ISOLATION OF CELLULOSE DEGRADING MICROORGANISMS IN OIL PALM EMPTY FRUITS BUNCH (OPEFB)

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ISOLATION OF CELLULOSE DEGRADING MICROORGANISMS IN OIL PALM EMPTY FRUITS BUNCH (OPEFB)

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Abstract

Tanda kosong kelapa sawit (TKKS) adalah salah satu limbah padat yang dihasilkan oleh pabrik industri. Tandan tersebut merupakan habitat dari bakteri selulolitik. Penelitian ini bertujuan untuk mengidentifikasi morfologi dan menentukan potensi mikroorganisme dalam mendegradasi limbah tandan kosong kelapa sawit. Metode yang digunakan dalam penelitian ini adalah isolasi dan pengamatan morfologi bakteri, skrining bakteri, pendegradasi limbah, uji degradasi limbah tandan kosong kelapa sawit, serta uji aktivitas enzim dengan metode DNS menggunakan spektrofotometer UV-Vis pada panjang gelombang 530 nm. Hasil yang diperoleh, yaitu terdapat 11 jumlah isolat dari proses isolasi tersebut. Zona bening terbesar ditunjukkan oleh isolat S_{10}. Potensi biodegradasi terbesar ditunjukkan oleh isolasi S_{10} selama masa inkubasi tujuh hari dengan persentasi degradasi sebesar 13,27%. Karakteristik morfologi bakteri yang dihasilkan ialah koloni melingkar dan berukuran sedang, koloni berwarna kuning, koloni bertepian gelombang dan rata, elevasi cembung, serta permukaannya halus. Aktivitas enzim yang dihasilkan adalah 0,1308 U mL^{-1}. Dengan demikian, isolat S_{10} diduga kuat berpotensi dalam mendegradasi plastik.

Keywords: degradasi plastik; enzim selulase; metode DNS; S_{10} bacterium isolate; TKKS

The oil palm empty fruit bunch (OPEFB) is one of the solid wastes produced by industrial factories. These bunches are the habitat of cellulolytic bacteria. This study aims to identify the morphology and determine the potential microorganisms in degrading oil palm empty fruit bunches waste. The methods used in this study were isolation and observation of bacterial morphology, bacterial screening, degradation test of empty oil palm fruit bunches waste, and enzyme activity test with DNS method using UV-Vis spectrophotometer at wavelength of 530 nm. The results obtained were 11 total isolates from the isolation process. The largest clear zone was shown by isolate S_{10}. The greatest potential for biodegradation was shown by the isolation of S_{10} during the seven-days incubation period with a degradation percentage of 13.27%. The morphological characteristics of the bacteria produced have circular and medium-sized colonies, yellow, colonies wave edges and flat colonies, elevation convex and smooth surface. The resulting enzyme activity was 0.1308 U/mL. Therefore, isolate S_{10} was suspected to have the potential to degrade plastic.

Keywords: Cellulase enzyme; degradation of plastic, DNS method; OPEFB; S_{10} bacterium isolate

Introduction

An Oil palm is a plant that is tolerant of the surrounding environment. There are many oil palm plantations in South Sulawesi, especially in East Luwu. There are many palm oil factories in the area. Every factory processes palm oil to crude oil which is then exported only to foreign countries for reprocessing. The fresh fruit will be processed to produce crude oil. In addition to producing oil, these products produce by-products, both in the form of liquids and solids. Liquid waste is waste generated from fruit washing and equipment used when processing palm oil, while solid waste is in the form of oil palm empty fruit bunch (OPEFB).

OPEFB is a very abundant solid waste of palm oil mills. Waste generated by EFB reached 5,050,367.60 tons in 2010 and 5,176,842.53 tons in 2011. The waste generated by factories will increase every year due to the increasing demand for palm oil nationally (Tarkono and Ali, 2015). If the OPEFB is in a pile within a few days, it will cause an unpleasant odor so that it can pollute the surrounding environment. OPEFB takes a long time to decompose because it contains cellulose(Rahmasita et al., 2017).

Cellulose is a polysaccharide which is hydrolyzed to produce cellobiose and glucose monomers. A single cellulose molecule is a straight chain polymer of β-1,4-glucoside linked by glycosidic bonds (Razie et al., 2011). Cellulose can be degraded using cellulase enzymes. Enzymes can generally be obtained from animals and plants that carry out reactions such as hydrolysis, oxidation, reduction, isomerization, addition, radical transfer, and termination of carbon chains (Supriyatna et al., 2015). In addition to plants and animals, enzymes can also be produced by microorganisms.

Several studies have used microorganisms as the producer of cellulase enzymes to degrade cellulose. Ekawati et al. (2012) reported five isolates of the genus *Pseudomonas* sp. which is able to degrade cellulose very well, namely *Cellvibrio* sp1. UV3 (74%), *Cytophaga* sp2. UV1 (77.6%), *Cytophaga* sp2. UV5 (78.6%); *Cellvibrio* sp2. UV4 (83.1%); and *Micrococcus* sp. UV2 (84.1%). Purkan et al. (2015) stated that Aspergillus niger reached a log phase of 0 to 4 hours, then significant growth was experienced up to 24 hours. The optimum activity of the cellulase enzyme at pH 4 was obtained at 0.324 IU/mL.

Murtiyaningsih and Hazmi (2017) obtained the daily activity of cellulase enzymes from bacteria isolated from waste soil on the 9th day. The value is about 0.135 nKat which produces a reducing sugar of 0.0874471 mg/mL. The results obtained are directly proportional to the cellulase activity and reducing sugar content.

Measurement of bacterial activity based on the amount of glucose produced using a UV-Vis spectrophotometer by measuring its absorbance at a wavelength of 540 nm using the di-nitro salicylic acid (DNS) method reacts with reducing sugars to produce 3-amino-5-dinitrosalicylate (Murtiyaningsih and Hazmi, 2017). DNS can be used to measure reducing sugars formed by microbes and can be applied to sugars that have even low levels (Argo and Yulianingsih, 2013).

Based on this background, the aim of this study is to screen bacterial isolates from OPEFB which have the potential to degrade cellulose.

Methodology

Materials

A sample, oil palm empty fruit bunch (OPEFB), originated from palm oil industry of PPTP XXVIII in Burau District, Luwu Timur Regency. The sampling was done with three points. Soil samples were taken with a depth of 7-15 cm measured from the soil surface.

Carboxymethyl cellulose (Merck, Germany), congo red 1% (Merck, Germany), yeast extract, glucose, dipotassium phosphate (K_2HPO_4), potassium nitrate (KNO_3), magnesium sulfate ($MgSO_4$), sodium chloride

(NaCl), nutrient agar (NA), nutrient broth (NB), and DNS (3,5-dinitro salicylic acid) reagents.
UV-Vis spectrophotometry Geneys 20 (Varian, USA), cold sentrifugator Z 366 K (Hermele, Germany), shaker MASQ 7000 (Thermo Scientific, USA), hareus incubator (Thermo Scientific, Germany), GmbH oven (Memmert, Germany), yx-280D autoclave (GEA, Germany), laminar air flow isocide 14644-1 (Esco, Singapore), magnetic stirrer (Health, United States), US 200 sieve shaker (Retsch, United States), vortex mixer wizard (Velp Scientifica, Italy).

Procedure

Isolation of cellulase-producing bacteria

Bacterial isolation was carried out using the dilution method. A sample of 1 g was put into 9 mL of 85% NaCl solution, then homogenized using a vortex. Dilution series was made up to 10^{-7} (Peristiwati et al., 2018). After that, about 0.1 mL of the sample was taken from dilution of 10^{-5},10^{-6}, and 10^{-7} were put into petri dishes that already contained selective media with the distribution method(Murtiyaningsih and Hazmi, 2017). The resulting morphology was inoculated on the same medium using the quadrant line method, then incubated at 37 °C for 48 hours (Arifin et al., 2019).

Screening of bacterial isolate using measurement of cellulolytic index

A total of one isolate was grown on CMC agar media and incubated at 37 °C for 48 hours. After that, the isolates were dripped with 0.1% congo red dye and allowed to stand for 20 minutes. Then the sample was washed with 1 M NaCl and the clear zone was observed. The presence of a clear zone shows a positive (+) result due to the degradation of cellulose by the cellulase enzyme (Peristiwati et al., 2018). The clear zone can be measured using a caliper and the value between the diameter of the medium clear zone and the diameter of the bacterial colony is calculated (Rawway et al., 2018). It is expressed as the Cellulolytic Index (IS).

$$CI = \frac{DCZ - DC}{DC}$$

which CI is cellulolytic index; DCZ is diameter of clear zone; DC is diameter of colony

Determination of dry weight percentage

The test was carried out to determine the ability to degrade cellulose. It was done using four pieces of OPEFB soaked in detergent for one minute and rinsed with distilled water. Removal of water content was cariied out at a temperature of 105 °C for five hours (Yusnia et al., 2019).
One oce of isolates with high clear zone was inoculated into 25 mL of CMC liquid media. The incubation of isolates was carried out at room temperature for 24 hours at a speed of 180 rpm. Approximately 10 mL of incubation results were inoculated into 100 mL of CMC liquid medium. Then the OPEFB known its initial dry weight was put into the CMC liquid medium. After that, the incubation process was run for 10 days at a speed of 180 rpm (Murtiyaningsih and Hazmi, 2017).

$$\Delta W\,(\%) = \frac{W_i - W_f}{W_i}$$

which ΔW is loss of dried weight; W_i is initial weight before degradation process; W_f is final weight after degradation process

Enzyme Activity Test using DNS Method

The test was carried out using 3,5-dinitro salissilic acid (DNS) reagent. A mixture of 1% CMC and 1 mL of crude extract enzyme was incubated for 60 minutes at 37 °C (Talantan et al., 2018). Then the mixture was added with 3 mL of DNS and heated for 10-15 minutes until a color change occurred. After cooling, the absorbance was at a wavelength of 540 nm using a UV-Vis Spectrophotometer. The manufacture of control and blank solutions were also carried out and the absorbances were measured (Murtiyaningsih and Hazmi, 2017).

$$EA = \frac{[glucose] \times V_t}{RM \times t \times V}$$

which EA is enzyme activity (U mL^{-1}); [glucose] is concentration of glucose; RM is relative mass (g mol^{-1}); t is time of incubation; V is enzyme volume (mL); V_t is total enzyme volume (mL)

Result and Discussion

Isolates of bacteria from OPEFB

Based on the result, there were nine bacterial isolates that were thought to be able to degrade cellulose. The observations were made aft two days of incubation for 2x24 hours. It can be seen in Figure 1.

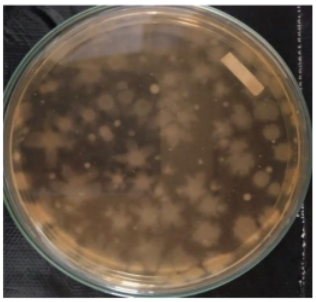

Figure 1. Bacterial colonies growing on CMC media

Selection of bacterial isolates can be seen based on the morphology formed. This is done by observing the macroscopic colony. The morphological observations of isolates that have been carried out include color, shape, edge, elevation, surface, and size. Isolates growing on the surface were observed for color, translucency, elevation, and edges (Talantan et al., 2018).

Table 1. The morphology of bacterial colonies

Codes of colony	Shape	Color	Edge	Elevation	Surface	Size
S_1	circle	slightly clear white	flat	flat	smooth	small
S_2	circle	yellowish	flat	convex	smooth	small
S_3	circle	slightly clear white	flat	flat	smooth	big
S_4 and D_3	circle	slightly clear white	flat	flat	smooth	medium
S_5	circle	yellow	flat	convex	smooth	medium
S_6 and D_7	irregular	milky white	flat	umbolate	smooth	medium
S_7 and D_1	circle	slightly clear white	flat	convex	smooth	medium
S_8	circle	yellow	wave	convex	smooth	medium
S_9	circle	yellow	wave	convex	smooth	medium
S_{10}	circle	milky white	flat	convex	smooth	big
S_{11}	circle	milky white	unflat	flat	smooth	small

Based on the results, the bacterial colonies obtained grew on CMC agar media. CMC was used because it is a good substrate that can affect the growth of bacteria in producing cellulase (Marina et al., 2018). Furthermore, macroscopic morphological observations were carried out to determine the differences that existed in each different colony. Colonies that were different from each other were selected based on colony shape, colony color, colony elevation, colony edge, and colony shape (Nofu et al., 2014).

Cellulolitic Index of Bacterial Isolates

Isolated colonies that were able to produce a clear zone were measured for their cellulolytic index. It can be worked by comparing the value of the diameter of the clear zone and the diameter of the bacterial colony (Nababan et al., 2019).

Table 2. Cellulolitic index of bacterial isolate

Isolate codes	Clear zone diameter	Colony diameter	Cellulolitic index
S_2	33.68	20.38	0.6526 mm
S_3	30.74	19.50	0.5728 mm
S_4	29.41	19.78	0.4873 mm
S_5	32.22	25.68	0.4422 mm
S_6	36.34	25.68	0.4112 mm
D_7	18.18	7.40	1.4567 mm
S_{10}	26.86	14.28	0.8809 mm
S_{11}	30.68	19.64	0.5621 mm

Based on the Table 2, there were only seven of isolates that were able to produce clear zones on CMC media that had been dripped with congo red among the eleven isolates suspected to be cellulolytic bacteria. They were S_{10}, S_2, S_3, S_{11}, and S_4.

Nababan et al. (2019) reported that congo red dye will enter the agar medium because the substance is absorbed by polysaccharide chains that have β-D-glucan bonds. Rahmawati and Rafdinal (2017) states that the resulting cellulolytic index value is categorized as small to medium because IS is worth less than 1 mm (Rahmawati and Rafdinal, 2017). According to Murtiyaningsih and Hazmi, (2017), the largest IS was owned by isolate of 6.1 whose chicken coop soil about 1,533 mm.

Percentage of Dry Mass Loss from Isolates of Bacteria

There were five isolates that had a higher clear zone, these isolates were indicated to degrade OPEFB. It can be seen in Figure 2.

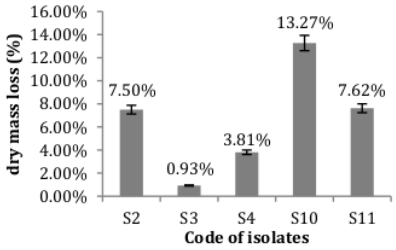

Figure 2. Percentage of dry mass from isolates of bacteria

The percentage of degradation in this study is in the medium category. According to Arifin et al. (2019), the reduced final weight resulted in a smaller potential for degradation. Irawati (2017) reported that the degradation of cellulose by bacteria was 9.4%-13.06%. Ahmad and KumarKhare (2018) reported the degradation of cellulose produced by 8%. While Lu et al. (2018) reported that vegetable compost isolation could degrade filter paper by 26.3%, 24.5%, and 19.4% after 14 days. Nababan et al. (2019) stated that the percentage of dry weight degradation obtained was 11.37% to 3.32%.

Enzyme Activity of Bacteria

The measurement of the absorbance of reducing sugar obtained by the equation y = 2.4635x - 0.1383 with a value of R^2 = 0.9987.

The activity of cellulolytic enzyme can be seen in Table 3.

Table 3. Activity of cellulase in bacterial isolate

Code Isolates	[glucose] (mg/mL)	Enzyme activity (U/mL)
S_2	0.5331	0.0493
S_3	0.4210	0.0389
S_4	0.3009	0.0278
S_{10}	1.4135	0.308
S_{11}	0.3910	0.0362

The Activity of cellulase enzyme Using DNS Method

Measurement of enzyme activity using the DNS method has the same results as screening for cellulolytic bacteria using the measurement of the diameter of the clear zone. The results obtained from both methods showed that S_{10} isolate had the largest clear zone diameter and this isolate also had the largest cellulase enzyme activity. Enzyme activity resulting from this research tends to be moderate to high. According to Nababan et al. (2019), the enzyme activity of crude extract from cellulolytic bacteria is in the moderate to high category. This can be seen from the activity value between 0.079 IU/mL to 0.069 IU/mL, the percentage of dry weight degradation obtained is 11.37% to 3.32%.

Mulyasari et al. (2015) obtained isolates from the digestive tract of gourami, crude extracts of these isolates can degrade the substrate from cassava leaves. The cellulase enzyme activity values obtained from isolates UG8, UG7, and UG3 were almost the same, namely 0.104 U/mL; 0.105 U/mL; and 0.107 U/mL. While Puspitasari and Ibrahim (2020) obtained bacterial isolates of EG2 which had been isolated from oil palm cake (Elaesis guineensis jacq), the enzyme activity obtained was 0.011 U/mL.

Conclusion

The morphological characteristics of microorganisms in degrading OPEFB were having a round colony shape, yellow color, convex elevation, and smooth and slippery surface, namely isolate S_{10}. The highest potential of microorganisms in degrading OPEFB was isolate S_{10}.

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