

ISSN: 1412-033X
E-ISSN: 2085-4722

BIODIVERSITAS

Journal of Biological Diversity

Volume 21 - Number 9 - September 2020



BUKTI KORESPONDENSI BIODIVERSITAS

Jurnal : Biodiversitas

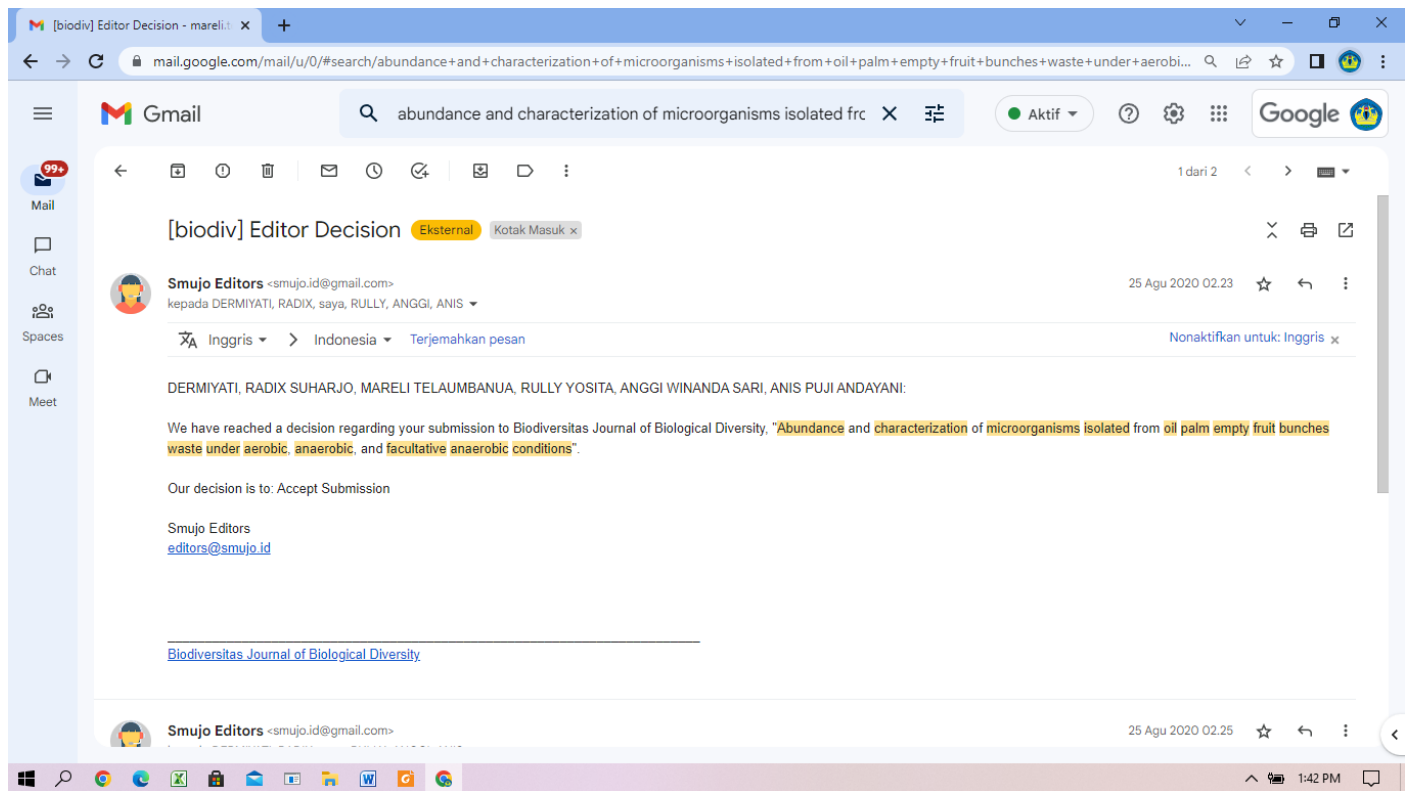
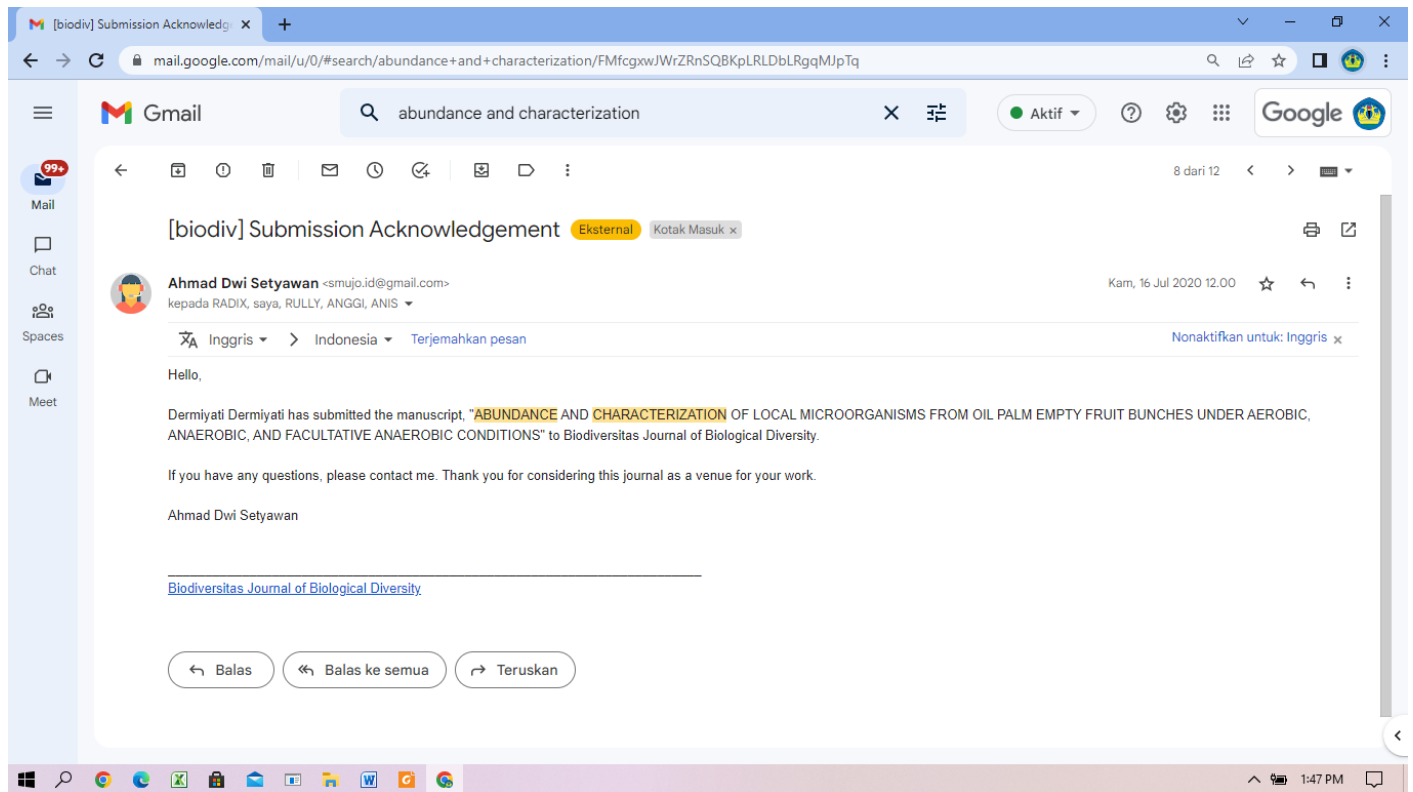
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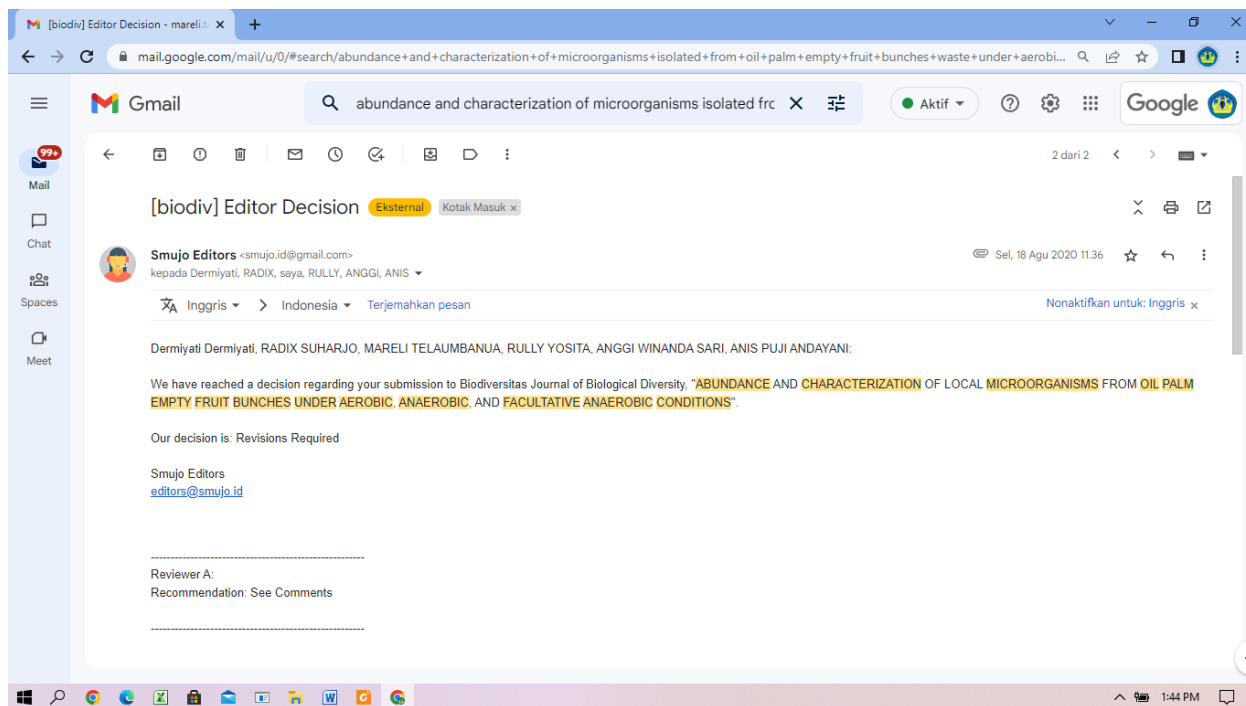
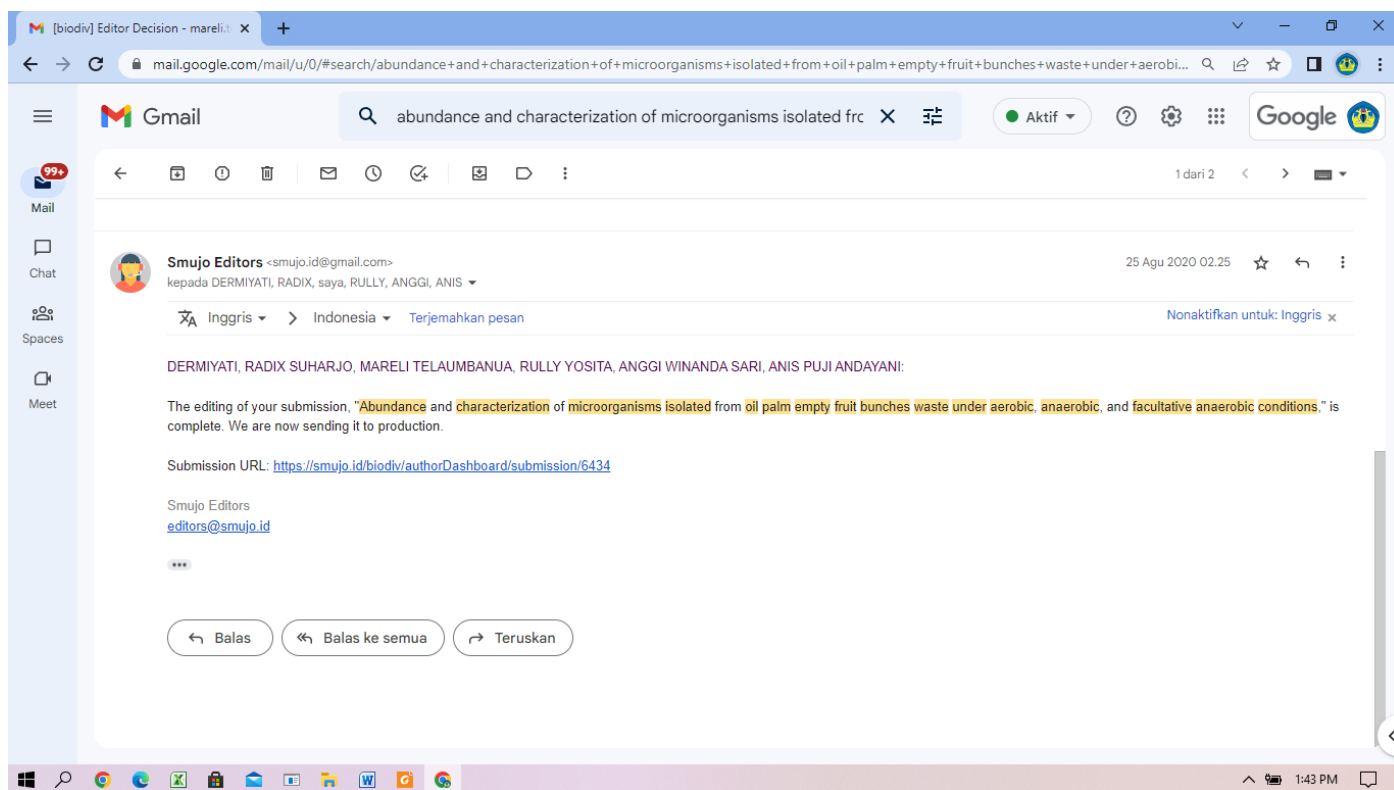
- SUBMIT (16 Juli 2020)
- REVISI (18 Agustus 2020)
- ACCEPTED SUBMISSION (25 Agustus 2020)
- TERBIT DI BIODIVERSITAS (September 2020)

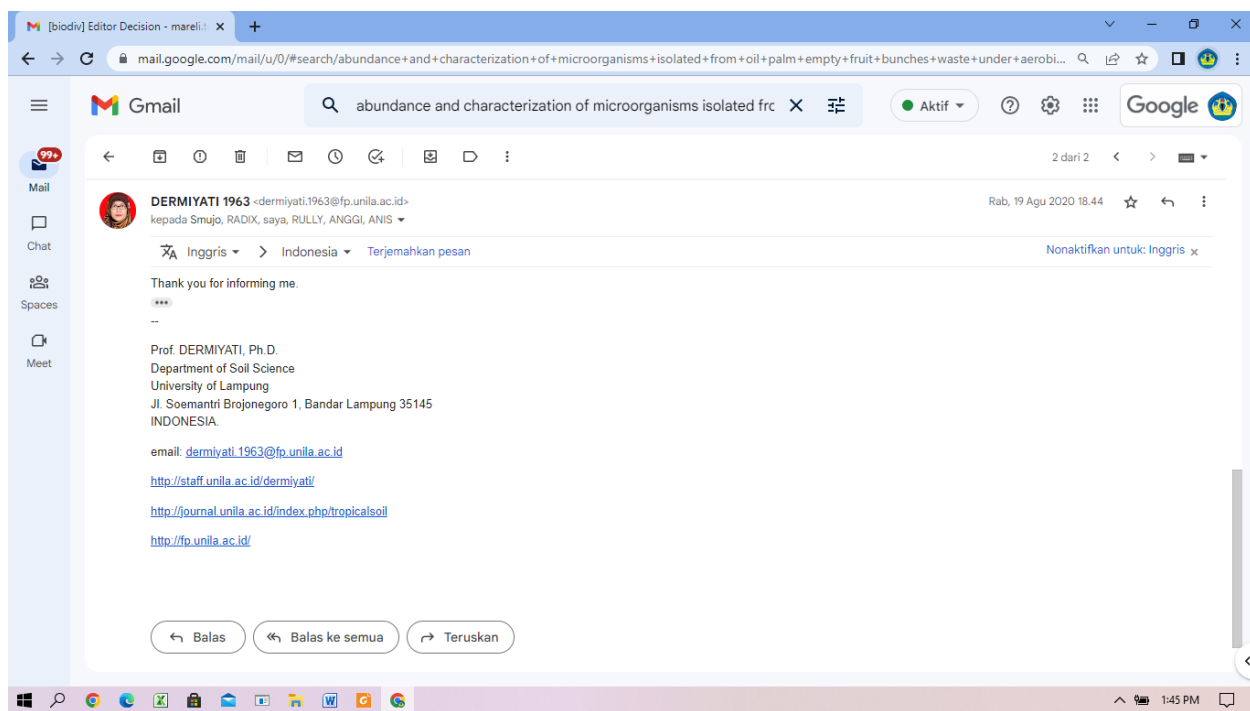
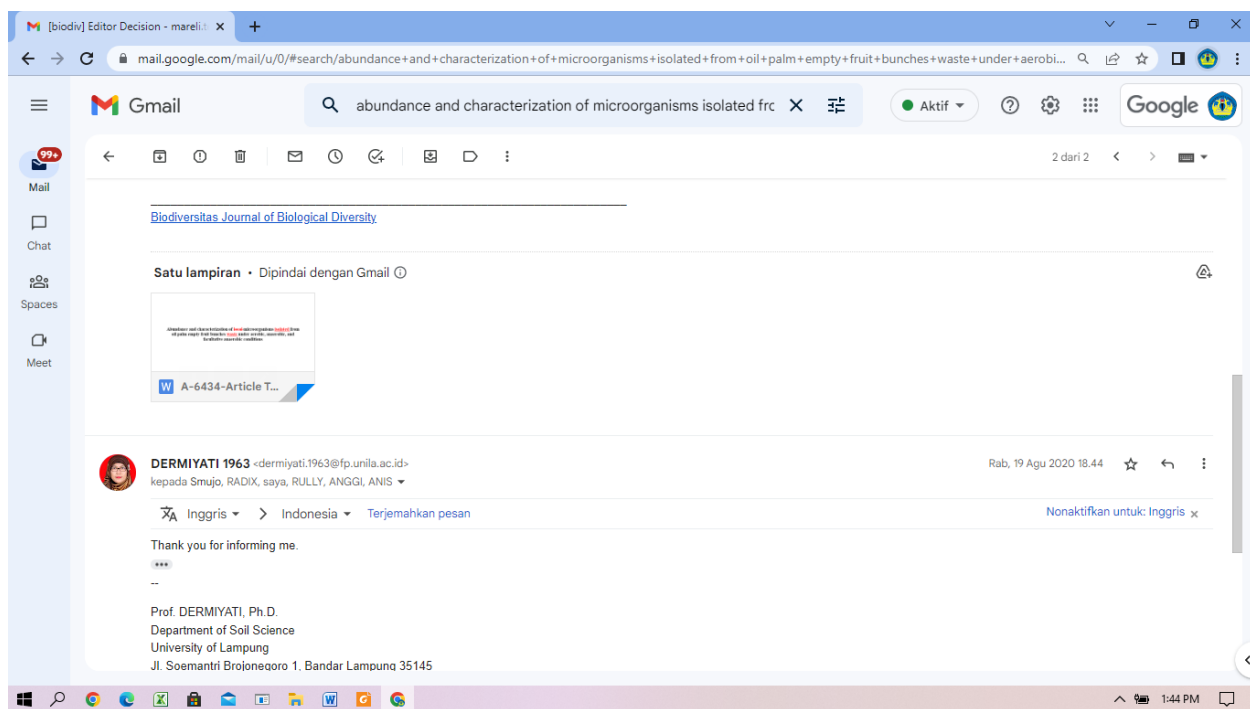
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Judul : Abundance and characterization of microorganisms isolated from oil palm empty fruit bunches waste under aerobic, anaerobic, and facultative anaerobic conditions







Abundance and characterization of ~~local~~ microorganisms isolated from oil palm empty fruit bunches waste under aerobic, anaerobic, and facultative anaerobic conditions

Abstract. Increased production of palm oil ~~production~~ causes an increase in oil palm empty fruit bunches ~~as a~~ waste, while the utilization of the waste has not been carried out optimally. Oil palm empty fruit bunches contains high cellulose content which promotes the development of ~~has the potential to produce the abundance of various types of~~ bacteria with different characteristics ~~and types due to its high cellulose content~~. This study was aimed to determine the abundance and the characterization of bacteria which ~~was were~~ isolated from extract suspension of oil palm empty fruit bunches in the form of a local microorganism solution under aerobic, anaerobic, and facultative anaerobic conditions. For isolation, the oil palm empty fruit extract suspension was inoculated on media Plate Count Agar Peptone and The bacterial abundance ~~was determined, was shown by bacterial population that was grown on the media Plate Count Agar Peptone.~~ Various Bbacterial characterization test included bacterial morphology test in the form of color and shape of macroscopic isolates, gram test using KOH 3%, fermentative oxidative test, soft rot test on potato tubers, hypersensitivity test on tobacco plant leaves, and hypo virulent test which was indicated by the presence of symptoms of disease in cucumber sprouts and indicated in the disease severity index (DSI) were carried out. The results of local microorganism solution isolation from oil palm empty fruit bunches obtained as many as 220 bacterial isolates consisting of 84 isolates in aerobic condition, 68 isolates in anaerobic condition, and 68 isolates in facultative anaerobic condition. The highest bacterial abundance in aerobic conditions was equal to 285.60×10^{10} CFU mL⁻¹. Each bacterial isolate had different characteristics. Most of the characteristics of bacteria were turbid white (34,55%), round (69,55%), gram-positive (76.36%), fermentative (89,55%), softrot negative (71,82%), hypersensitive negative (97,27%), and virulent (74,55%).

Key words: Abundance, Bacteria, Characteristics, Local microorganisms

INTRODUCTION

Oil palm empty fruit bunches (EFB) is a kind of biomass waste formed during the production process of palm oil ~~and it comprises 20–23% of the products produced during palm oil production~~ (Rahayu et al. 2019). Nowadays, the availability of oil palm empty fruit bunches as a waste is increasingly abundant in line with the increased oil palm production and land area. According to USDA (2019), with further expansion in mature area, palm oil production in Indonesia was forecast to reach 43 million tons in 2019/20, a 1.5 million ton increased from 2018/19. Moreover, as stated by Mariska (2019) that Indonesia's crude palm oil production was 34,7 million metric tons in August 2019, it rose by 14 percent compared to August 2018. Furthermore, Indonesia planting target at 2019 was 200,000 ha, ~~(however, the implementation was only about 20,000 ha)~~ and by 2022 the planting target is 700,000 ha. The increasing planting area would increase the palm oil production as well as their wastes. Furthermore, Santi et al. (2019) reported that until now the potential availability of oil palm empty fruit bunches as a waste can reach 47 million tons per year, but so the utilization of the waste has not been carried out optimally.

Oil palm empty fruit bunches can be utilized as a compost or an organic fertilizer which is useful to increase palm oil production and to improve physical, chemical, and biological soil properties (Yi et al. 2019). Furthermore, Yi et al. (2019) found that application of either compost or fertilizer alone increased fresh fruit bunch yield, from 23.24 to 25.49 or 25.55 t ha⁻¹yr⁻¹. Soil chemical properties such as soil pH, organic C, total N and P, available P, exchangeable cations (K⁺, Ca²⁺ and Mg²⁺) and cation exchange capacity were significantly increased by the application of compost, making compost an effective organic material and nutrient source for improving the health of soils under oil palm cultivation. Other than that, oil palm empty fruit bunches can also be used as bio fertilizer because it contains bacteria that can bind nitrogen and phosphate solvents (Hoe et al. 2016), as degraders lignin bacteria (Tahir et al. 2019), as organic materials decomposers (Chin et al. 2017), as well as a fuel for power plant (Han and Kim 2018). Oil palm empty fruit bunches also contain the

abundant bacteria, ~~this is because oil palm empty fruit bunches of~~ contain high cellulose so it can be used as a source of bacterial energy for the biosynthesis process (Md Yunus et al. 2015; Rosli et al. 2017). Moreover, Rosli et al. (2017) found that the oil palm empty fruit bunches contains the polymer ~~ic of~~ carbohydrates such as cellulose and hemicellulose. Also, the oil palm empty fruit bunches fiber were made up of 31.4% of glucan, 18.6% xylan and 2.7% arabinan with the lignin accounted around 23,9%.

Various studies have shown that bacterial isolates obtained from oil palm empty fruit bunches either in the fresh form, the weathered form, the compost form, the suspension extract form (local microorganisms solution) ~~will varied~~. The research of Ariana et al. (2017) resulted ~~the isolation of~~ as many as 430 microbes ~~which is isolated~~ from oil palm empty fruit bunches. Also, Harith et al. (2014) reported that 24 bacterial isolates were recovered from oil palm industry waste. Besides, Lai et al. (2017) obtained 34 bacterial isolates that were successfully isolated from compost oil palm empty fruit bunches. The abundance of bacteria can also be obtained through extract suspension of oil palm empty fruit bunches in the form of a local microorganism solution. A local microorganisms solution is a fermented liquid from plant or animal waste and contain microorganisms with different species in an area due to differences in environmental conditions (Adrizal et al. 2017). Besides, a local microorganisms ~~solutionsolution containscontains~~ macro and micro nutrient elements and also contains bacteria which potentially as a material overhaul organic, growth stimulant and as a pest and disease control agent plant. Astriani et al. (2017) isolated 3 kinds of local microorganism solution samples from vegetable waste, then they obtained 79 bacterial isolates with the characteristics of most bacteria that are round, uneven edges, and white colonies.

The diversity of abundance and characterization of bacteria isolated from extract suspension of local micororganisms solution depends on the source of raw material used as well as the conditions during fermentation. So far, the information regarding to the fermentation conditions under aerobic, anaerobic, and facultative anaerobic are not available yet. These conditions ~~will may~~ affect the growth of bacteria which is related to available oxygen during the fermentation processes. Therefore, this study was aimed to explore the abundance and characterization of indigenous bacteria isolated from the extract suspension based on oil palm empty fruit bunches under a different fermentation process namely aerobic, anaerobic, and facultative anaerobic.

MATERIALS AND METHODS

Development of local microorganism solutions from oil palm empty fruit bunches

Local Microorganism solution was developed using oil palm empty fruit bunches in three conditions, namely aerobic, anaerobic, and facultative anaerobic as described by Dermiyati et al. (2019). Making a suspension of local microorganisms was done by mixing 2.5 kg of finely chopped oil palm empty fruit bunches, 0.5 kg brown sugar, 2.5 L rice washing water, and 2.5 L coconut water, then put into the reactor machine and each fermentation condition contains the same ingredients. The process of making local microorganism solution was performed using a reactor machine developed by Telaumbanua et al. (2019). Oil palm empty fruit bunches were taken from PT Perkebunan Nusantara (PTPN) VII, Rejosari, Natar, Lampung Province.

Bacterial isolation and propagation

Bacterial isolation and bacterial propagation were performed as ~~per the method~~ described by Dermiyati et al. (2019).

Bacterial isolation

All the collected samples were diluted at 10^{-8} , 10^{-10} , and 10^{-12} . From each dilution series, 50 µl of the suspension were spread into sterile plastic petri dish (diameter 9 cm) containing Plate Count Agar Peptone (PCAP) media (plate count agar (PCA; OXOID®; England) 17.4 g, peptone (OXOID®; England) 2.5 g, agar 2 g, and 1000 mL distilled water). Observation of the total bacterial colonies which were emerged was performed every day for 7 days.

Bacterial propagation

The emerging bacteria were purified by placing into a sterile plastic Petri dish (9 cm in diameter) containing Yeast Peptone Agar (YPA) peptone media (OXOID®, England) 10 g, Yeast Extract (HIMEDIA®; India) 5 g, agar 20 g, and 1000 mL distilled water). For further analysis, the bacteria were streaked into Potato Peptone Glucose Agar (PPGA) slant media.

Bacterial abundance

Bacterial abundance was shown by the number of bacterial populations through observing the numbers of bacterial isolates that grew on Plate Count Agar Peptone media for 7 days after isolation such as that explained above.

Bacterial characteristics

Bacterial characteristics were seen based on bacterial morphology test, gram test with 3% KOH, [Oxidation-Fermentation or the OF test](#), ~~fermentative-oxidative (OF) test~~, soft rot test on potato tubers, hypersensitive test, and hypovirulent test.

Bacterial morphology was confirmed by observing the bacterial isolates ~~resulting from purification macroscopic isolate~~ for color and shape (Linda et al. 2018). The morphological characteristics test of the bacteria was carried out by observing the bacteria that grew on PCAP media for 7 days after spreading.

Gram test with KOH 3% was performed using the method by Chandra and Mani (2011). The nature of bacteria was divided into two, namely a gram-positive if it did not produce mucus when reacting with KOH 3% and a gram-negative which produced mucus when reacting with KOH 3%.

[Oxidation-Fermentation](#) ~~Fermentative-oxidative~~ test was performed based on the method described by Cymru and Wales (2010), it used 2 test tubes containing media with one of the tube was added as much as 1 mL of paraffin oil after bacterial isolates were inserted into the media and then it was observed for 7 days discoloration on the media. Change the color of the media from green to yellow on the both tube indicated the bacteria was fermentative, while if discoloration occurred in the tube which was given by paraffin oil then the bacteria was oxidative.

Soft rot test was done based on the method by Khlaif and Wreikat (2018) through scratching bacterial isolates in the middle of the [potato tubers](#) ~~potatoes~~ and the reaction was observed 24 hours. Negative reactions were indicated by ~~there~~ ~~was~~ not any changes in potato tubers, while positive reactions occurred when it was the decay and soft stabbed in the middle of a potato tuber carved by bacteria.

Hypersensitive test was prepared based on the method by Istiqomah and Kusumawati (2018) by injecting a bacterial isolate suspension into the tissue of the lower surface of the tobacco leaf. Positive response from tobacco plants if there was a necrosis of the leaves which was injected with a bacterial suspension at 24 hours after application.

Hypovirulent test was performed according to the method by Suharjo et al. (2018). The test used a cucumber as an indicator plant and observe the development of the symptoms of the disease in the hypocotyl or other parts of the sprouts for 14 days, then the results were stated as the disease severity index (DSI). If the isolate is not showing disease symptoms or symptoms caused by cucumber sprouts only a little (DSI<2,0), the isolate is categorized as hypovirulent.

RESULTS AND DISCUSSION

The results of bacterial isolates ~~which~~ were isolated from the extract suspension of oil palm empty fruit bunches with their isolate codes are shown in Table 1. This study obtained as many as 220 bacterial isolates consisting of 84 isolates under fermentation in aerobic conditions, 68 isolates in anaerobic condition, and 68 isolates in facultative anaerobic condition. The highest bacterial isolates were in the aerobic conditions which was related to the need of oxygen for the growth of the bacteria. Aerobic bacteria are the species of bacteria which require oxygen for their basic survival, growth, and the process of reproduction (Tortora 2019).

Table 1. Bacteria isolates from extract suspension of oil palm empty fruit bunches in aerobic, anaerobic, and facultative anaerobic conditions.

Aerobic		Anaerobic		Facultative Anaerobic	
No.	Isolate code	No.	Isolate code	No.	Isolate code
1	ASP1	1	ANSP1	1	SSP1
2	ASP2	2	ANSB1	2	SSP2
3	ASB1	3	ANSB2	3	SSP3
4	ASPKR1	4	ANSB3	4	SSP4
5	ASP3	5	ANSK1	5	SSPKR1
6	ASB2	6	ANSK2	6	SSPKR2
7	ASB3	7	ANSK3	7	SSK1
8	ASK1	8	ANSM1	8	SSP5
9	ASPKR2	9	ANSM2	9	SSPKR3
10	ASPKR3	10	ANSM3	10	SSPKR4
11	ASM1	11	ANSM4	11	SSP6
12	ASK2	12	ANSP2	12	SSPKR5
13	ASM2	13	ANSPKR1	13	SSPKR6
14	ASPKR4	14	ANSK4	14	SSPKR7
15	ASPKR5	15	ANSPKR2	15	SSP7
16	ASP4	16	ANSPKR3	16	SSP8
17	ASPKR6	17	ANSP3	17	SSB1
18	ASPKR7	18	ANSPKR4	18	SSPKR8
19	ASPKR8	19	ANSK5	19	SSM1

Aerobic		Anaerobic		Facultative Anaerobic	
No.	Isolate code	No.	Isolate code	No.	Isolate code
20	ASP5	20	ANSPKR5	20	SSM2
21	ASP6	21	ANSP4	21	SSPKR9
22	ASPKR9	22	ANSPKR6	22	SSK2
23	ASP7	23	ANSK6	23	SSP9
24	ASK3	24	ANSB4	24	SSPKR10
25	ASP8	25	ANSB5	25	SSPKR11
26	ASPKR10	26	ANSP5	26	SSB2
27	ASP9	27	ANSP6	27	SSB3
28	ASP10	28	ANSB6	28	SSB4
29	ASP11	29	ANSK7	29	SSPKR12
30	ASK4	30	ANSPKR7	30	SSPKR13
31	ASPKR11	31	ANSP7	31	SSPKR14
32	ASPKR12	32	ANSPKR8	32	SSP10
33	ASP12	33	ANSP8	33	SSPK1
34	ASK5	34	ANSPKR9	34	SSP11
35	ASPKR13	35	ANSP9	35	SSPKR15
36	ASK6	36	ANSK8	36	SSK3
37	ASM3	37	ANSK9	37	SSP12
38	ASK7	38	ANSM5	38	SSB5
39	ASB4	39	ANSPKR10	39	SSP13
40	ASB5	40	ANSB7	40	SSPKR16
41	ASB6	41	ANSP10	41	SSPKR17
42	ASP13	42	ANSB8	42	SSP14
43	ASPKR14	43	ANSP11	43	SSPKR18
44	ASPKR15	44	ANSB9	44	SSP15
45	ASPKR16	45	ANSPKR11	45	SSP16
46	ASK8	46	ANSPKR12	46	SSP17
47	ASB7	47	ANSP12	47	SSK4
48	ASPKR17	48	ANSKP1	48	SSP18
49	ASO1	49	ANSP13	49	SSP19
50	ASPKR18	50	ANSPK1	50	SSP20
51	ASK9	51	ANSP14	51	SSPKRL1
52	ASP14	52	ANSPKRL1	52	SSPKRL2
53	ASB8	53	ANSP15	53	SSPB1
54	ASPKR19	54	ANSPK2	54	SSPK2
55	ASP15	55	ANSPRL1	55	SSPRL1
56	ASPKR20	56	ANSP16	56	SSPKR19
57	ASP16	57	ANSPB1	57	SSB6
58	ASK10	58	ANSPK3	58	SSPB2
59	ASPK1	59	ANSP17	59	SSPKR20
60	ASP17	60	ANSPKR13	60	SSPB3
61	ASP18	61	ANSPKR14	61	SSPKR21
62	ASPKRL1	62	ANSPKR15	62	SSPKR22
63	ASP19	63	ANSPB2	63	SSB7
64	ASPK2	64	ANSPKR16	64	SSPKR23
65	ASP20	65	ANSPKR17	65	SSPKR24
66	ASKP1	66	ANSPKR18	66	SSPB4
67	ASP21	67	ANSPB3	67	SSPKR25
68	ASPRL1	68	ANSPKR19	68	SSB8
69	ASPB1				
70	ASPKR21				
71	ASPKR22				
72	ASPKR23				
73	ASPKR24				
74	ASPK3				
75	ASPKR25				
76	ASB9				
77	ASPKR26				
78	ASPB2				
79	ASPKR27				
80	ASP22				
81	ASB1				
82	ASB11				
83	ASBK1				

Aerobic		Anaerobic		Facultative Anaerobic	
No.	Isolate code	No.	Isolate code	No.	Isolate code
84	ASPKR28				

The highest bacterial population resulting from the isolation of the extract suspension of oil palm empty fruit bunches was in the aerobic condition of 285.60×10^{10} CFU mL⁻¹, followed by the anaerobic condition of 230.27×10^{10} CFU mL⁻¹, and the facultative anaerobic condition of 175.13×10^{10} CFU mL⁻¹ (Figure 1). This is supported by Zainudin et al. (2013) that bacteria contained in the compost of oil palm empty fruit bunches was dominated by bacteria *Bacillus* sp., *Paenibacillus* sp., *Pseudomonas* sp., and *Cellulomonas* sp, where the bacteria are included in the aerobic bacteria. Also, aerobic conditions will allow bacteria to grow properly with sufficient oxygen.

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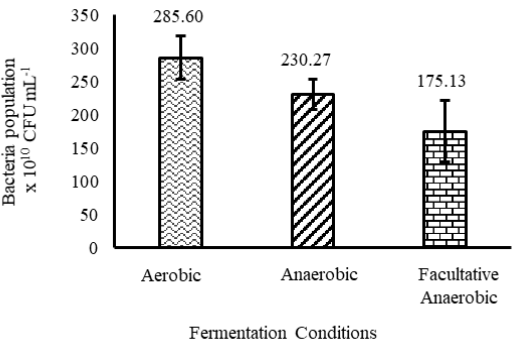
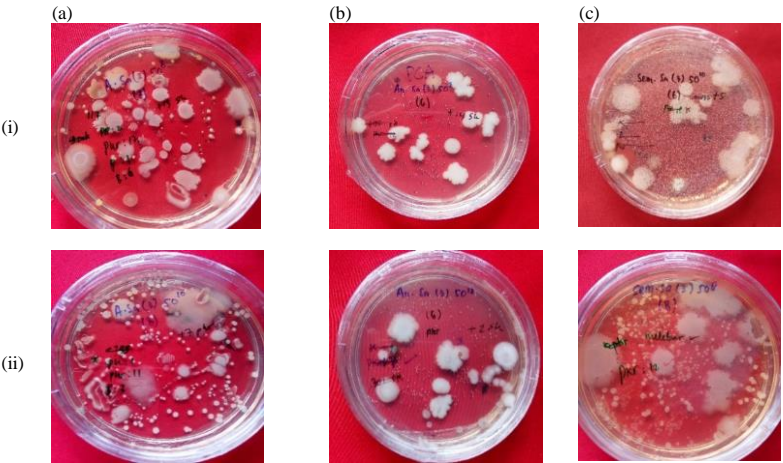


Figure 1. The population of the indigenous bacteria in the extract suspension of oil palm empty fruit bunches.

Some examples of the isolation results from the extract suspension of oil palm empty fruit bunches at three fermentation conditions are shown in Figure 2. Figures a (i, ii, and iii) are the results of isolation of bacteria in the aerobic condition, figures b (i, ii, and iii) in the anaerobic condition, while figures c (i, ii, and iii) in the facultative anaerobic condition. The three conditions of fermentation show an abundance of different bacteria.



(iii)

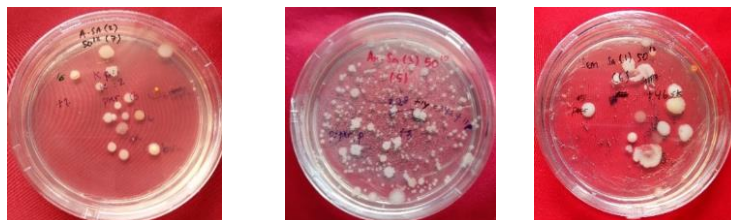


Figure 2. Examples of the abundance bacteria isolates from the extract suspension of EFB in aerobe, anaerobe, and facultative anaerobe

Examining the characteristics of bacterial isolates from the extract suspension of oil palm empty fruit bunches in the form of color and shape are presented in Table 2. Of the 220 bacterial isolates obtained, there were 13 types of characteristics in aerobic conditions, 14 characteristics-types in anaerobic conditions, and 11 characteristics-types in facultative anaerobic conditions. However, the characteristics of bacterial isolates obtained were dominated by round white followed by turbid white irregular-irregular and round white turbid under all conditions of fermentation. Characteristics of bacteria that are white and round in aerobic conditions are 20.23%, anaerobic conditions are 19.12%, and facultative anaerobic conditions are 27.94%.

Table 2. The characteristics of bacterial isolates from the extract suspensions of oil palm empty fruit bunches in the aerobic, anaerobic, and facultative anaerobic conditions.

No.	Fermentation Conditions ^{a)}	Characteristics ^{a)}
1	Aerobic (84)	Round White (17), Irregular White (6), Round Clear (10), Irregular Clear (1), Round Yellow (10), Round Red (3), Round Turbid White (14), No Turbid White Regular (15), Round Solid Yellow (1), Round Yellowish White (3), Round Clear White (2), Irregular Yellowish Clear (1), Round Orange (1)
2	Anaerobic (68)	Round White (13), Irregular White (5), Round Clear (6), Irregular Clear (3), Round Yellow (8), Irregular Yellow (1), Round Red (5), Round Turbid White (11), Irregular Turbidity White (9), Round Solid Yellow (1), Round Yellowish White (2), Irregular Yellowish White (1), Round Clear White (2), Irregular Clear White (1)
3	Facultative Anaerobic (68)	Round White (19), Irregular White (2), Round Turbid White (8), Irregular White Turbid (19), Round Yellow (4), Round Clear (7), Irregular Clear (2), Round Red (2), Round Yellowish White (2), Round Clear White (2), Irregular Clear White (1)

^{a)} The number in parentheses is the number of bacterial isolates.

The color and shape of bacterial isolates from the extract suspension of oil palm empty fruit bunches was dominated by white color and and round shape. Some examples the characteristics of bacterial isolates are shown in Figure 3.

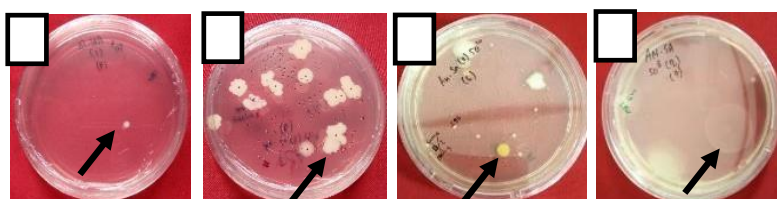


Figure 3. The characteristics of bacterial isolates from the extract suspensions of oil palm empty fruit bunches: (a) bacterial isolates with yellow color and round shape, (b) bacterial isolates with turbid white color and irregular shape, (c) bacterial isolates with yellow color and round shape, and (d) clear bacterial isolates and round shape.

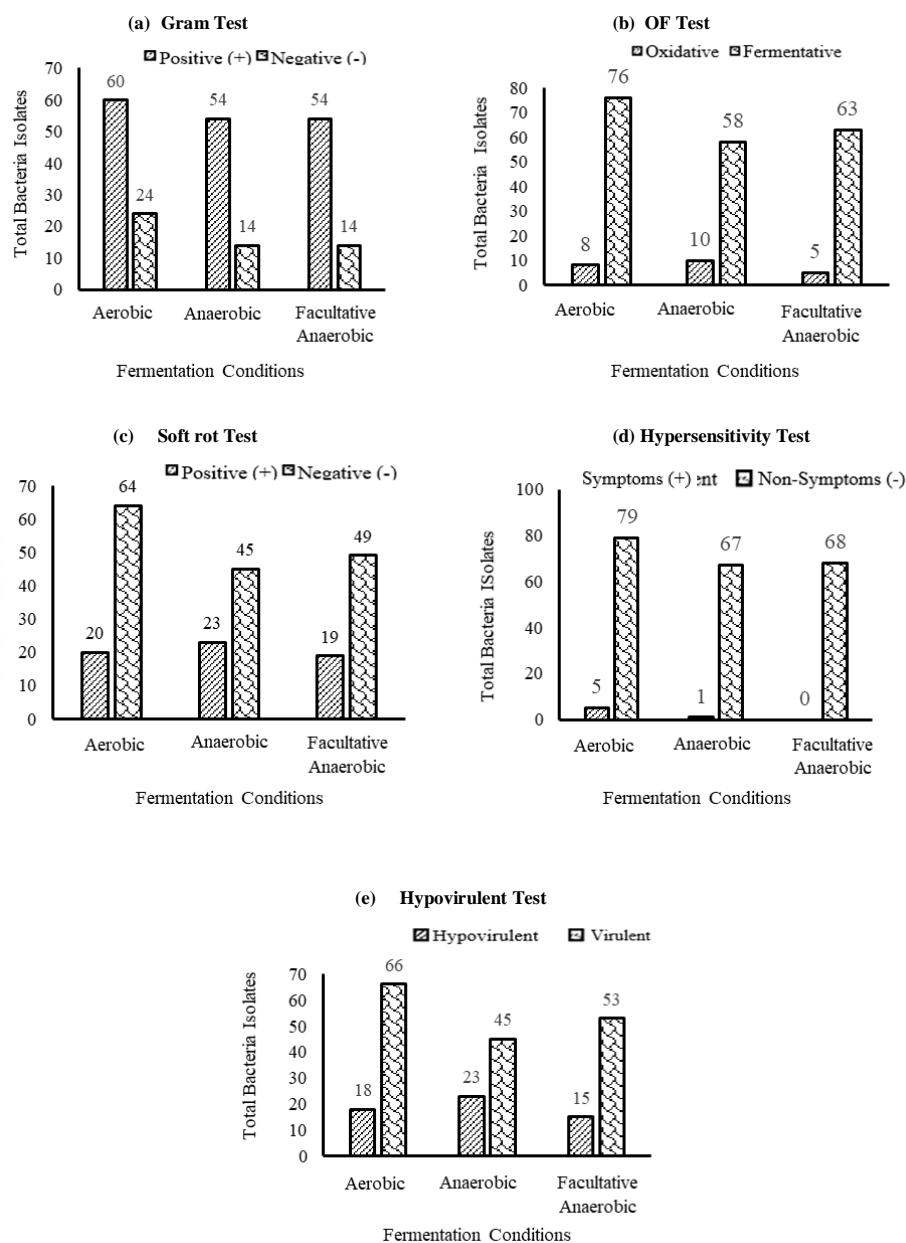


Figure 4. Bacterial characteristic based on the test results: (a) Gram Test, (b) OF Test, (c) Soft rot Test, (d) Hypersensitivity Test, and (e) Hypovirulent Test.

In the three fermentation conditions which were aerobic, anaerobic, and facultative anaerobic, dominating bacteria in the extract solutions of oil palm empty fruit bunches were 168 gram-positive bacterial isolates (76,36%), while gram-negative bacteria were 52 bacterial isolates (23,64%) (Figure 4a). Gram-positive bacterial isolates were 27,26% in the aerobic condition, 24,55% in the anaerobic condition, and 24,55% in the facultative anaerobic condition. On the other

hand, gram-negative bacterial isolates were 10,92% in the aerobic condition, 6,36% in the anerobic condition, and 6,36% in the facultative anaerobic condition. Gram-positive bacterial isolates were higher than gram-negative bacterial isolates. These results are in line with Lai et al. (2017) who also reported that the results of isolation of oil palm empty fruit bunches compost were dominated by gram-positive bacteria (8 bacterial isolates) followed by gram-negative bacteria (2 bacterial isolates). According to Mistou et al. (2016), gram-positive bacteria have cell walls that contain thick peptidoglycan while gram-negative bacteria have thin peptidoglycan content. Thin peptidoglycan content makes bacterial cells easily broken and destroyed when reacting with KOH (Chandra and Mani 2011). Gram negative bacteria have an outer membrane that contains lipopolysaccharide compounds, where these compounds play a role in influencing plant metabolic processes and damaging cell membranes, thus potentially as plant pathogens (Zhang et al. 2013).

The results of oxidative and fermentative tests indicated that the bacterial isolates were more fermentative compared to oxidative bacteria. Total 197 isolates (89,55%) Bacteria fermentative were 197 isolates (89,55%) and oxidative bacteria were 23 isolates (10,45%) (Figure 4b). Bacteria fermentative isolates were 34,55% in the aerobic condition, 26,36% in the anerobic condition, and 28,64% in the facultative anaerobic condition. On the other hand, the oxidative bacteria isolates were 3,64% in the aerobic condition, 4,54% in the anerobic condition, and 2,27% in the facultative anaerobic condition. According to Hadi et al. (2019), the oxidative bacteria included the aerobic bacteria, and the fermentative bacteria included the facultative anaerobic bacteria.

In the soft rot test, the bacterial isolates obtained showed a negative reaction (did not cause soft rot) more than a positive reaction (causing soft rot) (Figure 4c). Soft rot negative was as many as 158 isolates (71,82%) while soft rot positive was 62 isolates (28,18%). Bacterial isolates that showed soft rot positive reactions were 9,09% in the aerobic condition, 10,45% in the anerobic condition, and 8,64% in the facultative anaerobic condition. On the other hand, bacterial isolates that showed negative soft rot reactions were 29,09% in the aerobic condition, 20,46% in the anerobic condition, and 22,27% in the facultative anaerobic condition. According to Lee et al. (2014), bacteria that show positive reactions suspected as a pathogen because the bacteria produce the enzyme pectinase that can break down pectin and cellulose of the plant cell walls so that tuber system will be decomposed. Pectinolytic enzymes break down pectin which is an inter-cell adhesive in plant tissue. Factors affecting the development of soft rot in potato tubers are conditions (high temperature and humidity, low oxygen concentration, wound of host protective tissue) (Krejzar et al. 2008). Himel et al. (2016), *Dickeya* sp. and *Erwinia carotovora* are examples of pathogens that cause soft rot in fruits. From this statement, it is necessary to conduct a pathogenicity test in the form of the hypersensitive and hypovirulent test.

In the hypersensitive test, many bacterial isolates that react negatively were more compared to the bacterial isolates that react positively (cause symptoms necrosis). According to Balint-Kurti (2019), positive reactions occur due to cells that are physically penetration or direct contact with the pathogen so that the cells will die in the infection site. Thus, it was suspected that the bacterial isolates obtained were not dominated by pathogens because of the positive reaction bacteria were only 6 isolates (2,73%) out of 220 bacterial isolates obtained (Figure 4d). Bacterial isolates that showed hypersensitive symptoms were 2,27% in the aerobic condition and 0,46% in the anerobic condition. On the other hand, bacteria isolates that showed non hypersensitive symptoms were 35,91% in the aerobic condition, 30,45% in the anerobic condition, and 30,91% in the facultative anaerobic condition.

Furthermore, the experimental results showed that the characteristic of bacterial isolates from the extract suspension of oil palm empty fruit bunches were more virulent compared to hypovirulent. Virulent bacteria were 164 isolates (74,55%) and hypovirulent were 56 isolates (25,45%) (Figure 4e). Bacterial isolates that showed virulent were 30% in the aerobic condition, 20,46% in the anerobic condition, and 24,09% in the facultative anaerobic condition. On the other hand, bacterial isolates that show hypovirulent were 8,18% in the aerobic condition, 10,45% in the anerobic condition, and 6,82% in the facultative anaerobic condition. According to Sharma et al. (2018), hypovirulent is associated with a decreased ability of microbes in causing an illness, so it cannot produce a severe disease and can be used as a biocontrol agent.

Based on the results of this study, the extract suspension of oil palm empty fruit bunches the aerobic, anaerobic, and facultative anaerobic conditions produces various characteristics of bacterial isolates. Different bacterial characteristics indicate that each bacterial isolate came from different types so that the characteristics test can be used as a basis for identification of bacteria (Babu et al. 2017).

In conclusions, the bacterial abundance from the extract suspension of oil palm empty fruit bunches at the aerobic conditions were higher than the anaerobic conditions, and the anaerobic conditions were higher than the facultative anaerobic conditions. Moreover, the characteristics of most bacterial isolates from the extract suspension of oil palm empty fruit bunches were white color, round shape, including a gram-positive bacteria, fermentative, soft rot negative, did not cause necrosis of tobacco leaves, and virulent.

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260 ACKNOWLEDGMENTS

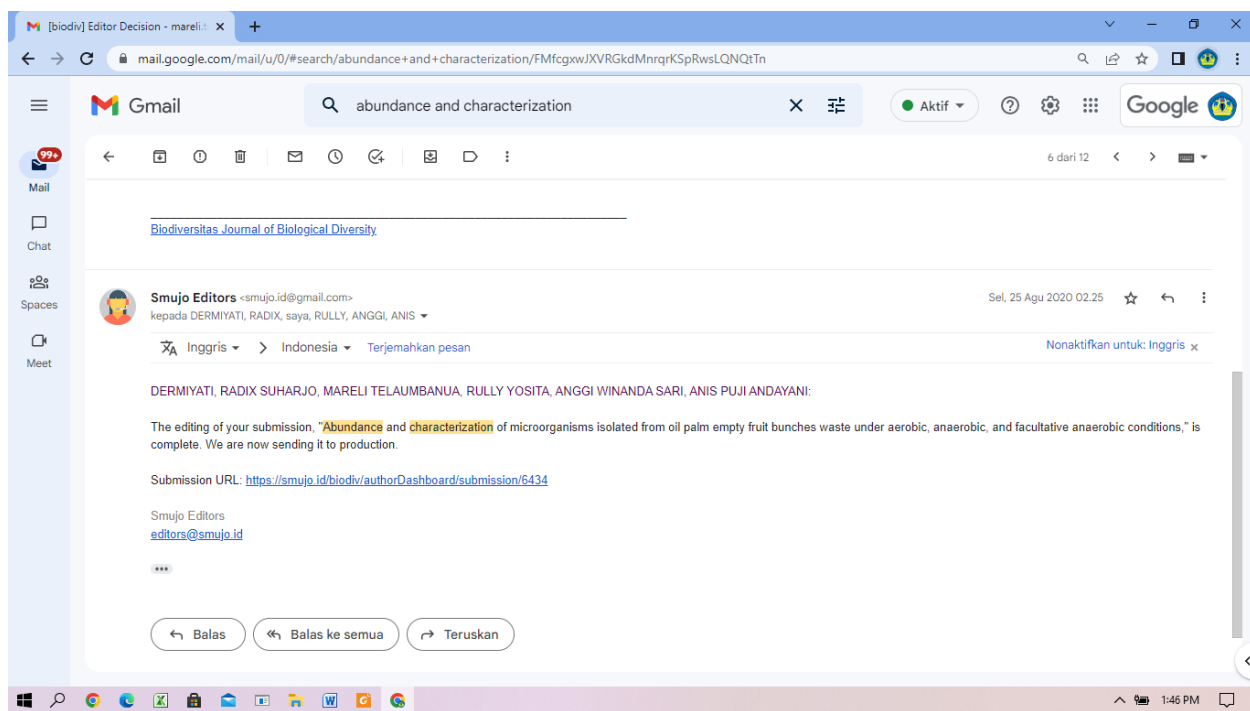
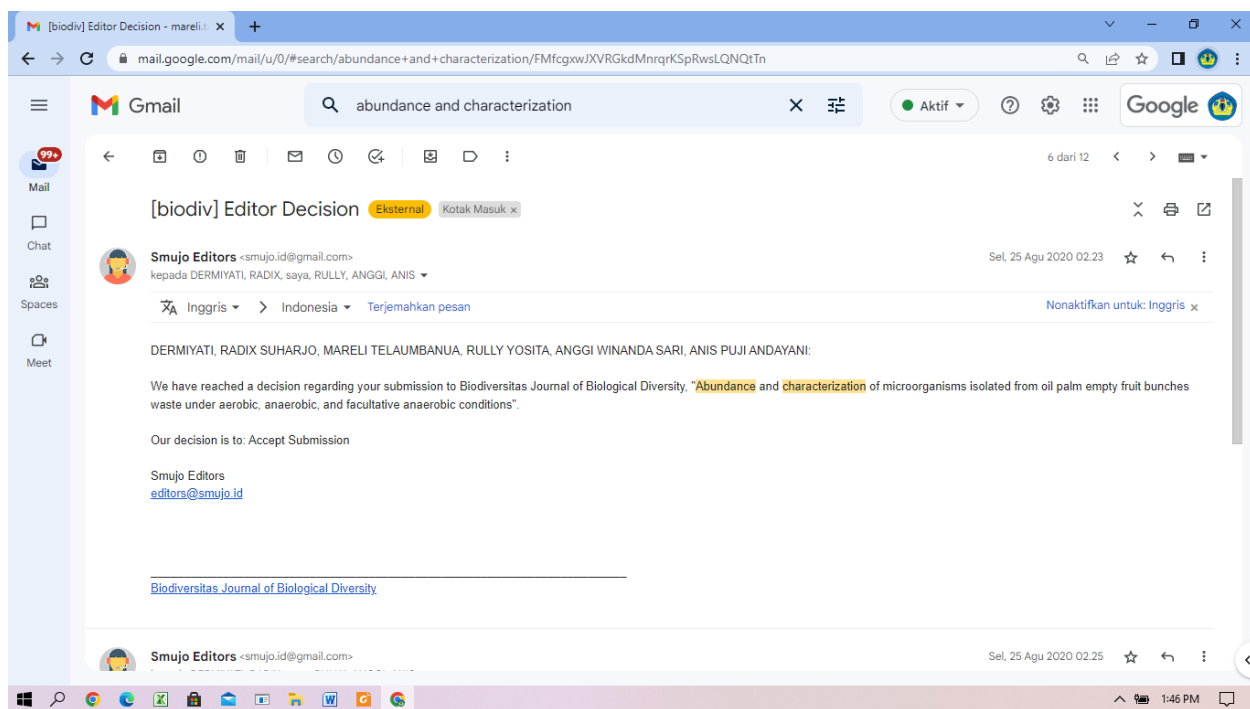
261 We are very grateful to the University of Lampung for "Hibah Profesor" from UNILA BLU's research grant. We also
262 thank the Research and Community Service Institution and the Faculty of Agriculture of the University of Lampung which

have facilitated the research and to PTPN VII, Rejosari, Natar, Lampung Province which has provided oil palm empty fruit bunches as research materials. We also would like to thank Dr. Kuswanta Futas Hidayat for his valuable comments.

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Abundance and characterization of microorganisms isolated from oil palm empty fruit bunches waste under aerobic, anaerobic, and facultative anaerobic conditions

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Manuscript received: 16 July 2020. Revision accepted: 21 August 2020.

Abstract. Dermiyati, Suharjo R, Telaumbanua M, Yosita R, Sari AW, Andayani AP. 2020. Abundance and characterization of microorganisms isolated from oil palm empty fruit bunches waste under aerobic, anaerobic, and facultative anaerobic conditions. *Biodiversitas* 21: 4213-4220. Increased production of palm oil causes an increase in oil palm empty fruit bunches waste, while the utilization of the waste has not been carried out optimally. Oil palm empty fruit bunches contain high cellulose content which promotes the development of various types of bacteria with different characteristics. This study was aimed to determine the abundance and the characterization of bacteria that were isolated from extract suspension of oil palm empty fruit bunches in the form of a local microorganism solution under aerobic, anaerobic, and facultative anaerobic conditions. For isolation, the oil palm empty fruit extract suspension was inoculated on media Plate Count Agar Peptone and bacterial abundance was determined. Various bacterial characterization test included bacterial morphology test in the form of color and shape of macroscopic isolates, gram test using KOH 3%, fermentative oxidative test, soft rot test on potato tubers, hypersensitivity test on tobacco plant leaves, and hypo virulent test which was indicated by the presence of symptoms of disease in cucumber sprouts and indicated in the disease severity index (DSI) was carried out. The results of local microorganism solution isolation from oil palm empty fruit bunches obtained as many as 220 bacterial isolates consisting of 84 isolates in aerobic condition, 68 isolates in anaerobic condition, and 68 isolates in facultative anaerobic condition. The highest bacterial abundance in aerobic conditions was equal to 285.60×10^{10} CFU mL⁻¹. Each bacterial isolate had different characteristics. Most of the characteristics of bacteria were turbid white (34.55%), round (69.55%), gram-positive (76.36%), fermentative (89.55%), softrot negative (71.82%), hypersensitive negative (97.27%), and virulent (74.55%).

Keywords: Abundance, bacteria, characteristics, local microorganisms

INTRODUCTION

Oil palm empty fruit bunches (EFB) is a kind of biomass waste formed during the production process of palm oil (Rahayu et al. 2019). Nowadays, the availability of oil palm empty fruit bunches as waste is increasingly abundant in line with the increased oil palm production and land area. According to USDA (2019), with further expansion in mature areas, palm oil production in Indonesia was forecast to reach 43 million tons in 2019/20, a 1.5 million ton increased from 2018/19. Moreover, as stated by Mariska (2019) that Indonesia's crude palm oil production was 34.7 million metric tons in August 2019, it rose by 14% compared to August 2018. Furthermore, Indonesia planting target in 2019 was 200,000 ha, however, the implementation was only about 20,000 ha and by 2022 the planting target is 700,000 ha. The increasing planting area would increase palm oil production as well as their wastes. Furthermore, Santi et al. (2019) reported that until

now the potential availability of oil palm empty fruit bunches as waste can reach 47 million tons per year, but so the utilization of the waste has not been carried out optimally.

Oil palm empty fruit bunches can be utilized as compost or an organic fertilizer which is useful to increase palm oil production and to improve physical, chemical, and biological soil properties (Yi et al. 2019). Furthermore, Yi et al. (2019) found that application of either compost or fertilizer alone increased fresh fruit bunch yield, from 23.24 to 25.49 or 25.55 t ha⁻¹yr⁻¹. Soil chemical properties such as soil pH, organic C, total-N and -P, available-P, exchangeable cations (K⁺, Ca²⁺, and Mg²⁺) and cation exchange capacity were significantly increased by the application of compost, making compost an effective organic material and nutrient source for improving the health of soils under oil palm cultivation. Other than that, oil palm empty fruit bunches can also be used as biofertilizer because it contains bacteria that can bind

nitrogen and phosphate solvents (Hoe et al. 2016), as degraders lignin bacteria (Tahir et al. 2019), as organic materials decomposers (Chin et al. 2017), as well as a fuel for power plant (Han and Kim 2018). Oil palm empty fruit bunches also contain abundant bacteria, high cellulose so it can be used as a source of bacterial energy for the biosynthesis process (Md Yunus et al. 2015; Rosli et al. 2017). Moreover, Rosli et al. (2017) found that the oil palm empty fruit bunches contain the polymeric carbohydrates such as cellulose and hemicellulose. Also, the oil palm empty fruit bunches fiber was made up of 31.4% of glucan, 18.6% xylan and 2.7% arabinan with the lignin accounted for around 23.9%.

Various studies have shown that bacterial isolates obtained from oil palm empty fruit bunches either in the fresh form, the weathered form, the compost form, the suspension extract form (local microorganisms solution) varied. The research of Ariana et al. (2017) resulted in the isolation of as many as 430 microbes from oil palm empty fruit bunches. Also, Harith et al. (2014) reported that 24 bacterial isolates were recovered from oil palm industry waste. Besides, Lai et al. (2017) obtained 34 bacterial isolates that were successfully isolated from compost oil palm empty fruit bunches. The abundance of bacteria can also be obtained through extract suspension of oil palm empty fruit bunches in the form of a local microorganism solution. A local microorganism solution is a fermented liquid from plant or animal waste and contain microorganisms with different species in an area due to differences in environmental conditions (Adrizal et al. 2017). Besides, a local microorganism solution contains macro and micronutrient elements and also contains bacteria which potentially as a material overhaul organic, growth stimulant, and as a pest and disease control agent plant. Astriani et al. (2017) isolated 3 kinds of local microorganism solution samples from vegetable waste, then they obtained 79 bacterial isolates with the characteristics of most bacteria that were round, uneven edges, and white colonies.

The diversity of abundance and characterization of bacteria isolated from extract suspension of local microorganisms solution depends on the source of raw material used as well as the conditions during fermentation. So far, the information regarding the fermentation conditions under aerobic, anaerobic, and facultative anaerobic are not available yet. These conditions may affect the growth of bacteria which is related to available oxygen during the fermentation processes. Therefore, this study was aimed to explore the abundance and characterization of indigenous bacteria isolated from the extract suspension based on oil palm empty fruit bunches under a different fermentation process namely aerobic, anaerobic, and facultatively anaerobic.

MATERIALS AND METHODS

Development of local microorganism solutions from oil palm empty fruit bunches

Local Microorganism solution was developed using

oil palm empty fruit bunches in three conditions, namely aerobic, anaerobic, and facultative anaerobic as described by Dermiyati et al. (2019). Making a suspension of local microorganisms was done by mixing 2.5 kg of finely chopped oil palm empty fruit bunches, 0.5 kg brown sugar, 2.5 L rice washing water, and 2.5 L coconut water, then put into the reactor machine and each fermentation condition contains the same ingredients. The process of making local microorganism solution was performed using a reactor machine developed by Telaumbanua et al. (2019). Oil palm empty fruit bunches were taken from PT Perkebunan Nusantara (PTPN) VII, Rejosari, Natar, Lampung Province, Indonesia.

Bacterial isolation and propagation

Bacterial isolation and bacterial propagation were performed as per the method described by Dermiyati et al. (2019).

Bacterial isolation

All the collected samples were diluted at 10^{-8} , 10^{-10} , and 10^{-12} . From each dilution series, 50 μ L of the suspension were spread into sterile plastic petri dish (diameter 9 cm) containing Plate Count Agar Peptone (PCAP) media (plate count agar (PCA; OXOID®; England) 17.4 g, peptone (OXOID®; England) 2.5 g, agar 2 g, and 1000 mL distilled water). Observation of the total bacterial colonies which were emerged was performed every day for 7 days.

Bacterial propagation

The emerging bacteria were purified by placing into a sterile plastic Petri dish (9 cm in diameter) containing Yeast Peptone Agar (YPA) peptone media (OXOID®, England) 10 g, Yeast Extract (HIMEDIA®, India) 5 g, agar 20 g, and 1000 mL distilled water). For further analysis, the bacteria were streaked into Potato Peptone Glucose Agar (PPGA) slant media.

Bacterial abundance

Bacterial abundance was shown by the number of bacterial populations through observing the numbers of bacterial isolates that grew on Plate Count Agar Peptone media for 7 days after isolation such as that explained above.

Bacterial characteristics

Bacterial characteristics were seen based on bacterial morphology test, Gram's test with 3% KOH, Oxidation-Fermentation, or the OF test, soft rot test on potato tubers, hypersensitive test, and hypovirulent test.

Bacterial morphology was confirmed by observing the bacterial isolates from color and shape (Linda et al. 2018). The morphological characteristics test of the bacteria was carried out by observing the bacteria that grew on PCAP media for 7 days after spreading.

Gram's test with KOH 3% was performed using the method by Chandra and Mani (2011). The nature of bacteria was divided into two, namely a gram-positive if it did not produce mucus when reacting with KOH 3% and a

gram-negative which produced mucus when reacting with KOH 3%.

Oxidation-fermentation test was performed based on the method described by Cymru and Wales (2010), it used 2 test tubes containing media with one of the tubes was added as much as 1 mL of paraffin oil after bacterial isolates were inserted into the media and then it was observed for 7 days discoloration on the media. Change the color of the media from green to yellow on both tubes indicated the bacteria was fermentative, while if discoloration occurred in the tube which was given by paraffin oil then the bacteria was oxidative.

Soft rot test was done based on the method by Khlaif and Wreikat (2018) through scratching bacterial isolates in the middle of the potato tuber and the reaction was observed 24 hours. Negative reactions were indicated by not any changes in potato tubers, while positive reactions occurred when it was the decay and soft stabbed in the middle of a potato tuber carved by bacteria.

Hypersensitive test was prepared based on the method by Istiqomah and Kusumawati (2018) by injecting a bacterial isolate suspension into the tissue of the lower surface of the tobacco leaf. Positive response from tobacco plants if there was a necrosis of the leaves which was injected with a bacterial suspension at 24 hours after application.

Hypovirulent test was performed according to the method of Suharjo et al. (2018). The test used a cucumber as an indicator plant and observe the development of the symptoms of the disease in the hypocotyl or other parts of the sprouts for 14 days, then the results were stated as the disease severity index (DSI). If the isolate is not showing disease symptoms or symptoms caused by cucumber sprouts only a little ($DSI < 2.0$), the isolate is categorized as hypovirulent.

RESULTS AND DISCUSSION

The results of bacterial isolates were isolated from the extract suspension of oil palm empty fruit bunches with their isolate codes are shown in Table 1. This study obtained as many as 220 bacterial isolates consisting of 84 isolates under fermentation in aerobic conditions, 68 isolates in anaerobic condition, and 68 isolates in facultative anaerobic conditions. The highest bacterial isolates were in the aerobic conditions which were related to the need for oxygen for the growth of the bacteria. Aerobic bacteria are the species of bacteria which require oxygen for their basic survival, growth, and the process of reproduction (Tortora 2019).

The highest bacterial population resulting from the isolation of the extract suspension of oil palm empty fruit bunches was in the aerobic condition of 285.60×10^{10} CFU

mL^{-1} , followed by the anaerobic condition of 230.27×10^{10} CFU mL^{-1} , and the facultative anaerobic condition of 175.13×10^{10} CFU mL^{-1} (Figure 1). This is supported by Zainudin et al. (2013) that bacteria contained in the compost of oil palm empty fruit bunches were dominated by bacteria *Bacillus* sp., *Paenibacillus* sp., *Pseudomonas* sp., and *Cellulomonas* sp, where the bacteria are included in the aerobic bacteria. Also, aerobic conditions will allow bacteria to grow properly with sufficient oxygen.

Some examples of the isolation results from the extract suspension of oil palm empty fruit bunches at three fermentation conditions are shown in Figure 2. Figure 2.A (i, ii, and iii) are the results of isolation of bacteria in the aerobic condition, Figure 2.B (i, ii, and iii) in the anaerobic condition, while Figure 2.C (i, ii, and iii) in the facultative anaerobic condition. The three conditions of fermentation show an abundance of different bacteria.

Examining the characteristics of bacterial isolates from the extract suspension of oil palm empty fruit bunches in the form of color and shape are presented in Table 2. Of the 220 bacterial isolates obtained, there were 13 types of characteristics in aerobic conditions, 14 types in anaerobic conditions, and 11 types in facultative anaerobic conditions. However, the characteristics of bacterial isolates obtained were dominated by round white followed by turbid white, irregular and round white turbid under all conditions of fermentation. Characteristics of bacteria that are white and round in aerobic conditions are 20.23%, anaerobic conditions are 19.12%, and facultative anaerobic conditions are 27.94%.

The color and shape of bacterial isolates from the extract suspension of oil palm empty fruit bunches were dominated by white color and round shape. Some examples of the characteristics of bacterial isolates are shown in Figure 3.

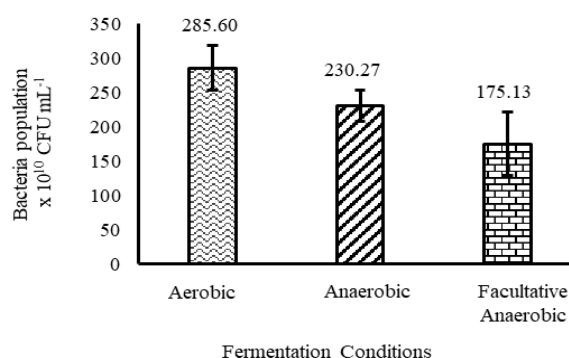


Figure 1. The population of the indigenous bacteria in the extract suspension of oil palm empty fruit bunches

Table 1. Bacteria isolates from extract suspension of oil palm empty fruit bunches in aerobic, anaerobic, and facultative anaerobic conditions

Aerobic condition		Anaerobic condition		Facultative Anaerobic condition	
No.	Isolate code	No.	Isolate code	No.	Isolate code
1	ASP1	1	ANSP1	1	SSP1
2	ASP2	2	ANSB1	2	SSP2
3	ASB1	3	ANSB2	3	SSP3
4	ASPKR1	4	ANSB3	4	SSP4
5	ASP3	5	ANSK1	5	SSPKR1
6	ASB2	6	ANSK2	6	SSPKR2
7	ASB3	7	ANSK3	7	SSK1
8	ASK1	8	ANSM1	8	SSP5
9	ASPKR2	9	ANSM2	9	SSPKR3
10	ASPKR3	10	ANSM3	10	SSPKR4
11	ASM1	11	ANSM4	11	SSP6
12	ASK2	12	ANSP2	12	SSPKR5
13	ASM2	13	ANSPKR1	13	SSPKR6
14	ASPKR4	14	ANSK4	14	SSPKR7
15	ASPKR5	15	ANSPKR2	15	SSP7
16	ASP4	16	ANSPKR3	16	SSP8
17	ASPKR6	17	ANSP3	17	SSB1
18	ASPKR7	18	ANSPKR4	18	SSPKR8
19	ASPKR8	19	ANSK5	19	SSM1
20	ASP5	20	ANSPKR5	20	SSM2
21	ASP6	21	ANSP4	21	SSPKR9
22	ASPKR9	22	ANSPKR6	22	SSK2
23	ASP7	23	ANSK6	23	SSP9
24	ASK3	24	ANSB4	24	SSPKR10
25	ASP8	25	ANSB5	25	SSPKR11
26	ASPKR10	26	ANSP5	26	SSB2
27	ASP9	27	ANSP6	27	SSB3
28	ASP10	28	ANSB6	28	SSB4
29	ASP11	29	ANSK7	29	SSPKR12
30	ASK4	30	ANSPKR7	30	SSPKR13
31	ASPKR11	31	ANSP7	31	SSPKR14
32	ASPKR12	32	ANSPKR8	32	SSP10
33	ASP12	33	ANSP8	33	SSPK1
34	ASK5	34	ANSPKR9	34	SSP11
35	ASPKR13	35	ANSP9	35	SSPKR15
36	ASK6	36	ANSK8	36	SSK3
37	ASM3	37	ANSK9	37	SSP12
38	ASK7	38	ANSM5	38	SSB5
39	ASB4	39	ANSPKR10	39	SSP13
40	ASB5	40	ANSB7	40	SSPKR16
41	ASB6	41	ANSP10	41	SSPKR17
42	ASP13	42	ANSB8	42	SSP14
43	ASPKR14	43	ANSP11	43	SSPKR18
44	ASPKR15	44	ANSB9	44	SSP15
45	ASPKR16	45	ANSPKR11	45	SSP16
46	ASK8	46	ANSPKR12	46	SSP17
47	ASB7	47	ANSP12	47	SSK4
48	ASPKR17	48	ANSKP1	48	SSP18
49	ASO1	49	ANSP13	49	SSP19
50	ASPKR18	50	ANSPK1	50	SSP20
51	ASK9	51	ANSP14	51	SSPKRL1
52	ASP14	52	ANSPKRL1	52	SSPKRL2
53	ASB8	53	ANSP15	53	SSPB1
54	ASPKR19	54	ANSPK2	54	SSPK2
55	ASP15	55	ANSPRL1	55	SSPRL1
56	ASPKR20	56	ANSP16	56	SSPKR19
57	ASP16	57	ANSPB1	57	SSB6
58	ASK10	58	ANSPK3	58	SSPB2
59	ASPK1	59	ANSP17	59	SSPKR20
60	ASP17	60	ANSPKR13	60	SSPB3
61	ASP18	61	ANSPKR14	61	SSPKR21
62	ASPKRL1	62	ANSPKR15	62	SSPKR22
63	ASP19	63	ANSPB2	63	SSB7
64	ASPK2	64	ANSPKR16	64	SSPKR23
65	ASP20	65	ANSPKR17	65	SSPKR24
66	ASPK1	66	ANSPKR18	66	SSPB4
67	ASP21	67	ANSPB3	67	SSPKR25
68	ASPKRL1	68	ANSPKR19	68	SSB8
69	ASPB1				
70	ASPKR21				
71	ASPKR22				
72	ASPKR23				
73	ASPKR24				
74	ASPK3				
75	ASPKR25				
76	ASB9				
77	ASPKR26				
78	ASPB2				
79	ASPKR27				
80	ASP22				
81	ASB1				
82	ASB11				
83	ASBK1				
84	ASPKR28				

Table 2. The characteristics of bacterial isolates from the extract suspensions of oil palm empty fruti bunches in the aerobic, anaerobic, and facultative anaerobic conditions

Fermentation Condition*)	Characteristics*)
Aerobic (84)	Round White (17), Irregular White (6), Round Clear (10), Irregular Clear (1), Round Yellow (10), Round Red (3), Round Turbid White (14), No Turbid White Regular (15), Round Solid Yellow (1), Round Yellowish White (3), Round Clear White (2), Irregular Yellowish Clear (1), Round Orange (1)
Anaerobic (68)	Round White (13), Irregular White (5), Round Clear (6), Irregular Clear (3), Round Yellow (8), Irregular Yellow (1), Round Red (5), Round Turbid White (11), Irregular Turbidity White (9), Round Solid Yellow (1), Round Yellowish White (2), Irregular Yellowish White (1), Round Clear White (2), Irregular Clear White (1)
Facultative Anaerobic (68)	Round White (19), Irregular White (2), Round Turbid White (8), Irregular White Turbid (19), Round Yellow (4), Round Clear (7), Irregular Clear (2), Round Red (2), Round Yellowish White (2), Round Clear White (2), Irregular Clear White (1)

Note: *) The number in parentheses is the number of bacterial isolates

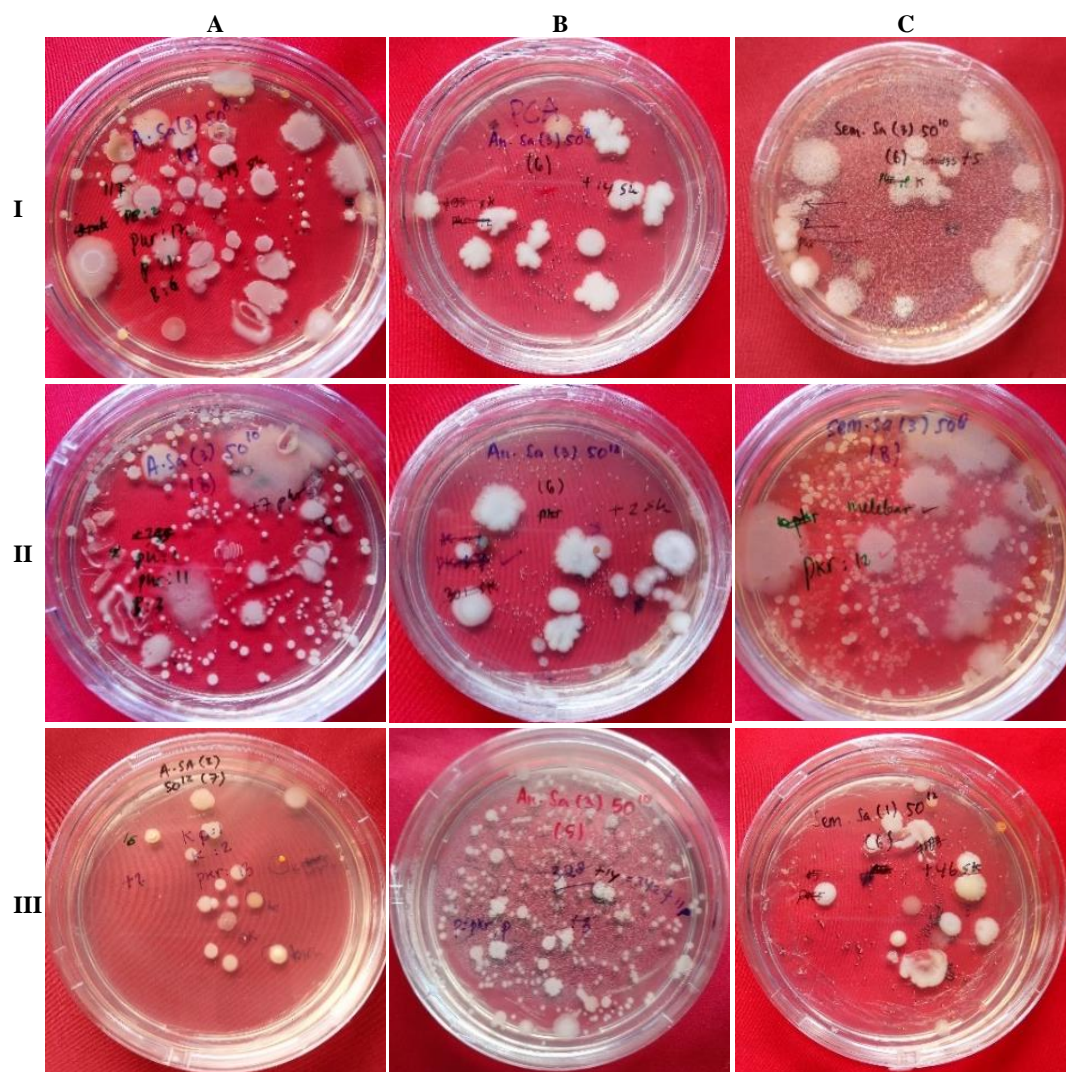


Figure 2. Examples of the abundance of bacteria isolate from the extract suspension of EFB in aerobe, anaerobe, and facultative anaerobe

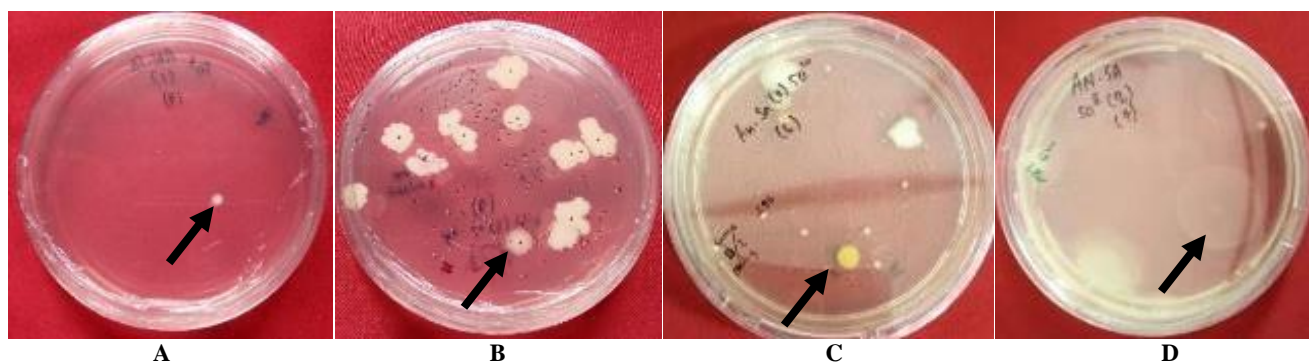


Figure 3. The characteristics of bacterial isolates from the extract suspensions of oil palm empty fruit bunches: A. Bacterial isolates with yellow color and round shape, B. Bacterial isolates with turbid white color and irregular shape, C. Bacterial isolates with yellow color and round shape, D. Clear bacterial isolates and round shape

In the three fermentation conditions which were aerobic, anaerobic, and facultative anaerobic, dominating bacteria in the extract solutions of oil palm empty fruit bunches were 168 gram-positive bacterial isolates

(76.36%), while gram-negative bacteria were 52 bacterial isolates (23.64%) (Figure 4.A). Gram-positive bacterial isolates were 27.26% in the aerobic condition, 24.55% in the anaerobic condition, and 24.55% in the facultative

anaerobic condition. On the other hand, gram-negative bacterial isolates were 10.92% in the aerobic condition, 6.36% in the anaerobic condition, and 6.36% in the facultative anaerobic condition. Gram-positive bacterial isolates were higher than gram-negative bacterial isolates. These results are in line with Lai et al. (2017) who also reported that the results of isolation of oil palm empty fruit bunches compost were dominated by gram-positive bacteria (8 bacterial isolates) followed by gram-negative bacteria (2 bacterial isolates). According to Mistou et al. (2016), gram-positive bacteria have cell walls that contain thick peptidoglycan while gram-negative bacteria have thin peptidoglycan content. Thin peptidoglycan content makes bacterial cells easily broken and destroyed when reacting with KOH (Chandra and Mani 2011). Gram-negative bacteria have an outer membrane that contains lipopolysaccharide compounds, where these compounds play a role in influencing plant metabolic processes and damaging cell membranes, thus potentially as plant pathogens (Zhang et al. 2013).

The results of oxidation-fermentation tests indicated that the bacterial isolates were more fermentative compared to oxidative. Total 197 isolates (89.55%) were bacteria fermentative and oxidative bacteria were 23 isolates (10.45%) (Figure 4.B). Bacteria fermentative isolates were 34.55% in the aerobic condition, 26.36% in the anaerobic condition, and 28.64% in the facultative anaerobic condition. On the other hand, the oxidative bacteria isolates were 3.64% in the aerobic condition, 4.54% in the anaerobic condition, and 2.27% in the facultative anaerobic

condition. According to Hadi et al. (2019), the oxidative bacteria included the aerobic bacteria, and the fermentative bacteria included the facultative anaerobic bacteria.

In the soft rot test, the bacterial isolates obtained showed a negative reaction (did not cause soft rot) more than a positive reaction (causing soft rot) (Figure 4.C). Soft rot negative was as many as 158 isolates (71.82%) while soft rot positive was 62 isolates (28.18%). Bacterial isolates that showed soft rot positive reactions were 9.09% in the aerobic condition, 10.45% in the anaerobic condition, and 8.64% in the facultative anaerobic condition. On the other hand, bacterial isolates that showed negative soft rot reactions were 29.09% in the aerobic condition, 20.46% in the anaerobic condition, and 22.27% in the facultative anaerobic condition. According to Lee et al. (2014), bacteria that show positive reactions suspected as a pathogen because the bacteria produce the enzyme pectinase that can break down pectin and cellulose of the plant cell walls so that tuber system will be decomposed. Pectinolytic enzymes break down pectin which is an inter-cell adhesive in plant tissue. Factors affecting the development of soft rot in potato tubers are conditions (high temperature and humidity, low oxygen concentration, wound of host protective tissue) (Krejzar et al. 2008). Himel et al. (2016), *Dickeya* sp. and *Erwinia carotovora* are examples of pathogens that cause soft rot in fruits. From this statement, it is necessary to conduct a pathogenicity test in the form of the hypersensitive and hypovirulent test.

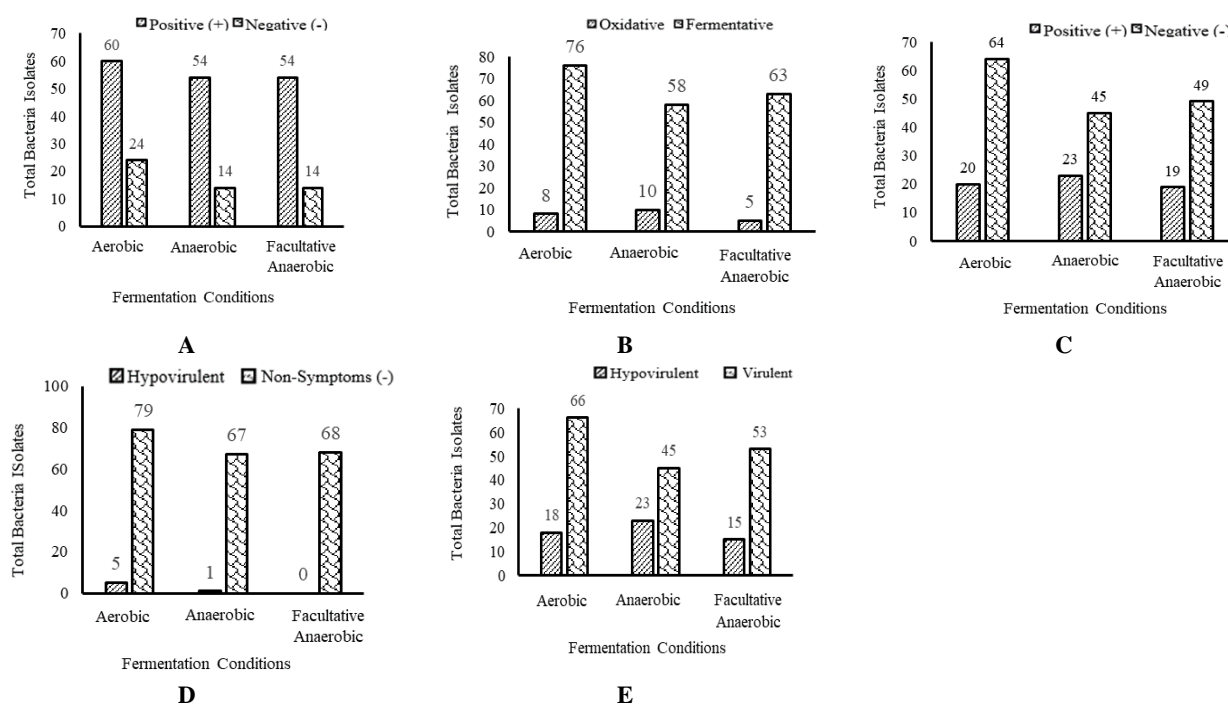


Figure 4. Bacterial characteristic based on the test results: A. Gram Test, B. OF Test, C. Soft rot Test, D. Hypersensitivity Test, E. Hypovirulent Test

In the hypersensitive test, many bacterial isolates that react negatively were more compared to the bacterial isolates that react positively (cause symptoms necrosis). According to Balint-Kurti (2019), positive reactions occur due to cells that are physically penetration or direct contact with the pathogen so that the cells will die in the infection site. Thus, it was suspected that the bacterial isolates obtained were not dominated by pathogens because of the positive reaction bacteria were only 6 isolates (2.73%) out of 220 bacterial isolates obtained (Figure 4.D). Bacterial isolates that showed hypersensitive symptoms were 2.27% in the aerobic condition and 0.46% in the anaerobic condition. On the other hand, bacterial isolates that showed non-hypersensitive symptoms were 35.91% in the aerobic condition, 30.45% in the anaerobic condition, and 30.91% in the facultative anaerobic condition.

Furthermore, the experimental results showed that the characteristic of bacterial isolates from the extract suspension of oil palm empty fruit bunches was more virulent compared to hypovirulent. Virulent bacteria were 164 isolates (74.55%) and hypovirulent were 56 isolates (25.45%) (Figure 4.E). Bacterial isolates that showed virulently were 30% in the aerobic condition, 20.46% in the anaerobic condition, and 24.09% in the facultative anaerobic condition. On the other hand, bacterial isolates that show hypovirulent were 8.18% in the aerobic condition, 10.45% in the anaerobic condition, and 6.82% in the facultative anaerobic condition. According to Sharma et al. (2018), hypovirulent is associated with a decreased ability of microbes in causing illness, so it cannot produce severe disease and can be used as a biocontrol agent.

Based on the results of this study, the extract suspension of oil palm empty fruit bunches the aerobic, anaerobic, and facultative anaerobic conditions produces various characteristics of bacterial isolates. Different bacterial characteristics indicate that each bacterial isolate came from different types so that the characteristics test can be used as a basis for identification of bacteria (Babu et al. 2017).

In conclusion, the bacterial abundance from the extract suspension of oil palm empty fruit bunches at the aerobic conditions was higher than the anaerobic conditions, and the anaerobic conditions were higher than the facultative anaerobic conditions. Moreover, the characteristics of most bacterial isolates from the extract suspension of oil palm empty fruit bunches were white color, round shape, including a gram-positive bacteria, fermentative, soft rot negative, did not cause necrosis of tobacco leaves, and virulent.

ACKNOWLEDGEMENTS

We are very grateful to the University of Lampung (Unila) Bandar Lampung, Indonesia for "Hibah Profesor" from UNILA BLU's research grant. We also thank the Research and Community Service Institution and the Faculty of Agriculture of the University of Lampung which have facilitated the research and to PTPN VII, Rejosari,

Natar, Lampung Province which has provided oil palm empty fruit bunches as research materials. We also would like to thank Dr. Kuswanta Futas Hidayat for his valuable comments.

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