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ISOLATION AND SELECTION FOR EDIBLE MUSHROOM PRODUCTION BY LIQUID FERMENTATION AS LACCASE BIOLEACHING SAGO STARCH

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ABSTRACT

Production sago starch from Indonesia reached 585,000 tons in 2015 has become the largest in the world. However sago starch has a weakness that is brownish color, unpleasant smells and soft texture. The weaknes can be overcome by adding laccase in the bleaching process. The purpose of this study is production of laccase from edible mushroom from market in liquid fermentation. Materials used are *Plurotusostreatus* mushroom, *Agaricusbisporus* and *Rhyzopusoligosporus* obtained from the market. The results showed that *Pleurotusostreatus* on petri dish led to a thick brownish red ring zone showing the ability of laccase to oxide guaicol and syringaldazine compounds. The result of 21st days fermentation of laccase production from *Pleurotus*, *Agaricus, Rhyzopus*respectively is 774 U/L, 484 U / L and 480 U / in 500 ml, erlenmeyer flask. Laccase production in 500 mL erlenmeyer flask *Pleurotus* have the highest result. These result indicate that *Pleurotus* has a potensial as a fungi for laccase production through liquid fermentation.

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INTRODUCTION

The vast potential of sago forests in Indonesia is estimated to reach 4.18 million hectares (Bintoro, 2010) but only a small portion is used for the production of sago starch. Indonesian sago starch production reached 585 thousand tons in 2015 and is the largest production in the world (Naim, 2016), along with Papua New Guinea controlled 94.6 percent of the world production of sago starch. Although Indonesia's largest production of sago starch, sago starch but less good quality because the color tends to be off-white (brownish). The quality of sago starch is determined by the color, viscosity and particle size (Hariyanto, 1992). Indonesia sago starch manufacturers face constraints in terms of starch that tend brownish color. This is because the content of polyphenol compounds in sago starch produced. All this time sago starch craftsmen using chemical bleaches such as alum, benzoyl, peroxide, potassium bromate, calcium iodate and ascorbic acid. Some phenol compounds are not allowed in food and chemical bleach can be harmful to health. It is necessary for sago processing that can meet the standards and maintained food security. Konuma *et al.* (2012) mention the color of the sago starch extraction results are affected by the condition of the soil acidity and sulfur where the sago palms grow. Acidity and sulfur content which leads to high ash and high phenolic compounds sago starch so that starch color becomes pink to brown. Laccase is a natural enzyme which can oxidize aromatic and non-aromatic compounds in a broad spectrum, formed by the activity of microbes and plants. Laccase is widespread in high-level plants, fungi Ascomycetes, Deuteromycetes, and Basidiomycetes, and bacteria (Brijwani *et al.,* 2010). Study of several types of fungi and bacteria that produce laccase that have the ability to reduce the color of the phenol compound mikropolutanwas done by Forootanfar *et al.* (2012) and Margot *et al.* (2013). Some types of fungi or bacteria that produce laccase include *Pleurotusostreatus*, *Penicilliumchrysogenum, Agaricusbisporus, Fusariumsolani* (Kumar *et al.,* 2011), *Aspergillus sp., Rhizopus sp*., *Fusarium*

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sp., Penicillium sp., Alternaria sp. (More *et al.,* 2011), *Lentinulaedodes* (Cavallazzi *et al.,* 2005), *Trametesversicolor* (Minussi *et al.,* 2007), *Trichoderma, Leptosphaerulina, Bacillus substilis* (Sheiki *et al.,* 2012), *Pseudomonas aeruginosa, Pseudomonas fluorescens* (Peter *et al.,* 2014), and *Lactobacillus*, which produce *oxidoreductase* compounds that are bioleaching. laccase is the main lignolitikenzyme capable of oxidizing Cu, involved in cross linking monomers, degradation of polymer compounds and opening aromatic complex compound rings (Pannu and Kapoor, 2014). Edible mushrooms can produce laccase and can be isolated from nature and cultivated to produce laccase used for bioleaching. Isolation and selection of *Pleurotusostreatus* mushroom, *Agaricusbisporus, Volvarielavolvacea* and mushrooms *Rhyzopusoligosporus* used as a model for the production of liquid fermentation fungal laccase bioleaching. Laccase enzyme has been applied to some stabilization in the food industry including the beverage industry fruit juice, tea, wine, and beer, improve the quality of starch in the bread industry, improving the performance and quality of overall food (Brijwani *et al.,* 2010).

Furthermore, Osma et al (2010) reported laccase can be promoted to control odors, improve taste and reduce the by-products (polyphenol compounds) that are not desired in some food products. Laccase is also used to reduce the bitter taste, improve the quality of the aroma and taste of chocolate based products and relieve unpopular in chocolate and enhance the flavor of the soybean oil product by removing the polyphenol compounds, improve the quality of aroma oils of plants by removing dissolved oxygen to prevent oxidation. Laccase can have new functionality, to improve quality, or lower production costs due to the reactivity of the components of food-related compounds such as carbohydrates, unsaturated fatty acids, phenol compounds and thiol-containing proteins. Some of them Suberzyme laccase formula and Flavoustar, used in the beer industry to eliminate polyphenol thereby extending the shelf life (Taylor & Francis, 2014). This study will perform the isolation and selection of edible mushroom fungus to produce laccase bioleaching sago. The purpose of this study is to obtain microbial laccase used to bioleaching sago starch and see the resulting enzyme activity.

MATERIALS AND METHODS

Chemical material

Chemicals for analysis using analytical grade materials: ABTS (Merck), Syringaldazine (Sigma), Guaiacol (Sigma), KH2PO4 (Merck), K2HPO4 (Merck), Ammonium sulfate (Merck), Sodium phosphate (Merck). While the fermenting materials used are combinations of pro analysis (PA) chemicals and technical materials.

Isolation of edible fungi on PDA (Potato Detrose Agar)

Isolation of edible mushrooms of the oyster mushroom (Pleurotusostreatus), button mushrooms (Agaricusbisporus), and (Rhyzopusoligosporus) that exist in the market, based on the method Desai et al (2011) and Das et al (2015) that were modified, using the potato dextrose agar (PDA) media containing 20% potato extract, dextrose 2% and agar 2% that dissolved in distilled water with a pH of 7.0 and a static incubation in a dark room with a temperature of 25 ± 2°C for 7 days.

Mycelia which grew further wassubcultured to obtain pure isolate of the fungus in question. Sub-culture fungal isolates of *A. bisporus*, *P. ostratus*, and *R. oligosporus* performed on Potato Dextrose Agar (PDA) refers to a modified Desai et al (2011) method. On the PDA 24 gL-1 enriched 0.5% yeast extract and micromineral planted pieces of *A. bisporus, P. ostratus*, and *R. oligosporus*, incubated at 30° C for 7 days. The results of the sub-fungal cultures were used as inoculum in liquid fermentation.

Laccase test on agar plate

Laccase test on agar using guaiacol and syringaldazine. Selection of edible mushroom fungus carried out based on the Kumar et al (2011) method. Pieces edible oyster mushroom (*Pleurotusostreatus*), button mushrooms (*Agaricusbisporus*), and fungi tempeh (*Rhyzopusoligosporus*) planted on Potato Dextrose Agar with addition of 0.02% guaiacoland 0.1% syringaldazine. Red-brown or orange color around the colony are positive indicator on the existence of laccase in guaiacol and purple on syringaldazine.

Liquid fermentation culture of edible mushrooms in the PDB (Potato Detrose Broth) media

Liquid fermentation culture of *A. bisporus*and *P. ostratus* edible mushroom using Potato Dextrose Broth (PDB) refers to the modified method of Kumar et al (2011). Media GDP enriched with micromineral dissolved in 100 mL of distilled water in a 250-ml Erlenmeyer flask. Media containing dextrose 1%, peptone 0.3%, KH_2PO_4 0.06%, 0.0001% $ZnSO_4$, K_2HPO_4 0.04%, FeSO4 0.0005%, $MnSO_4$0.5% and $MgSO_4$ 0.05% with a pH of 6.0 sterilized at 121° C for 15 minutes. Furthermore Erlenmeyer flask is inoculated with 1 cm^2*A. bisporus and P. ostratus* of PDA (each 3 pieces) and incubated at 30° C in a water bath incubator shaker 100 rpm in dark conditions for 7 days. Sampling is done from day 4 to day 7 after fermentation for laccase analysis. Tests were also performed on laccase fermentation on fungal cultures of*P.ostretus, A.bisporus*, and *R.oligosporus* using liquid fermentation medium referring to Kumar et al (2011) method modified by substituting a carbon source and a source of protein as follows (g/L): dextrose 5, yeast extract 3, KH_2PO_4 1.05, K_2HPO_4 0.7, $MgSO_4$ 0.5, soy flour 2.5, corn flour 1.5, tempeh flour 1.5, add demineralized water 1000 mL, pH 6, 0 to 6.5 sterilization at 121° C for 15 minutes. Furthermore Erlenmeyer flask is inoculated with *A. bisporus* and *P. ostratus* mycelium fungal from PDA (at size of 1cm^2, 3pieces for each fungal and incubated at 30°C in 100 rpm water bath shaker incubator in dark condition for 21 days by the sampling period 0, 3, 6, 9, 12, 15, 18, and 21.

Laccase Extraction

Laccase extraction is done gradually on the liquid fermentation culture on day 4, 5, 6, and 7 after fermentation referring to methods of Okamoto et al (2000), Trejo-Hernandez (2001) and modified Kumar et al (2011). Laccase Extraction on the fermentation broth is done by protein precipitation using a saturated solution of ammonium sulfate (80%), cooling at a temperature of 4° C for two hours and centrifuged at 10,000 x g for 15 minutes. Pellet concentrate is washed twice with a phosphate buffer solution with a ratio of 1: 1, followed by filtration on What man paper No. 1 and performed analysis on the filtrate using a Thermo scientific spectrophotometer.

Laccase Assay

Analysis of the filtrate by using a spectrophotometer refers to methods of Sandhu and Arora (1985), Okamoto et al (2000), Trejo-Hernandez (2001), Desai et al (2011), and Kalra et al (2013) that was modified. Analysis on the Thermo Scientific spectrophotometers carried out in a solution that is mixed in two ways: first, mixing 1 mL of laccase enzyme filtrate, 2 mL of 50 mMpH 6.0 phosphate buffer, and 1 mL of 2 mMGuaicol with incubation time of 20 minutes at room temperature and wave length of 470 nm. Second, mix 1 mL of laccase enzyme filtrate, 2 mL of 50 mMpH 6.0 phosphate buffer, and 1 mL of 0.45 mMABTS with incubation time of 20 minutes at room temperature and a wavelength of 420 nm. Calculation of enzyme activity is done in accordance with the method of Desai et al (2011), namely Enzyme Activity (U/mL) = Absorbance spectrophotometer x Total volume of solution / (volume

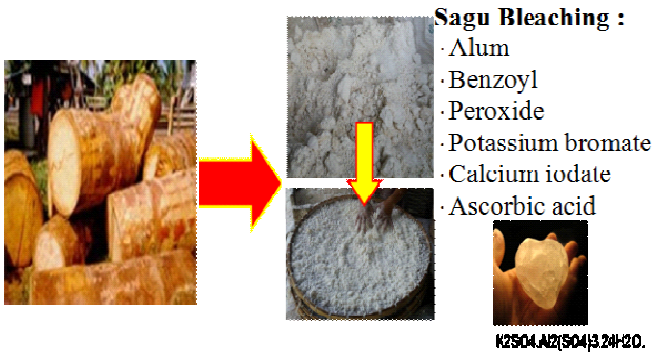

Figure 1. Schematic Process of Sago Tualto Sago Starch Using Chemical Bleaches

Sago pith naturally contains in digeneousmicrobes that function in the biosynthesis of natural materials sago through natural degradation process. Indigenous sago microbes can be isolated and cultured to be utilized in accordance with the potential, including the potential for the production of laccase enzyme. Selection and analysis of microbial diversity sago to do with approach bioleaching microbial DNA isolation metagenomik sago and isolation of edible mushroom fungi that produce laccase as bioleaching. The next test laccase production on agar and fermentation as well as analysis of laccase activity. Best strains isolated from both approaches are used as a starter inoculum in the fermenter scale production and purification laccase. enzyme x incubation time x coefficient guaiacolenzyme (12,100 $M^{-1}cm^{-1}$) or ABTS (36,000 $M^{-1}cm^{-1}$). Enzyme Activity (U/mL) = Absorbance spectrophotometer x Total volume of solution / (volume enzyme x incubation time x coefficient guaiacol enzyme (12,100 $M^{-1}cm^{-1}$) or ABTS (36,000 $M^{-1}cm^{-1}$)

RESULTS AND DISCUSSION

Sago starch is produced by grating sago, followed by extraction using running water. In sago starch extraction process, the color changes from white to pink pith and finally red-brown due to the oxidation reaction of polyphenol compounds in the pith of sago. The color change is very affected by soil conditions and acidity that occurs due to metal elements Mn, Fe, Mg and sulfur containing water to form the oxidation reaction. This phenomenon has been investigated by Konuma *et al* (2012) who mentioned the color of the sago starch extraction process is influenced by the condition of the soil acidity and sulfur where the sago palms grow. Acidity and high sulfur content lead to ash content and high phenolic compounds that make sago starch color becomes pink to brownish red and finally become dark brown/gray. Dark color of sago starch can be removed by oxidation using bleach. All these times traditional craftmendo sago starch bleaching using several chemical compounds such as alum, benzoyl, peroxide, potassium bromate, calcium iodate and ascorbic acid. Although classified in the food whitening chemicals, however large amounts usage can be detrimental to health. Bleaching sago schematically shown in Figure 1

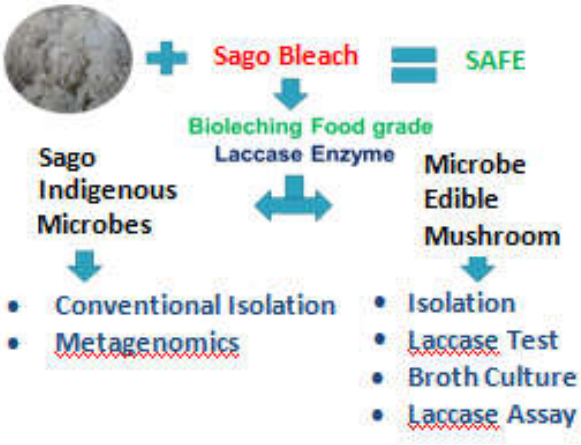

Figure 2. Schematic process of selection and analysis of microbial laccase bioleaching sago

Edible Mushroom Exploration

Button mushrooms (*Agaricusbisporus*) or champignon is edible mushroom-like white round buttons. Button mushrooms are most widely cultivated mushrooms in the world. In English referred to as table mushroom, or white mushroom, common mushroom or cultivated mushroom. Button mushrooms are harvested at 2-4 cm in diameter. Button mushrooms are sold in the form of fresh or canned mushroom, are used in a variety of Western dishes such as omelette, pizza, casserole, gratine, and salad. Button mushrooms can grow in obsolescentor husk wood by utilizing carbon eftover as an energy source. Because of that, it is also known as compost mushroom due to its ability to decipher lignosellulosa button mushrooms into a carbon source for the growth of mycelium and fruiting body formed into a button mushroom. Bioconversion capability lignosellulosa button mushroom button mushrooms is due to an enzyme capable of producing lignolitik groups, one of which is a laccase which is a multi copperextra cellular enzyme capable of oxidizing aromatic and non-aromatic structure components in lignin structure (Kumar, 2011).

Oyster mushroom (*Pleurotusostreatus*) is an edible Basidiomycota group mushroom, class Homobasidiomycetes with the general characteristics of the fruit body is white to cream and a semicircular oyster shells like hood with the middle slightly concave. The oyster mushroom has same family with *Pleurotuseryngii* and often known as the *King Oyster Mushroom*. Oyster mushrooms fruiting bodies have stalks that grow sideways (Latin: Pleurotus) and shaped like an oyster (ostreatus) so that the oyster mushroom have a *Pleurotusostreatus*binomial name. Covering part of the mushrooms colortransforms from black, gray, brown, to white, with a nearly smooth surface, 5-20 cm diameter smooth edged slightly curved hood.

In addition, oyster mushrooms also have a rod-shaped spores measuring 8-11 × 3-4 µm and white mycelia that can grow quickly. In the wild, oyster mushrooms can be found almost throughout the year in cool mountainous jungle area.

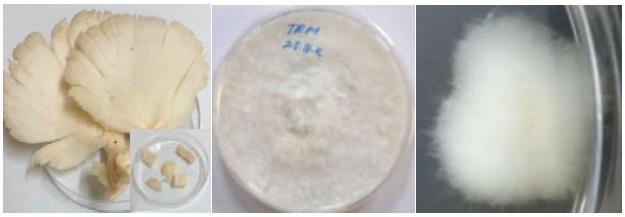

Figure 3. Oyster Mushroom (*Pleurotusostreatus*)

Fruiting bodies overlap each other on the surface of the rotten tree trunk or main trunks of trees that have been cut down because of the oyster mushroom is one type of wood mushrooms. Media commonly used for oyster mushroom farming is sawdust which is a waste of the sawmill. Oyster mushrooms also have the production capability in the extracellular laccase purified and molecularly characterized (Okamoto, 2000). Result of literature exploration has defined two types of edible mushrooms that will be used as a model in liquid culture fermentation and extraction of laccase. Further regeneration methods have been obtained edible mushroom *A. bisporus* and *P. ostreatus* as can be seen in the image below. Mushroom mycelial growth both aforementioned glance look the same, however, when observed more in the mikroskospis there is a difference in the form miselianya. Furthermore, the mycelia growth of *P. ostreatus* remain white while *A. bisporus* mycelia turn black. Speed *A. bisporus* mushroom mycelial growth faster than the fungus *P. ostreatus*. Karena mushroom *A. bisporus* faster form spores when nutrients are limited, the need for further mycelial growth. The study of kinetic mycelial growth of A. bisporus been done by Straatsma et al (1991), where the speed of mycelial growth could reach 30 mmd-1 in the exponential growth phase while stasionernya only accounted for 7.2 mmd-1.

Isolates edible mushroom fungi laccase test is then performed on the media agar plate containing 0.02% guaiacol and 0.1% syringaldazine. Testing the activity of laccase can also be done on a PDA medium plate so by adding a solution guaicol and syringaldehide. Furthermore, the sub-culture of fungal mycelia *A. bisporus* and *P. ostreatus* and incubated at 30 ° C for 7 days. Laccase activity is indicated by the formation of a yellowish brown color zones that are the result guaicol and syringaldehida substrate oxidation by laccase as can be seen in the image below. The greater the concentration of laccasenya will form a reddish brown zones are more extensive. The same method can also be done using paper disk dipped in laccase extract and put it on a paper disk media PDA agar plate added guaicol and syringaldehide.

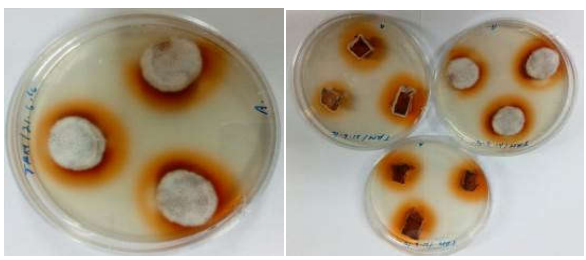

Figure 4. Testing laccase activity usingguaiacol on a saucer

The test results showed that *Pleurotus* laccase test has the ability to produce laccase and perform the oxidation of guaiacol and syringaldazine as can be seen in Figure 4.

Edible Mushroom Fermentation Test

At the trial,` liquid fermentation using of a piece of edible mushroom *A. bisporus* and *P. ostreatus* directly on liquid fermentation media did not succeed. This is due to the mycelia that is not able to grow or use a carbon source derived from simple sugars such as sucrose and dextrose. Fermentation media remained clear after fermentation lasts for four days, while the fermentation using inoculum from the second sub-cultured mycelia fungi on PDA agar media showed mycelial growth that is characterized by turbidity in the liquid fermentation culture medium as can be seen in the image below.

Figure 5. *Pleurotusostreatus* liquid fermentation culture, pre-fermentation and post-fermentation remain clear (left), post fermentation is turbid (middle) and *A. bisporus* remains clear despite of mycelia growing (right)

However, the level of turbidity liquid fermentation culture media does not always reflect the growth of fungal mycelia, but also influenced by the growth of bacterial contaminants symbiosis on fungal mycelia. In the culture of sterile fungal fermentation, showing no turbidity even though the growth of fungal mycelia looked lush and liquid fermentation culture medium remains clear. This phenomenon illustrates that the mushroom liquid fermentation culture does not always show the turbidity in the fermentation liquid media which showed growth of fungal mycelia but can also be liquid fermentation culture media may remain clear as can be seen in the picture above. Meanwhile, on a microscopic examination of the culture medium turbid liquid fermentation, does not indicate clearly the existence of bacteria, such as can be seen in Figure 6

Figure 6. Liquid Culture of *Agaricusbisporus* (left) and Microscopic *Agaricusbisporus* mycelia (right) spectrophotometer

Meanwhile, the results of laccase concentration testing in the extract liquid fermentation culture can be seen in the above table and graph below. The charts show an increase in the concentration of laccase since the days of fermentation 4th through 6th. While on the 7th day of fermentation the laccase concentration decreased sharp possibly due to laccase has run out or decomposed due to heat ambient temperature. The room temperature reached 34° C during spectrophotometer analysis, whereas the reccomended room temperature for analysis in the enzymatic reaction is 25° C. Another possibility is the production of laccase by *A. bisporus* is indeed small due to bacteria contaminated fungal growth *A. bisporus* when seen from the turbidity level of liquid fermentation culture medium. In the Erlenmeyer flask with JB code experienced turbidity in the media, while the Erlenmeyer flask A mycelial growth did not experienced contamination when viewed from the clarity of their liquid culture medium. The same thing is seen from the absorbance at spectrophotometer analysis showed a higher concentration of laccase when compared to culture with the JB code.

Results of analysis of the concentration on the absorbance of spectrophotometers laccase fermentation culture H-5JB and H-6JB by centrifuging speed difference provides significant influence. It can be seen in Table 1 above, which at 4000 xg centrifugation speed-H and H-6JB 5JB laccase concentration consecutively were 13.33 mL and 16.00 mL, while at the speed of 14,000 xg centrifugation were 2.33 µL and 3.67 µL. The differences in concentration values at that centrifugation speed mentioned above are caused by differences in the volume of centrifuged culture. At a speed of 4,000 x g using 50 mL falcon tube with 25 ml culture volume, whereas at a speed of 14,000 x g using ependof 2 mL filled with 1.5 mL volume. Although the results of the spectrophotometer analysis showed differencebased on the comparison figures in general percentage on using Eppendorf centrifuges laccase can harvest three times more compared to centrifuged culture volume. It shows speed centrifugation gives significant effect to produce a laccaseconcentrate.

Tabel 1. Laccase Analiysis Data on Spektrofotometer

Sampel (4000xg)	EA (µL)	
Blanko	Water	0.00
H-4 JB	Lac 4	2,67
H-5 JB	Lac 5	13.33
H-6 JB	Lac 6	16.00
H-7 JB	Lac 7	2,67
H-7 J	Lac 7 b	23,33
	14.000 x g	
H-5 JB	Lac 5	2,33
H-6 JB	Lac 6	3,67

Source: spectrophotometer analysis results batch 1, 2016

Meanwhile, the results of testing the concentration of laccase in the extract liquid fermentation culture can be seen in the above table. The room temperature reached 34° C during spectrophotometer analysis, whereas the recommended room temperature for analysis in the enzymatic reaction is 25° C. Another possibility is the production of laccase by *A. bisporus* is small, this is due to fungal growth *A. bisporus* contaminated with bacteria when seen from the turbidity level of liquid fermentation culture medium. In the Erlenmeyer flask with code JB experienced turbidity in the media, while mycelial growth in the Erlenmeyer flask A did not experienced contamination when viewed from the clarity of their liquid culture medium.

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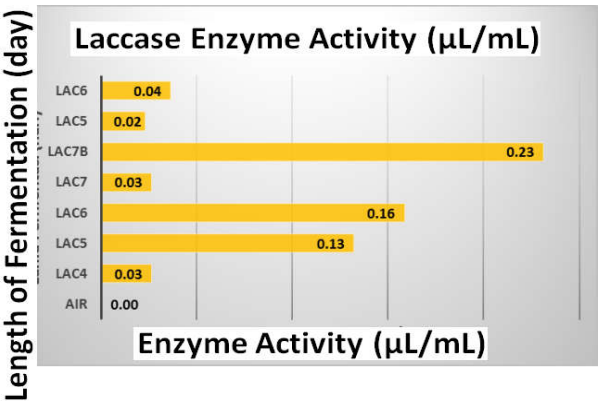

Figure 7. Test Result of *A. Bisporus Laccase* Activity on Spectrophotometer using Guaiacol Substrate on 470 nm Wavelength

Development of edible mushroom fungus fermentation test

Isolation edible mushroom done by taking samples of *Pleurotusostreatus, Agaricusbisporus, Volvarielavolvacea* and *Rhyzopusoligosporus* obtained three best strains using *Pleurotusostreatus, Agaricusbisporus,* and *Rhyzopusoligosporus*. The test results and the activity of laccase fermentation substrate ABTS showed the best activity of each are *Pleurotus* 774 U/L day 21, *Agaricus* 484 U/L day 9, and *Rhyzopus* 480 U/L day 21. Data can be seen in Table 7 and graph image 28. Generally fermentation test results mentioned above not yet using the precursor for the laccase induction is good enough when compared to results from other researchers.

Table 2. Spectrophotometer Laccase Data Analysis on Edible Mushroon Fermentation

Day of Fermentation	Enzyme Activity (U/mL)		
	Agaricus bisporus	*Rhyzopus oligosporus*	*Pleurotus ostreatus*
0	0.000	0.000	0.000
3	0.368	0.297	0.288
6	0.441	0.355	0.391
9	0.484	0.398	0.319
12	0.436	0.349	0.426
15	0.423	0.327	0.376
18	0.447	0.436	0.459
21	0.378	0.480	0.774

Source: results of the analysis spectrophotometer

Figure 8. Edible Mushroom Fermentation in liquid fermentation Before inoculation (left) and after inoculation (right)

laccase fungus Trichodermaspp which during the solid fermentation test laccase activity was 11.7 U / mL and 8.67 U/mL in the liquid fermentation with an incubation time of 9 days at the optimum temperature of 45-50° C and pH 4,5-5,5 enzyme activity ranges around 80%. While Kumar et al (2011) used Pleurotusostreatus fungi for the laccase production with 1 mM $CuSO_4$ induction obtained the best results 910 U/L during the 9 days of fermentation increased from 570 U/L without induction of copper sulfate.

Table 3. Total Sugar LevelData Analysis as Result of Edible Mushroom Fermentation on Reffractometer

Day Fermentation	Total Sugar Level (%)		
	Agaricus bisporus	*Rhyzopus oligosporus*	*Pleurotus ostreatus*
0	6.50	6.40	6.60
3	6.12	6.01	6.05
6	5.32	5.90	4.43
9	4.63	5.98	4.80
12	4.32	6.39	5.29
15	4.38	6.24	6.14
18	4.22	6.66	7.02
21	4.70	7.52	7.63

Source: results of the analysis refractometer batch 3, 2016

Table 4. Data Analysis pH fermented edible fungus mushroom on a pH meter

Day Fermentation	pH		
	Agaricus bisporus	*Rhyzopus oligosporus*	*Pleurotus ostreatus*
0	6.50	6.50	6.50
3	5.30	5.30	5.40
6	5.40	4.90	3.70
9	4.60	3.70	3.50
12	4.00	3.50	3.30
15	3.30	3.50	3.20
18	3.50	3.10	3.30
21	2.90	3.10	3.20

Resourch: Analysis Result on pH batch 3, 2016

Copper sulfateis a precursor compound that is better than alcohol veratril 7 mM and 10 mM benzyl alcohol. On the other hand, other researchers More et al (2011), using *PleurotusSsp*strain got opposite result for tannic acid induction, that is 83.83 U/mL and 112.88 U/mL without induction. This shows that not all types of precursors are suitable for the production of laccase. Kumar et al (2016) experimented on sourcing of carbon and nitrogen sources used strains of *Aspergillusflavus* obtained the best results in the production of laccase cellulose 8% carbon source, nitrogen peptone 2%, temp 35° C and pH 7 inoculum size of 1.5 cm.

During fermentation 21 days there will be a decrease in pH, as a result of acid formation. A description of the decrease in pH of the media during fermentation is presented in Table 4.

Conclusions and recommendations

From these researches, it can be concluded

Based on laccase concentration analysis in the bioleaching test mushroom broth fermentation, it yields that *Pleurotusostreatus*, *Agaricusbisporus* and *Rhizopusoligosporus* can produce laccase. Result of fermentation test and laccase activity on ABTS substrate shows that the best activity for each mushroom is Pleurotus 774 U/L on 21st day, Agaricus 484 U/L on 9th day and Rhyzopus 480 U/L on 21st day.

Suggestion

- The extraction result of crude enzyme should be followed by purification and characterization of laccase enzyme and tests of dye decolorizationand organoleptic
- Lab-scale fermentation and extraction of test results should be followed up with a fermentor-scale fermentation and a fermentor-scale laccase to get laccase enzyme protype.
- Laccase enzyme prototype results should be used in the bleaching test to get sago which meets the quality standards of Codex Stan 301R-2011 (regional standard for Asian edible sago flour)

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REFERENCES

Bintoro, D. 2008. Bercocok Tanam Sagu. IPB. Pres. Bogor

Bintoro, D. 2012. Potensi sagu di Papua dan Papua Barat. HasilKajian UP4B dan IPB. Bogor

Brijwani, K., Rigdon, A., and Vadlani, P.V. 2010. Review Article. Fungal Laccases: Production, Function, and Applications in Food Processing. SAGE-Hindawi Access to Research Enzyme Research Volume 2010, Article ID 149748, 10 pages doi:10.4061/2010/149748

Das, N., Dey, D., and Mishra, S. 2015. Isolation and physico-chemical characterization of extracellular lingo-cellulolytic enzymes of Pleurotuspulmonarius in submerged fermentation.International Journal of Applied Biology and Pharmaceutical Technology Volume-6, Issue-3, July-Sept-2015.Coden IJABFP-CAS-USA. Copyrights@2015, ISSN: 0976-4550. www.ijabpt.com

Demissie, A.G., Kumar, A. 2014. Isolation of Novel Bacteria Isolate from Soil for Production of Extra-Cellular Laccase Enzyme. International Journal of Emerging Technology and Advanced Engineering. Website: www.ijetae.com (ISSN 2250-2459, ISO 9001:2008 Certified Journal, Volume 4, Issue 11, November 2014)

Desai, S.S., Tennali, G.B., Channur, N., Anup, A.C., Deshpande, G., Azhar M, B.P.A. 2011. Isolation of laccase producing fungi and partial characterization of laccase. Research Article, Biotechnol. Bioinf.Bioeng. 2011. 1(4):543-549

Forootanfar, H., Moezzi, A., Aghaie-khozani, M., Mahmoudjanlou, Y., Ameri, A., Niknejad, F., and Faramarzi, M.A. 2012. Synthetic dye decolorization by three sources of fungal laccase. Iranian Journal of Environmental Health Sciences & Engineering 2012, 9-27.

Hariyanto, B. 2014. Perkembangan Teknologi Produk Pangan Berbasis Sagu Guna Mendukung Ketersediaan Pangan. Orasi Pengukuhan Profesor Riset Bidang Teknologi Pangan. BPPT-LIPI. Jakarta

Hariyanto, B. Dan, P. and Pangloli, 1992. Potensi dan Pemanfaatan Sagu. Kanisius. Yogyakarta

http://dx.doi.org/10.1155/2016/8319542

http://www.ijehse.com/content/9/1/27

José Renato, P., Cavallazzi, J.R.P., Catarina M. Kasuya, C.M., Marcos A. and Soares, M.A. 2005. Screening of inducers for laccase production by lentinulaedodes in liquid medium. *Brazilian Journal of Microbiology*, (2005) 36:383-387 ISSN 1517-8382

Kalra, K., Chauhan, R., Shavez, M. and Sachdeva, S. 2013. Isolation OfLaccase Producing *Trichoderma* Spp. And Effect Of PH And Temperature On Its Activity. *International Journal of ChemTech Research,* CODEN(USA): IJCRGG ISSN : 0974-4290 Vol.5, No.5, pp 2229-2235, July-Sept 2013.

Konuma, H., Rolle, R. and Boromthanarat, S. 2012. Color characteristics of sago starch as they relate to the growth environment of the sago palm (*Metroxylon sago* Robb). Journal of Agricultural Technology 2012 Vol. 8(1): 273-287 Available online http://www.ijat-aatsea.com

Kumar, R. Kaur, J., Jain, S. and Kumar, A. 2016. Optimation of laccase production from *Aspergillusflavus* by design of experiment technique: Partial purification and characterization. Journal of Genetic Engineering and Biotechnology (2016) 14, 125 – 131.

Kumar, V.V., Kirupha, S.D., Periyaraman, P. and Sivanesan, S. 2011. Screening and induction of laccase activity in fungal species and its application in dye decolorization. African Journal of Microbiology Research Vol. 5(11), pp. 1261-1267, 4 June, 2011.

Margot, J., Bennati-Granier, C., Maillard, J., Blanquez, P., Barry, D.A. and Holliger, C. 2013. Bacterial versus fungal laccase: potential for micropollutant degradation. AMB Express 2013, 3:63. http://www.amb-express.com /content/3/1/63

Minussi, R.C., Miranda, M.A., Silva, J.A., Ferreira, C.V., Aoyama, H., Marangoni, S., Rotilio, D., Pastore, G.M. and Durán, N. 2007. Research P Purification, characterization and application of laccase from Trametesversicolor for colour and phenolic removal of olive mill wastewater in the presence of 1hydroxybenzotriazole African Journal of Biotechnology Vol. 6 (10), pp. 1248-1254, 16 May 2007.http://www.academicjournals.org/AJB ISSN 1684–5315 © 2007

More, S.S., Renuka, P.S., Pruthvi, K., Swetha, M., Malini, S. and Veena, S.M. Isolation, Purification, and Characterization of fungal Laccase from *Pleurotus sp*. Enzyme Research Volume 2011, Article ID 248735, 7 pages doi:10.4061/2011/248735.SAGE-Hindawi Access to Research.

Naim, H.M., Yaakub, A.N. and Hamdan, D.A.A. 2016. Commercialization of Sago through Estate Plantation Scheme in Sarawak: The Way Forward. Hindawi Publishing Corporation International Journal of Agronomy Volume 2016, Article ID 8319542, 6 pages

Okamoto, K., Yanagi, S.O., Sakai, T. 2000. Purification and characterization of extracellular laccase from *Pleurotusostreatus*.Mycoscience 41: 7 – 13, 2000.

Osma, J.F., Toca-Herrera, J. and Rodr'iguez-Couto, S. 2010. Review Article Uses of Laccases in the Food Industry. SAGE-Hindawi Access to Research Enzyme Research Volume 2010, Article ID 918761, 8 pages doi:10.4061/2010/918761.

Pannu, J.S. and Kapoor, R.K. 2014. Microbial Laccases: A Mini-Review on Their Production, Purification and Applications. Review Article International Journal of Pharmaceutical Archive-3(12), 2014, 528-536.

Peter, J.K., Vandana, P., Masih, H. and Kumar, Y. 2014. Production Optimization and Partial Purification of Laccases from Bacterial Consortium. International Journal ofEngineering Research & Technology (IJERT) Vol. 3 Issue 6, June - 2014 ISSN: 2278-0181.

Sheikhi, F., Ardakani, M.R., Enayatizamir, N. and Rodriguez-Couto, S. 2012. The Determination of Assay for Laccase of Bacillus subtilis WPI with Two Classes of Chemical Compounds as Substrates. Indian J Microbiol (Oct–Dec 2012) 52(4):701–707 DOI 10.1007/s12088-012-0298-3

Trejo-Hernandez, M.R. and Lopez-Munguia, A., Quintero Ramirez, R. 2001.Residual compost of *Agaricusbisporus* as a source of crude laccase for enzymic oxidation of phenolic compounds. Process Biochemistry 36 (2001) 635–639.

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