## Invasion Of Endophytic Bacteria By Using Microencapsulation Technology As Stimulant in Cocoa Plants (*Theobroma cacao* L)

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#### Abstract

Fertilization is an important process in the growth of cocoa (Theobroma cacao L). Fertilizing cocoa usually used inorganic fertilizers. Using of inorganic fertilizers continuously could degrading soil fertility. One of efforts to replace inorganic fertilizers was using endophytic bacteria as biofertilizers. Some endophytic bacteria are abundant in healthy plant tissues. The aim of this research was to obtain endophytic auxin-producing bacteria in cocoa plants and determine effect of seed immersion and microcapsules addition of endophytic bacteria on cocoa plant growth. Research design used was Complete Randomized Design (CRD), 16 treatments and 3 replications. First factor was immersion of cocoa seed using endophytic bacteria suspension consisting of S0=0 hours; S1=5 hours; S2=6hours and S3 = 7 hours and second factor was microcapsules addition consisting of B0 = 0 gr; B1 =5 gr; B2=10 gr; B3=15 gr. Isolation from roots and stems cocoa obtained 5 isolates endophite bacteria. Auxin assays showed that five isolates were able produced auxin. Observations on plant height showed best treatment was treatment B1 (32.49 cm). Observation total leaves showed S3 treatment was highest data (11.83 strands). Observation rod diameter parameter, highest data was S3 treatment (4.01 mm). For leaf area parameter, highest value was B1 treatment ( $66.64 \text{ cm}^2$ ). For wet weight parameter, highest data was S3 treatment (18.41 g). Root length parameter, highest data was B3 treatment (15.78 cm). Test results showed that application of suspension and microcapsules endophytic bacteria significantly increased growth of cocoa.

Keywords: Auxin, Cocoa, Endophytic Bacteria, Microcapsules



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#### INTRODUCTION

Cocoa (*Theobroma cacao* L) is one of plantation commodities that are suitable for community also has considerable potential in increasing foreign exchange, and as a source of livelihood of farming families scattered in various provinces in Indonesia (Dekaindo, 2016). Cocoa cultivation can be done generatively by using cocoa beans. During breeding process, things to consider in cocoa cultivation was fertilization for fertility or plant health and productivity. Fertilizers commonly used in fertilization process was usually inorganic (chemical) fertilizers to increase production and quality of cocoa plants (Sriharti & Dyah, 2018).

According to Sabahannur et al., (2016) cocoa cultivation with inorganic fertilizer caused 90% of cocoa beans produced became low quality. Continuous use of inorganic fertilizers can also increase soil fertility, even changing physical, chemical and biological properties of soil (Maghfoer, 2018). Increase productivity due to use of inorganic fertilizers lasts only a short time and was not sustainable.

One of effort to increase cocoa production was using biofertilizer (Arifah et al., 2018). Application of biofertilizers and organic fertilizers in seedling phase was also necessary (Krisdayani et al., 2020). Biological fertilizer was microbes given into soil to increase nutrient uptake by plants from within land. This fertilizer contains superior soil microorganisms and it was useful to increase soil fertility as a result of soil biochemical processes.

One of innovations in biofertilizers is using of endophytic bacteria. Some excess endophytic bacteria are abundant in healthy plant tissue. Research (Puspita et al., 2019) reported that endophytic bacteria can be taken from almost all parts of plants. Foeh et al., (2019) mentioned that advantages of endophytic bacteria some of which was also able to increase plant growth. Saridewi et al., (2020) also reported endophytic bacteria had ability produced phytohormones such as auxin, increase phosphate availability, produced various enzymes such as amylase, selluase. Endophytic bacteria were also thought to be able to increase plant defense system against plant disease disorders due to their ability to produce antimicrobial compounds, enzymes, salicylic acid, ethylene and other secondary compounds (Sihombing, et al., 2019).

This research purposed to obtain isolates of endophytic bacteria from cocoa roots and stems, determine their ability to produce the auxin hormone and test their effectiveness in stimulating cocoa growth. Results of this study are expected to be useful as a reference and guidance for further research.

#### METHOD

#### **Tools and Materials**

Tools used in this study were petri dishes, test tubes, test tube racks, measuring cups, beakers, erlenmeyer, autoclaves, oven, spatulas, ose needles, incubators, hot plates, stirring rods, analytical scales, sprayers, laminar airflow, shakers, glass bottles, aluminium foil, cotton, cutter and polybag.

Materials used in this study are roots and stems of cacao, nutrient media Agar (NA), aquadest, alcohol 70%, chlorine solution, CaCl<sup>2</sup>, sodium alginate, inulin, poultry manure, top soil, rice husk charcoal, NaCl 0,9%, crystal violet, safranin, acetone alcohol, iodine, L-tryptopan, peptone, Salkowsky reagent.

#### **Research Methods**

This research was conducted using factorial CRD (Complete Randomized Design) method consisting of 2 factors, 16 treatments and 3 replications. Suspension immersion consists of: S0: 0 hour; S1: 5 hours; S2: 6 hours and S3: 7 hours. Microcapsule addition treatment consists of B0: 0 gr, B1: 5 gr; B2: 10 gr, B3: 15 gr. Data obtained were analyzed using ANOVA. Results of analysis were followed by Duncan's test.

### Isolation of Endophytic Bacteria

Endophite bacteria was isolated from cacao roots and stems. Isolation of endophite bacteria used method (Singh et al., 2022) modified. Before isolation process, surface of roots and stems of cocoa was sterilized.

#### Measurement of Auxin From Endophytic Bacteria

Auxin testing conducted according to (Walida et al., 2019) which has been modified. Isolates were inoculated on flat Nutrient Agar media supplemented with tryptophan at concentration of 100 ppm and incubated at room temperature for 48 hours. Salkowski reagent was dripped onto endophite bacterial colonies. Colonies that had been dripped with Salkowski reagent was stored in dark room for 30 minutes. Positive result was indicated by a change in color of colonies became red (Herlina et al., 2017).

#### Preparation and Sterilization of Planting Media

Planting media used top soil, broiler manure and rice husk charcoal with a ratio  $(1: \frac{1}{2}: \frac{1}{2})$ . Sterilization was carried out at 150°C for 10 hours.

#### Immersion Seeds with Suspension of Endophite Bacteria

Collection of endophite bacteria solution was carried out by adding 10 ml of NaCl 0,9% solution in 1 petri, and stirring using a triangular stir bar. Cacao seeds were soaked at ratio: 0 hours, 5 hours, 6 hours and 7 hours in a container covered with aluminum foil to keep it sterilized.

#### Producing of Microcapsules From Endophite Bacteria As Biofertilizers

Total of 14.7 g of CaCl<sub>2</sub> was dissolved with 1000 ml of distilled water in a volumetric flask, stirred until homogeneous. Solution was sterilized using autoclave at 121°C for 15 minutes. Sterile alginate solution containing suspension of endophite bacteria was put into a spit needle and then dropped into 0.1M CaCl<sub>2</sub> solution. To remove CaCl<sub>2</sub> residue, microcapsule was filtered and rinsed using distilled water (Panichikkal et al., 2021).

#### **Application of Microcapsules**

Application of microcapsule bacterial fertilizers was carried out in 2<sup>nd</sup> week after planting (WAP) plant shoots appear or when leaves appear with dose of predetermined treatment level.

#### **Observation Parameters**

Parameters observed in this research were plant height (cm), total of leaves (strands), stem diameter (mm), average leaf area (cm<sup>2</sup>), wet weight of plants (g), root length (cm).

#### **RESULTS AND DISCUSSION**

## Isolation of Endophytic Bacteria From Roots And Stems of Cocoa Plants

Isolates of endophytic bacteria from roots and stems of cocoa plants (*Theobroma cacao* L) obtained 5 isolates consisting of 2 isolates samples from cocoa stems and 3

isolates from cocoa roots. Each isolate had characteristics that vary in both morphology and coloring properties.

<b>Table 1</b> . Colony and cell morphology and gram staining properties of endophytic	
bacterial isolates of cacao roots and stems (Theobroma cacao L).	
Characterization	

			Char	acterization			_
Isolate		Morpholo	ogy Colony		Cell Mor	Gram	
	Color	Shape	Edge	Elevation	Shape	Setup	
GS1 BK	White	Irregulr	Irregulr	Placocoau	Basil	Mono	Positive
GS2 BK	White	Rhizoid	Lobate	Flat	Basil	Strepto	Negative
GS1 AK	White	Round	Irregulr	Flat	Basil	Strepto	Positive
GS2 AK	White	Irregulr	Irregulr	convex	Basil	Strepto	Positive
GS3 AK	White	Rhizoid	Lobate	margin	Coccus	Strepto	Negative

# Ability of Endophytic Bacteria From Roots and Stems of Cocoa Plants in Producing Auxin Hormone

Isolation results of endophytic bacteria, 5 isolates produced auxin hormones were marked with changes in transparent red and pink colors when dripped Salkowski reagent. Results of auxin hormone levels from root and stem endophytic bacteria were showed in Table 2.

Table 2.	Auxin hormone rate produced by endophytic bacteria from roots and stems of
	cocoa plant ( <i>Theobroma cacao</i> L).

Isolates	Auxin Rate
GS1 BK	+
GS2 BK	+
GS1 AK	+
GS2 AK	+
GS3 AK	+

## Plant Height (cm)

Cocoa plant height was observed at week 2, 4, 6, 10 weeks after planting (WAP). Based on results of observations and ANOVA test, it was known that immersion treatment of cocoa plant height growth (*Theobroma cacao* L) had no significant difference in plant height (cm) on observations of 2<sup>nd</sup> WAP, 4<sup>nd</sup> WAP, 6<sup>nd</sup> WAP, 8<sup>nd</sup> WAP, and had a markedly different influence on 10<sup>nd</sup> WAP. Addition of microcapsules had no significant difference on plant height at observation of 2<sup>nd</sup> WAP, 4<sup>th</sup> WAP, 6<sup>th</sup> WAP, 8<sup>th</sup> WAP, but giving a noticeable difference in observation at 10<sup>th</sup> WAP.

Interaction of influence of variations in immersion and microcapsule addition had unreal effect on measurement data of plant height (cm) on growth of cocoa plants (*Theobroma cacao* L). Duncan Distance test results were showed in Table 1.

Treatmont	Average Plant Height (cm)								
Treatment	2 <sup>nd</sup> WAP	4 <sup>th</sup> WAP	6 <sup>th</sup> WAP	8 <sup>th</sup> WAP	10 <sup>th</sup> WAP				
Immersion Treatment	(S)								
S0 = 0 Hours	13.61 <sup>aA</sup>	20.49 <sup>aA</sup>	22.27 <sup>aA</sup>	24.35 <sup>aA</sup>	27.55 <sup>bA</sup>				
S1 = 5 hours	13.42 <sup>aA</sup>	22.79 <sup>aA</sup>	25.68 <sup>aA</sup>	27.89 <sup>aA</sup>	31.84 <sup>aA</sup>				
S2 = 6 hours	$12.75 \ ^{aA}$	20.46 <sup>aA</sup>	24.60 <sup>aA</sup>	26.64 <sup>aA</sup>	30.98 <sup>aA</sup>				
S3 = 7 hours	13.25 <sup>aA</sup>	20.41 <sup>aA</sup>	23.71 <sup>aA</sup>	27.88 <sup>aA</sup>	31.22 <sup>aA</sup>				
Microcapsule Addition	n (B)								
B0 = 0 gr	13.80 aA	19.98 <sup>aA</sup>	23.35 <sup>aA</sup>	26.30 <sup>aA</sup>	29.78 <sup>aA</sup>				
B1 = 5 gr	13.38 <sup>aA</sup>	20.85 <sup>aA</sup>	23.89 <sup>aA</sup>	27.35 <sup>aA</sup>	32.49 <sup>abA</sup>				
B2 = 10 gr	12.21 <sup>aA</sup>	21.21 <sup>aA</sup>	24.07 <sup>aA</sup>	27.43 <sup>aA</sup>	$30.68 ^{\text{abA}}$				
B3 = 15 gr	13.64 <sup>aA</sup>	22.11 <sup>aA</sup>	24.95 <sup>aA</sup>	25.68 <sup>aA</sup>	28.64 <sup>aA</sup>				

 Table 3. Average Height Cocoa (*Theobroma cacao* L) by Immersion Treatment And Microcapsule Addition.

#### Total of Leaves (strands)

Total of leaves were observed at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 10<sup>th</sup> week after planting (WAP). Based on results of observations and ANOVA test, it was known that observation on total of leaves of cocoa plant (*Theobroma cacao* L) had unreal influence on the observation of 2<sup>nd</sup> WAP, 4<sup>th</sup> WAP, and 10 WAP. Observation on 6<sup>th</sup> WAP, and 8<sup>th</sup> WAP had very noticeable different influence on total of leaves (strands). However, it had an unreal influence on treatment of microcapsules addition at 2<sup>nd</sup> WAP, 4<sup>th</sup> WAP, 6<sup>th</sup> WAP, 8<sup>th</sup> WAP, 10<sup>th</sup> WAP. Interaction of influence of variations in immersion and addition of microcapsules had no real effect. Duncan test results were showed in Table 4.

**Table 4.** Average total of leaves cocoa (*Theobroma cacao* L) by immersion and<br/>microcapsule addition.

Treatment	Average Total of Leaves (strands)								
	2 <sup>nd</sup> WAP	4 <sup>th</sup> WAP	6 <sup>th</sup> WAP	8 <sup>th</sup> WAP	10 <sup>th</sup> WAP				
Immersion (S)									
S0 = 0 Hours	3.92 <sup>aA</sup>	5.17 <sup>aA</sup>	6.92 bcA	9.00 bB	11.08 <sup>aA</sup>				
S1 = 5 hours	4.08 aA	5.67 <sup>aA</sup>	6.92 bcA	8.50 bB	11.08 <sup>aA</sup>				
S2 = 6 hours	3.58 <sup>aA</sup>	5.17 <sup>aA</sup>	6.92 <sup>bA</sup>	8.75 bB	10.75 <sup>aA</sup>				
S3 = 7 hours	4.17 <sup>aA</sup>	6.08 <sup>aA</sup>	7.92 <sup>aA</sup>	$10.42 \ ^{\mathrm{aA}}$	11.83 <sup>aA</sup>				
Microcapsule Addition	. (B)								
B0 = 0 gr	4.33 <sup>aA</sup>	5.50 <sup>aA</sup>	7.17 <sup>Aa</sup>	5.75 <sup>aA</sup>	11.75 <sup>aA</sup>				
B1 = 5 gr	3.92 <sup>aA</sup>	5.58 <sup>aA</sup>	7.25 <sup>aA</sup>	5.75 <sup>aA</sup>	11.75 <sup>aA</sup>				
B2 = 10  gr	4.00 <sup>aA</sup>	5.58 <sup>aA</sup>	6.83 <sup>aA</sup>	5.75 <sup>aA</sup>	10.75 <sup>aA</sup>				
B3 = 15 gr	3.50 <sup>aA</sup>	5.42 <sup>aA</sup>	7.42 <sup>aA</sup>	5.63 <sup>aA</sup>	10.50 <sup>aA</sup>				

Treatment	Average Num	ber of Leaves (strands)
	6 <sup>th</sup> WAP	8 <sup>th</sup> WAP
S0B0	7 bcdCD	9 bcdeBCDE
S0B1	6.67 <sup>cdD</sup>	9 bcdeBCDE
S0B2	7.67 bcBC	9.33 bcdeBCD
S0B3	6.33 <sup>dD</sup>	8.66 <sup>cdefCDE</sup>
S1B0	7 bcdCD	8.33 defDEF
S1B1	7.67 bcBC	10  bcB
S1B2	6.33 <sup>dD</sup>	7.33 fF
S1B3	6.67 <sup>cdD</sup>	8.33 dcfDEF
S2B0	6.67 <sup>cdD</sup>	9 bcdeBCDE
S2B1	8 <sup>bB</sup>	9.66 bcdBC
S2B2	6.33 <sup>dD</sup>	8 efDF
S2B3	6.67 <sup>cdD</sup>	8.33 dcfDEF
S3B0	8 <sup>bB</sup>	10  bcB
S3B1	6.67 <sup>bB</sup>	8.33 dcfDG
S3B2	7 <sup>bB</sup>	10 <sup>bB</sup>
S3B3	10 <sup>aA</sup>	13 <sup>aA</sup>

Table 5. Average treatment of interaction of immersion and microcapsule addition on	
total of leaves cocoa (Theobroma cacao L).	

Based on observations from Table 4. showed that interaction of average amount of leaves from immersion treatment and addition of endophytic bacterial microcapsules had a markedly different influence on 6<sup>th</sup> WAP after being tested using Duncan Distance Test.

#### Rod Diameter (mm)

Based on results of observations and ANOVA test, it was known that effect of immersion and microcapsules addition on cocoa growth (*Theobroma cacao* L) has unreal different influence on diameter of plant stem on observations to 2<sup>th</sup> WAP, 4<sup>th</sup> WAP, 6<sup>th</sup> WAP, 8<sup>th</sup> WAP, and was very real on observations of 10<sup>th</sup> WAP.

Interaction effect of bacterial suspension immersion and microcapsule addition had unreal effect on measurement data of stem diameter (mm) on cocoa growth (*Theobroma cacao* L) after being tested using Duncan test were showed in Table 6.

The second	Α	Average Diameter of the Plant Stem (mm)							
Treatment	2 <sup>nd</sup> WAP	4 <sup>th</sup> WAP	6 <sup>th</sup> WAP	8 <sup>th</sup> WAP	10 <sup>th</sup> WAP				
Immersion (S)									
S0 = 0 Hours	2.66 <sup>aA</sup>	2.86 <sup>aA</sup>	3.09 <sup>aA</sup>	3.40 <sup>aA</sup>	$3.71 \ ^{\mathrm{bB}}$				
S1 = 5 hours	2.75 <sup>aA</sup>	2.96 <sup>aA</sup>	3.19 <sup>aA</sup>	3.53 <sup>aA</sup>	3.83 <sup>bAB</sup>				
S2 = 6 hours	2.86 <sup>aA</sup>	3.09 <sup>aA</sup>	3.35 <sup>aA</sup>	3.61 <sup>aA</sup>	$3.92^{abAB}$				
S3 = 7 hours	2.86 <sup>aA</sup>	3.05 <sup>aA</sup>	3.23 <sup>aA</sup>	3.70 <sup>aA</sup>	4.01 aA				
Microcapsule Addition	on (B)								
B0 = 0 gr	$2.84 \ ^{\mathrm{aA}}$	3.03 <sup>aA</sup>	3.27 <sup>aA</sup>	3.59 <sup>aA</sup>	3.91 <sup>aA</sup>				
B1 = 5 gr	2.87 <sup>aA</sup>	3.08 aA	3.32 <sup>aA</sup>	3.66 <sup>aA</sup>	3.99 abA				
B2 = 10 gr	2.74 <sup>aA</sup>	2.95 aA	3.16 <sup>aA</sup>	3.55 <sup>aA</sup>	3.88 abA				
B3 = 15 gr	2.68 aA	290 <sup>aA</sup>	3.11 <sup>aA</sup>	3.43 <sup>aA</sup>	3.70 <sup>aA</sup>				

Table 6.	Average	diameter	of ster	n cocoa	(Theobroma	cacao	L)	by	immersion	and
	microcap	psules add	ition.							

**Table 7**. Average interaction treatment of immersion and microcapsules addition on stem diameter of cocoa plant (*Theobroma cacao* L).

Treatment	Average Diameter of Plant Stem (mm)
S0B0	3.73 <sup>efgEF</sup>
S0B1	3.97 <sup>abcdeABCD</sup>
S0B2	3.56 <sup>Gg</sup>
S0B3	3.58 <sup>fgFG</sup>
S1B0	3.86 deCDE
S1B1	4 abcdABC
S1B2	3.94 <sup>abcdeCD</sup>
S1B3	3.52 <sup>gG</sup>
S2B0	3.90 <sup>cdeCDE</sup>
S2B1	4.12 abcAB
S2B2	3.86 deCDE
S2B3	3.79 deDE
S3B0	4.13 <sup>abA</sup>
S3B1	3.86 deCDE
S3B2	4.14 <sup>aA</sup>
S3B3	3.92 abcdeCDE

Based on observations from Table 7 showed that average interaction of stem diameters from immersion and microcapsules addition of endophytic bacterial microcapsules had a different influence on 10<sup>th</sup> weeks after planting (WAP) used Duncan test.

## Leaf Area (cm<sup>2</sup>)

Leaf area was observed at 10<sup>th</sup> weeks after planting (WAP). Based on results of observations and ANOVA test, it was known that of immersion and microcapsules addition of leaf area on cocoa plant (*Theobroma cacao* L) had unnoticeable different influence on leaf area at observation of 10<sup>th</sup> WAP.

**Table 8.** Average leaf area of cocoa plant (*Theobroma cacao* L) against of immersion and microcapsules addition.

Treatment	Leaf Area (cm <sup>2</sup> )
Immersion (S)	
S0 = 0 Hours	54.21 <sup>aA</sup>
S1 = 6 hours	58.25 <sup>Aa</sup>
S2 = 7 hours	74.71 <sup>aA</sup>
S3 = 8 hours	65.77 <sup>aA</sup>
Microcapsule Addition (B)	
B0 = 0 gr	63.94 <sup>aA</sup>
B1 = 5 gr	66.64 <sup>aA</sup>
B2 = 10  gr	61.79 <sup>aA</sup>
B3 = 15 gr	60.57 <sup>aA</sup>

## Plant Wet Weight (g)

Based on Duncan test results on plant wet weight parameter of cocoa (*Theobroma cacao L*), immersion treatment showed different very markedly and on the microcapsules addition got markedly different results and on interaction process got a real difference. Interaction of bacterial suspension immersion effect and microcapsule addition had a marked effect on plant wet weight measurement data (g) on cocoa growth.

Table 9	. Average plant wet weight of cocoa plants (Theobroma cacao L) of immersion and	
-	microcapsules addition.	

Treatment	Wet weight of the plant (g)
Immersion (S)	
S0 = 0 Hours	12.64 <sup>dD</sup>
S1 = 5 hours	15.96 °C
S2 = 6 hours	18.25 <sup>bB</sup>
S3 = 7 hours	18.41 <sup>aA</sup>
Microcapsule Addition (B)	
B0 = 0 gr	10.97 <sup>aA</sup>
B1 = 5 gr	11.53 <sup>aA</sup>
B2 = 10  gr	10.18 aA
B3 = 15 gr	9.89 <sup>aA</sup>

Treatment	Average wet weight of the plant (g)
S0B0	72.61 <sup>mM</sup>
S0B1	$12.25 ^{\text{dD}}$
S0B2	58.81 <sup>No</sup> .
S0B3	59.49 <sup>No</sup> .
S1B0	11.96 <sup>gG</sup>
S1B1	10.84 <sup>jJ</sup>
S1B2	12.09 <sup>eE</sup>
S1B3	67.81 <sup>nN</sup>
S2B0	11.21 <sup>fF</sup>
S2B1	14,4 ªA
S2B2	10,89 <sup>hH</sup>
S2B3	99.56 <sup>kK</sup>
S3B0	13.84 <sup>bB</sup>
S3B1	10.29 <sup>iI</sup>
S3B2	91.09 IL
S3B3	13.61 cC

 Table 10. Average interaction treatment of of immersion and microcapsules addition in plant wet weight of cocoa plants (*Theobroma cacao* L).

Based on observations from Table 10 showed that average plant wet weight interaction of immersion and microcapsules addition of endophytic bacterial microcapsules had markedly different effect after being tested using Duncan test.

#### Root Length (cm)

Based on results of observations and ANOVA test, it was known that effect of immersion and microcapsules addition of endophytic bacteria on cocoa growth (*Theobroma cacao* L) had different influence on the immersion and had noticeable difference in treatment of capsule microcapsules addition. Interaction effect of bacterial suspension immersion and microcapsules addition had unreal effect on root length measurement data (cm) on cocoa growth.

**Table 11**. Average roots length of cocoa plants (*Theobroma cacao* L) by immersion and microcapsules addition.

Treatment	Root length (cm)
Immersion (S)	
S0 = 0 Hours	14.37 <sup>aA</sup>
S1 = 5 hours	14.02 <sup>aA</sup>
S2 = 6 hours	13.72 <sup>aA</sup>
S3 = 7 hours	14.40 <sup>aA</sup>
Microcapsule Addition (B)	
B0 = 0 gr	12.96 <sup>Ba</sup>
B1 = 5 gr	14.34 <sup>abA</sup>
B2 = 10  gr	13.43 <sup>abA</sup>
B3 = 15  gr	15.78 <sup>aA</sup>

## Discussion

Isolation of Endophytic Bacteria From Roots and Stems of Cocoa (*Theobroma* cacao L) Cocoa plants had diversity of endophytic bacterial isolates. Auxin hormoneproducing endophytic bacterial isolated from cacao plants (*Theobroma* cacao L) had different characteristics such as irregular, rhizoid, round, lobate, plancocoau, flat, conrex, margin, bacil, coccus, mono, strepto. Cocoa plants had diversity of endophytic bacterial produced auxin hormones isolated from cocoa plants (*Theobroma cacao* L). These isolates dominant from genus of *Bacillus*. Salo & Novero, (2020) reported that plants of same type or species have endophytic bacteria that are not always same. Research (Giyanto & Anwar Arifin, 2019) reported that isolates of endophytic bacteria isolated from cocoa plants could spur growth of cocoa plants.

#### Test Potential of Endophytic Bacteria of Cocoa Plants In Producing Auxin

Based on results test there are 5 isolation of bacteria derived from roots and stems of cocoa plants, produced auxin hormones. Previous researchers have proven that physiological improvement of plant seeds and plant growth retardation by addition of endophytic bacteria or other microbial groups were related to the ability of microbes to increase nitrogen fixation, auxin production, and phosphate dissolving ability as per research (Khaeruni et al., 2020). Ability of microbes produced auxin was also influenced by type of microorganism species. From results of isolation *Bacillus* was most dominant. This was suspected because *Bacillus* sp. derived from plants produced auxin for plant growth. Results of analysis (Puspita et al., 2019) showed that 4 isolates of *Bacillus* sp. endophytic produces hormone auxin, which was characterized by formation of a pink. Same microorganism was not necessarily able to produce same auxin (Saputri et al., 2020).

## Plant Height (cm)

Results of plant height parameter obeservation of cocoa (Theobroma cocoa L), highest data at 10<sup>th</sup> WAP in S1 (31.84) cm and addition of microcapsules at 10<sup>th</sup> WAP in B1 (32.49 cm). These results was better than research (Warsito et al., 2022) showed application of chicken manure to plant heights, where the plant height at A1 (200g) was 23.20 cm and at A0 (control) it was 21.65 cm. Addition of coconut water showed different plant heights where plant height at K2 (200 ml / polybag) was 22.80 cm, K1 (100 ML/ polybag) 22.44 cm and K0 (control) 22.03 cm. In another research (Jamidi et al., 2021) reported that in pineapple peel liquid organic fertilizer activities with a concentration of 75 ml/ L, which showed highest plant height (31.97 cm). While in cow manure equipment, best dosage level was dose of 10 tons/ ha which showed value of plant height (32.32 cm). This research was also better than research (Aulia Meyuliana et al., 2022) that best equipment was 350 g/ polybag (cattle dirty cleaner) equipment with a value (25.06 cm). Research (Marwan et al., 2021) showed that endophytic bacteria was able to trigger plant height, in rice plant research. (Pradana et al., 2020) reported that tomato plants inoculated with endophytic bacteria were able to significantly increase height of different tomato plants compared to controls. (Prihatiningsih et al., 2021) also reported that endophytic bacteria directly promote plant growth including plant height and indirectly control pathogens.

## Total of Leaves (strands)

Observation total of leaves cocoa plants (*Theobroma cacao* L) showed that immersion treatment had highest yield of 6<sup>th</sup> WAP S3 (7.92 strands). Observation on 6<sup>th</sup> WAP showed that best yield on interaction in immersion (S3) and microcapsule addition B3 (10 strands). Observation on 8<sup>th</sup> WAP showed that best data on interaction treatment S3 and B3 (13.33 strands). This result was better than research (Aulia Meyuliana et al., 2022) with highest average number leaves found with a dose 25ml (liquid organic fertilizer from a mixture of tomato extract, coconut water and rice washing water) of 4.88 and lowest number leaves found in control of 3.25. (Ariana, 2016) reported that tobacco plants treated with endophytic bacteria were able to increase number tobacco leaves more than control plants.

## Stem Diameter (mm)

Observation on stem diameter of cocoa plant growth (Theobroma cacao L), best results were found at 10<sup>th</sup> WAP in immersion treatment S3 (4.01 mm) and microcapsule addition B1 (3.99 mm). Interaction treatment showed that best results was immersion treatment S3 and microcapsule addition B2 (4.14 mm). Results of this research was better than (Aulia Meyuliana et al., 2022) with addition of 25 ml (liquid organic fertilizer derived from a mixture of tomato extract, coconut water and rice washing water) gave highest average (0.41 mm). This research was also better compared (Aulia Meyuliana et al., 2022) with highest amount found in treatment of liquid tofu waste (80 ml/polybag) with an average (3.61 mm) and lowest in treatment of liquid tofu waste (60 ml/polybag) with an average (3.25 mm). (Permadi, 2021) reported biological agents auxin producingendophytic bacteria that served to spur plant stem growth. (Herlina et al., 2016) reported endophytic bacteria provided essential nutrients such as nitrogen, phosphorus, and other minerals, as well as growth hormones such as auxin, ethylene, and cytokinin by producing growth hormones including auxin, cytokinin, and peptide N synthase. Endophytic bacteria had ability to bind nitrogen, but some researchers reported that increase in plant growth was more in ability of microbes to produce growth regulators in form of hormones such as gibberellin (Zain et al., 2018).

## Leaf Area (cm<sup>2</sup>)

Best results of observation on 10<sup>th</sup> WAP by addition of microcapsules was B1 treatment (66.64 cm<sup>2</sup>) and for seed immersion best result was showed by S3 treatment (65,77cm<sup>2</sup>). This result was better than research (Yusuf et al., 2018) that highest yield of leaf area was found in 80% of soil layer :15% cow dung : 5% sand with an average of 56.11cm<sup>2</sup>. (Putri et al., 2016) reported that auxin produced by endophytic bacteria was known to spur the growth of cocoa seedlings compared to controls. Auxin produced by bacteria would be utilized by plants and passed metabolic processes in plant organ so that it could help growth process of cocoa seedlings extensively.

#### Plant Wet Weight (gr)

Observations of plant wet weight on cocoa (Theobroma cacao L) showed best result was in treatment S3 (18.41 g) for seed immersion and for addition of microcapsules was B1 treatment (11.97 g). Interaction of immersion treatment (S2) and microcapsule additon (B3) showed as best result (99.56 g). This results was better than (Aulia Meyuliana et al., 2022) used treatment of 350g/ polybag on cocoa (goat manure fertilizer) with highest value 14.13 g. This results also better than research (Usrin, U., Pamungkas, D. H., & Widata, 2019) used dose of 10 L liquid organic fertilizer and highest manure soil ratio of 1:1 with highest data 14.59 g. Results of this research support results of previous research that rubber plants treated with endophytic bacteria had significantly different wet and dry weights compared to controls (Moshinsky, 2020). This research was accordance with (Marwan et al., 2021) used treatment of endophytic bacteria on rice seedlings was also able suppressed severity of bacterial leaf blight by 76.17 to 86.61%, and had effect on plant height, total of saplings, total of panicles, grain weight contained and was able reduced percentage of empty grains. Therefore, it was also believed that increase in growth of cocoa seedlings significantly different than control treatment in this research was caused by immersion treatment and microcapsules addition of endophytic bacteria.

#### Root Length (cm)

Observations of cocoa root length (*Theobroma cacao* L) showed best result was treatment S3 (14.40 cm) for seed immersion and for microcapsule addition was B3 treatment (15.78 cm). This research was better than (Wati et al., 2021) used dosage of soil: manure : cocopeat (1:2:1) with root length average 16.65 cm). Based on ability test of bacteria produced auxin (Table 2), endophytic bacteria obtained were able to produce auxin. Auxin produced by endophytic bacteria plays an important role in root growth in cocoa plants. (Jia et al., 2018) reported rate of root growth and expansion of root structure in soil was important for nutrient solubilization and absorption. Auxins played direct role in root growth and development. Auxin production by bacteria has been directly associated with development of host plant's root system and increased root growth and branching. Auxin hormone played a role in process of division, enlargement and expansion of plant cells, especially in root area. Increased root hair growth was certainly influential in increasing area of nutrient absorption for plants (Rosyida & Nugroho, 2017).

#### Effectiveness of Microcapsules in Cocoa Plants

Microencapsulation was process in which a liquid was wrapped or coated by polymeric material. to produce micro-size, called microcapsules (RiauWati & Chaerunisaa, 2020). Principle of microencapsulation was mixing between water phase, core substance phase and coating material phase until a stable emulsion is formed (Ang et al., 2019). Encapsulation of inoculated cells in polysaccharide polymers such as alginate as a technique to ensure controlled release of beneficial plant microorganisms into soil (Vassilev et al., 2020). Bacteria microencapsulation used alginate has been tested to increase survival by 80-95% (Suryani et al., 2019).

The addition of alginates increases size and appearance of resulting capsules. But interactions between bacterial cells and matrix components may also had an important role especially in release behavior and survival stability of biofertilizer cells (Meftah Kadmiri et al., 2021). Bacterial encapsulation was an alternative technology intended to protect microbial cells introduced into soil and to ensure their slow and prolonged release, formulations with alginates are more efficient in stability of cell survival which was essential to guarantee a higher number of cells in capsule when applied to plants (Meftah Kadmiri et al., 2021). When released from the capsule the bacteria would colonized the root zone and allowed for more efficient absorption of water and nutrients as it produces growth hormones which enhance root growth. Similarly, the cyanobacterium, when released, will also enable nutrient bioavailability and nitrogen fixation, as well as the production of various growth hormones and bio-stimulants (El Semary et al., 2020).

Microbes derived from cocoa plant (*Theobroma cacao* L.) had potential as a potential biofertilizer that can be used as biofertilizer in a composition (Nurmayulis et al., 2023). Overall, biofertilizers are conducive for cocoa development seedlings in nursery, which guarantees good productivity (Djenatou et al., 2020). Application of biological fertilizer *Azosprilum brasilense* DSM1690 (Ab) packaged in alginate with addition of both types of mineral clay significantly increased plant growth parameters compared to control (Meftah Kadmiri et al., 2021). Research (Gandhi et al., 2021) showed changes in concentration of evective biofertilizers provide highest value for: 10 pods, 42.8 pods, and a yield of 0.71 kg per tree or equivalent to 591.67 kg/ha of dry cocoa beans. Another research demonstrated that in vivo application to lettuce plants of beads containing plant growth-promoting bacterium *Bacillus subtilis* CC-pg104 achieved significant growth promotion by increasing shoot length and roots and ensuring effective root and *Rhizospheric* colonization. Thus, humic acid added to this formulation boosted viability of these cells during storage, ensured progressive cell release, and protected bacteria against unfavorable environmental factors (De Melo et al., 2016).

## CONCLUSION

- 1. Result of isolation from cocoa roots and stems obtained 5 isolates of endophite bacteria. Auxin test showed that 5 isolates were able to produce auxin.
- 2. Observation of plant height showed that best treatment was S1 treatment (31.84 cm). Observation of total leaves showed that best treatment was S3 treatment (7.92 strands). Observation of stem diameter showed that best treatment was S3 treatment (4.01 mm). Observation of leaf area showed that best treatment was treatment B1 (66.64 cm2). For plant wet weight parameter, the highest data was on S3 treatment (18.41 g) and for root length parameter, highest data was S3 treatment (14.40 cm).
- 3. Results of this research showed that application of suspension and endophite bacteria microcapsules significantly increased growth of cocoa.

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