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Endophytics Bacteria from Pangi Fruit (*Pangium edule* REINW.) and Its Potential as Producer of Antibacterial Compound

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ABSTRAK

Antibakteri alami dapat berasal dari berbagai jenis tumbuhan, salah satunya tumbuhan Pangi (Pangium edule REINW.). Buah Pangi memiliki kandungan senyawa antioksidan dan antibakteri yang bisa menghambat pertumbuhan bakteri. Penelitian ini bertujuan untuk mengetahui isolat bakteri endofit yang terdapat dalam Buah Pangi dan potensinya sebagai penghasil senyawa antibakteri terhadap bakteri Escherichia coli dan Staphylococcus aureus. Penelitian ini menggunakan metode penelitian deskripsi eksploratif. Hasil penelitian isolasi bakteri endofit dari Buah Pangi diperoleh tujuh isolat. Setiap isolat mempunyai morfologi yang berbeda-beda baik dari bentuk, warna, tepian, dan elevasi koloni. Berdasarkan hasil pengujian aktivitas antibakteri, setiap isolat bakteri endofit memperlihatkan diameter zona bening yang berbeda-beda terhadap E. coli (0,75 mm - 4,5 mm) dan S. aureus (1 mm - 5 mm). Isolat bakteri endofit yang berpotensi sebagai antibakteri dengan diameter zona bening terbesar yaitu isolat PA 2.1 (4,5 mm) terhadap E. coli dan isolat PA 3.2 (5 mm) terhadap bakteri S. aureus. Berdasarkan hasil karakterisasi morfologi isolat PA 1.1 memiliki kemiripan dengan genus Basillus sp., isolat PA 1.2, PA 1.3 PA 2.2, PA 3.1 dan PA 3.2 memiliki kemiripan dengan genus Paracoccus alcaliphilus dan isolat PA 2.1 memiliki kemiripan dengan genus Alcaligenes sp.

Kata Kunci : *Pangium edule* REINW., Bakteri Endofit, Antibakteri

ABSTRACT

Natural antibacterials can be derived from various types of plants, one of which is the Pangi plant (Pangium edule REINW.). Pangi fruit contains an antioxidant and antibacterial compound that can inhibit bacterial growth. This study aims to determine the isolates of endophytic bacteria contained in Pangi fruit and its potential as a producer of antibacterial compounds against Escherichia coli and Staphylococcus aureus bacteria. This study used exploratory description research method. Results Isolation research of endophytic bacteria from Pangi fruit obtained seven isolates. Each Isolate has a different morphology both from the shape, color, edges, and colony elevation. Based on the results of antibacterial activity testing, each isolate of endophytic bacteria showed different clear zone diameters. Endophytic bacteria showed different clear zone diameters against E. coli (0.75 mm – 4.5 mm) and S. aureus (1 mm – 5 mm). Endophytic bacterial isolates That have the potential as antibacterial with the largest clear zone diameter are Isolate PA 2.1 (4.5 mm) against E. c oli and isolate PA 3.2 (5 mm) against the bacteria S. aureus. Based on the results of morphological characterization of isolate PA 1.1 has similarities with the genus Basillus sp., isolates PA 1.2, PA 1.3 PA 2.2, PA 3.1 and PA 3.2 have similarity with the genus Basillus sp. PA 3.2 has similarities with the genus Paracoccus alcaliphilus and isolate PA 2.1 has similarities with the genus Alcaligenes sp.

Keywords : *Pangium edule* REINW., *Antibacterial, Endophytic Bacteria*

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1. INTRODUCTION

Antibacterials are chemical or biological compounds that can inhibit the growth and activity of bacteria. Natural antibacterials can come from various types of plants, one of which is the Pangi plant. Pangi fruit contain compound antioxidant and antibacterial that can hinder growth bacteria[1,2]. Bacteria endophyte is bacteria used as alternative producer compound antibacterial, sized micro, can found on network plants, roots, stems, leaves and fruit[3,4]. Exploration of potential endophytic mikrobes an alternative to obtain new antibiotic compunds[5]. Antibacterial compounds can be obtained from endophytic bacteria so there is no need to extract plants because endophytic bacteria that live in plants have the potential to produce the same antibacterial compounds as their host plants[6,7]. Bacteria endophyte life symbiotic mutualism with plants[8], namely bacteria utilise nutrition from results metabolism plant[9,10] . Besides that, the plant it is attached to also gets profit from bacteria endophyte. Bacteria This capable protect plant in oppose herbivores, insects or network pathogen as well as stimulate growth plant[11]. Bacteria endophyte produce compound antibiotics, antimalarials and antifungal in scale big and potential to obtain component new bioactive with different conditions. Some bacteria endophytic from plant drug own efficacy strong antibacterial[12,13]. Endophytic bacteria can maintain the sustainability of medicinal plants, especially rare plant species, so that they are not exploited continuously which will eventually result in extinction[14].

Previous studies have reported antibacterial and antioxidant activities of pangi fruit extract, but until now there has been no report on endophytic bacteria from pangi fruit that have the potential to act as antibacterials.

Based on the information on the content of antibacterial compounds in Pangi Fruit, researchers are motivated to conduct research on endophytic bacterial isolates that have the potential to be antibacterial from Pangi Fruit with the title Endophytic Bacteria from Pangi Fruit (*Pangium edule* REINW.) and Its Potential as a Producer of Antibacterial Compounds.

2. RESEARCH METHODS

Isolation of Endophytic Bacteria

Endophytic bacteria were isolated from Pangi fruit and the parts of the fruit used were the flesh attached to the skin, the middle flesh of the fruit and the flesh attached to the fruit seeds. Sample then washed using running water until clean. Sample one by one soaked in 70% ethanol for 1 minute then soaked again use solution Sodium Hypochlorite (NaClO) then rinsed using 70% ethanol as much as three times.

Samples that have been ready planted in Nutrint Agar media containing nystatin then incubated with temperature 37°C in condition dark. Observation done every 1 x 24 hours. Bacteria endophyte growth purified one by one and cultivated in slanted NA media. Every isolate bacteria endophyte cultured on two slant NA media for stock culture and working culture[15].

Characterization of Endophytic Bacteria

Macroscopic includes observation of the shape, texture, appearance, edges, surface and color of the colony.

Microscopic examination includes observation of bacterial cell morphology including gram staining, citrate test, and catalase test[3].

Gram Staining

One loop of endophytic bacterial isolate is scratched on the object glass then fixed above the Bunsen flame. Crystal violet solution is dripped on the object glass then left for 1 minute then rinsed using running water. Drop lugol solution then let stand for 1 minute then rinsed. Drop alcohol let stand for 10 seconds then rinsed. Drop safranin solution, let stand for 1 minute then rinsed. Dry the object glass then observe using a microscope with a magnification of 100[3].

Citrate Test

A total of 5.75 grams of *Simmon Citrate Agar* (SCA) media was dissolved in 250 ml of distilled water and then heated in a hoplate while stirring until homogeneous. The media was sterilized for 15 minutes, at a temperature of 121°C and a pressure of 2 Atm. Pour the media into a test tube then tilt it to 45° and let it stand until solid. Inoculate the bacteria aseptically with an ose needle then scratch it on

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the surface of the slanted media. Incubate for 24 hours at a temperature of 37°C. Positive results are indicated by a change in the color of the media from green to blue[16].

Catalase Test

Isolate bacteria taken as much as 1 loop then scratched on the object glass then dripped H_2 O_2. Positive results are indicated by the presence of air bubbles on the surface[17].

Antibacterial Activity Test

Pure cultures of test bacteria (*S. aureus* and *E. coli*) were taken as much as 50 µl and put into semi-solid media (0.75%) agar as much as 10 ml then inoculated into solid MHA media and then leveled. The wells were made using sterile tip tips. Endophytic bacterial isolates as much as 50 µl were put into the wells that had been made and then incubated for 24 hours at a temperature of 37°C. Observe the clear zone around the wells[18].

3. RESULTS AND DISCUSSION

The results of the isolation of endophytic bacteria from pangi fruit obtained three isolates from the fruit flesh attached to the skin, namely PA 1.1, PA 1.2, PA 1.3 (Figure 1), two isolates from the middle fruit flesh, namely PA 2.1, PA 2.2 (Figure 2) and two isolates from the fruit flesh attached to the fruit seeds, namely PA 3.1 and PA 3.2 (Figure 3). The total number of endophytic bacteria obtained from pangi fruit was seven isolates.

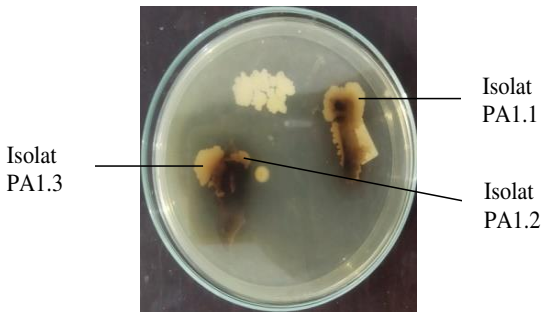

Figure 1. Endophytic Bacteria in Fruit Flesh Attached to Pangi Fruit Skin

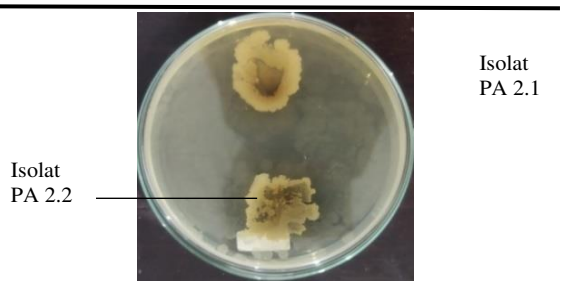

Figure 2. Endophytic Bacteria in the Middle of Pangi Fruit

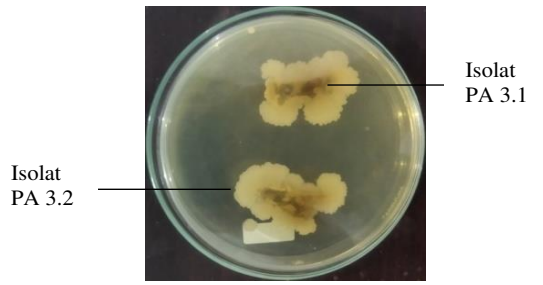

Figure 3. Endophytic bacteria in the flesh of the fruit attached to the seeds of the Pangi fruit

Endophytic bacteria can enter plant tissue and spread directly to all parts of the plant. Endophytic bacteria can live in vascular vessels or in intercellular spaces, roots, stems, leaves and fruits[3]. The number of endophytic bacteria in plants cannot be determined with certainty, but these bacteria can be detected by isolating them on agar media. The agar media used to isolate endophytic bacteria in this study was Nutrient Agar (NA) media. This media contains yeast extract, peptone, NaCl, and agar. Endophytic bacteria can grow on NA media because this media is complex and most likely has conditions similar to conditions in plants[19]. Endophytic bacteria from Pangi Fruit began to show growth after being incubated for ± 48 hours (2 days). The incubation time for at least 2 days aims to ensure that the body is endophytic bacteria, not contaminant bacteria. - The addition of nystatin (antifungal) to the media aims to maximize the isolation results and prevent contamination from fungi.

The results of cell morphology analysis, all isolates showed positive results in the catalase test and citrate test. While in the gram test, only isolate PA 1.1 showed gram positive while the other six isolates showed gram negative. The results of the morphology analysis obtained the alleged genus *Basillus* sp. (PA 1.1), *Paracoccus alcaliphilus* (PA 1.2, PA

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1.3, PA 2.2, PA 3.1 and PA 3.2) and *Alcaligenes* sp. (PA 2.1) (Table 1).

Biochemmical characterization was carried out with catalase and citrate test. Catalase test was carried out to determine the ability of bacteria to degrade hydrogen peroxide (H_2O_2) through enzyme production. Positive results are indicated by the formation of oxygen bubbles indicating that the microorganism is able to produce catalase enzyme which converts hydrogen peroxide into water H_2 and oxygen O_2. Citrate test was carried out to determine the ability of microorganisms to use citrate as the only carbon used.

Table 1. Results of Endophytic Bacterial Isolate Cell Morphology

Isolate Code	Cell Morphology			
	Gram	Citric	Catalase	Suspected Genus
PA 1.1	+	+	+	*Bacillus sp.*
PA 1.2	-	+	+	*Paracoccus alcaliphilus*
PA 1.3	-	+	+	*Paracoccus alcaliphilus*
PA 2.1	-	+	+	*Alcaligenes sp.*
PA 2.2	-	+	+	*Paracoccus alcaliphilus.*
PA 3.1	-	+	+	*Paracoccus alcaliphilus*
PA 3.2	-	+	+	*Paracoccus alcaliphilus*

Based on the results of colony morphology analysis, colony PA 1.1 which is suspected to be the genus *Bacillus sp.* has a *punctiform shape (dot), glossy* colony appearance, *entire* edge shape, *viscous* colony texture, milky white color, and a *raised surface* (the height looks a little but even on all parts of the surface) *flat* and *convex*. Colonies PA 1.2, PA 1.3, PA 2.2, PA 3.1 and PA 3.2 have *punctiform* and *circular colony shapes*, milky white and cream colors, *viscous* and butyrous textures (like butter), *dull* and *glossy appearance, convex* surface, *umbonate* (in the middle it looks a little protruding) and *flat* (Table 2).

Table 2. Results of Endophytic Bacterial Isolate Colony Morphology

Isolate Code	Colony Morphology					
	Form	Texture	Appearance	Edge	Surface	Color
PA 1.1	*Punctiform*	*Viscous*	*Glossy*	*Entire*	*Raised*	Milky white
PA 1.2	*Circular*	*Butyrous*	*Dull*	*Lobate*	*Covex*	Milky white
PA 1.3	*Circular*	*Viscous*	*Dull*	*Entire*	*Flat*	Beige
PA 2.1	*Circular*	*Viscous*	*Dull*	*Entire*	*Umbonate*	Milky white
PA 2.2	*Punctiform*	*Viscous*	*Glossy*	*Undulate*	*Flat*	Milky white
PA 3.1	*Circular*	*Butyrous*	*Dull*	*Entire*	*Umbonate*	Beige
PA 3.2	*Punctiform*	*Butyrous*	*Glossy*	*Undulate*	*Convex*	Milky white

Morphological characterization aims to observe the morphology of colonies and morphology of bacterial cells that have passed the selection. Endophytic bacterial colonies that grow have different characteristics, ranging from colony shape, color, edges, appearance, texture, and surface. This is in accordance with Shore's statement and Sathisha (2010)[20] that endophytic bacteria in a host plant generally consist of several genera and species. The diversity of bacteria is influenced by plant conditions, especially soil. In some cases, not all plants of the same genus and species have the same endophytic bacteria. In some plants there are distinctive and specific endophytic bacteria that inhabit the plant.

Testing the antibacterial activity of endophytic bacterial isolates was carried out using the well diffusion method and measuring the inhibition zone against *E. coli* and *S. aureus*

bacteria. Seven isolates of endophytic bacteria that have the potential to be antibacterial against *S. aureus* and *E. coli bacteria* were obtained, namely PA 1.1, PA 1.2, PA 1.3, PA 2.1, PA 2.2, PA 3.1, PA 3.2 (Table 3).

The seven endophytic bacterial isolates that have been identified based on morphological, physiological and biochemical characterization of the endophytic bacterial isolates were found to be three species that were harmonized according to *Bergey's book. Manual of Determinative Bacteriology Seventh Edition* (2005)[21].

1. *Bacillus sp.*

Isolate PA 1.1 was identified as a *Bacillus sp. species* because the colonies were in the form of bacilli and gram-positive. Data from biochemical tests that have been carried out showed that isolate PA 1.1 reacted positively to the catalase and citrate tests. This shows that the *Bacillus sp. species* is able to produce the catalase enzyme and use citrate to be able to reduce nitrate to free nitrogen.

Table 3. Diameter of Inhibition Zone of Endophytic Bacterial Isolates against Test Bacteria *E. coli* and *S. aureus* (mm)

Endophytic Bacterial Isolates	*Escherichia coli*	*Staphylococcus aureus*
PA 1.1	-	4
PA 1.2	0.75	1
PA 1.3	1	1
PA 2.1	4.5	1.5
PA 2.2	3	-
PA 3.1	-	-
PA 3.2	4	5

Fifi et al., 2017[22] have conducted research on the isolation and characterization of the morphology and physiology of endophytic *Bacillus sp. bacteria* from oil palm plants (*Elaeis guineensis* Jacq) and obtained the results of twelve isolates that were successfully isolated in this study.

2. Paracoccus alcaliphilus

Isolates PA 1.2, PA 1.3, PA 2.2, PA 3.1 and PA 3.2 were identified as Paracoccus alcaliphilus species because they have a coccus colony shape with gram-negative. Data from biochemical tests that have been carried out show that isolates PA 1.2, PA 1.3, PA 2.2, PA 3.1 and PA 3.2 reacted positively to the citrate and catalase tests. This shows that the genus *Paracoccus alcaliphilus* is able to produce the catalase enzyme and use citrate to reduce nitrate to free nitrogen. Arlita (2013)[23] has conducted a study on the identification of carotenoid pigments in marine symbiotic bacteria caulerpa cupressioddes (vahl) c. Agardh. The results obtained in the study showed that isolate cj.or from seaweed came from the genus *Paracoccus alcaliphilus*.

3. Alcaligenes sp.

Isolate PA 2.1 was identified as alcaligenes sp. which has a bacillus and gram-negative colony form. Data from biochemical tests that have been carried out show that isolate PA 2.1 reacts positively to the catalase test and citrate test. This indicates that the species *Alcaligenes sp.* is able to produce the catalase enzyme and use citrate to reduce nutrate to free nitrogen. Arlita (2013)[23] has conducted a study on the identification of carotenoid pigments in symbiotic bacteria of the seaweed caulerpa cupressioddes (vahl) c. Agardh. The results obtained in the study indicate that isolate cj.k from seaweed comes from the genus *Alcaligenes sp.*

4. CONCLUSION

Based on the results of the research that has been conducted, it can be concluded:

1. The results of the isolation of endophytic bacteria from Pangi fruit obtained seven isolates, namely PA 1.1, PA 1.2, PA 1.3, PA 2.1, PA 2.2, PA 3.1, and PA 3.
2. Endophytic bacterial isolates from Pangi fruit have antibacterial activity against *Staphylococcus aureus bacteria* with an inhibitory diameter of 0.75 - 4.5 mm and against *Escherichia coli bacteria* with an inhibitory diameter of 1 - 5 mm.

5. REFERENCES

1. Sangi, M. S., Koleangan, H.S., Kumaunang, M., & Dapas, SO (2023, April). Antioxidant s and antibacterial activity of Pangi fruit (*Pangium edule* Reinw). In *AIP Conference Proceedings* (Vol. 2694, No. 1). AIP Publishing.

2. Pangisian, J., Sangi, M. S., & Kumaunang, M. (2022). Analysis Compound Metabolites Secondary And Test Activity Antioxidants as well as Antibacterial Seed Fruit Pangi (Pangium) education Reinw). *LPPM Journal Field Science and Technology* , *7* (1), 11-19.
3. Lawalata, H. J., Rengkuan, M. Satiman U. 2019. Antibacterial Activities of Lactic Acid Bacteria From Langsat Fruit (*Lanisiumdomesticum)* against Phatogenic Bacteria and Spoilage Bacteria.
4. Fatimah, A. D. 2024. Isolasi dan Ientifikasi Bakteri Endofit dari Rimpang Temulawak (*Curcuma xanthorriza*) sebagai Penghasil Senyawa Antibakteri terhadap *Propiniobacterium acnes*. Doctoral Dissertation, Universitas Islam Negeri Maulana Malik Ibrahim.
5. Purwaningsih, D., Wulandari, D. 2021. Uji Aktivitas Antibakteri Hasil Fermentasi Bakteri Endofit Umbi Talas (*Colocasia esculenta* L.) terhadap Bakteri *Pseudomonas aeruginosa*. Jurnal Sains dan Kesehatan. 3(5), 750-75.
6. Hapida , Y., & Widjajanti , H. (2021). Biodiversity and antibacterial activity of endophytic fungi isolated from guava ball (Syzygium malaccent). *Biodiversity : Journal of Biological Diversity*, *22* (12).
7. Mao, Z., Zhang, W., Wu, C., Feng , H., Peng, Y., Shahid, H ., & Shan, T. (2021). Diversity and antibacterial activity of fungal endophytes from Eucalyptus exsert. *BMC microbiology* , *21* (1), 155.
8. Fitriana, M. N. 2021. Isolasi Bakteri Endofit dari Daun Kelor (*Maringa oleifera* Lam.) dan Uji Aktivitas Antibakteri Metabolit Sekundernya. Doctoral Disertation, Universitas Andalas.
9. Wu, W., Chen, W., Liu, S., Wu, J., Zhu, Y., Qin, L., & Zhu, B. (2021). Beneficial relationships between endophytic bacteria and medicinal plants. *Frontiers in plant science* , *12* , 646146.
10. Semuel , M. Y., Wurarah , M., & Tuegeh, R. S. (2022). Antagonistic and Antibacterial Activity of *Stapylocossus aureus* and Isolates of Oral Bacteria from the Endogenous Fungus Apis Dorsata Binghami Nest. *JOURNAL OF LEARNING AND NUCLEUS BIOLOGY (JPBN)* , *8* (2), 273-283.
11. Zahrina, Y. 2020. Isolation and Screening of Antibacterials from Nipah Fruit Endophytic Bacteria (*Nypa fruticans*) in Inhibiting Multidrug Resistant (MDR) Strains *of Escherichia coli* . Thesis. Ar-raniry Darussalam State Islamic University – Banda Aceh.
12. Nxumalo , C. I., Ngidi , L. S., Shandu, J. S. E., & Maliehe , T. S. (2020). Isolation of endophytic bacteria from the leaves of Anredera cordifolia CIX1 for metabolites and their biological activities. *BMC Complementary Medicine and Therapies*, *20* , 1-11.
13. Sagita D., Suharti N., Azzizah N. 2017. Isolation of Endophytic Bacteria from Betel Leaves (*Piper betle* L.) as Antibacterial Against *Escherichia coli* and *Staphylococcus aureus*. Journal of Applied Science and Education. *Research of Applied Science and Education* . V11.i1 (65-74).
14. Nursulisyarini, F and Ainy, EQ 2017. Isolation and Identification of Antibacterial Producing Endophytic Bacteria from Binahong Plant Leaves (*Anredera cordifolia* (Ten.) Steenis). UIN Sunan Kalijaga. Yogyakarta.
15. Fahdila, S. 2019. Isolation and Identification of Endophytic Bacteria in Chili Roots (*Capsicum annuum* L.) to Inhibit Fungal Growth (*Fusarium oxysporum*). Doctoral Dissertation, Medan Area University.
16. Sari, D. P. 2019. Detection and Identification of Coliform Bacteria Genera Isolated from Aloe Vera Drink. Jurnal Labora Medika. 3(1):29-35.
17. Sardiani, N., Magdalena, L., Risco, GB, Dody, P., Syahribulan and Zaraswati, D. 2015. Potential of Tunicates (*Rhopalaea sp.*) as a Source of Inoculum of Endosymbiont Bacteria Producing Antibacterials. Journal of

e-ISSN 2685-3167
p-ISSN 2828-6685

Nature and Environment, (6). 11: 1-10.

18. Lawalata, H. J., Kumajas, J., Tengker, S. M., Runtuwene, K. M., Hasani, R. S., Weken, M. M. 2023. Lactid Acid Bacteria as an Exopolysaccharides (EPS) Producing Starter from Pakoba Fruit (*Syzygium* sp.) Endemic Species at Minahasa, North Sulawesi. Journal of Pure & Applied Microbiology, 17 (4).
19. Hala, Y., Arifin, A. N. 2021. Isolasi dan Karakterisasi Bakteri Endofit dari Batang dan Akar Tanaman Mimba. Indonesian Journal of Fundamental Sciences Vol, 7(2), 67-76.
20. Shore, S. J., & Sathisha, G. (2010). Screening of endophytic colonizing bacteria for cytokinin-like compounds: crude cell-free broth of endophytic colonizing bacteria is unsuitable in cucumber cotyledon bioassay. World Journal of Agricultural Sciences, 6(4), 345-352.
21. Breed, R. S., Murray, E. G. D., Smith. 2005. Bergey's Manual of Determinative Bacteriology, Seventh Edition. Baltimore. The Wiliam and Wilkins Company. United States of America.
22. Puspita, F., Ali, M., & Pratama, R. 2017. Isolasi dan karakterisasi morfologi dan fisiologi bakteri Bacillus sp. Endofitik dari tanaman kelapa sawit (Elaeis guineensis Jacq.). Jurnal Agroteknologi Tropika, 6(2), 44-49.
23. Arlita, N. R., Radjasa, O. K., & Santoso, A. (2013). Identifikasi pigmen karotenoid pada bakteri simbion rumput laut Caulerpa cupressoides (Vahl) C. Agardh. Journal of Marine Research, 2(3), 68-77.