ABSTRACT

Amauroderma rugosum is a wild mushroom spesies widely distributed in tropics and is classified under class Basidiomycetes. The class Basidiomycetes is well-known for their abilities of producing lignocellulolytic enzymes such as lignin peroxidase (LiP), laccase (Lac) and manganese peroxidase (MnP). The *Amauroderma rugosum* produce lignin peroxidase in the medium supplemented with potato dextrose broth (PDB), 0.5 % (w/v) of yeast, 1 % (w/v) of saw dust and 150 µM (w/v) of copper with shaking speed of 120 rpm and 14 days duration gave the lignin peroxidase (LiP) activity of 105.68 ±5.87 U/ml (w/v). The utilization of the agricultural by-products (rubber wood saw dust) successfully induced a higher production of lignin peroxidase (LiP). The aim of this study was the primary extraction and recovery of lignin peroxidase (LiP) from *Amauroderma rugosum* using the rapid and low cost aqueous two phase system. There were a total of 25 systems from five PEG molecular weights; PEG600; PEG 1000, PEG 1500, PEG 3350 and PEG 8000 being tested. The system two of PEG 600 consisted 15% (w/v) polyethylene glycol (PEG) and 16 % (w/v) phosphate salts and tie-lie line 34.10 showed an increase of the top phase lignin peroxidase (LiP) activity to 89.29±19.35 U/ml (w/v) from the crude enzyme activity of 85.37±2.22 U/ml (w/v) . This system also gave the highest top phase purification factor (P_{FT}) of 1.33±0.62 with a yield of 72.18±8.50 % (w/v). In overall, the volume ratio of 0.62, pH 8.0 and 10 % (w/v) crude enzyme adding were able to produce the optimum result. The addition of salt to the system gave no significant difference to the purification factor. The optimized ATPs parameters gave the optimum top phase purification factor (P_{FT}) and yield of 6.25±3.21 and 81.08±4.92% (w/v) respectively. Lastly, SDS-PAGE and native-PAGE analysis of the purified lignin peroxidase from ATPS showed three distinctive bands sizes which were apparently 38 kDa,

45-46 kDa and 66kDa. The lignin peroxidase with band size of 66 kDa was never being reported in mushroom species to-date and this induced more interest to further investigate on this enzyme. In overall, the ATPs system was proven to be a promising method for primary extraction and purification of enzyme.

ABSTRAK

Amauroderma rugosum merupakan spesies cendawan asli yang tersebar luas di kawasan beriklim tropika dan diklasifikasikan sebagai kelas Basidiomycetes. Kelas Basidiomycetes terkenal dengan keupayaannya untuk menghasilkan enzim-enzim lignocellulolytic seperti lignin peroxidase (LiP), laccase (Lac) dan manganese peroxidase (MnP). *Amauroderma rugosum* menghasilkan lignin peroxidase dengan medium yang diperkayakan dengan potato dextrose broth (PDB), 0.5 % (w/v) yis, 1 % (w/v) habuk kayu getah dan 150 µM (w/v) dengan pengocokan sebanyak 120 rpm selama 14 hari mencatatkan aktiviti lignin peroxidase (LiP) sebanyak 105.68±5.87 U/ml (w/v). Penggunaan bahan sampingan agro(habuk kayu getah) berjaya menghasilkan enzim lignin peroxidase (LiP) yang tinggi. Matlamat penyelidikan ini bertujuan untuk menggunakan aqueous two phase system yang merupakan teknik yang pantas dan berkos rendah untuk mengekstrak dan menulenkan lignin peroxidase (LiP) daripada *Amauroderma rugosum.* Sejumlah 25 sistem daripada lima PEG; PEG600; PEG 1000, PEG 1500, PEG 3350 and PEG 8000 telah dikaji. Sistem dua bagi PEG 600 yang terdiri daripada 15% (w/v) polyethylene glycol (PEG) dan 16 % (w/v) phosphate salts and tie-lie line 34.10 menunjukkan peningkatan jumlah enzim lignin peroxidase (LiP) di fasa teratas kepada 89.29±19.35 U/ml (w/v) berbanding dengan bacaan awal bagi enzim mentah yang mencatatkan jumlah sebanyak 85.37±2.22 U/ml (w/v). Sistem ini juga berjaya menghasilkan faktor penulenan fasa teratas (P_{FT}) sebanyak 1.33±0.62 dengan hasil 72.18±8.50 % (w/v). Secara keseluruhan, nisbah jumlah sebanyak 0.62, pH8.0 dan 10% (w/v) penambahan enzim mentah mampu menghasilkan keputusan yang optimum. Penambahan garam NaCl didapati tidak memberi kesan terhadap faktor penulenan. Parameter sistem dua fasa air (ATPs) yang optimum ini mencatatkan faktor penulenan

fasa teratas (P_{FT}) dan hasil sebanyak 6.25 ± 3.21 dan 81.08 ± 4.92 % (w/v) secara respektif. Analisasi secara SDS-PAGE dan native-PAGE bagi lignin peroxidase tulen selepas proses penulenan mengaplikasikan teknik sistem dua fasa air (ATPs) memberikan tiga band iaitu 38 kDa, 45-46 kDa dan 66 kDa. Lignin peroxidase yang bersize 66 kDa tidak pernah dilaporkan di mana mana spesies cendawan dan mencungkil minat untuk terus menerokai enzim ini. Secara keseluruhan, sistem dua fasa air (ATPs) telah terbukti sebagai kaedah yang berkesan dalam pengekstrakan utama dan purifikasi enzim.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation and symbol	Full Title
ATPs	Aqueous two-phase system
β-mercapthoethanol	Beta-mercapthoethanol
PEG	Polyethylene glycol
TLL	Tie lie line
K_2HPO_4	Di-potassium hydrogen phosphate
KH_2PO_4	Potassium di-hydrogen phosphate
Mw	Molecular weight
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel
PDA	Potato dextrose agar
PDB	Potato dextrose broth
P_{FT}	Top phase purification factor
V_R	Volume ratio
K	Partition coefficient
U/ml	Unit/millimeter
U/mg	Unit/milligram
LiP	Lignin peroxidase
Lac	Laccase
MnP	Manganese peroxidase
H_2O_2	Hydrogen peroxide