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Characterization of Alpha-Amylase from *Aspergillus niger* Aggregate F Isolated from a Fermented Cassava Gatot Grown in Potato Peel Waste Medium

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The use of GRAS microorganisms isolated from fermented foods during amylase production using an economical food-waste medium provides more opportunities to produce amylase with a wider range of applications. Hence, this study aimed to isolate a good amylase-producing fungi from the traditional Indonesian fermented cassava, gatot, and to identify the amylase-producing capability of the isolate in a potato peel waste (PPW) medium. Black-colored fungi isolated from gatot was morphologically identified and the amylase produced was characterized using SDS-PAGE and Native PAGE. The isolate was then grown on PPW medium, and the amylase produced was further characterized. Morphological identification and enzyme characterization revealed that the *Aspergillus niger* aggregate F isolated from gatot secreted an active extracellular α-amylase with an optimum pH of 5–6. In conclusion, *Aspergillus niger* aggregate F isolated from gatot can be used to produce α-amylase using PPW as a medium.

Keywords: **Alpha-amylase, *Aspergillus niger*, fermented cassava gatot, potato peel waste**

Introduction

Amylase is one of the most popular enzymes with a great importance in industrial and biotechnological applications and an estimated share of 25% in the global enzyme market [1]. Amylases have a wide range of applications ranging from food, textile, detergent, paper, pharmaceutical, and biofuel industries. Amylase can be obtained from different sources such as plants, animals, and microbes. Microbial amylases both from bacteria and fungi possess higher popularity due to its viability to meet general industrial demands [1–3].

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The advantages of using microorganisms as the source of amylases depend on its cost effectivity, high productivity, stability, and the ease of cultivation, optimization, and modification. Among the microbe-producing amylase, *Aspergillus niger* has been reported as one of the best commercially producers of α-amylase and glucoamylase. Amylases produced by *Aspergillus niger* are known to have a wide range of pH activity and thermostability that is suitable for industrial purposes [4]. Moreover, *Aspergillus niger* has earned the Generally Recognized as Safe (GRAS) status that enables its application in food and feed industries [5].

Beside the fermenting microorganisms, the other vital factor that determines the cost-efficiency of amylase production is the selection of the culture medium. It is increasingly common to design a solid state fermentation (SSF) system for fungal amylase production by

using food wastes as substrate for amylase production [6–9]. Potato peel has been reported as the major waste of potato industry and at least 50 kg of them is produced from every ton of potatoes. Moreover, the management of this abundance waste has become an important issue to the potato industries [10]. Potato peel waste (PPW) is one of the potential culture medium that can be used to produce amylase due to its abundance in availability as industrial waste. The utilization of PPW for amylase production is very viable due to its high starch content and other nutrients to support the growth of microbes [6, 11]. As an added value from using food wastes for amylase production, the fermentation also yields smaller fermentable sugars from the breakdown of starch [8] which could be used as a substrate for other processes such butanol fermentation by *Clostridium* species [12].

Indonesia has plenty of fermented food products, which do not only serve as national delicacies but also the source of beneficial microbes [13]. One of them is gatot, a traditional dessert originated from the Special Region of Yogyakarta, which is made from spontaneous fermentation of cassava by indigenous fungi [14]. Aside from being a popular fermented food, the exploration of beneficial microbes from gatot and their potential use for industrial application is still limited. The high starch content of cassava plays a major role in selecting highly potential amylase-producing microbes involved in gatot fermentation. Isolation of microbes from fermented food like gatot offers a higher chance of finding generally recognized as safe (GRAS) microbes which potentially produce amylase with a wider range of application. Therefore, this study aimed to determine and characterize the ability of *Aspergillus niger* aggregate isolated from gatot to produce amylase using PPW as its culture medium.

Materials and Methods

Isolation and morphological identification of *Aspergillus* sp. isolated from gatot

Ten grams of gatot and 90 ml of sterilized distilled water were mixed and diluted to 10^{-3}. Afterward, 100 μl of the mixture was inoculated on PDA by spread plate method and incubated for three days at 30℃. Fungal colony grown on PDA then purified by streak plate method on the new PDA and incubated for another three days at 30℃. Morphological observation of the fungal colony was done by slide culture technique [15].

Enzyme extraction and purification

Fungal isolate was cultured in potato dextrose broth (PDB) medium and incubated at 30℃ overnight. Supernatant of the culture was then collected by centrifugation at 8,000 g for 15 min and stored as the crude extract of the enzyme. Acetone precipitation was used to partially purify and concentrate the protein content of the extract. Crude extract collected from the PDB culture was mixed with chilled acetone in 1:3 ratio then stored overnight at -20℃. The resulting precipitates were collected by centrifugation at 15,000 rpm for 5 min, air dried at room temperature for 1 h and suspended in 250 μl Phosphate-Buffered Saline (PBS) [16].

Molecular weight identification of amylase by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The crude and partially purified extracts of *Aspergillus niger* aggregate F were analyzed by SDS-PAGE using 4% stacking gel and 10% running gel according to the method of Laemmli [17]. Electrophoresis was run at 50 V for 60 min, followed by 100 V for another 60 min. Visualization of protein bands was done by staining the gel in Coomasie Blue solution followed by destaining procedure. Approximate molecular weight of *Aspergillus niger* aggregate F amylase was then determined against prestained protein ladder (Bioline state).

Identification of amylase activity by Native-PAGE and activity staining analysis

Native-PAGE analysis was carried out as SDS-PAGE without the addition of SDS. Samples were loaded into 4% stacking gel and 10% running gel and run at 50 V for 60 min, followed by 100 V for another 60 min. After the separation, activity staining was done by incubating the running gel in 1% starch solution for 45–60 min at 37℃. The gel was then stained with iodine solution to detect the area in which starch degradation has occurred.

Potato peel waste medium and inoculation of fungal culture

Potato peel waste (PPW) were collected from a potato home industry and autoclaved at 121℃ for 21 min. Semi-solid fermentation medium was achieved by mash-

ing the sterilized peels using a mortar until it reached a pulp-like consistency. *Aspergillus niger* aggregate F was inoculated into the PPW medium and incubated at 30℃ for seven days. A successful growth was indicated by the formation of cotton-like structure of *Aspergillus niger* aggregate F mycelia in the medium.

Identification of amylase hydrolytic activity products by thin layer chromatography (TLC)

Two percent (w/v) starch solution was mixed with crude and partially purified extracts of *Aspergillus niger* aggregate F as well as commercial α-amylase (Novozyme, Denmark) and glucoamylase (Novozyme). The mixtures were incubated at 60℃ for 1 h. Then, 2 µl of each mixture was spotted into the Si60 plate (Merck Milipore, USA) along with four sugar standards: starch, dextrose, maltose, and sucrose. After air-dried in the oven, the base of the plate was then placed inside a sealed chamber filled with eluent which consisted of n-butanol, 1-propanol, acetic acid, and distilled water in the ratio of 3:1:1:1. As soon as the eluent reached the top of the plate, the plate was removed from the chamber and then air-dried in the oven. In order to visualize the sugar spots, the plate was dip into the visualization solution consisted of 0.5 gram α-napthol, 2.5 ml H_2SO_4, and 47.5 ml ethanol absolute. The plate was heated in the oven to visualize sugar spots and then the retention factor (Rf) value of each spot was measured and compared to the standard sugars [18].

Characterization of amylase activity

The activity of amylase produced by *Aspergillus niger* aggregate F was characterized based on its optimum pH and substrate concentration. To determine the optimum pH of the amylase, 2 mg/ml of starch was dissolved in various buffers. For pH 3.5, 4 and 5, 0.1 M sodium acetate buffer was used. For pH 6 and 7, the same amount of starch was dissolved in 0.1 M phosphate buffer saline and for pH 9, the starch was dissolved in 0.1 M tris-HCl buffer. As much as 500 µl of that solution was then mixed with 20 µl of crude extract of *Aspergillus niger* aggregate F and incubated at 60℃ for 15 min. The amount of glucose resulted from the hydrolysis of starch by amylase contained in the extract were measured using DNS method, as decribed by Miller [17].

Optimum substrate concentration of the amylase was determined by mixing 500 µl of starch in various concentration (4, 6, 8, 10, 12, 14, 18, 22, and 30 mg/ml) with 20 µl of crude extract of *Aspergillus niger* aggregate F and incubated them at 60℃ for 15 min. The glucose level resulted from the hydrolysis of starch by amylase contained in the extract were measured using DNS method, as decribed by Miller [17].

Results and Discussion

In this study, the candidate of amylase-producing

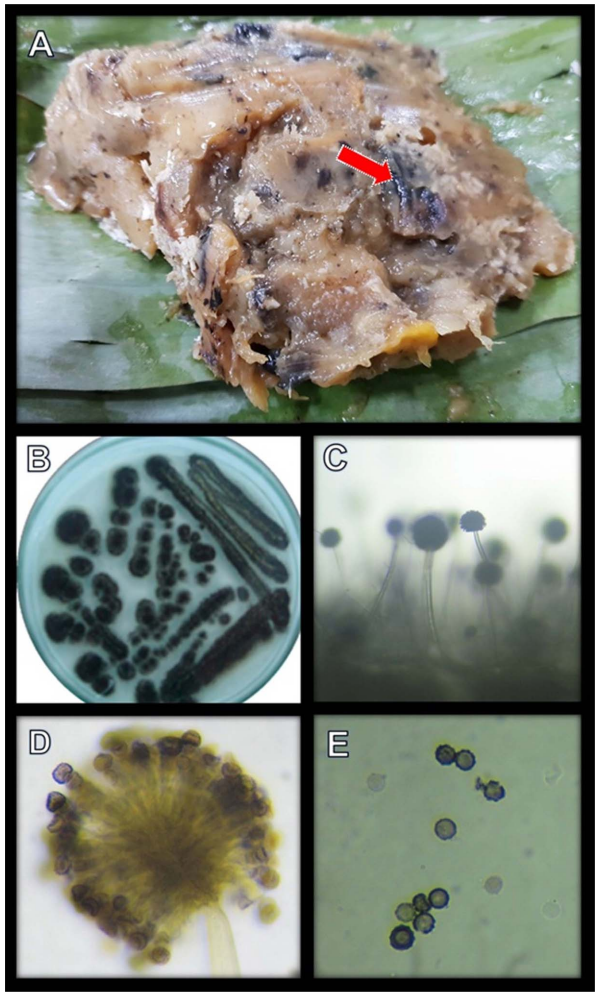

Fig. 1. Observation of *Aspergillus niger* aggregate F. (A) Gatot, a fermented cassava originated from the Special Region of Yogyakarta, Indonesia. Arrow showed black spot of *Aspergillus niger* colony on gatot; (B) *Aspergillus niger* aggregate F colony grown on PDA medium; (C) Conidiophore at 100x total magnification; (D) Conidial head at 400x total magnification; and (E) Conidia at 1,000x total magnification.

fungi were isolated from Indonesian traditional fermented food, gatot (Fig. 1A). Gatot is a product of spontaneous cassava fermentation by indigenous microorganisms, primarily fungi and lactic acid bacteria. The growth and activity of those microorganisms result in the alteration of the structure, flavor, and visible formation of black hyphae in the surface of the cassava tuber, which characterized the appearance of this particular fermented food [19]. Isolation of gatot's microorganisms has successfully selected a number of different multicellular fungi, including the one whose criteria matched the target candidate, *Aspergillus niger*. This fungus was denoted as isolate F and further used for morphological identification and amylase characterization in this study.

Morphological identification of isolate from gatot

For the morphological identification, isolate F was grown on PDA medium. Visual observation of the single colony separated from the streak showed that isolate F had a black surface color (Fig. 1B). The colony was round with entire margins and showed slight umbonate elevation which was in line with the morphology and feature of *Aspergillus niger* grown on agar plate as previously reported by Gautam *et al*. [20].

Observation under light microscope showed that isolate F was a biseriate species (Fig. 1C–E). Its vesicle shape was globose and metulae covered the whole vesicle. Both metulae and conidia were black colored. Stipes of the conidiophore was smooth and translucent. These descriptions matched the characteristics of *Aspergillus* section *Nigri* [21, 22]. The conidia surface looked uneven which indicated that it had warty conidia, similar to the one observed in *A. carbonarius, A. foetidus, A. niger* aggregate*, A. niger, A. tubingensis* and *Aspergillus* sp. UFLA DCA01 which were categorized to *Aspergillus* section *Nigri* [23]. Based on these morphological characteristics, isolate F was then denoted as *Aspergillus niger* aggregate F.

Characterization of amylase produced by *Aspergillus niger* aggregate F grown in commercial potato-based medium

As the main goal of this study was to isolate a potential amylase-producing fungi from gatot that can be applied in SSF with PPW as its sole medium, the ability of the isolate to produce extracellular amylase was first needed to be confirmed. Both crude and partially purified extracts of *Aspergillus niger* aggregate F grown in PDB were collected and their protein content were measured using Biuret assay. The crude extract contained

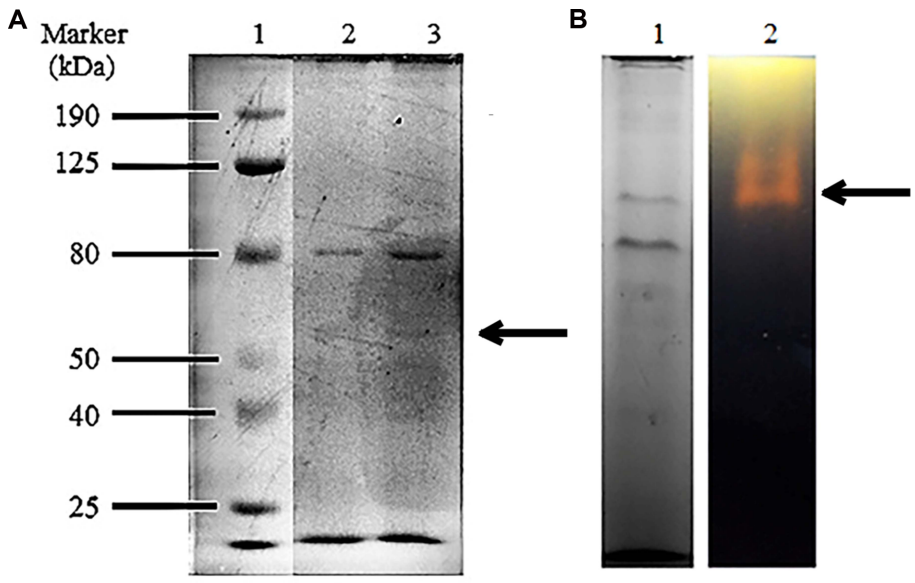

Fig. 2. Identification of amylase produced by *Aspergillus niger* aggregate F grown in PDB medium using (A) SDS-PAGE and (B) Native-PAGE and activity staining analysis. (A) Lane 1: HyperPage Prestained Protein Marker (Bioline); Lane 2: crude extract of *Aspergillus niger* aggregate F; Lane 3: partially purified and concentrated crude extract of *Aspergillus niger* aggregate F; (B) Lane 1: crude extract of *Aspergillus niger* aggregate F; Lane 2: a single band with an active amylase activity.

0.6148 mg protein per µl, while the partially purified extract protein concentration was 0.1522 mg/µl. The purification process recovery yield was 25%. Both extracts were analyzed using SDS-PAGE electrophoresis.

Two protein bands with molecular weight of approximately 60 kDa and 80 kDa were observed in both crude and partially purified extracts of *Aspergillus niger* aggregate F (Fig. 2A). The fainter 60 kDa protein bands might correspond to α-amylase originated from *Aspergillus* sp. which was reported to be in the range of 43–60 kDa [24, 25] whereas the 80 kDa might correspond to glucose oxidase which frequently isolated from *Aspergillus niger* [26].

Native-PAGE analysis was performed in order to confirm the presence and activity of amylase produced by *Aspergillus niger* aggregate F. The gel was incubated in 1% starch solution and stained with iodine to detect the starch degradation activity of the separated protein. The presence of a yellow band in iodine-stained gel (Fig. 2B) indicated the active amylase activity of the crude extract of *Aspergillus niger* aggregate F.

Characterization of amylase produced by *Aspergillus niger* aggregate F grown in potato peel waste medium

The ability of *Aspergillus niger* aggregate F to grow in commercial potato-based medium and produced extracellular amylase were important indicators of its potential to be used in a solid-state fermentation of PPW. The PPW medium was prepared by sterilizing the potato peel and mashing it to a pulp-like consistency. This treatment increased the surface area of the substrate, making it easier for the fungi to grow and utilize the nutrient [27]. Extracellular extract of *Aspergillus niger* aggregate F was then collected and filtered after seven days of incubation in potato peel waste medium. The supernatant was separated by centrifugation and subjected to partial purification by acetone precipitation.

Separation of starch hydrolysis products by TLC method was done in order to determine the specific type of amylase produced by *Aspergillus niger* aggregate F grown in PPW medium. The types of sugar present as the product of amylase activity could be used to reveal the unique hydrolyzing pattern that was possessed by each type of amylase. The substrate used in this method was 2% of starch solution mixed with crude and partially purified extracts of *Aspergillus niger* aggregate F. The types of sugar derivatives produced from the activity of the crude and partially purified extracts would also be compared with those resulted from the hydrolysis activity of commercial α-amylase and glucoamylase. Identification of each sugar spots were done by comparing its Rf values with the known sugar standards run under identical condition. Fig. 3 showed the results of starch hydrolysis by crude and partially purified extracts of *Aspergillus niger* aggregate F. At least six sugar spots identified as glucose, maltose, intermediate products, and starch were seen in lane 5 and 6. This indicated the presence of products derived from the hydrolysis of starch by crude and partially purified extract of *Aspergillus niger* aggregate F, respectively. The similarity of sugar spot pattern produced by commercial α-amylase in lane 7 suggested that *Aspergillus niger* aggregate F enzyme can be classified as α-amylase, which is a type of amylase that produced oligosaccharides of varying

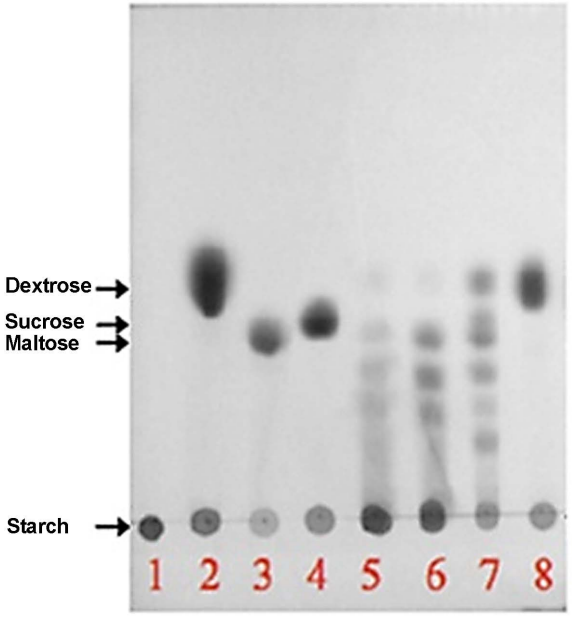

Fig. 3. Comparison of products derived from starch hydrolysis by crude and partially purified extract of *Aspergillus niger* aggregate F grown in PPW medium, commercial α-amylase, and commercial glucoamylase using TLC method.
1: Starch, 2: Dextrose, 3: Maltose, 4: Sucrose, 5-8: Starch degradation products derived from the activity of crude extract of *Aspergillus niger* aggregate F (5), partially purified extract of *Aspergillus niger* aggregate F (6), commercial α-amylase (7), and commercial glucoamylase (8).

lengths [28] as opposed to glucoamylase which only produced glucose as its products of hydrolysis (lane 8) [29].

Characterization of amylase activity from *Aspergillus niger* aggregate F Grown in potato peel waste medium

Characterization of amylase activity produced by *Aspergillus niger* aggregate F was done by determining its optimum pH and substrate concentration. In this study, DNS method was used to measure the level of glucose released when the crude extract of *Aspergillus niger* aggregate F was mixed with starch solution and incubated for 15 min. Based on prior experiment (data not shown), amylase produced by *Aspergillus niger* aggregate F had an optimum temperature of 60℃, thus substrate incubation for DNS analysis was done in that temperature.

The amylase activity of *Aspergillus niger* aggregate F on different pH can be observed in Fig. 4. The highest glucose concentrations were produced when hydrolysis of starch by the crude extract took place at pH 5–6. This data was in line with prior published results which stated that optimum pH for *Aspergillus niger*'s amylase activity ranged from 4 to 6.5 [4, 30, 31]. Generally, the *Aspergillus niger* aggregate F amylase activity increased linearly with the increase concentration of substrate until it reached its highest point at 22 mg/ml of starch,

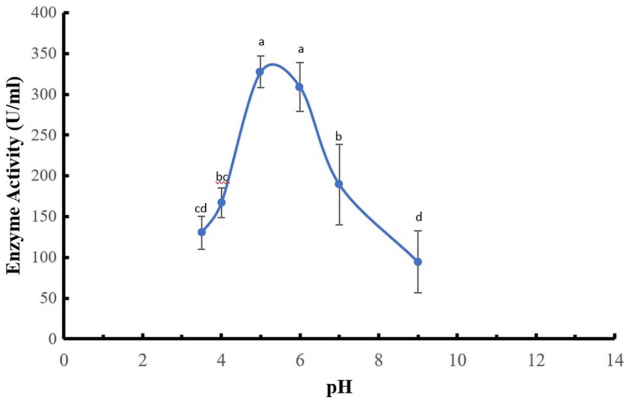

Fig. 4. The effect of pH on amylase activity of *Aspergillus niger* aggregate F grown in PPW. The hydrolysis of starch by the crude extract of *Aspergillus niger* aggregate F with varying pH were done in 60℃ for 15 min. Filled circles represented means obtained from nine repeats (n = 9) and vertical bars expressed standard deviation. Different superscript letters show significantly different means between treatments (p < 0.05). One unit of enzyme activity is defined as the amount of enzyme releases 1 μmol of glucose per minute.

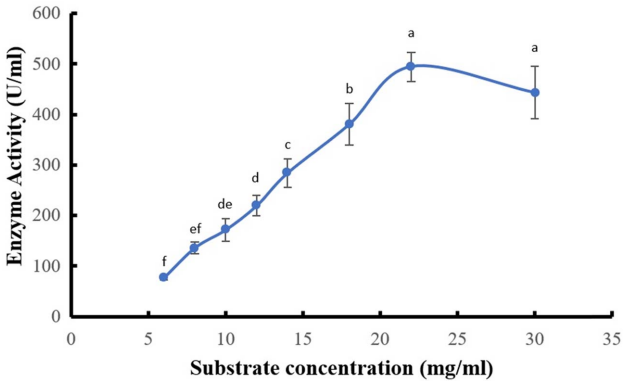

Fig. 5. The effect of substrate concentration on amylase activity of *Aspergillus niger* aggregate F grown in PPW. The hydrolysis of starch by the crude extract of *Aspergillus niger* aggregate F with varying substrate concentrations were done in 60℃ for 15 min. Filled circles represented means obtained from five repeats (n = 5) and vertical bars expressed standard deviation. Different superscript letters show significantly different means between treatments (p < 0.05). One unit of enzyme activity is defined as the amount of enzyme releases 1 μmol of glucose per minute.

upon which the activity started to plateau (Fig. 5).

Based on the results of this study, it was known that *Aspergillus niger* aggregate F isolated from gatot secreted extracellular amylase when grown both in commercial potato-based medium and PPW. In the spontaneous fermentation of gatot, the high starch content of the cassava (64–84% of dry weight) supports the growth of *Aspergillus niger*, a fungus most commonly found in the environment including food and feed ingredients [32, 33]. Similar to cassava, potato peel also has a high starch content, which might account to 67% of the dry weight [34, 35]. This might explain the ability of *Aspergillus niger* aggregate F to grow well on the PPW medium as well. The ability of *Aspergillus niger* aggregate F to secrete amylase enabled it to utilize the simpler sugar from starch to support its growth in the PPW medium.

The amylase produced by *Aspergillus niger* aggregate F on potato-based and PPW medium was predicted to be in the class of α-amylase based on the results of SDS-PAGE and TLC analysis. Fungal α-amylase is categorized as saccharifying enzymes as it is able to randomly cleave α-1,4-oligosaccharide links in the starch molecule from the non-reducing ends, resulting in the formation of varying degree of smaller unit oligosaccharides [1, 8, 36]. α-amylase is one of the most important indus-

trial enzymes commonly used both in food and biofuel industries. The production of this enzyme by generally recognized as safe (GRAS) microorganisms, including *Aspergillus niger*, offers a wider range of applications thus more preferable compared to the enzyme produced by the non-GRAS microorganisms [37].

All in all, the ability of *Aspergillus niger* aggregate F to grow on the PPW medium and produce extracellular α-amylase through solid-state fermentation may reveal its potential to be used for reducing the cost of amylase production while opening a new alternative in the waste management of potato peel.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. DeSouza PM. 2010. Application of microbial α-amylase in industry-A review. *Braz. J. Microbiol.* **41**: 850-861.
2. Rana N, Walia A, Gaur A. 2013. α-amylases from microbial sources and its potential applications in various industries. *Natl. Acad. Sci. Lett.* **36**: 9-17.
3. Li C, Du M, Cheng B, Wang L, Liu X, Ma C, *et al*. 2014. Close relationship of a novel Flavobacteriaceae α-amylase with archaeal α-amylases and good potentials for industrial applications. *Biotechnol. Biofuels*. **7**: 18-30.
4. Ramasamy S, Benazir JF, Ramalingam S, Kumar R, Hari A, Raman N, *et al*. 2011. Amylase production by *Aspergillus niger* under solid state fermentation using agroindustrial wastes. *Int. J. Eng. Sci. Technol.* **3**: 1756-1763.
5. Sewalt V, Shanahan D, Gregg L, La Marta J, Carrillo R. 2016. The Generally Recognized as Safe (GRAS) process for industrial microbial enzymes. *Ind. Biotechnol.* **12**: 295-302.
6. Mukherjee R, Paul T, Soren JP, Halder SK, Mondal KC, Pati BR, *et al.* 2017. Acidophilic α-amylase production from *Aspergillus niger* rbp7 using potato peel as substrate: A waste to value added approach. *Waste Biomass* **1**: 1-13.
7. Pereira CR, Resende JTV, Guerra EP, Lima VA, Martins MD, Knob A. 2017. Enzymatic conversion of sweet potato granular starch into fermentable sugars: Feasibility of sweet potato peel as alternative substrate for α-amylase production. *Biocatal. Agric. Biotechnol.* **11**: 231-238.
8. Xu H, Sun L, Zhao D, Zhang B, Shi Y, Wu Y. 2008. Production of α-amylase by *Aspergillus oryzae* As 3951 in solid state fermentation using spent brewing grains as substrate. *J. Sci. Food Agric.* **88**: 529-535.
9. Murthy PS, Naidu MM, Srinivas P. 2009. Production of α-amylase under solid-state fermentation utilizing coffee waste. *J. Chem. Technol. Biotechnol.* **84**: 1246-1249.
10. Arapoglou D, Varzakas T, Vlyssides A, Israilides C. 2010. Ethanol production from potato peel waste (PPW). *Waste Manag.* **30**: 1898-1902.
11. Shukla J, Kar R. 2006. Potato peel as a solid state substrate for thermostable α-amylase production by thermophilic *Bacillus* isolates. *World J. Microbiol. Biotechnol.* **22**: 417-422.
12. Arifin Y, Tanudjaja E, Dimyati A, Pinontoan R. 2014. A second generation biofuel from cellulosic agricultural by-product fermentation using *Clostridium* species for electricity generation. *Energy Procedia*. **47**: 310-315.
13. Tamang JP, Watanabe K, Holzapfel WH. 2016. Review: Diversity of microorganisms in global fermented foods and beverages. *Front. Microbiol.* **7**: 377.
14. Sugiharto S, Yudiarti T, Isroli I. 2015. Functional properties of filamentous fungi isolated from the indonesian fermented dried cassava, with particular application on poultry. *Mycobiology* **43**: 415-422.
15. Wijedasa MH, Liyanapathirana LVC. 2012. Evaluation of an alternative slide culture technique for the morphological identification of fungal species. *Sri Lankan J. Infect. Dis.* **2**: 47-52.
16. Bisht D, Yadav SK, Darmwal NS. 2013. An oxidant and organic solvent tolerant alkaline lipase by *P. aeruginosa* mutant: downstream processing and biochemical characterization. *Braz. J. Microbiol.* **44**: 1305-1314.
17. Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685.
18. Chidan Kumar CS, Chandraju S, Mythily R, Ahmad T, Made Gowda NM. 2012. Extraction of sugars from black gram peels by reversed-phase liquid chromatography systems and identification by TLC and mass analysis. *Adv. Anal. Chem. Sci. Acad. Publ.* **2**: 32-36.
19. Astriani A, Diniyah N, Jayus J, Nurhayati N. 2018. Phenotypic identification of indigenous fungi and lactic acid bacteria isolated from 'gatot' an Indonesian fermented food. *Biodiversitas* **19**: 947-954.
20. Gautam AK, Bhadauria R. 2012. Characterization of *Aspergillus* species associated with commercially stored triphala powder. *Afr. J. Biotechnol.* **11**: 16814-16823.
21. Samson RA, Pitt JI. 1986. *Advances in Penicillium and Aspergillus Systematics*. p. 60, Springer US, Boston, MA.
22. McClenny N. 2005. Laboratory detection and identification of *Aspergillus* species by microscopic observation and culture: the traditional approach. *Med. Mycol.* **43**: 125-128.
23. Silva DM, Batista LR, Rezende EF, Fungaro MHP, Sartori D, Alves E. 2011. Identification of fungi of the genus *Aspergillus* section nigri using polyphasic taxonomy. *Braz. J. Microbiol.* **42**: 761-773.
24. Sidkey N, Abo-Shadi M, Balahmar R, Sabry R, Badrany G. 2011.

Purification and characterization of α-amylase from a newly isolated *Aspergillus flavus* F 2 Mbb. *Int. Res. J. Microbiol.* **2**: 96-103.
25. Ciloci A, Bivol C, Stratan M, Reva V, Clapco S, Tiurin S, *et al.* 2012. Production and purification of α-amylase from *Aspergillus niger* 33-19 CNMN FD 02a mutant form. *Analele Univ. Din Oradea Fasc. Biol.* **19**: 74-79.
26. Derakshan FK, Darvishi F, Dezfulian M, Madzak C. 2017. Expression and characterization of glucose oxidase from *Aspergillus niger* in *Yarrowia lipolytica*. *Mol. Biotechnol.* **59**: 307-314.
27. Renge VC, Khedkar SV, Nandukar NR. 2012. Enzyme synthesis by fermentation method: A review. *Sci. Rev. Chem. Commun.* **2**: 585-590.
28. Dhital S, Warren FJ, Butterworth PJ, Ellis PR, Gidley MJ. 2017. Mechanisms of starch digestion by α-amylase—Structural basis for kinetic properties. *Crit. Rev. Food Sci. Nutr.* **57**: 875-892.
29. Xu Q-S, Yan Y-S, Feng J-X. 2016. Efficient hydrolysis of raw starch and ethanol fermentation: a novel raw starch-digesting glucoamylase from *Penicillium oxalicum*. *Biotechnol. Biofuels* **9**: 216-234.
30. Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B. 2003. Microbial α-amylases: a biotechnological perspective. *Process Biochem*. **38**: 1599-1616.
31. Passamani FRF, Hernandes T, Lopes NA, Bastos SC, Santiago WD, Cardoso M das G, *et al.* 2014. Effect of temperature, water activity, and pH on growth and production of ochratoxin A by *Aspergillus niger* and *Aspergillus carbonarius* from Brazilian grapes. *J. Food Prot*. **77**: 1947-1952.
32. Fakir MSA, Jannat M, Mostafa MG, Seal H. 2012. Starch and flour extraction and nutrient composition of tuber in seven cassava accessions. *J. Bangladesh Agric. Univ.* **10**: 217-222.
33. Frisvad JC, Larsen TO, Thrane U, Meijer M, Varga J, Samson RA, *et al*. 2011. Fumonisin and ochratoxin production in industrial *Aspergillus niger* strains. *PLoS One* **6**: 1-6.
34. Mahmood S, Shahid MG, Nadeem M, Irfan M, Syed Q. 2016. Production and optimization of α-amylase from *Aspergillus niger* using potato peel as substrate. *Pak. J. Biotechnol.* **13**: 101-109.
35. Sepelev I, Galoburda R. 2015. Industrial potato peel waste application in food production: a review. *Res. Rural Dev*. **1**: 130-136.
36. Kaur H, Arora M, Bhatia S, Alam MS. 2015. Optimization of α-amylase and glucoamylase production in solid state fermentation of deoiled rice bran (DRB) by *Rhizopus oryzae*. *Int. J. Pure Appl. Biosci.* **3**: 249-256.
37. Mobini-Dehkordi M, Javan FA. 2012. Application of alpha-amylase in biotechnology. *J. Biol. Todays World*. **1**: 15-20.