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Biological Detoxification of Lacquer Tree (*Rhus verniciflua* Stokes) Stem Bark by Mushroom Species

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Abstract The stem bark of *Rhus verniciflua* (RVSB) has been used in herbal medicine to treat diabetes mellitus and stomach ailments for thousands of years in Korea, despite its content of the plant allergen, urushiol. A new biological approach for the removal of urushiol from RVSB using mushrooms is described. All mushroom species (11 sp.) employed in this study were able to grow on RVSB, although the growth rate (mm/day) was lower than the control (sawdust). The components of urushiol congeners [C15 triene (*m/z* 314), C15 diene (*m/z* 316), C15 monoene (*m/z* 318), and C15 saturated (*m/z* 320)] were purified by HPLC and identified by GC-MS. A C15:3 (3-pentadecatrienly catechol) was found to be most abundant in RVSB. Urushiol analogues decreased remarkably from 154.15 to 10.73 mg/100 g (approximately 93%) by *Fomitella fraxinea*, whereas *Trametes vercicolor* showed only a 1.46% degradation capacity despite its 2 fold higher growth rate. Similarly, laccase activity was found to be high for *F. fraxinea* and low for *T. vercicolor*. Moreover, approximately 98% detoxification was accomplished by *F. fraxinea* cultivated on RVSB supplemented with 20%(w/w) rice bran. These findings suggest that mushrooms can be used in the detoxification of RVSB.

Keywords: detoxification, laccase, lacquer tree, mushroom, urushiol

Introduction

Rhus verniciflua Stokes (RVS) of the Anacardiaceae family is commonly known as the lacquer tree. It is an indigenous plant to China, Japan, and Korea, and is used as a food additive (1) and is a traditional herbal medicine for diabetes mellitus (2) and stomach diseases (3). Recently RVS has been found to have various biological activities, such as antioxidant (4, 5), anti-inflammatory (2), anti-mutagenic (6), antitumour (7, 8), anti-cancer (9-11), and anti-platelet effects (12).

However, its application as a food additive or source of pharmaceutical compounds has been limited because urushiol congeners in the plant cause irritation, inflammation, or blistering in sensitive individuals, a reaction known as urushiol-induced contact dermatitis (13). As a lipid-soluble compound, urushiol, derivatives of catechol with unsaturated C15 or C17 side chains at the C3 position, is a mixture of several compounds, and its allergenicity involves both the catechol ring and the side chain (14). It penetrates the skin, and when it bonds to skin cells initiates a T-cell mediated immune response in which T-cells activate macrophages to secrete mediators of inflammation. Previous studies have shown that 40% of human subjects respond to urushiol doses of 2 g or less (14), and over 30% of 171 Korean people who ate the stem bark of *Rhus verniciflua* (RVSB) containing chicken or duck foods developed contact dermatitis (15).

Therefore it is important to remove urushiol from the substrate before using it as a food additive or source of pharmaceutical compounds. Some methods have been suggested in connection with the detoxification of urushiol. These methods aim to either remove or reduce urushiol analogues in RVSB. They can be classified as chemical (either organic solvent or oxidizing agent), physical (pyrolysis at temperatures above 500°C) (17) and enzymatic methods. However, most of these methods are unfavorable because (i) of the formation of harmful chemicals or the denaturation of other compounds, (ii) smoke from burning plant debris is highly allergenic, (iii) of low efficiency of enzyme-urushiol complex formation due to the lipophilic properties of urushiol and 3-dimensional barriers of other lignolytic compounds in RVSB (18), and (iv) they are relatively expensive. Therefore, a more effective technique is needed to remove urushiol analogues from RVSB.

As shown Fig. 1, urushiol is subject to oxidation and polymerization through enzymatic procedures under aerobic conditions (16), and this mechanism leads to detoxification by reducing the active site. Basidiomycete fungi (mushrooms) are unable to use solar energy. Instead, they produce a wide range of extracellular enzymes including oxidoreductases (e.g., lignin peroxidase, manganese-dependant peroxidase and laccase) that enable them to degrade complex organic substrates for their nutrition (19, 20). These enzymes may play a role in the detoxification/removal of potential

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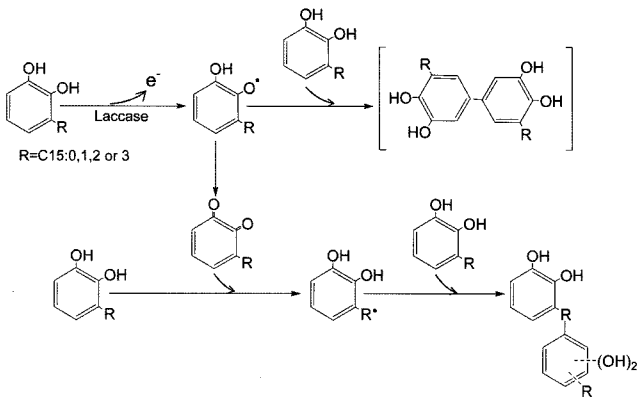

Fig. 1. Laccase-catalyzed oxidative coupling of urushiol.

inhibitory phenolic compounds present in the substrate.

In this respect, we hypothesized that mushrooms may be used as a biological agent to detoxify RVSB by removing urushiol from the plant, not only because they are safe and can metabolize urushiol compounds, but they also contain various health enhancing substances (21). In order to confirm this hypothesis, we investigated the effects of various mushrooms on the urushiol content of RVSB, and attempted to ascertain whether the mushrooms can be used as a biological method of detoxification.

Materials and Methods

Materials Sap and sun dried RVSB were purchased from individual farms located in Wonju, Korea. Eleven species of mushrooms (*Trametes trogii*, *Phellinus linteus*, *Fomitella fraxinea*, *Lyophyllum cinerascens*, *Ganoderma lucidum*, *Hericium erinaceus*, *Lentinus edodes*, *Ganoderma applanatum*, *Trametes versicolor*, *Pleurotus eryngii*, and *Tyromyces palustris*) were supplied from a microorganisms Lab. (Dept. Industrial Crop Production and Processing, Iksan National College, Iksan, Jeonbuk, Korea), and were pre-activated on potato dextrose agar (PDA, BD Difco™, Saprks, MD, USA) at 25°C for 6 days. They were then grown on broth (Table 1) with shaking (100 rpm) for liquid spawn, and 20%(w/w) rice bran supplemented sawdust (*Quercus variabillis*) containing 70% moisture for sawdust spawn, at 25°C for 7 and 20 days, respectively (Fig. 2).

Table 1. Composition of the basal medium

Glucose	10 g
Peptone	2 g
KH_2PO_4	1 g
$Na_2HPO_4 \cdot 2H_2O$	0.1 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$CaCl_2$	0.01 g
$FeSO_4 \cdot 4H_2O$	0.01 g
$MnSO_4 \cdot 4H_2O$	0.001 g
$ZnSO_4 \cdot 7H_2O$	0.001 g
$CuSO_4 \cdot 5H_2O$	0.002 g
Thiamine·HCl	50 μg
Distilled water	1,000 mL

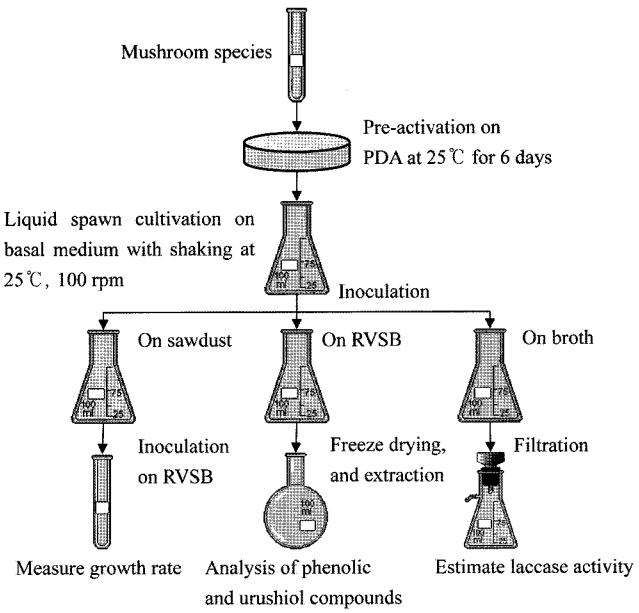

Fig. 2. Schematic diagram for the evaluation of biological detoxification of RVSB by mushroom species.

Sample preparation The sun dried RVSB was crushed to make a powder. The powder was then passed through a 10 mesh size sieve and the moisture content was adjusted to approximately 70% with distilled water.

Culture conditions ***Mycelial growth on RVSB***: The prepared RVSB was packed into test tubes (2.1×20 cm) at a height of approximately 15 cm and then autoclaved. Sawdust spawn was inoculated at the top of test tube as high as 1 cm, and incubated at 25°C for 28 days. As a positive control, sawdust of *Q. variabillis* containing 20%(w/w) of rice bran was employed. Growth was evaluated by measuring the height from the top (inoculated point) every 2 days (when the mycelium was grown on solid media (sawdust and RVSB) the hypha grew vertically from top to bottom of test tube).

Urushiol and phenolic compound analysis of mushrooms or non cultured-RVSB: The prepared RVSB (100 g) was placed in a 250 mL of Erlenmeyer flask, and then autoclaved for 25 min. The liquid spawn (5 mL) was then inoculated and cultured at 25°C for 20 days under standing conditions. It was then freeze dried and crushed to make a powder.

Laccase activity: The liquid spawn was homogenized under sterile conditions, and was inoculated (2 mL) into 100 mL of broth containing 2%(v/w) crushed RVSB supplemented as a putative laccase inducer, and cultured at 25°C, 100 rpm for 12 days. Crude enzyme was prepared by centrifugation (4°C, 600×g, 30 min) and filtration. Laccase activity was determined by measuring the oxidation of syringaldazine (4.47 mM, Sigma Co., St. Louis, MO, USA) in citrate (0.1 M)–Na_2HPO_4 (0.2 M) buffer (pH 4.6) at 525 nm. The reaction buffer (2.5 mL) and crude enzyme (0.5 mL) were mixed, and the reaction was triggered by the addition of 10 μL of syringaldazine solution. One unit of enzyme activity was defined as the change in absorbance per min under assay conditions (22).

Isolation, identification, and quantification of urushiols
Isolation: Urushiols were isolated from sap, RVSB or mushroom cultured RVSB, according to the methods described by Rivero-Cruz *et al.* (23) with the following minor modifications. The sap of lacquer tree, dried powder of RVSB and dried powder of mushroom cultured RVSB were soaked in 3 parts and 10 parts acetone (v/w), respectively, stirred at room temperature overnight, and filtered through No. 2 filter-paper. The solvent was removed in a rotary evaporator. The residue was dissolved in a volume of hexane equal to the volume of acetone used previously, centrifuged at 4°C, 14,000×g for 10 min to remove insoluble compounds, and dried under vacuum. The hexane soluble residue was finally dissolved in the same volume of acetonitrile as hexane and acetone, and separated in a similar way. Finally, acetonitrile soluble compounds were redissolved in 85% MeOH.
Identification: Urushiol congeners isolated from the sap were purified by high performance liquid chromatography (HPLC) and identified by GC-MS according to the method of Draper *et al.* (14) for use as standards since urushiol analogues are not commercially available. Absorption column chromatography was employed to separate compounds in a 4×200 mm preparative column (Grom-sil 120 ODS-4 HE; Grom Co., Rottenburg-Hailfinger, Germany) with 85% MeOH as the mobile phase (3 mL/min) on a preparative HPLC (Sycam Co., Gilching, Germany) instrument containing a 2 mL loop and a UV detector (273 nm). Each peak was separated by preparative HPLC and purified by analytical HPLC as described in the following section (*quantification*). It was then derivatized with *N*-methyl-*N*-(trimethylsilyl, TMS)-trifluoroacetamide (MSTFA) at 60°C for 30 min after 100 μL of sample in MeOH was combined with 100 μL of MSTFA. To identify each compound, GC-MS equipped with a QP-2010 mass spectrometer and with a 2010 series gas chromatograph (Shimadzu Co, Kyoto, Japan) was used. TMS derivatives were separated on a 30 m×0.32 mm (i.d.), 0.1 μm film DB-5HT capillary column (Agilent Co., Santa Clara, CA, USA). GC-MS conditions were as fellows: inlet temperature, 250°C; initial oven temperature 200°C (held for 10 min); 30°C/min ramp to 250°C (held for 10 min); 30°C/min ramp to 350°C (held for 20 min); source temperature 200°C; ionization voltage 70 Ev; carrier gas nitrogen (column flow, 1.29 mL/min).
Quantification: All isolated samples were filtered (0.45 μm) and pre-evaluated by TLC (Silica gel 60 F_{254}, 0.2 mm thickness, Merck Co., Damstadt, Germany) (22) with acetic acid:chloroform, 1:9 (v/v) as the solvent, and quantified by HPLC using isolated and identified urushiol from the above procedure as standards. The analytical HPLC system (Sycam Co.) consisted of a pump, an autoinjector with a 20 μL loop, a column oven, a diode array detector (DAD) and a 4.0×250 mm C18 column (Grom sil 120, 5 μm, ODS-5 ST, Grom Co.). The mobile phase was 85% MeOH, at a flow rate of 1 mL/min, UV detection was at 273 nm, and all samples were prepared through a C18 sep-pak cartridge (Waters Co., Milford, MA, USA) prior to injection.

Analysis of phenolic compounds Phenolic compounds were assayed by Folin reagent, and expressed as catechol (Sigma, Co.) amount with a standard curve. Samples in 0.5 mL of each solvent were combined with 5 mL of Na_2CO_3 and allowed to stand for 2 min. They were added to 0.5 mL of 50% Folin-Ciocalteu's reagent, allowed to stand for 30 min, and the absorbance was read at 750 nm (25).

Statistical analysis The experimental results are expressed as the means±SD. Data were assessed using analysis of variance (ANOVA). Duncan's multiple test was used to determine the difference among groups. Student's *t*-test was used in the 2 group comparison. A *p*-value less than 0.05 was considered statistically significant.

Detoxification ratio (%) was defined as decreasing ratio of total urushiol concentration (TUC) to non-cultured RVSB, and calculated by the equation: [(TUC of non-cultured RVSB-TUC of cultured RVSB)/TUC of non-cultured RVSB]×100.

Caution The RVSB contains urushiol, an allergen. Therefore it should be handled by using appropriate protective clothing, respiratory protection, or a fume hood.

Results and Discussion

Mycelial growth on RVSB The growth rate (mm/day) of each mushroom species on sawdust and RVSB was calculated by a linear plot of height versus time during the exponential phase (between 4 and 28 days), and the results are summarized in Table 2.

The values for each substrate had a high correlation coefficient ($r>0.97$, $p<0.05$) and showed significant differences in the growth rates for each species on the two substrates except for sample 3 (*F. fraxinea*). This may be due to a lack of nitrogen (wood usually contains less than 0.1% nitrogen by weight) and the presence of fungitoxic components such as terpenes, stilbenes, flavonoids, and tropolenes which are easily oxidized and attack cell membranes (26). However, one objective of this study was

Table 2. Growth rate of various mushroom species on sawdust and RVSB[1)]

No.	Strains	Growth rate of mycelium (mm/day)	
		Sawdust	RVSB
1	*T. trogii*	4.81±0.05[a2)]	3.47±0.20
2	*P. linteus*	3.13±0.14[a]	2.50±0.10
3	*F. fraxinea*	4.32±0.07[a]	2.80±0.21
4	*L. cinerascens*	2.26±0.07[a]	1.21±0.48
5	*G. lucidum*	5.37±0.06	5.12±0.28
6	*H. erinaceus*	4.36±0.13[a]	3.17±0.22
7	*L. edodes*	4.65±0.08[a]	3.61±0.33
8	*G. applanatum*	6.04±0.29[a]	4.61±0.51
9	*T. versicolor*	5.29±0.10	5.63±0.30
10	*P. eryngii*	3.85±0.17[a]	2.51±0.23
11	*T. palustris*	5.09±0.20	5.37±0.23

[1)]Experimental results are expressed as the means±SD.
[2)]Means the values for the same strain on each substrate are significantly different ($p<0.05$).

to identify mushrooms capable of degrading urushiol. From this point of view, all mushroom species used in this study grew well on RVSB, although the growth rates were lower than on sawdust medium.

Effect of mycelial growth on phenolic compounds and crude urushiol of RVSB A higher growth rate means faster cell growth and higher tolerance for particular fungitoxic compounds, but does not mean a higher capacity to degrade urushiol. Therefore the phenolic contents (Table 3) and crude urushiol contents (Fig. 3) in mushroom cultured RVSB were evaluated. The phenolic compounds in RVSB were reduced remarkably by 2.9 to 4.4 fold in the acetone layer, and by 1.3 to 5.9 fold in the acetonitrile layer, described in *isolation* section, due to the growth of mycelia. The lower proportion of phenolic compounds in the acetonitrile layer than the acetone layer indicates a higher specificity for urushiol because the phenolic compounds containing urushiol in RVSB were largely resolved in acetone, and eliminated by the organic pool formed during the extraction/isolation of urushiol. Finally, urushiol and urushiol-like chemicals remained in the acetonitrile layer. The ratio of phenolic compounds in the acetone layer (AT) relative to the acetonitrile layer (AN) may indicate urushiol-specific degradation, although this is not necessarily indicative of a high capacity for degradation. Three species of mushrooms, *G. applanatum*, *G. lucidum*, and *F. fraxinea*, were very effective because they contained a low concentration of phenolic compounds and show a higher specificity with at/an ratios of 4.93, 6.42, and 6.83, respectively.

On the other hand, *T. palustris* and *T. versicolor* were not effective since they contained a high concentration of phenolic compounds, although they were the most actively growing cultures. This result indicates that *T. palustris* and *T. versicolor* are applicable to other industries where higher resistance to oxidants is needed because they may have a higher tolerance towards oxidative stress.

As shown in Fig. 3, small bands/spots were detected from *F. fraxinea* (lane 3), *G. lucidum* (lane 5), and *G. applanatum* (lane 8) compared to the control (lane C). This indicates that these species may have a high urushiol degrading capacity because the urushiol compounds remained primarily in the extracted organic chemical.

Despite the low growth rate on RVSB, *F. fraxinea* showed the highest urushiol degrading activity. Interestingly, *T. versicolor* (lane 9) and *T. palustris* (lane 11) exhibited a single large spot indicating that they could not convert phenolic compounds into other chemicals for their nutrition or detoxification. Moreover, polymer bands of urushiol congeners were not detected on the TLC plate because they could be removed from the solvent by filtration and could reduce $FeCl_3$ binding of the active site of urushiol due to the polymerization of urushiols, thus reducing solubility.

Table 3. Effect of mushrooms on the content of phenolic compounds in RVSB

Strains	Concentration of phenolic compounds (mg catechol/100 g dry base)		
	Acetone layer (AT)	Acetonitrile layer (AN)	AT/AN ratio[1)]
RVSB (non-cultured, control)	181.48±1.60[a2)]	16.69±2.51[a]	9.20
T. trogii	53.92±3.14[dce]	5.00±0.38[dc]	9.27
P. linteus	48.69±1.55[dfe]	5.78±0.70[dc]	11.87
F. fraxinea	41.16±1.14[f]	2.83±0.23[d]	6.88
L. cinerascens	61.85±0.76[c]	13.06±1.98[b]	21.12
G. lucidum	44.68 ± 3.39[fe]	2.82±0.09[d]	6.31
H. erinaceus	50.90±3.92[dfe]	8.16±2.90[c]	16.03
L. edodes	55.82±1.36[dc]	4.09±2.24[d]	7.33
G. applanatum	48.19±1.09[dfe]	2.30±0.64[d]	4.77
T. versicolor	155.37±3.73[b]	19.86±3.82[a]	12.78
P. eryngii	56.63±1.49[dc]	7.94±0.45[c]	14.02
T. palustris	159.39±2.66[b]	18.03±2.24[a]	11.31

[1)]AT/AN ratio was calculated by the equation: (AN ×100)/AT.
[2)]The experimental results are expressed as the means±SD, and an upper case superscript means significantly different by paired Duncan's test at $p<0.05$.

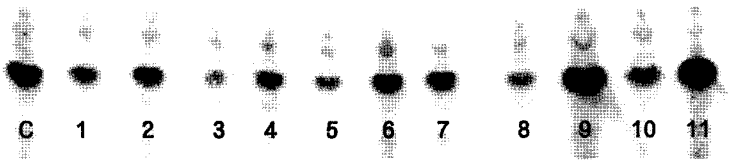

Fig. 3. Thin layer chromatographic (TLC) separation of urushiol congeners.
Bands were visualized by spraying with $FeCl_3$. Lane C, non-cultured RVSB; lane 1, *T. trogii*; lane 2, *P. linteus*; lane 3, *F. fraxinea*; lane 4, *L. cinerascens*; lane 5, *G. lucidum*; lane 6, *H. erinaceus*; lane 7, *L. edodes*; lane 8, *G. applanatum*; lane 9, *T. versicolor*; lane 10, *P. eryngii*; lane 11, *T. palustris*.

Changes in RVSB urushiol content by mycelial growth

It has been reported that *R. verniciflua* urushiol congeners are largely composed of C15 triene (*m/z* 314), C15 diene (*m/z* 316), C15 monoene (*m/z* 318), and C15 saturated (*m/z* 320) (27). These compounds were purified (assay, <95 %) by HPLC and identified by GC-MS (Fig. 4) for use as standards. Changes in urushiol concentrations in mushrooms cultured on RVSB are summarized in Table 4. As shown Fig. 5, the *n*-C15-substitued catechols were the most abundant components in RVSB. A similar result has been

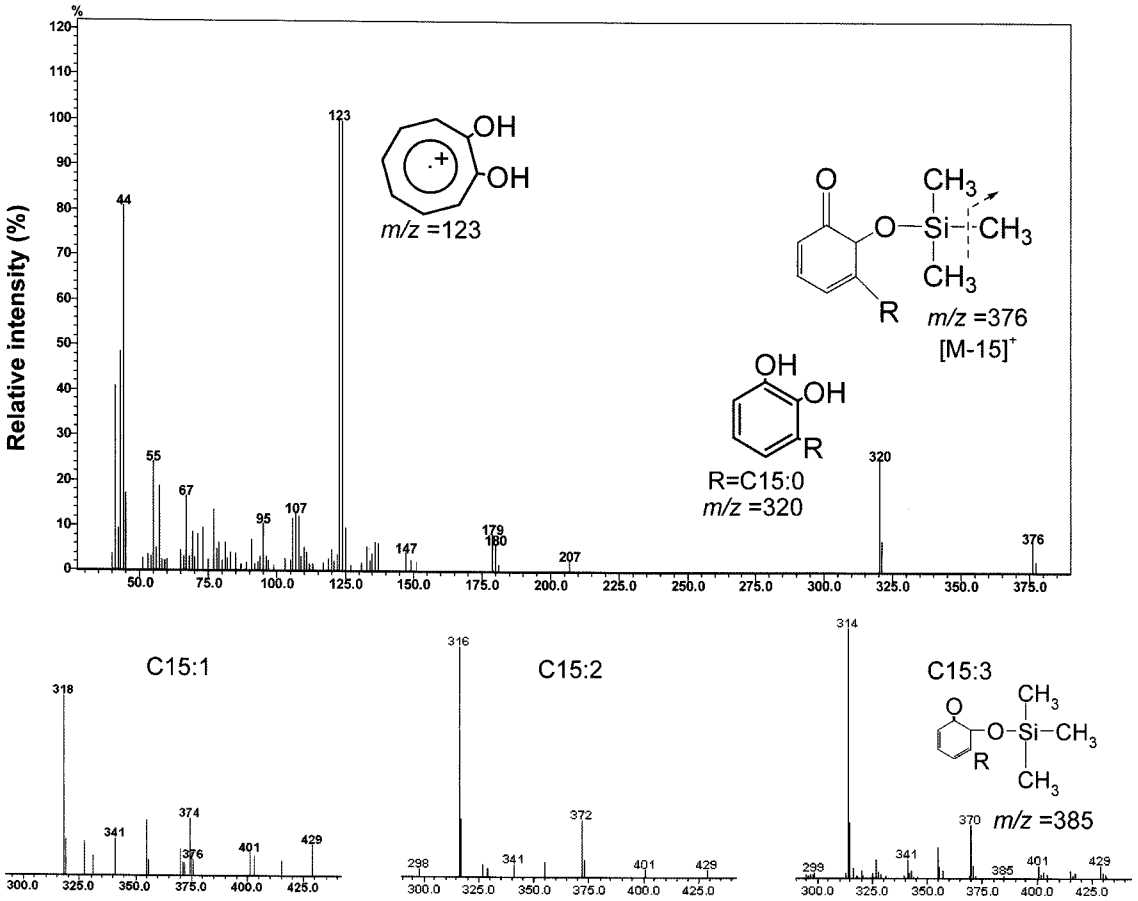

Fig. 4. GC-MS spectrum of urushiol congeners isolated from *Rhus verniciflua* sap.
Isolated compounds were derivatized with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) at 60°C for 30 min and the proposed fragmentation patterns are shown above the respective spectra.

Table 4. Content of urushiol analogues in mushrooms cultured on RVSB

Strains	Concentration of urushiol congers (mg/100 g)					
	C15:3	C15:2	C15:1	C15:0	Total	Detoxification ratio[1)] (%)
Non-cultured	95.70	9.09	42.57	6.79	154.15	-
T. trogii	9.50	0.54	6.88	1.30	18.22	88.2
P. linteus	16.71	0.79	11.01	2.29	30.8	80.0
F. fraxinea	5.10	0.53	4.35	0.75	10.73	93.0
L. cinerascens	46.65	4.10	26.75	4.58	82.08	46.8
G. lucidum	7.39	0.31	5.39	0.57	13.66	91.1
H. erinaceus	21.11	0.95	15.20	3.19	40.45	73.8
L. edodes	8.30	0.74	6.73	1.46	17.23	89.8
G. applanatum	10.49	0.56	5.89	0.88	17.82	88.4
T. versicolor	99.78	3.38	42.65	6.08	151.89	1.46
P. eryngii	19.57	0.85	11.61	2.58	34.61	77.6
T. palustris	81.42	6.18	39.95	7.56	135.11	12.4

[1)]Detoxification ratio (%) was defined as a decreasing ratio of total urushiol concentration (TUC) to non-cultured RVSB, and calculated by the equation: [(TUC of non-cultured RVSB-TUC of cultured RVSB)/TUC of non-cultured RVSB]×100.

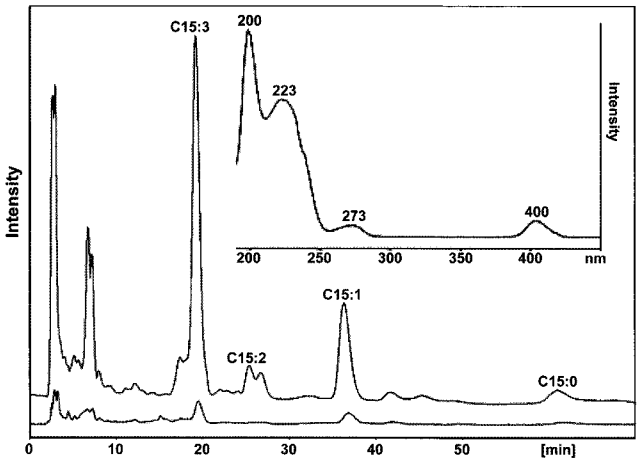

Fig. 5. Comparative HPLC chromatogram of non-cultured RVSB (upper) and *F. fraxinea*-cultured RVSB with a DAD absorption spectrum (inset) of C15:3.

Table 5. Laccase activity of various mushrooms cultured on broth medium supplemented with 2%(w/v) RVSB

Strains	Laccase activity (Unit/0.5 mL, day)		
	5	8	12
T. trogii	0.019	0.001	0.012
P. linteus	0.001	0.001	0.001
F. fraxinea	0.795	1.112	1.063
L. cinerascens	0.002	0.001	0.001
G. lucidum	0.408	0.593	0.865
H. erinaceus	0.055	0.065	0.081
L. edodes	0.366	0.176	0.068
G. applanatum	0.475	0.503	0.141
T. versicolor	0.001	0.002	0.001
P. eryngii	0.389	0.502	0.708
T. palustris	0.001	0.002	0.000

described for urushiols from the stem bark of *Metopium brownie* (23). All urushiol congeners from *R. verniciflua* have very similar UV absorption spectra (3 absorption maxima at ca. 200, 273, and 404 nm) because all of the urushiols share the 3-alkyl-1,2-benzenediol chromophore (14, 24) and yellow color. Moreover, the 223 nm peak leading to the shoulder was obscure in all congeners except triene. This result is similar to the findings of Draper *et al.* (14).

The concentrations of urushiol congers in RVSB (non-cultured) and mushroom cultured RVSB are summarized in Table 4. The approximate distributions of urushiol congeners in RVSB (non-cultured) were 95.70 (C15:3) : 9.09 (C15:2) : 42.57 (C15:1) : 6.79 (C15:0), whereas in sap were 64.5 : 10.9 : 16.5 : 4.5 (16), and in the bark of poison ivy (*Toxicodendron radicans*) were 59.9 : 31.3 : 8.1 : trace amount (28). The highest concentration (62.1% out of total urushiol) of urushiol congers in RVSB was observed in C15:3. This value is very similar to the sap of the same plant (*R. verniciflua*) (16). Urushiol congeners were remarkably decreased by mycelial growth, and decreasing urushiols is an indication of detoxification.

As shown in Table 4, more than 90% of the detoxification was accomplished by *F. fraxinea* and *G. lucidum*. Similarly, more than 80% of urushiol was detoxified by *L. edodes*, *G. applanatum*, *T. trogii*, and *P. linteus*. Interestingly, *F. fraxinea* showed the highest (93%) detoxification ratio, whereas *T. versicolor* and *T. palustris* had a low detoxification ratio (1.46 and 12.4%, respectively). It is worth noting that urushiol was able to be removed from RVSB through biological methods using mushroom species, and the degradation capacity depends on the species.

Laccase activity on broth To understand the relation between mycelial growth and urushiol content, laccase (one of ligninolytic enzymes) activity was evaluated (Table 5). As shown in Table 5, *F. fraxinea* and *G. lucidum* showed the highest laccase activity, whereas low activity was detected for *T. versicolor* and *T. palustris*. It should be noted that *T. trogii*, *P. linteus*, *H. erinaceus*, *L. edodes* and *G. applanatum* showed comparably high urushiol degrading capacity (Table 4). In contrast, low or no laccase activity was detected for these species. The reason for this could be that the degradation capacity of *F. fraxinea* and *G. lucidum* mainly depends on laccase activity, but for other mushrooms it may depend on other oxidoreductase enzymes.

In addition, white rot fungi produce unique extracellular oxidative enzymes that initiate the attack on nature's complex aromatic polymer, lignin, and the system depends on low molecular weight metabolites and cofactors; the secondary metabolite, veratryl alcohol (3,4-dimethoxybenzene) is the redox mediator for lignin peroxidase, whereas, Mn^{2+}, a metal ion naturally present in wood, is the redox mediator of manganese-dependent peroxidase (MnP). Also, fungal organic acid metabolites such as oxalate, glyoxylate, and lactate have an important role in MnP and lignin degradation (18).

Furthermore, it is well known that *T. versicolor* can release large quantities of laccase, which is enough for industrial supplies. It is therefore reasonable to assume that this species has excellent urushiol degrading capacity, however no such result was observed in this study. Although the basis is unclear, fungal laccase production is known to depend on growth conditions, in particular the nitrogen content of the substrate (29).

Changes of C15:3 content by *F. fraxinea* during the culture period In order to investigate the changes of urushiol degrading capacity, rice bran as a nitrogen source was added to 20%(w/w) just before culturing. As shown Fig. 6, C15:3 contents decreased sharply in the first 5 days after which it gradually decreased reaching a minimum level after 15 days of culture. The compound was actively reduced in both substrates (with and without rice bran) with a similar gap between 2 groups for the whole culture period. Approximately 98% detoxification (5% higher than RVSB) was recorded in rice bran supplemented substrate after 15 days. This could be due to the substrate composition, and mycelial growth (data not shown). Moreover, the initial concentration of urushiols could be an important factor for degradation, because the gap between the control and supplemented substrates was approximately 20% at the beginning and 25% at the end of the culture period. Further investigation is needed to

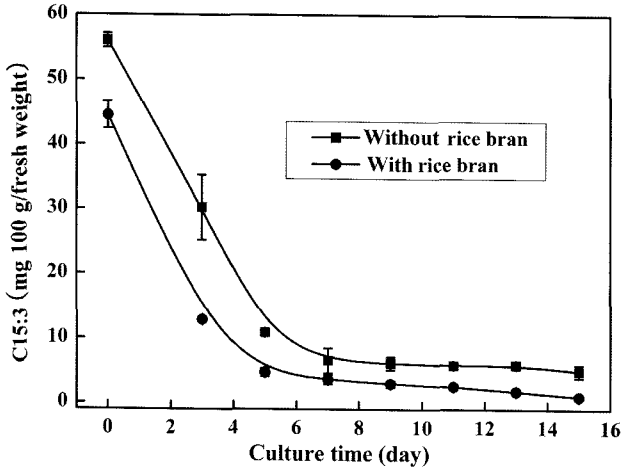

Fig. 6. Changes in RVSB urushiol content by *F. fraxinea*. RVSB was supplemented with rice bran (20%, w/w).

understand the effect of substrate composition on urushiol degrading capacity.

In this study, we have attempted to develop a new approach for the detoxification of RVSB by using edible mushrooms. To our knowledge this is the first study that reports a biological method for the reduction/removal of urushiol from RVSB. This biological method was found to offer a simple, effective, and economically viable process for the reduction/removal of urushiol from RVSB. In addition, this method can be applied to the detoxification of potentially inhibitory phenolic compounds, and mushrooms could be a potential biological source in this regard. Moreover, the mushrooms used for detoxification have a significant potential for food fortification (30) and nutraceutical applications.

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