy metals and other toxic components in the atmosphere poses threat to the mangrove ecosystem. As a result of these factors, mangroves in the arid regions are growing to their physiological limits, resulting in low productivity and slow growth (Santini *et al.,* 2012).

Despite all these, mangroves are also strongly affected by, rising temperatures, CO_2, sea-level rise (Gilman *et al.,* 2008; Alongi, 2018). The increasing temperatures (above 40°C) for a long period can affect the morphological and physiological in mangroves (Mafi-Gholami *et al.,* 2020). It was also reported that *A. marina* trees growing under saline conditions exhibited low growth rates (Santini *et al.,* 2012). Depending on the climatic types of mangroves can form different forest structures. The mangroves in

the arid regions are widely known as "scrub forests" as they form dense forests of low stature due to the high salinity of limited nutrients. The increased terrestrial vegetation can reduce groundwater levels and may increase the salinity in arid and semi-arid regions which adversely affect the growth of mangroves. In hyper-arid regions like the Middle East, high water extraction along with reduced rainfall will increase the salinity stress in mangroves (Ward *et al.,* 2016).

1.10 Bacterial Endophytes

A large range of microorganisms lives inside and outside of plant tissues. Many bacteria are present on the root surface and in the rhizosphere, many types of bacteria live endophytically inside living plant tissues. Which are involved in plant nutrition and plant resistance to stress. They can grow rapidly by utilizing nutrient sources (Oldroyd *et al.,* 2011).

Beneficial bacteria that can find in the roots and leaves of plants are called rhizosphere and phyllosphere bacteria, respectively. A high concentration of bacteria is always found in the rhizosphere due to the presence of high levels of amino acids, sugars, and organic acids that are exuded from the roots. Endophytic bacteria define as a microorganism that spends the whole or part of their life cycle within plant tissues without causing infections or symptoms of the disease. It may be either distributed throughout the plant or form specific structures such as nodules. Endophytic bacteria forming nodules are referred to as symbiotic bacteria and are utilized commercially for promoting plant growth. Thus, beneficial soil bacteria regardless of their region of action are commonly referred to as plant growth-promoting bacteria (PGPB) (Oldroyd *et al.,* 2011; Eljounaidi *et al.,* 2016). Several studies have shown that plant-associated with endophytes has enhanced growth tolerance against pathogens and pests improved efficiency for phytoremediation (Dasgupta *et al.,* 2020).

1.10.1 Diversity of Endophytes

Endophytic bacteria are in many plant species, and they are a ubiquitous part of all plant species. They have been isolated from different plant tissues, such as roots, stems, leaves, flowers, and seeds (Eljounaidi *et al.*, 2016; Ahmed *et al.,* 2020). These endophytic bacteria belonging to over 20 genera have been isolated from a variety of plants (Kobayashi & Palumbo, 2000). For example, the most common bacterial endophytes species that promote plant growth has been reported in multiple studies are *Arthrobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Methylobacterium*, *Microbacterium*, *Micrococcus*, *Paenibacillus*, *Pantoea*, *Phyllobacterium*, *Pseudomonas*, *Rhanella*, *Rhodanobacter*, *Sphingomonas*, and *Stenotrophomonas* (Santoyo *et al.*, 2016).

Several circumstances impact the endophytic communities in the plants. Such as host plant age, plant health, genotype and geographical location include the nature of the soil and its circumstances like its temperature, pH, and moisture (Santoyo *et al.*, 2016; Afzal *et al.*, 2019).

1.10.2 Colonization of the Endosphere

The endophytic colonization process usually starts from the roots (The rhizosphere zone) despite stiff competition from phytopathogens and other microorganisms for nutrients (Santoyo *et al.,* 2016). This process requires specific compounds in the root exudates which are rich in bioactive molecules which selectively attracts endophytes and mutualistic microorganisms for their ecological advantage. Exudates root compound includes amino acids, organic acids, sugar, phenolic compounds, and other bioactive secondary metabolites (Khare *et al.,* 2018). Some of those exudates are effective antimicrobial, which gives priority to the organisms that can produce specific detoxifying enzymes to inhabit the internal plant tissues.

Endophytic bacteria may also enter the plant roots through lenticels, stomata, wounds, cracks including broken trichomes, areas or emerging lateral roots, and the germinating radicle. Bacteria can also enter through undifferentiated meristematic root tissues and invagination of the root hair cell wall (Figure 4). Although endophytic bacteria usually enter the plants through the root zone, the aerial parts of the plants, including stems, leaves, flowers, and cotyledons, may also be used (Zinniel *et al.*, 2002).

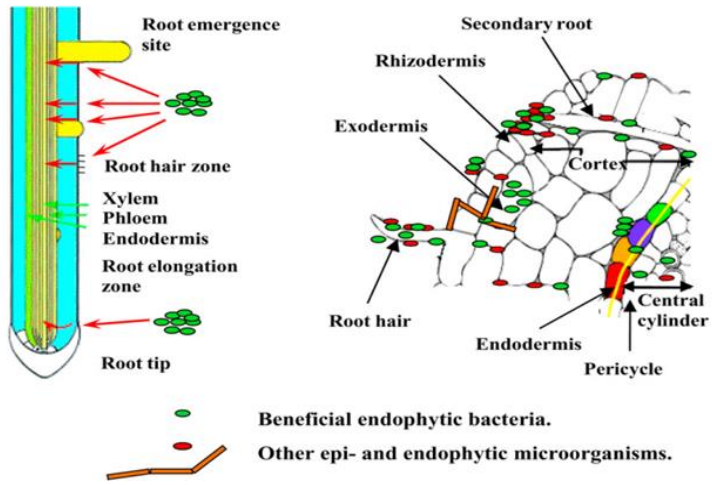

Figure 5: Sites of plant colonization by endophytic bacteria (Bajpai & Johri, 2019).

1.11 Actinobacteria

Actinobacteria are filamentous gram-positive bacteria with high Guanine - Cytosine content in its DNA that constitute one of the largest bacterial phyla (Barka *et al.*, 2016). They are unicellular like bacteria and cell walls made of peptidoglycan and it produces mycelium is non-septate and slenderer (Anandan *et al.*, 2016). The great majority of them are free-

living, aerobic, saprophyte, and they are widely distributed in soil, water, and colonizing in plants and gastrointestinal tract (Barka *et al.,* 2016; Adam *et al.,* 2018).

1.11.1 Habitat of Actinobacteria

Actinobacteria are found abundantly in soil such as alkaline soil and, desert soil. Besides the soil type, the pH, humus content, and the characteristics of the humic acid content of the soil affect actinobacteria distribution (Sharma *et al.,* 2014).

Moreover, actinobacteria are found in a watery environment. They have been isolated from freshwater as well as marine environments. Actinobacteria predominant in freshwater and marine environments or some of them being introduced from terrestrial habitats to water (Jose & Jebakumar, 2014).

Actinobacteria exist in the rhizosphere of plants. For example, actinobacteria reported from mangrove plant rhizosphere soil and mangrove endophytes are classified into 25 genera, 11 families, and 8 suborders (Jose & Jebakumar, 2014).

1.11.2 General Characteristics of Actinobacteria

Actinobacteria are a group of filamentous unicellular microorganisms, most of which are aerobic-forming mycelium known as substrate and aerial (Figure 6). They reproduce asexually via spores or binary fission. The morphological appearance of actinobacteria is various includes germination of spores, elongation, and branching of vegetative mycelium, the formation of aerial mycelium, the color of aerial and substrate mycelium, and pigment production have been used to identify actinobacteria on culture media (Barka *et al.,* 2016; Anandan *et al.,* 2016).

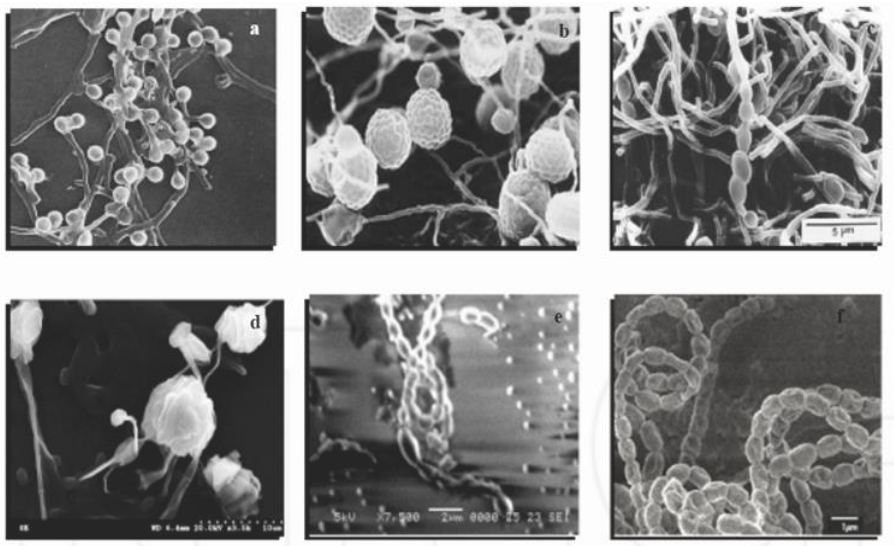

Figure 7: Scanning electron photographs of various actinobacterial isolates (Anandan *et al.,* 2016).

1.11.3 Actinobacteria in Medicine

Actinobacteria have made the most significant role in human health. Actinobacteria-derived substances span a wide range of chemistry, peptide, alkaloid, polyketides, and terpenoid with a comparable diversity of biotechnological properties, antimicrobial, antitumor, Cytotoxic, anti-parasitic, and immunosuppressive. In instance of that, actinobacteria, streptomyces can produce a varied range of secondary metabolites including antibiotics. Streptomyces species produce around 7600 secondary metabolites antibiotics. Also, several antifungal compounds are produced from actinobacteria (Anandan *et al.,* 2016; Hassan & Shaikh, 2017).

1.11.4 Actinobacteria for Sustainable Agriculture

In modern agriculture, there are a lot of challenges especially with increases in the human population in developing countries, where increases the demand for food products. Actinobacteria have their ability provide a

consistent and effective increase in the productivity of crops. There are referred to as beneficial plant-associated bacteria, plant-growth-promoting bacteria, or plant-growth-promoting rhizobacteria (Hayat *et al.,* 2010).

Actinobacteria have their ability to inhibit the growth of a wide range of phytopathogenic bacterial and fungal by producing different bioactive compounds are toxic to phytopathogens. It has been reported that around 60% of new insecticides and bioactive compounds were discovered in the past five years originate from actinobacteria Streptomyces (Anandan *et al.,* 2016).

1.12 Isolation and Cultivation of Endophytic Actinobacteria

Endophytic actinobacteria are isolated from various plants include tomato, neem, banana, wheat, and snake vine (Madhurama *et al.,* 2014). Many methods have been used for the isolation of endophytic bacteria includes surface disinfestation, trituration, centrifugation, and vacuum or pressure extraction. It depends on various factors including host plant species, age and type of the plant tissue, sampling season, geographical distribution, tissue sterility, and culture media (Jalgaonwala *et al.,* 2011).

Moreover, there are various types of culture media have been used to isolation of endophytic actinobacteria such as soybean, starch casein, starch casein nitrate (SCNA), chitin-vitamin B, humic acid vitamin B (HV), yeast extract casamino acid (YECA), modified Gausse and glycine–glycerol (Golinska *et al.,* 2015).

1.13 Role of Actinobacteria in Promote the Plant Growth

Plant growth promoting bacteria (PGPB) have been attracting attention recently as a promising approach to enhance the plant growth and development even under harsh environmental conditions (Numan *et al.,* 2018). PGPB will lead to sustained agriculture and forestry. Due to their

ability provides a consistent and effective increase crop productivity and enhance soil fertility and health without causes any toxic effect on the environment like chemical fertilizers (Hayat *et al.,* 2010; Ramakrishna *et al.,* 2020).

Endophytic actinobacteria have several biological mechanisms that enable them to promote plant growth, including production of phytohormones to increase the availability of nutrients such as Indole-3-Acetic Acid (IAA), production of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, production of siderophores compound, phosphate solubilization and nitrogen fixation. Bacterial endophytes that are referred to as symbiotic PGPB such as members of the genera *Azorhizobium*, *Bradyrhizobium*, *Devosia, Ensifer*, *Frankia*, *Mesorhizobium*, *Microvirga*, *Ochrobactrum*, *Phyllobacterium* and *Rhizobium* (Durand *et al.,* 2018).

1.13.1 Nitrogen Fixation

Nitrogen is an essential nutrient for plant growth. It is involved in the synthesis of chlorophyll, plants photosynthetic processes and in physiological and biochemical activities. Also, nitrogen involved in fertilizers (Pathania *et al.,* 2020). However, excessive use of these fertilizers is led to increase environmental pollution and health problems that threatening ecosystem sustainability (Batista *et al.,* 2018).

In the past two decades nitrogen fixing (diazotrophic) endophytes have been attention for providing biologically fixed nitrogen to plants (Patel *et al.,* 2018). The nitrogen-fixing microorganisms, using nitrogenase enzyme which is a complex enzyme encoded by nitrogenase gene (nif) to converting gaseous form of nitrogen (N_2) into combined forms like ammonia (NH_3) (Thatoi *et al.,* 2012; Ramakrishna *et al.,* 2020). More than 80% of the total

nitrogen fixed is done by help of microorganisms associated with the roots of the plants (Ramakrishna *et al.,* 2020).

1.13.2 Phosphate Solubilization

Phosphorus is an essential macronutrient for plants that exists in soil as inorganic and organic, and applied to the soil as a phosphatic manure (Ramakrishna *et al.,* 2020). However, the major amount of phosphorus applied in the soil is in insoluble forms and becomes unavailable for plants (Pathania *et al.,* 2020). In addition, phosphorus is required for photosynthesis, signal transduction, energy transfer, biosynthesis of macromolecules and respiration (Ramakrishna *et al.,* 2020).

Soil microorganisms play an important role in phosphorus transformation of soil. They solubilize soil phosphorus for plants growth (Numan *et al.,* 2018). Therefore, solubilization of phosphorus done by phosphate solubilizing bacteria such as *Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata*, and *Enterobacter* are reported as the most powerful phosphate solubilizers (Ayangbenro & Babalola, 2021).

Phosphate solubilizing bacterial can cause acidification of soil to solubilize the inorganic phosphate and becomes available for plants. Microorganisms could be synthesis an enzyme which can solubilize organic phosphate. For example, Streptomyces has solubilizing organic phosphate by secreting an enzyme called acid phosphatase (Numan *et al.,* 2018).

1.13.3 Siderophore Production

Iron is an important mineral for microorganisms and plants. Its concentration in the soil is low due to its low dissolvability (Amaresan *et al.,* 2018). Siderophores are iron chelating agents that help to make iron

available to the plants directly and deprives the other pathogenic bacteria of this iron as indirect promote plant growth (Ramakrishna *et al.,* 2020) also protect plants against fungal. Through inhibiting the establishment of phytopathogens through the sequestration of Fe_{3+} from the environment (Dimkpa *et al.,* 2009).

1.13.4 ACC Deaminase Production

The 1-aminocyclopropane-1-carboxylate (ACC) deaminase is an enzyme which first discovered by Honma & Shimomura (1978) has been shown to be involved PGPB in the promotion of plant growth (Santoyo *et al.,* 2016). PGPR with ACC deaminase can improve plant tolerance against various conditions such as high temperature, flood, drought, salinity, and acidity by reducing the level of stress triggered ethylene hormone inside the plants (Gupta & Pandey, 2019). Which when present in high concentrations can lead to inhibit plant growth or even death (Glick, 2014). There are several studies which have reported the positive impacts of plant growth promoting microbes with the ACC deaminase potential on plants growing under stressed conditions. In stance of that, multiple studies show the beneficial influences ACC deaminase on reducing salt stress in wheat, rice, ryegrass, and the medicinal plant *Limonium sinense* (Afridi *et al.,* 2019).

PGPB With ACC deaminase enzyme activity is a key factor in their ability to promote the growth of plant under stress conditions. This enzyme is responsible for the cleavage of the plant ethylene precursor by that catalyzes the degradation of ACC into ammonia and α-ketobutyrate. This would decrease ACC concentration in plant. Such decrease is likely to reduce the ethylene hormone in plant (Glick, 2014; Santoyo *et al.,* 2016).

1.13.5 Plant Hormone Production

Plant hormones are small molecules are known as plant growth regulators (PGR) that affect plant growth and development at low concentrations. Phytohormones are classified into five classes: auxins, gibberellins, cytokinins, ethylene, and abscisic acid (Pathania *et al.*, 2020). They effect seed growth, flowering, flowers sex, senescence of leaves, and ripening of fruits. Also, they enhance plant to be tolerance to abiotic and biotic that inhibit plant growth. By stimulate the density and length of root hairs and increase root surface area of a plant that enhances ability of the plant uptake its nutrient and water (Pathania *et al.*, 2020).

1.14 Seaweed Extract as a Plant Bio-stimulant

Crop production is under pressure due to increase in world population from the current 7.7 billion to 9.6 billion around 2050 (Zulfiqar *et al.*, 2020). Other biotic and abiotic stresses such as climate change, drought, salinity, pest, and disease, and weed infestations posing a major risk to the stability of the crop production (Zulfiqar *et al.*, 2020). The modern agricultural practices are largely usage of chemical fertilizers to improve crops production. The sustained use of these chemical fertilizers will disturb the soil efficiency and have serious impact on human health. In recent years, there is growing interest in the use of natural fertilizers to enhance crop growth and development in an eco-friendly manner (Vasantharaja *et al.*, 2019; Thriunavukkarasu *et al.*, 2020). Recently, the use of bio-stimulants in agriculture has increased to increasing the crops production in a sustainably way (Vasantharaja *et al.*, 2019).

In this context, plant bio-stimulants are excellent alternatives to improve crop yield under these pressures conditions that act to protect plants, minimizing the adverse effects caused by environmental stresses. According to the current European Union fertilizer regulation bio-stimulants

refers to substances that stimulate plant nutrition processes, increase availability of essential nutrients to plants growth in soils, improve the plant tolerance to abiotic stresses and improve the physiological and metabolic processes of plants (Anand *et al.,* 2016). Most of bio-stimulants promote plant growth by stimulating chlorophyll biosynthesis and improving, photosynthesis, stimulating root growth and enhancing soil water and nutrient absorption. Plant bio-stimulants are gaining widespread and are integrated into greenhouse production, fruit, vegetable, and floriculture increasing productivity and quality in a sustainable way (Mahmoud *et al.,* 2019).

Biostimulants that promote plant growth are classified in to seven classes including humic acid (HA) and fulvic acid (FA) protein hydrolysates (PHs), seaweed extracts, chitosan, inorganic compounds, beneficial fungi, and bacteria (Dong *et al.,* 2020).

1.14.1 Introduction to Seaweed Extract

Seaweeds one of an integral part of marine coastal ecosystems. They include the macroscopic, multicellular marine algae that found in the coastal regions (Khan *et al.,* 2009). Macroalgae are nearly 10,000 species and contribute to approximately 10% of the total world marine productivity (Battacharyya *et al.,* 2015). Seaweed's species play important role in marine ecosystems as they provide shelter and food to numerous marine species and can even contribute to the modification of physicochemical properties of seawater. Also, they are including in animal and human food and in agriculture as biofertilizer. In addition, humans have been used seaweeds virous purposes including food, medicine, agriculture, cosmetic products, coloring dyes, textiles (Anand *et al.,* 2016).

Seaweeds are classified depending on their pigmentation into three types; green algae (Chlorophyta) include *Ulva spp*. and *Cladophora spp*,

brown algae (Phaeophyta) include *Sargassum spp* and *Ascophyllum spp* and red algae (Rhodophyta), *Lithophyllum spp*. and *Asparagopsis spp* (Mahmoud *et al.,* 2019).

Brown seaweeds the most common used in modern, sustainable, and organic agriculture as natural plant growth stimulants or bio-fertilizers due to their consist of higher natural phytohormones, and micro and macro-nutrients contents than other types of algae (Dookie *et al.,* 2021). They are comprising about 2,000 species which occur on the rocky shores of the temperate zones. Around 15 million metric tons of seaweed products annually to use it as bio-stimulants or biofertilizers to increase plant growth and yield (Khan *et al.,* 2009).

1.14.2 Characterization of Seaweed Extract

Seaweeds are multicellular algae with a wide geographical distribution. Based on pigmentation type and morphological characteristics. They are divided into three categories, which include Phaeophyta (brown algae), Rhodophyta (red algae), and Chlorophyta (Yang *et al.,* 2021).

Seaweed extracts (SWE) are widely used on crops production and are available as liquid extracts or in a soluble powder form (Mahmoud *et al.,* 2019). It consists of a mixture of useful biologically active substances such as polyphenols, polysaccharides, alginates, polyamines, pigments, free amino acids, betaines, vitamins, micro and macro-nutrients and natural phytohormones such as auxins, cytokinins, gibberellins, abscisic acid and brassinosteroids (Battacharyya *et al.,* 2015; Supraja *et al.,* 2020).

1.15 Effects of Seaweed Extract on Plants

SWE as bio-stimulant cause many beneficial effects on plants due to contains growth promoting hormones such as indole-3-acetic acid (IAA), indole 3-butyric acid (IBA) and cytokinins, trace elements (Fe, Cu, Zn, Co,

Mo, Mn and Ni), vitamins and amino acids (Salim, 2016), mineral nutrients, and many other organic compounds besides compensating for the deficiency of N, P, and K (Civelek Yoruklu *et al.*, 2022). For instance, Seaweed liquid fertilizer is source of nitrogen, phosphorous, potassium, calcium and magnesium and plant growth regulators like cytokinin, auxin, and gibberellins (Thriunavukkarasu *et al.*, 2020).

In addition, SWE has ability to enhance plant growth and fruiting. It has been investigating that enhance seed germination and early seedlings growth. Moreover, it enhances leaf total chlorophyll content that reflected on the capacity and efficiency of photosynthetic process, as well as increasing organic carbon content in the soil and increase nutrient availability (Mahmoud *et al.,* 2019). Also, applied SWE elevated resistance to biotic and abiotic stress, and pathogens (Zulfiqar *et al.*, 2020). It became one of the important solutions to ensure sustainable agriculture, especially in arid and semi-arid regions where soils are poor in organic nutrients (Anli *et al.*, 2020).

1.15.1 Effects of Seaweed Extract on Nutrient Uptake

Plant absorbs nutrients by roots or from the leaf surface. SEW has been shown their ability to enhance impact on plant nutrient uptake by affecting soil processes include improvement of soil structure, improvement of micronutrient solubility in the soil or by directly affect the plant's physiology by changes in root morphology, and increased root colonization by arbuscular mycorrhizal fungi (Battacharyya, *et al.*, 2015; Halpern *et al.*, 2015). Moreover, they found that red alga *Kappaphycus alvarezii* seaweed increases the grain concentration of N, P, K, and S by up to 36%, 61%, 49% and 93%, respectively in soybeans grown (Halpern *et al.*, 2015).

SEW bio-stimulants change the physical, biochemical, and biological properties of the soil and may affect the architecture of plant roots

and increase efficient of uptake of nutrients from soil. In addition, brown seaweeds contain polyuronides such as alginates and fucoidans. Alginic acid showed soil-conditioning properties and chelated metal ions forming high molecular weight polymersso, The presence of highly cross-linked polymeric network improved water retention capacity of the soil and therefore, stimulated root growth and soil microbial activity. Moreover, SE kahydrin components derivative of vitamin K1 altered plasma membrane proton pumps and induced the secretion of H+ ions into the apoplast leading to acidification of the rhizosphere. The acidification changed the redox state of soil and the solubility of metal ions, making them available to plant (Battacharyya *et al.*, 2015).

SEW affect regulation of genes that played an important role in nutrient uptake. For example, *A. nodosum* extract upregulated the expression of a nitrate transporter gene NRT1.1. that improved nitrogen sensing and auxin transport resulting in enhanced lateral roots growth and improved nitrogen absorption (Battacharyya *et al.,* 2015).

1.15.2 Seaweed Extract Improves Soil Structure

Seaweed supports plant nutrition by enhance soil health by improving moisture-holding capacity and by promoting the growth of beneficial soil microbes. Instance for that, brown seaweed contains large amounts of polysaccharides such as alginates and fucoidans. Although, the Alginate occurs in the cell walls of seaweeds as a mixed salt with the major Na, Ca, Mg, and K together with a few minor metals counterions. These mixture of salts of alginic acid and metallic ions in the soil form high-molecular-weight complexes that absorb moisture, swell, retain soil moisture, and improve crumb structure. This improves soil aeration and capillary activity of soil pores which in turn stimulate the growth of the plant root system as well as boost soil microbial activity. In addition, the

polyanionic properties of seaweeds have proved valuable in remediation of soils, especially those with heavy metals (Khan *et al.,* 2009).

Moreover, SWE provide nutrients and affects bacterial diversity and community structure in plant rhizosphere soils. The result, those microbiota at the interface between plant roots and soil (rhizosphere) have been linked to improved plant growth and health (Chen *et al.,*2021; Hussain *et al.,* 2021). These beneficial bacteria are referred to as plant growth promoting bacteria (PGPB). PGPB may enhance plant growth through improve of plant nutrient uptake especially phosphorous nitrogen fixation and stimulation of transport systems in plants. Also, PGPB produce plant growth hormones such as auxins, cytokinins, gibberellins and polyamines and production of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. On other hand, PGPB effects plant growth indirect by the production of metabolites, such as iron-sequestering siderophores or antifungal metabolites where they reduce the growth of pathogenic microorganisms (El-Tarabily & Youssef, 2011).

1.15.3 Seaweed Extract Promote Symbiotic Relationship between Mycorrhiza Fungi and Roots

Arbuscular mycorrhiza fungi (AM) fungi are a ubiquitous symbiosis between the fungi a large majority of plants roots. It can enhance plant growth by extends beyond the root zone, promote the absorption of water and nutrients, in particular phosphorus (Anli *et al.,* 2020; Liu *et al.,* 2022; Cheng *et al.,* 2022) and enhanced the resistance plants to drought stress, salinity stress, low temperature stress, and pests and diseases (Cheng *et al.,* 2022). Moreover, AM fungi have been shown to decrease uptake of certain heavy metal (HM) in plant by supplying a HM barrier or excreting organic compounds (such as glomalin) to chelate HM ion or improving

phytochelatins production and the expression of phytochelatin synthase gene in plant (Zhang *et al.,* 2019).

As mentioned, brown seaweed contains alginates which influence soil properties and encourage growth of beneficial fungi. The alginate has been observed that significantly stimulated hyphal growth and elongation of (AM) fungi (Khan *et al.,* 2009).

1.15.4 Seaweed Extract Improve Plant Tolerant to Environmental Stresses

Abiotic stresses such as drought, salinity, and temperature extremes can negatively affect plant growth and productivity accounting for more than 50% losses in productivity of major crops (Rayirath *et al.,* 2009; (Khan *et al.,* 2009). For example, salinity and drought are becoming widespread in many regions, especially in Arabic countries with an estimated 50% possibly being salinized by 2050 (Khan *et al.,* 2009). In addition, the environmental stresses, such as heat, may reduce plants cytokinins, chlorophyll content, photochemical efficiency, and carbohydrate reserves and inhibit antioxidant defense systems (Zhang *et al.,* 2010).

The brown alga, *Ascophyllum nodosum* (L.) Le Jol. is the most seaweed used in commercial. Ascophyllum have been reported enhance plants resistance to diseases and tolerance to environmental stresses such as drought and salinity (Rayirath *et al.,* 2009). In addition, SWE contain biologically active concentrations of natural cytokinins such as trans-zeatin riboside (t-ZR) and isopentenyl-adenine. It has been reported that use of seaweed (*Ascophyllum nodosum Jol*.) increase leaf cytokinin content and delay senescence of creeping bent grass under heat and drought stress (Zhang *et al.,* 2010).

1.15.5 Seaweed Extract Improve Plant Resistance to Diseases

On other hand, biotic stress such as fungi, bacteria and viruses reduce plants growth and productivity. In stance for that, Fungi are a major cause of yield loss and responsible for 80% of plant diseases. Plant viruses cause huge yield loss and around, 30 types of viroids have been reported infect large number of plants. Moreover, some bacteria are very harmful to plants such as *Pseudomonas*, *Ralstonia*, *Agrobacterium*, *Xanthomonas*, *Erwinia*, *Xylella*, *Pectobacterium*, and *Dickeya*. However, generally the biotic stresses are controlled by use pesticides, fungicides and anti-microbial chemicals which are harmful to environmental (Agarwal *et al.*, 2021).

As mentioned, SWE is one of the important solutions to ensure sustainable agriculture (Anli *et al.*, 2020). It is rich of source of nutrients and bioactive compounds, which can improve the disease tolerance in plants. It has been reported the efficiency of SWE towards disease tolerance in plants to control fungal, bacterial, and viral pathogens. Through the different algal polysaccharides such as carrageenans, fucans, laminarans and ulvans is molecules to protect plants against various diseases (Agarwal *et al.*, 2021).

Chapter 2: Methods

2.1 Soil Characteristics, Plant Material and SWE

In the current study, dark grayish-black sediments were collected from the east coast of Abu Dhabi-UAE (24° 26′ 48.5″ N; 54° 26′ 40.6″ E). Viviparous propagules of gray mangrove (*A. marina*) were obtained from either mother trees or freshly fallen ones collected from the same above-mentioned location. Similar sized-propagules were surface-sterilized using 70% ethyl alcohol (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), and 20% Clorox bleach. All surface-sterilized propagules were then washed 10 times with 0.22 µm filter-sterilized (Millipore Corporation, Burlington, MA, USA) full-strength seawater (salinity of 40) and left to air dry for 30 min.

In the current study, a commercial Acadian soluble SWE powder (Acadian Sea plants limited, Dartmouth, Nova Scotia, Canada) was used as a Biostimulants. This organic seaweed concentrate is derived from the kelp, *Ascophyllum nodosum.* Physiochemical properties, macronutrients, micronutrients of SWE can be found in Table (1). Mangrove sediment was amended with 400 ml of SWE pot^{-1} according to the manufacturer's recommended rate (0.3 g SWE L^{-1}water) every 14 days for the entire period of the in *vivo* experiments.

Table 1: Physical, chemical, and biochemical analyses of the commercial Acadian soluble SWE powder.

Item [a]	Value	Item [a]	Value
Physical analyses		**Amino Acids (%)**	
Appearance	Brownish-black crystals	Ala	0.32
Odor	Marine	Arg	0.04
Solubility in water	100%	Asp	0.62
pH	10.0-10.5	Cys	0.01
Biochemical analyses		Glu	0.93
(i) Macronutrients (%)		Gly	0.29
Total N	0.8-1.5	His	0.08
Available P_2O_5	1.0-2.0	Ile	0.26
Soluble K_2O	17.0-22.0	Leu	0.41
S	1.0-2.0	Lys	0.16
Mg	0.2-0.5	Met	0.11
Ca	0.3-0.6	Phe	0.25
(ii) Micronutrients (ppm)		Pro	0.28
Na (%)	3.0-5.0	Ser	0.08
B	75-150	Thr	0.04
Fe	75-250	Tyr	0.17
Mn	8-12	Val	0.28
Cu	1-5	Try	0.07
Zn	25-50	Total	4.4
Chemical analyses (%)			
Maximum moisture	6.5		
Organic matter	45.0-55.0		
Ash (minerals)	45.0-55.0		
Carbohydrates	Alginic acid, mannitol, laminarin		
PGRs	Auxins, CKs, GA_3		

[a] All analyses were according to Acadian Agritech, Dartmouth, Nova Scotia, Canada.

SWE, seaweed extracts; N, nitrogen, P_2O_5, phosphoric acid; K_2O, potash; S, sulfur; Mg, magnesium; Ca, calcium; Na, sodium; B, boron; Fe, iron; Mn, manganese; Cu, copper; Zn, zinc; CK, cytokinin; GA3, gibberellins; Ala, alanine, Arg, arginine; Asp, aspartic acid; Cys, cystine; Glu, glumatic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalnine; Pro, proline; Ser, serine; Thr, threonine; Try, tyrosine; Val, valine; Trp, tryptophan.

2.2 Isolation of Endophytic Actinobacteria

Mangrove propagules (Section 2.1) sown in plastic pots (23 cm diameter × 17 cm depth) containing sediment from the area described above were watered daily with full strength seawater under greenhouse conditions (temperature of 25±2°C; relative humidity of 60±5%; average daily photosynthetic photon flux density of 700±150 µ mol m^{-2} s^{-1}). Eight pots (each containing two propagules) were prepared.

After 5 weeks, 16 seedlings from eight pots were collected and transferred to the laboratory. Roots were cut, washed and fresh weight (FW) was recorded. Roots were soaked in sterile phosphate-buffered saline (PBS) solution (pH 7.0) for 10 min (Rennie *et al.,* 1982) and surface-disinfested. For the surface-sterilization, roots were firstly exposed to propylene oxide (Sigma-Aldrich) vapour for 25 min (Sardi *et al.,* 1992). Roots were soaked in 70% EtOH for 4 min and 1.05% NaOCl for 4 min; followed by rinsing ten times in PBS. In order to verify no transmission of biological contamination into the root tissues during maceration (El-Tarabily *et al.,* 2019).

The slurry was filtered through a sterile cotton cloth, and the filtrate was serially diluted (10^{-2}, 10^{-3}, 10^{-4}). Aliquots (200 µl) were spread on plates containing inorganic salt starch agar (Küster, 1959). For each root sample dilution, three replicated plates were dried for 15 min followed by 7-day-incubation at 28±2°C in dark (El-Tarabily *et al.,* 2019). Population density (PD; log10 colony-forming units (cfu) g root FW^{-1}) of endophytic actinobacteria was calculated (Hallmann *et al.,*1997). PD counted and recorded by the following formula: colony forming unit (CFU/g) = number of colonies × dilution factor/volume of culture plate.

The Colonies of streptomyces actinobacteria (SA) and non-streptomyces actinobacteria (NSA) were purified and identified on oatmeal agar plates supplemented with 0.1% yeast extract (Shirling & Gottlieb, 1966).

Hyphae/spores of actinobacterial isolates were stored in 20% glycerol at −70°C (Williams, 1977).

Culture characteristics such as color of aerial and substrate mycelia, and the production of diffusible pigments in addition to the presence or absence of aerial mycelia, the distribution of spores both on aerial and substrate mycelia, the formation of sporangia, and the stability/fragmentation of substrate mycelia were used to differentiate between SA and NSA (Cross, 1989). Filter-sterilized full-strength seawater was used in the preparation of all microbiological media in the current study.

2.3 *In vitro* Screening for PGP Traits

All endophytic isolates were streaked on ISSA medium supplemented with 80 g l^{-1} (8%) NaCl (Williams *et al.*, 1972). Plates were incubated at 28°C in dark for 7 days. Strong growth and heavy sporulation of actinobacterial isolates indicated high salt tolerance.

The high salt tolerant SA and NSA were preliminary tested for the production of indole-3-acetic acid (IAA). Briefly, 2 ml of each isolate (108 cfu ml^{-1}) was incubated on 250 rpm orbital shaker incubator in flasks containing 50 ml inorganic salt starch broth (Küster, 1959) supplied with 5 ml of 5% L-tryptophan (Sigma-Aldrich) for 7 at 28°C in dark (Khalid *et al.*, 2004; El-Tarabily *et al.*, 2019). Suspensions were centrifuged at 12,000 x g, and 4 ml of Salkowski reagent was added to the collected supernatants (Gordon & Weber, 1951). IAA-equivalents (µg ml-1) were quantitatively determined using spectrophotometer (UV-2101/3101 PC; Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan) at 530 nm. All promising IAA-producing isolates were further grown for 10 days at 28°C in dark in glucose peptone broth (Di Menna, 1957) supplemented with 5 ml of 5% L-tryptophan to detect IAA and indole-3-pyruvic acid (IPYA), and on Strzelczyk and Pokojska-Burdziej (1984) medium to detect gibberellic acid

(GA3) and cytokinins (CKs) including isopentenyl adenine (iPa), isopentenyl adenoside (iPA) and zeatin (Z), using reverse-phase high performance liquid chromatography (HPLC; Spectra Lab Scientific Inc., Ontario, Canada). Auxins, GAs, and CKs were separated by using two isocratic solvent systems, according to Tien *et al.* (1979). Waters Associates HPLC with a differential ultraviolet detector was used to analyze the chromatograms, which were created by injecting 10 µl of the methanol dissolved extract onto a 10-m reverse phase column (Waters Associates Bondapak C18, 4 mm x 30 cm) (El-Tarabily *et al.*, 2020). To calculate the concentrations of PGRs in the unknown sample, their respective peak areas were compared with those obtained with authentic samples (Sigma-Aldrich) of a known concentration.

For ACCD assay, all isolates were plated on Dworkin and Foster's (DF) salts minimal agar medium (Dworkin & Foster, 1958) supplemented with either 0.3033 g l-1 ACC (DF-ACC; Sigma-Aldrich) or 2 g l-1 ammonium sulfate (DF-(NH4)2SO4; control) for 7 days at 28°C in dark (El-Tarabily *et al.*, 2019). Growth/sporulation on DF-ACC plates indicated that the isolate could produce ACCD. We also quantified the enzymatic activity of ACCD by measuring the amount of α-keto-butyrate (Honma & Shimomura, 1978). Protein concentrations were determined according to Bradford (1976).

To test the puteriscine (Put) production, plates containing Moeller's decarboxylase agar medium (MDAM) was supplemented with 2 g l^{-1} of L-arginine-monohydrochloride (Sigma-Aldrich) and phenol red (Sigma-Aldrich) (Arena & Manca de Nadra, 2001). MDAM plates were incubated for 2 days in dark at 28°C (El-Tarabily *et al.*, 2020). Dark red halo surrounding the colonies indicated a Put-producing isolate. We also quantitatively test these Put-producing actinobacterial isolates for their production of the polyamines Put, spermidine (Spd) and spermine (Spm) using reverse-phase HPLC (Marino *et al.,* 2000). Positive Put-producers were placed in Moeller's

decarboxylase broth medium (MDBM) amended with 2 g l^{-1} L-arginine-monohydrochloride (Arena & Manca de Nadra, 2001). Using a 254-nm UV detector (Smith & Davies, 1985), an aliquot of 10 µl of the sample was injected onto a Bondapak C18 column (4 mm x 30 cm) in a liquid chromatograph (Waters Associates) as described by Marino *et al*. (2000).

For siderophores production, plates of chrome azurol S (CAS) agar (Schwyn & Neilands, 1987) were inoculated with isolates and incubated for 3 days at 28°C in dark. Actinobacterial isolates that were considered as siderophore-producers, developed yellow-orange halo zone around the colony.

Phosphate-solubilizating actinobacteria (PSA) were assayed using Pikovskaya (PVK) agar medium (Pikovskaya, 1948) supplemented with rock phosphate (Tianjin Crown Champion International Co. Limited, Tianjin, China) and amended with bromophenol blue. The appearance of clear zone underneath the colony indicated a PSA isolate. The same isolates were also grown in 20-ml of sterilized National Botanical Research Institute Phosphate (Nautiyal, 1997) broth at 28°C for 2 days on a shaker at 150 rpm. For each isolate, aliquots (1 ml) were transferred to a flask containing 250 ml NBRIP medium and incubated with continuous shaking at 28°C. Sterilized un-inoculated medium served as a control. After 3 days, a 10 ml sample of each culture or control was centrifuged at 12,000 x g for 15 min. The supernatant was used to determine the drop in pH and the amount of P released into the medium. The pH was recorded using a pH-meter, whereas P availability was determined using phospho-molybdate blue color method (Murphy & Riley, 1962).

Acetylene-*reduction assays* (Dye, 1962) and Nessler's reagent (Holguin *et al.,* 1992) were used to measure nitrogenase activity and NH3 production,

respectively. In all in *vitro* assays, eight independent replicates were used for each strain.

2.4 Evaluation of PGP Parameters of PGPA Isolates under Gnotobiotic Conditions

Of all PGP traits tested in *vitro*, the strongest PGPA isolates (#11, #12 and #20) were selected for the preliminary growth promotion experiment under gnotobiotic conditions. In a greenhouse, surface-sterilized mangrove propagules were sown in plastic pots containing sediment for 10 days and watered daily with full strength seawater.

In order to prepare the inoculum for all the gnotobiotic experiments, 4 ml aliquots of 20% glycerol suspension of the selected endophytes were individually inoculated into 250-ml ISSB and shaken at 250 rpm on orbital shaker incubator for 5 days. Cells were centrifuged at 12,000 x g for 15 min at 20°C, and the pellet was suspended in 10 ml PBS and re-centrifuged (El-Tarabily *et al.*, 2019). For each suspension, 0.1 ml of each of the dilutions of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} was made in PBS and spread on ISSA. After 5 days of incubation, a final concentration of ~108 cfu ml^{-1} of each isolate was used as an inoculum.

Selected endophytic actinobacterial isolates were introduced inside the young seedlings using the pruned-root dip method (Musson *et al.,* 1995). Briefly, root tips (3 mm) from germinated propagules were trimmed and young seedlings were placed in sterile plastic cups containing the inoculum suspension (108 cfu ml-1) of each isolate for 3 h at 25°C. Seedlings of mangrove with or without the actinobacterial inoculum were aseptically planted into glass tubes (300 x 35 mm) filled with sediment and moistened with seawater. Control treatment was represented by seedlings that were placed in autoclaved ISSB.

All seedlings maintained in a growth chamber (day-time cycle: 16/8-h light/night; temperature: 25/20°C; fluorescent light: 180-200 µmol m-2 s^{-1}) were daily irrigated with full strength sterilized seawater. After 6 weeks of transplantation, plants were harvested, washed, and separated into roots and shoots. Dry weight (DW; g) and length (cm) of shoot and root tissues were measured. Each treatment representing one seedling was independently replicated eight times.

2.5 *In planta* Population Density of Selected Actinobacterial Isolates

To quantification determined of the internal Colonization by actinobacterial Isolates. Rifampicin resistant mutants of the promising PGPA (#11, #12 and #20) and non-PGPA control (#7) isolates were selected on ISSA medium supplemented with rifampicin (100 µg ml^{-1}; Sigma-Aldrich) and compared to the corresponding wild type strains (Misaghi & Donndelinger, 1990). Features, such as morphology growth and PGP, of these mutants were found to be similar to the parental strains.

The pruned-root dip method was used to inoculate 10-day-old seedlings of mangrove with the endophyte inoculum (Section 2.4) in order to evaluate the colonization of internal root and stem tissues by isolates. Free draining pots (36-cm in diameter) were filled with 14 kg of sediments (Section 2.1) and watered daily with full strength seawater to container capacity in the greenhouse (Section 2.2). Roots and stems were sampled, washed and surface-sterilized (Section 2.1) every 3 weeks (for 15 weeks) after planting. Samples were homogenized to determine the PD of isolates on ISSA amended with rifampicin. Each replicate represents a single pot containing one seedling, and each treatment was replicated eight times.

For light microscopy (LM) and transmission electron microscopy (TEM), specimens (6-week-old mangrove seedlings inoculated with the selected PGPA isolate) were fixed in freshly prepared karnovsky's fixative

(2% paraformaldehyde (Sigma-Aldrich) + 2.5% glutaraldehyde (Sigma-Aldrich) in a 0.17 M Sorensen's phosphate buffer, pH 7.2) for 24 h at 4°C. After washing three times with the buffer, tissues were post-fixed with 1% aqueous osmium tetroxide for 2 h at 25°C, dehydrated with ascending grades of ethanol (30%-100%) and dipped into the propylene oxide (Sigma-Aldrich). Finally, samples were infiltrated, embedded in epoxy resin (Epon 812, Agar Scientific, UK) and polymerized at 60°C in embedding oven for 24 h (Millonig, 1976). Blocks were trimmed into semi-thin sections (1.5 µm) and ultra-thin sections (95-nm) with Leica EM7 ultra microtome (Vienna, Austria). Slides of selected heat-dried, semi-thin sections stained with 1% toluidine blue, and 1% borax (Sigma-Aldrich) were examined using Olympus BH-2 (Olympus Optical Co. Ltd, Tokyo, Japan) LM equipped with a digital camera and software (Jenoptik ProgRes Camera, C12 plus, Frankfurt, Germany). For TEM study, ultra-thin sections were collected on 200 mesh copper grids, stained with 10% uranyl acetate and 3% lead citrate, and examined using Tecnai Spirit G2 Biotwin TEM (FEI Co., Eindhoven, Netherlands).

2.6 Identification of the Most Potent PGP Endophytic Isolate

The most potent isolate was identified based on the 16S rRNA gene sequencing, performed by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ)-Germany. Primers targeting 16S rRNA gene: 900 R (5'CCGTCAATTCATTTGAGTTT3'); 800 F (5'ATTAGATACCCTGGTAG-3') and 357 F (5'-TACGGGAGGCAGCAG-3'). Were used (Rainey *et al.,* 1996; Saeed *et al.,* 2017; Kamil *et al.,* 2018). All 16S rRNA gene sequences of 16 representatives from the genus Streptomyces were retrieved from the NCBI database (http://www.ncbi.nlm.nih.gov/nucleotide/). Multiple sequence alignment of 16S rRNA genes was carried out using CLUSTAL-X (Thompson, 1997) implemented in the Molecular Evolutionary Genetics

Analysis 7.0 (MEGA7) software with default parameters (Kumar *et al.*, 2016). A phylogenetic tree of 16S rRNA genes was reconstructed using the maximum-likelihood method (Felsenstein, 1981) implemented in the MEGA 7.0 software. Bootstrap values were calculated with 1,000 resamples.

The spore chain morphology and spore surface were examined using Philips XL-30 scanning electron microscopy (SEM; FEI Co., Eindhoven, The Netherlands) of 15-day old cultures grown on ISP-3 (Kumar *et al.*, 2011).

2.7 Assessment of Growth Promotion, Photosynthetic Pigments and Endogenous PGRs in Mangrove under Greenhouse Conditions

In free draining pots filled with sediments (Section 2.5), seedlings were inoculated with the most promising endophytic isolate #12 using the pruned-root dip method (Section 2.4). A total of four treatments were carried out: (1) Control (seedlings inoculated with autoclaved ISSB medium only; neither SWE nor isolate was applied); (2) *St* (seedlings inoculated with the endophytic isolate #12); (3) SWE (seedlings supplemented with only SWE); and (4) *St*+SWE (seedlings inoculated with the endophytic isolate #12 in a sediment supplemented with SWE). SWE was added as per the manufacturer's recommended rate (Section 2.1). Seedlings were watered daily with full strength seawater to container capacity (Section 2.5). In the greenhouse, a randomized complete block design (RCBD) was used, where each replicate (total of eight replicates) was determined by a single pot containing one seedling. Trials were independently repeated twice with similar results.

The dry weight (DW) and length of roots and shoots, number of branches and leaf surface area (cm^2) were recorded at the end of 9 months post planting (mpp) of propagules. Chlorophyll (chl) fluorescence measurements were performed at 645 (for chl *a*) and 663 nm (for chl *b*) (Holden, 1965*).* Carotenoid pigments *were* measured at 470 nm according to Davies *(*1965).

The PAs (Put, Spd and Spm) extracted from tissues of apical root and shoot tissues (Flores & Galston, 1982) were quantitatively determined using benzoyl chloride (Sigma-Aldrich) and normal internal standard of PAs (Redmond & Tseng, 1979). Reverse-phase HPLC chromatograms were produced onto a 10-µm reverse-phase column (Marino *et al*., 2000; section 2.3).

To quantitatively measure the endogenous PGRs (auxins, CKs, GA_3 and ABA, abscisic acid [ABA]), crude extracts from mangrove root and shoot tissues (Shindy & Smith, 1975; Guinn *et al.,* 1986; Machàckovà *et al*., 1993) were detected with the *UV* at *254 nm using reversed-phase* HPLC (Waters Associates). This method provides quantification of phytohormones in a single run from 50 mg of fresh plant tissue. *ACC* content was assayed by the method of Concepcion *et al. (*1979*)*. Derivatization of ACC was carried out by the addition of phenylisothiocyanate (Sigma-Aldrich) and the reverse-phase HPLC chromatograms were produced as described by Lanneluc-Sanson *et al.* (1986).

All measurements were taken on seedlings at the end of 9 mpp of propagules. Sixteen replicate samples from two independently repeated experiments were analyzed for all the tested parameters for each treatment.

2.8 Analyses of Plant Nutrients

We selected the tissues of the terminal part of the root and shoot systems at the end of 9 mpp of propagules, washed them in deionized water and cut them into small pieces, which were then dried overnight at 70°C. Mineral nutrients were then analyzed in the roots and shoots as follows. Nitrogen was measured using a LECO FP-428 nitrogen analyzer by combusting finely ground plant material at 950°C in oxygen. As the sample passed through a thermal conductivity cell, the amount of N released from it was measured (Sweeney & Rexroad, 1987). Plant samples were digested in a

9:1 solution of nitric acid and perchloric acid to measure phosphorus (P), K, sulfur (S), magnesium (Mg), calcium (Ca), sodium (Na), Fe, manganese (Mn), copper (Cu) and zinc (Zn) (McQuaker *et al.,* 1979). For the measurement of boron (B), plant material was dry-ashed, extracted with dilute acid, and B was colorimetrically measured with azomethine H (Gaines & Mitchell, 1979). Analyses were performed for all the nutrients in 16 replicates from two independently repeated experiments. Assessment of *in vivo* Growth Promotion under Open Field Nursery Conditions

In free draining pots filled with sediments (Section 2.5), seedlings were inoculated with the promising endophytic isolate #12 using the pruned-root dip method (Section 2.4). A total of four treatments were carried out as descried above (2.7). For each treatment, eight separate pots each containing one seedling were arranged in a RCBD. The container open field nursery experiments were carried out in the Arabian Gulf coast of Abu Dhabi in the same location described in 2.1 and the nursery trials were independently repeated twice. Control and inoculated seedlings were maintained under natural conditions between February to October (relative humidity range =22-43%; daytime length range=11.0-13.5h day; average temperature =35.0±9°Cday /23.0±8.0°C night; average precipitation= 6.7 mm). Irrigation was carried out naturally from the sea during the high and low tide time of the day. The DW and length of roots and shoots, number of branches and leaf surface area (cm^2) were recorded from 16 samples from two independently repeated experiments at the end of 12 mpp of propagules.

2.9 Assessment of in vivo Growth Promotion under Open-field Nursery Conditions

In free draining pots filled with sediments (Section 2.5), seedlings were inoculated with the promising endophytic isolate #12 using the pruned-root dip method (Section 2.4). A total of four treatments were

carried out as descried above (2.7). For each treatment, eight separate pots each containing one seedling were arranged in a RCBD. The container open-field nursery experiments were carried out in the Arabian Gulf coast of Abu Dhabi in the same location described in 2.1 and the nursery trials were independently repeated twice. Control and inoculated seedlings were maintained under natural conditions between February to October (relative humidity range =22-43%; daytime length range=11.0-13.5h day; average temperature=35.0±9°Cday/23.0±8.0°C night; average precipitation =6.7 mm). Irrigation was carried out naturally from the sea during the high and low tide time of the day. The DW and length of roots and shoots, number of branches and leaf surface area (cm2) were recorded from 16 samples from two independently repeated experiments at the end of 12 mpp of propagules.

2.10 Estimation of the Sediment Total Microbial Activity

To study the effect of SWE on the growth of PGPA, *including S. tubercidicus* UAE1, a newly SWE agar (SWEA) medium was developed. This medium was prepared by dissolving 40 ml SWEA in 1 l of filter-sterilized full-strength seawater (pH 7.5). All endophytic isolates were streaked on SWEA, and the plates were incubated at 28°C in dark for 7 days. Strong growth and sporulation of actinobacterial isolates on SWEA indicated the ability of SWE to support the growth of the endophytic isolates.

To compare the effect of SWE individually or in combination with *S. tubercidicus* UAE1 on mangrove sediment ecosystem, total microbial activity was assessed at the end of the greenhouse and open-field nursey experiments. The microbial activity was measured using the fluorescein diacetate (FDA) hydrolysis technique (Schnurer & Rosswall, 1982). Briefly, 5 g of each sediment was added to 20 ml of 60 mM potassium phosphate buffer (8.7 g K2HPO4 and 1.3 g KH2PO4 in 1 l distilled water, pH 7.6) in 250 ml flasks. The FDA was dissolved in acetone and

stored as a stock solution (2 mg ml-1) at -20°C. The reaction was started by adding 0.2 ml of FDA (400 µg) from the stock solution to a buffer-sediment mix. The reaction flasks were shaken (250 rpm) at 25°C for 20 min on orbital shaker incubator. The reaction was then stopped by adding 20 ml acetone to all samples. Sediment residues were centrifuged at 12,000 x g for 15 min and filtered through using Whatman filter paper (Whatman, Maidstone, England). The filtrate was collected in a test tube, covered with Parafilm and placed into an ice bath to reduce volatilization of the acetone. The concentration of fluorescein was determined by reading the optical density at 490 nm using spectrophotometer (Shimadzu Corporation). For each treatment, the background absorbance was corrected with the blank sample run under identical conditions but without the addition of FDA. Standard curves were prepared according to Chen (1988). The results were converted to µg hydrolyzed FDA g dry sediment ^{1}. All analyses were collected from sediments at the end of 9 and 12 mpp of propagules of the greenhouse and open-field nursery experiments, respectively. Sixteen replicate samples from two independently repeated experiments were analyzed for each treatment.

2.1 Statistical Analyses

In all experiment, RCBD was performed. Gnotobiotic greenhouse and nursery experiments were repeated with similar results. All data were combined and analyzed using analysis of variance (ANOVA) procedure of SAS Software version 9 (SAS Institute Inc., NC, USA). Mean values of treatments were compared using Fisher's protected least significant difference (LSD) test at P=0.05 levels. The PD of the selected PGPA isolates were transformed into log10 cfug root or stem FW^{-1}.

Chapter 3: Results

3.1 *In vitro* Assessment of PGP Traits of Purified Endophytic Actinobacteria

The purified endophytic actinobacterial isolates were in *vitro* assessed for their salinity tolerance and different PGP traits. From the 26 endophytic actinobacteria, 20 SA and 6 NSA were isolated from the surface-sterilized root samples, seven (6 SA and 1 NSA) isolates (#3, #11, #12, #17, #20, #23 and #25) were able to perform different PGP activities with an ability also to tolerate 8% NaCl (Table 2; Figure 5). Regardless of their PGP traits, the rest of isolates were not included in further experiments because they did not grow or sporulate on ISSA containing 8% NaCl.

We first tested the highly salt tolerant isolates for the production of PGRs, ACCD, siderophores, nitrogenase, NH3, and for their ability to solubilize insoluble P. The identified isolates synthesized significantly ($P<0.05$) different levels of auxins (IAA and IPYA), GA3 and CKs (iPa, iPA and Z) (Table 1). Three isolates (#11, #12 and #20) produced higher amounts of auxins (Table 2; Figure 5); whereas five (#11, #12, #20, #23 and #25) produced higher levels of GA3 than any other tested isolates (Table 2). Although isolate #3 produced the lowest levels of auxins and GA3, isolate #17 did not produce GA3 in the culture extracts. By comparing the in vitro production of CKs among the tested actinobacteria, isolate #12 produced the highest amounts of the three types (Table 2). Thus, other isolates synthesized comparable amounts of iPa (#20) and iPA (#17) to isolate #12. Except of #3, #23 and #25, all other isolates produced significantly ($P<0.05$) high levels of Z (Table 1).

The production of enzymes ACCD and nitrogenase known for their PGP activities (El-Tarabily *et al.,* 2019) by the actinobacterial isolates were also assessed in vitro. Although isolates #11, #12, #17, #20 and #25

produced the highest level of ACDD (Table 2; Figure 5); none of these isolates produced nitrogenase (Table 2).

We also qualitatively and quantitatively determined the production of PAs on culture extracts of the seven isolates. The amounts of Put, Spd and Spm synthesized by these isolates significantly ($P<0.05$) varied (Table 1). By using the HPLC, isolates #11, #12, #20, #23 and #25 produced significantly ($P<0.05$) higher levels of Put than the other tested isolates (Table 1; Figure 5). Isolates #11, #12 and #20 synthesized the highest amounts of Spd and Spm (Table 2). When we checked the production of siderophores and NH3 in vitro, #17 and #25 were the only actinobacterial isolates that showed these PGP traits. The remaining produced either one or none of the two traits (Table 2).

Six isolates (#3, #11, #12, #17, #20 and #23) were able to solubilize P (Table 1; Figure 5). Since we tend to find the isolate(s) showing most, if not all, PGP activities, with the highest values in all the PGRs and ACCD tested, only isolates #11, #12 and #20 were further included for their endophytic existence and abundance in planta.

Table 2: In *vitro* production of PGRs, ACCD, siderophores, nitrogenase enzyme and NH3, and effect of inoculation with RP amended PVK broth with the selected halotolerant endophytic PGPA strains isolated from within surface sterilized mangrove roots.

Activity [a]		Isolate [b]							
		#3	#7	#11	#12	#17	#20	#23	#25
Production of									
Auxins	**IAA equivalents**	3.14 *a*	-	20.72 *c*	22.17 *c*	16.17 *b*	23.17 *c*	17.28 *b*	5.67 *a*
	IAA	7.50 *a*	-	54.82 *c*	56.14 *c*	36.14 *b*	56.24 *c*	39.20 *b*	7.25 *a*
	IPYA	3.78 *a*	-	11.66 *c*	12.22 *c*	9.74 *b*	12.40 *c*	11.84 *c*	3.59 *a*
GA_3		4.94 *a*	-	6.96 *b*	7.42 *b*	-	7.42 *b*	7.14 *b*	7.65 *b*
CKs	**iPa**	4.85 *b*	-	7.18 *c*	8.10 *d*	7.22 *c*	7.92 *cd*	3.25 *a*	4.47 *b*
	iPA	2.15 *a*	-	3.36 *b*	3.94 *c*	3.96 *c*	3.44 *b*	3.54 *b*	2.28 *a*
	Z	1.24 *a*	-	2.56 *b*	2.64 *b*	2.44 *b*	2.44 *b*	1.12 *a*	1.32 *a*
ACCD		122.25 *b*	-	438.54 *d*	503.12 *e*	328.54 *b*	449.92 *d*	17.22 *a*	314.33 *c*
PAs	**Put**	200.18 *b*	-	454.04 *d*	452.20 *d*	37.58 *a*	430.94 *d*	315.44 *c*	331.49 *c*
	Spd	98.66 *b*	-	176.12 *c*	185.52 *c*	12.45 *a*	166.66 *c*	104.38 *b*	91.24 *b*
	Spm	38.01 *b*	-	72.40 c	75.04 *c*	4.10 *a*	71.82 *c*	33.28 *b*	35.20 *b*
Siderophores		+	-	+	+	+	+	-	+
P sol.	**Concentration (Control 13.11)**	175.3 *b*	-	314.70 *c*	288.67 *d*	300.20 *c*	330.0 *c*	277.36 *d*	12.60 *a*
	pH (Control=7.72)	5.97 *b*	-	4.46 *c*	3.36 *d*	4.40 *c*	4.33 *c*	3.41 *d*	7.02 *a*
Nitrogenase		-	-	-	-	-	-	-	-
NH_3		-	+	-	-	+	-	-	+

[a] PGRs (auxins, GA_3, CKs and PAs), ACCD and P were measured in µg ml^{-1}, nanomoles α-keto-butyrate mg^{-1} protein h^{-1} and mg l^{-1}, respectively.

[b] Only isolate #3 belonged to non-streptomycete actinobacteria, whilst isolates # 7, #11, #12, #17, #20, #23, and #25 belonged to streptomycete actinobacteria. Isolate #3 was identified as *Micromonospora* sp. Isolates #7 (positive control), #11, #12 (*Streptomyces tubercidicus* UAE1), #17, #23, and #25 were identified as *Streptomyces* spp. Isolates #11, #12 and #20 were selected for further studies. All isolates (#3, #7, #11, #12, #17, #20, #23, and #25) were found to be halotolerant isolates. They sporulated heavily on inorganic salt starch agar amended with 8% NaCl.

Data were from 8 independent replicates. Values with same letter in a row are not significantly (P>0.05) different according to Fisher's Protected LSD Test.

PGRs, plant growth regulators; ACCD, 1-aminocyclopropane-1-carboxylic acid deaminase; NH_3, ammonia; RP, rock phosphate; PVK, Pikovskaya; PGPA, plant growth promoting actinobacteria; IAA, indole-3-acetic acid; IPYA, indole -3-pyruvic acid; GA_3, gibberellic acid; iPa, isopentenyl adenine; iPA, isopentenyl adenoside; Z, zeatin; +, PA, polyamine; Put, putrescine; Spd, spermidine; Spm, spermine; P, phosphorus; P sol., P solubilization; producing/active; -, non-producing/inactive.

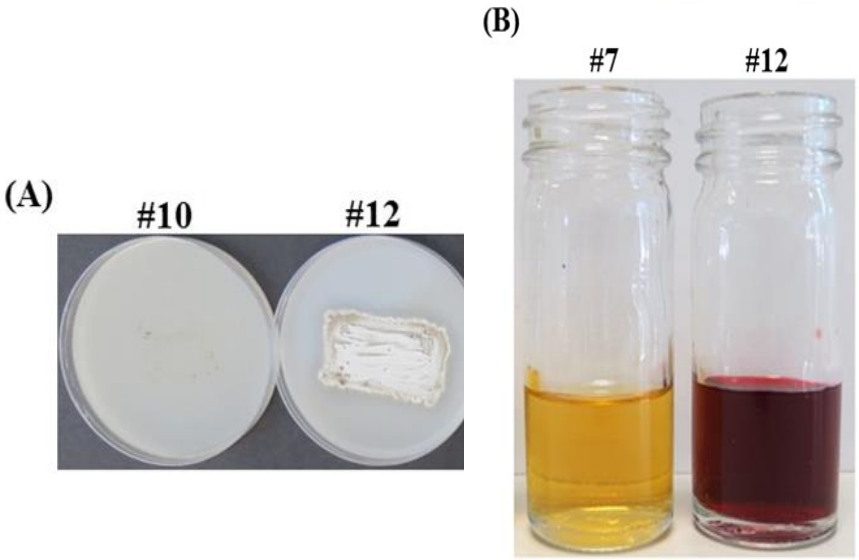

Figure 8: In *vitro* plant growth promoting characteristics of the promising endophytic actinobacterial isolate. The selected endophytic actinobacterial strain isolated from *Avicenna marina* root tissues possessing (A) tolerance to salinity; production of (B) IAA. In (A), growth and sporulation of isolate #12 on ISSA supplemented with 8% NaCl indicated a halotolerant isolate. In (B), formation of red color of isolate #12 after addition of Salkowski reagent to cultures grown in ISSB amended with L-tryptophan indicates production of IAA.

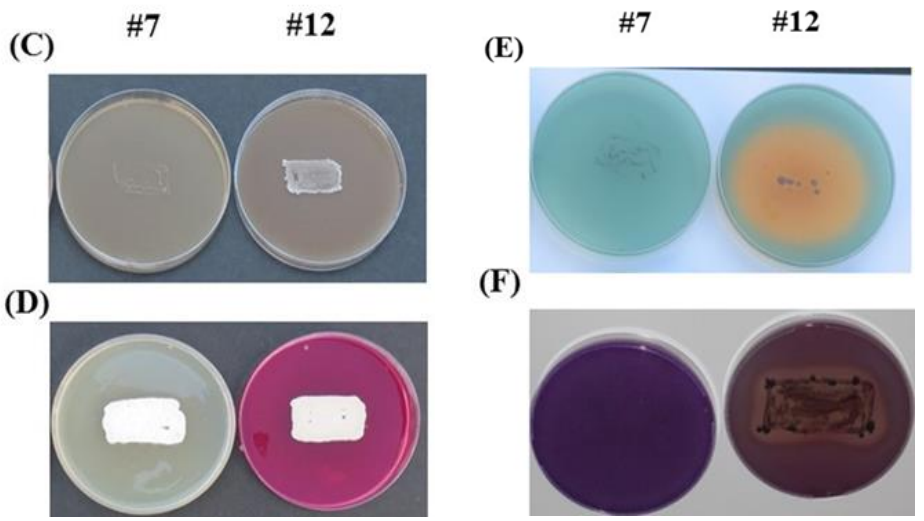

Figure 5: In *vitro* plant growth promoting characteristics of the promising endophytic actinobacterial isolate. The selected endophytic actinobacterial strain isolated from *Avicenna marina* root tissues possessing (C) growth and sporulation of isolate #12 tested on DF-ACC indicated the efficiency to utilize ACC and production of ACCD. In (D), change to red color of the phenol-red of isolate #12 tested on MDAM amended with L-arginine-monohydrochloride indicated production of Put. In (E), yellow halo surrounding the colony of isolate #12 tested on CAS agar plates indicated the excretion of siderophores. In (F), production of clear zone surrounding the colony of isolate #12 tested on PKA medium amended with rock phosphate and bromophenol blue indicated P-solubilization. Actinobacterial strain #7 (non-PGPA) was used as a positive control isolate. Actinobacterial strain #10 was a non-halotolerant isolate. IAA, indole-3-acetic acid; ACC, 1-aminocyclopropane-1-carboxylic; ACCD, ACC deaminase; Put, putrescine; P, phosphorus; ISSA/B, inorganic salt starch agar/broth; DF, Dworkin and Foster's salts minimal agar medium amended with ACC; MDAM, Moeller's decarboxylase agar medium; CAS, chrome azurol S; PKA, Pikovskaya's agar, PGPA, plant growth promoting actinobacteria (continued).

3.2 Growth Promotion and Tissue-specific Colonization of Selected Endophytic PGPA

The strongest actinobacterial isolates showing multiple in vitro PGP traits were selected to determine growth promotion of mangrove under gnotobiotic conditions. First, the chemical characteristics of the sediment collected earlier (Section 2.1) were analyzed and recorded as the following: pH (8.36), electrical conductivity (5.81 dSm^{-1}), organic carbon (6.24%), total P (85 and 8.83 mg kg^{-1} sediment), available P (8.83 mg kg^{-1}), N (4 and 6.4 mg kg^{-1} as nitrate and ammonium, respectively), bicarbonate extractable potassium (K; 241 mg kg^{-1}), amorphous iron (Fe) oxides (331 mg kg^{-1}) and sulfate (414 mg kg^{-1}).

Table 3: Effect of endophytic PGPA isolates on growth parameters of mangrove (*Avicennia marina*) under gnotobiotic conditions.

Treatment [a]	Length (cm)		FW (g)	
	Root	**Shoot**	**Root**	**Shoot**
Control	5.98±0.34 *a*	10.56±0.54 *a*	1.09±0.09 *a*	2.81±0.10 *a*
#7	6.19±0.17 *a*	11.76±0.29 *a*	1.18±0.13 *a*	2.95±0.15 *a* a
#11	11.46±0.43 *b*	15.79±0.21 *c*	2.78±0.29 *b*	4.32±0.27 *c*
#12	17.51±0.25 *c*	19.34±0.81 *d*	4.87±0.15 *c*	5.07±0.11 *d*
#20	10.16±0.15 *b*	13.12±0.40 *b*	2.99±0.12 *b*	3.62±0.24 *b*

[a] Isolates #11, #12, and #20 and were the strongest PGPA obtained. Isolates #11, and #20 were identified as *Streptomyces* spp., whilst isolate # 12 was identified as *Streptomyces tubercidicus* UAE1. The non-PGPA isolate #7 identified as *Streptomyces* sp. was used as a positive control. Values are means ±SE of 8 replicates for each treatment. Values with the same letter for each growth measurement within a column are not significantly (P>0.05) different according to Fisher's Protected LSD Test. Plants were harvested after 6 weeks. PGPA, plant growth promoting actinobacteria; FW, fresh weight.

To determine the longevity of the promising endophytic isolates in planta, we compared their PD in the internal tissues of mangrove roots and stems on a triweekly basis (up to 15 weeks). In our greenhouse experiments, isolates #11, #12 and #20 recovered in all tissues albeit the sampling time. This suggests an endophytic nature of these PGPA isolates without causing harm to the host plant i.e., mangrove. The total population of isolates, except of #11, increased significantly (P<0.05) from the beginning until the end of the experiment in both tissues (Figure 6). Following the significant (P<0.05) increase in the first 6 weeks of colonization, there was a drop in PD of isolate #11 inside root and stem tissues of mangrove by the end of the experiment. We also noticed that the mean of the total population of isolate #20 in

mangrove stems in Weeks 6-15 was insignificant ($P>0.05$; Figure 6). Together, our data suggested that isolate #11 did not sufficiently recover from the tissues of mangrove seedlings after Week 6, and that isolate #20 inefficiently colonized stem tissues. Thus, the two isolates were excluded from further experiments. Our results imply, on the other hand, that #12 can be a potential PGPA isolate to be considered as a potential endophytic PGPA isolate. This was evident from the PD of this isolate that strikingly ($P<0.05$) increased for the period of colonization in root and stem tissues throughout the greenhouse trials (Figure 6). Compared to isolate #12, we noticed similar increase patterns in the PD of the non-PGPA isolate #7 (control). Overall, the increased growth and high PD consistently found in seedlings by isolate #12 under controlled gnotobiotic and greenhouse conditions, respectively, made this isolate ideal at a large-scale experiment i.e., non-controlled open-field nursery.

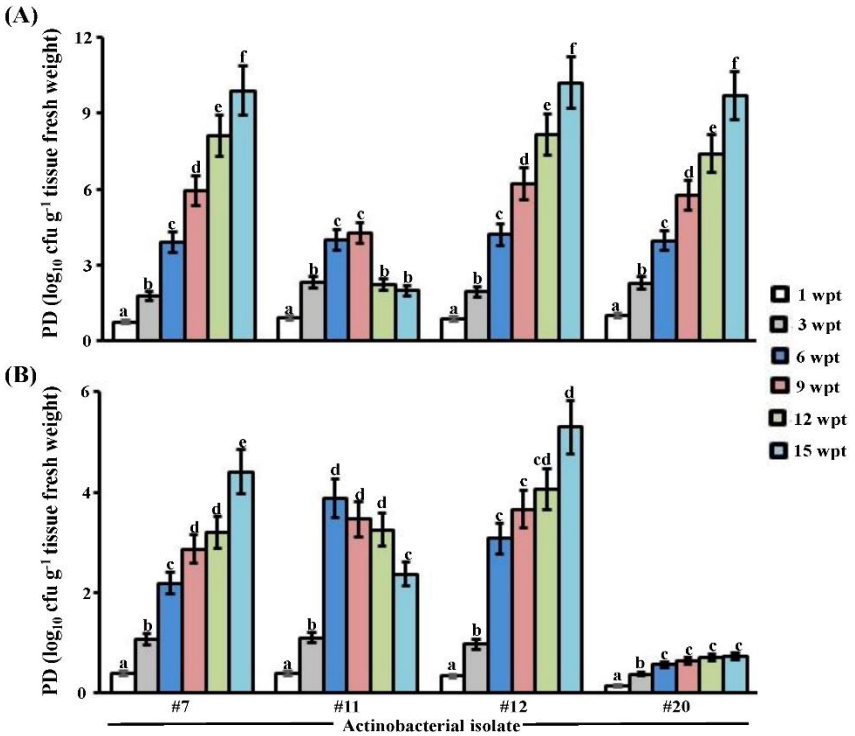

Figure 9: Population density of the promising endophytic actinobacteria within mangrove tissues. Total population of the endophytic isolates (#11, #12 and #20) showing multiple PGP traits in *vitro* residing in (A) root and (B) stem tissues of grey mangrove grown under greenhouse conditions. Tissues were sampled every 3 wpt. Values are means of 8 replicates ±SE for each sampling per treatment. Mean values followed by different letters are significantly ($P<0.05$) different from PD of each strain in that particular tissue according to Fisher's Protected LSD Test. Isolate #7 represents the endophytic Streptomyces sp. that does not show any PGP trait in vitro; while isolate #12 represents *Streptomyces tubercidicus* UAE1. Isolates #11 and #20 belonged to Streptomyces spp. PGP, plant growth promoting; PD, population density; wpt, weeks post treatment.

To confirm the endophytic lifestyle of isolate #12, we used microscopic analyses to visually investigate the presence of spores and/or mycelia within the internal tissues of mangrove roots and stems at 6 weeks post inoculation (wpi). Using LM, the spores of actinobacterial isolate #12 colonized the root tissues intercellularly within the parenchyma cells of the cortex and the xylem (Figure 7A). In addition, mycelial growth carrying the spiral spore chains of isolate #12 was detected within the cortical cells of roots (Figure 7B). Interestingly, the germinated spores and the formed germ tubes indicated the ability of isolate #12 to colonize the intracellular spaces of root cells (Figure 8). Similarly, the endophytic isolate could successfully reside within the stem cells (Figure 7C). This was confirmed by the growth and survival of isolate #12 as part of its lifecycle in vascular structures with its host plant (Figure 7D). The obtained results suggested that isolate #12 can translocate between the root and shoot systems through conductive tissues of xylem.

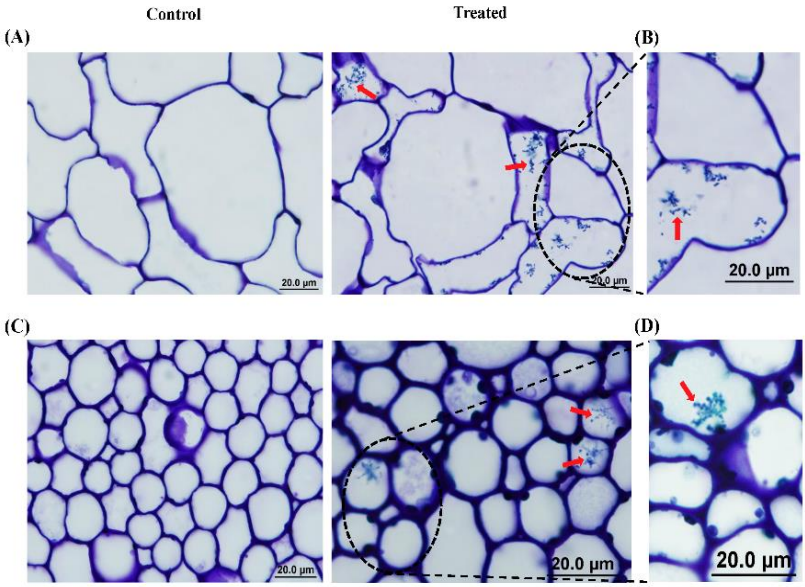

Figure 10: Colonization of mangrove tissues by actinobacterial isolate #12. Light micrograph of semi-thin sections of mangrove (A) root and (C) stem not inoculated (control; left) or inoculated with isolate #12 (treated; right) (1000x). Close-up views of mangrove (B) root and (D) stem inoculated with isolate #12 (1000x). In (A-D), all sections were stained with 0.1% toluidine blue showing the distribution of spores (red arrows) within cells of root and shoot tissues of mangrove. Bars: 20 µm. Isolate #12 represents *Streptomyces tubercidicus* UAE1.

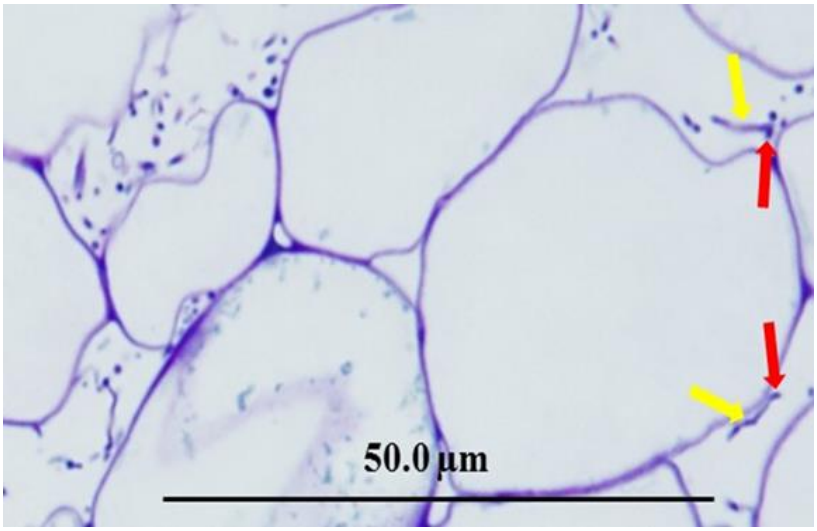

Figure 11: Intracellular colonization of mangrove root tissues by isolate #12. Light micrograph of semi-thin sections of mangrove root cells penetrated by mycelial growth of isolate #12 carrying spores (1000x). The section was stained with 0.1% toluidine blue showing the spore germinated spore (red arrows) and substrate mycelium (yellow arrows) within the root's cells of mangrove. Bar: 50µm. Isolate #12 represents *Streptomyces tubercidicus* UAE1.

Using TEM, the presence of the spore chains and substrate mycelia of isolate #12 was determined within the cortex of roots (Figure 9A) and stems (Figure 3B). We also figured out individual spores colonizing the cortical cells (Figure 9C) and attaching the cell membrane of host plant roots (Figure 9C) and stems (Figure 9D). This indicates a beneficial association between isolate #12 and mangrove seedlings. It is worth to mention that we did not observe plant cell defects in any of the imaged samples in these microscopic studies. Overall, our data suggested that isolate #12 is an actinobacterial endophyte that inhabits within living root and stem tissues of mangrove.

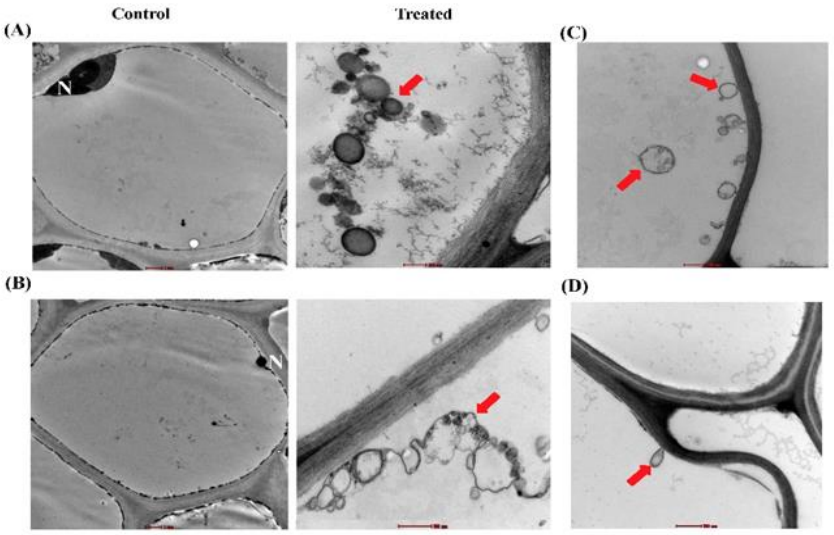

Figure 12: Intercellular colonization of mangrove root and stem tissues by actinobacterial isolate #12. Transmission electron micrograph of ultra-thin sections of mangrove (A) root and (B) stem not inoculated (6000x; control; left) or inoculated (treated; right) with isolate #12 (top: 43000x; bottom: 26500x). (C) Distribution of individual spores in root cells (16500x), and (D) attachment of spores on the cell membrane (20500x). In (A-D), all sections were stained with uranyl acetate and lead citrate showing the distribution of spore chains (red arrows). Bars: (A, left & B, left) 1 µm, (B, right, C & D) 500 nm, (A, right) 200 nm. Isolate #12 represents *Streptomyces tubercidicus* UAE1. N, nucleus.

3.3 Taxonomic, Cultural and Morphological Identification of *Streptomyces tubercidicus* UAE1

Genomic DNA was extracted from the promising endophytic actinobacterial isolate, and the 16S rRNA gene was amplified (1,523 bp), sequenced and deposited in GenBank under accession number MT883495. Next, the amplified fragment was used to perform a comparative sequence analysis with sequences available in GenBank. Our results identified actinobacterial species belonging to the genus Streptomyces. The identity

between the 16S rRNA gene sequences obtained from isolate #12 and those available in GenBank ranged from 97.9 to 100.0%, of which *Streptomyces nigrescens* NBRC12894, *Streptomyces libani* NBRC13452 and *Streptomyces tubercidicus* NBRC13090 showed the highest similarities (Figure 10A). To distinguish it from other Streptomyces species, we described the pure cultures of this particular strain and the morphological characteristics of its spore chains. On ISP medium 3, the actinobacterial isolate developed light brownish gray mass color and yellowish-brown substrate mycelium with the production of yellow pigment on the reverse side of cultures (Figure 10B). The spore chains belonged to section Spirals, consisting of 3-10 mature, smooth-surfaced spores per chain (Figure 10C). Together, the molecular phylogeny, culture characteristics and morphology of spores classified isolate #12 as *Streptomyces tubercidicus* Nakamura 1961 Strain UAE1.

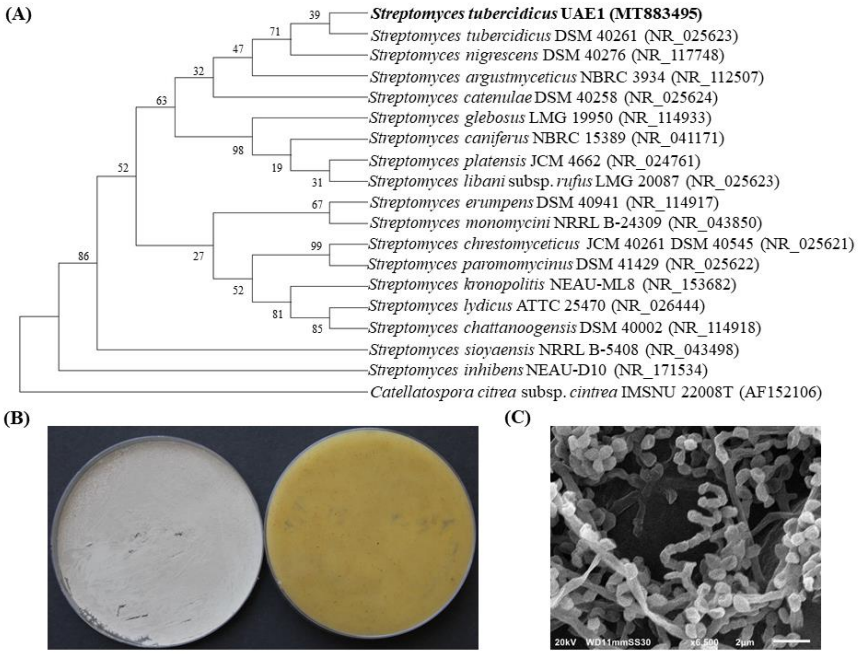

Figure 13: Taxonomic determination of *Streptomyces tubercidicus* UAE1. Based on phylogenetic, cultural and morphological characteristics. (A) The tree showing the phylogenetic relationships between *S. tubercidicus* UAE1 (MT883495; 1,523 bp) and other members of Streptomyces species on the basis of 16S rRNA sequences. (B) Aerial mycelia (left) and substrate mycelia (right) growing on ISP medium 3 supplemented with yeast extract, and (C) scanning electron micrograph (6,500X) of the spiral-shaped chains and smooth-surfaced spores of the strain of *S. tubercidicus* UAE1. In (A) numbers at nodes indicate percentage levels of bootstrap support based on a maximum likelihood analysis of 1000 resampled datasets. Bar, 0.002 substitutions per site. GenBank accession numbers are given in parentheses.

3.2 Valuation of Growth Promotion of Mangrove Seedlings under Greenhouse Conditions

To determine their effect on growth promotion, mangrove seedlings were grown in sediment supplemented with SWE and/or inoculated with *S.*

tubercidicus UAE1 (*St*) under greenhouse conditions. After SWE application or *St* inoculation, mangrove grew and developed into health plants (Figure 11A). The DW (Figure 11B) and length (Figure 11C) of roots and shoots apparently increased with either SWE or St compared to non-treated plants at the end of 9 mpp of the inoculated mangrove propagules, thus significantly ($P<0.05$) varied between the two treatments. Seedlings of mangrove treated with the combination of SWE +*St*, however, showed the greatest DW and length of root and shoot systems. In line with that, the number of branches significantly ($P<0.05$) increased by 40.6% and 55.3% in St-inoculated plants and SWE-treated, respectively (Figure 11D). We also noticed that the total leaf area was larger in plants treated with SWE by 29.0% or St by 18.3% than in non-inoculated plants, (Figure 11E). The number of branches and total leaf area per plant were recorded at the highest values with SWE +*St* by 64.8% and 38.0%, respectively.

In *planta* photosynthetic pigments largely determine the photosynthetic capacity and hence plant growth (Li *et al.,* 2018). Our results showed that contents of *chl a* and *chl b* pigments were significantly ($P<0.05$) higher in SWE-treated plants than in non-inoculated or St-inoculated plants at 9 mpp the inoculated mangrove propagules (Figure 11 F). Among all the treatments, the combined treatment of SWE and St had the highest chlorophyll contents. Although there was no significant difference in the amounts of carotenoids in plants treated with SWE, *St* or SWE + *St*, we observed that any of these treatments significantly ($P<0.05$) stimulated the production of this particular photosynthetic pigment compared to the control. Our data suggested that growth of mangrove can be enhanced as aresult of the photosynthesis and biomass production, more in plants treated with the combined treatment than the individual treatments of SWE or *St*.

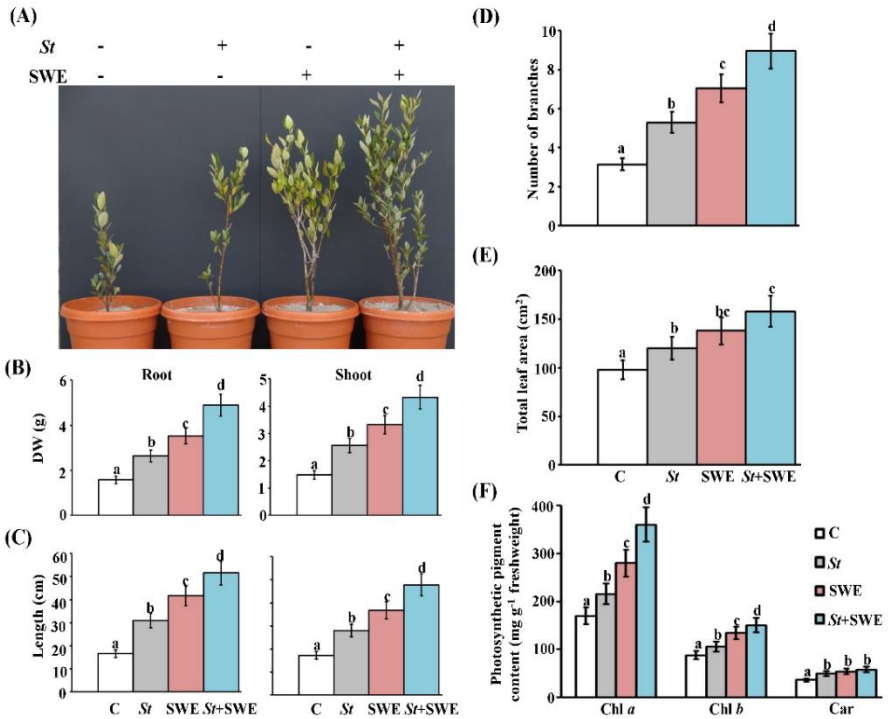

Figure 14: Effect of the application of seaweed extract and *Streptomyces tubercidicus* UAE1 on mangrove growth under greenhouse conditions. Effect of the inoculation of the endophytic isolate *Streptomyces tubercidicus* UAE1 (St) and application of SWE bio-stimulant, on the (A) formation of the vegetative growth; (B) DW and (C) length of root (left panel) and shoot (right panel); (D) number of branches; (E) total leaf area; and (F) photosynthetic pigment contents of *Chl a*, *Ch l b* and Car of mangrove. In (A-F), non-supplemented/non-inoculated seedlings with either *St* or SWE were used as the control treatment. In (B-F), measurements were taken at the end of 9 months post planting the inoculated mangrove propagules. Values are means of 16 replicates± SE for each sampling from two independent experiments. Mean values followed by different letters are significantly ($P<0.05$) different from each other according to Fisher's Protected LSD Test. Bars represent standard error. C, control (non-inoculated inorganic salt starch broth); *St*, *Streptomyces tubercidicus* UAE1 (isolate #12); SWE, seaweed extract; *St*+ SWE, combination of *S. tubercidicus* UAE1 and SWE; DW, dry weight; *Chl a*/*b*, *chlorophyll a/b*, Car, carotenoids.

3.5 Effect of SWE and *St* on PGRs and Nutrient Contents in Mangrove Tissues

In the greenhouse, we also studied the effect of SWE and/or *St* application on the endogenous contents of PGRs (phytohormones and ACCD) and mineral nutrients in roots and shoots at 9 mpp the inoculated propagules associated with growth promotion of mangrove. In general, all PGRs levels investigated in this study were relatively higher in shoot than in root tissues. The concentration of auxins (IAA and IPYA) in the tissues of plants grown in sediment supplemented with SWE, inoculated with St or both were significantly ($P<0.05$) different from the control treatment and from each other (Figure 12A; Figure 13). Seedlings grown in sediment supplemented with SWE or inoculated with St were characterized by about 28.3-30.6% or 16.7-17.3% higher IAA concentration in root and shoot, respectively, than those in control treatments. IAA levels increased by 42% in root and 33% in shoot tissues in plants supplied with the combined two treatments. Compared to control, there were greater contents of IPYA in roots (22.3-41.4%) and shoots (18.0-28.0%) in seedlings supplied with any of the three treatments, of which SWE +*St* was the highest (Figure 6A; Figure 13). Similarly, the concentration of three types of CKs (iPA, iPa and Z) varied significantly ($P<0.05$) among all treatments in root tissues (Figure 13). Although iPA and iPa contents increased significantly ($P<0.05$) by SWE, *St* and SWE +*St* treatments, we did not find significant ($P>0.05$) differences in Z concentrations between control and St in shoots (Figure 12 B). Treatments of SWE or SWE +*St* on mangrove seedlings increased Z in shoot tissues to similar levels, which were significantly ($P<0.05$) higher than that with or without *St* (Figure 12 B). There was no significant ($P>0.05$) difference in the endogenous levels of ABA in mangrove root and shoot tissues in the four treatments (Figure 14). Our data suggested that growth promotion of mangrove can be increased by the application of SWE or inoculation of St by increasing the endogenous levels

of auxins and CKs in tissues, thus these can be greatly enhanced by SWE + *St* treatment.

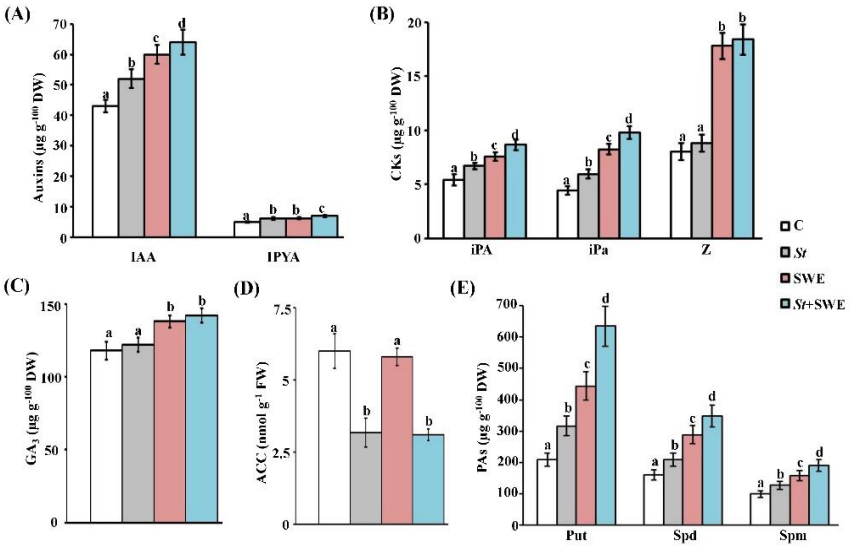

Figure 15: Effects of *Streptomyces tubercidicus* UAE1 and seaweed extract on PGRs in mangrove shoots. Endogenous contents of (A) auxins, (B) CKs, (C) GA3, (D) ACC, and (E) PAs in mangrove shoot tissues after treatment with SWE and/or St. Mangrove seedlings were grown in an evaporative-cooled greenhouse and maintained at 30±2°C. Values are means± standard error of 16 replicates for each treatment from two different independent experiments. Mean values followed by different letters are significantly ($P<0.05$) different from each other according to Fisher's Protected LSD Test. Bars represent standard error. Endogenous contents of all PGRs were measured at the end of 9 months post planting the inoculated mangrove propagules. C, control (non-inoculated inorganic salt starch broth); *St*, *Streptomyces tubercidicus* UAE1 (isolate #12); SWE, seaweed extracts; *St*+ SWE, combination of *S. tubercidicus* UAE1 and SWE; DW, dry weight; IAA, indole-3-acetic acid; IPYA, indole-3-pyruvic acid; iPA, isopentenyl adenine; iPa, isopentenyl adenoside; Z, zeatin; GA3, gibberellic acid, ACC, 1-aminocyclopropane-1-carboxylic; PA, polyamine, Put, putrescine; Spd, spermidine; Spm, spermine.

In addition, the contents of GA3 increased and ACC decreased significantly ($P<0.05$) by St whether it was individually inoculated or combined with SWE in the examined tissues of mangrove (Figure 12C & D; Figure 13). In general, there was a drop in ACC contents of 40-50% in root and shoot tissues upon applying *St*. This suggests that the ACCD secreted by the local strain of *S. tubercidicus* UAE1 efficiently relieves plants from stress.

Under controlled conditions, there was significant ($P<0.05$) difference in the endogenous levels of Put, Spd and Spm in tissues of all treatments (Figure 6E; Figure S3). When *St* was inoculated or SWE was applied in pots containing seedlings of mangrove, roots had significantly ($P<0.05$) higher levels of Put (34.2% or 53.2%, respectively), Spd (23.4% or 44.4%, respectively) and Spm (22% or 37.3%, respectively) than those grown without any bio-stimulant/bioinoculant. The treatment of SWE+*St*, however, increased Put by 62.7%, Spd by 61.1% and Spm by 47.0% in the same tissue. Likewise, the pattern of increase in the three PAs was clearly demonstrated in shoot tissues as follows (from the highest to the lowest): SWE+*St*>SWE>*St*>control (Figure 12 E; Figure 13).

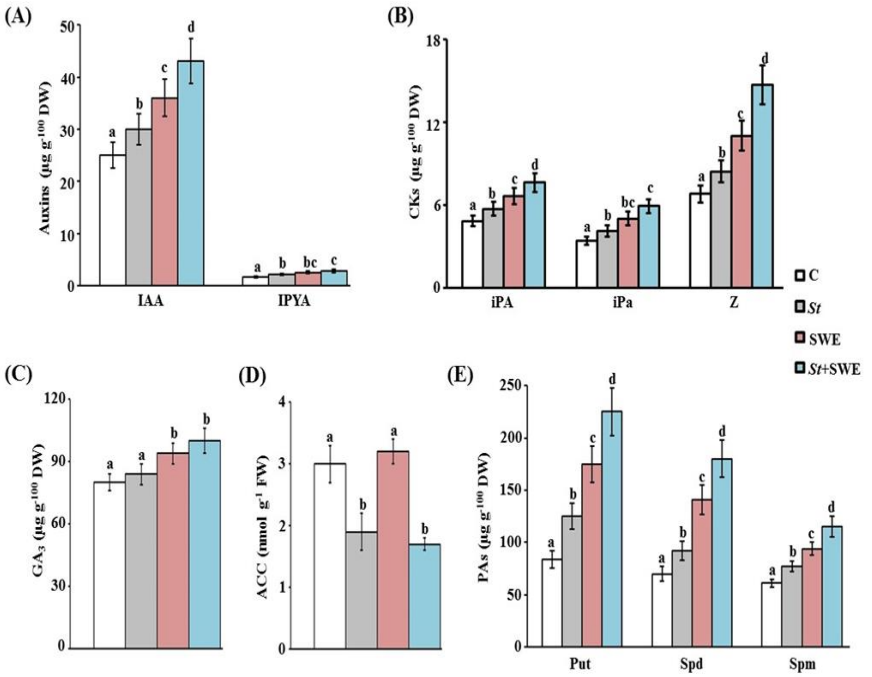

Figure 16: Effects of *Streptomyces tubercidicus* UAE1 and SWE on PGRs in mangrove roots. Endogenous contents of (A) auxins, (B) CKs, (C) GA3, (D) ACC, and (E) PAs in mangrove root tissues after treatment with *St* and/or SWE. Mangrove seedlings were grown in an evaporative-cooled greenhouse and maintained at 30±2°C. Values are means± standard error of 16 replicates for each treatment from two different independent repeated experiments. Mean values followed by different letters are significantly ($P<0.05$) different from each other according to Fisher's Protected LSD Test. Bars represent standard error. Endogenous contents of all PGRs were measured at the end of 9 months post planting the inoculated mangrove propagules. C, control (non-inoculated inorganic salt starch broth); *St*, *Streptomyces tubercidicus* UAE1 (isolate #12); SWE, seaweed extract; *St*+ SWE, combination of *S. tubercidicus* UAE1 and SWE; DW, dry weight; PA, polyamine, Put, putrescine; Spd, spermidine; Spm, spermine; IAA, indole-3-acetic acid; IPYA, indole-3-pyruvic acid; iPA, isopentenyl adenine; iPa, isopentenyl adenoside; Z, zeatin; GA3, gibberellic acid, ACC,1-aminocyclopropane-1-carboxylic.adenine;iPa,isopentenyl adenoside; Z, zeatin; GA3, gibberellic acid, ACC, 1-aminocyclopropane-1-carboxylic.

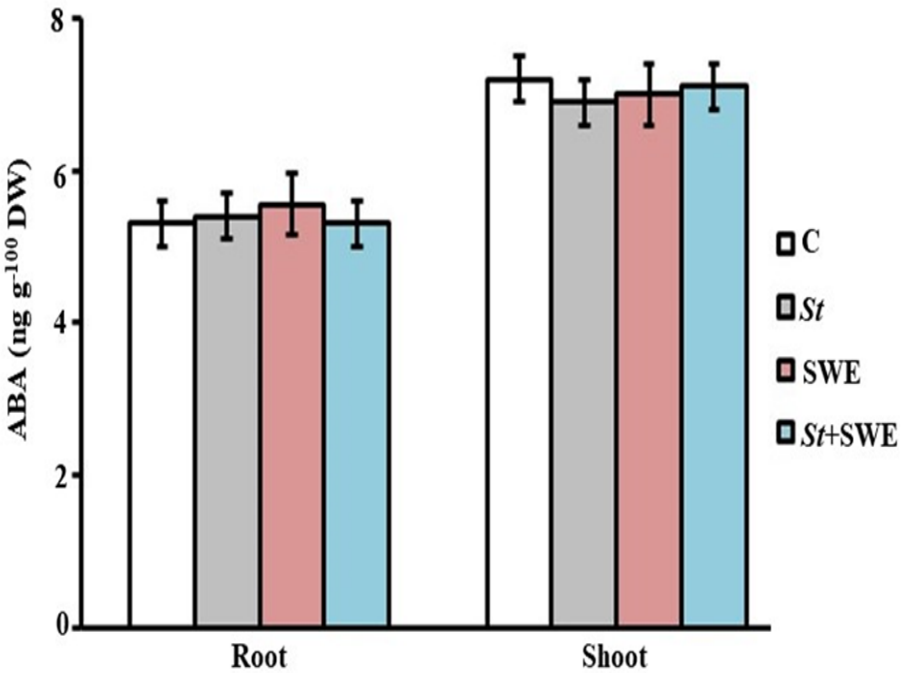

Figure 17: Effect of *Streptomyces tubercidicus* UAE1 and SWE on ABA contents in mangrove tissues. Endogenous contents of ABA in mangrove root and shoot tissues after treatment with St and/or SWE. Values are means±standard error of 16 replicates for each treatment from two different independent repeated experiments. Bars represent standard error. Endogenous contents of ABA were measured at the end of 9 months post planting the inoculated propagules of mangrove. C, control (non-inoculated inorganic salt starch broth); *St*, *Streptomyces tubercidicus* UAE1 (isolate #12); SWE, seaweed extract; *St*+ SWE, combination of *S. tubercidicus* UAE1 and SWE; DW, dry weight; ABA, abscisic acid. There were no significant differences (P>0.05) among all treatments.

In addition, we examined if other growth promoting substances (e.g., macro- and micro-nutrients) of *St* and SWE have a role in plant growth and development. In general, we found significant (P<0.05) differences in

the tissue contents of all examined macro- and micro-nutrients in plants treated with SWE (Table 4). We also noticed that there could be an additive effect of SWE when provided simultaneously with St. For instance, SWE or *St* significantly ($P<0.05$) increased N in both tested tissues compared to control plants (Table 4). Thus, these three nutrients increased to the highest levels when SWE and *St* were applied together compared to their corresponding individual treatments. Notably, there was a significant ($P<0.05$) increase in the measured available P in sediments and contents of P and K in roots and shoots in plants treated with SWE and *St* + SWE compared to those non-treated (control) or inoculated with *St* only (Table 2). This suggests that SWE, but not *St*, can positively regulate nutrient (e.g., P) availability in sediments; thus, increasing P and other nutrients in mangrove tissues for improved growth characteristics.

In addition, there were significant ($P<0.05$) differences in the concentrations of S, Mg, Ca, Na, B, Fe, Mn, Cu and Zn in the shoots and roots of SWE- or *St* + SWE-treated plants compared to those non-inoculated or inoculated with *St* (Table 4). Though, all the measured macro- and micro-elements were insignificantly ($P>0.05$) different between control vs. *St* treatments. This suggests that *St* does not have a direct effect on the uptake of these mineral nutrients. In general, *St* + SWE-treated plants showed comparable effect in the concentrations of the above-mentioned elements in tissues compared to those SWE treated. The data indicated that the bio-stimulant SWE and the bioinoculant *S. tubercidicus* UAE1 can compensate the lack/deficiency of mineral nutrients in sediments and regulate endogenous PGRs in planta; thus, enhancing growth of mangrove and improving the efficiency of photosynthesis.

Table 4: Effect of seaweed extracts and/or *Streptomyces tubercidicus* UAE1 on available P concentration in sediments and tissue nutrient contents in mangrove grown in the greenhouse.

Nutrient [a]	Sample	Treatment [b]			
		C	***St***	**SWE**	***St*+SWE**
N	**Root**	5.48 *a*	9.08 *b*	13.64 *c*	19.02 *d*
	Shoot	8.88 *a*	12.20 *b*	16.66 *c*	19.98 *d*
P	**Sediments**	8.18 *a*	8.30 *a*	18.82 *b*	19.14 *b*
	Root	0.41 *a*	0.47 *a*	2.16 *b*	2.28 *b*
	Shoot	0.82 *a*	0.91 *a*	2.32 *b*	2.41 *b*
K	**Root**	2.96 *a*	3.02 *a*	9.64 *b*	10.06 *b*
	Shoot	4.62 *a*	4.74 *a*	12.74 *b*	13.12 *b*
S	**Root**	2.76 *a*	2.80 *a*	6.42 *b*	6.59 *b*
	Shoot	3.86 *a*	4.12 *a*	9.96 *b*	10.12 *b*
Mg	**Root**	1.40 *a*	1.32 *a*	3.90 *b*	4.08 *b*
	Shoot	2.42 *a*	2.06 *a*	4.54 *b*	4.88 *b*
Ca	**Root**	2.05 *a*	2.13 *a*	4.63 *b*	4.73 *b*
	Shoot	3.32 *a*	3.40 *a*	5.88 *b*	6.01 *b*
Na	**Root**	1.72 *a*	1.81 *a*	3.15 *b*	3.24 *b*
	Shoot	2.87 *a*	2.96 *a*	4.41 *b*	4.53 *b*
B	**Root**	12.22 *a*	14.13 *a*	25.71 *b*	26.85 *b*
	Shoot	23.44 *a*	24.97 *a*	35.39 *b*	37.73 *b*
Fe	**Root**	29.32 *a*	32.34 *a*	130.12 *b*	142.44 *b*
	Shoot	43.22 *a*	43.60 *a*	156.06 *b*	159.00 *b*
Mn	**Root**	17.34 *a*	19.43 *a*	35.61 *b*	39.05 *b*
	Shoot	25.77 *a*	28.13 *a*	43.18 *b*	45.33 *b*
Cu	**Root**	1.26 *a*	1.36 *a*	5.46 *b*	5.73 *b*
	Shoot	1.96 *a*	1.80 *a*	3.88 *b*	4.22 *b*
Zn	**Root**	5.74 *a*	6.04 *a*	13.84 *b*	14.62 *b*
	Shoot	6.18 *a*	6.50 *a*	12.84 *b*	13.06 *b*

aN, P, K, K, S, Mg, Ca and Na in root and shoot tissues were measured in g kg^{-1}. P in sediments and B, Fe, Mn, Cu and Zn in root and shoot tissues were measured in mg kg^{-1}.
bMangrove seedlings were grown in sediments amended with SWE and/or inoculated with *St* in the greenhouse. Plants were sampled at the end of 9 months post treatment.
Values are means of 16 replicates± SE from two independent repeated experiments. Within rows, values followed by the same letter are not significantly (P>0.05) different according to Fisher's Protected LSD Test.
C, control (inorganic salt starch broth only); SWE, seaweed extracts; *St*, *Streptomyces tubercidicus* UAE1 (isolate #12); SWE+ *St*, combination of SWE and *S. tubercidicus* UAE1; N, nitrogen; P, phosphorus; K, potassium; S, sulfur; Mg, magnesium; Ca, calcium; Na, sodium; B, boron; Fe, iron; Mn, manganese; Cu, copper; Zn, zinc.

3.6 Mangrove Growth Promotion under Open Field Nursey Conditions

The DW and length of roots and shoots were clearly increased with either *St* or SWE compared to non-treated plants at 12 mpp of the inoculated mangrove propagules under non-controlled open-field nursery conditions; thus, these parameters significantly ($P<0.05$) varied between the two treatments (Table 5). Mangrove plants treated with *St* + SWE demonstrated; however, the greatest effect on their DW and length. Along with that, the number of branches significantly ($P<0.05$) increased by 1.7 and 2.2 times in *St*-inoculated and SWE-treated plants, respectively (Table 5). In addition, the total leaf area was larger in plants treated with *St* by 21.6% or SWE by 38.1% times than in non-inoculated plants. The number of branches and total leaf area per plant were recorded the highest with *St*+ SWE, reaching to three-fold and 63.1%, respectively.

Table 5: Effect of the application of seaweed extracts and *Streptomyces tubercidicus* UAE1 on the growth of mangrove under open field nursery conditions.

Trait [a]	Sample	Treatment [b]			
		C	***St***	**SWE**	***St*+SWE**
DW	**Root**	3.2±0.6 *a*	4.1±0.8 *b*	6.4±0.5 *c*	8.5±0.7 *d*
	Shoot	3.6±0.7 *a*	4.8±0.5 *b*	7.0±0.8 *c*	9.1±0.6 *d*
Length	**Root**	28.2±1.5 *a*	43.3±3.2 *b*	51.5±4.2 *c*	63.7±5.3 *d*
	Shoot	30.1±1.8 *a*	47.3±2.4 *b*	56.1±3.5 *c*	68.9±5.6 *d*
Number of branches		4.2±0.5 *a*	7.1±0.8 *b*	9.4±1.0 *c*	12.9±1.6 *d*
Total leaf area		115.1±10.6 *a*	140.0±13.0 *b*	159.0±15.0 *c*	187.7±17.2 *d*

[a]Tissue DW, length and total leaf area were measured in g, cm and cm^3, respectively.[b] Mangrove seedlings were grown in sediments amended with SWE and/or inoculated with *St* in the greenhouse. Plants were sampled at the end of 9 months post treatment.
Values are means of 16 replicates±SE from two independent repeated experiments. Within rows, values followed by different letters are significantly (P>0.05) different according to Fisher's Protected LSD Test.
C, non-supplemented/non-inoculated seedlings (control); SWE, seaweed extracts; *St*, *Streptomyces tubercidicus* UAE1 (isolate #12); SWE+*St*, combination of SWE and *S. tubercidicus* UAE1; DW, dry weight.

3.7 Stimulation of Microbial Activity in Mangrove Sediments Upon SWE Treatment

Endophytic PGPA isolates grew and sporulated on SWEA. This suggests that SWE can serve as a nutrient/food base for the growth of PGPA isolates without showing any adverse effect on growth and multiplication of isolates. Microbial activity in sediment amended with SWE was found to be significantly (P<0.05) higher than the non-amended control sediment in both the greenhouse and open-field nursery experiments (Table 6). An increase of 66.4% after 9 mpp in the greenhouse and 75.3% at 12 mpp in the open-field nursery of mangrove seedlings supplemented with SWE compared to the control sediment. Thus, there was no significant (P>0.05) difference between SWE treatments, with or without inoculation with St. When comparing *St*-

inoculated and non-inoculated (control) sediments under controlled (i.e., greenhouse) and non-controlled (i.e., open-field nursery) conditions, no significant (P>0.05) difference was detected in the microbial activities (Table 6). This suggests that SWE can increase the number of soil microbiota, in addition to its growth benefits when applied to marine plants, such as mangrove.

Table 6: Effect of UAE1, SWE and their combination on sediment microbial activity (µg hydrolyzed FDA g dry sediment-1).

Treatment	**Microbial activity** [a]	
	Greenhouse	**Open field nursery**
C	35.38 *a*	41.25 *a*
St	38.74 *a*	43.76 *a*
SWE	105.23 *b*	167.36 *b*
St+SWE	108.17 *b*	169.73 *b*

[a] Microbial activity in sediment collected at the end of 9- and 12-months post planting the inoculated mangrove propagules in the greenhouse and open field nursery, respectively. Microbial activity was assayed by the fluorescein diactete (FDA) hydrolysis technique. Within rows, values followed by different letters are significantly (*P*>0.05) different according to Fisher's Protected LSD Test. Values are means of 16 replicates±SE from two independent repeated experiments. C, control (non-inoculated inorganic salt starch broth); *St*, *Streptomyces tubercidicus* UAE1 (isolate #12); SWE, seaweed extract; *St*+SWE, combination of *S. tubercidicus* UAE1 and SWE.

Chapter 4: Discussion

Biostimulants are widely studied for their role in improving plant growth and productivity. They are derived from a range of natural resources including manure compost, SWE and beneficial PGPR (Yakhin *et al.*, 2017) and are mostly applied solely on plants. However, the combination effects between different bio-stimulants are seldom investigated. To preserve and increase mangrove forest coverage in the UAE and the Arabian Gulf, I determined -for the first time- the interactive effect of the endophytic actinobacterial isolate *St* and the commercial SWE-based bio-stimulant, applied alone and in combination on mangrove plants. My aim was to evaluate the effect of *St* and SWE on growth characteristics and phytochemical contents of mangrove plants.

Previously, in *vitro* investigations followed by preliminary in *vivo* studies have successfully identified rhizosphere-competent and endophytic PGPA of various mechanisms in Salicornia (El-Tarabily *et al.*, 2019; Mathew *et al.*, 2020), mangrove (El-Tarabily, Ramadan *et al.*, 2021) and other plant species (Al Hamad *et al.*, 2021; Al Raish *et al.*, 2021; Alblooshi *et al.*, 2021). An extensive in *vitro* screening was carried out in the current study to find the suitable salt tolerant endophytic PGPA strains producing high levels of PGRs (auxins, CKs, GA3 and PAs) and possessing ACCD activity. The potential isolates were also assessed for their effects on growth as well as their endophytic nature in plant tissues under gnotobiotic and greenhouse conditions. Accordingly, *S. tubercidicus* UAE1 was identified based on the similarity of the 16S rRNA gene to others of Streptomyces species (Figure 10). Consistent with the positive effects of PGPA on the growth of mangrove as a halophytic plant in the current study, other reports have shown the involvement of PGPR on growth promotion of non-halophytic plant species. For example, Ansari and Ahmad (2019) have demonstrated

that the inoculation with *B. licheniformis* and *P. fluorescens* can improve growth and photosynthetic efficiency in wheat. In alignment with that, P. fluorescens-inoculated plants enhanced yields and resistance against pathogens in rice (Nehal, 2015).

The rise of the global seaweed industry and its environmental consequences has, with no doubt, positive impacts on agriculture. SWE-based bio-stimulants have been commercially used for their plant growth promotion as fertilizers and soil conditioning agents (Rouphael & Colla, 2020). Application of *A. nodosum*-based bio-stimulants can stimulate plant growth, stress tolerance and disease management (Shukla *et al.,* 2019). In the current study, a commercial *A. nodosum*-based SWE was tested on mangrove seedlings; thus, suggesting its contribution to plant growth promotion. This could be attributed to the presence of various nutrients, organic matter and PGRs in SWE. Previously, SWE-treated okra (*Abelmoschus esculentus*) showed increased length and weight of shoots, and numbers of leaves and roots under P- and K-deficiencies (Papenfus *et al.,* 2013). Spraying of SWE on sugarcane seedlings enhanced growth, and increased cane yield and sucrose content (Chen *et al.,* 2021). The SWE, originally derived from *Sargassum horneri*, also increased yield and fruit hardness, and shortened ripening time; thus, achieving high net returns of tomato (Yao *et al.,* 2020). SWE can also promote chlorophyll biosynthesis or minimize its breakdown (Sharma *et al.,* 2014). In the current study, contents of chlorophyll and carotenoids were highly abundant in SWE-treated seedlings of mangrove (Figure 11F), which could potentially increase photosynthetic rates in leaves (Bulgari *et al.,* 2019). Similarly, Rengasamy *et al.* (2016) have detected an elevation in photosynthetic pigments in cabbage treated with eckol (a phenolic SWE compound).

Plant improvement can be associated with the application of the seaweed fertilizer by enhancing N uptake and synthesis of chlorophyll to increase photosynthetic rates of *Arachis hypogaea* (Prakash *et al.,* 2014). In the present study, SWE-treated plants showed an increase in tissue N and S acquisition (Table 4). In addition, the roots and shoots were able to uptake more P, K, Mg, Ca, Na, B, Fe, Mn, Cu and Zn after SWE application than in control or *St*-treated seedlings of mangrove. Similar observations have been reported in corn leaves that absorbed more nutrients in plants treated with SWE originated from *A. nodosum* or *Laminaria spp*. than in non-treated control plants (Ertani *et al.,* 2018). Mustard (Brassica rapa L. ssp. sylvestris) and switchgrass (*Panicum virgatum* L.) supplied with SWE significantly increased K uptake in leaves (Fei *et al.,* 2017; Di Stasio *et al.,* 2018). Brown and Saa (2015) have proposed that SWE-based bio-stimulants are not nutrients per se; instead, they facilitate the uptake of nutrients or contribute to growth promotion or stress resistance. We argue that SWE supplemented to mangrove sediments can enhance soil nutrient contents (Table 5). Hence, this improves plant health and aids plant response during periods of stress. It has also been reported that SWE can cause reduction in electrolyte leakage and lipid peroxidation, decreased Na+/K+ ratio and increased Ca content; thus, reducing ionic disparity (Ali *et al.,* 2021). In line with that, we noticed a decrease in Na+/K+ ratio and an increase in Ca concentration by at least two-fold in seedlings treated with *St* + SWE compared to the control.

Compared to individual treatments, *St* + SWE had additive and synergistic effects to improve growth and productivity in mangrove. Similar observations have been reported in crop plants using a combination of PGPR bioinoculants and SWE bio-stimulants (Ngoroyemoto *et al.,* 2019; Aremu *et al.,* 2022). Despite the benefits of PGPR on growth and yield of Salicornia and mangrove (Bashan *et al*., 2000; Komaresofla *et al.,* 2019;

Mathew *et al.*, 2020; El-Tarabily, Ramadan *et al*., 2021), the impact of the co-application of SWE and rhizosphere and/or endophytic PGPA in marine agriculture has been rarely studied is still meager. Thus, the novelty of the present study is the use of SWE combined with *S. tubercidicus* UAE1 to provide more of nutritional values and PGRs to govern all the factors of growth and development within mangrove for sustainable marine farming. The overlapping growth promotion and stress relief effects between these two stimulants may offer an opportunity for synergy if applied together. *Actinoplanes deccanensis*, *Streptomyces euryhalinus*, *Streptomyces polychromogenes* and *Streptomyces bacillaris* are PGPA strains that promote growth and enhance biochemical properties in Salicornia and mangrove by stimulating the endogenous levels of PGRs (El-Tarabily *et al.*, 2020; El-Tarabily, Ramadan *et al.*, 2021). Aligned with that, the endophytic *S. tubercidicus* UAE1 developed its abilities to produce relatively high levels of PGRs and increased the enzymatic activity of ACCD. Similar to other ACCD-producing PGPA isolates (El-Tarabily *et al.*, 2019; Mathew *et al.*, 2020; El-Tarabily, Sham *et al* 2021), *St* reduced ACC levels in both shoot and root tissues in mangrove plants. This suggests that ACCD is a major mechanism utilized by *St* to lower ET levels and to reduce environmental stresses in planta; thus, this agrees with other reports (Glick *et al.*, 2007; El-Tarabily *et al.*, 2019).

The nutrient uptake was, however, most pronounced in tissues of plants treated with *St*+SWE (Table 4). This indicates that SWE can enhance plant growth (Figure 11) directly through the regulation of macro- and micro-nutrients (Table 4) and endogenous PGRs (Figure 12; Figure 13), or indirectly through enhancement of other microorganisms in the rhizosphere to promote growth (Table 5). This was evident when P levels significantly ($P<0.05$) increased in sediments and plant tissues upon the application of

SWE with or without *St* (Table 4). Some studies have suggested that SWE treatments may cause significant changes in the microbiome of the soil and plant; thus, contributing to plant growth (Renaut *et al.,* 2019). For instance, SWE supplements enrich the diversity of rhizosphere bacteria, which in turn, enhances soil nutrient level, and increases yield and quality of rice (Chen *et al.,* 2022). The current study not only demonstrated the potential of SWE to serve as a nutrient base for PGPA, but also supported the overall increase of the microbial activity in mangrove sediments (Table 5). These results agreed with a previous study on the bio-stimulant fish emulsion which was successfully used as a food base for PGPR/PGPA to promote growth and productivity of radish (El-Tarabily *et al.,* 2003). The combined treatment of *St* + SWE, used in the present study, resulted in a significant improvement on growth of mangrove, indicating that the positive effect of SWE complemented that of the PGPA isolate.

It is worth mentioning that not all combinations of bio-stimulants are synergistic; they might have antagonistic effects instead. For example, the combination of either *B. licheniformis* or *P. fluorescens* and smoke-water (containing naturally occurring stimulant karrikinolide) were antagonistic, albeit their slight rooting improvement of grapevine cuttings when applied individually (Papenfus *et al.,* 2015). This might be attributed to the overlapping modes of action; thus, disrupting hormone levels in the plant. In the current study, the presumed mode of action involved in the stimulation of growth performance and chlorophyll biosynthesis was linked to enhanced nutrient availability and PGRs driven by the synergistic action of St and SWE applied in combination. Thus, this could be due to the different types of signal transduction pathways activated by the two stimulants to mediate shared signaling components (e.g., hormones) and to regulate the expression level of a pathway-specific component by the other pathway (Mengiste *et al.,* 2009; AbuQamar *et al.,* 2021). Elucidating

regulatory mechanisms employed by the endophytic *St* Al Hamad and bio-stimulant SWE in enhancing growth of mangrove is on top of our priorities.

Chapter 5: Conclusion

The current study demonstrated, for the first time, the remarkable effect of *S. tubercidicus* UAE1 (an endophytic PGPA isolate) and *A. nodosum* (a commercial SWE-based bio-stimulant) on growth of mangrove. The individual *St* and SWE treatment had varying effects on growth, endogenous PGRs and mineral element content in mangrove tissues. Compared to *St* treatment, SWE treatment enhanced growth parameters, photosynthetic pigments, PGRs levels (IAA, CKs, GA_3 and PAs) and nutritional quality of mangrove. Even though *St* enhanced growth at a lesser extent than SWE, it did not inhibit the growth parameters relative to control. Overall, *St* had positive impact on mangrove growth due to the increased auxins, iPA, iPa, PAs and N, and decreased ACC *in planta*; thus, mitigating the adverse effects of stress generating ET. However, the combined treatments of *St* +SWE was relatively superior in enhancing the growth of mangrove over any of the single treatments. The novelty of this research lies in the fact that SWE can act as a conducive environment for PGPR/*PGPA* to grow. This research also proves that mangrove plants treated with *St* and SWE can increase nutrients and endogenous PGRs levels in plant tissues, for better growth of marine plants (e.g., gray mangrove) under greenhouse and open-field nursery conditions. Thus, this novel combination can be used on a commercial applied basis. In the long-run objective is to foster propagation of mangrove in open areas along the Arabian Gulf coastline, thus, helping the UAE implement a mangrove reforestation program.

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List of Publications

Alkaabi, A. K., Ramadan, G. A., Elddin, A. M. T., El-Tarabily, K. A., & AbuQamar, S. F. (2022). The Multifarious Endophytic Actinobacterial Isolate, Streptomyces tubercidicus UAE1, Combined with the Seaweed Biostimulant Further Promotes Growth of Avicennia marina. *Frontiers in Marine Science*, 9, 896461. https://doi.org/10.3389/fmars.2022.896461.

Appendix

Media

The following media have been used in the present study. The media composition is listed below

1. Inorganic salt-starch agar (starch nitrate agar) (SNA)
2. Oat-meal yeast extract agar (OMYEA) (Küster, 1959).
3. Dworkin and Foster's salts minimal agar medium (DF) (Dworkin and Foster, 1958).
4. Moeller's decarboxylase agar medium (MDAM) (Arena and Manca de Nadra, 2001).
5. Modified chrome azurol agar (CAS agar) (Alexander & Zuberer, 1991).
6. Glucose peptone broth (GPB) (Di Menna, 1957).

Composition of Media

1- Inorganic salt-starch agar (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 mL-1
Nystatin (Sigma)	50 µg mL-1
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- Oat-meal yeast extract agar (OMYEA) (Küster, 1959)

Twenty grams of oatmeal were steamed in 1 liter of distilled water for 20 min and filtered the oats through cheese cloth, and distilled water was added to continue the filtrate to 1 liter. Yeast extract (1 g) (Sigma) and agar (Sigma) (20 g) were added, and the final medium pH was adjusted to 7.2.

3- 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

4- Moeller's decarboxylase agar medium (MDAM) (Arena and Manca de Nadra, 2001)

Peptone (Sigma)	5 g
Yeast extract (Sigma)	3 g
Glucose (Sigma)	1 g

Pyridoxal-5-phosphate (Sigma)	0.03 g
Manganese sulfate	0.03 g
Phenol red (pH dye indicator) (Sigma)	0.02 g
L-arginine-monohydrochloride (Sigma)	2.00 g
Distilled water	1 L
Agar	20 g

5- Modified chrome azurol agar (CSA agar) (Alexander and Zuberer, 1991)

CAS agar was prepared from four sterilized solutions which were sterilized separately before mixing. The Fe-Chrome azurol S indicator solution (solution 1) was prepared by mixing 10 mL of 1 mM FeCl3 (in 10 mM HCl) with 50 mL of an aqueous solution of Chrome azurol S (CAS) (1.21 mg mL-1) (Sigma). The resulting dark purple mixture was added slowly to 40 mL of an aqueous solution of hexadecyltrimethylammonium bromide (HDTMA) (1.82 mg mL-1) (Sigma). The resulting dark blue solution. Then, the solution was autoclaved separately and cooled to 50°C.

The buffer solution (solution 2) was prepared by dissolving 30.24 g of PIPES buffer (Piperazine-N,N-bis [2-ethanesulonic acid) (Sigma) in 750 ml of a slat solution containing 0.3 g potassium di-hydrogen phosphate,

0.5 g sodium chloride, and 1 g ammonium chloride. The pH was set to 6.8 and water was added to bring the volume to 800 ml. Then, adding 15 g of agar. The solution was autoclaved and then cooled to 50°C.

Solution 3 was prepared by adding 2 g glucose, 2 g mannitol, 493 mg magnesium sulfate, 11 mg calcium chloride, 1.17 mg manganese sulfate, 1.4 mg boric acid, 0.04 mg copper sulfate, 1.2 mg zinc sulfate, and 1 mg sodium molybidate in 70 ml of water. Then, Solution 3 was autoclaved and cooled to 50°C.

Solution 4 was containing of 30 ml of Millipore membrane sterilized (pore size 0.22 µm, Millipore Corporation, MA, USA) 10% (w/v) casamino acids (Sigma). For the final solution, solution 3 was added to solution 2 and solution 4. Finally, Solution 1 was added with sufficient mixing to mix the ingredients of the four solutions without forming bubbles. The medium color was dark blue after mixing the fours solutions.

6. Glucose peptone broth (GPB) (Di Menna, 1957)

Glucose (Sigma)	10 g
Peptone (Sigma)	5g
L-Tryptophan (L-TRP) (Sigma) 5%	5 ml
Distilled water	1 L

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In this thesis, the endophytic actinobacteria isolated from the roots of mangrove (Avicenna marina) to find suitable isolates that can promote plant growth and increase mangrove productivity. The improvement of the efficiency of the actinobacteria using seaweed extracts bio-stimulants. To determine the impact of SWE bio-stimulant and endophytic actinobacterial isolates on growth performance and endogenous hormonal levels of mangroves.

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