Diverse \( p \)-terphenyl compounds, named curtisians, have been isolated from the fungus \( Paxillus curtisii \), and degradation of wood by this fungus is thought to be progressed by iron chelation of \( p \)-terphenyl curtisians. In this study, the iron chelation ability of \( p \)-terphenyls has been proved by chrome azurol S (CAS) assay, reducing power, and UV–visible spectroscopic analyses. The catechol moiety of \( p \)-terphenyl is an essential factor for the potent iron chelation ability, and thus deacylated curtisian with a tetrahydroxyl moiety in the central ring of \( p \)-terphenyl is more effective than acylated curtisians.

**Key words:** Curtisian, iron chelation, \( Paxillus curtisii \), \( p \)-terphenyl

Basidiomycetes, which cause brown and white rots of wood, are major recyclers of lignocelluloses in terrestrial ecosystems. It is suggested that these fungi produce extracellular hydroxyl radicals, which attack wood cellulose and hemicelluloses and break down their polymeric structures [2]. Hydroxyl radical is produced by the iron-catalyzed Fenton reaction, where iron plays an important role in biological degradation of wood as an essential element for fungal metabolism and growth. However, the low solubility of many iron-containing minerals greatly limits their availability. To sequester iron, fungi utilize an efficient acquisition system, including siderophores, which are of low molecular weight and generally produced under conditions of low iron availability. Siderophores are usually classified by the ligands used to chelate the ferric iron, such as catecholates, hydroxamates, and carboxylates [9]. Besides siderophores, another strategy to enhance iron solubility and uptake is the reduction of ferric iron into more soluble ferrous iron. Therefore, the reduction of iron is considered to be a prerequisite for hydroxyl radical production via the Fenton reaction in wood-degrading fungi [2].

\( Paxillus curtisii \), belongings to the family Paxillaceae, brown rot wood fungi, produced mainly \( p \)-terphenyl compounds such as curtisians that were reported as antioxidant and neuroprotective agents [6, 8]. Curtisians A–D have previously been reported to show potent inhibition of lipid peroxidation, Fenton reaction, and glutamate-induced neurotoxicity, which have been implicated in iron-mediated oxidative damage, but are free from any role as free radical scavengers. Their neuroprotective activity was dependent on their iron-chelation properties [6]. However, it was difficult to make a decision that curtisians act as an iron chelator. In this study, we proposed the functions of \( p \)-terphenyls through the iron chelating ability and structure–activity relationship.

The \( p \)-terphenyl compounds tested included curtisians D, E, and I from fungus \( Paxillus curtisii \) [6, 8], betulina A from fungus \( Lenzites betulina \) [7], polyozellin from \( Polyozellus multiplex \) [4], and deacylated curtisian obtained by alkaline hydrolysis of curtisian N [11]. Curtisian N (12 mg) was added to KOH (10 mg) in 2 ml of 50% aqueous methanol and the mixture was stirred at room temperature for 3 h. The reaction mixture was neutralized by 1 N HCl to pH 7 and then subjected to a column of Sephadex LH-20, eluting with methanol to give deacylated curtisian (2 mg); \(^{1}H\) NMR (600 MHz, CD\(_{3}\)OD) \( \delta \) 7.28 (d, \( J = 8.6 \) Hz), 6.74 (d, \( J = 8.6 \) Hz), \(^{13}C\) NMR (125 MHz, CD\(_{3}\)OD) \( \delta \) 156.7, 133.1, 126.7, 115.6, 114.4; their structures are shown in Fig. 1. Deferoxamine (DFO), a traditionally used iron chelator, and antioxidants BHA (butylated hydroxyanisole) and Trolox were taken as controls. For the purpose of evaluating the iron chelating ability of \( p \)-terphenyl compounds, the CAS (chrome azurol S) assay was conducted according to the method described in the literature [10]. In brief, CAS liquid was prepared using 60.5 mg of CAS dissolved in 50 ml of distilled water, and mixed with 10 ml of iron(III) solution (1 mM FeCl\(_{3}\)-6H\(_{2}\)O, 10 mM HCl). Under stirring, this solution was slowly added to 72.9 mg of HDTMA

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(hexadecytrimethylammonium bromide) dissolved in 40 ml of water. The resultant dark blue liquid of 90 µl was added to 10 µl of sample, and the mixture was incubated for 12 h at room temperature. Absorbance was measured at 630 nm using a microplate reader. For the negative control, only the iron solution was replaced by the same volume of distilled water. Iron chelation ability was calculated using the following equation: Iron chelation ability (%) = \[1 - \frac{(\text{Abs (sample)} - \text{Abs (negative control)})}{\text{Abs (positive control)} - \text{Abs (negative control)}}\] × 100. The iron chelating ability of \(p\)-terphenyls was compared with that of BHA, Trolox, and DFO. The CAS assay has been widely used for the detection of siderophores from microorganisms. This assay is based on the competition for iron between the ferric complex of an indicator dye, chrome azurol S, and an iron chelator. Iron is removed from CAS by the iron chelator, which apparently has a higher affinity for iron than CAS. The positive reaction results in a color change of the CAS reagent, in which the dark blue of the positive control with iron changes to pink of the negative control without iron. As a result of the CAS assay (Fig. 2), the iron chelation ability of polyozellin and deacylated curtisian was significantly powerful and distinguishable from those of the acylated curtiains E, D, and I. This result suggested that the catechol moiety of \(p\)-terphenyl provides a potent iron chelation effect. It is assumed that there is an occurrence of the redox cycle, where two hydroxyl groups can be converted by one electron reduction into a quinone or semiquinone radical and reduce ferric iron into ferrous iron for the release of iron.

Iron chelators in wood rotting fungi are known to possess reductive capabilities, which might provide a
promotion of redox cycling activity to continually provide iron by iron chelators. Thus, the reducing power of \( p \)-terphenyls was evaluated using the potassium ferricyanide reduction method [1]. In brief, different concentrations of

![Graph showing reducing power of \( p \)-terphenyls.](image)

**Fig. 3.** Reducing power of \( p \)-terphenyls.

![Graphs showing UV–visible absorption spectra.](image)

**Fig. 4.** UV–visible absorption spectra of 50 \( \mu \)M deacylated curtisian and curtisian E in 20 mM potassium phosphate buffer at pH 7.2, in the absence or presence of Fe(III) and Fe(II).
samples were mixed with 25 µl of phosphate buffer (200 mM, pH 6.6) and 25 µl of 1% potassium ferricyanide. The mixture was incubated for 20 min at 50°C. After incubation, 25 µl of 10% trichloroacetic acid was added to the mixture, followed by centrifugation at 650 ×g for 10 min. The upper layer (50 µl) was mixed with 50 µl of distilled water and 10 µl of 0.1% ferric chloride, and the absorbance of the resultant solution was measured at 700 nm using a microplate reader. The reducing power was compared with that of BHA, Trolox, and DFO. Polyozellin and deacylated curtisian exhibited higher reducing power than that of curtisians D, E, and I (Fig. 3). Although antioxidant Trolox also exhibited relatively high iron chelating ability and reducing power, there were significant differences between deacylated and acylated \( p \)-terphenyls in the tests. Therefore, it was suggested that deacylated curtisian had more affinity with iron.

Formation of the curtisian–iron complex was investigated by UV–visible spectrophotical analysis. Fifty micromoles of curtisian E or deacylated curtisian was mixed with 20, 50, and 100 µM FeSO\(_4\), and FeCl\(_3\), respectively, in 20 mM potassium phosphate buffer at pH 7.2, and their UV spectra were obtained. The influences of Fe(III) and Fe(II) on the spectral characteristics of the curtisians are shown in Fig. 4. For the deacylated curtisian, Fe(III) and Fe(II) ions induced concentration-dependent shift towards the blue with great change, whereas curtisian E showed little shift. These results provide evidence for the formation of a curtisian–iron complex and demonstrate that the deacylated curtisian is more effective than acylated curtisian in iron chelation. It has been reported that siderophore is modified at the same rate as iron is transported into the cell, and that the modified form has little affinity to iron. Modification of siderophore is thought to prevent reformation of the complex with reoxidized iron. One method of modification would be the acylation of the hydroxyl group, which impedes formation of iron chelation [3].

There are plenty of \( p \)-terphenyls with different acyl substituents in basidiomycetes Paxillus curtisii. They are prevalently acylated on the central ring of the \( p \)-terphenyl moiety and, consequently, exhibit outstandingly less affinity than deacylated curtisian in iron chelation. Therefore, it is proposed that \( p \)-terphenyl with no acyl group acts as an iron chelator for the transfer and release of iron to the lignocellulose substrate through reduction of iron and production of hydroxyl radicals to attack wood cells and to proceed with wood degradation. After transfer of iron into cells, deacylated curtisian might be modified by acylation to give curtisians E, D, and I with outstandingly low iron chelation affinity. Therefore, iron chelation affinity seems to depend on the catechol moiety in the central ring of \( p \)-terphenyl. This suggestion is also supported by other phenolic compounds including antioxidant flavonoids, which require the presence of a catechol group for high iron-binding efficiency [5]. To the best of our knowledge, this is the first report about the iron chelating ability and structure–activity relationship of fungal \( p \)-terphenyls and their proposed role in fungi.

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