MYCELIAL AMYLASE AND CELLULASE CHARACTERIZATION AS WELL AS BASIDIOMA PHYSICOCHEMICAL ANALYSIS OF LINGZHI MUSHROOM

Karakterisasi Amilase dan Selulase Miselial Serta Analisis Fisikokimia Basidioma Jamur Lingzhi (*Ganoderma lucidum*)

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ABSTRACT

*Lingzi mushroom (*Ganoderma lucidum*) synthesizes enzymes which have anti-hyperglycemic and anti-diabetic activities. This preliminary study aims to characterize the amylase and cellulase activities of mycelial culture, and to analyze the physicochemical compounds in the basidioma of* G. lucidum *InaCC F11 and* G. lucidum *InaCC F106. The enzymes were characterized spectrophotometrically using DNS method, and the basidioma was subjected to proximate and high performance liquid chromatography (HPLC) analysis, as well as microstructural observation using scanning electron microscope. Results showed both strains demonstrated amylase activity, but not cellulase activity. The optimum activity of amylase in* G. lucidum *InaCC F11 mycelial cultures was achieved on the 3rd incubation day, at pH 5.5, 35 °C temperature, and 1.5% substrate concentration, whereas that of* G. lucidum *InaCC F106 on the 7th incubation day, at pH 5, 40 °C temperature, and 1.75% substrate concentration. Dried basidioma of* G. lucidum *InaCC F11 contained 93.72% carbohydrates, 3.06% protein, 0.85% fat, 0.768% crude fiber, 0.54% ash, and 1.83% moisture. In addition, HPLC detected the presence of phenols (0.036%), steroids (0.014 mg 100 mL^{-1}), and active triterpenoid compounds.*

Keywords: *amylase, basidioma, cellulase,* Ganoderma lucidum*, physicochemical*

ABSTRAK

Jamur lingzi (*Ganoderma lucidum*) mensintesis enzim yang memiliki aktivitas anti-hiperglikemik dan anti-diabetes. Studi pendahuluan ini bertujuan mengkarakterisasi aktivitas amilase dan selulase kultur miselium, serta menganalisis senyawa fisikokimia pada basidioma *G. lucidum* InaCC F11 dan *G. lucidum* InaCC F106. Enzim dikarakterisasi secara spektrofotometri menggunakan metode DNS. Basidioma dianalisis secara proksimat, menggunakan kromatografi cair kinerja tinggi (KCKT), serta diamati mikrostrukturnya menggunakan mikroskop elektron. Hasil menunjukkan kedua strain tersebut memiliki aktivitas amilase, dan tidak ada aktivitas selulase. Aktivitas amilase optimum pada kultur miselium *G. lucidum* InaCC F11 dicapai pada inkubasi hari ke-3, pH 5,5, suhu 35 °C, dan konsentrasi substrat 1,5%, sedangkan pada kultur miselium *G. lucidum* InaCC F106 dicapai pada inkubasi hari ke-7, pH 5, suhu 40 °C, dan konsentrasi substrat 1,75%. Basidioma kering *G. lucidum* InaCC F11 mengandung karbohidrat 93,72%, protein 3,06%, lemak 0,85%, serat kasar 0,768%, abu 0,54%, dan kadar air 1,83%. Selain itu, KCKT mendeteksi adanya fenol (0,036%), steroid (0,014 mg 100 mL^{-1}), dan senyawa triterpenoid aktif.

Kata Kunci: amilase, basidioma, fisikokimia, *Ganoderma lucidum*, selulase

INTRODUCTION

Enzymes offer a number of competitive advantages over the use of chemical catalysts (Robinson 2015). This is because they are environmentally friendly, and do not produce toxic residues due to their biological nature (Chapla et al. 2012; Choi et al. 2015; Chapman et al. 2018). In application, enzymes are used to catalyze reactions in the production process in different sectors, including agriculture, bioremediation, industrial bioconversion, and biotransformation of various compounds, such as flavonoids (Choi et al. 2015; Chapman et al. 2018).

Mushroom is a taxa group of micro-organisms which includes regnum. One of their uniqueness is high biodiversity, which is the second-highest after insects. Several mushroom types have been widely used by humans as a source of food and medicine. Furthermore, lingzhi or reishi with the scientific name *Ganoderma lucidum* (Curtis) P. Karst is a type that is widely cultivated in Indonesia. Presently, this variety has reached 120 species globally. Systematically, lingzhi belongs to the porous Basidiomycota taxa or Polyporales. However, *G. lucidum* is not classified as an edible mushroom due to its bitter taste and wood texture. Nevertheless, for the past few decades, it has been used as a functional food to prevent and treat immunological diseases (Hapuarachchi et al. 2016). Physiologically and biochemically, lingzhi contain about 400 different bioactive compounds, which include amino and fatty acids, nucleotides, proteins, polysaccharides, β-glucans, lectins, natural germanium (Ge), alkaloids, adenosine, phenols, steroids, lignin, vitamins, and triterpenoids (Cör et al. 2018; Kumar 2021).

Also, they are widely used as raw materials in the manufacture of medicines and dietary supplements to prevent and treat various immunological diseases (Wang et al. 2012; de Silva et al. 2013; Tan et al. 2015; Yang et al. 2019). This variety is known to have various pharmacological effects such as immunomodulating, anti-inflammatory, anti-cancer, anti-diabetic, anti-oxidant, anti-premature aging, and free radical scavenger (Cör et al. 2018).

Some of the functional enzymes produced during the fermentation process include amylase, lipase, phytase, protease, cellulase, and xylanase (Srilakshmi et al. 2015). In the industrial sector, these enzymes undergo hydrolytic reactions (Choi et al. 2015). Furthermore, the three commercial enzymes (amylase, lipase, and protease) are widely used as active ingredients in detergents and dish washing soap (de Souza et al. 2015). Also, amylase is one of the widely used enzymes during the preparation of fermented foods (Sharma et al. 2020). Besides food and starch industry, they are also used in various other manufacturing companies such as paper and pulp, textiles and more (Ahmad et al. 2019).

Based on utilization and product development, lingzhi mushroom is divided into three groups, which are fruiting bodies (basidioma), mycelia, and powdered spores. The spore powder is the most popular product used by consumers (Li et al. 2016). Meanwhile, amylase and cellulase are commercially available enzymes, and are produced through a fermentation process using microbes. For example, *G. lucidum* produces a number of enzymes, including α-amylase and/or α-glucosidase which have anti-hyperglycemic activity, and useful for controlling blood sugar (anti-diabetic). The first step in bringing these compounds from the field to health institutions is by adopting standard pharmaceutical methods for quality assurance, safety, and testing their efficacy (Bulam et al. 2019). This study aims to characterize the activity of amylase and cellulase enzymes produced by *in vitro* culture of lingzhi mushroom mycelia, and to analyze the physicochemical compounds in the InaCC collection.

MATERIALS AND METHODS

Place and time of research

This study was conducted at the Micro-organism Physiology and Biochemistry Laboratory, Microbiology Division, Biology Research Center, Indonesian Institute of Sciences (LIPI), Cibinong, West Java. Furthermore, it was conducted from April to September 2019.

Materials

The materials used were *G. lucidum* (Curtis) P. Karst isolates obtained from the Indonesian Culture Collection (InaCC),

Biology-LIPI Research Center, Cibinong with accession numbers InaCC F11 and InaCC F106. Furthermore, the chemicals used were potato dextrose agar (PDA), distilled water, yeast extract, bacteriological peptone, soluble starch, carboxymethyl cellulose (CMC), KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4 \cdot 7H_2O$, $CaCl_2$, NaCl, Agar Bacto, and 3,5-dinitrosalicylic acid (DNS) reagent.

In vitro culture maintenance

The lingzhi mushroom isolates InaCC F11 and InaCC F106 stored in a deep freezer of -80 °C for inactive metabolic condition were grown from their dormant states through a series of thawing and reviving. Subsequently, the viability, purity, and identity of the grown isolates were analyzed (Ilyas and Soeka 2019). A well grown culture with verified purity and identity was rejuvenated on a number of PDA slanted tubes as a backup and working culture.

Enzyme extraction

The mycelia of lingzhi mushroom were inoculated into 150 mL of basal culture medium consisting of 0.05% KH_2PO_4, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.1% $CaCl_2$, and 1% (g/v) soluble starch. The acidity (pH) of the basal culture medium was adjusted to 5.5 for amylase and 6 for cellulase. Furthermore, the culture was incubated in an incubator shaker at a speed of 120 rpm and temperature of 27 °C for 8 days. To isolate the enzyme, the culture was centrifuged at 3500 rpm for 15 minutes at 4 °C. The supernatant was separated from the pellet using a 5 mL micropipette, placed in a closed container and stored in the freezer until it was ready for further analysis.

Enzyme activity test

Amylase activity test used a soluble starch substrate, while cellulase activity used 1% (g/v) of carboxymethyl cellulose (CMC) substrate. This was carried out by reacting 62.5 µL of the sample with 62.5 µL of 1% soluble starch substrate dissolved in 0.1 M acetate buffer of pH 5.4. As a control, 62.5 µL of the sample was added to 62.5 µL of 0.1 M acetate buffer of pH 5.4, and for the blank, 62.5 µL of distilled water was added to 62.5 µL of 0.1 M acetate buffer. The sample, control, and blank tubes were incubated for 30 mins at 40 °C. Subsequently, 125 µL of

DNS reagent was added to the tubes, heated in boiling water for 5 mins, and cooled to room temperature. Moreover, it was diluted with 1.25 mL distilled water, and the absorbance was measured using a microplate reader at a wavelength of 540 nm. The corrected absorbance value was entered into the linear regression equation to determine the glucose concentration. Subsequently, DNS reagent was added to stop the reaction (Kar et al. 2006). The sample was immediately cooled under running water and 1.25 mL of distilled water was added. The sample absorbance was measured using a Microplate Reader BioSpec-1601 (Shimadzu) at λ 540 nm. In addition, the blank and control were measured to calculate the corrected absorbance. Amylase/cellulase activity was obtained by the formula equation:

$$A_{corrected} = [(As - Ab) - (Ak - Ab)]$$

As = Absorbance sample,
Ab = Absorbance blank
Ak = Absorbance control

The corrected absorbance result was entered into the *y* equation, therefore, the *x* equation was obtained as the concentration of reducing sugar in the sample. The calculation of cellulase activity was carried out with the formula:

$$Cellulase\ activity\ (U\ mL^{-1}) = \frac{Sample\ glucose\ concentration}{V.T.BM}$$

Description:
V = Enzyme volume (62.5 µL)
T = Incubation time
BM = Anhydrous glucose molecular weight (180 g/mol)

One unit of cellulase activity is defined as the amount of enzyme that produces 1 µmol of glucose in one minute under test conditions.

Enzyme characterization

The enzyme characterization includes determining the optimum pH, temperature, substrate concentration, and the effect of several metal ions. The optimum pH was carried out according to the procedure for determining enzyme activity by varying the pH of the 0.1 M acetate buffer to 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 (Carvalho et al. 2008).

Meanwhile, the effect of the optimum temperature was carried out in accordance with the procedure for determining enzyme activity by varying the incubation temperature to 30, 35, 40, 45, and 50 °C (Carvalho et al. 2008). The optimum substrate concentration was carried out in accordance with the procedure for determining enzyme activity by varying the substrate concentration to 1, 1.25, 1.5, 1.75, and 2% at optimum pH and temperature. Also, Ca^{2+}, Co^{2+}, Cu^{2+}, K^+, and Na^+ in the form of $CaCl_2$, $CoCl_2$, $CuCl_2$, KCl, and NaCl salts with a concentration of 0.01 M of 10 µL were reacted with 52.5 µL of enzyme solution at pH, temperature, and the optimum substrate concentration. This was done to determine the effect of these metal ions on enzyme activity (Carvalho et al. 2008).

Cultivation on baglog media

To form the structure of basidioma, isolates of *G. lucidum* InaCC F11 and InaCC F106 were grown on baglog media made of sengon wood sawdust (*Albizia chinensis*), rice bran, TSP, and dolomite lime ($CaCO_3$) which had been sterilized. The baglog was inoculated with *G. lucidum* isolates on F3 generation, and subsequently incubated in a mushroom house (*kumbung*). Lingzhi mushroom cultured on the baglog was treated by maintaining the humidity and temperature of the *kumbung* in the range of 80–85% RH and 25–28 °C. In fact, Lingzhi mushroom cultures that grow well and are uncontaminated with other microorganisms started forming basidioma at 2 to 2.5 months from the initial inoculation. The basidioma was harvested after 15 days of formation on the baglog.

Flour preparation and physiochemical analysis

The fruiting bodies of the harvested lingzhi mushroom were oven-dried at 50 °C, and the dried basidioma was mashed in a blender to form flour. Subsequently, the flour was analyzed for its proximate (moisture, ash, carbohydrate, fat, and protein content), crude and dietary fiber. Further analysis to ascertain the content of the active substances in the basidioma flour includes the determination of phenols, steroids, and triterpenoids levels using HPLC (Sa-Ard et al. 2015).

Microstructural observation

The microstructure of lingzhi mushroom basidioma flour was observed and documented using a scanning electron microscope (SEM). The SEM used was the JEOL JSM-5000 (JEOL Ltd.), with a specimen magnification scale of 1,000× and a voltage of 20 kV.

RESULTS AND DISCUSSION

The results for amylase and cellulase activity test in the mycelia of *G. lucidum* InaCC F11 and InaCC F106 through standard glucose calculations showed the two isolates only demonstrated amylase activity, and no cellulase activity (Figure 1 and 2). The test results of negative cellulase activity on both strains caused the enzyme characterization to be carried out only on amylase. The analysis results for the characterization of amylase activity with considerable test parameters on the two strains are presented in Figures 3 to 7.

According to Figure 3, the characterization of amylase activity against incubation time showed the optimum amylase activity of *G. lucidum* InaCC F11 was on the 3rd day, with a value of 2.9 U mL^{-1}, while the amylase enzyme activity of the lingzhi mushroom incubation on the 7th day had a value of 1.95 U mL^{-1}. The activity of *G. lucidum* InaCC F11 continuously decreased after the 3rd day of incubation, whereas in *G. lucidum* InaCC F106, decreased activity occurred after the 8th day. In addition, Jo et al. (2011) found that after 5 days of incubation at 25 °C, enzyme activity was qualitatively observed on the clear zones formed around mushroom colonies resulting from the reactions between secreted enzymes in the chromogenic substrate. However, little information about the quantitative extracellular enzyme activity of Ganoderma was reported.

The amylase characterization results of the two lingzhi mushroom strains against pH showed *G. lucidum* InaCC F11 had the optimum amylase activity at pH 5.5 with a value of 2.9 U mL^{-1}. Furthermore, *G. lucidum* InaCC F106 had an optimum activity at pH 5 with a higher value of 3.45 U mL^{-1} which is more than InaCC F11 strain (Figure 4). The activity of an enzyme is closely related to its structure and active site; therefore, an

alteration will cause changes in its activity. According to Robinson (2015), at optimum pH, the enzyme conformation is at ideal conditions. This causes the interaction between the enzyme and the substrate to be maximized. Generally, acidic or alkaline conditions will change the conformation of the enzyme, hence, disrupting its activity. In addition, changes in the acidity level will cause a decrease in activity.

Characterization of amylase against several temperature treatments showed the mycelia of *G. lucidum* InaCC F11 had optimum amylase enzyme activity at 35 ºC with a value of 3.22 U mL^{-1}. Meanwhile, tests conducted for other temperatures, specifically at 30 °C, showed a fairly low activity value and at temperatures above 35 °C, the activity value continued to decline (Figure 5). Different results were obtained for the mycelia of *G. lucidum* InaCC F106 where at 30 ºC, the amylase activity was higher than InaCC F11. At 35 ºC, the activity value decreased but increased again at 40 ºC. The enzyme activity showed an increase in accordance with the increment in temperature with a value of 3.26 U mL^{-1} at 45 ºC and 3.84mL at 50 ºC, but the changes were insignificant. The highest and most significant increase in activity rate occurred when the temperature changed from 35 ºC with a value of 1.62 U mL^{-1} to 40 ºC with a value of 2.97 U mL^{-1} (Figure 5).

The characterization of amylase activity of *G. lucidum* InaCC F11 mycelial with various concentrations of soluble starch had a high activity value at a substrate concentration of 1.5% with a value of 3.99 U mL^{-1}. Generally,

the activity showed a gradual increase from 1 to 1.5% substrate concentration, fixed at 1.75% with a value of 4.06 U mL^{-1}, and decreases at 2% with a value of 3.78 U mL^{-1} (Figure 6). From these observations, it can be concluded that the optimum substrate concentration in the mycelial amylase of *G. lucidum* InaCC F11 is 1.5%.

Furthermore, the observation results of amylase activity on the substrate concentration in *G. lucidum* InaCC F106 showed the activity increased gradually in line with substrate increment. The highest increase in activity occurred at the transfer of substrate concentration from 1.5% with a value of 2.33 U mL^{-1} to 1.75% with a value of 4.7 U mL^{-1}. In fact, the amylase activity tends to be fixed at the addition of a 2% substrate. This showed the optimum substrate concentration in the mycelial amylase of *G. lucidum* InaCC F106 is 1.75% with a value of 4.7 U mL^{-1}.

Substrate concentration is one of the factors affecting enzyme activity. At a fixed enzyme concentration, the addition of substrate will increase activity until it reaches the optimum value (Robinson 2015). At optimum conditions, all the enzymes are saturated, therefore the addition of substrate will not increase activity. Meanwhile, the addition of metal ions at optimum concentration will increase concentration of the substrate-metal complex, create an equilibrium in the desired area and change the electrokinetic potential of the enzyme, hence, optimizing the activation process. Conversely, when the concentration is above or below the optimum, the equilibrium and
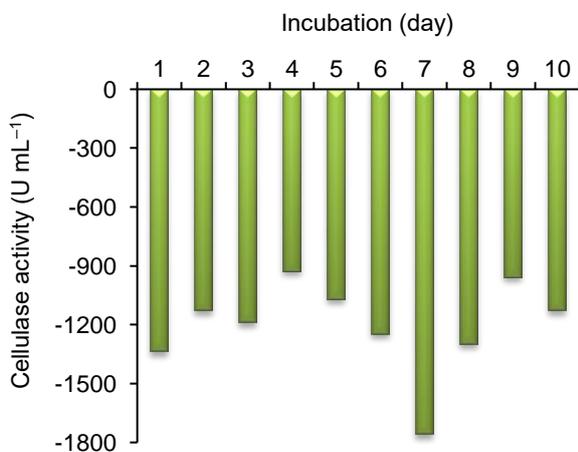
Figure 1. Effect of incubation time on cellulase activity of lingzhi mushroom cellulase *G. lucidum* InaCC F11
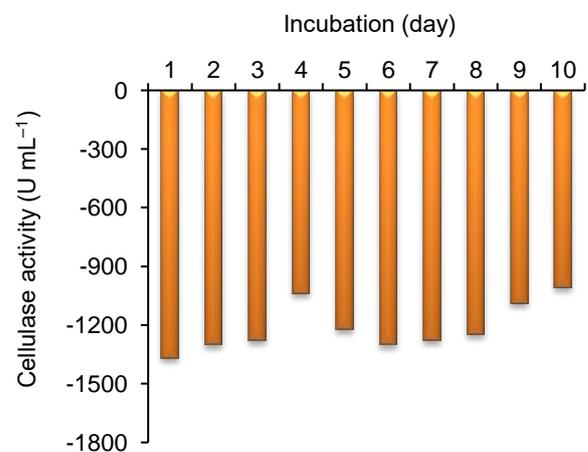
Figure 2. Effect of incubation time on cellulase activity of lingzhi mushroom cellulase *G. lucidum* InaCC F106

electrokinetic potential does not reach or exceed the desired area, hence, causing the activation process not to be optimal and even inhibit or reduce enzyme activity (Soeka 2017).

Bednarska (2015) stated that at low substrate concentrations, the active site only accommodates a small amount of substrate. In this condition, the concentration of the enzyme-substrate complex is small and
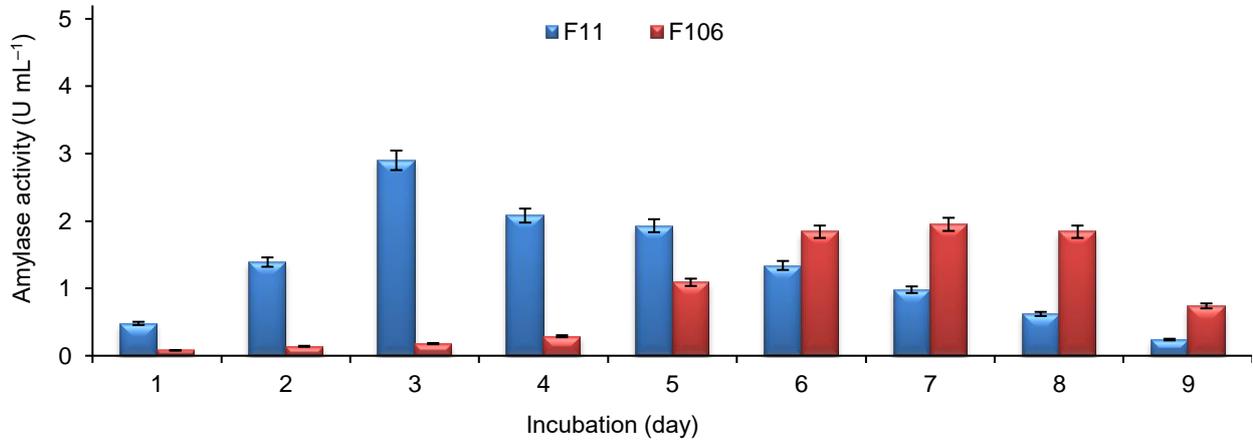
Figure 3. Effect of incubation time on the amylase activity of *G. lucidum* InaCC F11 and InaCC F106 mycelia
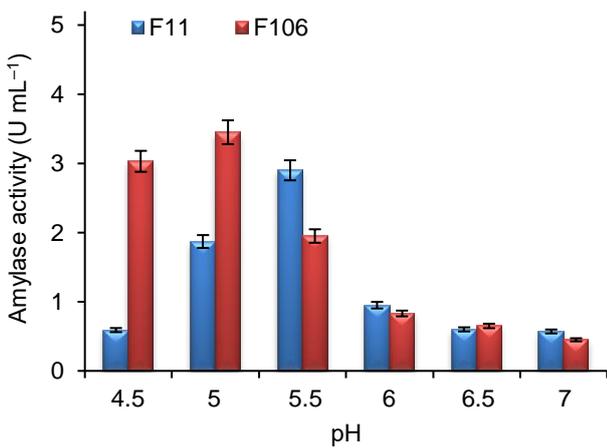
Figure 4. Effect of the degree of acidity (pH) on the amylase activity of *G. lucidum* InaCC F11 and InaCC F106 mycelia
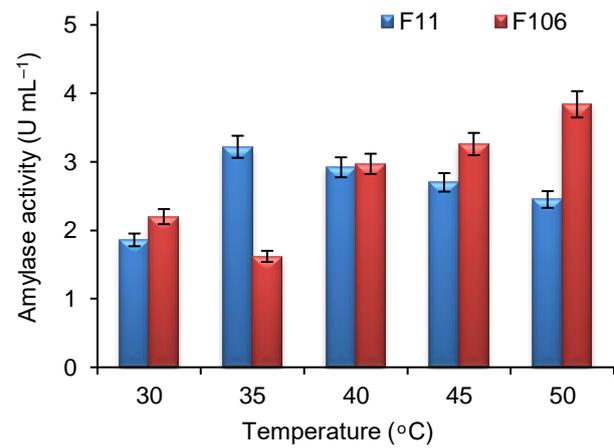
Figure 5. Effect of temperature on the amylase activity of *G. lucidum* InaCC F11 and InaCC F106 mycelia
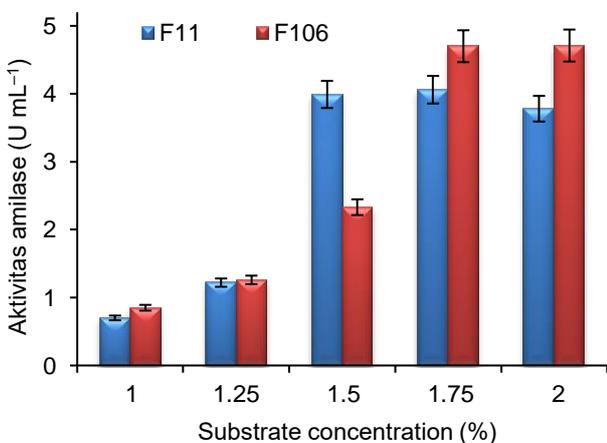
Figure 6. Effect of soluble starch substrate concentrations on the amylase activity of *G. lucidum* InaCC F11 and InaCC F106 mycelia
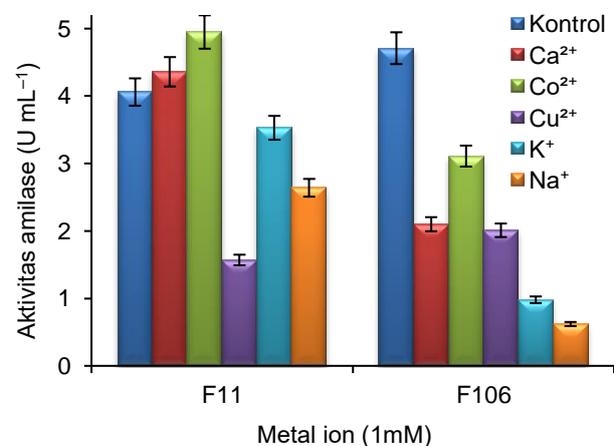
Figure 7. Effect of the addition of metal ions on the amylase activity of *G. lucidum* InaCC F11 and InaCC F106 mycelia

causes low enzyme activity. Meanwhile, with increase in concentration, the better the substrate binds with the enzyme active site. Hence, the enzyme-substrate complex and activity will increase.

The observation results of amylase activity against a number of metal ions showed the addition of Ca^{2+} and Co^{2+} ions in the mycelial culture of *G. lucidum* InaCC F11 increased the enzyme activity over control while, the addition of Cu^{2+}, K^+, and Na^+ ions caused the activity to be under control (Figure 7). This showed that in the mycelial amylase activity of lingzhi mushroom *G. lucidum* InaCC F11 the Ca^{2+} and Co^{2+} ions act as activators, while the Cu^{2+}, K^+, and Na^+ ions as inhibitors. However, in the mycelial amylase of *G. lucidum* InaCC F106, the addition of Ca^{2+}, Co^{2+}, Cu^{2+}, K^+, and Na^+ metal ions lower the enzyme activity below control. This showed that in the mycelial amylase activity of *G. lucidum* InaCC F106, Ca^{2+}, Co^{2+}, Cu^{2+}, K^+, and Na^+ metal ions act as inhibitors. Most of the amylases are known as enzymes which depend on divalent metal ions such as Ca^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, and Pb^{2+} to increase activity (Okwuenu et al. 2017). Also, several monovalent, divalent, and trivalent metal ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+}, and Fe^{3+} can act as both inhibitors and activators of enzyme activity (Pereira et al. 2017).

Physicochemical analysis of basidioma was carried out on isolates of *G. lucidum* grown on baglog. During the cultivation, only *G. lucidum* InaCC F11 cultures could form fruiting bodies, whereas InaCC F106 did not. This is similar to Luangharn et al. (2019) with *G. tropicum* KUMCC18-0046 from Chiang Rai Province, Thailand. The successful growth conditions for producing mycelium on PDA (Potato Dextrose Agar), MEA (Malt Extract Agar), and YPD (Yeast extract Peptone Dextrose) media were pH 7–8 and temperature of 25–28 ºC, but fertilization was not achieved.

The basidioma of *G. lucidum* InaCC F11 formed on the baglog is kidney-shaped, reddish-brown, 10–15 cm in diameter, with a thickness of 2–5 cm (Figure 8A). Furthermore, the basidioma formed was harvested after 15 days and processed into flour for physicochemical analysis (Figure 8B). A scanning electron microscope (SEM) was used to analyze the microscopic structure of the basidioma flour. The results can be seen in Figure 8C.

The proximate analysis results of basidioma flour of *G. lucidum* InaCC F11 is seen in Table 1, while the analysis results for the active substance content is seen in Table 2. Sharif et al. (2016) found that the proximate analysis results of *G. lucidum* dry weight showed 15.04% crude protein, 0.53% crude fat, 54.12% fiber, 2.01% ash, and 82.47% carbohydrates. This differs from the analysis of this study, where the crude protein was 3.06%, crude fat 0.85%, fiber 0.768%, ash 0.54%, and total carbohydrates 93.72%. These results need to be improved by considering the composition of the growing medium, planting method, harvest time interval and stage, measurement method, as well as the specific part of the basidioma used for analysis.

Figure 8. (A) Morphology of fruiting bodies or basidioma lingzhi mushroom *G. lucidum* InaCC F11 aged 15 days since basidioma formation in baglog, (B) lingzhi mushroom basidioma flour *G. lucidum* InaCC F11, and (C) microscopic structure of lingzhi mushroom basidioma flour *G. lucidum* InaCC F11 under SEM

Table 1. Results of proximate analysis of basidioma flour of lingzhi mushroom *G. lucidum* InaCC F11

Component	Method	Content (%)
Moisture	Thermogravimetry	1.83
Ash	Gravimetry	0.54
Fat	Soxhlet	0.85
Protein	Kjeldahl	3.06
Carbohydrate	By difference	93.72
Crude fiber	Gravimetry	0.768
Dietary fiber	Enzymatic	–

G. lucidum mushrooms have a high moisture content, which is 90% of their fresh weight (Bijalwan et al. 2020). In this study, the moisture content in mushrooms was > 98%. The ash content of a material describes the amount of mineral (Hung and Nhi 2012), and the fat varies but is mostly low. Mushrooms are low-fat, low-calorie foods and are important source of nutrients or bioactive compounds (Agarwal and Fulgoni 2021). Hung and Nhi (2012) found the carbohydrate content of *G. lucidum* with a value of 82.3%. This is different from this study where carbohydrate content was 93.72%. The main source of fiber is cellulose and other indigestible cell wall polymers, which help to cleanse and maintain motility in the intestinal tract (Mukhopadhyay and Guha 2015). According to Hu et al. (2013), edible mushrooms are excellent foods included in a balanced diet due to their low fat, high dietary fiber and protein content. In fact, their low protein, carbohydrates, essential minerals, and energy make many wild mushrooms an excellent source of functional food. Therefore, it is concluded that mushrooms can be a substitute as a good source of nutrition, which is cheaper than meat, eggs, and milk, especially in developing countries. Besides, it has prospective therapeutic value in controlling blood glucose and lipids due to its high fiber content.

Tong et al. (2008) stated that the immunomodulatory activity of *G. lucidum* protein can be maintained under processing conditions (heating to 100 ºC for 30 mins, autoclaving for 15 mins, and vacuum drying), and its stability can be maintained at pH of 2–12, but not beyond pH 13. This allows mushroom proteins to be used in functional foods and nutraceuticals.

Table 2. HPLC analysis results of the active substance in the basidioma flour of lingzhi mushroom *G. lucidum* InaCC F11

Active Substance	Content
Phenol	0.036 (%)
Steroids	0.014 mg/100 mL
Triterpenoids	Positive

Ganoderma species are good sources of anti-oxidant compounds (Cör 2018). Furthermore, the bioactive peptids in edible mushrooms are used to treat medical and biotechnological problems (Keypour et al. 2019). Hung and Nhi (2012) found that *G. lucidum* showed a high anti-oxidant capacity (7.5%), and that of free phenolic extracts was much higher (40.7%) than bound phenolic extracts (7.5%). The capacity of phenolic extract indicates that mushrooms are not only consumed for nutrition but also as medicine and functional food. These results showed the basidioma of *G. lucidum* contains bioactive molecules, although phenol, steroids, and triterpenoids levels are still far below the results of Sharif et al. (2016). The differences in proximate and bioactive molecule content in the basidioma of the mushroom are caused by several factors, such as differences in species and strains, the composition of growing media, cultivation techniques, harvesting time stages and intervals, availability of carbon and nitrogen sources and its ratio, measurement method, and specific basidioma subset used for analysis (Hoa et al. 2015; Sarnthima et al. 2017).

More than 200 triterpenes have been identified from the fruiting bodies, spores, and mycelia of *G. lucidum*, and over 20 types of sterols are divided into ergosterol and cholesterol (Xia et al. 2014; Baby et al. 2015). According to Mishra et al. (2018), *G. lucidum* is medicinal because many phenolic compounds are detected in this species. Hennicke et al. (2015) stated that this mushroom has been cultivated commercially with two strains of *G. lucidum*, M9720 and M9724 from the company Mycelia bvba (Belgium).

Polysaccharides, steroids, and triterpenoids are the main group of active substances found in *G. lucidum* followed by alkaloids, fatty acids, glycoproteins, inorganic

elements, lignins, nucleosides, nucleotides, peptides, phenols, proteins, sterols, and vitamins (Boh et al, 2007). Also, Ganoderma species are good sources of raw material for anti-oxidant compounds (Sarnthima et al. 2017). The presence of phenolics is responsible for the anti-oxidant activity of *G. lucidum* basidioma and other mushrooms (Keypour et al. 2019; González et al. 2020). Meanwhile, polysaccharides extracted from *G. lucidum* strains GL-1 and GL-2 showed potential anti-microbial activity against pathogenic bacteria (Kaur et al. 2015). Therefore, the extract is found in many dietary supplements used in improving human health (Valverde et al. 2015). These species contain a number of bioactive complex compounds, with high and low molecular weight, most of which are classified as triterpenoids (Sandargo et al. 2019).

Hennicke et al. (2016) stated that the high content of triterpenic acid causes a bitter taste in *G. lucidum*. Furthermore, the triterpenoids have good nutritional value as well as health benefits, and have been shown to improve human health (Yu et al. 2020). Also, *G. lucidum* has hepatoprotective, anti-hypertensive, hypocholesterolemic, anti-histamine, anti-tumor, anti-genogenic, and anti-HIV-1 effects which have significant anti-complement activity (Boh et al. 2007; Ma et al. 2011). According to Yang et al. (2019), secondary metabolites isolated from *G. lucidum* can be used as anti-melanogenesis, anti-aging, and protective skin repair activities.

CONCLUSION

The results of amylase and cellulase tests on the isolates of *G. lucidum* InaCC F11 and InaCC F106 showed both strains had amylase activity, but no cellulase activity. The optimum amylase activity in *G. lucidum* InaCC F11 mycelial culture was achieved on the 3rd incubation day, with a pH of 5.5, temperature of 35 ⁰C, 1.5% substrate concentration, as well as Ca^{2+} and Co^{2+} metal ions, which act as activators, while Cu^{2+}, K^+, and Na^+ as inhibitors. Meanwhile, in *G. lucidum* InaCC F106 mycelial cultures, the optimum amylase activity was achieved on the 7th incubation day, with a pH of 5, temperature of 40 ⁰C, substrate concentration of 1.75%, and Ca^{2+}, Co^{2+}, Cu^{2+}, K^+, Na^+ act as

inhibitors. Based on the analysis, proximate dried basidioma of *G. lucidum* InaCC F11 contained 93.72% carbohydrates, 3.06% protein, 0.85% fat, 0.768% crude fiber, 0.54% ash, and 1.83% moisture content. In addition, it contained 0.036% phenol, 0.014 mg/100mL steroids, and positive for triterpenoid active substances.

AUTHOR CONTRIBUTION

Yati Sudaryati Soeka, as main contributor, and Muhammad Ilyas as member contributor.

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