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Amylase enzyme production with variation of carbon sources and molecular identification of thermophilic fungus *Aspergillus* sp. LBKURCC304 from Bukik Gadang, West Sumatra, Indonesia

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Abstract. *Saryono, Devi S, Nugroho TT, Fadhila WF, Lorenita L, Nasution FS, Suraya N. 2023. Amylase enzyme production with variation of carbon sources and molecular identification of thermophilic fungus* Aspergillus *sp. LBKURCC304 from Bukik Gadang, West Sumatra, Indonesia. Biodiversitas 24: 1200-1205.* Amylase is an enzyme used to hydrolyze starch into smaller molecules. Starch degradation is very difficult because of the presence of 1-4 glucoside bonds from complex polysaccharides and the presence of enzyme accommodation centers, so the production of amylase is in great demand by industry. The production of amylase is strongly influenced by carbohydrates which act as inducers to stimulate the production of enzymes. This research was conducted to determine the effect of different carbohydrate sources on the production of amylase enzyme from thermophilic fungus *Aspergillus* sp. LBKURCC304. Different carbon sources used were cassava, corn, taro, purple sweet potato, potato, breadfruit, canna, gembili, gadung, and sago. The effect of different carbohydrate sources on enzyme production was statistically tested using Duncan's Multiple Range Test (DMNRT) at a significant level of 5% and Principal Component Analysis (PCA). The results of molecular identification showed that carbohydrates from sago were a relatively better carbon source than other carbon sources, with an activity of 0.0391±0.0017 U/mL, and a specific activity of 0.0874±0.0049 U/mg. The highest (0.7651±0.0096 mg/mL) protein content was recorded from canna. Molecular identification showed that LBKURCC304 isolate was *Aspergillus fumigatus*.

Keywords: *Aspergillus fumigatus*, amylase, canna, carbon source, sago, thermophilic

INTRODUCTION

Amylase is an extracellular enzyme that can be produced by microorganisms. Amylase is a starch-degrading enzyme that cleaved -1-4 glucoside bonds from complex polysaccharides (Pandey et al. 2000). This enzyme catalyzes the hydrolysis reaction of starch into smaller molecules, such as maltose, dextrin, and glucose as the smallest unit (Simair et al. 2017). The amylase group of enzymes is divided into three, namely α-amylase, β-amylase, and γ-amylase, based on the type of bond they cleave (Liu and Kokare 2017). α-amylase breaks down starch molecules by reducing the viscosity of starch by breaking bonds randomly, resulting in glucose chains of varying sizes. β-amylase breaks the glucose-glucose bond by removing two lactose units at once, by producing maltose. γ-Amylases breaks α(1-6) glycosidic linkages, in addition to cleaving the last α(1-4) glycosidic linkages at the nonreducing end of amylose and amylopectin (Bharathiraja et al. 2016; Liu and Kokare 2017). This enzyme is widely used in the health and industrial fields, such as food industry, textile, and paper industries (Pandey et al. 2000).

Amylase enzymes can come from several sources, including plants, animals, and microorganisms (Gomez-Villegas et al. 2021). However, the production of amylase from microorganisms has succeeded in replacing the chemical hydrolysis of starch in the starch processing industry (Pandey et al. 2000). Thermophilic microorganisms, especially fungi, are widely used in the production of enzymes. These microorganisms can live at an optimum temperature of >55°C and are generally polyextremophiles i.e. they are able to live in extreme environmental conditions, such as those related to pH, redox potential, salt concentration, or the presence of a broad spectrum of toxic compounds (Ovando-Chacon et al. 2020). These microorganisms have several advantages, including the ability to produce thermotolerant extracellular enzymes that are stable and active at high pH and temperature (Saryono et al. 2022).

Enzyme production using thermophilic bacteria and fungi has been widely carried out, including by Saryono et al. 2022, which has succeeded in producing cellulase enzymes from thermophilic fungi from Sungai Pinang, Riau, Indonesia. The cellulase enzyme activity obtained was 2.6 x 10 IU/mL, and the specific enzyme activity was 8.0 x 10-3 IU/mg protein with an incubation time of 96 hours. Ardhi et al. (2020) have succeeded in producing amylase enzyme from thermophilic bacteria isolated from Bukit Gadang, West Sumatra, Indonesia. The optimum amylase enzyme production obtained was 231.33 U/mL, and the specific enzyme activity was 101.79 U/mg after 36 hours of incubation. Ovando-Chacon et al. (2020) performed the isolation, identification, and characterization of

thermophilic strain-produced lipase enzyme from the geothermal water of the El Chichón volcanic crater lake. The production of extracellular lipase with the highest activity obtained was 143 U/mL at 8.3 hours of incubation. Unal (2018) has succeeded in producing amylase enzyme from the thermophilic fungi *A. niger*, *A. oryzae*, and *A. terreus* using the flask shaking method and culture and optimization of activity. The results revealed that *A. niger* exhibited the highest production and activity, with an optimum temperature of 65°C and an optimum pH of 8.

One of the important factors in the production of enzymes is carbohydrates. Carbohydrates act as a carbon source in fungal metabolism (Abd-elhalem et al. 2015; Mahmood et al. 2016). In addition, the presence of starch carbohydrates in the production medium acts as an inducer in stimulating the production of extracellular enzymes so that in the composition of the extracellular amylase production medium, carbohydrates are the component that has the greatest influence (Dojnov et al. 2015). In this research, an analysis of amylase production from thermophilic mushrooms from Bukik Gadang, West Sumatra, was carried out. The analysis was carried out by replacing the use of starch sources with carbohydrates sourced from nature, which are easily and economically available, such as flour, grains and tubers. The purpose of this research is to obtain potential natural carbohydrate sources and increase the utilization of biological natural resources, one of which is Riau sago, which is one of the largest sago producers in Indonesia.

MATERIALS AND METHODS

Microorganisms

The isolate used in this study was *Aspergillus* sp. LBKURCC304 collected from the Enzyme, Fermentation, and Biomolecular Research Laboratory, Faculty of Mathematics and Natural Sciences, Riau University. *Aspergillus* sp. was isolated from the Bukik Gadang hot spring, West Sumatra, Indonesia.

Substrates

The source of carbohydrates used in this study was obtained from one of the traditional markets in Pekanbaru, Riau. The substrates used as carbon sources were cassava, corn, taro, purple sweet potato, potato, breadfruit, canna, gembili, gadung and sago.

Moisture content of carbon sources

The percentage of water content in carbohydrate sources was determined by the Gravimetric method. Water content was determined by inserting the carbon samples into an oven at a temperature of 90°C until a constant weight was obtained. Water content can be calculated by the following formula (Rosmawati et al. 2019):

$$\%\ water\ content\ = \frac{(B1 - B2)}{B1} \times 100\%$$

Where:
B1: initial mass of carbohydrate sources (g)
B2: final mass after dried (g)

Amylase enzyme production

The stock fungus of *Aspergillus* sp. LBKURCC304 was rejuvenated on Potato Dextrose Agar (PDA) medium at 50°C for seven days. *Aspergillus* isolate was then spread on Petri plates and incubated at 50°C for three days. 10 different types of carbon sources (starch: cassava, corn, taro, rice, sorghum, purple sweet potato, potato, breadfruit and sago) were used to produce amylase. For the production of amylase in liquid media, two plugs of inoculum of 1 cm size were inoculated in each production medium and incubated at 50°C for 11 days. After eleven days, the production medium in each Erlenmeyer flask was filtered with Whatman No. 1 filter paper and the filtrate was used to determine the activity of amylase enzyme, protein content, and specific activity of enzyme.

Liquid state fermentation

The liquid media was made by dissolving all components of composition in 100 mL containing carbohydrates (carbon source) 0.5 g, and Yeast extract 0.2 g, KH_2PO_4 0.1 g, $MgSO_4.7H_2O$ 0.05 g, Phosphate buffer 0.05 M pH 7 100 mL (Unal 2018). Furthermore, medium was sterilized using an autoclave at a temperature of 121°C and a pressure of 15 psi for 20 minutes.

Determination of amylase enzyme activity

Amylase enzyme activity was measured by reducing sugars formed from the hydrolysis of starch by amylase using Nelson-Somogyi method. The unit value of amylase enzyme activity was defined as the amount of enzyme that released 1 mol of reducing sugar per minute. This value can be calculated using the formula:

$$Enzyme\ activity = \frac{reducing\ sugars - control}{Volume\ of\ enzyme\ crude\ extract \times incubation\ time}$$

Assay of protein concentration and enzyme-specific activity

Protein content was estimated by Lowry method (Lowry et al. 1951). The calculation of protein content as done by substituting the absorbance of solution obtained in determining the protein content of enzyme into the regression equation of the calibration curve of protein standard solution. The specific activity of enzyme was determined using the following equation of Page (1989):

$$As = \frac{Enzyme\ activity}{Total\ protein}$$

Where: As: Specific activity of enzymes in mg protein (U/mg)

Molecular identification

Pure fungal isolate was identified using Internal Transcribed Spacer (ITS) rDNA and universal primers: ITS 4 primer(TCC TCC GCT TAT TGA TAT GC) and ITS 5 (GGA AGT AAA AGT CGT ACA AGG). The genomic DNA of fungal mycelia was isolated when the fungus was two days old, using the Wizard Genomic DNA Purification Kit (Promega) containing a lyticase enzyme as a breaker of fungal cell walls. PCR amplification of the ITS region

containing 2 µL of genomic DNA, 10 µL primer ITS4 (2 pmol), 10 µL primer ITS5 (2 pmol), 5 µL (2 mM) dNTP mix, 10 µL buffer, 5 µL $MgCl_2$, 7.75 µL H_2O and 0.25 µL GoTaq DNA Polymerase (Promega). PCR thermocycler was programmed: pre-denaturation was performed at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 45°C for 30 seconds, extension at 72°C for 1 minute, and the final extraction at 72°C for 10 minutes. The PCR products were visualized using 1% agarose gel. Sequences in the ITS rDNA region were analyzed using the NCBI database. Phylogenetic and molecular evolutionary analyses were performed with the Clustal X version 2.1 program. The phylogenetic tree of the ITS rDNA gene sequences was constructed by neighbor-joining with 10.000 Bootstrap analysis, and the alignment results were viewed using the Mega version 6 program.

Statistical analysis

Data on amylase enzyme activity, protein content, and enzyme-specific activity obtained from this study was displayed in the form of figures and tables. Results were statistically analyzed with Analysis of Variance (ANOVA), Duncan's New Multiple Range Test (DNMRT) at 5% level, and Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

Percentage of water content

The percentage of water content in carbohydrates sources in amylase production medium can affect the mass of carbohydrates. Moisture content can also affect the stability and quality of material. The percentage of water content of each carbohydrate source can be seen in Table 1. The result showed that potato starch had the highest moisture content of 14.220%, while the lowest had in canna starch 5.349%. It was also observed that high water content did not significantly affect the enzyme activity, protein content, and specific activity.

Determination of enzyme activity, protein content, and specific activity

The results of enzyme activity, protein content, and specific activity of crude amylase extract can be seen in Table 1.

The obtained results showed the highest amylase activity of *Aspergillus* sp. LBKURCC304 was produced in media containing carbon sources of sago, purple sweet potato, and breadfruit. The highest amylase activity was recorded in sago (0.0391±0.0017 U/mL), and in purple sweet potato (0.0205±0.0040 U/mL), which was in the first cluster, while in breadfruit it was (0.0267±0.0009 U/ mL), which was in second cluster, and differ significantly with each other. The lowest amylase activity was obtained in media without carbohydrate carbon source with an activity of 0.0029±0.0007 U/mL, located in the fourth cluster. The division of clusters through the Principal Component Analysis (PCA) test can be seen in Figure 2.

The highest amylase crude extract activity was produced by medium containing carbon sources, such as sago, purple sweet potato (first cluster), and breadfruit (second cluster). The amylase activity of sago was (0.0391±0.0017 U/mL), followed by purple sweet potato (0.0205±0.0040 U/mL), and breadfruit (0.0267±0.0009 U/mL) were significantly different ($p<0.05$) based on Duncan's multiple-range test. Based on the results obtained, it can be seen that the production of amylase enzyme from *Aspergillus* sp. LBKURCC304 was strongly influenced by variations in the amylose content of the carbon source used. The higher amylose content induces the production of more amylase enzymes in the production medium. The chemical composition of amylose is relatively different in sago, breadfruit, and purple sweet potato plants. Sago starch has an amylose content of 25-45% (Khatijah and Patimah 1995; Ahmad et al. 1999; Uthumporn et al. 2014). Breadfruit starch has an amylose content of 26-38% (Rincon et al. 2004), while purple sweet potato starch has an amylose content of 19-35%, which is the cultivar with the highest amylose content of all sweet potato cultivars (Swinkles 1985; Aina et al. 2012).

Table 1. Enzyme activity, protein content, and specific activity of crude amylase extracted from *Aspergillus* sp. LBKURCC304

Carbon sources	Water content (%)	Enzyme activity (U/mL)	Protein content (mg/mL)	Specific activities (U/mg)
Sago	11.234%	**0.0391±0.0017^{a}**	0.4471±0.0115^{e}	**0.0874±0.0049^{a}**
Purple sweet potato	6.704%	**0.0205±0.0040^{c}**	0.5325±0.0040^{c}	0.0384±0.0076^{c}
Breadfruit	7.925%	**0.0267±0.0009^{b}**	0.3436±0.0009^{h}	0.0778±0.0026^{b}
Canna	5.349%	0.0068±0.0005^{ef}	**0.7651±0.0096^{a}**	0.0089±0.0006^{e}
Taro	6.643%	0.0065±0.0010^{ef}	0.6017±0.0198^{b}	0.0108±0.0019^{e}
Gembili	5.467%	0.0070±0.0004^{def}	0.5951±0.0231^{b}	0.0118±0.0005^{e}
Cassava	9.257%	0.0092±0.0019^{d}	0.5178±0.0345^{c}	0.0179±0.0041^{d}
Potato	**14.220%**	0.0078±0.0006^{de}	0.4701±0.0275^{d}	0.0167±0.0017^{d}
Gadung	8,452%	0,0078±0,0022^{de}	0,3968±0,0046^{g}	0.0198±0.0054^{d}
Corn	8.147%	0.0049±0,0008^{fg}	0,5174±0,0035^{c}	0.0095±0.0017^{e}
Without carbon source	-	0.0029±0.0007^{g}	0.4190±0.0048^{f}	0.0069±0.0016^{e}

Note: The superscript of same letter in one column indicates that values are not significantly different at 5% level ($p<0.05$) based on Duncan's multiple-distance test. The color difference between the rows represents the cluster division based on the PCA test

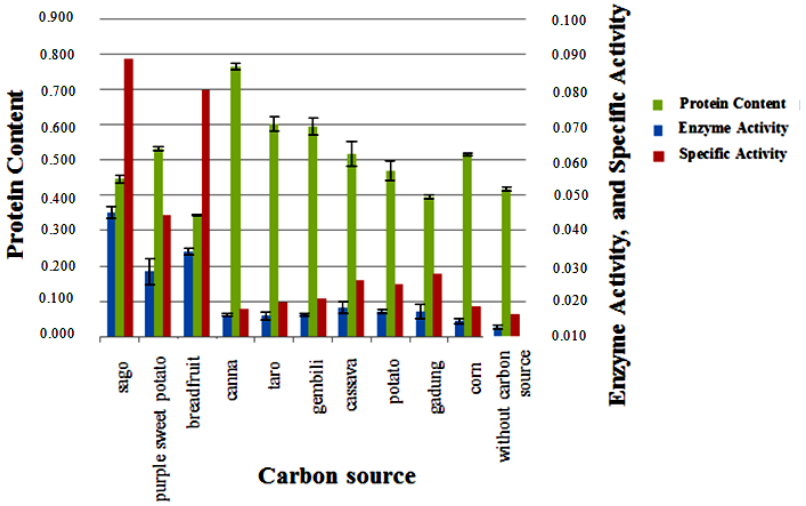

Figure 1. Protein content, enzyme activity and specific activity of enzyme *Aspergillus* sp. LBKURCC304 after 11 days

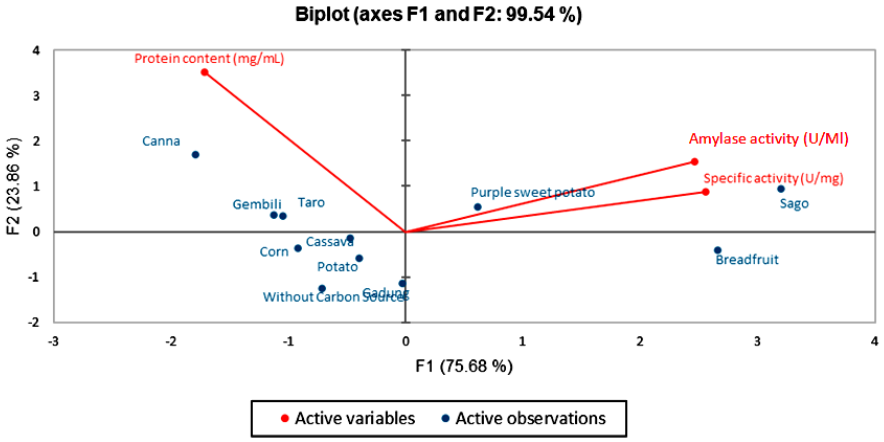

Figure 2. Cluster division of 11 different carbon source media based on the value of enzyme activity, protein content, and specific activity

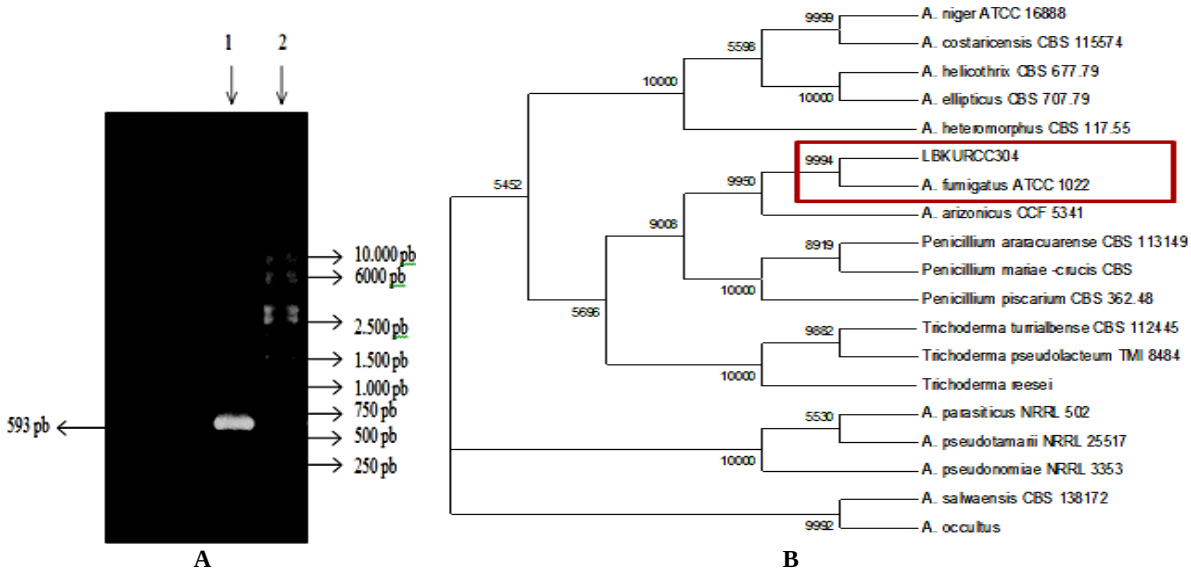

Figure 3. A. PCR product of LBKURCC304 isolate. B. Phylogenetic tree showing the relationship between LBKURCC304 and the representative *A. fumigatus* ATCC 1022

In the third and fourth clusters, value of enzyme activity in each carbohydrate was not significantly different ($p<0.05$) from each other. Amylase from production media with carbon source type was as follows: cassava > potato > gadung > gembili > canna > taro > corn > non-carbohydrate. This is similar to the research results of Nuwamanya et al. (2011) and Karmakar et al. (2014), showing that the reduced sugar content resulted from the hydrolysis of starch by amylase in cassava starch > potato > taro. Likewise, the research of Piyachomkwam et al. (2001) and Thitipraphunkul et al. (2003) showed greater amylase activity in the carbon source of cassava starch than in canna starch. This is due to the higher amylose content in cassava starch compared to potatoes and taro. It is also influenced by the high viscosity value of taro starch rather than potato and cassava. This high viscosity is proportional to the size of the starch granules (Otegbayo et al. 2013; Fauziah et al. 2016). The particle size of starch granules affects the ability of fungi to utilize nutrients and adhesion to starch. Generally, smaller starch particles provide a larger surface area for fungal penetration (Pandey et al. 2000). In the fourth cluster, amylase activity obtained from the production medium without the addition of carbohydrates was 0.0029±0.0007 U/mL. This was the smallest amylase activity of the overall amylase activity produced and was significantly different ($p<0.05$) from other carbon sources. The amylase activity produced from the medium without addition of carbohydrates as a carbon source was not an inductive enzyme but a constitutive extracellular enzyme, an enzyme produced during metabolism in the absence of an inducer. This is supported by the research of Poli et al. (2006) and Lucas et al. (2012), who found the activity of amylase produced from the production media of bacterium *Anoxybacillus amylolyticus* sp. and fungus *Aspergillus phoenicis* without the addition of a carbon source (starch), this amylase enzyme is expressed as constitutive extracellular amylase. The data in Table 1 showed that the highest protein content of crude amylase extract came from media with canna carbohydrates (0.7651±0.0096) mg/mL, taro (0.5951±0.0019 mg/mL), and gembili (0.5951±0.0231 mg/mL). The measured total protein content included the protein of production medium and the protein of enzyme produced by *Aspergillus* sp. LBKURCC304. This is supported by the research of Piyachomkwam et al. (2001), who reported that canna starch and taro have much higher protein content than cassava. Based on the results of statistical tests using PCA, crude extracts of enzymes derived from canna, taro, and gembili were grouped in the same cluster i.e. in the 3rd cluster, whereas with Duncan's multiple range test, the protein content of the crude enzyme extract from canna was significantly different from the protein content from taro media ($p<0.05$) and the protein content from taro medium was not significantly different from the protein content from gembili media. The results of Duncan's test with a level of 5% indicate that differences in carbohydrate sources affect the protein content produced.

The specific activity of the enzyme represents a measure of the purity of enzyme. The higher the enzyme activity and the lower the protein content, the higher the specific activity. The specific activity of crude extract of amylase enzyme cannot be used as a benchmark for its purity. The highest specific activity was found in production media containing sago as carbon source (Table 1). This was due to the high amylase activity produced in media containing sago and relatively low protein content, resulting in high specific activity values compared to other carbon sources.

Molecular identification

The level of similarity of DNA sequences was carried out using BLAST (Basic Local Alignment Search Tool). BLAST analysis determined the highest level of similarity of DNA sequences obtained in the present study with other sequences contained in the NCBI GenBank database. The level of sequence similarity was expressed by percent identity. Based on BLAST analysis, thermophilic fungus *Aspergillus* sp. LBKURCC304 had the highest percent identity of 100% with *Aspergillus fumigatus*. Phylogenetic analysis of isolate LBKURCC304 (Figure 3) was performed using the neighbor-joining tree method with 10,000 bootstrap repetitions. Bootstrap analysis is carried out to test the reliability of branches of the phylogenetic tree (Saryono et al. 2022). Based on the phylogeny of 18 strains, isolate LBKURCC304 belonged to the genus *Aspergillus* with the species *Aspergillus fumigatus*. Isolate LBKURCC304 had the closest relationship with *Aspergillus fumigatus* ATCC 1022, with a branching level of 9994. *Aspergillus fumigatus* is one of the most abundant thermophilic fungal species isolated from various habitats with high-temperature conditions. A similar study was conducted by Odilia et al. (2018), who succeeded in isolating thermophilic fungi from hot springs in Bogoria, Kenya. Sequence analysis obtained showed that isolate had 99% homology with *Aspergillus fumigatus*. Previously Chen et al. (2001) also succeeded in isolating *Aspergillus fumigatus* from the soil in Northern Taiwan hot springs.

In conclusion, the sources of carbohydrates affect the production of enzymes produced. Of the ten carbon sources used, medium with the carbon source of sago had the highest amylase activity of 0.0391±0.0017 U/mL, and specific activity of crude extract of amylase was 0.0874±0.0049 U/mg.Whereas, the highest protein content of amylase crude extract produced from media of canna was 0.7651±0.0096 mg/mL. The result of molecular identification in the ITS rDNA area showed that LBKURCC304 isolate was *Aspergillus fumigatus*, with the closest relationship to *Aspergillus fumigatus* ATCC 1022, with a branching level of 9994.

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REFERENCES

Abd-elhalem BT, Gamal RF, Abou-taleb KA. 2015. Production of amylases from *Bacillus amyloliquefaciens* under submerged fermentation using some agro-industrial by-products. Ann Agric Sci 60 (2): 193-202. DOI: 10.1016/j.aoas.2015.06.001.

Ahmad FB, Williams PA, Doublier JL, Durand S, Buleon A. 1999. Physicochemical characterisation of sago starch. Carbohydr Polym 38 (4): 361-370. DOI: 10.1016/S0144-8617(98)00123-4.

Aina AJ, Falade KO, Akingbala JO, Titus P. 2012. Physicochemical properties of caribbean sweet potato (*Ipomoea batatas* (L.) Lam) starches. Food Bioproc Technol 5: 576-583. DOI: 10.1007/s11947-009-0316-6.

Ardhi A, Sidauruk AN, Suraya N, Pratiwi NW, Pato U, Saryono. 2020. Molecular identification of amylase-producing thermophilic bacteria isolated from Bukit Gadang Hot Spring, West Sumatra, Indonesia. Biodiversitas 21 (3): 994-1000. DOI: 10.13057/biodiv/d210319.

Bharathiraja B, Jayakumar M, Nithyananthi MJT, Muthukumar PV, Saravanaraj A, Senthilkumar K. 2016. Production and kinetics of amylase from starch using mutant strain of *Bacillus* sp MTCC 1434. J Chem Pharma Sci 9 (1): 281-286.

Chen K, Huang D, Liu C. 2001. The mycoflora of hot spring soil in Northern Taiwan. Taiwania 48 (3): 203-211. DOI: 10.6165/tai.2003.48(3).203.

Dojnov B, Grujić M, Perčević B, Vujčić Z. 2015. Enhancement of amylase production by *Aspergillus* sp. using carbohydrates mixtures from triticale. J Serbian Chem Soc 80 (10): 1279-1288. DOI: 10.2298/JSC150317043D.

Fauziah, Mas S, Hendrian. 2016. Study on starch granules of local varieties of *Dioscorea hispida* and *Dioscorea alata*. J Trop Life Sci 6 (1): 47-52. DOI: 10.11594/jtls.06.01.09.

Gomez-Villegas P, Vigara J, Romero L, Gotor C, Raposo S, Gonçalves B, Léon R. 2021. Biochemical characterization of the amylase activity from the new Haloarchaeal Strain *Haloarcula* sp. HS isolated in the Odiel Marshlands. Biology (Basel) 10: 337. DOI: 10.3390/biology10040337.

Karmakar R, Ban DK, Ghosh U. 2014. Comparative study of native and modified starches isolated from conventional and nonconventional sources. Intl Food Res J 21 (2): 597-602.

Khatijah I, Patimah H. 1995. Physico-chemical properties of sago starch in Malaysia. MARDI Res J 23 (1): 93-97.

Lowry OH, Rousenbough HI, Fair AL, Randall RI. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-275. DOI: 10.1016/S0021-9258(19)52451-6.

Liu X, Kokare C. 2017. Microbial Enzymes of Use in Industry. In: Brahmachari G, Demain AL, Adrio JL. Biotechnology of Microbial Enzymes: Production, Biocatalysis and Industrial Applications. Academic Press, Cambridge. DOI: 10.1016/B978-0-12-803725-6.00011-X.

Mahmood S, Shahid MG, Nadeem M, Irfan M, Syed Q. 2016. Production and optimization of a-amylase from *Aspergillus niger* using potato peel as substrate. Pak J Biotechnol 13 (2): 101-109.

Nuwamanya E, Baguma Y, Wembabazi E, Rubaihayo P. 2011. Comparative study of the physicochemical properties of starches from root, tuber and cereal crops. Afr J Biotechnol 10 (56): 12018-12030. DOI: 10.5897/AJB10.2310.

Odilia AS, Makonde HM, Kasili RW, Boga, H.I. 2018. Isolation and characterization of fungi from a hot-spring on the shores of Lake Bogoria, Kenya. J Yeast Fungal Res 9 (1): 1-13. DOI: 10.5897/JYFR2018.0182.

Otegbayo B, Oguniyan D, Akinwumi O. 2013. Physicochemical and functional characterization of yam starch for potential industrial applications. Starch/Staerke 66 (3-4): 235-250. DOI: 10.1002/star.201300056.

Ovando-Chacon SL, Tacias-Pascacio VG, Ovando-Chacon GE, Rosales-Quintero A, Rodriguez-Leon A, Ruiz-Valdiviezo VM, Servin-Martinez A. 2020. Characterization of thermophilic microorganisms in the geothermal water flow of El Chichón Volcano crater lake. Water 12: 2172. DOI: 10.3390/w12082172.

Page DS. 1989. Principles of Biological Chemistry. Second Edition. Erlangga, Jakarta.

Pandey A, Nigam P, Soccol C, Thomaz-Soccol V, Singh D, Mohan R. 2020. Advances in microbial amylases. Biotechnol Appl Biochem 31: 135-152. DOI: 10.1042/BA19990073.

Poli A, Esposito E, Lama L, Orlando P, Nicolaus G, de Appolonia F, Gambacorta A, Nicolaus B. 2006. *Anoxybacillus amylolyticus* sp. nov., a thermophilic amylase producing bacterium isolated from Mount Rittmann (Antarctica). Syst Appl Microbiol 29 (4): 300-307. DOI: 10.1016/j.syapm.2005.10.003.

Rincon AM, Padilla FC. 2004. Physichochemical properties of breadfruit (*Artocarpus altilis*) starch from Margarita Island, Venezuela. Arch Latinoam 54 (4): 449-456.

Rosmawati A, Rumhayati B, Srihardyastutie A. 2019. Biorecovery of chitin from shrimp shell waste (*Litopenaeus vanamme*) using fermentation and co-fermentation of *L. plantarum* and *B. thuringiensis*. IOP Conf Ser Materials Sci Eng 546: 022019. DOI: 10.1088/1757-899X/546/2/022019.

Saryono, Novianty R, Suraya N, Piska F, Devi S, Pratiwi NW, Ardhi A. 2022. Molecular identification of cellulase-producing thermophilic fungi isolated from Sungai Pinang hot spring, Riau Province, Indonesia. Biodiversitas 23 (3): 1457-1465. DOI: 10.13057/biodiv/d230333.

Simair AA, Qureshi AS, Khushk I, Ali CH, Lashari S, Bhutto MA, Mangrio GS, Lu C. 2017. Production and partial characterization of α-amylase enzyme from *Bacillus* sp. BCC 01-50 and potential applications. Biomed Res Intl 2017: 9173040. DOI: 10.1155/2017/9173040.

Swinkles JJM. 1985. Source of starch, its chemistry and physic. In: Van Beynum GMA, Roels JA. Starch Conversion Technology. Marcel Dekker Inc, New York.

Thitipraphunkul K, Uttapap D, Piyachomkwan K, Takeda Y. 2003. A comparative study of edible canna (*Canna edulis*) starch from different cultivars. Part I: Chemical composition and physicochemical properties. Carbohydr Polym 53 (3): 317-324. DOI: 10.1016/S0144-8617(03)00081-X.

Unal A. 2015. Production of α-amylase from some thermophilic *Aspergillus* species and optimization of its culture medium and enzyme activity. Afr J Biotechnol 14 (47): 3179-3183. DOI: 10.5897/AJB2015.14924.

Uthumporn U, Wahidah N, Karim AA. 2014. Physicochemical properties of starch from sago (*Metroxylon sagu*) palm grown in mineral soil at different growth stages. IOP Conf Ser Materials Sci Eng 62 (1): 012026. DOI: 10.1088/1757-899X/62/1/012026.