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Determination of the 16S rRNA Gene Sequence in F1C Isolates Producing Amylase and Lipase Enzymes from Initial Phase Composting

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Abstract:
Background*: The existence of enzyme activity in the cow manure composting process will provide its own advantages. Compost from cow manure can be used not only in planting but also in industry as a new alternative for potential sources of enzymes. This study aimed to identify lipase and amylase-producing F1C bacteria during the cow manure composting process by means of 16S gene sequencing analysis*.
Materials and Methods*: This research uses amylase and lipase screening methods with specific substrates and gene sequence determination using the 16S rRNA method*.
Results*: From the identification results, it was found that the F1C bacteria had the closest homology to Bacillus cereus strain GXBC-3 with 98.66% similarity*.
Conclusion: *Lipase and amylase enzymes obtained from composting cow manure have been screened. F1C potential bacteria that have amylase and lipase activity were selected.*
Key Word*: amylase; compost; lipase*

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I. Introduction

One way of processing livestock wastes such as those from livestock activities, slaughterhouses, and livestock product processing is composting[1]. The composting process is an activity that converts organic material into a more stable material containing humus through a thermophilic stage and mesophilic stage[2]. The composting process has 4 main phases: the initial mesophilic phase, the thermophilic phase, the final mesophilic phase and the cooling or maturation phase. The changes that occur during the phase change process are the occurrence of a significant temperature change pattern that causes changes in the microorganism community. Other factors that cause changes in microorganisms in each phase are pH, water availability, light and oxygen[3]. In addition, the type and amount of nutrients also determine the niche for certain organisms. The degradation of compost organic matter is carried out by different groups of microorganisms at each composting phase. In aerobic composting conditions, bacteria, fungi, actinomycetes are the most active[4]. The composting process of organic material involves bacteria, fungi, fungi and other living microorganisms. During the composting process, it is marked by changes in the abundance of the biological population[5]. The main microorganism community in compost consists of fungi, actinomycetes and unicellular bacteria. The microorganisms involved in the degradation process of compost generally produce enzymes. This organic waste composting is one of the sources in finding enzyme-producing microorganisms. The activity of protease, amylase, esterase, cellulase, lipase, ligninase and xylanase enzymes in compost with wood chips from pharmaceutical industrial waste was see[6 7 8]. With the activity of these enzymes in the composting process of cow manure will provide its own advantages. Compost from cow manure can also be used not only in planting but also in industry as a new alternative for potential sources of enzymes. F1C bacteria were isolated from cow manure compost in the final thermophilic phase. These bacteria are known to produce lipase and amylase enzymes. This study aimed to identify lipase and amylase-producing F1C bacteria during the cow manure composting process by means of 16S gene sequencing analysis.

II. Material And Methods

The samples used in this study were from composting cow manure mixed with rice straw at a ratio of 3: 1. The composting process includes 5 composting phases, namely the initiation phase (temperature 28^{o}C), the first mesophilic (temperature 50^{o}C), thermophilic (temperature 55 ^{o}C), the second mesophilic (temperature 50^{o}C) and the maturation phase (temperature 37 ^{o}C). However, the sample used in this study came from the

initiation phase samples taken with 3 positions, namely the top, middle and bottom. This is to obtain an overview of the dynamics of enzyme activity in each phase in the compost-making process. The sample was then dissolved with distilled water with a ratio of 1:10. Then 0.1 mL was taken and inoculated into 10 mL of liquid LB medium. The isolates were incubated at a temperature suitable for the composting temperature for 16 hours using a shaker with a speed of 150 rpm. The culture was diluted using 0.9% physiological NaCl. Afterward, the culture was grown in LB media by means of a spread. The culture was then incubated at 28 ^{o}C for 16 hours. The morphology of the microbes that grew was observed. Colonies with different morphologies were regrown in new solid LB media and used as microbial collections.

Lipase screening

Single colonies were grown in selective LB media containing olive oil and rhodamine B emulsion. Olive oil emulsion was made by mixing olive oil, tween 80 and ddH_2O. The mixture is vortexed to form a white emulsion and 0.1 ml of rhodamine B is added to the mixture to form a pink emulsion. The colonies were then grown on LB media that had been added with emulsion and incubated for 16 hours at 28 ^{o}C. Bacteria that grow and have lipase activity will glow when irradiated with UV 350 nm[9].

Amylase screening

Pure cultures were streaked and incubated at 50^{o}C for 16 hours on starch agar. After 16 hours, the plates were immersed with Lugol's iodine, and clear zone indicates a positive amylase-producing colony[10].

DNA Isolation

Identifying bacteria is carried out through the isolation of chromosomal DNA, amplification by PCR, agarose gel electrophoresis, sequencing and then homology analysis using the blast program. The Isolation of Chromosomal DNA was carried out by the Klein method[11]. A total of 3 µl of bacterial culture was centrifuged at 8000 rpm for 5 minutes. As much as 300 µl of extraction buffer (100 mM Tris-HCl pH 8, 100 mM Na-EDTA pH 8) and 5 mg lysozyme were added to the remaining pellets, and was then incubated at 37 ^{o}C for 1 hour. Cells are lysed with lysis buffer and were then incubated at 50^{o}C for 30 minutes. As much as 30ml 5M CH3COOK and 5.75 ml glacial acetic acid were added, and were then incubated at cold temperature for 5 minutes. The supernatant from centrifugation at a speed of 12,000xg for 20 minutes was precipitated with isopropanol and was then incubated at room temperature for 1 hour, and recentrifuged for 15 minutes. The centrifuged pellets were then mixed with 70% ethanol, and recentrifuged for 5 minutes. The centrifuged pellets were then air dried overnight. Chromosomal DNA amplification process is carried out using PCR. The primers used were 10 mM Uni B1 primer (5'-GGTTAC (G / C) TTGTTACGACTT-3 '), 10 mM BactF primer (5'-AGAGTTTGATC (A / C) TGGCTCAG-3'), reacted in a PCR condition 30 repetitions. The results of DNA amplification can be seen through agarose gel electrophoresis

16S rRNA sequencing

The nucleotide sequence determination was carried out at First BASE. For one determination, a DNA template of 100 µl and primers of BactF, UniB1, ComF, ComR each of 10 pmol are needed

Homology anaysis

The nucleotide sequence of the 16S rRNA gene obtained was then analyzed by the BLAST program (www.blast.ncbi.nlm.nih.gov/blast.egi) to identify bacterial groupings. The BLAST program compares the resulting nucleotide sequences and looks for matches with the nucleotide sequences that are on GenBank (www.ncbi.nlm.nih.gov/genbank/)

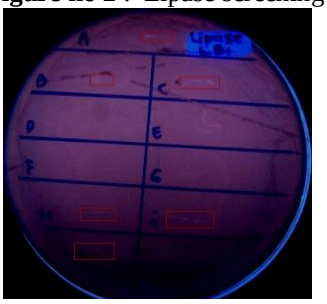
III. Result

From phase 1, the bacteria count was 3.5 x 107 CFU/mL. Eleven colonies with different morphologies were selected. Those with different morphologies were then grown back in solid LB media with an incubation temperature corresponding to the composting temperature, 28 ^{o}C.

Figure no 1 : Selected colony

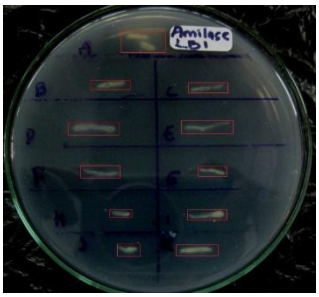
Lipase Screening
Bacterial colonies that have lipase activity produce a glow when viewed under UV light as seen in the image below

Figure no 2 : Lipase screening



Amylase Screening
Bacterial colonies that have amylase activity produce a clear zone around the colony[12] as shown in the image below

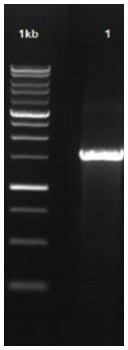
Figure no 3 : Amylase screening



From the screening results, the enzyme activity of each colony could be seen. Bacterial colonies that showed lipase activity in this phase were F1A, F1B, F1C, F1H, F1I, F1J. Meanwhile, amylase activity can be shown in all colonies. F1C colonies are the selected colonies that will be tested further, namely identification of the type of bacteria. Amylase enzyme is produced by all colonies; this shows higher activity than the lipase.

DNA isolation
F1C bacteria were chosen because of their potential in producing lipase and amylase. The potential bacteria representing each composting phase were then isolated for their chromosomal DNA for further identification. The isolated DNA was then electrophoresed using agarose gel. The results of electrophoresis showed that the DNA had a size above 20,000 bp. The 16S rRNA gene amplification was carried out using universal primers (Bact F primers and UniR primers). From the results of this study, it was found that the size of DNA after amplification was ± 1500 bp

Figure no 4 : PCR amplification



16S rRNA sequencing

Based on the results of the 16S rRNA gene sequencing, the nucleotide sequence data of the primers used were obtained. The nucleotide sequences obtained were analyzed using the BLAST program (www.blast.nbi.nlm.nih.gov/). The BLAST program compares the resulting nucleotide sequences with those in GenBank (www.ncbi.nlm.nih.gov/genbank).

Homology Analysis

Phylogenetic analysis was carried out using the Mega X program. Based on the results of the phylogenetic tree analysis, it was found that F1C bacteria had the closest homology to Bacillus cereus strain GXBC-3 with 98.66% similarity.

Figure no 5: Phylogenetic tree of F1C bacteria

IV. Discussion

In the first phase of composting, primary decomposers such as fungi, actinobacteria, and bacteria will convert energy-rich compounds such as sugars and proteins[2]. Bacterial colonies that have amylase activity produce a clear zone around the colony[10]. From the screening results, the enzyme activity of each colony could be seen. Bacterial colonies that showed lipase activity in this phase were F1A, F1B, F1C, F1H, F1I, F1J. Meanwhile, amylase activity can be shown in all colonies. F1C colonies are the selected colonies that will be tested further, namely identification of the type of bacteria. Amylase enzyme is produced by all colonies; this shows higher activity than the lipase. In this phase, the compost is decomposed by amylase, which is the breakdown of starch into simpler compounds where the starch comes from cow feed in the form of cassava. Meanwhile, lipase degrades lipids from concentrate feed in the form of peanut and coconut cake. The identification of bacteria was carried out to determine the bacterial species based on the 16S gene sequence.

The sequence of nucleotides at both ends of the gene is sustainable so that almost all genes can be amplified. F1C bacteria were chosen because of their potential in producing lipase and amylase. The potential bacteria representing each composting phase were then isolated for their chromosomal DNA for further identification. The isolated DNA was then electrophoresed using agarose gel. The results of electrophoresis showed that the DNA had a size above 20,000 bp. Chromosomal DNA amplification was carried out using PCR techniques and the results were electrophoresed with agarose gel. The isolated chromosome DNA was used as a template in the PCR process. PCR 16S rRNA was performed with the aim of amplifying the whole 16S rRNA gene. The 16S rRNA gene amplification was carried out using universal primers (Bact F primers and UniR primers). From the results of this study, it was found that the size of DNA after amplification was ± 1500 bp. This indicates that the amplification process has been successfully carried out because the gene amplified is the 16S gene which has a size of about 1500 bp[13].

Phylogenetic analysis was carried out using the Mega X program. Based on the results of the phylogenetic tree analysis, it was found that F1C bacteria had the closest homology to *Bacillus cereus* strain GXBC-3 with 98.66% similarity. Based on[10], the dominant bacteria through molecular of 16S rRNA genes amplified from the mesofilic (30^{o}C) phase composting is *Bacillus*

V. Conclusion

From the results of this study, it was found that the lipase and amylase-producing F1C bacteria during the initiating composting process of cow manure were closest to Bacillus cereus strain GXBC-3 with 98.66% similarities.

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