Churapa Teerapatsakul 2007: Physiological Aspects of Lignin-Degrading Enzymes from a Macrofungus Isolated in Thailand. Doctor of Philosophy (Microbiology), Major Field: Microbiology, Department of Microbiology. Thesis Advisor: Associate Professor Lerluck Chitradon, Dr.Agr. 268 pages.

Physiological aspects of lignin-degrading enzymes, in particular laccase, were studied from a new isolated Polyporaceae designated as KU-Alk4. Molecular taxonomy and morphological studies indicated that KU-Alk4 is a new species of *Ganoderma*. Regulation of laccase of KU-Alk4 was induction and catabolite repression controlled. Types of carbon source and inducers, concentration of both and Cu^{2+}, pH and aeration affected the enzyme production. In the medium without pH control, the fungus produced laccase and MnP at initial pH 4.5 and 7.0 but only laccase at pH 8.0. The highest laccase, 218 IU/mg CDW or 328 IU/mL, was in pH 8.0 medium with 1% glucose, 0.2 mM $CuSO_4$, 0.85 mM veratryl alcohol and shaking at 120 rpm. It was 16 times higher than the basal medium. In the medium with pH control, a seven-level Box–Behnken factorial design was used to engineer the optimal medium. The optimized medium was predicted and proved to maintain its pH at 6.0 with the compositions of 40 g/L glycerol, 0.02 mM $CuSO_4$ and 0.85 mM veratryl alcohol. The correlation of the experiments to the statistical design was 98%. Laccase achieved from the statistical design was 240 IU/mL that was 12 times improved. At pH 8.0, 2 isozymes, KULac 1 and 2 were produced with 1% glucose, while 3 different isozymes, KULac 3, 4 and 5, were produced with 4% glucose. The KULacs differed in molecular mass, 53-112 kDa. KULac 3 had the extremely high optimum temperature, 90ºC, and stable to high temperature of 60°C for 1 h. Those of the other KULacs were, relatively high, 55-70°C and stable at 40-45°C. All KULacs were 80-100% stable to pH 3.0-10.0 for 1 h. KULac 2 was a new laccase according to its novel N-terminal amino acid sequence.

The free laccase of *Ganoderma* sp. KU-Alk4 degraded various phenolic compounds including dyes. A novel immobilization system was developed, for Indigo Carmine decolorization, using Latin Square design. Maximal dye decolorization was achieved using Cu-alginate immobilized enzyme. The statistical and experimental designs suggested the best immobilized conditions with 3.6% w/v low mannuronate alginate and 0.15 M $CuSO_4$. The immobilized laccase, $6x10^4$ IU, in a 5 L-airlift bioreactor with 4 L/min airflow rate provided the most efficiency by 100% dye decolorization of 14 successive batch runs. The system was non-bufferized. Total dye removed was 1.8 g in 20 days. This is the first description of the development of an effective method for the immobilization of laccase for use in dye decolorization.

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