Indian Journal of Science and Technology, *Vol 10(7), DOI: 10.17485/ijst/2017/v10i7/111224, February 2017*
ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

Bacteria Isolation from Landfill for Production of Industrial Enzymes for Waste Degradation

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Abstract

Background/Objectives: The purpose of this study is to isolate the potential bacteria for the production of industrial enzyme cellulose and xylanase for waste degradation. **Methods/Statistical Analysis:** The bacteria were isolated from three different sources (landfill surface, 10 cm depth soil and moist soil) collected from Sungai Ikan Landfill, Kuala Terengganu, Malaysia using serial dilution and spread plate technique. Primarily screening for the enzyme production was carried out. The bacteria isolates which shows a good clearing zone diameter in ranged 2.6-8.8 cm were selected for further quantitative screening for cellulase and xylanase production by performed submerged fermentation using Carboxymethyl Cellulose (CMC) and xylan as a substrate. **Findings:** A total of 130 potential bacteria were isolated on Nutrient Agar (NA). Only 50 and 30 of the isolates exhibited cellulase and xylanase, respectively, by showing clear zone on CMC and Xylan agar plates after stained with iodine dye. From the observation of the quantitative screening of enzyme activities, B6.2 isolate exhibited the highest cellulase activity (endoglucanase19.797 U/m Land FPase 7.384 U/mL) and M29 isolate exhibited the highest activity of xylanase (5399 U/mL). Overall, all isolates showed good cellulase and xylanse activity ranged between 0.491-19.797 U/mL and 2075-5399 U/mL, respectively. **Application/Improvements:** For future study, the enzyme produce will be used to degrade waste into fermentable sugars which used as a feedstock for bioethanol production.

Keywords: Cellulose- Degrading Bacteria, Cellulase, Landfill Soil, Xylanase, Xylan- Degrading Bacteria

1. Introduction

Currently, most of the wastes in Malaysia are land filled in both sanitary and unsanitary landfills. These landfills mostly are about to reach their limit. In addition, creating new landfill sites were far from possible due to land shortage and high cost as a result of fluctuation in the country's economic growth. The recycling efforts are still low with only selected waste involved. On the other hand, the composting are preceded at a very low rate with virtually no incineration done except on the Islands. Since most of the solid wastes mostly contain organic matters which can be easily biodegraded, the conversion of solid waste into bioenergy such as bioethanol or biohydrogen should be considered. Biologically conversion of solid waste to bioenergy will reduce the volume of waste in landfill and also vary the energy sources[1]. The conversion technique normally involves a various types of microorganisms to degrade the organic matters into bio-products via a series of biodegradation process.

Waste treatment processes through biology methods consist of the waste degradation microorganism such as bacteria or fungi which consume biodegradable waste as a food sources for growth and propagation. Both bacteria and fungi have long been studied for their abilities to produce a wide variety of cellulase and xylanase[2]. Currently, more researchers have interest on bacteria as a cellulase and xylanase producer compared to fungi due to their high growth rate, the enzyme is more complex and can adapt with extreme environment[2,3]. Since solid waste might be consist high content of cellulose and xylan, the isolation of the potential cellulolytic and xylanolytic enzyme producing bacteria is highly demanding.

Hence, the aim of this study is to isolate the potential solid waste degrading bacteria from landfill soil.

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2. Materials and Methods

2.1 Sample Collection and Bacterial Isolation

Soil sample from Sungai Ikan Landfill, Kuala Terengganu, Malaysia, were used for isolation of the solid waste degrading bacteria. Soil sample was collected from three different resources which were landfill surface, 10-cm deep soil and moist soil. Moist soil was collected next to the leachate pond. It has more moisture (more than 11% wt/wt) than that other soil samples. Approximately one kilogram of soil was collected at each resource within the area of the landfills' life cell. The samples were kept at 4˚C until used.

2.2 Serial Dilution and Spread Plate Method

One gram of samples was suspended in 50 mL sterile distilled water. A series of dilutions from 10^{-1} to 10^{-10} were made, these dilutions were used in spread plate method. The diluted samples (100 µL) were pipette and spread on Nutrient Agar (NA) plates and incubated for 24 h at 37˚C. The bacteria colonies were sub-cultured and purified on NA plates. The pure cultures were sub-cultures in NA slant, incubated at 37˚C to achieve vigorous growth and then preserved in 15% glycerol vials at -80˚C for further used.

2.3 Morphological Study

Gram staining was conducted by using Gram staining kit (Sigma Aldrich) and the morphological characteristic of the bacterial isolates was observed under the light microscope (Carl Zeiss Axiostar plus) at 1000x total magnification.

2.4 Qualitative Screening of Cellulase and Xylanase Activities

Individual pure culture of bacterial isolates were aseptically transferred into Carboxymethyl-cellulose and Xylan agar plates containing 10g/L peptone; 2g/L K_2HPO_4; 0.3g/L $MgSO_4$; 2.5g/L $(NH_4)_2SO_4$; 15g/L Agar and 10g/L CMC or Xylan [4]. After incubated for 48 h, the agar plates were flooded with gram iodine stain suggested by Gohel *et. al.*[5]. The plates were incubated for 10-12 min and then washed with water. The stains were washed with 1 M NaCl solution. The clear zone present around growing bacteria indicated the hydrolysis of CMC or xylan.

2.5 Inoculum Preparation

Pure cultures of bacterial isolates were maintained individually on NA slants at 4˚C until further used. Selected pure bacterial isolates were inoculated into broth medium containing 0.03% $MgSO_4$; 0.2% K_2HPO_4; 1% glucose; 0.25% $(NH_4)_2SO_4$ and 1% peptone at pH 7 for 18 h of cultivation period. After 18 h of cultivation period these exponential growth cells were used as inoculum sources.

2.6 Crude Enzyme Preparation

Large clearing zones presenting potential cellulolytic and xylanolytic enzyme producing bacteria in Iodine test were used in enzyme production for quantitative screening. Broth medium containing 0.03% $MgSO_4$; 0.2% K_2HPO_4; 0.25% $(NH_4)_2SO_4$; 1% peptone and supplemented with 1% CMC or xylan at pH 7. The cultivation was conducted in 250 mL shake flask with 30 mL working volume. 10% (v/v) of inoculum were inoculated into the culture medium. The fermentation was performed in incubator shaker for 48 h at 37˚C and agitated at 150 rpm. After 48 h fermentation period, the broth culture were collected and centrifuged at 4000 rpm for 15 min. The clear supernatant was separated from the pellet and stored as crude enzyme at -20 ˚C for further enzyme analysis.

2.7 Endoglucanase Assay

Endoglucanase activity was determined by measuring the amount of reducing sugar liberated from amorphous cellulose. The enzyme activity was determined following the methods by International Union of Pure and Applied Chemistry (IUPAC) commission on biotechnology[6]. A mixture of 0.5 mL of crude enzyme with 0.5 mL of 1% Carboxymethyl-Cellulose (CMC) in 0.05 M sodium citrate buffer (pH 4.8) was incubated at 50˚C for 30 min. After 30 min incubation period, the reaction was stopped by adding 3 mL of DNS solution and boiled at 100˚C for 5 min. Sugars produced were determined by measuring absorbance at 540 nm. Endoglucanase production was estimated by using glucose standard curve. One Unit (U) of enzyme activity is expressed as the quantity of enzyme which required releasing 1 µmol of glucose per minute under standard assay conditions.

2.8 Total Cellulase (FPase) Assay

Total cellulase activity (FPase) was determined by measuring the amount of reducing sugar liberated from filter paper. The enzyme activity was determined by incubating 0.5 mL of crude enzyme with 1.0 mL of 0.05 M sodium citrate buffer (pH 4.8) supplemented with What man no.1 filter paper strip 1.0 x 6.0 cm (=50 mg) as a substrate. The mixture was incubated at 50˚C for 60 min. After incubation period, the reaction was stopped by adding 3 mL DNS solution and boiled at 100˚C for 5 min. Reducing sugars produced were determined by measuring absorbance at 540 nm.

2.9 Xylanase Assay

Xylanase activity was determined following the method suggested by Kim et al.[7]. Xylanase activity was determined using Corn core xylan as a substrate. A mixture containing 0.2 mL of crude enzyme, 0.5 mL of 1% xylan solution in 0.05 M phosphate buffer (pH 6.0) and 0.3 mL of buffer (pH 6.0) was incubated at 50˚C for 10 min. After incubation period, the reaction was stopped by adding 3 mL of DNS solution and boiled at 100˚C for 5 min. Xylose released were determined by measuring absorbance at 520 nm. D- Xylose was used as a standard for the preparation of a calibration curve. One unit of xylanase activity was defined as the quantity of enzyme which required releasing 1 μmol of xylose per minute under standard assay conditions.

3. Results and Discussion

3.1 Bacterial Isolation and Qualitative Screening of Enzyme Production

The potential cellulase and xylanase producing bacteria were successfully isolated from landfill surface, 10-cm depth soil and moist soil of Sungai Ikan Landfill, Kuala Terengganu, Malaysia. Moist soil was the A total of 130 bacteria isolates were obtained on NA plates (49 isolates from landfill surface; 51 isolates from 10 cm depth soil and 30 isolates from moist soil). Their cellulolytic and xylanolytic activities were detected by the formation of clear zone on CMC and Xylan agar plates. Among 130 isolates obtained, a total of 50 and 30 isolates showed positive cellulolytic and xylanolytic ability respectively, indicating by clear zone on agar plate after staining with Iodine dye (Table 1).

Table 1. Numbers of isolate with positive cellulolytic and xylanolytic enzyme production

Sample source	Numbers of Isolates		
	Total Isolates on NA plate	(+) Cellulolytic	(+) Xylanolytic
Landfill Surface	49	7	17
10 cm Depth Soil	51	34	-
Moist Soil	30	9	13
Total Isolates	130	50	30

Since CMC and xylan was used as the only carbon source in the CMC and Xylan agar, the presence of clear zone after staining with Iodine dye is a clear evident that cellulase or xylanase secreted by bacteria culture in order to hydrolysed the CMC and xylan [3,8]. The results showed that the clearing zone diameter ranged in between 1.3 to 8.8 cm for all positive cellulolytic and xylanolytic bacterial isolates. However, only isolates that showed good clearing zone diameter ranged in between 2.6 to 8.8 cm were selected for further study. All of the selected isolates were inspected for their cell shape, colony appearance, and gram reaction after incubation at 37˚C for 24 h. Table 2 summarized the clear zone diameter, morphological and physiological characteristic of selected potential cellulolytic and xylanolytic enzyme producing isolates. Most of bacterial isolates are gram positive and bacilli in shape.

There are numerous bacteria have been reported with cellulolytic and xylanolytic activities. Most commonly studied cellulolytic producing bacteria belong to the genera *Clostridium, Cellulomonas, Cellulosimicrobium, Thermomospora, Bacillus, Ruminococcus, Streptomyces, Fibroacter, Paenibacillus, Erwinia, Acetovibrio* and *Microbispora*[3,9–12].

3.2 Determination of Cellulase Production

The six selected bacteria isolates (M2, M6, B6.2, K1, S3.1, D1, and T2) were examined for the endoglucanase and total celulase (FPase) production after 48h cultivation in 30 mL liquid medium supplemented with 1% CMC as a substrate. The activities ranged from 1.574 to 19.797 U/mL for endoglucanase and 0.491 to 7.384 U/mL for FPase assay. From the observation, the B6.2 isolate exhibited the highest endoglucanase and FPase activities, 19.797 U/mL and 7.384 U/mL respectively, than that the others. Figure 1 shows the enzyme activity performed by all six selected isolates.

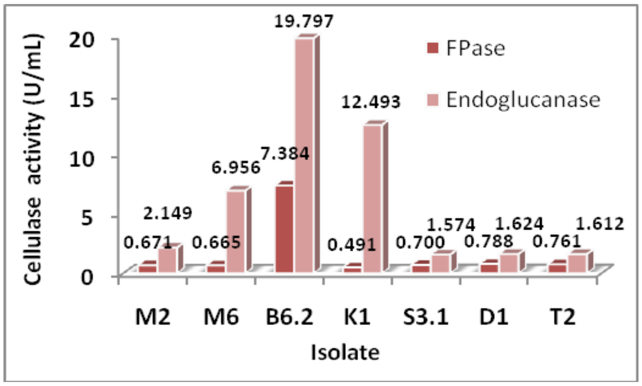

Figure 1. Cellulase activities of two enzymes (endoglucanase and FPase) of six selected isolates after 48 h cultivation in CMC medium. Values in figure are means of duplicates.

Table 2. Characteristic of selected potential xylanolytic and cellulolytic enzyme producing bacteria

Characteristic					
Gram	Shape	Colour	Form	Clear zone diameter (cm)	
				Xylanolytic	Cellulolytic
+	Bacilli	Cream	Circular	N/A	3.5
+	Bacilli	Cream	Irregular	N/A	3.8
-	Cocci	Cream	Circular	4.5	N/A
+	Bacilli	Cream	Irregular	4.4	N/A
+	Bacilli	Cream	Irregular	4.3	N/A
+	Bacilli	Cream	Irregular	3.9	N/A
+	Bacilli	Cream	Circular	4.0	N/A
+	Cocci	Cream	Circular	N/A	2.6
+	Bacilli	Cream	Filamen-tous	N/A	3.8
+	Bacilli	Cream	Rhidzoid	N/A	2.7
+	Bacilli	White	Irregular	N/A	3.5
-	Bacilli	Cream	Irregular	3.7	3.5
+	Bacilli	Cream	Circular	3.7	N/A
-	Strepto-bacilli	Cream	Circular	4.0	N/A
+	Bacilli	White	Circular	5.0	N/A

The activities of endoglucanase and FPase for all isolates are comparable with the previous studies reported by Samira et. al.[8] where *Streptomyces* BRC1 and BRC2 showed maximum endoglucanase activity at 6.4-6.6 U/mL after 72-88h cultivation. CMC was served as sole carbon source might be the reason of higher activity of cellulase. Narasimha et al.[13] who found that CMC was the best choice for the carbon source followed by cellulose for cellulase production.

3.3 Determination of Xylanase Production

The twelve selected isolates were grown in xylan medium for 48 h of cultivation period at 37˚C. Overall, all isolates shows good xylanase activity in ranged of 2075 to 5399 U/mL. Figure 2 illustrated the xylanase activity of isolates. Among 12 isolates, isolate M29 exhibited the highest xylanase activity (5399 U/mL) compared to the other isolates.

The activity of xylanase for all isolates is in agreement with reported studies. *Aspergillus niger* IME-216 exhibited 74800 U/mL xylanase activity when grew on xylan as a sole carbon source[15].

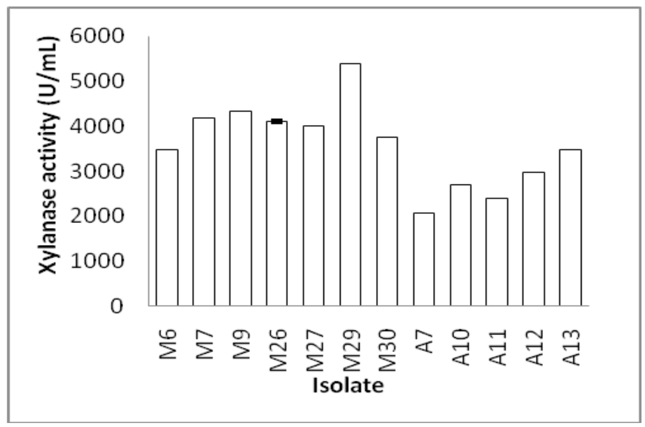

Figure 2. Xylanase activity of 12 selected isolate after 48 h cultivation in medium supplemented with 1% (w/v) xylan. Values in figure are means of duplicates with (±) standard deviation.

4. Conclusion

The bacterial isolates showed potential to produce cellulase and xylanase to hydrolyse cellulose and xylan into fermentable sugars which could be readily used in many applications like feed stock for the production of valuable product such as bioethanol. The International Conference on Fluids and Chemical Engineering (FluidsChE 2017) is the second in series with complete information on the official website[14] and organised by The Center of Excellence for Advanced Research in Fluid Flow (CARIFF)[15]. The publications on products from natural resources, polymer technology, and pharmaceutical technology have been published as a special note in volume 2[16]. The conference host being University Malaysia Pahang[17] is the parent governing body.

5. Acknowledgement

The authors gratefully acknowledge Siti Nor Amira Rosli, Muhammad Yusreen Ismail, Siti Aisyah Azhar, Nur Suhaila Anang Japar and Nur Idayu Wahab for their works on bacteria isolation.

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