Key Engineering Materials
ISSN: 1662-9795, Vol. 884, pp 165-170
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Submitted: 2020-04-20
Revised: 2020-12-02
Accepted: 2020-12-02
Online: 2021-05-04

Pectinase Production by Using Coffee Pulp Substrate as Carbon and Nitrogen Source

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Keywords: coffee pulp, optimization of pH, pectinase, pH stability, solid-state fermentation

Abstract. About thirty-five percent of coffee pulp waste is pectin. It may potentially be a source to be used in the bioprocessing industry. For example, it can be used as a substrate to produce pectinase from microorganisms under solid-state fermentation (SSF). In this investigation, an isolated fungus VTM4 with density 10^7 spores/mL was grown on coffee pulp medium-based, and after 0-168 hours incubation at 30 °C, pectinase activity was detected. The activity was measured based on reducing sugar released by crude pectinase against 0.5% alkali extract pectin substrate in 20 mM buffer acetate pH 5. The highest reducing sugar produced was 223.34 μg/mL after 72 hours of incubation at 30 °C. The optimum pH on enzyme activity was 4 with the maximum activity 0.747 U/mL and was stable (more than 80%) at a pH range of 4-5.5. The results revealed that the coffee substrate could be utilized as a carbon and nitrogen source to produce pectinase. Further research on purification and characterization of the enzyme to improve pectinase yield production was needed.

Introduction

In coffee production, about 50% residues as coffee pulp of the total coffee fruit were produced. Coffee production in 2018 was estimated at 169 million bags, and Indonesia produced 9.4 million bags. Indonesia placed 4th country as a coffee producer globally after Brazil, Vietnam, and Colombia [1]. Coffee processing resulted in residues causing major environmental, economic, and social problems. The main residue of coffee processing is coffee pulp; it represents about 40% mass of the ripe coffee fruit [2].

Coffee pulp is rich in sugars, protein, minerals, and that are potential to be used as raw material in bioprocessing industry. Coffee pulp is organic material that can be used as a source of carbon and nitrogen for the synthesis of microbial metabolism, including enzyme secretion. Growth fungi condition in solid-state fermentation (SSF) is suitable with the natural habitat filamentous fungi than in liquid culture [3]. It is known that the mycelium of fungi could grow well in solid-state fermentation (SSF) [4]. This process generates higher productivity, lower cost, simpler, and easier downstream [5].

Pectin is one of the components present in the plant's cell wall and the fruit peel. In general, pectin content in the cell wall of dicotyledonous plants is approximately 35%, grass 2-10%, and almost fruit peel contains pectin [3]. The citrus peel contains pectin 20-30%, while pomelo citrus peel contains pectin 42.5% [6]. The coffee pulp contains 35% pectin; thus, it is potential to be used as a substrate for pectinolytic fungi to produce pectinase [7]. In this case, coffee pulp waste is utilized as a substrate that contains a pectin source for the cultivation of fungal enzymes. In other cases, the world market has reported that pectinase accounts for 10% of global industrial enzymes produced [8]. Pectinase sales reached $75 million out of $1 billion industrial enzymes [9]. Pectinase is widely used in food fermentation, the textile industry, and animal feed [7].

Pectinase is produced from various microorganisms such as, bacteria, fungi, and yeast. The fungal enzyme has been developed to produce enzymes in industrial sector. Fungi utilization for

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producing enzyme does not need expensive cost because the culture medium originated from organic waste. Fungi will be induced to secrete enzyme if cultivated in a pectin medium [10]. The pectinase production by solid-state fermentation (SSF) resulted in an abundant yield than submerged fermentation (SmF). To convince that isolate VTM4 secrets pectinase, it conducted cultivation in extract pectin medium [11]. The preliminary investigation revealed that isolate VTM4 is a positively hydrolyzed pectin medium. In the present study, isolate VTM4 were cultured on coffee pulp in solid-state fermentation. The aim was to know the optimum incubation time for isolate VTM4 to produce pectinase, optimum pH of pectinase, and pH stability of pectinase.

Material and Method

Inoculum preparation. Isolate VTM4 was pre-cultured on slant Potato Dextrose Agar. It was incubated at 30 °C for 3 days of incubation. After that, cultured isolate VTM4 was moved to extract pectin + M9 (NaCl, $MgSO_4$, KH_2PO_4, NH_4Cl, Na_2HPO_4) medium and incubated at 37 °C for 0-168 hours. The spore of inoculum was observed using a Petroff-Hauser counting chamber to get spore density at 10^7 spores/mL [12].

Optimization production on coffee pulp substrate under SSF. Hydrolysis optimization was performed by inoculated 1 mL inoculum with a spore density of 10^7 spores/mL from isolate VTM4 into 10 g coffee pulp with a water content of 20 mL (1:2) [13], then it was incubated at 30 °C for 0-168 hours. The crude enzyme was harvested daily and measured for the enzyme activity.

Enzyme Extraction. The enzyme was harvested by adding 20 mL of distilled water containing 1% NaCl and 0.01% sodium azide (NaN_3) and shaking it for 12 hours at room temperature. The suspension was filtered by synthetic fabric filter and centrifuged for 10 minutes to separate the supernatant.

Enzyme assay. Enzyme activity was determined by measuring reducing sugars with Somogyi–Nelson method. Pectin substrate 0.5% in buffer acetate 20 mM pH 5 was incubated at 37 °C for 20 hours. Then crude enzyme 50 µL added into the test tube. The test tube and control tube were incubated at 37 °C for 2 hours. Both of them were then added 500 µL Somogyi reagent to stop the reaction between enzyme and substrate. A tightly stoppered tube then incubated in boiling water for 15 minutes; after cooling, added 500 Nelson reagent and 2.5 mL distilled water. Each sample was centrifuged at 8000 rpm for 10 minutes and was measured using a spectrophotometer with a wavelength of 500 nm [14].

Enzyme properties. The effect of pH on the enzyme activity and stability was determined at the pH range of 3 to 8 [15]. The crude enzyme was dissolved in buffer acetate (pH 3-5) and buffer phosphate (pH 5.5-8). The effect of pH optimization on the enzyme activity was measured after 2 hours incubation at 37 °C in 500 µL 0.5% substrate pectin with various range of pH (3-8). While the effect of pH stability was measured after 4 hours incubation and 2 hours incubation at 37 °C in 500 µL 0.5% substrate pectin with various range of pH (3-8) [16]. Then, the activity was determined by measuring reducing sugars by Somogyi–Nelson method.

Result and Discussion

Optimization of spore density. Density spore is one of the factors that affect the SSF process and yield. Here, isolate VTM4 was cultured in extract pectin + M9 (NaCl, $MgSO_4$, KH_2PO_4, NH_4Cl, Na_2HPO_4) medium and then resulted spore density 5.3×10^7 spores/mL after 96 hours incubation time (Fig. 1).

The incubation time to produce a density of spores in maximum will be used as a reference in the optimization of pectinase production of VTM4. As shown in Fig. 1, the maximum density spore was produced by isolate VTM4 after 168 hours incubation, with the density of spore reached 8.5×10^7 spores/mL. For optimization, pectinase production used 96 incubation times, although maximum density spore was found in 168 hours due to the time efficiency. The inoculum concentration of 10^6-10^8 spores/mL had known contributed to the maximum enzyme activity [18]. Higher cells for inoculum inhibiting fungal growth and enzyme production. The spore concentration

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and inoculum must be enough to colonize substrate particles because higher or lower can hinder the penetration of oxygen into a solid medium, inhibiting fungal growth from producing enzymes [17].

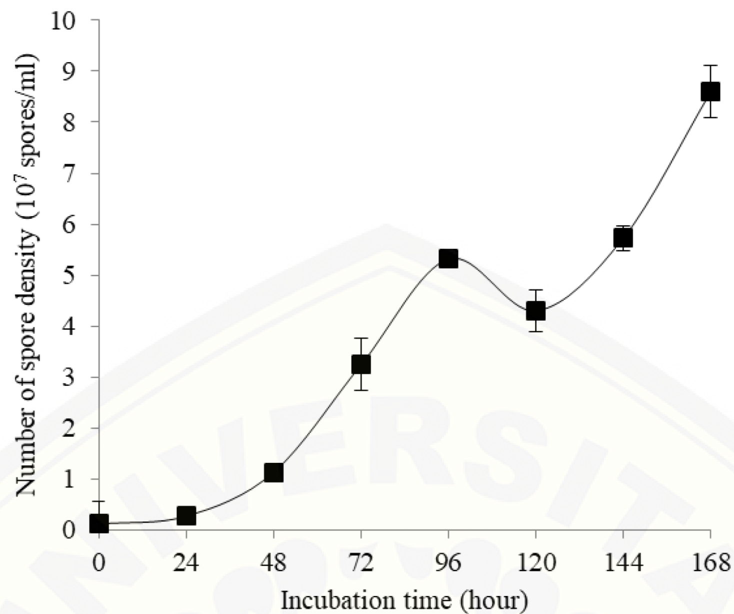

Figure 1. Spore density of isolate VTM4 in extract pectin + M9 medium

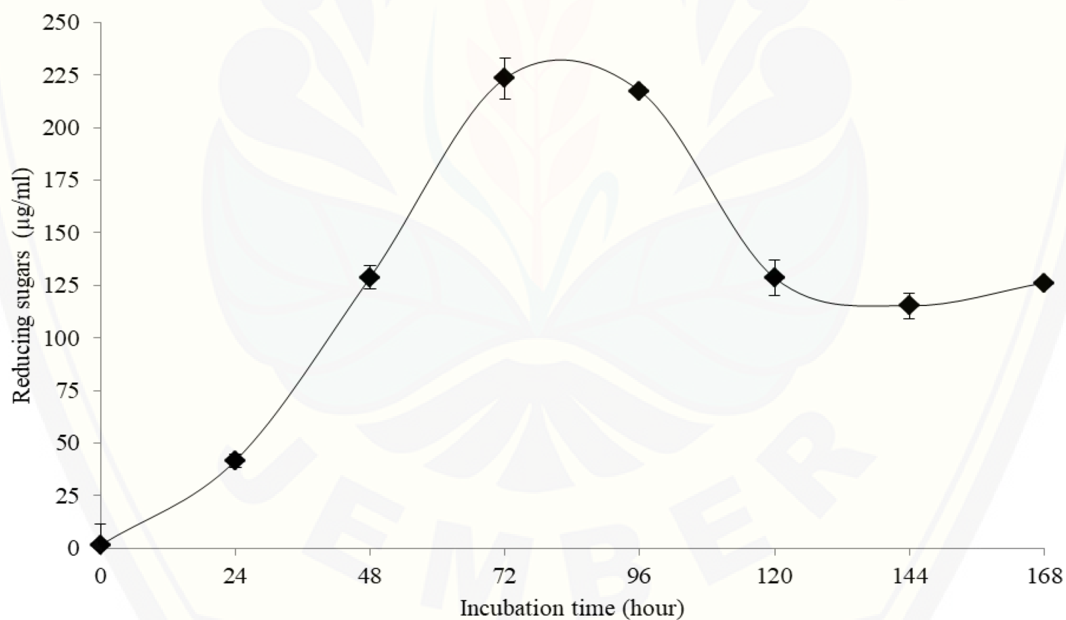

Figure 2. Reducing sugar curve of isolate VTM4 on coffee pulp substrate during SSF.

Optimization of pectinase production by isolate VTM4. Inoculum from isolate VTM4 1 mL was inoculated in coffee pulp substrate by a solid-state fermentation process. The water content of this substrate is 50%, and this condition was proposes to create a humidity substrate same as coffee pulp in nature. Extracellular enzyme activity was released by isolate VTM4 during the SSF process with coffee pulp substrate. The optimum activity to produce pectinase at 72 hours incubation (Fig. 2). Pectinase hydrolyzed coffee pulp was indicated by releasing reducing sugar against 0.5% alkali extract pectin in 20 mM buffer acetate pH 5. The optimum reducing sugar is obtained at 72 hours incubation time with an amount of 223.34 μg/mL; it can be used as a reference for large-scale pectinase production. After reducing sugar increased at an optimum point, then reducing sugars were decreasing. It may be caused by microbial using the simpler sugar from hydrolyzed first, so microbial less active to break down the complex substrate [19]. An increasing amount of product

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may occur in feedback inhibition. The amount of the product will be inhibitor bound at the allosteric site and change the enzyme's active conformation site [20]. Isolate VTM4 is able to utilized coffee pulp as a carbon and nitrogen, even mineral source [13].

Enzyme properties. The effect of pH pectinase was studied by using different range of pH (3, 3.5, 4, 4.5, 5, 5.5, 6,6.5, 7, 7.5; and 8) at 37 °C. The crude enzyme from isolate VTM4 was incubated for 96 hours in media coffee pulp. The optimal pH and stability were determined by measuring reducing sugars using Somogyi–Nelson method. The pH effect on the activity and stability of the enzyme was measured at the pH of 3 to 8. The optimum pH was 4, with an enzyme activity of 0.747 U/mL (Fig. 2). According to Pedrolli et al. [21] the optimum activity of pectinase occurred at pH 4.5. While the effect of pH stability was measured after 4 hours incubation and 2 hours incubation at 37 °C in 500 µL 0.5% substrate pectin.

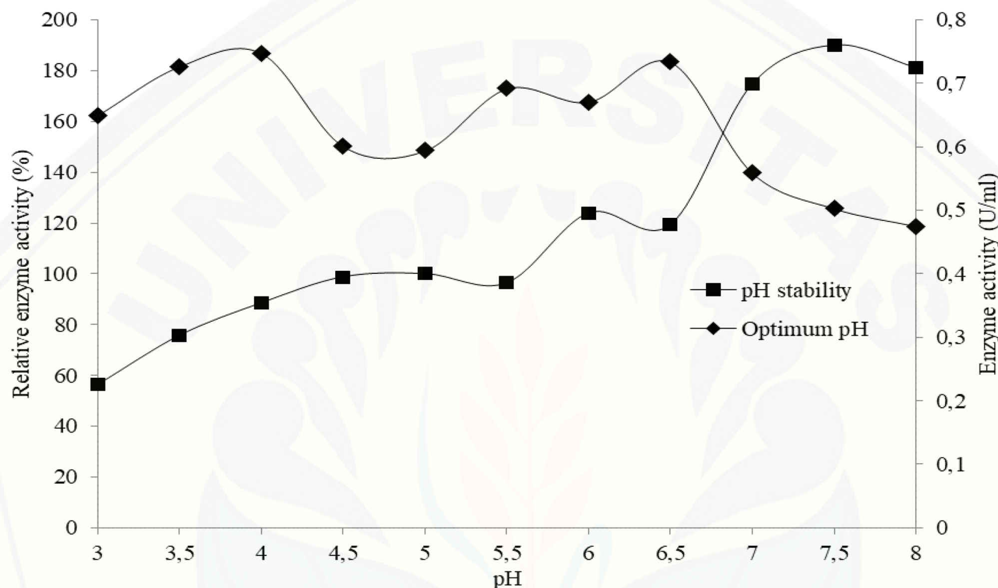

Figure 3. Enzyme properties (stability and optimum pH) of pectinase by isolate VTM4.

As illustrated in Fig.3, pectinase was stable (more than 80%) at a pH value of 4-5.5. The result revealed that pectinase was stable at a slightly acidic condition. This enzyme is stable under alkaline conditions, so this enzyme is potential for alkaline pectin degradation in coffee processing, the paper and pulp industry, and wastewater treatment.

Enzymes are affected by changes in pH. The suitable value of pH pectinase will activate the enzyme. While extremely low or high value of pH causes an inactive enzyme and may affect the production. Therefore each enzyme has a region of optimum pH and pH stability.

Summary

Coffee pulp is used as a substrate for fermentation isolate VTM4 that produce pectinase. Carbon and nitrogen in coffee pulp effective for fungi growth, so hydrolyze is fermented by isolated VTM4 formed reducing sugars. This enzyme has optimum reducing sugar 223.34 µg/mL, while optimum pH at value 4 and pH stability at value 4-5.5. However, it still needed purification and characterization of the enzyme to improve yield production.

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References

[1] ICO, Coffee production by exporting countries, International Coffee Organization, London, 2019.

[2] J.S. da Silveira, N. Durand, S. Lacour, M-P. Belleville, A. Perez, G. Loiseau, M. Dornier, Solid-state fermentation as a sustainable method for coffee pulp treatment and production of an extract rich in chlorogenic acids, Food Bioprod. Process. 115 (2019) 175-184.

[3] D. Silva, K. Tokuioshi, E.D.S. Martins, R. da Silva, E. Gomes, Production of pectinase by solid-state fermentation with *Penicillium viridicatum* RFC3, Process Biochem. 40 (8) (2005) 2885-2889.

[4] K. Muzakhar, A consortium of three enzymes : xylnase, arabinofuranosidase and cellulase from *Aspergillus sp.* which liquefied pulp waste, IOP Conf. Ser.: Mater. Sci. Eng. 546 (2) (2019) 022013.

[5] E.S. Martins, D. Silva, R. da Silva, E. Gomes, Solid state production of thermostable pectinases from thermophilic *Thermoascus aurantiacus*, Process Biochem. 37 (9) (2002) 949-954.

[6] B. Rivas, A. Torrado, P. Torre, A. Converti, J.M. Domínguez, Submerged citric acid frmentation on orange peel autohydrolysate, J. Agric. Food Chem. 56 (7) (2008) 2380-2387.

[7] U. Hasanah, M. Setyowati, Edwarsyah, R. Efendi, E. Safitri, R. Idroes, L.Y. Heng, N.D. Sani, Isolation of Pectin from coffee pulp Arabica Gayo for the development of matrices membrane, IOP Conf: Ser.: Mater. Sci. Eng. 523 (2019) 012014.

[8] K. Roy, S. Dey, Md.K. Uddin, R. Barua, Md.T. Hossain, Extracellular pectinase from a novel bacterium *Chryseobacterium indologenes* strain SD and its application in fruit juice clarification, Enzyme Res. 2018 (2018) 3859752.

[9] D.R. Kashyap, P.K. Vohra, S. Chopra, R. Tewari, Applications of pectinases in the commercial sector: a review, Bioresour. Technol. 77 (3) (2001) 215-227.

[10] G. Aguilar, C. Huitrón, Conidial and mycelial-bound exo-pectinase of *Aspergillus* sp., FEMS Microbiol. Lett. 108 (2) (1993) 127-132

[11] K. Muzakhar, Sutoyo, A.B. Saragih, Phosphate Solubilizing Bacteria Adaptive to Vinnasse, J. Math. Fundam. Sci. 47 (2) (2015) 219-225

[12] L. Ikasari, D.A. Mitchell, Protease production by Rhizopus oliigosporus in solid-state fermentation, World J. Microbiol. Biotechnol.10 (1994) 320-324 .

[13] K. Muzakhar, R. Winarsa, Activity of α-L-Rhamnosidase proced by Aspergilluus niger during solid state fermentation of coffee pulp wastes, J. Biodjati. 4 (1) (2019) 105-111.

[14] S. Ubaidillah, K. Muzakhar, Sugar-rich hydrolyzates from coffee pulp waste which produced under solid state fermentation by *Pestalotiosis* sp. VM9 and *Aspergillus* sp. VTM 5, and its effenciency as medium for single cell protein *Saccharomyces cerevisiae*, IOP Conf. Ser.: Mater. Sci. Eng. 546 (6) (2019) 062033.

[15] K. Muzakhar, Masruroh, Siswoyo, R. Winarsa, Sutoyo, Sugar-rich hydrolysates of palm oil empty fruit bunch production through two step solid state fermentations and its conversion to ethanol, Adv. Sci. Lett. 23 (3) (2017) 2533-2535.

[16] Z.W. Strain, G. Coral, Some Properties of crude carboxymethyl cellulase of *Aspergillus niger* Z10 wild-type strain, Turk. J. Biol. 26 (4) (2002) 209-213.

[17] V.H. Vu, T.A. Pham, K. Kim, improvement of fungal cellulase production by mutation and optimization of soolid state fermentation, Mycobiology. 39 (1) (2011) 20-25.

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[18] K. Mojsov, Experimental investigations of submerged fermentation and synthesis of pectinolytic enzymes by *Aspergillus Niger*: effect of inoculum size and age of spores, Appl. Technol. Innov. 2 (2) (2010) 40-46.

[19] S.C. Sati, S. Bisht, Utilization of various carbon sources for the growth of waterborne conidial fungi, Mycologia. 98 (5) (2006) 678-681.

[20] T. Sander, N. Farke, C. Diehl, M. Kuntz, T. Glatter, H. Link, Allosteric feedback inhibiton enables robust amino acid biosynthesis in *E.coli* by enforcing enzyme overabundance, Cell Syst. 8 (1) (2019). 66-7.

[21] D.B. Pedrolli, A.C. Monteiro, E. Gomes, E.C. Carmona, Pectin and pectinase: production, characterization and industrial application of microbial pectinolytic enzymes, Open Biotechnol. J. 3 (1) (2009) 9-18.