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Isolation and Properties Biochemical of Amylase Producing-Bacteria from Compost

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Abstract:- Amylase bacteria are enzymes that can hydrolyze starch into sugars. Amylase producing thermophile bacteria can be obtained from hot springs, geothermal, and from compost Compost is a solid mature product obtained from composting process. The search and study of biochemical properties of amylase-producing bacteria originated from have not been done. Compost is rich with various substrates and C/N elements for bacterial growth. This allows the high diversity of amylase-producing bacteria derived from compost. The aims of this research are to find out and understand the biochemical properties of amylase-producing bacteria derived from compost. The result of bacteria isolation and screening obtained 25 isolates and 5 isolates of amylase-producing bacteria. From 5 isolates of amylase-producing bacteria seen isolate CS3.1 with widest amylolytic index, ie 3.38 mm. CS3.1 bacteria isolates belong to Gram-positive bacteria and have endospores. Based on the results of biochemical properties test showed that bacteria isolate CS3.1 belong to *Bacillus* sp.

Keywords:- *bacterial isolation; biochemical test; amylase; compost.*

I. INTRODUCTION

Bacteria is one of the microbes producing various enzymes, such as protease, lipase, xylanase, and amylase. Amylase produced by bacteria or bacteria amylase is an enzyme that can hydrolyze starch to sugar [14]. Amylase is important due to their commercial applications in starch liquefaction, paper, textile fabrics, brewing industry, sugar induction by the production of sugar syrups, pharmaceuticals, and preparing cold water dispersible laundry [17]. Amylases derived from thermophile bacteria are particularly attractive in many industries because they are resistant to high temperatures during industrial processes. Amylase-producing bacteria can be obtained from hot springs, geothermal, and from compost. Compost is a solid mature product obtained from composting process. Composting is the recycling of solid waste. By the action of bacteria and fungi, organic waste change into humus [1]. During composting, the starting material is transformed through a variety of biological and biochemical processes in which enzymes play a role [7]. The search and study of biochemical properties of amylase-producing bacteria have not been done. Whereas compost is a good place for the growth of amylase-producing thermophile bacteria. Compost is rich with various substrates and C/N elements for bacterial growth. This allows the high diversity of amylase-producing bacteria derived from compost. The compost used in the research comes from organic waste that has been composting for 2 months. The aims of this research are to find out and understand the biochemical properties of amylase-producing bacteria derived from compost.

II. METHODE

A. Sampling and sample dilution

The compost sample was taken and put into a sample bottle and taken to the laboratory. Next dilution, 1 gram of soil derived from the compost is fed into the test tube, in addition to 9 ml of distilled water and in the vortex until the homogenous suspension is formed. One ml was taken from a 10^{-1} dilution suspension using a sterile dropper and inserted into a second reaction tube and added 9 ml of aquades to form a 10^{-2} suspension. The same procedure was performed until 10^{-7} dilution was obtained.

B. Isolation and screening of amylase–producing bacteria

Bacteria isolation was done by spread plate method, where 1 ml of suspension from 10^{-7} was spread on the medium surface in petri dish and leveled by using drill glass. Subsequently, the petri dish was incubated in an upside position for 24 hours at 37°C. Petri dish that has contained medium for sterilized starch. The bacteria were then fed into the NA medium and incubated at 50°C for 24 hours. Drop the iodine solution over the growing colony to see its amylolytic index. Isolates that produce amylase will produce clear zones around bacterial colonies. The clear zone is measured using a sliding range. Bacterial isolates with the widest amylolytic index were tested for their characterization and biochemical properties.

C. Characterization of amylase producing bacteria

Characteristics of the colony morphology observed include shape, edges, and elevation [9].

D. Biochemical properties of bacteria

Biochemical properties of bacteria were performed by bacterial staining and biochemical tests, such as TSIA, Methyl Red, Voges Proskauer, SIM (Sulfide Indol Motility), Urea, Citrate, Sugar (glucose, lactose, sucrose, and maltose), Fermentation, Oxidation, and Nitrate test [6].

III. RESULT AND DISCUSSION

The result of bacterial isolation and screening obtained 25 isolates and 5 isolates of amylase-producing bacteria (Figure 1).

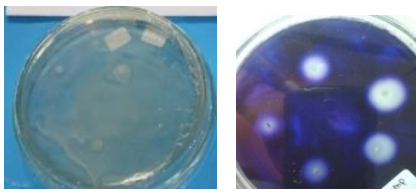

Fig 1:- The result of isolation and screening amylase-producing bacteria

Figure 1 shows a clear zone around bacterial growth. The clear zone around the growth of the thermophile bacteria indicates that starch has been hydrolyzed by amylases that are fed by the thermophile bacteria into simple sugars that do not show the color reaction with iodine [18]. According to [2], the difference of enzyme activity in each isolate is caused by the amount and activity of enzyme from each isolate which is secreted on a different medium. The enzyme activity is determined by enzyme concentration, enzyme conformation, amino acid-forming enzyme sequence and various enzyme-building amino acids. Screening and testing of amylase activity have also been performed by [4], which obtained R-SAII-1b isolates from the hot springs of Lejja and [11]. managed to obtain BT5.9 isolate from Changar hot spring.

From 5 isolates of amylase-producing bacteria seen isolate CS3.1 with widest amylolytic index, ie 3.38 mm. According to [2], the difference in enzyme activity in each isolate is due to the amount and activity of the enzyme from each isolate secreted on a different medium. The enzyme activity is determined by enzyme concentration, enzyme conformation, amino acid-forming enzyme sequence and various enzyme-building amino acids.

The macroscopic characterization results show white CS3.1 bacterial isolates, irregular shapes, grooved edges, and elevated elevations. Colony and form its size vary greatly depending on its type. Gram staining CS3.1 bacteria isolates belong to Gram-positive bacteria and have endospores (Figure 2).

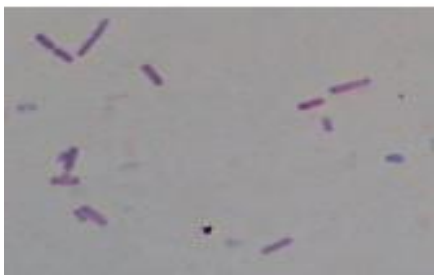

Fig 2:- Staining Gram

The endospora produced by *Bacillus* aims protecting bacteria from a state that is not profitable such as drought, nutrient deficiency, freezing, as well as chemicals. The bacterial endospora types this is resistant to environmental changes, resistant to heat, and chemical disinfectants certain in a long time [12]. If the environment is good, then endospores will experience sporogenesis and be forming a vegetative cell [15].

Based on the results of biochemical properties test showed that bacteria isolate CS3.1 belong to *Bacillus* sp (Table). *Bacillus* is motile (nonmotile reaction occasionally), producing spores that are usually resistant to heat, aerobes (some facultative anaerobes), positive catalase, oxidation varies, and are Gram-positive.

No.	Biochemical assay	Result
1	SIM	+
2	Urea	-
3	Citrate	-
4	Lactose	-
5	Glucose	+
6	Sucrose	-
7	Maltose	-
8	MR	+
9	VP	-
10	Oxidase	+
11	Fermentasion	-
12	Nitrate	+
13	TSIA	R/Y

Table 1. Biochemical properties test results of bacteria isolate CS3.1

The acid is produced from the hydrolysis of sucrose, lactose, and maltose, but the gas is produced through positive tests on glucose, citrate, and Voges-Proskauer. Similar results reported by [16]; [10]. on the biochemical test of *Bacillus*.

Test motility with using media SIM show that bacterial isolate CS3.1 is motile. This is seen with the presence of traces movement of bacteria after media inoculated in the SIM media, which means this bacterium has a motion device.

According to Pelczar [13] that *Bacillus* move with peritricus flagel where this peritrikus is a flagellum which is located throughout the bacterial specimens.

The urea test on bacterial isolate CS3.1 was obtained negative results, this is it because there is no color change on the medium from red-orange to red-purple. Some of the genus *Bacillus* can produce urease, but in general bacteria of this genus *Bacillus* cannot producing urease [6].

Glucose testing in positive CS3.1 bacterial isolates ferment glucose, this is the case marked by the occurrence of the color change on medium to yellow. Color yellow due to the presence Brom Timol Blue (BTB) indicator inside the medium. MR test on isolate CS3.1 showed positive result marked with the color on MR medium remains red and negative results are marked with changes the color on the medium becomes yellow, if methyl red indicator added on culture with a pH of 5 or less then the indicator will be a

meaningful red the bacteria are acidic fermentation mixed acid fermenter. Some *Bacillus* bacteria is an acidic ferment mixed and partly not fermented acidic acid [6].

The VP test has negative results for CS3.1 marked by no change the color on the medium becomes red, Based on that study has been done for the test the ability of bacteria to reduce nitrate become nitrite, show positive reaction due to the color change medium to red. In general most of *Bacillus* cannot be forming nitrates and much more can produce nitrate [6], this enzymatic reaction is controlled by an enzyme called *nitratase*.

On observation of biochemical test for TSIA test (Trepton Soya Iron Agar), the part slant is red, this is due because the formation of a basic compound indicating that *Bacillus* did not ferment lactose, maltose, and sucrose. While the TSIA media color on the yellow butt part is meaningful *Bacillus* ferment glucose. According to [5] *Bacillus* can fermenting glucose for the source energy.

Most *Bacillus* are mesophyll bacteria that grow at optimum temperatures between 30-45℃, although there are some that belong to thermophile with the optimal temperature at 65℃. *Bacillus* is known to produce a variety of extracellular enzymes and can be used in various industries [3].

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