Jurnal Pembelajaran Dan Biologi Nukleus p-ISSN: 2442-9481
Vol 9 (1): 165-180, March 2023 e-ISSN: 2685-7332

Effectiveness Microcapsules From Auxin Producing-Endophite Bacteria As Biofertilizer in Tea (*Camellia sinensis* L)

Sigit Indra Zit, Kabul Warsito(*), Refnizuida
Department of Agrotetechnology, University of Pembangunan Panca Budi, Medan, Jl. Gatot Subroto km, 4.5, Indonesia

***Corresponding author:** kabulwarsito@dosen.pancabudi.ac.id

Submit February 08th 2022 and Accepted February 28th 2023

Abstract

Tea (Camellia sinensis L*), is a popular agricultural product enjoyed by people around the world. Tea quality is determined by good growth process starting from the seedling stage until fertilization. Fertilization is the most important stage to determine quality of tea. Biofertilizers that use bacteria found in plant tissue called endophytes, have proven effective for increasing growth and plant productivity. Endophite bacteria can also produce growth hormones such as auxins, ethylene, and cytokinins. This research aims to determine the effectiveness of auxin-producing endophytic bacterial microcapsules on tea growth. Research design used was completely randomized design (CRD), 16 treatments with 2 replications. First factor was immersion of endophite bacterial suspension consisting of S0: 0 hours; S1: 24 hours; S2: 36 hours and S3: 48 hours and second factor was addition of microcapsules consisting of I0: 0 gr; I1: 5 gr; I2: 10 gr; I3: 15 gr. Isolation from tea roots and stems obtained 6 isolates of endophite bacteria. Auxin test showed that the six isolates were able to produce auxin. Observation of plant height showed best treatment was treatment I3 (17.04 cm). Observation of leaves total was treatment I3 (6.88 strands). For the leaf area parameter, highest number was S1 treatment (22.8 cm^2). For stem diameter parameter, highest data was in treatment I2 (1.69 mm). Test results showed that the application of suspension and endophite bacteria microcapsules significantly increased growth of tea.*

Keywords: *Auxin, Endophite Bacteria, Microcapsule, Tea*

https://doi.org/10.36987/jpbn.v9i1.3904

INTRODUCTION

Tea (*Camellia sinensis* L) is an agricultural product that is in great demand by wider community. Cultivating tea tree seedlings is a step that needs to be done before returning to green planting (Hindersah et al., 2018). Results of tea plantations are one of mainstays of Indonesia's export commodities. Currently Indonesia is one of largest tea exporting countries in world. Indonesian black tea or green tea has been recognized and accepted by world community because of its distinctive aroma and taste. Apart from being used as a refreshing drink, tea also has health benefits due to secondary metabolism it contains (Syahbanuari et al., 2020).

Fertilization is an important factor to obtain plants that can grow and develop properly. Many farmers still used inorganic (chemical) fertilizers. Long-term use inorganic fertilizers have a negative impact on environment such as decreased production and organic matter, soil microbial populations and increased soil acidity (Sulaeman et al., 2017). Several research have used endophite bacteria which have been proven effective as a source of nitrogen nutrition. Endophite bacteria also produced growth hormones such as auxins, ethylene, and cytokinins, which help plants meet their hormone needs (Herlina et al., 2016).

(Panichikkal et al., 2021) reported that survival of encapsulated probiotic bacteria was greater than that of free bacteria (not encapsulated) in about 1 log cycle, during storage and number of probiotic bacteria. Capsules were stored at 4°C and their viability was recorded for 63 days. According to (Mardikasari & Puspitasari, 2020) these microcapsule granules can be formed due to a cross-linking reaction between the sodium alginate polymer and $CaCl_2$ solution which acts as a cross-linking agent. Cross-linking occurs when sodium alginate droplets are dropped into the $CaCl_2$ medium. When sodium alginate is dropped into a solution containing calcium ions, calcium ions will replace sodium ions in the polymer to form a three-dimensional gel network and is described as an "egg-box" model.

Auxin hormone is an endogenous auxin which plays a role in cell enlargement and formation of xylem and phloem tissue, inhibits the growth of side shoots, stimulates abscission, and affects development and elongation of roots. In preparing the biofertilizer formula, researchers tried to find potential auxin hormone-producing microbes by exploring various places. auxin a natural auxin group of phytohormones and plays a role in stimulating plant growth by increasing processes of cell elongation, cell division and differentiation in plants (Herlina et al., 2016).

Xie et al., (2020) showed that endophytic bacteria isolated from tea plants contained several types of bacterial species, including *Bacillus amyloliquefaciens*, *Bacillus sapensis*, *Bacillus subtilis* which were capable of producing hormone auxin. Some endophytic bacteria promote plant growth by providing auxins, gibberellins, cytokinins, siderophores, enzyme phosphate solvents. (Yan et al., 2018) tested plant growth promoter activity of endophite bacteria obtained from two tea cultivars Zijuan and Yunkang-10, and found that *Herbaspirillum* spp., *Methylobacterium* spp., and *Brevundimonas* spp. Demonstrate PGP capability. Endophite bacteria Strain *Burkholderia cepacia* G3 isolated from tea plants have significant PGP ability on seed germination and growth of wheat.

Plant-bacterial associations can affect plant productivity directly and indirectly. Directly, bacteria could act as growth promoters or stimulants by synthesizing growth regulators. Endophite bacteria played a role in increasing plant growth, providing nutrition, producing growth hormones and inducing plant resistance (Putri et al., 2016).

METHOD

Tools and Materials

Tools in this research were petri dishes, test tubes, test tube racks, measuring cups, beaker glass, Erlenmeyer, autoclave, oven, spatula, needle loops, incubator, hot plate, stir bar, analytical balance, sprayer, laminar air flow, shaker, glass bottle, aluminum foil, cotton, knife and polybag. Materials in this research were tea roots and stems, media Nutrient Agar (NA), distilled water, alcohol 70%, chlorine solution, $CaCl_2$, sodium alginate, inulin, poultry manure, top soil, rice husk charcoal, NaCl 0,9%, crystal violet, safranin, acetone, alcohol, iodine, L-tryptopan, peptone and salkowsky.

Research Methods

This research was conducted using factorial CRD (Completely Randomized Design) method consisting of 16 treatments with 2 replications. First factor was immersion of endophite bacterial suspension consisting of S0: 0 hours; S1: 24 hours; S2: 36 hours and S3: 48 hours. Second factor was the addition of microcapsules consisting of I0: 0 gr; I1: 5 gr; I2: 10 gr; I3: 15 gr. Application of microcapsule was carried out on 4^{th} week after planting. Data were analyzed using ANOVA. Results of the analysis of variance were continued with Duncan's multiple range test.

Isolation of Endophite Bacteria

Endophite bacteria was isolated from tea roots and stems. As for types of types. Isolation of endophite bacteria using the method (Singh et al., 2022) modified. Before isolation process, surface of roots and stems of tea was sterilized.

Measurement of Auxin from Endophite Bacteria

Potential test of endophite bacteria in auxin producing used streak plate method. Isolates were inoculated on flat Nutrient Agar media supplemented with tryptophan at a concentration of 100 ppm and incubated at room temperature for 48 hours. Salkowski reagent was dripped onto endophite bacterial colonies. Colonies that has been dripped with Salkowski reagent was stored in a dark room for 30 minutes. Positive result was indicated by a change in the color of colonies became red (Herlina et al., 2017).

Preparation and Sterilization of Planting Media

Planting media used in this research were top soil that had been cleaned of weeds, broiler manure and rice husk charcoal. With a ratio (1: ½ : ½), top soil: 50%, broiler manure: 25% and rice husk charcoal: 25%. Sterilization was carried out at 150^0C 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. Tea seeds were soaked at ratio of 24 hours; 36 hours and 48 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 $CaCl_2$ was weighed and then dissolved with 1000 ml of distilled water in a volumetric flask, stirred until homogeneous. Solution was sterilized by autoclaving at 121°C for 15 minutes. Sterile alginate solution containing a suspension of endophite bacteria was put into a spit needle and then dropped into a 0.1M $CaCl_2$

solution. Formed microcapsule was allowed for 1 hour. To remove $CaCl_2$ residue, microcapsule was filtered and rinsed using distilled water (Panichikkal et al., 2021).

Observation Parameters

Parameters observed in this research are auxin measurment of endophite bacteria, plant height (cm), total of leaves (strands), leaf area (cm^2) and stem diameter (mm).

RESULTS AND DISCUSSION

Isolation of Endophite Bacteria from Roots and Stems of Tea Plants

Isolation of auxin-producing endophite bacteria from the roots and stems of tea plant (*Camellia sinensis* L) was obtained as 6 isolates. Consists of 3 isolates from plant roots and 3 isolates from plant stems. These 6 isolates showed varying characteristics, both morphology and coloring properties. Results of characteristics of endophite bacteria showed in table 1.

Table 1. Colony and cell morphology and gram staining properties of endophytic bacterial isolates of tea plant roots and stems (*Camellia sinensis* L).

Isolate	Characterization						Grams
	Colony Morphology				Cell Morphology		
	Color	Form	edge	elevation	Form	Setup	
SI1 AT	White	Irregular	Irregular	Raised	Basil	Diplo	+
SI2 AT	White	Rhizoids	Rhizoids	Flat	Basil	Diplo	+
SI3 AT	White	Irregular	Lobate	Raised	Basil	Diplo	+
SI1 BT	White	Rhizoids	Rhizoids	Flat	Basil	Diplo	+
SI2 BT	White	Irregular	Lobate	Flat	Cocus	Mono	+
SI3 BT	White	Irregular	Irregular	Raised	Cocus	Mono	+

The Ability of Root and Stem Endophite Bacteria In Tea Plants to Produce Auxin Hormones

Bacteria originating from roots and stems of tea plant can produce auxin hormone which was indicated by formation of transparent pink and results of auxin rate in six isolates produced positive auxin rate. Results of auxin hormone rate from root and stem endophite bacteria showed in table 2.

Table 2. Auxin hormone rate produced by endophite bacteria from the roots and stems of tea plant (*Camellia sinensis* L).

Isolate	Auxin Rate
SI1 AT	+
SI2 AT	+
SI3 AT	+
SI1 BT	+
SI2 BT	+
SI3 BT	+

Plant Height (cm)

Plant height was observed on the 50 days, 60 days, 70 days, 80 days, 90 days after planting (DAP). Based on results of observations and analysis of variance, it was known that immersion treatment affected height growth of tea (*Camellia sinensis* L) had a significantly different effect on plant height (cm) at 50 DAP, 60 DAP, 70 DAP, 90 DAP and had a highly significant different effect at 80 DAP. However, Effect was not significantly different in treatment of microcapsules addition at 50 DAP, 60 DAP, 70 DAP, 80 DAP and 90 DAP.

Interaction of effect of immersion variations and microcapsules addition had no significant effect on plant height measurement data (cm) on growth of tea (*Camellia sinensis* L). Duncan's distance test results were showed in Table 3.

Table 3. Average height tea (*Camellia sinensis* L) to immersion treatment and addition of microcapsules.

Treatment	Average Plant Height (cm)				
	50 DAP	**60 DAP**	**70 DAP**	**80 DAP**	**90 DAP**
Immersion Treatment (S)					
S0 = 0 hours	4.39 bB	8.05 bB	9.28 bB	11.59 bB	13.06 bB
S1 = 24 hours	5.44 aAB	10.63 Aa	12.46 aA	15.49 aA	17.03 aA
S2 = 36 hours	6.19 aA	9.10 aBA	11.29 aBA	14.74 aAB	16.31 aAB
S3 = 48 hours	6.00 aA	8.89 aBA	10.9 aBA	15.35 aA	17.00 aAB
Microcapsules Addition (I)					
I0 = 0 gr	5.31 aA	7.90 bA	9.79 bA	13.33 aA	15.08 aA
I1 = 5 gr	5.46 aA	9.15 abA	10.84 abA	13.88 aA	15.36 aA
I2 = 10 gr	5.58 aA	10.19 aA	12.21 aA	14.77 aA	15.95 aA
I3 = 15 gr	5.66 aA	9.43 abA	11.09 abA	15.20 aA	17.04 aA

Total of Leaves (strands)

Total of leaves was observed on 50, 60, 70, 80, 90 day after planting (DAP). Based on results of observations and analysis of variance, it was known that immersion treatment with suspension of endophite bacteria on total of leaves of tea plant (*Camellia sinensis* L) had no significant effect on total of leaves (strands), then at 50 DAP, 60 DAP and 70 DAP observations gave very significant different effect at 80 HST and 90 HST. However it had no significant effect on treatment of endophite bacterial microcapsules at 50 DAP, 60 DAP, 70 DAP, 80 DAP and 90 DAP.

Interaction effect of variations of immersion endophite bacteria suspension and administration of endophite bacterial microcapsules had a significant effect at 80 DAP and 90 DAP. Duncan's distance test results were showed in Table 4.

 p-ISSN: 2442-9481
e-ISSN: 2685-7332

Table 4. Average total of leaves tea (*Camellia sinensis* L) to immersion treatment and addition of microcapsules.

Treatment	Average Total of Leaves (strands)				
	50 DAP	60 DAP	70 DAP	80 DAP	90 DAP
Immersion Treatment (S)					
S0 = 0 hours	2.50 aA	4.00 aBA	4.00 aBA	6.00 aA	6,38a A
S1 = 24 hours	2.13 aA	4.25 aA	4.25 aA	6.00 aA	6.75 aA
S2 = 36 hours	2.38 aA	3.50 abA	3.63 abA	4.88 bB	5.50 bB
S3 = 48 hours	2.25 aA	3.25 aA	3.50 bA	6.00 aA	6.63 aA
Microcapsules Addition (I)					
I0 = 0 gr	2.75 aA	4.00 aA	4.00 aA	5.75 aA	6.25 aA
I1 = 5 gr	2.25 aA	3.75 aA	4.00 aA	5.75 aA	6.00 aA
I2 = 10 gr	2.13 aA	3.63 aA	3.63 aA	5.75 aA	6.13 aA
I3 = 15 gr	2.13 aA	3.63 aA	3.75 aA	5.63 aA	6.88 aA

Table 5. Average immersion treatment and addition of microcapsules to total of leaves tea (*Camellia sinensis* L) at 80 DAP and 90 DAP.

Treatment	Average Total of Leaves (strands)	
	80 DAP	90 DAP
S0I0	6bcAB	6.5bcABC
S0I1	6bcAB	6cdBCD
S0I2	5.5cdBC	5.5deCD
S0I3	6.5abA	7.5aA
S1I0	5.5cdBC	6.5bcABC
S1I1	5.5cdBC	6.5bcABC
S1I2	7aA	7.5aA
S1I3	6bcAB	6.5bcABC
S2I0	4.5efCD	5eD
S2I1	6bcAB	6bcdBCD
S2I2	4fD	5eD
S2I3	5deBCD	6bcdBCD
S3I0	7aA	7aAB
S3I1	5.5cdBC	5.5deCD
S3I2	6.5abA	6.5bcABC
S3I3	5deBCD	7.5aA

Based on the observations from Table 5, showed that interaction of mean total of leaves from immersion treatment and addition of endophite bacteria microcapsules had a significantly different effect at 80 DAP and 90 DAP after being tested using Duncan's Range Test.

Leaf Area (cm^2)

Leaf area was observed at 90 days after planting (DAP). Based on the results of observations and analysis of variance, it was known that the immersion treatment of leaf area of tea (*Camellia sinensis* L) gave a significantly different effect on leaf area at 90 DAP and had a significantly different effect on treatment of microcapsules addition at 90 DAP. Duncan's distance test results were showed in Table 6.

Table 6. Average leaf area of plants tea (*Camellia sinensis* L) to immersion treatment and addition of microcapsules.

Treatment	Average Leaf Area (cm^2)
Immersion Treatment (S)	
S0 = 0 hours	11.74^{cC}
S1 = 24 hours	22.80^{aA}
S2 = 36 hours	19.80^{bAB}
S3 = 48 hours	21.19^{abAB}
Microcapsules Addition (I)	
I0 = 0 gr	16.75^{bB}
I1 = 5 gr	19.35^{abAB}
I2 = 10 gr	18.69^{abAB}
I3 = 15 gr	20.74^{aA}

Table 7. Average immersion treatment and microcapsules addition to leaf area of tea (*Camellia sinensis* L) at 90 DAP.

Treatment	Average Leaf Area (cm^2)
S0I0	10.5^{hG}
S0I1	15^{gF}
S0I2	10.74^{G}
S0I3	10.7^{hG}
S1I0	18^{efDEF}
S1I1	21.8^{bcBC}
S1I2	28.7^{aA}
S1I3	22.6^{bB}
S2I0	19.5^{cdeBCDE}
S2I1	20.5^{bcdBCD}
S2I2	18.8^{deCDE}
S2I3	20.4^{bcdeBCD}
S3I0	19^{deCDE}
S3I1	20^{cdeBCD}
S3I2	16.5^{fgEF}
S3I3	29.3^{aA}

Based on the observations from Table 6, showed that interaction of leaf area from immersion treatment and addition of endophite bacterial microcapsules had a highly significant different effect at 90 DAP after being tested using Duncan's Range Test.

Stem Diameter (mm)

Based on results of observations and analysis of variance, it was known that effect of immersion and addition microcapsules on the growth of tea (*Camellia sinensis* L) had no significant effect on stem diameter. Interaction between effect of Immersion and addition microcapsules had no significant effect on stem diameter measurement data (mm) on tea growth (*Camellia sinensis* L) after being tested using Duncan Distance Test were showed in Table 8.

Table 8. Average diameter of stems tea (*Camellia sinensis* L) to immersion treatment and addition of microcapsules.

Treatment	Average Stem Diameter of Plants (mm)				
	50 DAP	60 DAP	70 DAP	80 DAP	90 DAP
Immersion Treatment (S)					
S0 = 0 hours	0.77 ^{aA}	1.15 ^{aA}	1.33 ^{aA}	1.51 ^{aA}	1.62^{aA}
S1 = 24 hours	0.83 ^{aA}	1.23 ^{aA}	1.36 ^{aA}	1.55 ^{aA}	1.68^{aA}
S2 = 36 hours	0.79 ^{aA}	1.10 ^{aA}	1.26 ^{aA}	1.55 ^{aA}	1.68^{aA}
S3 = 48 hours	0.67 ^{aA}	0.98 ^{aA}	1.24 ^{aA}	1.51 ^{aA}	1.59^{aA}
Microcapsules Addition (I)					
I0 = 0 gr	0.72 ^{aA}	1.06 ^{aA}	1.31 ^{aA}	1.55 ^{aA}	1.67^{aA}
I1 = 5 gr	0.75 ^{aA}	1.03 ^{aA}	1.20 ^{aA}	1.47 ^{aA}	1.58^{aA}
I2 = 10 gr	0.77 ^{aA}	1.17 ^{aA}	1.33 ^{aA}	1.58 ^{aA}	1.69^{aA}
I3 = 15 gr	0.82 ^{aA}	1.19 ^{aA}	1.35 ^{aA}	1.53 ^{aA}	1.62^{aA}

Discussion

Isolation of Endophite Bacteria from Roots and Stems of Tea (*Camellia sinensis* L)

Pure endophite bacterial isolates were identified morphologically based on colony color, colony margin shape, colony elevation and colony growth. Endophite bacterial isolates produced auxin hormone had different characteristics. These characteristics include the shape of bacterial colonies were dominated by irregular shapes and there were also rhizoid shapes in SI2 AT and SI1 BT samples with irregular colony edges in SI1 AT and SI3 BT samples, then lobate edges in SI3 AT and SI2 BT samples, and rhizoid edges. In SI2 AT and SI1 BT samples with white color, with a raised elevation in the SI1 AT, SI3 AT, and SI3 BT samples, then with a flat elevation in SI2 AT, SI1 BT and SI2 BT samples. Other researches using solid media, the growth of microorganisms was characterized by different colony shapes such as circular, irregular (Maulani et al., 2019). Results of other research include four phyla, seven classes, 13 orders, 24 families and 32 genera. Strains of *Micrococcales* of *Actinobacteria*, *Bacillales* of *Bacilli*, *Rhizobiales* of *Alphaproteobacteria*, and *Burkholderiales* of *Betaproteobacteria* were dominant species. Several endophite fungi and bacteria had not been identified and classified (Xie et al., 2020). Results of other research, endophite bacteria isolated from tea plants had several species such as *Bacillus paramycoides, Bacillus endophyticus, Bacillus pseudomycoides , Bacillus cereus Bacillus altitudinis , Bacillus flexus, Lysinibacillus xylanilyticus, Paenibacillus aceris, Brevibacterium sediminis, Bacillus subtilis, Bacillus pseudomycoides, Bacillus altitudinis, Bacillus weidmannii* (Borah et al., 2019).

Ability of Root and Stem Endophite Bacteria in Tea Plants to Produce Auxin Hormones

Results of auxin measurements of endophite bacteria showed 6 isolates of endophite bacteria. Endophytes from roots and stems, were capable of producing hormone auxin. Other research showed that production of auxin was recorded in 93.5% and 21.7% of all isolates, respectively. Overall, these results indicate that actinomycetes endophite from tea plant are a good source of bioactive metabolites with antibacterial, anti fungal, and plant growth promoting properties (Shan et al., 2018). Results of research (Borah et al., 2019) used bacteria *Bacillus subtilis* D7XPN1 to produce 6.5 ppm of auxin, *Bacillus paramycoides* NH 24A2 produced 6.6 ppm of auxin, *Bacillus altitudinis* 41KF2B to produce 11.0 ppm of auxin with mass of incubation for 25 minutes using 1 mg/ml L-tryptophan. Auxin was synthesized as secondary metabolite produced under suboptimal bacterial growth conditions or when tryptophan was available as amino acid precursor (Singh et al., 2022).

Plant Height (cm)

Results of observing height of tea plant showed that growth in height of tea plant (*Camellia sinensis* L) in immersion treatment had highest at 90 DAP in S1 treatment (17.03 cm) and for lowest plant in treatment S0 (13.06 cm) and for treatment of giving capsules highest was at 90 DAP in treatment I3 (17.04 cm) and for lowest in treatment I0 (15.08 cm). This result was better than other research using organic fertilizer with green tea liquid waste with a tea plant height of 11.69 cm and for tea plant height without treatment with a height of 10.50 cm (Muningsih & Ciptadi, 2019). In another research using tea fluff compost and *Azotobacter* sp (8.4 cm) in F2 treatment (60% topsoil + 40% tea fluff compost) and in addition of 3 ml *Azotobacter* sp (7.6 cm) at 16 WAP (Dewi & Wulansari, 2023). Effect of endophite bacteria on length of rice plant canopy showed that almost all isolates tested were able to stimulate the growth of rice plant canopy compared to control (Munif et al., 2016). In research (Khaeruni et al., 2020) treatment of endophite bacterial isolates significantly affected height of cocoa plants and was significantly different compared to controls. *Arabidopsis* plants treated with *Bacillus aryabhattai* showed increased growth compared to control plants. Analysis showed that bacterial inoculation significantly increased size of *Arabidopsis* plants (4.55 cm) compared control plants (3.10 cm). Plant growth of *Nicotiana tabacum*. Height of *Nicotiana tabacum* inoculated by plants was significantly greater (4.05 cm) than that of non-inoculated plants (2.25 cm) (Xu et al., 2022). Auxin hormone played a role in cell enlargement, inhibits growth of side shoots, stimulates abscission, plays a role in formation of xylem and phloem tissue (Herlina et al., 2017).

Total of Leaves (strands)

Obeservation on total of leaves showed that growth in total of tea plant leaves (*Camellia sinensis* L) in immersion treatment had highest data at 90 DAP in S1 treatment (6.75 strands) and for lowest total of tea plant leaves in treatment S0 (6.38 strands). Treatment of microcapsules addition, highest data was at 90 DAP in treatment I3 (6.88 strands) and for lowest in treatment I1 (6.00 strands). This results was better than those of other research using organic fertilizers with green tea liquid waste with 3.92 strands and 3.23 strands without treatment (Muningsih & Ciptadi, 2019). Another research used tea fluff and *Azotobacter* sp. compost with result that total of leaves was 3.10 strands in F1 treatment (70% topsoil + 30% tea fluff compost) and in of 3 ml of *Azotobacter* sp. total of leaves was 2.47 strands at 16 WAP (Dewi & Wulansari, 2023). In a previous research (Afiati & Purnamasari, 2019), total of purple egg plant leaves at age of 28 DAP showed that endophite bacteria with dose of 40 ml were higher than endophite with a smaller dose. Increase in total of leaves was strongly influenced by nutrient nitrogen which was responsible for preparation of chlorophyll and cell turgidity as well as increase in total of leaves.

Based on observations from Table 5, showed that interaction of average total of leaves at 80 DAP with immersion treatment and endophite bacteria microcapsules addition had highest data on treatment, S1I2, and S3I0 (7.00 strands) and lowest data on S2I2 (4.00 strands). Interaction of average total of leaves at 90 DAP with immersion treatment and endophite bacterial microcapsules addition had highest data in S3I3, S1I2 and S0I3 treatments (7.5 strands) and lowest data in S2I2 and S2I0 treatments (5,0 strands).

Leaf Area (cm^2)

Observation leaf area of tea plant at 90 DAP showed that leaf area of tea (*Camellia sinensis* L) in immersion treatment had highest at 90 DAP in S1 treatment (22.8 cm^2) and for lowest leaf area in treatment S0 (11.74 cm^2). Results treatment of microcapsules addition, highest tea leaf area at 90 DAP was in treatment I3 (20.74 cm^2) and for lowest in treatment I0 (16 .75 cm^2). Research (Afiati & Purnamasari, 2019) on purple egg plant plants with 40 ml of endophite suspension addition had a higher leaf area at all ages of observation. It could be due to provision of endophite bacteria will ensure fulfillment of nutrient needs, such as elements of N, especially in leaves. Nitrogen was primary macro element which is main component of various compounds in plant tissue. Growing plants must contain nitrogen in forming new cells. Research (Tangapo, 2020) reported that endophite bacteria could enhance plant growth by providing nutrients for plants such as nitrogen, phosphate and other minerals as well as producing growth hormones such as ethylene, auxin and cytokinins.

Based on observations from Table 7, showed that interaction of average leaf area with immersion treatment with endophite bacteria and treatment with endophite bacterial microcapsules had highest data in S3I3 treatment (29.3 cm^2) and lowest data in S0I0 treatment (10,5 cm^2). Another research described a three-year experiment with bacterial formulation treatment and fertilizer application significantly affecting parameters studied compared to controls. Among treatments tested, inoculation with a mixture of BF4

(*Bacillus atrophaeus* RC36, *Paenibacillus polymyxa* 28/3, *Pseudomonas fluorescens* 51/2) and BF6 (*Bacillus subtilis* 39/3, *Bacillus subtilis* RC63, *Pseudomonas fluorescens* 53/6) bioformulation increased leaf area of tea plant, chlorophyll and anthocyanin (ACI) content of tea plant was significantly different compared to control (Cakmakci et al., 2018).

Stem Diameter (mm)

Observations on diameter of tea plant stems showed that diameter growth of tea (*Camellia sinensis* L) in immersion treatment obtained significantly different results and S2 treatment (1.68 mm). Lowest data was in treatment S3 (1.59 mm) and microcapsules addition had no significant effect on stem diameter measurement on tea growth (*Camellia sinensis* L). Highest data in treatment I2 (1.69 mm), then lowest data was in treatment I1 (1.58 mm). This result was better than other research using urea fertilizer with a tea plant stem diameter of 0.49 mm (Pamungkas dan Supijatno., 2017). This finding was in line with previous research (Sudiarti, 2017) which found that use of microbes included in biological fertilizers did not have a significant effect on plant growth. Therefore, a longer observation period (more than two months) was needed to detect a statistically significant effect of endophite microbial application on oil palm seedlings.

Effectiveness of Microcapsules on Tea (*Camellia sinensis* L) Growth

Bacterial microencapsulation uses a matrix in the form of alginate. Alginate has been tested to increase life recovery by 80-95% (Suryani et al., 2019). Alginate also acts as a protective layer for microbial cells from abiotic stresses. Microbial survival can be increased by immobilization in alginate polymers compared to conventional liquid bacterial cells which do not provide adequate protection for microorganisms (Stella et al., 2019). Microencapsulation makes it possible to control the number of live and active cells trapped and the number of cells released to reduce the death rate during storage (Balla et al., 2022). Especially during long-term storage or even when applied to plants; maintaining the effectiveness of traits related to long-term plant growth (Keswani et al., 2016).

Encapsulation to overcome the problem of physical instability or chemical compounds. This can inhibit reduction and protect the encapsulated material against unfavorable environmental conditions (Zabot et al., 2022). Encapsulation materials consist of various synthetic or natural polymers (Luiz De Oliveira et al., 2018). Hydrolyzed starch as an agent in encapsulating pesticides (metabolites produced by Bacillus thuringiensis) because it protects environmental factors and improves the formulated product. In addition, encapsulation can be efficient for the formulation of biofungicides, biopesticides, and/or biological fertilizers in agriculture (Do Nascimento Junior et al., 2021). Cell encapsulation in polysaccharide polymers such as alginate as a technique for ensuring the controlled incorporation of beneficial plant microorganisms into the soil. Results of research on soil gel and the effects of cell-free carriers on plants and rhizosphere microbiota (Vassilev et al., 2020). to improve plant growth and health are oligosaccharides derived from natural polysaccharides because they act as signaling molecules that regulate plant development and defense (Liao et al., 2019).

Encapsulation causes the gradual capture of bacteria and secures from the alginate matrix the bentonite gel beads after inoculation into the soil and maximizes the formation of stable PGPB populations and the possibility of population closure over time can be minimized. Important advantages of alginate inoculants are their degradation in the soil, their non-toxicity and slow retention of bacteria trapped in the soil (Saberi-Rise & Moradi-Pour, 2020). First step, the microorganisms are mixed and adsorbed in the polymer matrix. Then second step, which is a mechanical operation, the liquid solution is dispersed by agitation, where solid particles are formed. In the third stage, particles formed in the previous stage undergo polymerization and physicochemical stabilization (Chanratana et al., 2018).

The application of biological fertilizer *Azosprilum brasilense* DSM1690 (Ab) packaged in alginate with the addition of both types of mineral clay significantly increased plant growth parameters compared to the control. Plant height was 25% higher, and root and shoot biomass increased by more than 100% compared to control plants. These findings confirm the ability of the biological fertilizer *Azosprillum brasilense* DSM1690 (Kadmiri et al., 2021). The same finding was reported by Wu et al., which revealed that a synergistic effect was formed by *Pseudomonas putida* Rs-198 microcapsules and sodium alginate Bentonite, resulting in an increase in fresh and dry weight of cotton plants. Encapsulation matrix exhibits different levels of degradation which directly affect the activity of soil microorganisms. They could also have suitable permeability to control bacterial growth metabolism and thus exhibit beneficial effects on plant growth (Wu et al., 2019).

CONCLUSION

From this research showed that,

a. The isolation from tea roots and stems obtained 6 isolates of endophite bacteria. Auxin test showed that six isolates were able to produce auxin.
b. Observation of plant height showed that best treatment was in treatment I3 (17.04 cm). Observation of leaves total was in treatment I3 (6.88 strands). For leaf area parameter, highest number was in S1 treatment (22.8 cm^2). For stem diameter parameter, highest data was in treatment I2 (1.69 mm).
c. Results of this research showed that application of suspension and endophite bacteria microcapsules significantly increased growth of tea.

REFERENCES

Afiati, I., & Purnamasari, R. T. (2019). Pengaruh pemberian bakteri endofit terhadap pertumbuhan dan hasil tanaman terung ungu (*Solanum melongena* L). *J. Agroteknologi Merdeka Pasuruan*, *3*(1), 32–37.

Balla, A., Silini, A., Cherif-Silini, H., Chenari Bouket, A., Alenezi, F. N., & Belbahri, L. (2022). Recent Advances in Encapsulation Techniques of Plant Growth-Promoting Microorganisms and Their Prospects in the Sustainable Agriculture. *Applied Sciences*

(Switzerland), *12*(18). https://doi.org/10.3390/app12189020

Borah, A., Das, R., Mazumdar, R., & Thakur, D. (2019). Culturable endophytic bacteria of *Camellia* species endowed with plant growth promoting characteristics. *Journal of Applied Microbiology*, *127*(3), 825–844. https://doi.org/10.1111/jam.14356

Cakmakci, R., Atasever, A., Erat, M., Erturk, Y., Haliloglu, K., Haznedar, A., Kotan, R., Sekban, R., Turkyilmaz, K., & Varmazyari, A. (2018). The Effect of Bacteria-Based Formulations On Tea (*Camellia sinensis* L.) growth, yield, and enzyme activities. *Annals of Warsaw University of Life Sciences - SGGW - Horticulture and Landscape Architecture*, *38*, 5–18. https://doi.org/10.22630/ahla.2017.38.1

Chanratana, M., Han, G. H., Melvin Joe, M., Roy Choudhury, A., Sundaram, S., Halim, M. A., & Sa, T. (2018). Evaluation of Chitosan And Alginate Immobilized *Methylobacterium oryzae* CBMB20 On Tomato Plant Growth. *Archives of Agronomy and Soil Science*, *64*(11), 1489–1502. https://doi.org/10.1080/03650340.2018.1440390

Dewi, C., & Wulansari, R. (2023). Pengaruh Aplikasi Kompos *Tea Fluff* dan *Azotobacter* Sp. Terhadap Sifat Fisik Tanah dan Pertumbuhan Bibit Pada Persemaian Teh. *Jurnal Tanah Dan Sumberdaya Lahan*, *10*(1), 135–142. https://doi.org/10.21776/ub.jtsl.2023.010.1.15

Do Nascimento Junior, D. R., Tabernero, A., Cabral Albuquerque, E. C. de M., & Vieira de Melo, S. A. B. (2021). Biopesticide encapsulation using supercritical CO^2: A comprehensive review and potential applications. *Molecules*, *26*(13). https://doi.org/10.3390/molecules26134003

Herlina, L., Pukan, K. K., & Mustikaningtyas, D. (2016). Kajian Bakteri Endofit Penghasil IAA (*Indole Acetic Acid*) untuk Pertumbuhan Tanaman. *J. FMIPA, Universitas Negeri Semarang*, *14*(1), 51–58.

Herlina, L., Pukan, K. K., & Mustikaningtyas, (2017). The Endophytic Bacteria Producing IAA (*Indole Acetic Acid*) In *Arachis hypogaea*. *Cell Biology and Development*, *1*(1), 31–35. https://doi.org/10.13057/cellbioldev/v010106

Hindersah, R., Adityo, B., & Suryatmana, P. (2018). Populasi Bakteri Dan Jamur Serta Pertumbuhan Tanaman Teh (*Camellia sinensis* L.) Pada Dua Jenis Media Tanam Setelah Inokulasi *Azotobacter*. *Agrologia*, *5*(1), 1–9. https://doi.org/10.30598/a.v5i1.191

Keswani, C., Sarma, B. K., & Singh, H. B. (2016). Agriculturally Important Microorganisms: Commercialization And Regulatory Requirements In Asia. In *Agriculturally Important Microorganisms: Commercialization and Regulatory Requirements in Asia*. https://doi.org/10.1007/978-981-10-2576-1

Khaeruni, A., Nirmala, T., Siti Anima Hisein, W., Gusnawaty, G., Wijayanto, T., & Kade Sutariati, G. A. (2020). Potensi dan Karakterisasi Fisiologis Bakteri Endofit Asal Tanaman Kakao Sehat sebagai Pemacu Pertumbuhan Benih Kakao. *Jurnal Ilmu Pertanian Indonesia*, *25*(3), 388–395. https://doi.org/10.18343/jipi.25.3.388

Liao, N., Luo, B., Gao, J., Li, X., Zhao, Z., Zhang, Y., Ni, Y., & Tian, F. (2019).

Oligosaccharides as co-encapsulating agents: effect on oral *Lactobacillus fermentum* survival in a simulated gastrointestinal tract. *Biotechnology Letters*, *41*(2), 263–272. https://doi.org/10.1007/s10529-018-02634-6

Luiz De Oliveira, J., Ramos Campos, E. V., & Fraceto, L. F. (2018). Recent Developments and Challenges for Nanoscale Formulation of Botanical Pesticides for Use in Sustainable Agriculture. *Journal of Agricultural and Food Chemistry*, *66*(34), 8898–8913. https://doi.org/10.1021/acs.jafc.8b03183

Maulani, B. I. G., Rasmi, D. A. C., & Zulkifli, L. (2019). Isolation And Characterization Of Endophytic Bacteria From Mangrove *Rhizophora Mucronata* L. And Antibacterial Activity Test Against Some Pathogenic Bacteria. *Journal of Physics: Conference Series*, *1402*(3). https://doi.org/10.1088/1742-6596/1402/3/033038

Meftah Kadmiri, I., El Mernissi, N., Azaroual, S. E., Mekhzoum, M. E. M., Qaiss, A. E. K., & Bouhfid, R. (2021). Bioformulation of Microbial Fertilizer Based on Clay and Alginate Encapsulation. *Current Microbiology*, *78*(1), 86–94. https://doi.org/10.1007/s00284-020-02262-2

Miftah Anugrah Pamungkas dan Supijatno. (2017). Pengaruh Pemupukan Nitrogen Terhadap Tinggi dan PercabanganTanaman Teh (*Camelia Sinensis* L.) O. Kuntze) untuk Pembentukan Bidang Petik. *BMC Public Health*, *5*(1), 1–8. https://ejournal.poltektegal.ac.id/index.php/siklus/article/view/298%0Ahttp://repositorio.unan.edu.ni/2986/1/5624.pdf%0Ahttp://dx.doi.org/10.1016/j.jana.2015.10.005%0Ahttp://www.biomedcentral.com/1471-2458/12/58%0Ahttp://Ovidsp.Ovid.Com/Ovidweb.Cgi?T=JS&P

Munif, A., Wiyono, S., & Suwarno, S. (2016). Isolasi Bakteri Endofit Asal Padi Gogo dan Potensinya sebagai Agens Biokontrol dan Pemacu Pertumbuhan. *Jurnal Fitopatologi Indonesia*, *8*(3), 57–64. https://doi.org/10.14692/jfi.8.3.57

Muningsih, R., & Ciptadi, G. (2019). Analisis Kandungan Unsur Hara Limbah Cair Teh Hijau Sebagai Bahan Pupuk Organik Pada Bibit Teh. *Mediagro*, *14*(01), 25–32. https://doi.org/10.31942/md.v14i01.2615

Panichikkal, J., Prathap, G., Nair, R. A., & Krishnankutty, R. E. (2021). Evaluation Of Plant Probiotic Performance of *Pseudomonas* sp. Encapsulated In Alginate Supplemented With Salicylic Acid And Zinc Oxide Nanoparticles. *International Journal of Biological Macromolecules*, *166*, 138–143. https://doi.org/10.1016/j.ijbiomac.2020.10.110

Saberi-Rise, R., & Moradi-Pour, M. (2020). The effect of *Bacillus subtilis* Vru1 encapsulated in alginate – bentonite coating enriched with titanium nanoparticles against *Rhizoctonia solani* on bean. *International Journal of Biological Macromolecules*, *152*, 1089–1097. https://doi.org/10.1016/j.ijbiomac.2019.10.197

Shan, W., Zhou, Y., Liu, H., & Yu, X. (2018). Endophytic *actinomycetes* from tea plants (*Camellia sinensis*): Isolation, abundance, antimicrobial, and plant-growth-promoting activities. *BioMed Research International*, *2018*. https://doi.org/10.1155/2018/1470305

Singh, R., Pandey, K. D., Singh, M., Singh, S. K., Hashem, A., Al-arjani, A. B. F., Abd_allah, E. F., Singh, P. K., & Kumar, A. (2022). Isolation and Characterization of Endophytes Bacterial Strains of *Momordica charantia* L. and Their Possible Approach in Stress Management. *Microorganisms*, *10*(2), 0–13. https://doi.org/10.3390/microorganisms10020290

Stella, M., Theeba, M., & Illani, Z. I. (2019). Organic fertilizer amended with immobilized bacterial cells for extended shelf-life. *Biocatalysis and Agricultural Biotechnology*, *20*(September 2018), 101248. https://doi.org/10.1016/j.bcab.2019.101248

Sudiarti, D. (2017). The Effectiveness of Biofertilizer On Plant Growth Soybean Edamame (*Glycin Max*). *Jurnal SainHealth*, *1*(2), 97. https://doi.org/10.51804/jsh.v1i2.110.97-106

Sulaeman, Y., , M., & Erfandi, D. (2017). Pengaruh Kombinasi Pupuk Organik Dan Anorganik Terhadap Sifat Kimia Tanah, Dan Hasil Tanaman Jagung Di Lahan Kering Masam. *Jurnal Pengkajian Dan Pengembangan Teknologi Pertanian*, *20*(1), 1. https://doi.org/10.21082/jpptp.v20n1.2017.p1-12

Suryani, N., Suzanti Betha, O., Fakultas, M., Kesehatan, I., Syarif, U., & Jakarta, H. (2019). Uji Viabilitas Mikroenkapsulasi *Lactobacillus Casei* Menggunakan Matrik Natrium Alginat Microenkapsulation Viability Test of *Lactobacillus Casei* Using Sodium Alginate Matric. *Jurnal Farmasi Lampung*, *8*(1), 1–8. http://digilib.utb.ac.id/index.php/jfl/article/download/80/75

Syahbanuari, Yusniwati, & Efendi, S. (2020). Bioma: jurnal biologi makassar. *Jurnal Biologi Makasar*, *5*(1), 47–59.

Tangapo, A. M. (2020). Potensi Bakteri Endofit Ubi Jalar (*Ipomoea batatas* L.) dalam Menghasilkan Hormon Indole Acetic Acid (IAA) dengan Penambahan L-triptofan.

Vassilev, N., Vassileva, M., Martos, V., Garcia del Moral, L. F., Kowalska, J., Tylkowski, B., & Malusá, E. (2020). Formulation of Microbial Inoculants by Encapsulation in Natural Polysaccharides: Focus on Beneficial Properties of Carrier Additives and Derivatives. *Frontiers in Plant Science*, *11*(March), 1–9. https://doi.org/10.3389/fpls.2020.00270

Wu, Z., Li, X., Liu, X., Dong, J., Fan, D., Xu, X., & He, Y. (2019). Membrane Shell Permeability Of Rs-198 Microcapsules And Their Ability For Growth Promoting Bioactivity Compound Releasing. *RSC Advances*, *10*(2), 1159–1171. https://doi.org/10.1039/c9ra06935f

Xie, H., Feng, X., Wang, M., Wang, Y., Kumar Awasthi, M., & Xu, P. (2020). Implications of Endophytic Microbiota In *Camellia sinensis*: a review on current understanding and future insights. *Bioengineered*, *11*(1), 1001–1015. https://doi.org/10.1080/21655979.2020.1816788

Xu, H., Gao, J., Portieles, R., Du, L., Gao, X., & Borras- Hidalgo, O. (2022). Endophytic bacterium *Bacillus aryabhattai* induces novel transcriptomic changes to stimulate plant growth. *PLoS ONE*, *17*(8 August), 1–20. https://doi.org/10.1371/journal.pone.0272500

Yan, X., Wang, Z., Mei, Y., Wang, L., Wang, X., Xu, Q., Peng, S., Zhou, Y., & Wei, C. (2018). Isolation, diversity, and growth-promoting activities of endophytic bacteria from tea cultivars of Zijuan and Yunkang-10. *Frontiers in Microbiology*, *9*(JUL), 1–11. https://doi.org/10.3389/fmicb.2018.01848

Zabot, G. L., Schaefer Rodrigues, F., Polano Ody, L., Vinícius Tres, M., Herrera, E., Palacin, H., Córdova-Ramos, J. S., Best, I., & Olivera-Montenegro, L. (2022). Encapsulation of Bioactive Compounds for Food and Agricultural Applications. *Polymers*, *14*(19). https://doi.org/10.3390/polym14194194

How To Cite This Article, with *APA style* :
Zit, S.I., Warsito, K., & Refnizuida. (2023). Effectiveness Microcapsules from Auxin Producing Endophite Bacteria As a Biofertilizer in Tea (*Camellia sinensis* L). *Jurnal Pembelajaran dan Biologi Nukleus,* 9(1), 165-180. https://doi.org/10.36987/jpbn.v9i1.3904