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Screening of Xylanase and Cellulase from Bacillus cereus F1C

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Abstract:
Background*: The increase in livestock production on the other hand can have a negative impact on the environment in the form of waste. One method of processing livestock waste is by composting. The composting process has 4 main phases, namely the initial mesophilic phase, the thermophilic phase, the late mesophilic phase and the cooling or maturation phase.. This study aims to screen for xylanase and cellulase enzymes from Bacillus cereus F1C isolates.*
Materials and Methods*: In this research, extraction of xylan from corn cobs, cultivation of F1C bacterial isolate, xylanase screening and cellulase screening have been carried out.*
Results*: From the screening results obtained using specific media, it is known that F1C isolates can produce xylanase and cellulase enzymes. Xylan substrate was obtained from the extraction of corn cobs.*
Key Word*: Bacillus cereus ; Cellulase ;Compost; Screening ; Xylanase.*

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I. Introduction

Xylanase is a hydrolytic enzyme that randomly cleaves the β-1,4 backbone of the xylan polysaccharide complex[1] (Toni colins2005). Xylanases are produced by microorganisms like bacteria, fungi , protozoa. Xylanase is widely applied to the paper and pulp industry, animal feed processing, beverage industry, and the production of biofuels from agro residues[2 3 4 5].

Cellulase (EC 3.2.1.4) is an enzyme complex consisting of several enzymes that break down cellulose into glucose by hydrolyzing β-1,4 bonds in cellulose[6]. Cellulase enzymes are widely applied in the textile industry, in detergents as agents to increase the brightness and softness of fabrics, improve nutritional quality and digestibility of animal feed, and manufacture bioethanol [7]. Cellulase can be produced from microorganisms such as bacteria and fungi [8 9].

Composting is a process of recycling organic waste that involves a number of microbes that occur under controlled conditions. Composting organic waste is one of the sources in finding enzyme-producing microorganisms. The research results conducted by Glavica [10] showed the activity of protease, amylase, esterase, cellulase, ligninase and xylanase enzymes in compost made from wood chips and pharmaceutical industry waste. Cow manure is widely used as fertilizer because it can change soil structure, increase root development, increase soil binding capacity and absorption of water, increase the life of organisms in the soil and add nutrients to the soil[11]. Composted cow manure also contains beneficial bacteria, which convert nutrients into an easily accessible form to be released slowly without burning the tender plant roots. Composting cow manure also produces about a third fewer greenhouse gases, making it environmentally friendly

In previous studies, bacterial isolation from cow manure compost was carried out in the mesophilic and thermophilic phases[3 12]. In Fenti, 2021 [13] screening for amylase and lipase enzymes was carried out from samples of F1C isolated cow manure compost obtained in the initiation phase. From the results of the identification of 16S, it was known that the isolate was *Bacillus cereus*. The same isolate sample will be screened for xylanase and cellulase enzymes. This study aims to screen for xylanase and cellulase enzymes from *Bacillus cereus* F1C isolates.

II. Material And Methods

Material

The material used in this study was a sample of F1C isolates derived from cow manure compost in the early stages of composting.

Method

Extraction of xylan from corn cobs

Xylan was extracted from corn cobs by delignification using 1% sodium hypochlorite (NaOCl) solvent. After that, the corn cobs were baked for 48 hours at 50oC. Then it was soaked with 15% NaOH solution. Xylan was then precipitated with HCl[14].

Cultivation of F1C isolate

F1C isolate was obtained from the isolation of cow manure compost samples mixed with straw in a ratio of 3:1. This compost sample was taken from the initial composting phase (initiation phase) with a temperature condition of 28^oC [13]. The isolates were grown on solid Luria Bertani media. The isolates were incubated at the same temperature as the composting temperature of 28^oC for 16 hours using a shaker at 150 rpm.

Xylanase screening

The composition of solid media for the selection of xylanase-producing bacteria were 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). v), 0.5% Na_2HPO_4 (w/v), 2% bakto agar, 0.2% (w/v) yeast extract and 0.5% (w/v) xylan. The F1C isolate was scratched on Agar media and then incubated at 28 ^oC for 16 hours. Xylanase selection was carried out by looking at the clear zone that was produced around the colony after the addition of Congo red solution [15].

Cellulase screening

F1C isolates were grown on CMC media (CMC, $MgSO_4.7H_2O$, KNO_3, FeSO4.7H_2O, $CaCl_2.2H_2O$, K_2HPO_4, yeast extract, bakto agar, glucose), and then the culture was scraped on solid media and then incubated at 28 ^oC for 16 hours. After the microbes grew well, they were stained using Congo red (1mg/mL) for 15 minutes and destained using 1 M NaCl solution for 15 minutes[16].

III. Result

In the composting process, microorganisms secrete extracellular enzymes to break down components that have a significant molecular weight into pieces that can be assimilated and converted into humus or compost. While intracellular enzymes play a role in helping components in compost with a small molecular weight for cellular metabolic processes, provide energy and re-form larger polymers[10]. The feed is given to cow, generally consists of forage and concentrate. Forage is feed derived from plants that are given to cattle in fresh form. The concentrate is a fortifying feed composed of grains and waste from the food industry process that increase the nutritional value to meet the normal needs of livestock to grow and develop healthily.

In previous studies, it was known that F1C isolates had amylase and lipase enzyme activity. This isolate was taken from cow manure compost samples from the initial phase of composting with a temperature of 28oC. DNA isolation, DNA amplification by PCR, sequencing of F1C isolates and then the homology analysis of these isolates was carried out[13]. This isolate was close to Bacillus cereus strain GXBC-3 with 98.66% similarity.

The isolates then were grown on solid Luria Bertani media. The isolates were incubated at the same temperature as the composting temperature of 28^oC for 16 hours using a shaker at 150 rpm.

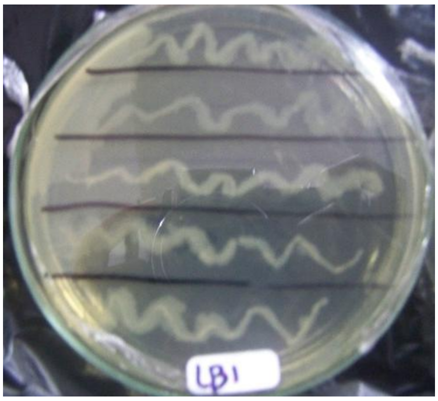

Figure 1. F1C isolates

This research used corn cobs which are the most considerable corn waste, contain 12% xylan in them. Xylan was extracted and used as a xylanase substrate. In the early stages, the delignification process occurs, removing lignin from lignocellulosic materials. Xylan was used as a substrate for the growth medium of isolate F1C in screening the xylanase. The incubation results showed that the isolate could produce xylanase enzyme. The results can be seen from the clear zone produced around the colony (Figure 2).

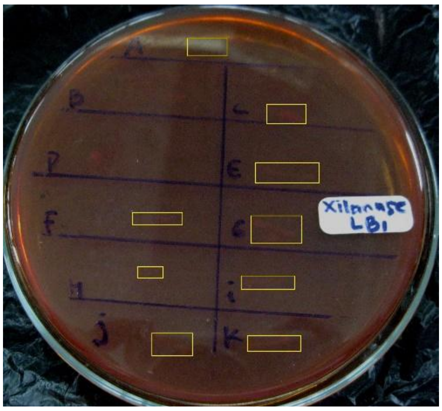

Figure 2. Xylanase screening results

F1C isolates were also grown on CMC-specific media to determine whether this isolate could produce cellulase enzymes. From the screening results, it can be seen that there is a clear zone around the bacteria. This condition shows that F1C isolates can produce cellulase enzymes (Figure 3).

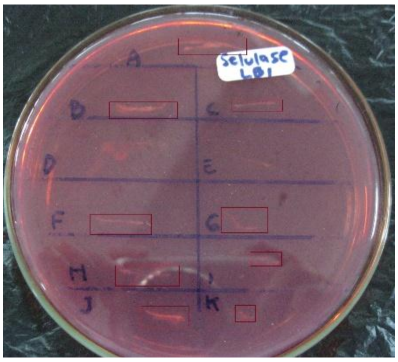

Figure 3. Cellulase screening results

The study results of Pantaya[17] show that rumen fluid obtained from abattoirs is rich in fiber-degrading enzymes and vitamins. Rumen fluid contains the enzymes -amylase, galactosidase, hemicellulase, cellulase, and xylanase. This is in line with the results of this study, which isolates F1C, close to *Bacillus cereus.*

IV. Conclusion

From the screening results obtained using specific media, it was known that F1C isolates can produce xylanase and cellulase enzymes. Xylan substrate was obtained from the extraction of corn cobs.

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