Applications of lignin-degrading enzymes

Lignin-degrading enzymes have showed their potential in various applications, ranging from effluent decoloration and detoxification to pulp bleaching, removal of phenols from wines and dye, transfer blocking functions in detergents and washing powders, many of which have been patented. The enzymes from fungi are of interest in synthetic chemistry and medicine, oxidizing steroid hormones and transforming antibiotics and alkaloids. The importance of these enzymes for biotechnology, results also from their considerable retention of activity in organic solvents with applications in organic synthesis, and their participation in biosensors and immunoenzyme assay. The examples of some applications are discussed below:

1. Application in textile industry

The textile industry accounts for two-thirds of the total dyestuff market (Riu *et al*., 1998) and consumes large volumes of water and chemicals for wet processing of textiles. The chemical reagents used are very diverse in chemical composition, ranging from inorganic compounds to polymers and organic products (Mishra and Tripathy, 1993; Banat *et al*., 1996; Juang *et al*., 1996). There are more than 100,000 commercially available dyes with over 7×10^5 ton of dyestuff produced annually (Meyer, 1981; Zollinger, 1987). Due to their chemical structure dyes are resistant to fading on exposure to light, water and different chemicals (Poots and McKay, 1976; McKay, 1979) and most of them are difficult to decolourise due to their synthetic origin.

Government legislation is becoming more and more stringent, especially in the more developed countries, regarding the removal of dyes from industrial effluents (O'Neill *et al*., 1999). Concern arises, as several dyes are made from known carcinogens such as benzidine and other aromatic compounds (Baughman and Perenich, 1988). Most currently existing processes to treat dye wastewater are ineffective and not economical

(Cooper, 1995; Stephen, 1995). Therefore, the development of processes based on laccases seems an attractive solution due to their potential in degrading dyes of diverse chemical structure (Abadulla *et al*., 2000; Blánquez *et al*., 2004; Hou *et al*., 2004), including synthetic dyes currently employed in the industry (Couto *et al*., 2005).

2. Application in organic synthesis

The capability of laccase to catalyze the oxidation of various compounds makes its applications promising in organic synthesis. An example of laccase application is the biotransformation of resveratrol which is a compound from plant. Laccase plays a role in prevention of carcinogenesis. Those laccases are from *Myceliophtora thermophyla* and *Trametes pubescens* which generated trans-dehydrodimers or resveratrol dimer. Wide-array of biological activities such as antimicrobial, anti-HIV, anti-inflammatory, exhibited from resveratrol oligomers, the synthesized compounds of which can lead to develop new drugs and nutraceuticals (Nicotra *et al*., 2004)

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Milstein and co-workers attempted to utilize laccase for the transformation of aromatic and lignin-related compounds in organic solvents (Milstein *et al*., 1992). They utilized free and immobilized *Trametes versicolor* laccase in a two phase system in which most of the water was replaced with n-hexane, acetonitrile, or dioxane. They demonstrated that the enzyme in either free or immobilized form was able to convert aromatic or lignin-related compounds in organic solvents in the presence of sufficient amount of water.

3. Application in pulp and paper industry

Traditionally, the lignin-degrading enzymes can remove residuals of lignin from pulp and paper. The residual lignin from the pulp was removed using alkaline and chloride, the process of which produced waste to environment. Biological delignification with lignin-degrading enzymes has been approached. Treatment of wood pulp with the extracted lignin-degrading enzymes from *Trametes versicolor* (Paice *et al*., 1993) or Polyporaceae strain (Poonpairoj *et al*., 2001a, b) increased brightness of pulp.

4. Application in food industry

Phenol-removal activity of lignin-degrading enzymes has been proved to be useful in the production and treatment of beverages includes wine, fruit juice and beer. Several phenolic compounds (coumaric acids, flavans and anthocyanins) usually are present in these beverages and may, during their shelf-life, cause undesirable and deleterious changes. Laccase was an effective stabilizer of wine, apple and parry pear juices (Minussi *et al*., 2002; Gianfreda, 1999).

5. Soil bioremediation

Polycyclic aromatic hydrocarbons (PAHs) together with other xenobiotics are a major source of contamination in soil, therefore, their degradation is of great importance for the environment. The catalytic properties of laccases can be used to degrade such compounds. Thus, laccases were able to mediate the coupling of reduced 2,4,6-trinitrotoluene (TNT) metabolites to an organic soil matrix, which resulted in detoxification of the munition residue (Durán and Esposito, 2000). Moreover, PAHs, which arise from natural oil deposits and utilization of fossil fuels, were also found to be degraded by laccases (Pointing, 2001). Recently, Nyanhongo *et al*. (2006) showed that a

laccase from *Trametes modesta* was involved in immobilisation of TNT degradation products.

The use of lignin-degrading enzymes is promising with a great potential application in several areas. The use of this enzyme could improve productivity, efficiency and quality of products without high investment costs and has advantage of being a mild technology. Studies on lignin-degrading enzymes production, purification and immobilization techniques at lower costs (Table 11) are also needed to improve the industrial applications of this enzyme.

***Ganoderma* sp. KU-Alk4**

As commonly known that the paper making process, especially pulping and bleaching, causes environmental pollution, therefore, biotechnology is concerned as one of the way to improve not only the paper quality but also environmental management. Poonpairoj *et al*. (2001) used the lignin-degrading enzymes from an isolated fungus in Thailand for biopulping of paper mulberry. Eighty-four samples of mushroom and filamentous fungi were collected from various sources in Thailand in order to isolate lignin degradable strains by screening on wood meal agar. From the eighty-four samples of mushroom and filamentous fungi, a strain of Polyporaceae designated as KU-Alk4 was selected according to its high activities of lignin-degrading enzymes in Kirk's liquid medium (Tien and Kirk, 1988).

Table 11 Some prices of commercially available lignin-degrading enzymes

	Quantity (Units)	Price
(1) Julich Fine Chemicals		
(www.juelich-chemicals.com)		
Lacc from *Agaricus bisporus*	10 KU	305.00 (US$)
	100 KU	1,560.00 (US$)
Lacc from *Coriolus versicolor*	10 KU	250.00 (US$)
	100 KU	1,290.00 (US$)
(2) Tienzyme™		
(www.tienzyme.com)		
Lacc from *Pleurotos ostreatus*	10,000 U (concentrate)	150.00 (US$)
	10,000 U (purified)	400.00 (US$)
	100.000 U (concentrate)	650.00 (US$)
	100.000 (purified)	1,600.00 (US$)
MnP from *Phanerochaete chrysosporium*	100 U	75 (US$)
LiP from *Phanerochaete chrysosporium*	100 U	100 (US$)
(3) Sigma-Aldrich		
(www.sigma-aldrich.com)		
Lacc From *Rhus vernificera*	10,000 U	72.30 (US$)
Lacc From *Agaricus bisporus* (≥1.5 U/mg)	1 g	30.50 (US$)
	5 g	120.90 (US$)
Lacc From *Coriolus versicolor* (≥1 U/mg)	1 g	44.00 (US$)
	10 g	358.20 (US$)

U = Units

Source: Minussi *et al*. (2002)

KU-Alk4 was isolated from a living tree, *Terminalia bellerica* Roxb., at Kasetsart University, Thailand. Morphological characterization of the fruiting body was carried out for strain identification (Figure 8A). The fruiting body was found to grow out laterally like a shelf on the bark of tree. The dorsal surface showed numerous pores. Hence, it was concluded that the strain KU-Alk4 belongs to family Polyporaceae. The fruiting body was fleshy, leathery and the surface had a hard crust formed by thick walled elongated cells. All the observations suggest that the strain KU-Alk4 belongs to genus *Ganoderma*.

Colony of *Ganoderma* sp. KU-Alk4 on potato dextrose agar (PDA) was a white mycelium (Figure 8B). The fungus could grow on wood meal agar containing guaiacol, lignin related compound, used for screening of lignin-degrading enzymes. Positive result with red zone on wood meal-guaiacol agar that showed guaiacol-degradable ability of *Ganoderma* sp. KU-Alk4.

Lignin-degrading enzymes from *Ganoderma* sp. KU-Alk4 showed success in removing lignin from paper mulberry bark in pulp process prior alkaline used and decreasing alkaline concentration in chemical process. This leads to efficiency improvement of pulping process that far better to the environment.

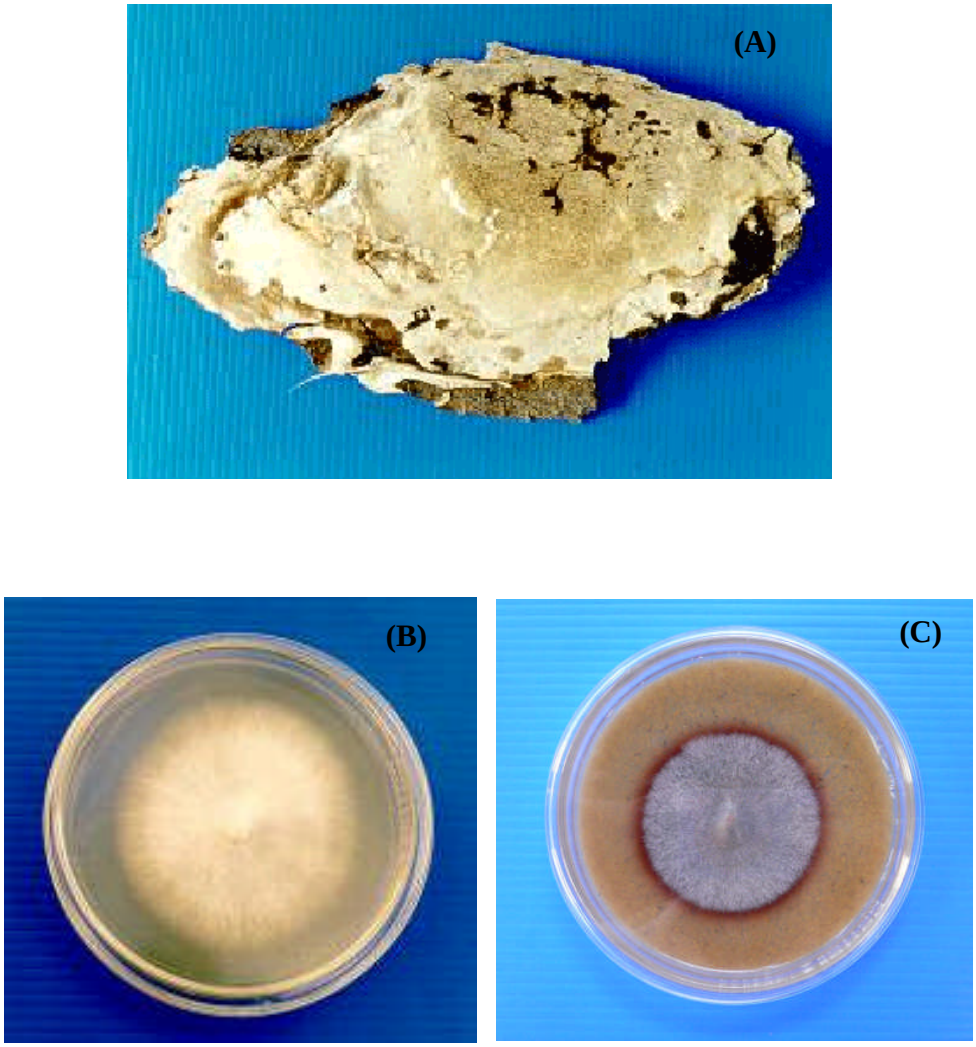

Figure 8 *Ganoderma* sp. KU-Alk4. (A), fruiting body in nature. (B), colony on potato dextrose agar. (C), colony on wood meal-guaiacol agar.