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Original Research

Relief from Salt Stress by Plant Growth-Promoting Bacteria in Hydroponic Leaf Lettuce (*Lactuca sativa* L.)

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Abstract

Indonesia is a large archipelago with a long coastline. Climate change and saltwater intrusion both endanger freshwater availability as a source of drinking water and agricultural resources. Therefore, the saline and brackish water demand for irrigated farm goods should be considered a method for producing salt-resistant crop products. This study aimed to monitor Indole-3-acetic acid (IAA) and biofilm by selected bacterial isolates and its impact on the growth of lettuce (*Lactuca sativa* L.) during salt stress. Several isolates are employed such as *Streptomyces hygroscopicus* GGF4-i18, *Streptomyces* sp. AB8, *Micrococcus luteus*, *Serratia marcescens* MBC1, and *Streptomyces hygroscopicus* subsp. *Jinggangensis* InaCC A497. A 1.0 percent L-tryptophan is used as a precursor of IAA. The impact of IAA and biofilm produced by bacteria was demonstrated by inoculating bacterial isolates on *Lactuca sativa* (lettuce) plants in the hydroponic system. The nutrient film technique (NFT) is the selected hydroponic cultivation technique. The result shows that Streptomyces genera were able to maintain higher IAA hormone production within a week. On the other hand, *Serratia marcescens* MBC1 and *Micrococcus luteus* tended to have stable IAA levels. The administration of MBC1 strain bacteria increased lettuce growth as measured by the number of leaves and leaf length, compared to isolate AB8, i18, and *Micrococcus luteus*. However, the growth was not greater than the control treatment, which grew in fresh water, when it was observed in root length. The ability of bacterial isolates to tolerate salt levels is needed, in addition to the capacity to produce IAA to help plants survive and grow in brackish water media. In nature, the interaction between plants and microbes does not take place alone. In this study, the treatment was still given in the form of a bacterial monoculture to plant. In the future, it is necessary to develop how the treatment is given in the form of a consortium and consider other

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growth- supporting factors. In addition, the use of simple technology in the form of adding bacterial inoculum to a simple NTF reactor is easy to apply to developing countries.

Keywords: actinomycetes, brackish water, IAA, lettuce, *Serratia* sp.

Introduction

Water is a resource that is often considered abundant and available in the long term. Water scarcity is rarely acknowledged until a problem develops [1]. Climate change and seawater intrusion threaten the supply of fresh water appropriate for use, both as an agricultural medium and as drinking water. Researchers are interested in using seawater and brackish water as a medium for farming and drinking water [2]. Many plant crops cannot be grown with both types of water properly. The salt concentration is one of the barriers and stress for plants to live in salinity environments. Alternatives to this problem include adapting food crops to tolerate salt levels and water filtration [3]; however, this approach is time and money-consuming. The employment of aquaculture and hydroponics is a consideration that is impossible to avoid due to a shortage of clean water resources in salt water [4, 5]. Although reverse osmosis and water dialysis are being developed [6, 7], designing merely water as a medium without studying microbial and plant interactions is insufficient. Moreover, technological adoption is more challenging among developing-country populations due to a lack of experience and access to technology. A natural strategy based on microbial interaction with plants appears as an easier to execute and share with the community option. Microbial associations help plants adapt to salt stress [8].

Salt stress has a slew of obstructive effects on the bio-physicochemical parameters of plants, as well as a reduction in output [9]. Bacteria associated with plants are producers of bioactive compounds. They produced exogenous phytohormones that promoted growth and metabolism under stressful conditions [10, 11]. These stresses included drought, salinity, and contamination of soil by petroleum [12]. Indole-3-acetic acid (IAA) was the most widely used auxin phytohormone. IAA-producing bacteria included *Serratia marcescens* [13], *Micrococcus yunnanensis* [14], *Micrococcus luteus* [15], dan *Streptomyces* sp., [16]. This microorganism was tolerant to salty water. Since human population growth and climate change were depleting groundwater resources, brackish water was a promising solution [17].

Indonesia's territory with an expansive coastline and preparedness efforts against groundwater scarcity requires a breakthrough in utilizing plant cultivation using brackish water. Extensive maintenance is necessary to address these problems. Hydroponics on lettuce is one of them. This method uses water dissolved in nutrients as a medium for plant growth to replace the soil [18]. Furthermore, the utilization of simple technologies, such as introducing microorganisms cell suspension to a simple NTF reactor, is straightforward to deploy in developing countries. This study aimed to produce Indole-3-acetic acid (IAA) and biofilm and its impact on the development of lettuce (*Lactuca sativa* L.). *L. sativa* L. was a popular vegetable among Indonesians. It also contains a lot of antioxidants, potassium, folate, and carotene. It could also assist in the generation of white and red blood cells in the bone marrow structure, lower critical illness, and improve the human digestion system [19].

Material and Methods

Design Study

Seawater collected from the Lampung Marine Aquaculture Center (BBPBL Lampung). Seawater is combined with distilled water to form a brackish water mixture medium for hydroponic cultivation. Lettuce is selected and cultivated because it propagates easily using hydroponic techniques. Lettuce is transferred to the hydroponic installation after it has had seeded for two weeks. There were four media tanks, each containing nutrition media plus 1 L inoculum liquid media for *Streptomyces* sp. i18, Streptomyces sp. AB8, S. marcescens MBC1, and M. luteus bacteria, and one control tank with fresh water media but no bacteria. Bacterial isolates were evaluated for their potential to produce IAA and biofilms in order to explore bacteria's capability to encourage lettuce growth during salt stress. Plant height, number of leaves, and root length were all measured for lettuce plants (Fig. 1).

Bacterial Culture

A single loop colony was inoculated into an agar plate medium. Bacteria were incubated at 37°C for three days, except for actinomycetes, which were set for seven days. *Serratia marcescens* strain MBC1 was cultured on Trypticase soy agar (TSA) medium consisting of 15 g pancreatic digest of casein, 5 g peptic digest of soybean meal, NaCl 5.0 g, 15 g agar, 1 L dH_2O, with final pH 7.3 +/- 0.2 at 25ºC. *Micrococcus luteus* grown on nutrient agar and *Streptomyces hygroscopicus* strain GGF4-i18 maintained using ISP2 media (Difco™) (4 g yeast extract, 10 g malt extract, 4 g dextrose broth, 20 g bacteriological agar, and 1 L dH_2O). *Streptomyces hygroscopicus* subsp. *jinggangensis* InaCC A497 and *Streptomyces* sp. strain AB8 cultivated on International Streptomyces Project medium 4 (Difco™) [20], also named inorganic salts-starch agar with the composition are 10 g Soluble Starch (1 g $MgSO_4$ x $7H_2O$, 1 g NaCl,

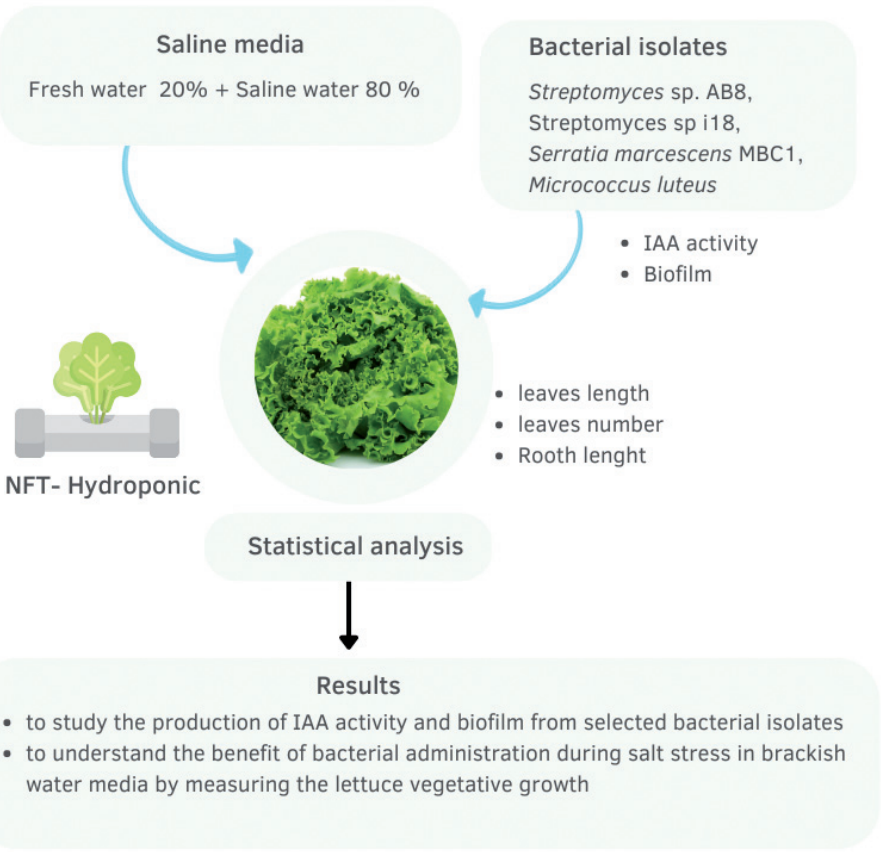

Fig. 1. Methodology for bacterial administration and lettuce observation through NFT-hydroponic to see salt stress effect.

2 g $(NH_4)_2SO_4$, 2 g $CaCO_3$, 1 L dH_2O, 20 g agar, and 1 mL Trace Salts solution; 0.1 g $FeSO_4$ x $7H_2O$, 0.1 g $MnCl_2$ x $4H_2O$, 0.1 g $ZnSO_4$ x $7H_2O$, 100 mL dH_2O). The concentration was quantified by taking two bacterial inoculums every two days until day 9. The measurement was conducted using a spectrophotometer at a wavelength of 600 nm [21].

Screening of Indole-3-acetic Acid Production

Bacterial culture fermented using Gause's medium for production. The composition consists of soluble starch, KNO_3, NaCl, $MgSO_4.7H_2O$, K_2HPO_4, and $FeSO_4$. $7H_2O$, and aquades [22]. One loop of bacterial inoculum was administered to 100 mL of Gause's medium, using L-tryptophan as a precursor. Gause media without tryptophan was utilized as a control. Fermentation occurs in the dark at 150 rpm at room temperature [23]. Bacterial growth was measured using a spectrophotometer at a wavelength of 600 nm per day for nine days. With triplicate sampling measurements in the morning and afternoon, observations were conducted twice.

First, the metabolites were separated from the culture using centrifugation at 6000 rpm for 10 minutes. Next, 1 ml of free-cell metabolite in a test tube with 4 ml of Salkowski reagent and incubated for 60 minutes in the dark was obtained for IAA screening. The color shift was detected at a wavelength of 530 nm and recorded the absorption. The first day of fermentation is considered day 0 [16]. The findings of bacterial IAA production optimization are used to estimate the production age of the inoculum before it is fed to the hydroponic installation.

Vegetable Cultivation

Bacteria were cultured for six days on Gause No. 1 medium (approximately 5.6×10^6CFU/mL). The inoculum was produced using 10% of the volume of the hydroponic nutrient media tank – the Nutrient-film technique (NFT) used for hydroponic cultivation. There were five NFT hydroponic installations, each for two combination treatments, five positive controls, and one negative control. In one tool, 2 m long gutters are employed with a plant spacing of 20 cm apiece, resulting in 10 plants in one installation tool. The planting media was Rockwool which measured 23.5 cm x 9 cm x 3.5 cm, was cut into 3 cm x 2 cm x 3 cm pieces, and was perforated. The lettuce seeds (3-4 cm) were put in the planting medium after 14 days of the seedling. Nutrient solution [24] is composed as 150 mg/L^{-1} N, 48 mg/L^{-1} P, 216 mg/L^{-1} K, 139 mg/L^{-1} Ca, 31 mg/L^{-1} Mg, 3 mg/L^{-1} Fe, 0.05 mg/L^{-1} Mn, 0.15 mg/L^{-1} Zn, 0.50 mg/L^{-1} B, 0.15 mg/L^{-1} Cu, and 0.10 mg/L^{-1} Mo. The capacity of the tanks was about 30-40 gallons. The growth period

and nutrient solution pH were kept between 7 and 9. The salt concentration in the tank is evaluated and turned to less than 3%. The flow rate is set up between 1.5 and 2.5 mS/cm – the greenhouse is built without extra illumination. The temperature follows the room temperature. Nutrient-rich growth mediums are created using diluted salt water (80% seawater, 20% distilled water, or brackish groundwater) and (C- controls) [25]. The leaves' length and roots were measured using a caliper, while the number of leaves was counted and recorded. This measurement is conducted every two days, beginning on the 16th day of the plant's life after planting and ending on the 28th day.

Biofilm Formation Analysis

Bacterial isolates were examined in plates for their potential to create biofilms, mainly following established techniques [26] with slight alterations. Liquid cultures were incubated overnight and then diluted to an OD600 of 0.4. An amount of 100 microliters was taken and seeded into each well of a 96-well plate. Each culture was cultivated in triplicate to eliminate position effects at random spots on the plate. The dish was then sealed and incubated at 30ºC for 24 h (without shaking). The liquid medium was carefully removed, and the wells were dyed for 20 minutes at room temperature with 100 µL of 0.01 percent crystal violet. The dye was released, the wells were rinsed twice with sterile distilled water, and the leftover paint in each well was eluted by adding 100 µL of 30 percent acetic acid and pipetting up and down to suspend and mix the coloring thoroughly. Finally, the plate was scanned at OD 570 nm to determine the biofilm levels in each sample.

Data Analysis

A randomized block design with a factorial pattern had adopted in this study. Factors observed include vegetative growth and the addition of bacteria to the media composition, besides the amounts of IAA generated by bacterial isolates. The vegetative growth of lettuce plants was measured by the amount of leaf length, leaf blade number, and root length, with 3 replications and 10 plants for each treatment. Finally, the data had evaluated statistically.

Results and Discussion

The study was concerned with observing the effect on bacterial isolates granting in lettuce NFT-hydroponic using briny media. The ability of bacterial isolates to encourage plant growth during salt stress evaluation through IAA measurement and biofilm formation, while plant growth is determined using vegetative monitoring.

The production rate of IAA increased on day two and day 5. Genera *Streptomyces* spp was superior to *Serratia* sp. and Micrococcus sp. isolates in the results of IAA generation with the administration of L-tryptophan precursor at a concentration of 2 mg/mL (Fig. 2). Compared to the data in Fig. 3, *Streptomyces* sp. AB8 has the most excellent density. Bacterial cell growth was observed by calculating the increased cell density. *Serratia* sp. peak growth reached no more than 24 hours. On the other hand, the actinomycetes group, including *Micrococcus* sp., could grow optimally for a longer time, more than 24 hours.

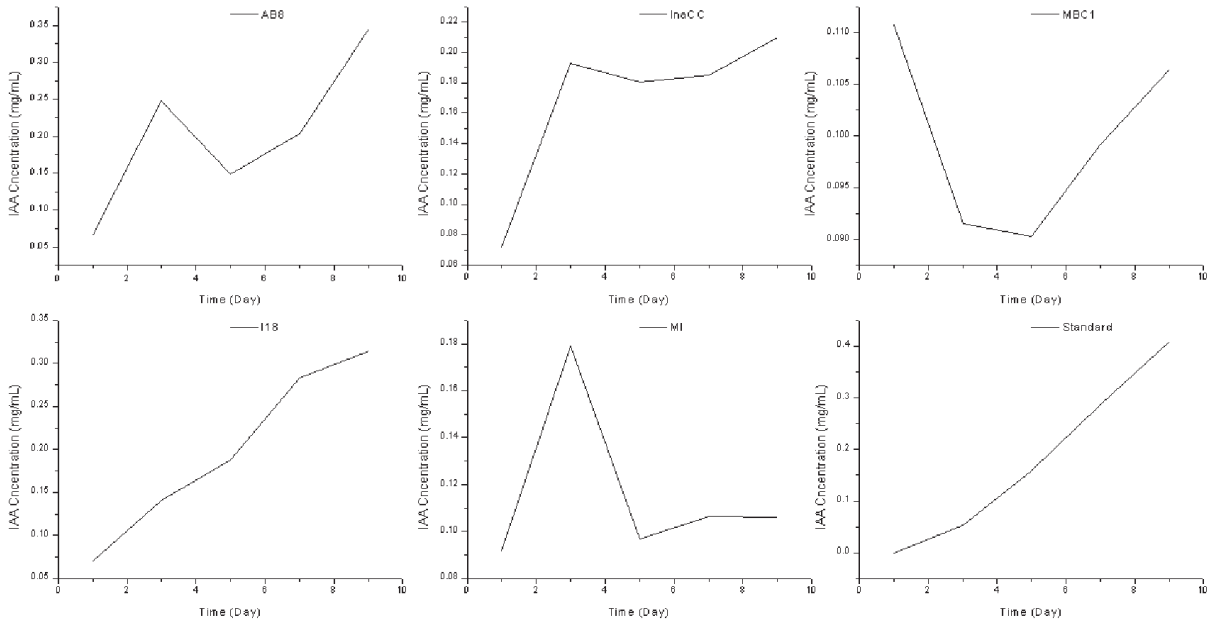

Fig. 2. *Streptomyces* sp AB8 bacteria, *Streptomyces* sp InaCC A497, *Streptomyces* sp i18, *S. marcescens* MBC1, and *Micrococcus luteus* days 1 to 9 were used to optimize the indole-3-acetic acid synthesis.

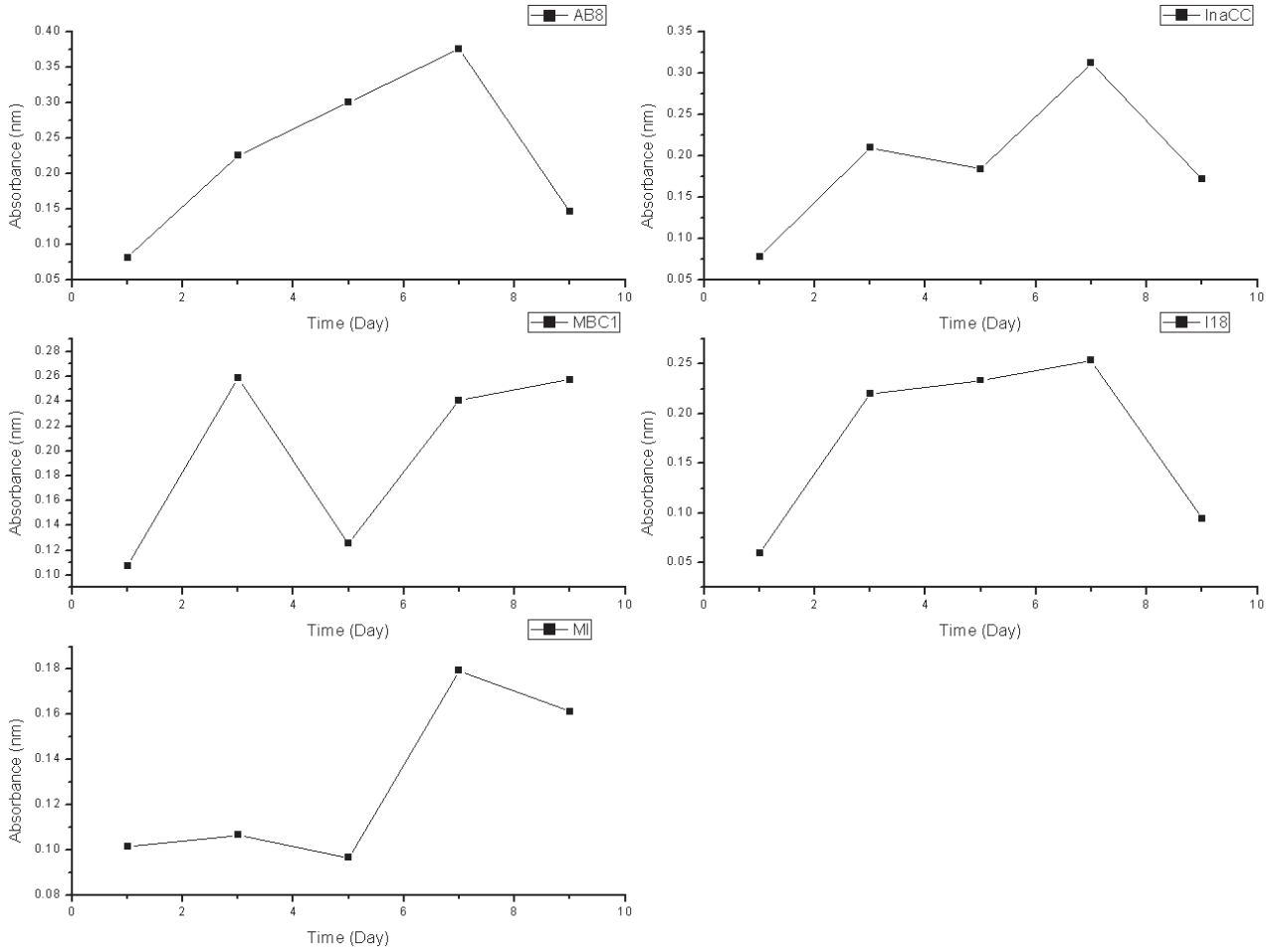

Fig. 3. The density of bacterial cells *Streptomyces* sp AB8, *Streptomyces* sp InaCC A497, *Streptomyces* sp i18, *S. marcescens* MBC1, *Micrococcus luteus* on observation days 1 to 9.

The growth of lettuce grown hydroponically with brackish water stress was unaffected because of the presence of bacteria in hydroponic media (Fig. 5). The treatment utilizing *Serratia* sp. strain MBC1 produced the highest growth rate in the lettuce category, followed by the control group came in second place. The leaf number category also revealed that the impact of supplying non-*Streptomyces* sp. bacteria, particularly *Serratia* sp. and *Micrococcus* sp, was better (Fig. 6). The control group was superior in root growth compared to other treatments. Salt stress in brackish water affects root growth in lettuce plants.

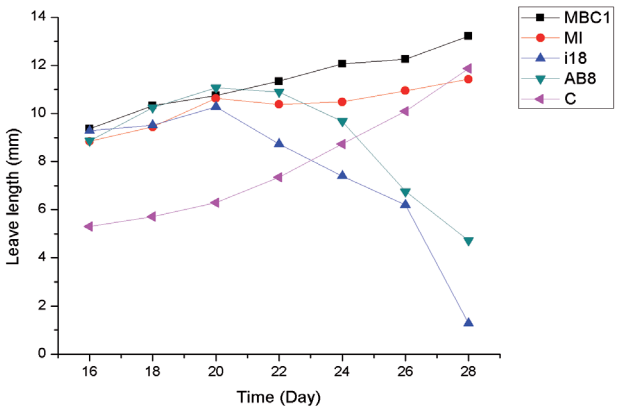

Fig. 4. Lettuce leaf growth on days 16-28 after spreading in hydroponic installation.

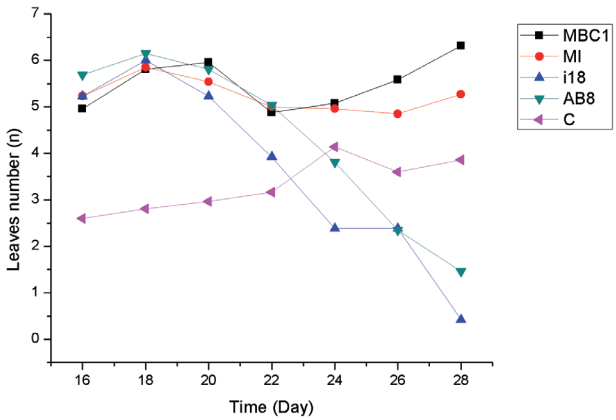

Fig. 5. Growth in the number of lettuce leaves on days 16-28 after spreading in hydroponic installations.

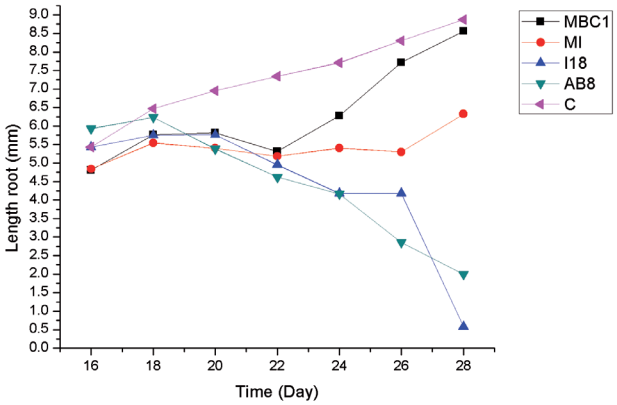

Fig. 6. Growth of lettuce root length on days 16-28 after spreading in hydroponic installations.

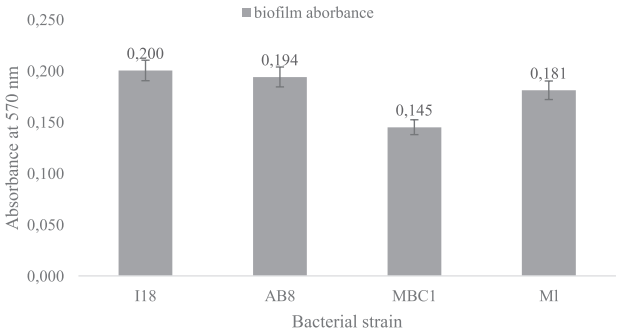

Fig. 7. Measurement of biofilm absorbance on *Streptomyces* sp AB8 bacteria, *Streptomyces* sp i18, *Micrococcus luteus*, and *Serratia marcescens* MBC1.

The MANOVA-test-of between-subjects effects data derived a significance value of <0.05 (Table 4). Therefore, it is possible to deduce that bacterial administration during hydroponic lettuce cultivation influences the number of leaves and both leaf and root length. The data from the Levene test indicates that there is data inhomogeneity with a P-value of 0.05 (Table 3). The Games-Howell test is employed to analyze the MANOVA findings. According to the data in the table on the study of the effect of supplying plant-promoted regulating hormone bacteria, treatment group 1 employing MBC1 bacteria exhibited variations in leaf length parameters from the treatments with I18, AB8, and control groups. There was no measurable difference between MI administration and MBC1 or AB8. When it came to lettuce leaf length growth, the administration of I18, AB8, and the treatment of the control group made no difference. The main result inferred that the control group appeared to be considerably different compared to MBC1 and MI bacteria (Table 5). From the test of homogeneity of variances table, it can be seen that the variances of the three groups are the same (P-value = 0.463) (Table 6). Hence, the ANOVA test is valid for testing this relationship. In the ANOVA table, the column Sig. obtained P-value = 0.683 (Table 7). At the significance level = 0.05, it can be concluded that there is no significant difference in biofilm ability as measured by absorption at a wavelength of 570 nm (Fig. 7).

In overcoming the phenomenon of salt stress, there are several solutions offered, namely:

– Engineered saline media to produce non-saline water for use as an agricultural growth medium. Water can be purified via membrane filtration, reverse osmosis, or dialysis technique [7, 27, 28]. Molecularly altering the ability of food crops to enhance salt stress tolerant crops [29-31].
– Conducting a research on the interaction of food crop hosts with microorganisms to induce salt stress resistance [9, 32, 33].

Engineering saline water medium, both physically and electrochemically, gives a costly option [34]. Access to and implementation of this technology is particularly challenge in developing countries, creating a unique obstacle in its deployment [35, 36]. Meanwhile, genetically modified foods have long been the subject of disputes over their safety, biodiversity, and health hazards [37, 38]. Microbes-Host plant interaction

Table 1. IAA concentration and cell abundance for Levene's Test of Equality of Error Variances[a]

		Levene Statistic	df1	df2	Sig.
IAA_concentration	Based on Mean	4.535	4	45	.004
	Based on Median	3.529	4	45	.014
	Based on Median and with adjusted df	3.529	4	30.338	.018
	Based on trimmed mean	4.219	4	45	.006
Abundance	Based on Mean	1.039	4	45	.398
	Based on Median	.826	4	45	.515
	Based on Median and with adjusted df	.826	4	38.105	.517
	Based on trimmed mean	1.017	4	45	.409

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a. Design: Intercept + Strain

Table 2. The IAA concentration and cell abundance for tests of Between-Subjects Effects.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	IAA_concentration	762.680[a]	4	190.670	3.240	.020
	Abundance	121270208513155648.000[b]	4	30317552128288912.000	2.742	.040
Intercept	IAA_concentration	13154.420	1	13154.420	223.554	.000
	Abundance	488138639515321920.000	1	488138639515321920.000	44.154	.000
Strain	IAA_concentration	762.680	4	190.670	3.240	.020
	Abundance	121270208513155440.000	4	30317552128288860.000	2.742	.040
Error	IAA_concentration	2647.900	45	58.842		
	Abundance	497495485786445120.000	45	11055455239698780.000		
Total	IAA_concentration	16565.000	50			
	Abundance	1106904333814921980.000	50			
Corrected Total	IAA_concentration	3410.580	49			
	Abundance	618765694299600770.000	49			

a. R Squared = .224 (Adjusted R Squared = .155)
b. R Squared = .196 (Adjusted R Squared = .125)

Table 3. Bacterial administration upon lettuce growth on Levene's Test of Equality of Error Variances[a]

		Levene Statistic	df1	df2	Sig.
Leaves_length	Based on Mean	12.920	4	45	.000
	Based on Median	5.414	4	45	.001
	Based on Median and with adjusted df	5.414	4	26.111	.003
	Based on trimmed mean	12.665	4	45	.000
Leaves_number	Based on Mean	9.105	4	45	.000
	Based on Median	7.632	4	45	.000
	Based on Median and with adjusted df	7.632	4	31.153	.000
	Based on trimmed mean	8.851	4	45	.000
Root_length	Based on Mean	7.726	4	45	.000
	Based on Median	4.924	4	45	.002
	Based on Median and with adjusted df	4.924	4	31.065	.003
	Based on trimmed mean	7.684	4	45	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a. Design: Intercept + Bacteria_administration

involving osmotic adjustment, antioxidant defense, nutritional recovery, and ionic homeostasis. This association benefits plants by increasing nutrient uptake, balancing hormones, combating reactive oxygen species (ROS), and preventing ionic toxicity and osmotic stress [9, 39, 40].

The formation of indole-3-acetic acid (IAA) is a significant feature of rhizosphere bacteria that promotes and accelerates plant development. Plants produce IAA endogenously, whereas microorganisms generate it exogenously. It regulates development by many biological pathways, including changes in cell orientation, organ development, fertility, and cell elongation, even in salt stress [41]. Fig. 2 presents that the *Streptomyces* sp. genera create a maximum IAA concentration by never more than 0.35 ppm, whereas MBC1 and *Micrococcus luteus* strains must only manufacture IAA at a concentration of not less than 0.10 ppm. Different types of bacteria affect the levels of hormones synthesized and their specifications [42].

Table 4. Bacterial administration upon lettuce growth for Tests of Between-Subjects Effects.

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[d]
Corrected Model	Leaves_length	5638942.120[a]	4	1409735.530	17.031	.000	68.125	1.000
	Leaves_number	1940340.480[b]	4	485085.120	16.770	.000	67.082	1.000
	Root_length	2911152.080[c]	4	727788.020	13.756	.000	55.024	1.000
Intercept	Leaves_length	31556745.680	1	31556745.680	381.245	.000	381.245	1.000
	Leaves_number	13057072.020	1	13057072.020	451.411	.000	451.411	1.000
	Root_length	17092535.120	1	17092535.120	323.070	.000	323.070	1.000
Bacteria administration	Leaves_length	5638942.120	4	1409735.530	17.031	.000	68.125	1.000
	Leaves_number	1940340.480	4	485085.120	16.770	.000	67.082	1.000
	Root_length	2911152.080	4	727788.020	13.756	.000	55.024	1.000
Error	Leaves_length	3724782.200	45	82772.938				
	Leaves_number	1301624.500	45	28924.989				
	Root_length	2380794.800	45	52906.551				
Total	Leaves_length	40920470.000	50					
	Leaves_number	16299037.000	50					
	Root_length	22384482.000	50					
Corrected Total	Leaves_length	9363724.320	49					
	Leaves_number	3241964.980	49					
	Root_length	5291946.880	49					

a. R Squared = .602 (Adjusted R Squared = .567)
b. R Squared = .599 (Adjusted R Squared = .563)
c. R Squared = .550 (Adjusted R Squared = .510)
d. Computed using alpha = 0.05

This is supported by the test-of between-subjects effects data using the MANOVA method, and the significance value is <0.05 (Table 2). Thus, the strain type showed an effect on the concentration of IAA produced and the abundance of cell density during the IAA production process.

The IAA concentration was not homogeneous in the Levene test, with a significance value of <0.05. Therefore, the MANOVA output was continued using post hoc readings with the Games-Howell test. There was a significant difference in the concentration of IAA produced by the MBC1 strain compared to the InaCC A497 isolate. In contrast, the IAA concentration in other bacteria did not significantly differ. There was also no significant difference in bacterial cell abundance during IAA fermentation. Based on this reason, the InaCC A497 strain was not used in the treatment of adding bacteria in hydroponic installations.

According to the findings of this study, lettuce cultivated through hydroponic methods exhibited a growth increase despite using a brackish water medium. The addition of MBC1 strain bacteria resulted in a tremendous increase in root length, number of leaves, and leaf length, followed by the control group and *Micrococcus luteus*. This phenomenon illustrates the assumption that even though growth-promoting bacteria can produce IAA, they may not be able to modulate the expected plant growth until the harvest period optimally. The density of inoculated-*Streptomyces* in the rhizosphere reduced considerably as the tomato grew. *Streptomyces* may enhance plant yields by modifying the microbial population in the rhizosphere [43]. Unfortunately, this research experiment did not use brackish water media and salt stress.

The use of levels and salt stress point out an intriguing reason that raising the salt concentration over 1% to 5% lowers the quantity of generated IAA [44]. The type of carbohydrate source has an impact on IAA production. Sucrose has been shown to influence greater levels of IAA production than other forms of supplementary sugar sources. Finally, the degree of acid-base impacts the effectiveness of IAA synthesis, although this is due to an ecological reason, which prefers alkaline or acidic environments for microbes.

The IAA generated in the hydroponic installation was not assessed in this investigation, nor was the concentration of bacteria in the hydroponic facility. Before introducing the nutrient tank, IAA levels were evaluated in vitro. As a result, we only have a limited understanding of why the bacteria that have been demonstrated to make IAA could not control growth until the lettuce harvest time of 28 days. Although the ability of bacteria to produce biofilms has been measured, it turns out that this ability is insufficient

Table 5. Multiple Comparisons of lettuce growth using MANOVA.

Dependent Variable		(I) Bacteria_ administration	(J) Bacteria_ administration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Leaves_ length	Games-Howell	MBC1	Ml	171.4000	93.48874	.395	-121.1470	463.9470
			I18	700.7000*	153.88030	.003	226.7014	1174.6986
			AB8	589.5000*	148.82657	.009	132.6103	1046.3897
			Control	901.7000*	86.10106	.000	620.5673	1182.8327
		Ml	MBC1	-171.4000	93.48874	.395	-463.9470	121.1470
			I18	529.3000*	136.70741	.018	87.0629	971.5371
			AB8	418.1000	130.99281	.053	-4.3755	840.5755
			Control	730.3000*	49.22561	.000	576.6878	883.9122
		I18	MBC1	-700.7000*	153.88030	.003	-1174.6986	-226.7014
			Ml	-529.3000*	136.70741	.018	-971.5371	-87.0629
			AB8	-111.2000	179.15948	.970	-653.0704	430.6704
			Control	201.0000	131.76557	.571	-236.2802	638.2802
		AB8	MBC1	-589.5000*	148.82657	.009	-1046.3897	-132.6103
			Ml	-418.1000	130.99281	.053	-840.5755	4.3755
			I18	111.2000	179.15948	.970	-430.6704	653.0704
			Control	312.2000	125.82672	.174	-104.8608	729.2608
		Control	MBC1	-901.7000*	86.10106	.000	-1182.8327	-620.5673
			Ml	-730.3000*	49.22561	.000	-883.9122	-576.6878
			I18	-201.0000	131.76557	.571	-638.2802	236.2802
			AB8	-312.2000	125.82672	.174	-729.2608	104.8608
Leaves number	Games-Howell	MBC1	Ml	57.2000	36.70979	.545	-57.5408	171.9408
			I18	341.0000*	79.46969	.007	89.1784	592.8216
			AB8	281.7000*	79.62002	.026	29.3572	534.0428
			Control	-202.0000*	60.13474	.029	-387.2009	-16.7991
		Ml	MBC1	-57.2000	36.70979	.545	-171.9408	57.5408
			I18	283.8000*	74.55861	.022	38.4577	529.1423
			AB8	224.5000	74.71883	.078	-21.3909	470.3909
			Control	-259.2000*	53.47656	.004	-432.0140	-86.3860
		I18	MBC1	-341.0000*	79.46969	.007	-592.8216	-89.1784
			Ml	-283.8000*	74.55861	.022	-529.1423	-38.4577
			AB8	-59.3000	102.71672	.977	-369.8946	251.2946
			Control	-543.0000*	88.47352	.000	-813.8808	-272.1192
		AB8	MBC1	-281.7000*	79.62002	.026	-534.0428	-29.3572
			Ml	-224.5000	74.71883	.078	-470.3909	21.3909
			I18	59.3000	102.71672	.977	-251.2946	369.8946
			Control	-483.7000*	88.60858	.000	-755.0336	-212.3664
		Control	MBC1	202.0000*	60.13474	.029	16.7991	387.2009
			Ml	259.2000*	53.47656	.004	86.3860	432.0140
			I18	543.0000*	88.47352	.000	272.1192	813.8808
			AB8	483.7000*	88.60858	.000	212.3664	755.0336

Table 5. Continued.

Root_ length	Games-Howell	MBC1	Ml	134.3000	65.04464	.293	-71.6670	340.2670
			I18	404.2000*	97.33595	.005	107.8189	700.5811
			AB8	391.0000*	91.89099	.004	112.3249	669.6751
			Control	-232.4000	121.86328	.358	-611.7806	146.9806
		Ml	MBC1	-134.3000	65.04464	.293	-340.2670	71.6670
			I18	269.9000*	81.69540	.044	6.2336	533.5664
			AB8	256.7000*	75.12527	.035	15.7812	497.6188
			Control	-366.7000*	109.77483	.046	-727.0756	-6.3244
		I18	MBC1	-404.2000*	97.33595	.005	-700.5811	-107.8189
			Ml	-269.9000*	81.69540	.044	-533.5664	-6.2336
			AB8	-13.2000	104.34216	1.000	-329.0052	302.6052
			Control	-636.6000*	131.50663	.001	-1038.2960	-234.9040
		AB8	MBC1	-391.0000*	91.89099	.004	-669.6751	-112.3249
			Ml	-256.7000*	75.12527	.035	-497.6188	-15.7812
			I18	13.2000	104.34216	1.000	-302.6052	329.0052
			Control	-623.4000*	127.52906	.001	-1015.3480	-231.4520
		Control	MBC1	232.4000	121.86328	.358	-146.9806	611.7806
			Ml	366.7000*	109.77483	.046	6.3244	727.0756
			I18	636.6000*	131.50663	.001	234.9040	1038.2960
			AB8	623.4000*	127.52906	.001	231.4520	1015.3480

Based on observed means.
The error term is Mean Square (Error) = 52906.551.
*. The mean difference is significant at the

Table 6. Test of Homogeneity of Variances.

		Levene Statistic	df1	df2	Sig.
Biofilm_absorbance	Based on Mean	.947	3	8	.463

Table 7. ANOVA for Biofilm_absorbance.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4498.000	3	1499.333	.516	.683
Within Groups	23228.667	8	2903.583		
Total	27726.667	11			

to help the *Streptomyces* group survive and modulate lettuce growth under salt-stress conditions. On the other hand, the MBC1 strain that produced lower levels of IAA than the Streptomyces genera increased lettuce growth until harvest. It turns out that MBC1 can adapt to saline habitats, as reported in a previous study [45].

Auxin has increased crop growth and production by stimulating root development. Furthermore, auxin might increase plant biotic and abiotic stress tolerance. As a result, auxin-producing bacteria have been explored as a biological resource for agricultural purposes [46]. Plants can adapt to high salt content environmental circumstances by leveraging rhizobacteria's role that may produce biofilms. This confirms that bacteria associated with plants must be tolerant to stress, including salt, in addition to creating phytohormones [26].

However, microbial administration in this experiment allows the prospect of using hormone-producing microorganisms that might tolerate stress caused by a brackish water growth medium [47]. Another point to consider is that plants require IAA under specific conditions which are sufficient but not excessive [48]. The outline of why the *Serratia marcescens* strain MBC1 is preferable to the Actinomycetes group, on the other hand, supports earlier comparable results by other researchers. Inoculation of *Serratia liquefaciens* KM4 significantly improved osmoregulation, maize growth, antioxidant defense system, and nutrient absorption under salt stress. These findings are accompanied by the upregulation of stress-related genes (APX, CAT, SOD, RBCS, RBCL, H+-PPase, HKT1, and NHX1) and the downregulation of critical genes in ABA biosynthesis (NCED). *Micrococcus* sp. is also acknowledged as a bacteria strain tolerant to salt stress, besides *Serratia* spp genera [49]. Although many species of *Streptomyces* sp. have been recorded to be kind of salt levels [50], strains i18 and AB8 were shown to be intolerant of salt levels in this study. This was indicated by the occurrence of death in lettuce plants in the addition of the two isolates before the harvest period. The scope of this study is currently restricted to studying IAA, biofilm, and plant growth. Nonetheless, because the technology employed is simple, its applicability is straightforward for individuals in developing countries.

Conclusions

The microorganisms utilized in this investigation were shown to be capable of producing the hormone IAA. The generation of the IAA was able to modify the growing of lettuce under salt stress in brackish water media, as evaluated by the number of leaves, leaf length, and roots. The capacity to endure salt levels is critical since it can encourage IAA production, allowing host plants to survive salt stress levels in the environment using simple technology. Although the application of bacterial isolates to hydroponic culture using a brackish water medium proved to trigger the development of lettuce plants, with the support of IAA and biofilm production, the yields of lettuce plants were no better than control lettuce grown on freshwater media in root growth. The findings of this study are still in the early phases of development. Even while the natural interaction conditions do not just entail the interaction of a single microbe, but several species engaged, the formulation of bacteria is still a single colony, not in a consortium. Monitoring bacterial ability is still currently confined to IAA and biofilm hormone factors, despite the fact that there are many additional indicators that may be examined in the future, such as the mechanism of salt tolerance modifications and expression of the resultant proteins. Protein plays an important role because alterations in enzyme metabolism may be observed in both plants and microbes.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. JURY W.A., VAUX H. The role of science in solving the world's emerging water problems. Proceedings of the National Academy of Sciences, **102** (44), 15715, **2005**.
2. KO S.H., ISHIDA K., OO Z.M., SAKAI H. Impacts of seawater intrusion on groundwater quality in Htantabin township of the deltaic region of southern Myanmar. Groundwater for Sustainable Development, **14**, 100645, **2021**.
3. CONG V.H. Desalination of brackish water for agriculture: Challenges and future perspectives for seawater intrusion areas in Vietnam. Journal of Water Supply: Research and Technology - AQUA, **67** (3), 211, **2018**.
4. ABOU-SHADY A., EL-ARABY H. Electro-agric, a novel environmental engineering perspective to overcome the global water crisis via marginal water reuse. Natural Hazards Research, **1** (4), 202, **2021**.
5. GODDEK S., DELAIDE B., MANKASINGH U., RAGNARSDOTTIR K.V., JIJAKLI H., THORARINSDOTTIR R. Challenges of Sustainable and Commercial Aquaponics. Sustainability, **7** (4), 4199, **2015**.
6. GREENLEE L.F., LAWLER D.F., FREEMAN B.D., MARROT B., MOULIN P. Reverse osmosis desalination: Water sources, technology, and today's challenges. Water Research, **43** (9), 2317, **2009**.
7. JARIN M., DOU Z., GAO H., CHEN Y., XIE X. Salinity exchange between seawater/brackish water and domestic wastewater through electrodialysis for potable water. Frontiers of Environmental Science & Engineering, **17**, **2022**.
8. OTLEWSKA A., MIGLIORE M., DYBKA-STĘPIEŃ K., MANFREDINI A., STRUSZCZYK-ŚWITA K., NAPOLI R., BIALKOSWKA A., CANFORA L., PINZARI F. When Salt Meddles Between Plant, Soil, and Microorganisms. Frontiers in Plant Science, **11**, **2020**
9. KUMAR A., SINGH S., GAURAV A.K., SRIVASTAVA S., VERMA J.P. Plant Growth-Promoting Bacteria: Biological Tools for the Mitigation of Salinity Stress in Plants. Frontiers in Microbiology, **11** (July), 1, **2020**.
10. EGAMBERDIEVA D., WIRTH S.J., ALQARAWI A.A., ABD-ALLAH E.F., HASHEM A. Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. Frontiers in Microbiology, **8** (October), 1, **2017**.
11. KUMAWAT C., KUMAR A., PARSHAD J., SHARMA S.S., PATRA A., DOGRA P., YADAV G. K., DADHICH S.K., VERMA R., KUMAWAT G.L. Microbial Diversity

and Adaptation under Salt-Affected Soils: A Review. Sustainability (Switzerland), **14** (15), 1, **2022**.
12. KUDOYAROVA G., ARKHIPOVA T., KORSHUNOVA T., BAKAEVA M., LOGINOV O., DODD I.C. Phytohormone Mediation of Interactions Between Plants and Non-Symbiotic Growth Promoting Bacteria Under Edaphic Stresses. Frontiers in Plant Science, **10** (October), 1, **2019**.
13. DEVI K.A., PANDEY P., SHARMA G.D. Plant Growth-Promoting Endophyte *Serratia marcescens* AL2-16 Enhances the Growth of *Achyranthes aspera* L., a Medicinal Plant. HAYATI Journal of Biosciences, **23** (4), 173, **2016**.
14. DAR G.H., SOFI S., PADDER S.A., KABLI A. Molecular characterization of rhizobacteria isolated from walnut (*Juglans regia*) rhizosphere in Western Himalayas and assessment of their plant growth promoting activities. Biodiversitas, **19** (2), 632, **2018**.
15. NAMWONGSA J., JOGLOY S., VORASOOT N., BOONLUE S., RIDDECH N., MONGKOLTHANARUK W. Endophytic Bacteria Improve Root Traits, Biomass and Yield of *Helianthus tuberosus* L. Under normal and deficit water conditions. Journal of Microbiology and Biotechnology, **29** (11), 1777, **2019**.
16. DE FRETES C.E., SEMBIRING L., PURWESTRI Y.A. Characterization of *Streptomyces* spp. Producing Indole-3-acetic acid as Biostimulant Agent. Indonesian Journal of Biotechnology, **18** (2), 83, **2013**.
17. MATHEW B.T., TORKY Y., AMIN A., MOURAD A.H.I., AYYASH M.M., EL-KEBLAWY A., HILAL-ALNAQBI A., ABUQAMAR S.F., EL-TARABILY K.A. Halotolerant Marine Rhizosphere-Competent Actinobacteria Promote *Salicornia bigelovii* Growth and Seed Production Using Seawater Irrigation. Frontiers in Microbiology, **11** (April), **2020**.
18. HAKIM M.A.R., SUMARSONO S., SUTARNO S. Pertumbuhan dan produksi dua varietas selada (*Lactuca sativa* l.) pada berbagai tingkat naungan dengan metode hidroponik. Journal of Agro Complex, **3** (1), 15, **2019**.
19. WARDHANA I., HASBI H., WIJAYA I. Respon Pertumbuhan dan Produksi Tanaman Selada (*Lactuca savita* L.) pada Pemberian Dosis Pupuk Kandang Kambing dan Interval Waktu Aplikasi Pupuk Cair Super Bionik. Agritop Jurnal Ilmu-ilmu Pertanian, **14** (2), 165, **2016**.
20. LESTARI M.D., AR M.M., SETIAWATI U.N., NUKMAL N., SETYANINGRUM E., ARIFIYANTO A., AENY T. N. Bioaccumulation and Resistance Activity of Lead by *Streptomyces* sp. strain I18. Jurnal Sumberdaya Alam dan Lingkungan, **9** (1), 1, **2022**.
21. FITRIANI M., ARIFIYANTO A., LANDE M.L., EKOWATI N., AENY T.N., AFRIYANI H. *Streptomyces hygroscopicus* Subspecies *hygroscopicus* Strain I18: Incubation Time and Tryptophan Concentration Effects on Indole-3-Acetic Acid (IAA) Hormone Production. In The 2nd Universitas Lampung International Conference On Science, Technology, and Environment (ULICoSTE) 2021 **2563** (1), 080001, **2022**.
22. MYO E.M., GE B., MA J., CUI H., LIU B., SHI L., JIANG M., ZHANG K. Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth. BMC Microbiology, **19** (1), 1, **2019**.
23. LIN Y.B., WANG X.Y., FANG H., MA Y.N., TANG J., TANG M., WEI G.H. *Streptomyces shaanxiensis* sp. nov., a blue pigment-producing streptomycete from sewage irrigation soil. International Journal of Systematic and Evolutionary Microbiology, **62** (8), 1725, **2012**.
24. THAKULLA D., DUNN B., HU B., GOAD C., MANESS N. Nutrient Solution Temperature Affects Growth and °Brix Parameters of Seventeen Lettuce Cultivars Grown in an NFT Hydroponic System. Horticulturae, **7** (9), **2021**.
25. DE SOUSA L.L., DA HORA D.S., SALES E.A., PERELO L.W. Cultivation of *Nannochloropsis* sp. in brackish groundwater supplemented with municipal wastewater as a nutrient source. Brazilian Archives of Biology and Technology, **57** (2), 171, **2014**.
26. KEARL J., MCNARY C., LOWMAN J.S., MEI C., AANDERUD Z.T., SMITH S.T., WEST J., COLTON E., HAMSON M., NIELSEN B.L. Salt-tolerant halophyte rhizosphere bacteria stimulate growth of alfalfa in salty soil. Frontiers in Microbiology, **10** (August), **2019**.
27. VENZKE C.D., GIACOBBO A., FERREIRA J.Z., BERNARDES A.M., RODRIGUES M.A.S. Increasing water recovery rate of membrane hybrid process on the petrochemical wastewater treatment. Process Safety and Environmental Protection, **117**, 152, **2018**.
28. MENGESHA A., SAHU O. Sustainability of membrane separation technology on groundwater reverse osmosis process. Cleaner Engineering and Technology, **7**, 100457, **2022**.
29. RASHEED A., LI H., NAWAZ M., MAHMOOD A., HASSAN M.U., SHAH A.N., GILLANI S.F.A., MAJEED, Y., QARI S.H., WU Z. Molecular tools, potential frontiers for enhancing salinity tolerance in rice: A critical review and future prospective. Frontiers in Plant Science, **13** (July), 1, **2022**.
30. AZIZ M., BRINI F., ROUACHED H., MASMOUDI K. Genetically engineered crops for sustainably enhanced food production systems. Frontiers in Plant Science, **13**, 1027828, **2022**.
31. LÓPEZ-ARREDONDO D., GONZÁLEZ-MORALES S., BELLO-BELLO E., ALEJO-JACUINDE G., HERRERA-ESTRELLA L. Crop Engineering food crops to grow in harsh environments. F1000 Research. **4** (651), 1, **2015**.
32. KAUSHAL M. Insights Into Microbially Induced Salt Tolerance and Endurance Mechanisms (STEM) in Plants. Frontiers in Microbiology, **11** (August), **2020**.
33. MUNIR N., HANIF M., ABIDEEN Z., SOHAIL M., EL-KEBLAWY A., RADICETTI E., MANCINELLI R., HAIDER G. Mechanisms and Strategies of Plant Microbiome Interactions to Mitigate Abiotic Stresses. Agronomy, **12** (9), 1, **2022**.
34. GBENRO FOLARANMI M.B., PHILIPPE SISTAT M.C. and F.Z. Towards Electrochemical Water Desalination Techiniques, Membranes, **10** (5), 96, **2020**.
35. MAC MAHON J., GILL L.W. Sustainability of novel water treatment technologies in developing countries: Lessons learned from research trials on a pilot continuous flow solar water disinfection system in rural Kenya. Development Engineering, **3**, 47, **2018**.
36. TREACY J. Drinking Water Treatment and Challenges in Developing Countries. In N. Potgieter & A. N. T. Hoffman (Eds.), Intechopen (p. Ch. 5). Rijeka: IntechOpen. **2019**.
37. BAWA A., ANILAKUMAR K. Genetically modified foods: Safety, risks and public concerns - A review. Journal of Food Science and Technology, **50** (6), 1035, **2013**.
38. ITO N. Genetically modified foods: Hazard identification and risk assessment. Experimental and toxicologic pathology: official journal of the Gesellschaft für Toxikologische Pathologie, **54**, 57, **2002**.
39. GR S., YADAV R.K., CHATRATH A., GERARD M., TRIPATHI K., GOVINDSAMY V., ABRAHAM G.

Perspectives on the potential application of cyanobacteria in the alleviation of drought and salinity stress in crop plants. Journal of Applied Phycology, **33** (6), 3761, **2021**.
40. POUDEL M., MENDES R., COSTA L.A.S., BUENO C.G., MENG Y., FOLIMONOVA S.Y., GARRET K.A., MARTINS S.J. The Role of Plant-Associated Bacteria, Fungi, and Viruses in Drought Stress Mitigation. Frontiers in Microbiology, **12** (October), 1, **2021**.
41. LABEEUW L., KHEY J., BRAMUCCI A.R., ATWAL H., DE LA MATA A.P., HARYNUK J., CASE R.J. Indole-3-Acetic Acid Is Produced by *Emiliania huxleyi* Coccolith-Bearing Cells and Triggers a Physiological Response in Bald Cells. Frontiers in Microbiology, **7** (828), 1, **2016**.
42. SPAEPEN S., VANDERLEYDEN J., REMANS R. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiology Reviews, **31** (4), 425, **2007**.
43. HU D., LI S., LI Y., PENG J., WEI X., MA J., ZHANG C., JIA N., WANG E., WANG Z. *Streptomyces* sp. strain TOR3209: a rhizosphere bacterium promoting growth of tomato by affecting the rhizosphere microbial community. Scientific Reports, **10** (1), 20132, **2020**.
44. XA L.T., NGHIA N.K., TECIMEN H.B. Environmental Factors Modulating Indole-3-Acetic Acid Biosynthesis by Four Nitrogen Fixing Bacteria in a Liquid Culture Medium. Environment and Natural Resources Journal, **20** (3), 279, **2022**.
45. ARIFIYANTO A., AFRIANI H., PUTRI M.H., DAMAYANTI B., RIYANTO C.L.R. The biological prospective of red-pigmented bacteria cultured from contaminated agar media. Biodiversitas, Journal of Biological Diversity, **22** (3), 1152, **2021**.
46. PARK S., KIM A.-L., HONG Y.-K., SHIN J.-H., JOO S.-H. A highly efficient auxin-producing bacterial strain and its effect on plant growth. Journal, genetic engineering & biotechnology, **19** (1), 179, **2021**.
47. GAMALERO E., BONA E., TODESCHINI V., LINGUA G. Saline and Arid Soils: Impact on Bacteria, Plants, and their Interaction. Biology, **9** (6), **2020**.
48. MSIMBIRA L.A., SMITH D.L. The Roles of Plant Growth Promoting Microbes in Enhancing Plant Tolerance to Acidity and Alkalinity Stresses. Frontiers in Sustainable Food Systems, **4**, 106, **2020**.
49. MASUO N., YOSHIMUNE K., ITO K., MATSUSHIMA K., KOYAMA Y., MORIGUCHI M. *Micrococcus luteus* K-3-type glutaminase from *Aspergillus oryzae* RIB40 is salt-tolerant. Journal of Bioscience and Bioengineering, **100** (5), 576, **2005**.
50. SADEGHI A., SOLTANI B.M., JOUZANI G.S., KARIMI E., NEKOUEI M.K., SADEGHIZADEH M. Taxonomic study of a salt tolerant *Streptomyces* sp. strain C-2012 and the effect of salt and ectoine on lon expression level. Microbiological Research, **169** (2-3), 232, **2014**.