EVALUATION OF THE CAPABILITIES OF SEVERAL REDOX MEDIATORS TO PROMOTE LACCASE-MEDIATED OXIDATION OF XENOBIOTIC PHENOLIC COMPOUNDS

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Abstract

Purified laccase from *Ganoderma lucidum* KMK2 oxidized several xenobiotic phenolic compounds. The reaction rates of laccase were dramatically increased by synthetic mediators such as ABTS and HBT. In addition, naturally occurring redox mediator screening was performed to find cheaper and eco-friendly mediators. Syringaldehyde showed the strongest effect whereas *p*-coumaric acid slightly inhibited laccase oxidation activity. These findings suggest the capabilities of natural redox mediators directly depend on the target substrates.

Introduction

Xenobiotic phenolic compounds can enter the environment from several sources, including the partial degradation of phenoxy herbicides, the use of wood preservative, and the byproducts of disinfection of drinking water by chlorination. These phenolic compounds are toxic to human and aquatic organisms and their degradation and detoxification in the environment are therefore important. [1] One way to remove the toxic effect of xenobiotic phenolic compounds is to use oxidative enzymes. Oxidative enzyme-based methods generally have low energy requirements, are easy to control, can operate over a wide range of conditions. Furthermore, enzyme-based treatments used alone could be sufficient when the enzymes transform toxic compounds to less harmful products. Many oxidative enzymes have been described that could potentially help to decrease the toxicity of xenobiotic phenolic compounds. [2]

Laccases are multi-copper oxidative enzymes produced by most white-rot fungi, which can oxidize a variety of aromatic compounds, having high activity on phenols. Some of chlorophenols such as 2,4-dichlorophenol, 2,4,5-thrichlorophenol and pentachlorophenol were oxidized and detoxified by laccases. [1,3,6] However, the reaction efficiency was relatively low because of insufficient redox potential of laccases. This limitation may be overcome by using redox mediators like ABTS(2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)) and 1-hydroxybenzotriazole(HBT) and natural phenolic redox mediators like syringaldehyde, acetovanillone, vanillin and p-coumaric acid. Especially, natural phenolic redox mediators would present environmental and economical advantages. These kinds of mediators are cheaper and eco-friendly. [4,5]

The purpose of this study was to determine the ability of the laccase purified from *Ganderma lucidum* KMK2 to oxidize aromatic compounds including various xenobiotic chlorophenols and 2,4-dibromophenol and the capabilities of several redox mediators including natural mediators present in soil to promote the laccase-mediated oxidation of them.

Materials and Methods

Laccase preparation from *G. lucidum* KMK2 was carried out by solid state fermentation (SSF) as described earlier[8]. The crude laccase was purified through ammonium sulphate precipitation, Ion-exchange and gel filtration chromatography using FPLC system (BIO-RAD BIOLOGIC). The purified laccase was filter sterilized and stored in refrigerator for further use. Laccase activity was estimated as described in previous report[8] using ABTS as a substrate. In the aromatic compound oxidation experiment, reaction mixtures contained the individual compound (40uM) ABTS (1 mM), and HBT (1 mM) in 15% citric-phosphate buffer , pH 4 containing 1% (v/v) acetonitrile with 10 units *G. lucidum* laccase in a 500µl reaction volume. The assay was started by the addition of enzyme and terminated by addition of 500µl acetonitrile. 20µl samples were analyzed by HPLC using Agilent 1100 series (Agilent, Waldbronn,

Germany) and a ZORBAX SB C-18 column (Applied Biosystems) with isocratic elution using acetonitril:water(40:60 v/v). All compounds were monitored and quantified by UV absorbance at 210nm. Screening for natural redox mediators was based on the removal of 2,3,4,6-tetrachlorophenol (monitored by HPLC described above) by 5U units *G. lucidum* laccase in the presence of each compound (1mM). The chemical structures of tested compounds as mediators are listed in Table 1. Each mediator was incubated in the buffer described above for 24h at room temperature in 2ml glass tube.

Results and Discussion

To evaluate the oxidation potential of laccase purified from *G. lucidum*, we employed several aromatic compounds listed in Table2. In the case of nonphenolic compounds, no reaction occurred in the absence of synthetic mediators. However, synthetic mediators such as HBT and ABTS strongly enhanced carbazole oxidation. Carbazole was completely oxidized by laccase in the presence of synthetic mediators. Previous study showed that the oxidations of some of polycyclic heterocycles by laccase directly depend on their ionization potentials.[7] Although dibenzodioxin, 2-monochlorodibenzo-*p*-dioxin and biphenyl have almost similar ionization potentials with carbazole, no one showed detectable oxidations even in the presence of synthetic mediators. This result shows there is only a limited relationship between laccase activity and ionization potentials of target compounds. All of phenolic compounds tested in this study except 2,4-dinitrophenol were significantly oxidized by laccase and the presence of synthetic mediators dramatically increased the reaction rates. Although many studies have also shown that laccase has the ability to oxidize and detoxify several chlorophenolic compounds, we herein firstly demonstrated purified laccase has potential to oxidize 2,4-bromophenol.

In general, synthetic mediators are expensive and even cause environmental pollution.[4,5] From this concern, the mediating capabilities of naturally occurring phenols were studied. Syringaldehyde among the four kinds of natural redox mediators showed the best capability. However, any mediators couldn't overcome the mixture of synthetic mediators in terms of the reaction rate and *p*-coumaric acid slightly inhibited the oxidation activity of laccase on 2,3,4,6-tetrachlorophenol. This result is interesting since the removal of anthracene is strongly enhanced by *p*-coumaric acid.[5] Our data shows the mediating capabilities of naturally occurring phenols directly linked to the target compound.

Acknowledgements

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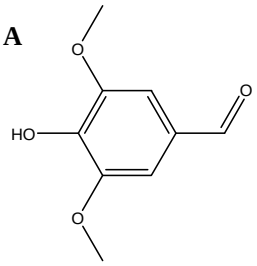
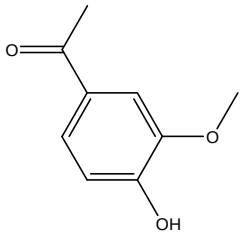
Table 1. Chemical structures of compounds (A to D)for the natural redox mediator screening

A



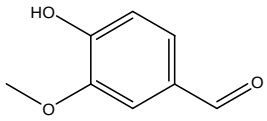
Syringaldehyde

B



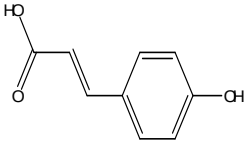
Acetovanillone

C



Vanillin

D



p-coumaric acid

Table 2. laccase oxidation experiment with various aromatic compounds including xenobiotic phenols

Target chemicals (non-phenolic)	**Laccase + ABTS and HBT**	**Laccase only**
Carbazole	**O**	**X**
Dibenzodioxin	**X**	**X**
2-MCDD	**X**	**X**
Biphenyl	**X**	**X**
Target chemicals [Phenolic)	**Laccase + ABTS and HBT**	**Laccase only**
2,4-dinitrophenol	**X**	**X**
2,3-dichlorophenol	**O**	**O**
2,4-dichlorophenol	**O**	**O**
2,6-dichlorophenol	**O**	**O**
2,4-dibromophenol	**O**	**O**
2,3,4,6-tetrachlorophenol	**O**	**O**

O : complete oxidation, X : No significant reaction

Figure 1. HPLC profiles of the reaction of 2,4-dibromophenol(A) and 2,3,4,6-tetrachlorophenol(B) by laccase with HBT(1mM) and ABTS(1mM)

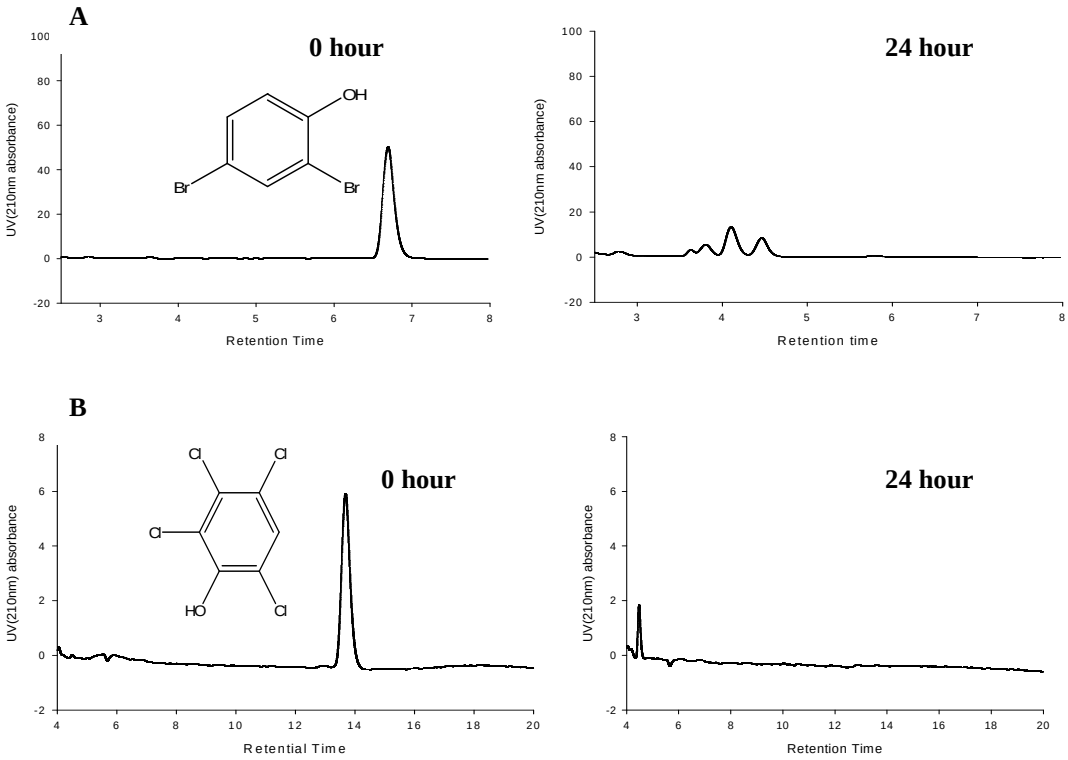


Figure 2. Efficiency of various natural redox mediators on the removal of 2,3,4,6-tetrachlorophenol