Isolation Bacteria Producing $\alpha$-Amylase from Black Soldier Fly larvae ($Hermetia illucens$) L.

Luthfia Hastiani Muharram*, Nelis Hernahadini, and Muhammad Fauzi
Departement of Biotechnology, Universitas Muhammadiyah Bandung

*hastiani.0209@gmail.com

Abstract. Black Soldier Fly ($Hermetia illucens$) larvae used as a large-scale organic waste bioreduction agent with ability reduce organic waste up to 200 tons per day. The potency of BSF larvae to process organic waste was related to the function of phisiology, microbial and biochemical processes. The gut extracts had high amylase, lipase and protease activities. $\alpha$-amylase is one of the important enzyme in development of biotechnology and belongs to the main enzyme class on the world enzyme market. $\alpha$-amylase used to replace chemical hydrolysis process in starch processing industry. $\alpha$-amylase from microorganism was more prospective than plant and animal amylase, because it was more easily manipulated to produces targetted enzymes, characteristics and more economical. The purpose of this study was to isolate bacteria producing $\alpha$-amylase from BSF larvae. The research method was extracted of 200 BSF larvae in phosphate buffer pH 7.4. Homogenate was isolated in Lysogeny Broth (LB) by serial dilution and fourway strick. Each culture was screened based on the activity of $\alpha$-amylase by Fuwa method. The results of isolation obtained 8 single colonies bacteria. The $\alpha$-amylase activity assay for Fuwa method obtained the highest $\alpha$-amylase activity unit was 10.86 units/ml, produced by the 8th colony.

1. Introduction
The Black Soldier Fly (BSF), $Hermetia illucens$ (L.) (Diptera: Stratiomydae), is one of the most important insects for bioconversion and waste bioreduction [1]. The use of BSFL as an organic waste bioreduction agent has been proven on a large scale with the ability to reduce organic waste up to 200 tons per day. As an illustration, 1 kg of fresh BSF larvae can reduce 3 kg of Palm Kernel Meal (PKM) [2]. The ability of BSF larvae to process organic waste is an interesting research focus. Previous studies have explained the effects of macronutrient organic waste, microbes and food control, some studying physiology, microbiology, and biochemical processes [3].

$\alpha$-amylase is one of the important enzyme in development of biotechnology and belongs to the main enzyme class on the world enzyme market. $\alpha$-amylase from microorganism was more prospective than plant and animal amylase, because it was more easily manipulated to produces targetted enzymes, characteristics and more economical [4]. The larvae eats on diets with a large number and they have microbes with high diversity. The microbes in the larvae gut have so many functions that are crucial for larvae development [5], [6]. It is because of the great potential of BSFL for bioconversion enzymatic based microbes, this study was therefore to bacteria bioprospecting that optimal produce $\alpha$-amylase from BSFL.
2. Experimental
This research conducted in microbiology laboratory of Universitas Muhammadiyah Bandung and PUSRIS (Pusat Riset Bioinformatika dan Bioteknologi) UNPAD. BSFL samples were obtained from organic waste treatment center based BSF in Padasuka village, Cimahi City West Java Province.

2.1. Isolation
The samples, 200 BSFL was cleaned and turned off nerves on ice then dissected in ice batch. The contents of body was separated from the shells, stored in phosphate buffer 7.4 (pH). As much as 1ml of homogenate solution was carried out serial dilution into 9ml of physiological NaCl up to $10^{-7}$ dilution. Culture dilution then plate counted on LB agar and stricked fourway method, incubation on 37°C 48hours. This isolation step obtained 8 single colonies.

2.2. Crude Enzyme Extraction
Each colony was inoculated into 25ml of LB media, incubation 37°C for 48h with agitation 150rpm. Each culture was harvested by centrifugation at 5,000 x g for 10 minutes at 4°C. The supernatant was crude enzyme.

2.3. Activity assay of α-amylase (Fuwa methode)
0.1% starch solution was prepared by dissolving it in distilled water, then heated until the starch dissolves. 100 μL crude enzyme samples were added with 100 μL 0.1% dissolved starch, incubated at 50 °C for 10 minutes. A total of 100 μL of 1 N Hydrochloric Acid (HCl) solution was added. A total of 100 μL of Potassium Iodide (KI-I₂) solution (0.2% I₂ in 2% KI) was added. Then diluted with distilled water to a volume of 2mL. The absorbance of the sample is measured at a wavelength (λ) of 600nm [7].

3. Result & Discussion
The ability of BSF larvae to bioconversion organic waste is an interesting research focus. As the other insects and mammals, fly larvae feed to obtain nutrients for metabolic requirements. The monomer of carbohydrates, glucose, is used as building block for tissues, fuel, also carbohydrate chitin for insect phase [8]. Preparation study have been carried out in this research, to investigate activity of amylase from body content and gut (figure1).

figure1. Fresh BSFL (a), component body of BSFL : shells, body content and gut (b).

α-amylase activity assay (Fuwa method) from body content extract is bit higher than gut extract (figure2). This data explained that α-amylase produce besides in gut, also in the other organ. The biochemical process of BSF larvae starts from the mouth, the salivary glands secrete the enzymes amylase and maltase. Beside that, food decomposition occurs in the middle of the stomach [9].
Microbial decomposition of biowaste material involves the metabolism of a microbes consorsium from BSFL bioreactor. Microbes produce some enzymes include hydrolytic enzyme that function to hydrolyse macromolecule (carbohydrates, proteins, and lipids). α-amylases (EC 3.2.1.1) hidrolyse starch (polysaccharide) as the main substrate to be small units of glucose (monosaccharide) and maltose (disaccharide). Microbial amylases obtained from bacteria, fungi, and yeast [10]. This study have been isolated bacteria from body extract of BSFL (excepted the shells), there were eight different single colonies. Each colonies grown in shaker incubator (150rpm, 37 °C, 48h) to produced enzymes. The culture harvested by sentrifugation (5000 xg, 10 minutes), obtained eight cure enzyme extrects (supernatants). α-amylase is an extracellular enzyme, so the enzyme obtained in supernatant. The enzyme extracts have been investigated of α-amylase activity by Fuwa method and gave result as in figure3.

![Figure2](image)

**Figure2.** α-amylase Activity from gut extract and body content extract of BSFL.

![Figure3](image)

**Figure3.** α-amylase Activity from crude extract and eight single colonies bacteria from body content of BSFL.
This data describe that each bacteria colony gave high value of α-amylase activity, the highest was obtained by 8th colony (M8) with value activity is 10.9 U/ml. The activity from crude extract was very high because there were whole component of body BSFL including microbes consortia.

Fuwa method assay tested the remaining starch content. The enzyme activity of α-amylase was carried out using iodine solution. Iodine solution was basically brownish yellow. However, iodine solutions can form blue complexes with remaining starch. The blue absorbance measured shown the amount of starch that didn’t hydrolyzed by the enzyme.

**Figure4.** visualisation of activity α-amylase assay (Fuwa method) from crude extract and isolated bacteria of BSFL.

This result shown that each isolated bacteria from BSFL have different characteristics. It can be used as preliminary research to explore bacteria biodiversity from BSFL. Previous research reviewed, gut bacteria dominated by phyla Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria [11], [12], [13]. The level of amylase production various depending on genus, species, and strain. Beside that the source of the microbe’s origin and environmental factor such as pH, temperature (fermentation condition) can effected production of amylase. Microbes that isolated from starch- or amylose-rich environments naturally have higher amounts of enzyme [10]. Base on source of BSFL samples, the feed of BSFL was organic waste from hometown, dominate by foodsrap such as rice, fruits and vegetable residu.

4. Conclusion
Microbial enzyme is significant research field to explore biodiscovery and bioprospecting enzyme for industrial application. The present study clearly illustrated that Black Soldier Fly Larvae have great ability bioconversion and it’s associated with microbial diversity, primary in the gut. This study have been isolated bacteria colonies from BSF larvae and proved the value of α-amylase activity. The highest activity level of α-amylase was obtained by 8th isolated bacteria with the value was 10.9 U/ml (by Fuwa method). This isolated bacteria can be further research to identify genus and spesies, also genetic engineering studies to get optimal perform enzyme for industrial requirement.

References

[1] H. P. Makkar, G. Tran, V. Heuzé and P. Ankers, "State of the-art on use of insects as animal feed," Animal Feed Science and Technology, vol. 197, pp. 1-33, 2014.

[2] R. Rachmawati, B. Damayanti, P. Hidayat, S. Hem and M. R. Fahmi, "Perkembangan dan
Kandungan Nutrisi Larva Hermetia illucens (Linnaeus) (Diptera: Stratiomyidae) pada Bungkil Kelapa Sawit," J. Entomol. Indon., vol. 7, pp. 28-41, 2010.

[3] M. Fauzi and L. H. Muharram, "Karakteristik Bioreduksi Sampah Organik oleh Maggot BSF (Black Soldier Fly) pada Berbagai Level Instar: Review," JSTE, vol. 1, p. 134 – 139, 2019.

[4] P. M. de Souza and P. d. O. Mag, "Application of microbial α-amylase in industry – A review," Braz J Microbiol, vol. 41, pp. 850-861, 2010.

[5] M. Gold, J. K. Tomberlin, S. Diener, C. Zurbrügg and A. Mathys, "Decomposition of biowaste macronutrients, microbes, and chemicals in," Biowaste management, vol. 82, p. 302–318, 2018.

[6] A. E. Douglas, The Symbiotic Habit, Orincetcon, 2010.

[7] H. Fuwa, "A new method of microdetermination of amylase activity by the use of amylase as the substrate," J. Biochem., vol. 41, p. 583–603, 1954.

[8] A. C. Cohen, Insect Diets, CRS Press, 2005.

[9] W. R. Terra and B. P. Jordão, "Final digestion of starch in Musca domestica larvae. Distribution and properties of midgut α-d-glucosidases and glucoamylase," Insect Biochemistry, vol. 19, pp. 285-292, 1989.

[10] C. B. G. Subash, A. Periasamy, M. A. M. K., L. Thangavel, H. V. Chun, H. Uda and V. C. Suresh, "Biotechnological Processes in Microbial Amylase Production," BioMed Research International.

[11] I. V. Boccazzi, M. Ottoboni, E. Martin, F. Comandat, L. Vallone, T. Spranghers, M. Eeckhout, V. Mereghetti, L. Pinotti and S. Epis, "A survey of the mycobiota associated with larvae of the black soldier fly (Hermetia illucens) reared for feed production," PlosOne, vol. 12, 2017.

[12] C. S. Seung, K. Sung-Hee, Y. Hyejin, K. Boram, C. K. Aeri, L. Kyung-Ah, Y. Joo-Heon, R. Ji-Hwan and L. Won-Jae, "Drosophila Microbiome Modulates Host Developmental and Metabolic Homeostasis via Insulin Signaling," Science, vol. 334, no. 6056, pp. 670-674.

[13] Z. L. S. C and W. W. D, "Diversity and Contribution of the Intestinal Bacterial Community to the Development of Musca domestica (Diptera: Muscidae) Larvae," Journal of Medical Entomology, vol. 37, no. 6, p. 924–928, 2000.

[14] L. B. and M. Aliaga, "The digestive tract of Drosophila melanogaster.," Annu-rev-Genet, vol. 4, pp. 377-404, 2013.