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Research Paper

Identification of Mesophilic Bacteria Producing Hydrolase Enzymes from Cow Manure Compost

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ABSTRACT: *Five bacterial isolates were obtained from compost sample isolation. The isolates were F1C, F2E, F4D, F4E, and F5D, each taken from a different temperature. The aim of this research was to screen for mesophilic bacteria that can produce enzymes from the hydrolase class. F2E was a potential mesophilic bacterium that produced 3 enzymes from the hydrolase class: cellulase, xylanase, and amylase. The ribotyping technique was used to identify the species of bacteria. DNA extraction revealed that the bacteria were larger than 20,000 bp. PCR successfully amplified a DNA size of 1500 bp. Homology studies showed that Bacillus cereus and F2E bacteria are 99% similar.*

KEYWORDS: *Hydrolase, Cow Manure Compost, Bacillus cereus*

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I. INTRODUCTION

Hydrolases are a class of hydrolytic enzymes that utilize water as a hydroxyl group donor when breaking down their substrate. This enzyme has a role in catalyzing the hydrolysis of chemical bonds in biomolecules so that smaller molecules. Hydrolases are important for the environment because they can digest large molecules into small fragments for synthesis, and biopolymers as well as for the degradation of toxins[1]. Hydrolase has subclasses that act on ester bonds, Glycosylases, ether bonds, peptide bonds (peptidases), carbon-nitrogen bonds, other than peptide bonds, acid anhydrides, carbon-carbon bonds, halide bonds, phosphorus-nitrogen bonds, sulfur-nitrogen bonds, carbon-phosphorus bonds, sulfur-sulfur bonds, carbon-sulfur bonds.

The amylase, which is a hydrolase class enzyme, has been isolated from soil[2]. Xylanase and cellulase which are hydrolase class enzymes have been isolated from soil[3]. Hydrolase enzyme-producing bacteria have also been successfully isolated from the initiation compost phase[4], thermophilic[5], and ripening phase[6]. In this research, mesophilic bacteria were isolated which can produce hydrolase enzymes.

Cow manure compost contains grass, legumes, and concentrates so it can be predicted that there are enzymes that can degrade large compounds into small molecules such as xylanase, cellulase, and amylase. Ribotyping is a molecular technique that uses data from rRNA-based phylogenetic analysis to identify bacteria quickly and precisely. This study aimed to identify mesophilic bacteria producing hydrolase enzymes from cow manure compost.

II. RESEARCH METHOD

Isolate

In this study, samples were taken from cow manure compost which had been carried out previously[7]. Bacteria have been isolated from compost samples with the names F1C, F2E, F4D, F4E and F5D. This isolate was then grown on solid media (Nutrient agar) for 16 hours. The isolate was used for enzyme screening.

Ezyme screening

The five isolates were grown on enzyme selective media (xylanase, cellulase, and amylase selective media) for 16 hours. In amylase screening, isolates were grown on media containing 25 grams of nutrient agar, 5 grams of tryptone, 2 grams of yeast extract, 1.3 grams of KH_2PO_4, 0.5 grams of $MgSO_4.7H_2O$, 0.1 grams of $CaCl_2.2H_2O$, 20 grams of dissolved starch (in 1 L of media solution). Colonies were grown by scratching on

solid media and then incubated for 16 hours. Microbes that grow on the media are then dripped with iodine solution (1 gram of iodine plus 2 grams of KI) on the surface. Microbes with amylase activity produce a clear zone around the microbial colony, while microbes that do not have amylase activity produce a blue zone around the colony[8].

In cellulase screening, the same single colony was also grown on selective media (1 gram CMC, 0.02 gram $MgSO_4.7H_2O$, 0.075 gram KNO_3, 0.002 gram $FeSO_4.7H_2O$, 0.004 gram $CaCl_2.2H_2O$, 0.05 gram K_2HPO_4, 0.2 gram nutrient agar, 0.1 gram glucose) by streaking the bacterial culture on solid media and incubated for 16 hours. After the microbes grew well, staining was carried out using Congo red (1mg/ml) for 15 minutes, and destaining was carried out using 1 M NaCl solution for 15 minutes. Microbes with cellulase activity produce a clear zone around the colony[9].

In xylanase screening, the composition of the media for selecting xylanase-producing bacteria was K_2HPO_4 1.5% (w/v), $MgSO_4.7H_2O$ 0.025% (w/v), NaCl 0.25% (w/v), NH_4Cl 0.5% (w/v), Na_2HPO_4 0.5% (w/v), bacto agar 2%, yeast extract 0.2% (w/v) and xylan 0.5% (w/v). Cultivated bacteria on solid media by streaking the culture from the bacterial stock that has been collected. Selection of bacterial colonies is carried out in stages based on the clear zone produced around the colony after adding the Congo red solution[10]. Xylans are hemicelluloses and the second most abundant natural polysaccharide[11].

DNA preparation

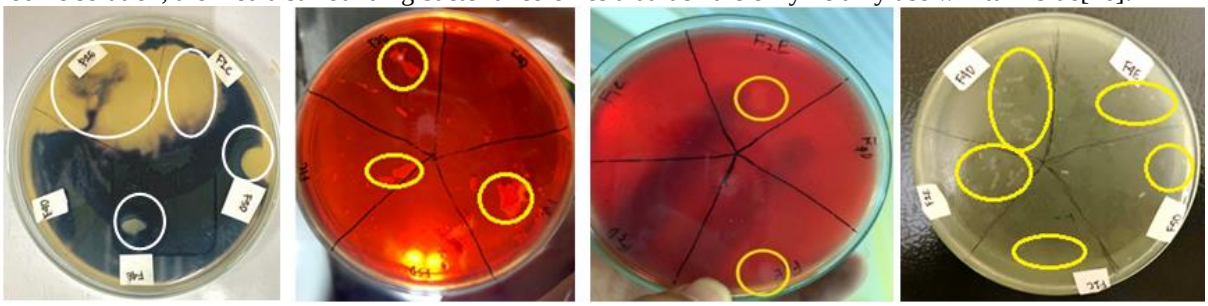
To identify bacterial species using a molecular genetic approach, DNA preparation, sequencing, and Bioinformatics analysis were carried out. The DNA preparation was carried out by isolating DNA, amplifying DNA by PCR, and agarose gel electrophoresis. The Genaid kit was used for the bacterial DNA isolation. Before DNA was isolated, the bacterial isolate was grown in nutrient broth for 16 hours using an incubator shaker at a speed of 150 rpm. This liquid isolate was centrifuged at 14000 rpm for 1 minute and the pellet was taken. The DNA isolate was then amplified using PCR (results can be seen in [7]).

Sequencing

The DNA amplicon was sequenced and then bioinformatics analysis was carried out to identify the species of bacteria by analyzing the homology of the sample compared to references in the gene bank using the BLAST program from https://www.ncbi.nlm.nih.gov/ and the application Mega X[12].

III. RESULTS AND DISCUSSION

The five bacterial isolates were rejuvenated by growing at a temperature of 37°C for 16 hours. The five colonies, namely F1C, F2E, F4D, F4E, and F5D, were grown on nutrient agar and three enzyme-selective media (Figure 1). In cellulase enzyme screening, CMC (cellulose derivative) was used as a carbon source. CMC is an enzyme substrate to produce glucose which can be used as a growth medium for cellulolytic bacteria. In screening for xylanase enzymes, xylan was used as the main carbon source[13]. Xylanolytic and cellulolytic bacteria can be identified by looking at the clear zone after adding Congo red. Congo red dye will diffuse into the agar medium and will only be absorbed by long chains of polysaccharides that have β-D-glucan bonds so that Congo red can be used as an indicator for breaking β-D-glucan bonds on xylan substrates[14][15]. Rinsing with NaCl solution will enhance the visibility of the hydrolysis clear zone. In amylase screening, starch is a carbon source that is hydrolyzed into glucose. The presence of a clear zone around the bacterial colony indicates that the enzyme (amylase) produced by the bacteria has digested the starch in the medium into simple sugar molecules that do not show a color response to the iodine solution. With carbohydrate polymers containing less than five monosaccharide groups, such as glucose, the iodine solution does not exhibit color. When dripped with iodine solution, the media surrounding bacterial colonies that lack the enzyme amylase will turn blue[16].



a **b** **c** **d**

Figure1: Agar Media : a (amylase), b (xylanase), c (cellulase), d (nutrient agar)

Only F2E and F4E bacteria could grow on all selective media, according to the results of the enzyme screening (Table 1). F4D colonies could not grow on all selective media. In F1C, cellulase enzymes were not produced. F5D colonies cannot produce xylanase and cellulase enzymes. F2E was considered as a potential bacteria for the identification of bacterial species.

Table 1. Enzyme screening

	Amylase	Xylanase	Cellulase
FIC	√	√	X
F2E	√	√	√
F4D	X	X	X
F4E	√	√	√
F5D	√	X	X

The results of DNA isolation and PCR amplification of F2E isolates showed that the DNA size was above 20,000 bp and the amplification results were 1500 bp[7]. This shows that the amplification process has been successful. The gene that was amplified was the 16S gene which has a size of around 1500 bp. As a result of sequencing, nucleotide sequence data and chromatograms of base peaks were obtained as in Figures 2 and 3. These peaks were selected from high peaks and did not overlap. Short peaks were obtained from the sequencing results, possibly caused by thin DNA amplification results.

Figure2: Nucleotide sequence

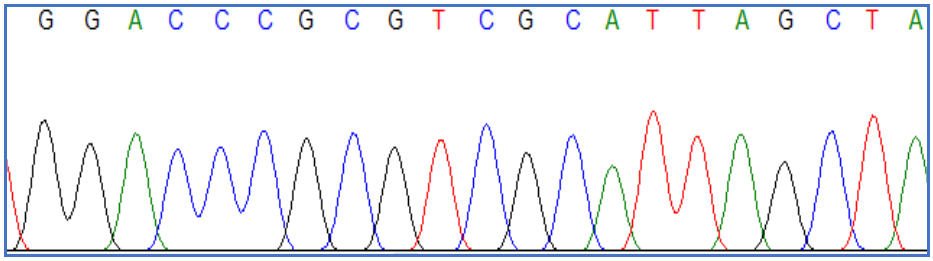


Figure3: Cromatogram of the F2E

The nucleotide sequences obtained were then analyzed using the BLAST program (www.blast.nbi.nlm.nih.gov/). The BLAST program compares the resulting nucleotide sequences with existing nucleotide sequences in GenBank (www.ncbi.nlm.nih.gov/genbank). Based on homology analysis obtained from BLAST, the percentage of similarity between the target bacteria and other bacteria can be determined.

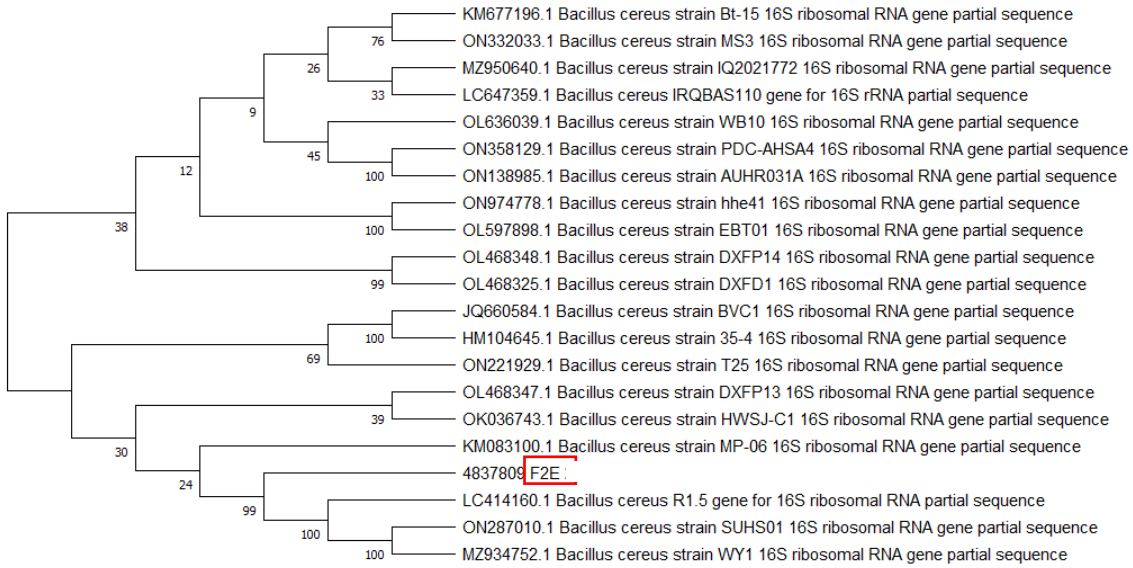


Figure4: Filogenetic tree

Homology analysis of F2E bacteria was carried out using BLAST which showed that F2E bacteria were identified as *Bacillus Cereus*. It can be seen in Figure 4 that the F2E bacteria have the closest homology to *Bacillus Cereus* with a similarity of 99%, this shows that the F2E bacteria are *Bacillus Cereus* bacteria.

IV. CONCLUSION

In this research, mesophilic bacteria were successfully isolated and identified that can produce hydrolase enzymes from cow dung compost. This isolate was an F2E isolate that can produce the enzymes amylase, xylanase, and cellulase. This isolate was close to *Bacillus cereus*.

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