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CHARACTERISTICS AND DEGRADATION ABILITY OF CELLULOLYTIC BACTERIA FROM PLIEK-U AS FIBROUS FEED BIOACTIVATOR

Agricultural Science

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ABSTRACT

The isolation of microorganisms from Pliek-U fermentation products has been carried out mainly in lactic acid producing bacteria (LAB), but the cellulolytic microbial (bacterial) isolation has not been explored particularly in terms of its ability to produce cellulase enzymes to degrade fiber. The purpose of this study was isolation, characteristics, and degradation of the ability of cellulolytic bacteria from Pliek-U as fibrous feed bioactivation. Materials and Methods: Cellulolytic bacteria isolate colonies grown on each treatment were calculated and different isolates were selected to be isolated on nutrient agar medium. Characterization was performed by growing selected pure isolates in 1% CMC medium then dripped with 0.1% Congo red to test for cellulolytic potential. The results obtained, from 20 isolates isolated there are 5 potential bacteria isolates in degrading cellulose ie isolate YNH.B3, YNH.B6, YNH.B11, YNH.B13, and YNH.B16. Isolate YNH.B11 is the best isolate in degrading cellulose, which is indicated by the highest hydrolysis value index compared to other isolates. Isolate YNH.B11 is a cellulolytic bacteria of Pliek-U that potential and can be used as bioactivator for fibrous feed.

KEYWORDS

Cellulolytic Bacteria, Bioactivator, Characteristics, Degradation

INTRODUCTION

This Pliek u is one of Aceh's traditional food, made from fermented coconut. Pliek u can also be an alternative fed additive for poultry [1]. From Pliek-U obtained isolates of lactic acid producing bacteria that produced bioactive compounds (bacteriocin) having inhibitory activity against L. monocytogenes [2]. While finding 6 types of fungi and 3 groups of bacterial colonies in the form of bacilli (stem) and coccus (round) dominated by groups of gram-positive bacteria that are pathogenic (harmless to health) [3].

Researchers have previously found several groups of lactic acid-producing bacteria. However, studies of potential bacteria from Pliek-U as fiber degradation have not been studied. Allegedly Pliek-U in addition to producing lactic acid-producing bacteria also produces fiber degrading bacteria. One group of bacteria degrading fiber is cellulolytic bacteria.

Cellulolytic bacteria are bacteria that have the ability to hydrolyze cellulose complexes into smaller oligosaccharides and eventually become glucose by using cellulase enzymes [4]. These microorganisms can degrade cellulose because it produces enzymes with different specifications that work together. The enzyme will hydrolyze the bond (1.4) -β-D-glucose in cellulose [5]. Cellulosic degradation occurs along with an increase in crude protein content, in which the more and more thickness of the mycelium formation in the substrate [6]. To form mycelium requires energy degradation of glucose polymer chain from cellulose by the action of cellulase enzyme [7].

MATERIALS AND METHODS

Material: Materials used include: sample of isolate source (pliek- U). The general medium of NA, selective medium is selective medium modified according to [8] ie 0.5% peptone; 0.5% yeast extract; 0.1% K2HPO4; 0.02% MgSO4.7H2O; 1% Na2CO3; 2% agar and 1.0% (respectively CMC, xylan, lignin and mannan), pH 6.8. Aquades, alcohol, congo red reagent, CMC (Carboxyl methylcellulose) medium, 0.85% NaCl solution, gram dye (crystal violet, iodine, safranin, and 96% alcohol), tryptophan medium, Simon citrate medium, TSIA medium Fermentation of glucose, MR-VP medium (Methyl Red- Voges Proskeur),

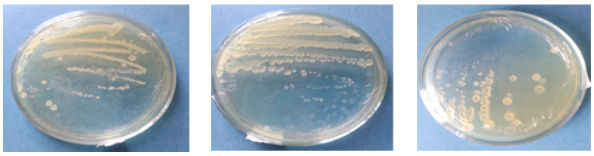
Method: Isolation of cellulolytic bacteria: As the source of indigenous isolates used oil palm waste (oil palm frond, palm carnel cake, and sludge). One gram each of samples collected aseptically were serially diluted in distilled water from 10-3 to 10-7 and spread plated on modified selective agar medium containing (0.5% peptone; 0.5% yeast extract; 0.1% K2HPO4; 0.02% MgSO4.7H2O; 1% Na2CO3; 2% agar dan 1.0% (each for CMC, xylan, lignin dan mannan), pH 6.8. in 1000 mL distilled water. The plates were incubated at 37°C for 1-2 days and the growing bacteria colonies were sub-cultured to obtain pure cultures.

Qualitative screening of cellulase producing from cellulolytic bacteria: cellulase producing bacteria were screened on agar medium containing 1% CMC. The screening process is performed to determine the potential of cellulolytic by dripping congo red 0.1%, which is marked by the formation of a clear zone around the colony. Plates were spot inoculated with a spore suspension of pure cultures and incubated at 37°C. After 48 h, plates were flooded with 1% Congo red solution for 15 min. The diameter of the zone of decolorization around each colony was measured. Hydrolysis index was determined and expressed by the ratio between the diameter of the degradation halo and the diameter of the colony and than identified. Colony morphology observation includes observation on the shape and color colonies.

RESULT AND DISCUSSION

Isolates of pliek-u cellulolytic bacteria: The result of calculating the number of bacteria from pliek-U obtained ranged from $5.25x10^6$ to $7.83x10^8$ CFU / gram. The result of bacteria exploration from pliek-U obtained 20 isolates capable of growing on a selective medium containing CMC (Carboxyl Methyl Cellulosa). This indicates that bacteria can produce cellulase enzymes, which means that bacterial isolates are capable of degrading cellulose so that these bacteria can be grouped into cellulolytic bacteria. Cellulolytic bacteria have the ability to hydrolyze materials from nature that contain cellulose into simpler products. The presence of cellulose from pliek-U will induce the synthesis of cellulase enzymes so that bacteria secrete cellulase enzymes. The purification result using the scratch method of bacterial isolate morphology characteristics can be seen in Table 1 and Figure 1.

Figure 1. Isolation of cellulolytic bacteria from pliek-U



Morphology characteristic and Physiological of cellulolytic bacteria: From isolation resulted in 20 isolates cellulolytic bacteria have morphological characteristics that vary both shape, size, elevation/surface, edges/borders and color of bacterial colonies. It is shown the Table 1.

Table 1. Morphology and Physiological Charasterictic of Cellulolytic Bacteria Isolate of Pliek-U Origin

Isolate Code	Morphology Of Bacteria Colony					Bacteria isolate physiology		
	Shape	Size	Elevasi	Edge	Color	Gram reaction	Cellulose enzyme	Oxidative/fermentative test
YNH.B1	CIrcular	Medium	Umbonate	Filamentous	Milky White	-	+	Fakultatif aerob
YNH.B2	Circular	Large	Umbonate	Filamentous	Milky White	+	+	Fakultatif aerob
YNH.B3	Irregular	Medium	Umbonate	Undulate	Yellowish White	+	+++	Fakultatif aerob
YNH.B4	Rhizoid	Small	Flat	Filamentous	Clear White	-	+	Fakultatif aerob
YNH.B5	Irregular	Medium	Convex	Undulate	Clear White	-	++	Fakultatif aerob
YNH.B6	Circular	Large	Convex	Entire	Yellowish White	+	+++	Fakultatif aerob
YNH.B7	Rhizoid	Large	Raised	Filamentous	Yellowish White	+	++	Fakultatif aerob
YNH.B8	Rhizoid	Large	Umbonate	Lobate	Yellowish White	+	+	Fakultatif aerob
YNH.B9	Circular	Small	Umbonate	Curled	Yellowish White	-	+	Fakultatif aerob
YNH.B10	Irregular	Small	Raised	Undulate	Yellowish White	+	+	Fakultatif aerob
YNH.B11	Circular	Medium	Flat	Lobate	Yellowish White	+	+++	Fakultatif aerob
YNH.B12	Circular	Medium	Umbonate	Entire	Milky White	+	+	Fakultatif aerob
YNH.B13	Irregular	Large	Umbonate	Undulate	Yellowish White	+	+++	Fakultatif aerob
YNH.B14	Rhizoid	Small	Flat	Filamentous	Yellowish White	-	+	Fakultatif aerob
YNH.B15	Circular	Large	Flat	Entire	Clear White	+	++	Fakultatif aerob
YNH.B16	Rhizoid	Medium	Convex	Filamentous	White	-	+++	Fakultatif aerob
YNH.B17	Circular	Medium	Convex	Entire	Yellowish White	-	+	Fakultatif aerob
YNH.B18	Circular	Medium	Raised	Entire	Yellowish White	+	+	Fakultatif aerob
YNH.B19	Irregular	Medium	Flat	Undulate	White	-	++	Fakultatif aerob
YNH.B20	Circular	Large	Flat	Entire	Clear White	-	+	Fakultatif aerob

Based on the observations, the morphology of 20 isolates these cellulolytic bacteria have morphological characteristics that vary both shape, size, elevation/surface, edges/borders and color of bacterial colonies. The growth of bacteria from this diverse pure culture has a morphology of colonies (irregular, circular, rhizoid), colony size (small, medium and large), colony color (milky white, white, yellowish white and translucent white), colony surface Umbonate, convex, raised, flat), and colony edges (entire, filamentous, undulate, lobate, curled). Different growth forms of each bacterial isolate on the agar medium indicate that all isolates are of different bacteria. Some bacteria have faster growth than others, resulting in larger bacterial colonies.

The growth of bacteria is influenced by nutrients and various physical factors such as temperature, pH, atmospheric gas, osmotic pressure, and others[21]. The observation of morphology of Pliek-U isolates on the same nutrition showed the different morphological response of bacterial colonies. This indicates that the isolate bacteria are suspected to come from different strains. Based on observation of physiological characteristics of isolate of cellulolytic bacteria from Pliek-U, 9 isolates were gram negative bacteria characterized by red / pink cells after gram staining.

From the screening of the cellulolytic activity index, it was showed that 20 bacterial isolates have the potential to degrade cellulose. There are 5 isolates with very strong activity, 4 isolates have strong activity, 11 isolates have weak activity in secreting selullase enzyme. All of these cellulolytic bacterial isolates have facultative aerobic properties. Each bacterial isolate produces different cellulase enzyme complexes, depending on the genes possessed and the carbon source used.

Ability of bacteria isolates in degrading cellulose: The test ability of bacteria isolates in degradation cellulose can see on Table 2. The results showed that the clear zone formed ranged from 11 mm - 36 mm with the highest index of cellulolitik 5.00 and the lowest was 0.83. The size of the index of cellulolytic activity produced showed strong or weak ability of bacteria in degrading cellulose. The ability of bacteria in hydrolyzing cellulose is expressed in the form of cellulolytic activity index or hydrolysis index. The activity of cellulase enzyme secretion is characterized by the cellulotic activity index value which is the ratio of the diameter of the clear zone to the isolate colony diameter grown on CMC containing medium (Figure 2). The ability to form a clear zone on CMC medium shows the presence of an endo-1.4-β-glucanase enzyme that can break 1.4-β-glucoside bonds on cellulose fibers randomly and the amount of amorphous on the substrate causes the CMC to be hydrolysed more efficiently.

Table 2.Diameter of The Clear Zone and Hydrolysis Index from Isolates on CMC Medium

Isolate Code	Clear zona (mm)	Hydrolysis/cellulolytic index
YNH.B1	13	1.17
YNH.B2	15	1.50
YNH.B3	34	4.67
YNH.B4	14	1.33
YNH.B5	20	2.33
YNH.B6	30	4.00
YNH.B7	20	2.33
YNH.B8	18	2.00
YNH.B9	12	1.00
YNH.B10	16	1.67
YNH.B11	36	5.00
YNH.B12	14	1.33
YNH.B13	35	4.83
YNH.B14	11	0.83
YNH.B15	22	2.67
YNH.B16	26	3.33
YNH.B17	19	2.17
YNH.B18	17	1.83
YNH.B19	22	2.67
YNH.B20	13	1.17

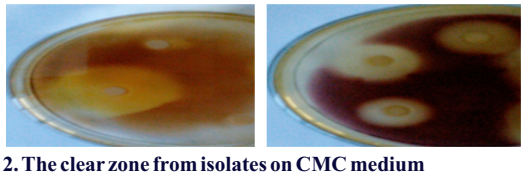


Fig 2. The clear zone from isolates on CMC medium

Cellulase enzyme activity will be formed by microorganisms when cellulose is the only substrate. The formation is inhibited by another substrate or decreases when the medium contains other substrates so that the cellulase-producing potential will appear together with other enzymes [8].

The result of cellulolytic index test on bacteria isolate of pliek-U indicates that isolates are able to degrade cellulose by producing cellulase enzyme. This is indicated by the formation of a clear zone in bacterial isolates grown on a medium to contain CMC. The presence of CMC as a carbon source induces bacteria to synthesize the cellulase enzyme (Endo-1.4 β-D-Glucan Glucanohydrolase). This enzyme will actively randomly cut the amorphous side of the polysaccharide chain. It is assumed that the amorphous side-cutting ability is expressed through the clear zone formed on the CMC agar medium. Thus, bacterial isolates having high cellulolytic indexes are potential isolates to be applied as inoculums in degrading cellulose.

CONCLUSIONS

The result of bacteria isolation from pliek-U from Aceh founded the existence of cellulolytic bacteria group which have high ability in degrading cellulose and can be used as bioactivator for fibrous feed.

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REFERENCES

1. Nurliana, M., Sudarwanto, L.I. Sudirman, A.W. Sanjaya. 2008. Initial Testing Of The Antibacterial Activity Of The Pliek U Oil: Traditional Acehnese. J. Kedokteran Hewan. 2:150-156.
2. Nurliana, Suzanna Rabfiani dan M. Hanafiah. 2002. Karakteristik awal bakteriosin produksi bakteri asam laktat yang diisolasi dari pliek U. J.Sain Vet. Vol.XX No.1.
3. Rivan R., R. Hayatillah, Amirunnas, Nurul A'la Dan Manna Wassalwa. 2015. Isolasi Dan Identifikasi Organisme Fermentor Pada Proses Pembuatan Pliek-U.Program Kreativitas Mahasiswa- Penelitian (PKM-P). Universitas Syiah Kuala (Unsyiah).
4. Ibrahim, A.S.S. and Al. Dewany. 2007. Isolation And Identification Of New Cellulases Producing Thermophilic Bacteria From An Egyptian Hot Spring And Some Properties Of The Crude Enzyme". Australian Journal Of Basic And Applied Sciences. 1(4): 473-478.
5. Galbe, M and Zacchi, G. 2007. Petreatment Of Lignocellulolitic Materials For Efficient Bioethanol Production. Adv Biochem Engine/Biotechnol 108:(41-65).
6. Yunilas., Lili Warly., Yetti Marlida., and Irsan Riyanto. 2014. Quality Improvement of Oil Palm Waste- Based Feed Product Through Indigenous Microbial Fermentation to Reach Sustainable Agriculture. International Journal On Advanced Science Engineering Information Technology. Vol.4 (4): 72-75.
7. Yunilas. 2016. Peran Mikroorganisme Indigenous YL (MOIYL) Sebagai Inokulum Pendegradasi Serat Berbasis Limbah Perkebunan Sawit. Prosiding Seminar Nasional Peternakan Berkelanjutan 8, 16 November 2016, Sumedang, Indonesia.
8. Yunilas, Lili Warly, Yetti Marlida., and Irsan Riyanto. 2013. Potency of Indigenous Bacteria from Oil Palm Waste in Degrades Lignocellulose as A Sources of Inoculum Fermented to High Fibre Feed. Pakistan Journal of Nutrition. 12(9) : 851-853.