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Hydrolytic and *Transglycolation* Characteristics of *Xanthomonas campestris* and *Bacillus megaterium* in Several Substrates

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***Abstract*— Some microbes can produce *hydrolytic* enzymes and have *transglycosylation* capacity at the same time, including *Xanthomonas campestris* and *Bacillus megaterium*. The enzyme characteristics of the microbes can be observed from their activity by using several types of substrates. This research aimed to characterize the hydrolytic and *transglycosylated CGTase* enzyme activity from *Xanthomonas campestris* and *Bacillus megaterium* on *glucose*, tapioca, and corn starch media at several concentrations. Stages of research included bacterial rejuvenation, growth on the substrate, and analysis (hydrolytic activity, carbohydrate concentration, and *transglycosylation* activity). The research design used was a Completely Randomised Design (CRD). The data from observations were then analyzed using Analysis of Variance (ANOVA) with the Duncan Multiple Range Test (DMRT) at a 5% level to determine the effect of treatment on all observational variables. Data analysis was carried out using the SPSS Statistics 17.0 program. The analysis results showed *Xanthomonas campestris* and *Bacillus megaterium* with tapioca and *cornstarch* substrates of 2%, 4%, and 6%, respectively, had hydrolytic activity. *CGTase* enzymes produced from *Xanthomonas campestris* and *Bacillus megaterium* with *glucose*, tapioca, and corn starch substrates 2%, 4%, and 6%, had intramolecular *transglycosylation* activity. The *CGTase* hydrolytic activity test's analysis showed that the CGTase hydrolytic test's significance value was significantly different between treatments with an incubation time of 24 to 120 hours. The substrate, a carbon source, greatly influences the rate of enzyme production produced by microorganisms.**

***Keywords*— Substrate; hydrolytic; *transglycosylation*; *Xanthomonas campestris*; *Bacillus megaterium*.**

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I. INTRODUCTION

Microbes have the potential to produce enzymes that are useful for food processing and pharmaceutical processing. In addition to transfer activity, *cyclodextrin glycosyltransferase* (*CGTase*) can *hydrolyze* starch or *cyclodextrin* into compounds with medium or simpler chains. *CGTase* enzyme is an extracellular enzyme produced by *bacteria* and *archaea*, which converts starch to *cyclodextrin* (CD) used by various industries, such as pharmaceuticals, chemicals, cosmetics, textile, foods, and environmental preservation. CD is widely used to improve solubility, stability, and the bioavailability of difficult-to-dissolve drug compounds, improve odor and bad taste, protect compounds that are easily oxidized, and reduce the volatility of volatile substances in the pharmaceutical industry [1]. In the food and cosmetics industry, it is used as an antioxidant and improves the texture and stability of product flavors. Several studies related to cyclodextrin's application as a matrix for drug delivery, sponge formation, oilfield, hydrogel, and ease of solubility have been studied and developed [2]-[10].

One of them is the production of *hydrolytic* enzymes and *CGTase*. *CGTase* under the systematic name of [1,2-α-D-*glucan-4- α-D-* (*1,4-glucan0*) *-transferase* (cyclic), EC 2.4.19] is an enzyme that has *transglycosylation* activity, both intermolecular (transferring *glucosyl* groups from the donor to the appropriate acceptor) and intramolecular (converting starch to *cyclodextrin*). *Transglycosylation* reaction is the reaction of the transfer of sugar units to the acceptor, which has the -OH group, using the *CGTase* enzyme on the *polyphenol* compound as an acceptor [11]. Several hydrolytic and *transglycosylated* enzymes from *amylosucrase* can be produced from several types of bacteria, including *Xanthomonas campestris* [12]. Hargono *et al*. [13] carried out cassava starch hydrolysis using the α-amylase and glucoamylase enzymes. Liu *et al*. [14] analyzed the *amylopullulanase* activity and *transglycosylation* enzymes of *Bacillus megaterium*. *B. megaterium* able to grow on a wide variety of carbon sources. They were found

in many ecological niches such as waste from the meat industry or *petrochemical* effluents [15]. The process of *transglycosylation* and hydrolysis can be done using bacteria that produce the enzyme. Tu et al. [16] conducted research and identified *B. megaterium* capable of producing an enzyme that can *hydrolyze* rice starch and wheat starch. This strain indicates this capacity were found in the cultures up to 72-hour-old and at incubation temperature up to 45°C. Rojas et al. (2019) [17] recovery starch used cassava bagasse for *cyclodextrin* production by sequential treatment with α-amylase and *cyclodextrin glycosyltransferase. CGTase* enzymes are produced by a variety of bacteria, namely *Bacillus*, *Thermoanaerobacterium,* and *Brevibacterium*. The most natural *cyclodextrin* production is produced by the *Bacillus bacterium* with the extracellular *CGTase* enzyme. Sources of *CGTase*-producing enzymes are found in many microorganisms that live on the ground. The largest microorganisms that live in the soil are bacteria due to the availability of nutritional needs such as water, minerals, and carbon [18].

The bacteria that have been known to produce *CGTase* enzymes are *Candida rugosa*, *Bacillus coagulans*, *Bacillus polymyxa*, *Bacillus megaterium*, *Bacillus macerans* [19], *Xanthomonas campestris*, *Bacillus sp.TS1*, *NR1s.d NR11UPM* isolated from local Malaysian soil, *Paenibacillus graminis* isolated from the rhizosphere of corn and wheat roots in agricultural fields in France and Australia, *B. clarkia 7364* isolated from Japanese soil, *Bacillus sp. BGG-1* isolated from the UNPAD lawn park, Js-1 isolates isolated from Sumedang, West Java [1], *Bacillus sp* [18], *TRU* isolates, *SSJ1* isolates, *SSJ2* isolates, *HPU1* isolates, and *Bacillus sp* [20], *TRU* isolates, *SSJ1* isolates, *SSJ2* isolates, *HPU1* isolates, and *BGG1* isolates isolated from Jatinangor UNPAD soil. *Bacillus megaterium* and *Xanthomonas campestris* also have the hydrolytic capacity [17], [21]. The purpose of this research activity was to characterize the hydrolytic and *transglycosylated* activities of the *CGTase* enzyme from *Xanthomonas campestris* and *Bacillus megaterium* on media containing *glucose*, tapioca, and corn starch at several concentrations.

II. Materials and Method

A. Materials

The materials used in this study were: *alginate* Himedia® MB114, *nutrient broth* OXOID CM0001, *nutrient agar* OXOID CM0003, yeast extract OXOID LP0021, *malt* extract, *tripton* OKOID LP0042*, peptone* OXOID LP0034, OXOID *agar* CM0003, NaClC4040 OXOID MERCID .1000, (NH) 2SO4, MgSO4, CaSO4, MnSO4, FeSO4, H2SO4 MERCK 1,00731.2500, HCl MERCK 1,00317.2500, *phosphate* buffer, *iodine dye*, *glucose* SIGMA G8270, fructose SIGMA F0127, MERCK C1307, c*yclolstrule methanol* MERCK 1.06009, soluble starch MERCK 1.01252.0250, *tapioca* flour SPM, *Maizeena Maize flour®, MERCK resorcinol* 1.07593.0100, MERCK *ethyl acetate* 1,00868.2500, MERCK acetate acid 1,00244*, Maizeaku® cornstarch* enzyme, MERCK *resorcinol* 1.07593.0100, MERCK ethyl acetate 1,00868.2500, MERCK acetic acid 1,00244, *Maizeaku® cornstarch enzyme*, MERCK *resorcinol* 1.07593.0100, *MERCK ethyl acetate* 1.00868.2500, *acetic* acid MERCK 1.00244, enzyme α-*aminoglycoside, xanthan* Sigma® gum, and distilled water.

The equipment used in the research process were as follows: KERN ABJ-NM/ABS-N analytical balance, HIRAYAMA HICLAVETM HVE-50 autoclave, Fisher Scientific hotplate stirrer, Fisher Scientific spectrophotometer, Vis Agilent Teknologies 60 UV-Vis, BRUKER infrared spectrophotometer (IR) TENSOR 37, TLC (thin layer chromatography), pH meter HANNA Instruments HI 2211, micro-pipette JENCONS SEALPETTE, Memmert Oven UN110, IKEDA MFP-300N furnace, Memmert INB400 incubator, STUART SCIENTIFIC SI 50 micro-pipette, TOMY TX-160 centrifugation furnace, IKEDA MFP-300N furnace incubator, Memmert INB400 incubator, STUART SCIENTIFIC SI 50, pipette incubator TOMY TX-160, laminar centrifugation ESCO airflow, Petri dishes, Bunsen burners, measuring cups, goblets, Erlenmeyer, measuring flasks, and Whatwann filter paper.

B. Methods

The research carried out included rejuvenation of *Xanthomonas campestris* and *Bacillus megaterium*, bacterial growth on *CGTase* media, *CGTase* hydrolytic activity testing, total dissolved solids and *transglycosylation* activity tests. The study used two bacteria, namely *Xanthomonas campestris* and *Bacillus megaterium* on *CGTase* media with tapioca substrate and 2%, 4%, and 6% cornstarch, respectively. Furthermore, the samples were identified XT (*X.campestris* on tapioca substrate), XM (*X.campestris* on tapioca substrate), XG (*X.campestris* on *glucose* substrate) BT (*B. Megaterium* on *tapioca* substrate), BG (*B.megaterium* on cornstarch substrate) and *BM (B. Megaterium on cornstarch* substrate). Whereas 2,4 and 6 show the concentration of the substrate added to the media.

1) Bacterial Rejuvenation: Rejuvenation of bacteria *Xanthomonas campestris* and *Bacillus megaterium* was conducted by growing them on Luria Broth Agar (LA) media. Rejuvenation media consisted of 5 g yeast, 10 g *tripton*, 5 g NaCl, and 15 g agar dissolved in 1 L distilled water and then sterilized at 121^0C. A total of 10 mL of bacterial culture was grown on rejuvenation media with a *scratchplate* method and then incubated at 37^0C for 24 hours. A total of 2 bacterial cultures from LA media were inoculated in *Nutrient Broth* (NB) media, then incubated at 37^0C for 24 hours.

2) Growth of Xanthomonas Campestris and Bacillus Megaterium on CGTase Media: Media for *CGTase* enzyme growth were made from standard media (NH) 2SO4 3 g, MgSO4 0.5 g, CaSO4 0.1 g, MnSO4 0.01 g, FeSO4 0.1 g, malt extract 3 g, peptone 0.5 g dissolved into 1 L dose of 0.05 mM *phosphate* buffer pH 7. Standard dissolved media were put into 18 250 mL tubes of 55.55 mL each and were added tapioca flour substrate (2%, 4%, and 6%), cornstarch (2%, 4%, and 6%), and *glucose* (2%, 4%, and 6%). All tubes were sterilized using an autoclave temperature of 121^0 C pressure of 1 atm.

The culture media of *CGTase* enzymes added by culture was incubated for 72-120 hours in a rocking incubator at 240 rpm, the temperature of 37^0C, and then centrifuged at 10,000

rpm for 15 minutes at 4^0C. Furthermore, the supernatant was used as a source of the crude *CGTase* enzyme [12].

3) CGTase Hydrolytic Activity Test: Supernatants containing crude *CGTase* enzyme were then tested for their hydrolytic activity. The reaction mixture consisted of 100 µL of crude *CGTase* enzyme solution and 450 µL of 50 mM phosphate buffer containing 0.5% soluble starch incubated at 45^0C for 10 minutes. The mixture of crude *CGTase* enzyme and partially buffered phosphate was then tested for pH and total dissolved solids (TDS). The reaction was stopped by the addition of 0.5 N HCl of 1 mL, and then reacted with 2.5 mL iodine dye (0.05% KI containing 0.002% I2). A spectrophotometer measures color absorption at a wavelength of 660 nm. One enzyme activity unit is expressed as the amount of enzyme that can reduce the absorption unit by 0.5 at a wavelength (λ) of 660 nm [12]. Furthermore, serial measurements are carried out for five days every 24 hours.

4) Measurement of Total Dissolved Solids: The reaction mixture consisted of 100 µL of crude *CGTase* enzyme solution and 450 µL of 50 mM phosphate buffer containing a 0.5% soluble starch incubated 45^0C for 10 minutes. It was then measured using a refractometer. Furthermore, serial measurements were carried out for five days every 24 hours.

5) Carbohydrate Concentration Test: The carbohydrate test was determined by the sulfur-phenol acid rule [22]. As much as 0.5 mL of 5% phenol plus 2.5 mL of concentrated H2SO4, then 0.5 mL of *CGTase* growth media sample was added, which was diluted ten times with *Aquades*. The mixture was left for 20 minutes at room temperature. Furthermore, it was determined through a standard *glucose* curve. *Carbohydrate* standard curves were made by measuring the absorbance of carbohydrate content that is known for its absorption. Standard concentrations were made by dissolving *glucose* at a concentration of 0.10,20,30,40.50 µg each dissolved into distilled water until the volume of the solution reaches 5 mL. The solution was then reacted with 0.5 mL phenol and 2.5 mL concentrated H2SO4. Then the reaction mixture was measured its absorbance at a wavelength of 490 nm, starting from the smallest concentration to the largest concentration.

6) Transglycosylation Activity Test: The reaction mixture was 2 mL, consisting of 1.5 mL 0.05 M phosphate buffer pH 7 containing 5% soluble starch and 2% resorcinol and 0.5 mL of crude *CGTase* enzyme incubated at 45 0C for 24 hours. *CGTase* enzyme activity was stopped at 100^0C for 10 minutes. After being cooled, the enzyme α-amyloglucosidase was produced by Sigma® as much as 0.005% and incubated for 45 minutes.

The resulting transfer product was identified by thin-layer chromatography (TLC), using a solution of ethyl acetate-acetic acid-distilled water (3: 1: 1, v / v). TLC plates were dried at 100 0C for 1 hour, then sprayed with a generator solution containing 20% H2SO4 in methanol, then TLC plates were burned in an oven at 150 0C for 5-10 minutes.

In this research, the research design used was a Completely Randomized Design (CRD). Data from observations were then analyzed using Analysis of Variance (ANOVA) to determine the effect of treatment on all observational variables. The analysis proceeded with the Duncan Multiple Range Test (DMRT) at a 5% level if there were significant differences. Data analysis was carried out using the SPSS Statistics 17.0 program.

III. Results and Discussions

A. Hydrolytic Activity

The *CGTase* hydrolytic activity test's analysis showed that the CGTase hydrolytic test's significance value was significantly different between treatments with an incubation time of 24 to 120 hours. Because there is a real difference between the treatments in the *CGTase* hydrolytic test (24-120 hours), the pH test of the *CGTase* hydrolytic activity (0-120 hours), and the total dissolved solid *CGTase* test (0-72 hours), so the Duncan test continued to see the effect between treatments.

TABLE I
CGTase Hydrolytic Activity

Sample	Incubation Time (unit/mL)				
	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours
XT2	1.72± 0.05^{cd}	2.35± 0.52^{de}	2.40± 0.50^{ef}	2.42± 0.47^{e}	2.49± 0.48^{fg}
XT4	1.75± 1.74^{cd}	3.81± 0.48^{ab}	3.89± 0.47^{abc}	3.92± 0.49^{abc}	4.01± 0.47^{abc}
XT6	2.94± 0.11^{abcd}	3.80± 0.27^{ab}	3.86± 0.30^{abc}	3.87± 0.31^{abc}	3.99± 0.33^{abc}
XM2	1.26± 0.63^{c}	1.59± 0.23^{e}	1.63± 0.24^{f}	1.64± 0.24^{f}	1.73± 0.34^{g}
XM4	3.29± 0.01^{abc}	3.48± 0.25^{bc}	3.55± 0.21^{bcd}	3.57± 0.21^{bcd}	3.59± 0.20^{bcde}
XM6	3.48± 0.39^{abc}	3.51± 0.36^{bc}	3.71± 0.35^{abc}	3.75± 0.35^{bc}	3.80± 0.33^{bcd}
BT2	4.53± 0.36^{a}	4.57± 0.74^{a}	4.57± 0.73^{a}	4.63± 0.73^{a}	4.79± 0.71^{a}
BT4	2.86± 0.36^{abc}	3.17± 0.74^{bcd}	3.16± 0.73^{cde}	3.18± 0.73^{cde}	3.21± 0.71^{cde}
BT6	1.28± 1.78^{d}	2.52± 0.19^{d}	2.55± 0.17^{e}	2.63± 0.06^{e}	2.77± 0.12^{ef}
BM2	2.48± 0.41^{bcd}	2.86± 0.12^{cd}	2.87± 0.12^{de}	2.89± 0.10^{de}	2.95± 0.15^{def}
BM4	3.94± 0.21^{ab}	4.38± 0.30^{a}	4.39± 0.29^{ab}	4.41± 0.25^{ab}	4.42± 0.25^{ab}
BM6	2.64± 0.02^{abcd}	2.73± 0.06^{cd}	2.79± 0.01^{de}	2.74± 0.02^{e}	2.75± 0.16^{ef}

Note: X: *Xanthomonas campestris*, B: *Bacillus megaterium*, G: glucose, T: tapioca, M: corn-starch values followed by different letters show significant difference at a 95% significance level

Duncan test results on *CGTase* hydrolytic activity on 24 hours *Bacillus megaterium* with tapioca substrate 2% (BT2) had the highest activity of 4.53 units / mL. Still, they were not significantly different from *Bacillus megaterium* with maize substrate 4% (BM4), *Xanthomonas campestris* with *maize* substrate 6% (XM6), *Xanthomonas campestris* with corn starch substrate 4% (XM4), *Xanthomonas campestris* with tapioca substrate 6% (XT6), *Bacillus megaterium* with tapioca substrate 4% (BT4), and *Bacillus megaterium* with *maize* substrate 6% (BM6). Meanwhile, *Xanthomonas campestris* with maise substrate, 2% had the lowest 1.26 units / mL activity.

The differences in substrate type and concentration affect the *hydrolytic CGTase* activity. The longer incubation time increases the ability of *CGTase* activity as the results are presented in Table 1. Between the 24 and 48 hours, incubation time shows a significant increase compared to the increase that occurs at the incubation time of 48 hours and above. The differences vary considerably between types and substrate concentrations. An increase in substrate concentration indicated a decrease in the *hydrolytic CGTase* activity in *B. megaterium* using a substrate from tapioca. Meanwhile, using cornstarch substrate showed a maximum fluctuation of 4%. On the *cornstarch* substrate using X. Campestris, an increase in concentration resulted in increased hydrolytic CGTase activity and 6% tapioca substrate slightly decreased compared to the 4% concentration.

Madonna [23] analyzed the production of amylolytic enzymes from *sago* starch using the bacterium *Bacillus megaterium*. The highest amylolytic enzyme activity, equal to 0.076 (units/ml), was found in the fermentation medium treatment with *sago* starch content of 2% (w / v). It was not significantly different from treatment with *sago* starch content of 0.5% (w / v) and treatment with *sago* starch content of 1% (w / v). The substrate, a carbon source, greatly influences the rate of enzyme production produced by microorganisms.

Microbes can produce *exopolysaccharides* by degrading waste or using several types of substrates. *Exopolysaccharide* implementation can use sugar-beets substrate materials: *molasses*, sugar syrup, *sucrose*, or from corn: starch, *hydrolyze*d starch, glucose syrup, glucose. Furthermore, differences in the type and concentration of substrates will affect the growth of *X. campestris* in producing metabolites [6]. *Xanthomonas campestris* and its production yield can be further increased at suitable physical conditions, i.e., temperature, pH, agitation, carbon, and nitrogen sources.

B. Total Dissolved Solids (TDS)

The TDS of crude *CGTase* enzyme significantly different between treatments. The Duncan test results of TDS of crude *CGTase* enzyme with the medium of *Bacillus megaterium* glucose 6% (BG6) had the highest 7.6° Brix value. Whereas *Xanthomonas campestris* with *maize* substrate, 2% (XM2) had the lowest 2.4° Brix value.

TDS (Total Dissolved Solids) (see Table 2) shows microbes' activity related to the ability to produce *CGTase Hydrolytic* activity. This activity is illustrated by its ability to *hydrolyze* and *transglycolate* substrates. The influence of the type and concentration of the substrate and the incubation time affected the resulting TDS. The turbidity level of the solution described by TDS shows the magnitude of the microbial ability combined with the substrate concentration and incubation time related to the ability of the resulting *Hydrolytic CGTase* activity.

TABLE II
TOTAL DISSOLVED SOLIDS (TDS) OF *CGTASE* HYDROLYTIC ACTIVITY

Sample	Incubation Time (°Brix)					
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours
XG2	1.0± 0.00^{d}	1.1± 0.07^{cde}	1.0± 0.00^{ef}	1.0± 0.00^{efg}	1.3± 0.00	1.0± 0.00
XG4	1.2± 0.00^{c}	1.3± 0.00 ^{bcde}	1.1± 0.00^{de}	1.1± 0.00^{def}	1.8± 0.49	1.4± 0.57
XG6	1.5± 0.14^{b}	1.5± 0.21^{abc}	1.3± 0.21^{cde}	1.5± 0.28^{abcd}	1.9± 0.35	1.5± 0.42
XT2	1.0± 0.00^{d}	1.0± 0.00^{ed}	0.8± 0.07^{fg}	0.8± 0.00^{fg}	1.2± 0.00	1.6± 0.00
XT4	1.5± 0.00^{b}	1.2± 0.07^{cde}	1.1± 0.14^{de}	1.2± 0.14^{cdef}	1.5± 0.07	1.3± 0.07
XT6	1.4± 0.28^{b}	1.2± 0.21^{cde}	1.1± 0.14^{de}	1.2± 0.21^{def}	1.5± 0.21	1.4± 0.07
XM2	1.0± 0.00^{d}	1.3± 0.71^{bcde}	0.7± 0.07^{g}	0.7± 0.07^{g}	1.0± 0.00	0.8± 0.00
XM4	1.2± 0.00^{c}	0.9± 0.00^{e}	1.0± 0.42^{ef}	1.4± 0.57^{bcde}	1.7± 0.85	2.0± 1.48
XM6	1.4± 0.00^{b}	1.0± 0.00^{de}	1.0± 0.00^{ef}	1.0± 0.00^{efg}	1.3± 0.00	1.1± 0.00
BG2	1.2± 0.00^{c}	1.2± 0.00^{bcde}	1.4± 0.00^{bcd}	1.2± 0.00^{cdef}	1.2± 0.00	1.2± 0.00
BG4	1.4± 0.00^{b}	1.6± 0.00^{ab}	1.6± 0.00^{ab}	1.6± 0.00^{abc}	1.4± 0.00	1.4± 0.00
BG6	1.8± 0.00^{a}	1.8± 0.00^{a}	1.9± 0.14^{a}	1.7± 0.14^{ab}	1.7± 0.14	1.4± 0.00
BT2	1.0± 0.00^{d}	1.2± 0.00^{bcde}	1.2± 0.00^{cde}	1.4± 0.00^{bcde}	1.3± 0.14	1.0± 0.00
BT4	1.4± 0.00^{b}	1.4± 0.00^{abcd}	1.5± 0.14^{bc}	1.7± 0.14^{ab}	1.6± 0.00	2.4± 1.70
BT6	1.7± 0.14^{a}	1.6± 0.00^{ab}	1.7± 0.14^{ab}	1.9± 0.14^{a}	1.8± 0.28	1.1± 0.14
BM2	1.0± 0.00^{d}	1.0± 0.00^{de}	1.2± 0.00^{cde}	1.2± 0.00^{cdef}	1.2± 0.00	1.0± 0.00
BM4	1.2± 0.00^{c}	1.1± 0.14^{cde}	1.2± 0.00^{cde}	1.4± 0.00^{bcde}	1.4± 0.00	1.0± 0.00
BM6	1.4± 0.00^{b}	1.4± 0.00^{abcd}	1.2± 0.00^{cde}	1.4± 0.00^{bcde}	1.2± 0.00	1.0± 0.00

Note: X: *Xanthomonas campestris*, B: *Bacillus megaterium*, G: glucose, T: tapioca, M: corn starch values followed by different letters show significant difference at a 95% significance level

The TDS of the glucose substrate showed little change over the incubation period. This change is because the glucose substrate is already in the form of monomers, so that very little hydrolytic activity may occur, and is more likely due to the transformation of other activities of these microbes. Meanwhile, tapioca and cornstarch showed an increase in fluctuation, which was highly dependent on combining the type and concentration of the substrate and incubation time. The highest TDS was obtained from a combination of treatments using *B.megaterium* microbes on tapioca substrate with a concentration of 4% (BT4), which was incubated for 120 hours. Further implementation still requires further optimization related to the effect of TDS on enzyme activity.

C. Carbohydrate Concentration

The analysis results of *CGTase* media carbohydrate density showed a significance value (P-value) from the treatment of 0.00 for the incubation parameters of 0 hours to 120 hours. The significance value of the *CGTase* media carbohydrate concentration test of the six parameters was < 0.05. It can be concluded that there were significant differences between treatments with an incubation time of 0 to 120 hours. There was a real difference between treatments in the *CGTase* media carbohydrate concentration test from 0 hours to 120 hours, so the Duncan test continued to see the effect between treatments.

TABLE III
THE *CGTASE* MEDIA CARBOHYDRATE DENSITY

Sample	Incubation Time µg/mL					
	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours
XG2	136.50± 6.19^{d}	135.53± 5.97^{fg}	131.78± 1.72^{ef}	129.28± 0.22^{e}	127.66± 1.64^{d}	126.34± 1.10^{ef}
XG4	160.66± 3.23^{ed}	154.66± 7.91^{ef}	150.84± 6.85^{d}	147.31± 5.21^{d}	143.03± 0.13^{c}	140.75± 0.62^{d}
XG6	173.38± 0.18^{c}	160.94± 0.09^{e}	147.37± 7.16^{de}	145.59± 6.58^{d}	144.41± 5.79^{c}	137.94± 12.55^{de}
XT2	133.72± 0.75^{d}	129.97± 2.08^{g}	127.56± 2.39^{f}	126.81± 1.68^{e}	125.03± 0.22^{d}	124.31± 0.44^{f}
XT4	143.50± 4.41^{d}	139.69± 0.27^{ef}	137.28± 0.04^{def}	132.66± 0.57^{e}	130.94± 1.86^{d}	129.22± 0.04^{def}
XT6	151.13± 2.91^{cd}	142.13± 5.75^{efg}	140.63± 3.80^{def}	134.97± 3.31^{e}	132.66± 0.57^{d}	126.34± 7.20^{ef}
XM2	139.78± 11.62^{d}	137.44± 8.66^{efg}	134.4± 6.81^{def}	132.53± 4.55^{e}	128.63± 6.36^{d}	123.81± 1.50^{f}
XM4	148.97± 1.54^{cd}	141.56± 4.51^{efg}	137.44± 4.51^{def}	136.22± 5.35^{e}	134.84± 1.64^{cd}	133.94± 2.74^{def}
XM6	156.66± 5.78^{cd}	150.19± 13.79^{efg}	136.09± 7.20^{def}	132.50± 4.24^{e}	129.66± 4.73^{d}	128.59± 5.97^{def}
BG2	216.31± 13.79^{b}	199.69± 4.95^{cd}	195.75± 1.59^{bc}	165.53± 8.97^{c}	174.31± 3.54^{ab}	167.31± 8.49^{abc}
BG4	239.09± 14.63^{ab}	222.47± 5.79^{abc}	206.72± 7.20^{abc}	181.94± 0.09^{ab}	173.28± 2.25^{ab}	166.44± 6.28^{abc}
BG6	257.22± 1.81^{a}	240.59± 10.65^{a}	219.81± 5.83^{a}	184.00± 4.51^{a}	180.09± 3.31^{a}	177.28± 5.17^{a}
BT2	214.59± 21.70^{b}	197.97± 12.86^{d}	195.50± 0.62^{c}	174.69± 1.77^{abc}	167.84± 8.71^{b}	156.94± 2.65^{bc}
BT4	218.88± 7.15^{b}	202.25± 8.31^{cd}	197.25± 13.44^{bc}	176.59± 0.49^{ab}	173.06± 2.83^{ab}	167.34± 4.38^{abc}
BT6	235.91± 24.70^{ab}	219.28± 15.87^{abcd}	205.56± 2.47^{bc}	178.31± 6.98^{ab}	176.06± 9.99^{ab}	168.63± 7.16^{ab}
BM2	218.13± 1.41^{b}	201.50± 10.25^{cd}	196.13± 6.54^{bc}	174.00± 1.50^{bc}	171.34± 0.66^{ab}	155.156± 2.52^{c}
BM4	233.13± 21.83^{ab}	210.25± 21.83^{bcd}	198.34± 14.63^{bc}	175.25± 4.24^{ab}	171.84± 4.02^{ab}	164.22± 1.99^{bc}
BM6	243.81± 13.70^{ab}	230.31± 9.28^{ab}	213.03± 11.53^{ba}	174.56± 1.33^{abc}	171.72± 4.29^{ab}	156.56± 6.81^{cb}

Note : X: *Xanthomonas campestris*, B: *Bacillus megaterium*, G: glucose, T: tapioca, M: corn starch values followed by different letters show significant difference at a 95% significance level

The Duncan test results of *CGTase carbohydrate* concentration in 0 hours of *Bacillus megaterium* with *glucose* substrate 6% (BG6) had the highest *carbohydrate* content of 257,219 µg / mL. Still, it was not significantly different from *Bacillus megaterium* with *maize* substrate 6% (BM6), *Bacillus megaterium* with glucose substrate 4 % (BG4), and *Bacillus megaterium* with *tapioca* substrate 6% (BT6). Meanwhile, *Xanthomonas campestris* with *tapioca* substrate, 2% had the lowest *carbohydrate* content of 133,719 µg / mL.

Analysis of the determination of carbohydrates produced a reaction between carbohydrates with phenols and concentrated *sulfuric* acid to form a solid black color if the carbohydrate content were high. The lower the carbohydrate content, then the reaction mixture would fade. The test was carried out 24 days for five days. From the 18 treatments, the *carbohydrate* levels decreased due to the *Xanthomonas campestris* b*acterium's* nature, which can use various carbohydrates and organic salts as a carbon source [6], [12]. In this study, it was also known that *Bacillus megaterium* could use various carbohydrates as a carbon source. B. *megaterium* also has the activity of producing amylase and cellulase enzymes on specific substrates [21].

D. TDS of CGTase Crude Enzyme

Based on the TDS of crude *CGTase* enzyme, the significance value (P-value) obtained from the treatment was 0.00. The significance value of the TDS of crude *CGTase* enzyme was <0.05. It can be concluded that there were significant differences between treatments. Hence, further Duncan's test was performed to see the effect between treatments.

Based on the Duncan test results of TDS of crude *CGTase* enzyme, *Bacillus megaterium* with glucose 6% (BG6) had the highest 7.6° Brix value. Meanwhile, *Xanthomonas campestris* with cornstarch substrate 2% (XM2) has the lowest 2.4° Brix value. It shows that increasing microbial activity would increase the capacity of the enzymes it produced. The type of substrate and incubation time affected the growth of *X. campestris* [24]. To produce *CGTase*,

several things need to be considered to produce optimal enzyme concentrations [25].

TABLE IV
TOTAL DISSOLVED SOLIDS (TDS) OF CRUDE *CGTASE* ENZYME

Sample	(°Brix)
XG2	2.7 ± 0.14 [l]
XG4	4.4 ± 0.00 [h]
XG6	5.2 ± 0.00 [d]
XT2	3.0 ± 0.00 [k]
XT4	4.7 ± 0.00 [fg]
XT6	4.8 ± 0.00 [ef]
XM2	2.4 ± 0.07 [m]
XM4	4.0± 0.00 [i]
XM6	4.7 ± 0.00 [fg]
BG2	4.0 ± 0.00 [i]
BG4	6.0 ± 0.00 [c]
BG6	7.6 ± 0.28 [a]
BT2	3.8± 0.00 [i]
BT4	5.0 ± 0.00 [de]
BT6	6.8± 0.00 [b]
BM2	3.3 ± 0.14 [j]
BM4	4.5 ± 0.14 [gh]
BM6	4.7± 0.14 [fg]

Note: X: *Xanthomonas campestris*, B: *Bacillus megaterium*, G: *glucose*, T: *tapioca*, M: corn starch numbers followed by different letters show significantly different at a 95% significance level

TDS is used to help identify the ability to produce crude enzymes as TDS increases. TDS can reflect the turbidity level in the resulting supernatant so that it is identified with the increasing amount of crude enzymes produced. With the lower TDS reflects, the crude enzyme levels contained therein are also getting lower. This activity is closely related to the type of microbe, the type and concentration of the substrate used. The TDS produced by the microbe *B. megaterium* was higher than that of *X. campestris.* This result may imply that microbial species' capacity affects the ability to produce enzyme levels from *CGTase*. The suitability of microbes with the type of substrate also showed differences. The research results show that the increasing substrate concentration can generally increase the TDS produced. This result can be explained that the increasing number of substrates used can increase the amount of crude *CGTase* enzyme produced, based on the level of turbidity identified from the TDS results. *CGTase* is highly influenced by the type, substrate concentration, incubation time, and pH, which affects the production process [26], [27].

E. CGTase Transglycillation Activity

One of the enzymes that have transfer activity is *CGTase*. This enzyme can convert starch to *cyclodextrin* through intramolecular *transglycosylation* reactions and *glucosyl* groups' transfer to OH acceptors through intermolecular reactions. Tests were carried out on *CGTase* enzymes produced from various types of substrates that can transfer *glycosyl* groups to resorcinol acceptors. The results of resorcinol-glycoside products were then identified using thin-layer chromatography (TLC), marked by a spot that has a Retention factor (Rf) value close to the Rf value of Arbutin standards. *Arbutin* is a *polyphenol glycoside* compound known as *4-hydroxyphenyl-D-glucopyranoside* and has been reported to have biological activities like antimicrobial, anti-inflammatory, and antioxidant compounds [19].

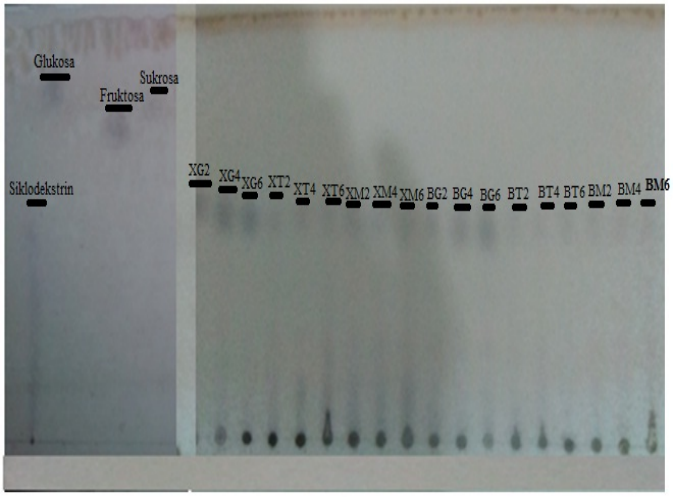

Fig. 1 Chromatogram results of *CGTase transglycosylation* activity test results. X: *Xanthomonas campestris*, B: *Bacillus megaterium*, G: glucose, T: tapioca, M: corn starch, with substrate concentration 2% - 6%

The TLC stationary phase was polar silica, while the mobile phase used was the developer solution consisting of *ethyl acetate-acetic acid-Aquades* (3: 1: 1, v / v). The mobile phase will carry compounds with low polarity levels to have a high Rf pillar, while compounds with high polarity levels will be restrained so that they tend to have smaller Rf values [12].

Assay for *transglycosylation* reaction activity of the *CGTase* enzyme derived from some culture media containing some kinds of carbohydrates. The carbon source was corresponding to enable transferring *glycosyl* to acceptor polyphenol on the synthesis of polyphenol-glycosides as transfer product determined by using TLC and identified by the presence of spot showed of Retention factor (*Rf*) value was approaching to *Rf* value of arbutin [12].

In this study, the standards used were *cyclodextrin*, glucose, fructose, and sucrose. *CGTase* enzymes from *Xanthomonas campestris* and *Bacillus megaterium* consisting of 18 samples distinguished by substrate types and substrate concentrations had intramolecular *transglycosylation* activity that is changing starch into *cyclodextrin*. This is indicated by the TLC spot, which had an Rf value close to the standard *cyclodextrin* with an Rf value of intramolecular *transglycosylation* (converting starch into *cyclodextrin*).

*Cyclodextrin*s are cyclic oligosaccharides composed of six, seven or eight D-glucose (*α*-, *β*-, and *γ-cyclodextrin*s, resp.) Which are joined by *α*- (1, 4) *glycosidic* bonds. *Cyclodextrin* is produced through an intramolecular *transglycosylation* (*cyclisation*) reaction during the starch degradation process by the *CGTase* enzyme [26]. Rajput et al. (2016) [26] used six types of starch, namely: soluble starch, potato starch, *sago* starch, corn starch, corn flour, and rice flour to produce β-*Cyclodextrin*. Based on the research results, the use of enzymes, substrates, and microbes affects cyclodextrin productio*n*. *Cyclodextrin glucotransferase* produced from

Microbacterium terrae KNR 9 can degrade gelatinized form as well as the raw form of all the different starch substrates tested except corn starch.

IV. Conclusion

CGTase enzymes produced from the bacteria *Xanthomonas campestris* and *Bacillus megaterium* with tapioca and corn starch substrate of 2%, 4%, and 6%, respectively, had hydrolytic activity. *CGTase* enzymes produced from the bacteria *Xanthomonas campestris* and *Bacillus megaterium* with *glucose*, tapioca, corn starch substrate of 2%, 4%, and 6% had intramolecular *transglycosylation* (converting starch to *cyclodextrin*). Research results showed that both bacteria have activities to produce hydrolytic and *transglycosylated* enzymes and can be utilized to further process technology development.

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