# Characterization of Lignin Peroxidase from the Suspected Novel Strain *Phanerochaete chrysosporium* ITB Isolate

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**Abstract:** *This study was aimed to characterize lignin peroxidase (LiP) obtained from* Phanerochaete chrysosporium *ITB isolate. The characterizations included molecular weight, the pH and optimum working temperature of the crude extract of the enzyme, the temperature stability, the effect of metal ions and inhibitors, their precipitation with ethanol, and the storage stability. The LiP of* P. chrysosporium *ITB isolates was 34 kDa. The crude extract of LiP displays high activity at pH between 3 until 5 and 26–32 °C, has good thermal stability at 26–32 °C for 20 h. The activity is affected by $Pb^{2+}$, $K^{+}$, $Co^{2+}$, $Fe^{2+}$, $Cd^{2+}$, $Mg^{2+}$, and $Cu^{2+}$ EDTA, $Na^{+}$, $Cr^{3+}$, $Hg^{2+}$, $NaN_3$, $Ni^{2+}$, and $Ca^{2+}$ ions, is not affected by $Mn^{2+}$ and $Zn^{2+}$ ions, precipitated with the optimum ethanol at 64% ethanol saturation which results in an increase in specific activity of 2.3 times. The crude extract storage at 0 °C is more stable than the precipitate resulting from ethanol precipitation and resuspension from ethanol precipitation. These results strengthen that LiP from* P. chrysosporium *is another LiP isoenzyme that can be used for bioremediation processes. Unfortunately, the concentration using the ethanol precipitation method has not been effective, so further studies using other methods should be required.*

**Keywords:** *lignin peroxidase;* Phanerochaete chrysosporium*; ITB isolate; characterization*

## ■ INTRODUCTION

*Phanerochaete chrysosporium* is known to degrade lignin and various aromatic pollutants during secondary metabolism in the stationary phase. Peroxidases (manganese peroxidase and lignin peroxidase) and the extracellular $H_2O_2$-producing enzyme system of oxidase produced by these organisms are the main enzymes involved in the metabolism of lignin degradation, synthesized in response to the limited levels of nitrogen, carbon, and sulfur; also adopted various metabolic strategies to degrade complex polymeric substrates [1-5]. Many studies have shown that the type of strain and medium culture conditions affected the type of isoenzyme of LiP produced. *Phanerochaete chrysosporium* has multiple LiP-encoding genes and secretes the enzyme as numerous isozymes (H1, H2, H6, H7, H8, and H10), where H8 has been well characterized. It is an extracellular globular glycoprotein of approximately 343 amino acid residues with a molecular size of 38–42 kDa. Lignin peroxidases have unique properties, such as a low optimum pH of 3.0–4.5 and a theoretical isoelectric point (pI) of 3.3–4.7, depending on the isozyme [6]. However, LiP of *P. chrysosporium* Karnataka India isolates was 55 kDa [7].

*Phanerochaete chrysosporium* ITB isolate is a group of white rot fungus owned by the Microbiology Laboratory of the Bandung Institute of Technology since 1999. Although this strain has been widely used for various studies, such as improving the quality of pulp from kapok wood [8] and improving the quality of animal feed from lignocellulosic waste [9-10], neither its ligninolytic enzyme, especially LiP, has been investigated. In fact, the application of LiP in various industrial fields is intensively being developed, including for delignification of feedstock for ethanol production, textile effluent treatment, dye decolorization, coal depolymerization, treatment of hyperpigmentation, skin-lightening through melanin oxidation and catalytic elimination of pharmaceutical and endocrine-disrupting compounds [11-12].

The phylogenetic analysis using the ITS sequence showed that *P. chrysosporium* ITB isolate had the highest similarity of 99.6% with *P. chrysosporium* BKM-F-1767, RP78, PV1, KCTC 6728, SF-4, ATCC MYA-476, FCL208, FCL236, and Gold-9-419-4 and it was suspected as a new strain of *P. chrysosporium* [13]. This was reinforced by the fact that the ability of this strain to produce MnP lowest than LiP even in specific media for production MnP whereas *P. chrysosporium* BKMF-1767 (ATCC 24725), which had been widely studied, can generate MnP with high activity. On the other side, it was also known that the highest specific activity of LiP from *P. chrysosporium* ITB isolate in modified Kirk's medium containing sawdust 1% (w/v), ammonium sulfate 20 mM, Tween-80 0.025%, and veratryl alcohol 300 ppm in aqua demineralized, cultivated to $1 \times 10^5$ spore/mL, and were grown at 37 °C and 50 rpm for five days was 77.4 ± 13.1 U/mg [14]. The activity was high enough that this isolate had a high potential to be used as a source of LiP. It was essential to observe and determine the characteristics of the LiP enzyme from *P. chrysosporium* ITB isolate. The characterizations included molecular weight, the pH and the optimum working temperature of the crude extract of the enzyme, the temperature stability, the effect of metal ions and inhibitors, their precipitation with ethanol, and the storage stability.

## ■ EXPERIMENTAL SECTION

### Materials

Chemicals used were of analytical grade, purchased from Merck including Tween-80, glasswool, $KH_2PO_4$, $MgSO_4{\cdot}7H_2O$, $CaCl_2$, $MgSO_4{\cdot}7H_2O$, NaCl, $FeSO_4{\cdot}7H_2O$, $CoCl_2$, $ZnSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$, $AlK(SO_4)_2{\cdot}12H_2O$, nitriloacetate, thiamine-HCl, $CH_3COOH$, $CH_3COONa$, $(NH_4)_2SO_4$, veratryl alcohol, Tween-80, HCl, KCl, citric acid, sodium citrate, Folin Ciocalteu, NaCl, $MnSO_4$, Pb-acetate, $ZnSO_4$, $Cd(NO_3)_2$, $CaCl_2$, $HgCl_2$, $Cr(NO_3)_3$, EDTA, $NaN_3$, ethanol absolute, bovine serum albumin (BSA), $Na_3C_6H_5O_7{\cdot}2H_2O$, sodium carbonate, NaOH, $K_2HPO_4$, and $KH_2PO_4$. Potato dextrose agar was purchased from Difco, while the demineralized water and sawdust were obtained from a local brand.

### Instrumentation

The equipment used in this study included glass apparatus, analytical balance (Precisa XT 120 A), thermo scientific water bath shaker incubator, vortex laboratory shaker (Dragon Lab MX-5), shaking water bath (Medfuture), soft incubator (Eyela SLI-600ND), drying oven (Memert UNB 400), autoclave (Tomy), universal indicator (Merck), amicon ultrafiltration, laminar airflow, microcentrifuge (Tomy MX-105), and magnetic stirrer (B -One). The used instrument for analysis was Vis 50 DA spectrophotometer.

### Procedure

#### *Production of crude extract of lignin peroxidase (LiP)*

The *P. chrysosporium* ITB isolate was obtained from the Culture Collection of Microbiology Laboratory of Institut Teknologi Bandung, Indonesia. This strain was purified with monospore technique on potato dextrose agar (Difco) and was cultured on potato dextrose refers to [14]. As many as $1 \times 10^6$ spores/mL of suspension spore were inoculated to the production medium of LiP, were modified from Kirk's Medium (Basal medium 1 X, sawdust 1%, trace element solution 1 X, Thiamine-HCl 0.01%, 20 mM acetate buffer pH 4.5, ammonium sulfate 20 mM, Tween-80 0.025%, and veratryl alcohol 300 ppm in aqua demineralized), were

grown at 37 °C and 50 rpm for 5 d. The mixture was centrifuged at 10000 rpm at 4 °C for 10 min. The filtrate obtained was the crude extract of the LiP enzyme and was determined for its protein level and LiP activity. The protein levels were determined by referring to the Lowry assay [14].

### *Lignin peroxidase assay*

Determination of the activity of the LiP enzyme in this experiment was carried out by a modified method referring to [3,14] as follows: 800 µL of 10 mM veratryl alcohol, 1,000 µL of 0.2 M tartaric acid, and 1,680 µL of aquaDM and 200 µL of enzyme solution were mixed in a test tube. The mixture was incubated for 2 min, and then the reaction was initiated by the addition of 320 µL $H_2O_2$ 5 mM. The absorbance was measured at a wavelength of 310 nm at the $0^{th}$ and $1^{st}$ min. The activity of the LiP enzyme in this experiment was calculated using Eq. (1).

$$\text{Activity of LiP (U/mL)} = \frac{(\text{At} - \text{Ao}) \times V_{total}\,(\text{mL}).10^6}{\varepsilon_{max} \times \text{d} \times V_{enzym}\,(\text{mL}) \times \text{t}} \quad (1)$$

where $\varepsilon_{max}$ = veraryl aldehyde molar extension coefficient (9300 $M^{-1}$ $cm^{-1}$), d = cuvette width (1 cm), $V_{total}$ = 4 mL, $V_{enzym}$ = 0.2 mL, t = 1 min

### *Characterization of LiP peroxidase from* Phanerochaete chrysosporium *ITB isolate*

The activity of the LiP enzyme was determined at various pH by replacing the tartaric acid in the LiP assay with 0.2 M HCl-KCl buffer (pH = 1 and 2), 0.2 M citrate buffer (pH = 3), 0.2 M acetate buffer (pH = 4 and 5), 0.2 M phosphate buffer (pH = 6 and 7).

The temperature was optimized at, 32, 38, 50 and 58 °C, whereas the other conditions were remained the same.

The thermal stability assay of LiP was carried out by incubating the crude extract of LiP for 0, 20, 24, and 30 h at various temperatures.

The effect of metal ions and inhibitor was observed by measuring the LiP activity in the reaction mixture containing 10 mM metal ions. The tested inhibitor that were $K^+$, $Na^+$, $Mn^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Mg^{2+}$, $Cd^{2+}$, $Ca^{2+}$, $Hg^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Fe^{2+}$, $Cr^{3+}$, EDTA and $NaN_3$. The enzymes that were not added with metal ions and inhibitors were used as controls.

The thermal stability of the LiP enzyme was expressed as the relative activity of LiP or the activity remaining after incubation for the n th hour according to Eq. (2).

$$\text{Relative activity of LiP (\%)} = \frac{\text{LiP activity at n} - \text{hour}}{\text{LiP activity at 0}} \times 100\% \quad (2)$$

The effect of metal ions and inhibitors on the activity of the LiP enzyme was expressed as the relative activity of LiP which was determined using Eq. (3).

$$\text{Relative activity LiP (\%)} = \frac{\text{Activity of LiP with addition compounds}}{\text{Activity LiP as control}} \times 100\% \quad (3)$$

The crude extract was precipitated by adding ethanol until the ethanol saturation was 33, 50, 55, 60, 64, and 67%. The mixture was stored in the refrigerator (4 °C) overnight. The mixture was centrifuged at 0 °C at 10000 rpm for 30 min. The pellets obtained were air-dried at room temperature for 2 h and resuspended in 1.5 mL acetate buffer pH 4.5 for approximately 3 h. Each measured protein content, LiP activity, and storage stability for 0, 2, 9, 16, and 23 days at 0 °C. The protein profile of each treatment was traced by SDS-PAGE to identify the molecular weight of LiP.

### *Data analysis*

All treatments were repeated in triplicate. The results were expressed as mean ± standard deviation of three repetitions. The statistical analysis was conducted using the Statistical Package for The Social Science (SPSS) v.16 software. The one-way analysis of variance (ANOVA) followed by Tukey's HSD test was used to determine the real difference from each variation. The differences at $p < 0.05$ were considered statistically significant.

## ■ RESULTS AND DISCUSSION

### Optimum pH of LiP from *P. chrysosporium* ITB Isolate

The results showed that the LiP of *P. chrysosporium* ITB isolate was affected by the pH of the reaction (the farther away the alphabetical differences are, the more significant the difference due to the treatment). At very acidic pH (pH 1.0), the activity was low. The activity was

increased with increasing of the reaction pH until it reached pH 5.0. However, the LiP activity dramatically decreased at pH 6, and no LiP enzyme activity was detected at pH = 7 and 8 (Fig. 1). The optimum pH value for the oxidation reaction of veratryl alcohol by LiP from *P. chrysosporium* ITB isolate was in the pH range of 3–5 because the high activity of the LiP enzyme at this pH range did not statistically differ. These results were in line with previous studies, which stated that the most dominant LiP isoenzyme, LiPH8, from *P. chrysosporium* BKMF-1767 wildtype, had the highest catalytic ability at pH 3.0, and low at pH < 3.0, but differed at pH 4.0 and 5.0 [15]. In that study, the activity of LiPH8 at pH 4.0 and 5.0 tended to decrease although was still considered stable, while in this study, it tended to increase. This study was in line with the purified LiP of immobilized *P. chrysosporium* Indian Karnataka isolate, which has high activity at pH 3.0 and was still stable up to pH 5.0, then decreased at high pH [7]. The high activity of LiP at low pH was in line with the computational studies showing that at low pH, the heme redox potential value of LiP was higher so that LiP can oxidize veratryl alcohol at that pH [15]. Based on this comparison, LiP from *P. chrysosporium* ITB isolates offered the advantage that the crude extract had a fairly wide pH working range of 3.0 to 5.0.

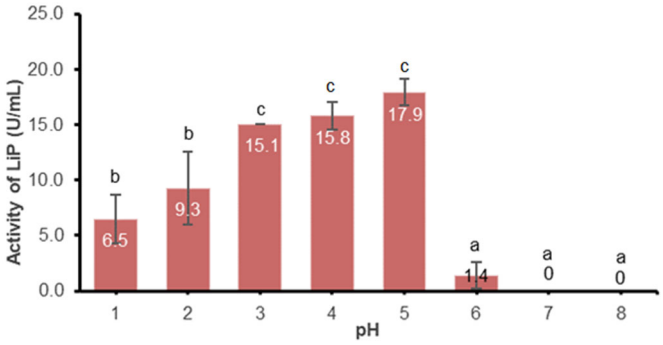


**Fig 1.** LiP activity at various pH of enzymatic reactions (the farther away the alphabetical differences are, the more significant the difference due to the treatment)

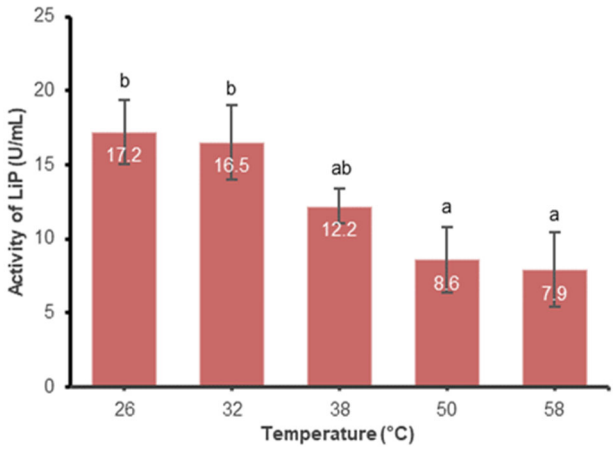


**Fig 2.** LiP activity at various temperatures of enzymatic reactions

## The Optimum Temperature of LiP from *P. chrysosporium* ITB Isolate

Temperature is a factor that greatly affects enzyme activity. Certain temperatures (generally more than 40 °C) can change the conformation of the three-dimensional structure of the enzyme because the heat breaks some of the noncovalent bonds that stabilize the three-dimensional structure of the enzyme [16]. The LiP enzyme from *P. chrysosporium* ITB isolates had high activity and did not differ significantly at 26 and 32 °C, while at temperatures higher than 32 °C the activity of the LiP enzyme decreased, as shown in Fig. 2. These results were not in line with the optimum temperature of LiP production [14]. The production of LiP at 37 °C was much higher than at 30 °C.

On the contrary, these results showed that the activity of the LiP enzyme decreased at 38 °C, indicating that the growth temperature for LiP enzyme production did not automatically reflect the optimum reaction temperature carried out by the LiP enzyme. However, the results of this study were in line with the optimum reaction temperature of LiP enzymes from various other fungi, namely *P. chrysosporium* ATCC-24725 (26 °C), *Penicillium citrinum* (30 °C), *Gloeophyllum sepiarium* (25 °C), and *Pycnoporus sanguineus* (25 °C) as summarized in [17], also in line with [2] and [7] which stated that the LiP enzyme from *P. chrysosporium* BKM-F-1767 and *P. chrysosporium* Karnataka India isolate had optimum activity at 30 °C.

### Thermal Stability of LiP from *P. chrysosporium* ITB Isolate

The thermal stability profile of LiP from *P. chrysosporium* ITB isolate was shown in Fig. 3. The LiP enzyme was stable at 26 and 32 °C after 20 h of incubation and decreased slightly after 24 and 30 h of incubation. The thermal stability of the enzyme at a temperature of more than 32 °C decreased by varying degrees after 20, 24, and 30 h of incubation. The LiP enzyme activity after incubation for 20, 24, and 30 h at the highest temperature of 58 °C still displayed the activity of 58, 53, and 42%, respectively, indicating that LiP from *P. chrysosporium* isolate ITB was a moderate thermostable enzyme. The results were better than the LiP enzyme from *P. chrysosporium* BKMF-1767, which had an activity of only 38% after 24 h of incubation at 60 °C [2], and much better than the purified LiP from *P. pulmonarius*, where the activity became 25 and 20% after 2 h incubation at 40 and 60 °C [18].

### Effect of Metal Ions and Inhibitors on LiP from *P. chrysosporium* ITB Isolates

Lignin peroxidase (LiP) is expected to be applied for the bioremediation of xenobiotic compounds in the environment. The waste in the environment certainly contains various metal ions and some enzyme inhibitors. Actually, it is necessary to have information about the effect of these compounds, which may also be present in the waste, on LiP activity. The results obtained indicated that the data distribution was not normal because the significance value is < 0.05. Therefore, the Kruskal Wallis test was performed. The results of the analysis showed that the addition of metal ions and inhibitors affected the decolorization ability of the LiP enzyme. The $Pb^{2+}$ ion eliminated the activity of the LiP enzyme; EDTA, $Na^{+}$, $Cr^{3+}$, and $Hg^{2+}$ ions decreased LiP enzyme activity to less than 40%; $K^{+}$, $Co^{2+}$, $Fe^{2+}$, $Cd^{2+}$, $Mg^{2+}$, and $Cu^{2+}$ ions decreased the activity of the LiP enzyme to 78–90%; $Mn^{2+}$ and $Zn^{2+}$ ions did not affect LiP enzyme activity, while $Ni^{2+}$, $NaN_3$, and $Ca^{2+}$ ions increased LiP enzyme activity (Fig. 4). These results were similar to those [2] in the case that $Ca^{2+}$ ions increased LiP activity and $Hg^{2+}$, $K^{+}$, $Fe^{2+}$, and $Mg^{2+}$ ions decreased LiP activity, while $Na^{+}$ and $Co^{2+}$ ions in this study increased LiP activity. These results were also in line with [18] in case of $K^{+}$, $Na^{+}$, $Hg^{2+}$, $Mn^{2+}$, and $Zn^{2+}$ ions, but opposite to the effect of $Fe^{2+}$, EDTA, $Cu^{2+}$, and $Mg^{2+}$ [18]. These results were presumably because the LiP isoenzyme produced in this experiment was different from the LiP isoenzyme. The LiP enzyme had a protoporphyrin heme group at its active center, so it was suspected that the presence of other metal ions affected the stability of the $Fe^{2+}$ ion in the heme group. The stability of the heme group greatly affected the activity of LiP.

### Fractionation Profile of Crude Extract of LiP Enzyme from *P. chrysosporium* ITB Isolate with Ethanol Precipitation

Although the crude extract of the LiP enzyme has a relatively high specific activity (± 77 U/mg) but the protein content is very low (± 150 ug/mL). Concentration

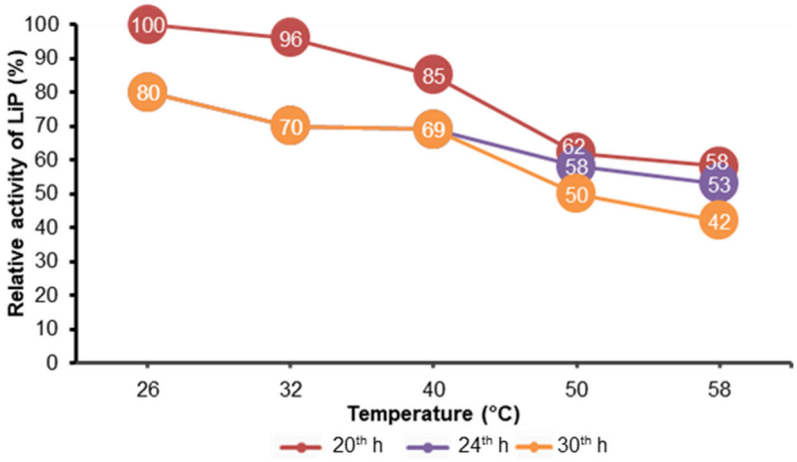


**Fig 3.** Thermal stability of LiP from *P. chrysosporium* ITB isolate

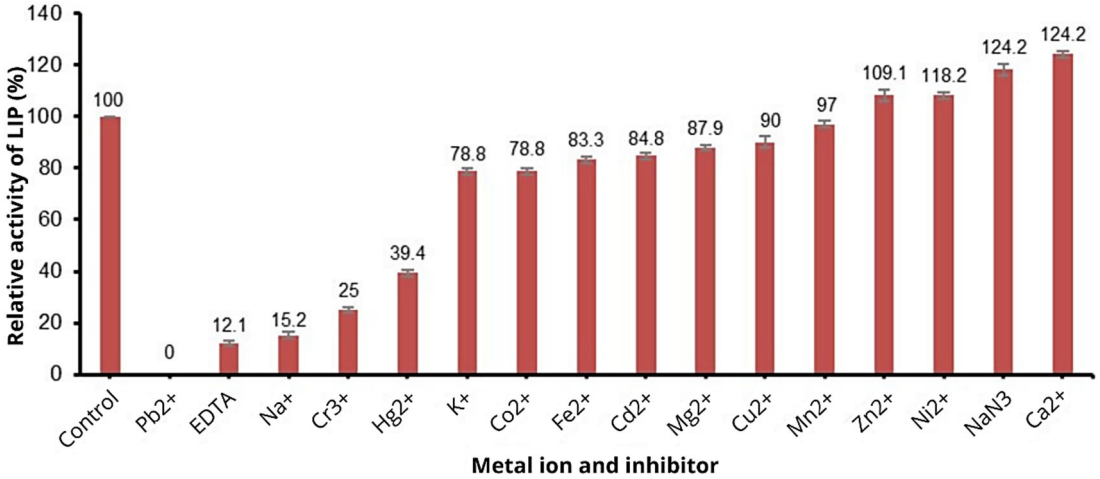


**Fig 4.** The effect of metals ion and inhibitor on LiP of *P. chrysosporium* ITB isolate

of the sample will reduce the volume of the sample, making it easier for packaging and storage. Concentration is the first step in enzyme purification as an effort to increase enzyme activity and stability, and it is also required for molecular analysis of enzymes such as SDS-PAGE. The addition of organic solvents will reduce the interaction between proteins and water molecules because water will immediately surround the organic solvent molecules through hydrogen bonds. This situation encourages electrostatic interactions between protein molecules so that they form aggregations and precipitate [19].

In this study, it appeared that the concentration of the added ethanol affected the deposition of the LiP, which was indicated by changes in the specific activity value of LiP produced at each concentration of ethanol used (Fig. 5). The greater the concentration of ethanol added, the higher the specific activity of LiP produced because more LiP enzymes were deposited. Although the use of ethanol at concentrations of 55, 60, 64, and 67% did not statistically and significantly affected the value of specific LiP activity, the use of 64% ethanol produced the highest specific activity value of LiP. Under these conditions, from five milliliters of the sample, which has a specific activity of 48.36 U/mg, precipitated with 64% ethanol, then the precipitate obtained was resuspended in 1.5 mL of acetate buffer pH 5.0, the specific activity of LiP was 112.82 U/mg on average. It indicated that there was a volume concentration of 3.3 times and an increase in specific activity of 2.3 times.

The storage stability test was carried out on three samples of LiP enzyme, namely LiP enzyme in the form

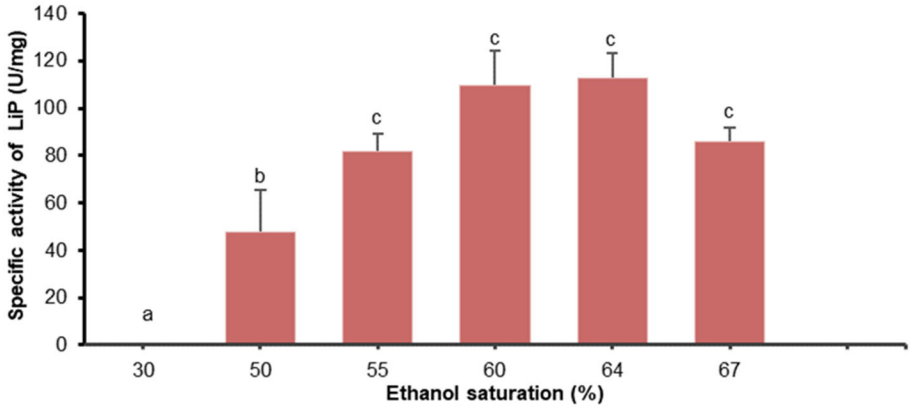


**Fig 5.** Effect of ethanol concentration on crude extract fractionation of LiP with ethanol precipitation method

of crude extract of LiP, resuspension of ethanol fractionated precipitate, and ethanol fractionated precipitate, which were stored at 0 °C. On day of 0, 2, 9, 16, and 23, each sample was placed at room temperature, allowed to melt, and the activity of the LiP enzyme was measured. The timing was chosen to observe the stability of the enzyme in short (2 d), medium (8 and 16 d), and long-term (23 d) storage. So far, no similar research had been found, whereas for large-scale applications, information on enzyme stability during storage was very necessary. Fig. 6 shows the crude extract has the best enzyme stability compared to the precipitate resulting from the fractionation of ethanol and the resuspension of the precipitate from the fractionated ethanol. The crude extract had LiP enzyme activity which remained high until the 23$^{rd}$ day. At the same time, the ethanol fractionated precipitate started to gradually decrease since the 9$^{th}$ day, and the resuspension of the ethanol fractionated precipitate experienced a sharp decrease in LiP enzyme activity since the 2$^{nd}$ day and lost its activity on the 16$^{th}$ day of storage. The decreased activity of the LiP in the ethanol fractionated precipitate and the loss of LiP enzyme activity in the resuspension of the ethanol fractionated precipitate during storage at 0 °C were unexpected results. This was presumably because, during the deposition process with ethanol, there was a change in the dielectric constant of the medium, which led to changes in protein conformation so that the structure was less stable. Before being stored, the precipitate resuspended in acetate buffer pH 5.0 might experience a change in the dielectric constant of the medium. It allowed a change in protein conformation again so that when the sample was thawed or subjected to freeze-thaw, it triggers denaturation so that the enzymes in the sample lose their activity. Buffering in protein solution will result in dramatic changes in pH during the freezing process, which can result in protein denaturation [20]. The LiP enzyme in the precipitate resuspended in acetate buffer pH 5.0 was not only denatured due to conformational changes due to changes in the dielectric constant of the medium but it was also thought to be due to drastic changes in pH during freezing so that there was no LiP activity after 16 d of storage. On the other hand, the crude enzyme extract sample remained stable because denaturation did not occur before and during the freezing process at 0 °C, so when the sample was thawed, the LiP activity remained high. Therefore, it was suggested that it was not necessary to concentrate the sample with ethanol precipitation methods, to maintain the stability and activity of the crude extract of the LiP from *P. chrysosporium* ITB isolates. However, this suggestion should be further studied, considering that the higher the concentration of purified protein, the higher its stability of protein. Unfortunately, there was no similar research that can be used as a comparison. Recent studies have used the filtration method at the

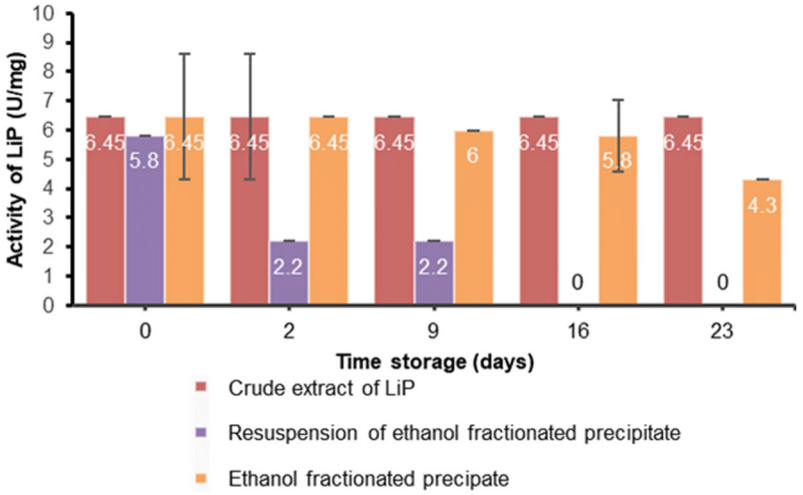


**Fig 6.** Stability of the storage of LiP

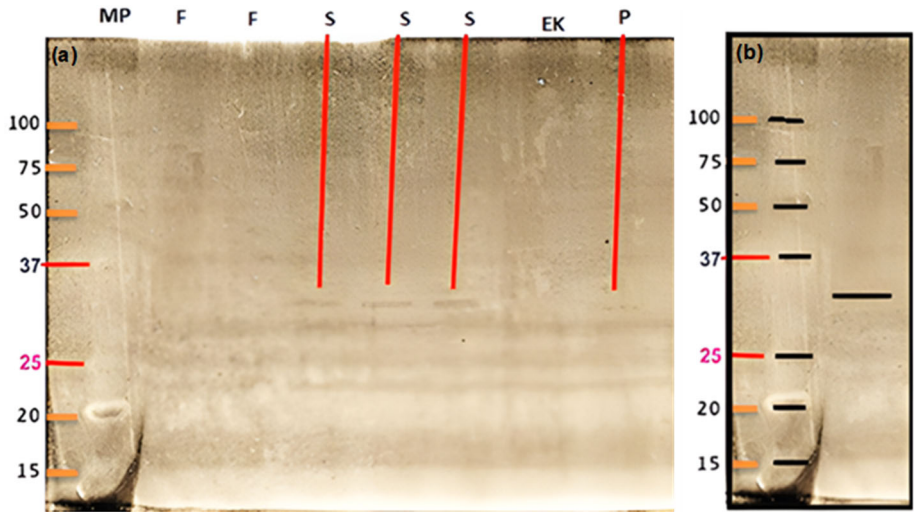


**Fig 7.** SDS-PAGE profile of LiP enzymes from *P. chrysosporium* ITB isolate. Electropherogram photo from SDS-PAGE with MP: standard protein, F: filtrate before ultrafiltration, S: filtrate from ultrafiltration, EK: crude extract of LiP enzyme, P: result of concentration with ethanol fraction (a). Illustration of LiP enzyme band versus standard protein band (b)

stage of concentration of crude LiP extract [18]. The use of this method minimized the occurrence of denaturation due to changes in the dielectric constant of the medium as happened in this study. But the ultrafiltration technique was still quite expensive for large-scale enzyme purification.

### The Molecular Size of LiP from *P. chrysosporium* ITB Isolate

Electropherogram of SDS-PAGE shows that the MP well (which were the standard protein bands used, ranging from 15 kDa to 100 kDa), the F well (containing the filtrate before ultrafiltration) and the EK well (containing the crude extract of the LiP enzyme) did not show any bands, while the S well (containing the filtrate from ultrafiltration) and the P well (as the result of concentration by ethanol fractionation) showed a thin band measuring ± 34 kDa. This band was suspected to be the LiP enzyme because both the filtrate and the ethanol fractionation results showed LiP activity. Although the crude extract measured the activity of the LiP enzyme, there was no visible bands because the concentration was too small. These results were slightly different from previous studies. The pure LiP enzymes from *P. chrysosporium* ATCC 20696, namely LiP1 and LiP2 measuring 38 and 40 kDa [21], displayed three bands measuring between 35–45 kD in the extracellular protein of *P. chrysosporium* RP28, which was thought to be an H1-ligninase isoenzyme. The H5 LiP isoenzyme [22] and the pure LiP enzyme from *P. chrysosporium* BKMF-1767 measuring at 36 kDa [2]. This confirmed that *P. chrysosporium* isolate ITB was a different strain from the previous *P. chrysosporium* strains, namely *P. chrysosporium* ATCC 20696, *P. chrysosporium* RP28, and *P. chrysosporium* BKMF-1767.

## ■ CONCLUSION

The LiP was suspected to be a LiP isoenzyme with a molecular weight of 34 kDa. The crude extract of LiP displayed high activity at pH 3–5, at 26–32 °C, had good thermal stability at 26–32 °C as long as 20 h. The activity was lost due to the presence of $Pb^{2+}$ ion, decreased to 78–90% due to $K^{+}$, $Co^{2+}$, $Fe^{2+}$, $Cd^{2+}$, $Mg^{2+}$, and $Cu^{2+}$ ions, decreased to less than 40% due to EDTA, $Na^{+}$, $Cr^{3+}$, and $Hg^{2+}$ ions, was not affected by $Mn^{2+}$ and $Zn^{2+}$ ions, but increased in the presence of $NaN_3$, $Ni^{2+}$, and $Ca^{2+}$ ions. The crude extract precipitated with the optimum ethanol at 64% ethanol saturation which resulted in an increase in specific activity of 2.3 times. The storage of the crude at 0 °C was more stable than the precipitate resulting from ethanol precipitation and resuspension from ethanol precipitation. With these characteristics, the crude extract of LiP from *P. chrysosporium* ITB isolate has good potential to be used as industrial enzymes.

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## ■ AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception, design, data collection and draft manuscript preparation: ES; analysis and interpretation of data: ES and A. All authors reviewed the results and approved the final version of the manuscript.

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