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Micromorphological Features of Oak Wood Cultivated With Shiitake Mushroom, *Lentinus edodes*(Berk) Sing.[*1]

Yoon Soo Kim[*2]· Byung Dae Park[*2]·Jae Ki Lee[*2]

표고 재배 졸참나무 椙木의 微觀形態的 變化 樣相[*1]

金 潤 受[*2]· 朴 炳 埰[*2]· 李 載 棋[*2]

要 約

표고버섯 (*Lentinus edodes*) 균에 의한 졸참나무 (*Quercus serrata*)椙木의 미시형태적 변화를 주사형전자현미경 (SEM) 을 통해 관찰하였다. 표고 재배 5년후 골목의 중량 감소율은 50%에 달했다. 구성세포중 목섬유는 표고균사에 의해 쉽게 분해되었으나 도관과 유세포는 저항성을 나타냈다.

SEM 관찰 결과 표고균사는 세포內腔에서부터 세포벽을 서서히 분해 薄壁化시켰으며, 또한 Cell corner 가 중간층보다 먼저 공격을 받아 분해 되었으나 중간층은 쉽게 분해되지 않았다. 흥미롭게도 軟腐朽의 전형적 형태인 2차막에서의 空洞역시 백색부후균인 표고균사에 의해 형성되었다. 이상의 관찰결과 표고균은 백색부후와 연부후균이 갖는 미시형태적 특징을 동시에 보여주었다. 아울러 중간층 보다 Cell corner 가 먼저 선택적으로 분해되며, 도관세포벽의 분해저항성을 세포벽 구성 리그닌의 화학적 특성과 연관시켜 논의했다.

1. INTRODUCTION

Deliberate conversion of solid wood as unmodified lignocellulosics by the agent of biodecomposition is done commercially for the production of various mushroom. The "Shiitake" mushroom (*Lentinus edodes* Berk.) has been cultivated and used as human food for centries in far eastern countries. This mushroom has an important over the common champinon *Agricus bisporus* in that it can be cultivated on wood. Thus it has potential for the direct bioconversion of lignified residues and low-quality wood into fungal protein[16,26] . Oaks (*Quercus*) are the preferred species for the cultivation of this mushroom.

Biological decomposition of oakwood by *L. edodes* usually leads to an increase rather than a decrease in the value of wood; a good source of protein for human consumption but also the increase of the ruminant digestibility of wood. The ruminant digestibility of Shiitake bedlog

Key words:*Lentinus edodes*, *Quercus serrata*, SEM, Micromorphology, progressive thinning, cell corner, cavity formation.

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*2. 全南大學校 農科大學 College of Agriculture, Chonnam National University, Kwangju 500-757 Korea

was increased to over 60%[23]. In contrast, our preliminary investigation revealed that the increase of ruminant digestibility was at best 30% (Myung and Kim, unpublished data). This discrepancy leads us to investigate the chemical and micromorphological characteristics of oakwood cultivated with *L. edodes*. The objectives of the present studies are to 1) determine the micromorphological changes that occur in oakwood under natural condition by *L. edodes* and 2) to compare the decay pattern of it with other white rot fungi.

2. MATERIALS AND METHODS

Wood blocks were obtained from *Quercus serrata*-bedlog inoculated with Shiitake-spawn and cultivated for 1,2,5 and 6 years in the College Forest of Chonnam National University. Sections were cut on a slide microtome after the fixation with FAA. Sections were stained with safranin and astrabule for the detection of microorganisms and of woody cell wall[1]. For histochemical studies, some sections were also stained with phloroglucinol/HCl and with Höpfner-Vorsatz for lignin and polyphenolics respectively[15].

For SEM sections were also removed asceptically from the oak-bedlog and fixed in FAA and washed through with distilled water. After the dehydration through a graded ethanol series, samples were critical point dried and coated with gold in a spuutering apparatus. The specimens were observed with a JEOL SEM at 15kV.

The weight loss of degraded wood was determined using the specific gravity of sound and degraded wood using techniques previously described by Kawase[22].

3. RESULTS

Losses in dry weight during the 6-year cultivation of Shiitake in oakwood are summarized in Table 1. A 35% loss in total dry weight occurred mostly within 2 years and about the half of total weight was losed within 5 years. After the six-year cultivation, about 80% of cell wall substances were dissolved.

Table 1. Loss in dry weight of Oakwood during the cultivation of Shiitake

Cultivation time (year)	1	2	5	6
Weight loss (%)	1.2	34.1	54.1	81.2

Macroscopically the oak wood inoculated with Shiitake spawn appeared more bleached in color than the control. Figure 1 shows the partial discoloration of dark-colored heartwood after the three-year cultivation. Almost all the dark color of heartwood was bleached after the 5-year cultivation. Some oak bedlog had narrow dark streaks within the degraded wood (Fig. 2) which were filled with the unidentified substances (Fig. 3). The cells in this black line were initially intact to *L. edodes*. However, this zone line was also degraded by *L. edodes* as the cultivation of mushroom progresses.

Distribution of hyphae and initiation of attack *L. edodes* in oakwood is shown in Figures 4,6, and 7. Initailly hyphae of *L. edodes* are concentrated in the vessels and ray cells, although other cells are also invaded in early stages (Fig. 4). The vessels provide the major longitudinal pathways of hyphae of *L. edodes*. At more advanced stages of cultivation, almost all the fiber cells were decomposed, while vessels and axial parenchymas were resistant to the attack of fungi (Fig. 5).

In the early stage of colonization, hyphae grow from cell to cell through natural pits openings rather than by boring through lignified cell walls (Fig. 6). Cell wall lysis by *L. edodes* in oak wood is usually initiated from hyphae gromwing in the cell lumen. Along the young hyphae, lysis furrows are produced (Figs. 6,7). At the early stage of cultivation, these erosion troughs are localized to the immediate areas around the

fungus.

As the cultivation of Shiitake progresses, *L. edodes* attacked the cell wall both from within the lumen and from the cell corners. Micromorphological studies indicate that *L. edodes* caused a progressive thinning of the cell wall preceeding from the S_3 layer of the secondary wall towards the middle lamella (Fig. 8). The degraded cell wall surface adjacent to the lumen in oak wood was occasionally irregularly eroded (Figs. 8,9,15) and in some cells it appeared serrate (Figs. 19, 20). In Fig. 12, it can be seen that the fungus attacks the space between the S_1 and S_2, which seems to make these transition layers be susceptible to attack by the fungus. Fig. 12 shows also that S_1 layer is degraded without attack of compound middle lamella or adjacent S_2 layer. In some cells, the S_3 layer was the last cell wall components remaining in the secondary wall (Fig. 11).

In addition to this thinning of secondary wall, the hyphae of *L. edodes* penetrated the cell wall (Fig. 9) and enlarged bore holes at the later stage of cultivation (Fig. 10). The constriction of hyphae at the point of penetration into cell wall which is a characteristic of penetration hyphae, could not be observed in the present study.

Another characteristics which are very obious for the Shiitake mushroom are that 1) they can cause the cavity in the cell walls (Figs. 13,14,18) which is usually associated with soft-rot fungi, and 2) the cell corners are preferentially attacked especially during the late stage of cultivation. In contrast, the highly lignified middle lamella is resistant to attack until late stage of degradation.

Cell corners were especially susceptible to degradation (Figs. 12, 15,17,19). Degradation was locally restricted, but it was very intense and it caused by direct contact with hyphae in some cells (Figs. 12,15). In Figs. 15-17, it can be seen that cell corners were severly degraded without appreciable degradation to adjacent cell wall and compound middle lamella. In advanced stage of cultivation, hyphae produce cavities and fissures not only in fibre but also in vessel and middle lamella (Figs. 18-20). However, the cell walls adjacent to the intensely degraded areas often are not noticeable degraded (Figs. 12,15,17).

Degradation of cell wall in oak wood, in general, occurred in close contact with the hyphae (Figs. 6-10,15,16,19). Hyphal sheaths are present around hyphae of *L. edodes* within the lumina of wood cells (Figs. 8,16). The sheath extended some distance from the hyphae and cell walls were degraded beneath it (Figs. 8,16). Based on the chemohistological studies, lignin in secondary wall and polyphenolics in the axial parenchyma were not detected at the advanced stage of cultivation, whereas middle lammela showed the positive reaction in the phloroglucin/HCl staining test.

4. DISCUSSION

The SEM techniques have been used to study fungal growth in wood[2,5,13]. The present studies have been undertaken on the micromorphological level using SEM in order to understand how the attack on oak wood is carried out by *L. edodes*.

Microscopical investigations showed that the hyphae of *L. edodes* first colonized the vessels extensively and then entered neighboring tissues. Barvery[7] suggested that hyphae grow preferentially through the largest available voids in the substrates during the initial period of passive vegetative growth. The natural pits in the wood elements appeared to be preferred for the ramification of hyphae from the initial major pathways into the adjacent tissues. Four possibilities of propagation of the mycelium have been proposed by Radtke et al[35]. However these possibilities could be changed according to the fungus and the substrate. Pits are not essential for the passage for hyphae of *L. edodes* which can produce bore holes perpendicular to the cell axis. The formation of bore holes was active during the late stage of cultivation as shown in Figs. 9 and 10.

Micromorphological changes caused by *L. edodes* in oakwood are quite complex in comparision with other white rot fungi. There are

similarities in the pattern of attack in other white rotters. These include the erosion troughs beneath the hyphae at the early stage of cultivation, the progressive thinning of cell walls, the cell wall separation, and the formation and enlargement of bore holes at more advanced stage of degradation[2,9,13,29,36,40]. The formation of hyphae impression in cell wall at the early stage of degradation suggests that enzyme diffusion is restricted to the immediate vicinity of the hyphae[29].

There are also differences, particularly in cavity formation and the preferential attack of cell corners. The formation of cavities in the S_2 layer are usually associated with soft-rot fungi. However, in the present studies such cavities are formed by white rot fungi. It is well known that one fungus is capable of different types of attack in wood and this might be attributed to host cell type and nutrients as well as genetic and physiologic differences in fungus[32]. Such cavity-shaped fissures have been also found in the walls of white-rotted and brown-rotted wood[8,12,20,30].

SEM observations indicate that the attack on cell corners in oak wood by *L. edodes* was considerably faster than that on middle lamella. Our observations conflicted with those of other authors, who reported that the cell corners were the last to be destroyed[4,29,32,36,40]. The faster degradation of cell corners may be attributed to their chemical nature of lignin found in these regions. Recent investigations indicate that the chemical nature of lignin in wood may be more important than their amount[14,18]. It is apparent that the guaiacyl: syringyl ratio of lignin is different in the different morphological regions. According to the studies published so far, cell corner-lignin consisted entirely of guaiacylpropane unit[6,10,17,34,38,39]. Despite of high amount of guaiacyl lignin in cell corner, these regions were considerably attacked by *L. edodes*. This result suggests that the chemical nature of the lignin in cell corner might be different from that in middle lamella in oak wood. One point to be mentioned is that the values on lignin composition in wood were varied with the determining methods[11].

The high guaiacyl lignin contents in vessel of oakwood could be also responsible for the lack of degradation in these areas, while the easier degradation of fibre walls for a syringyl-rich lignin fraction. Recent studies suggested that white-rot fungi were better adapted to utilize the syringyl lignin residues than guaiacyl residues[18,21,25,28]. Thus, the slower attack to vessel walls and middle lamella may result from difficulty in utilization of the guaiacyl lignin in these tissues.

In contrast, the marked reduction of colonization and degradation of axial parenchyma seems to be not related with the lignin composition, but with the presence of polyphenolics deposited in these cells. Oakwood in general has a high content of phenolic compounds and that many of them are colored. Due to their resistance to the *L. edodes*, it could be deduced that the polyphenolic substances in parenchyma cells would have an effect on the restriction of growth of *L. edodes*.

The micromorphological changes in cell wall also provide important information regarding the mechanism of ligninolytic activity, which is key to wood degradation by white rot fungi. *L. edoes* is classified as a simultanous white-rotter because it dagrades and metabolizes lignin and carbohydrates at approximately the same rate[22,27,31]. Blanchette et al[5] have provided a schematic drawing of the decay process caused by simultaneous white rot fungi. Our results indicate that the degradation by *L. edodes* does not follow this type of attack. From the preceding observations it could be deduced that ligninolytic activity of *L. edodes* might be increased up to 3 year-cultivation and them decreased with the time of cultivation. Generally, simultaneous white-rotters have not a high activity in lignin decomposition[3,24,27,36].

The act of hyphal sheath around the hyphae of *L. edodes* was not investigated in this study. However, it should be mentioned that sheaths around wood decay fungi hyphae may serve as media for housing and transporting lignocellulose

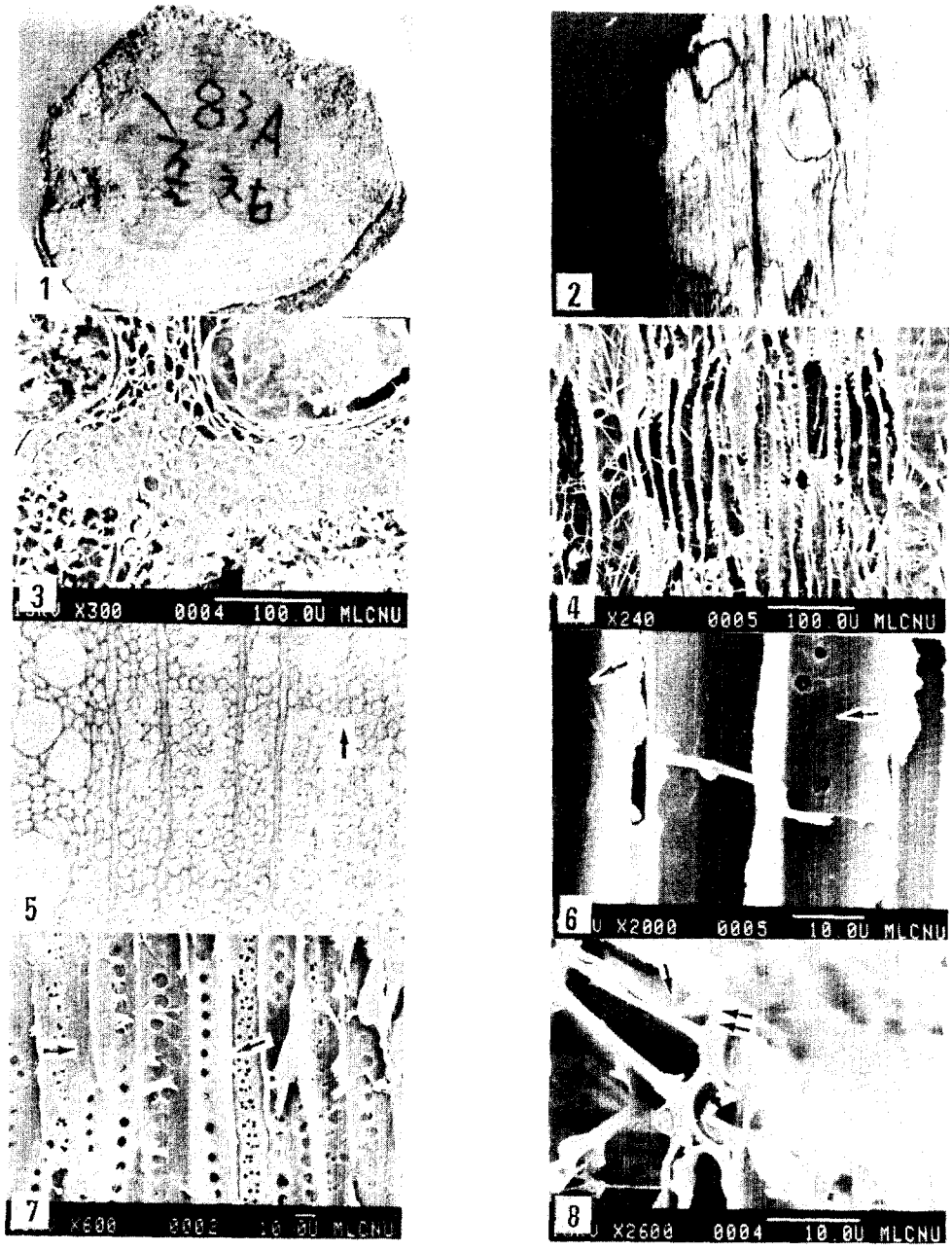
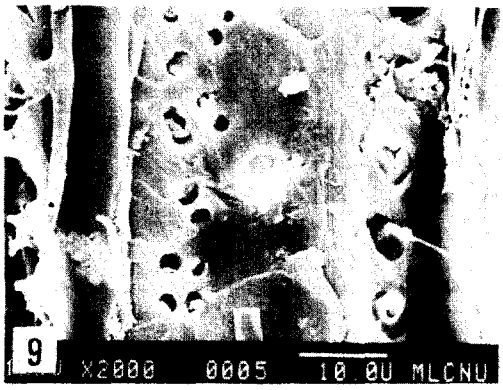


Fig. 1. Bleaching out of dark-colored heartwood after three-year cultivation with Shiitake. Fig. 2. Zone line around the degraded areas. 3. SEM-micrograph of zone line. Note the cells filled with melanin-like substances in zone line, (x 300). 4. Distribution of hyphae at early stage of cultivation, (x 240). Fig. 5. Preferential degradation of fiber cells. Vessels and parenchyma cells (arrow) are not intensely decomposed, (x 200). Fig. 6 Penetration of hyphae through pit and lysis furrows (arrow) along the cell wall surface (x 2,000). Fig. 7 Erosion troughs (arrow) in fiber and parenchyma, (x 600). Fig. 8. Thining of secondary wall from the cell lumen towards the middle lamella. Note the extened hyphal sheath (arrow) and irregular eroded cell wall (double arrows), (x 2, 600).



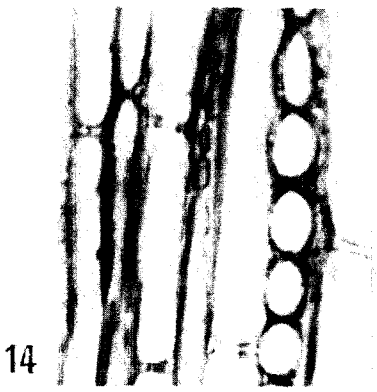
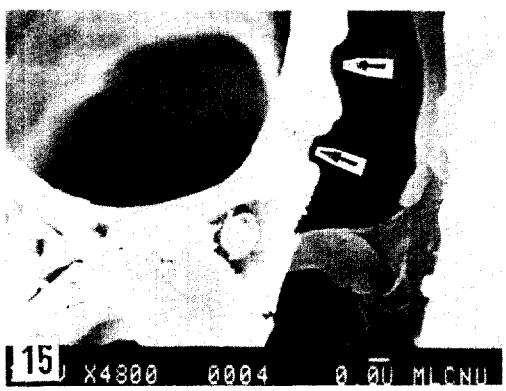
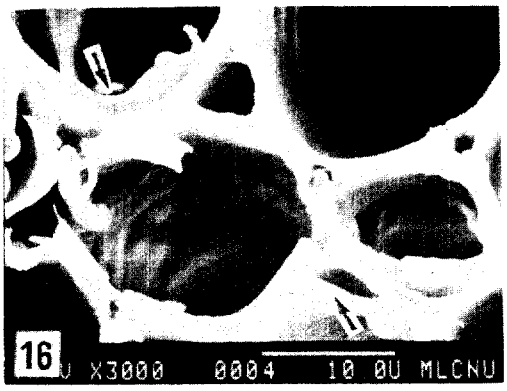
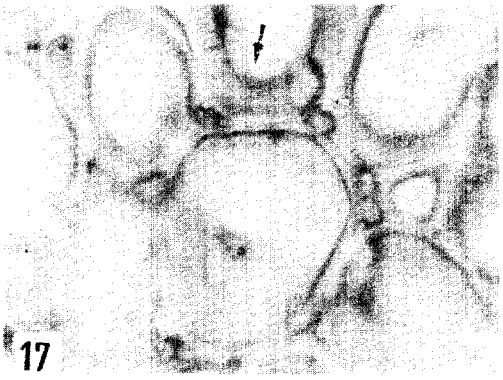


Fig. 9. Formation of bore holes through cell walls (x 2,000). Fig. 10. Enlargement of bore holes (x 2,000). Fig. 11. Preferential degraduation of S_2 layer (arrow). Note the intact S_3 layer (x 1,200). Fig. 12. Fungal attack to the transition layer between S_1 and S_2 layers. Note the perforations in S_2 layer (arrow) and the presence of hyphae in cell corner (double arrows) (x 4,000). Fig. 13 and 14. Cavities in vessels (x 600) and fiber walls (x 600).







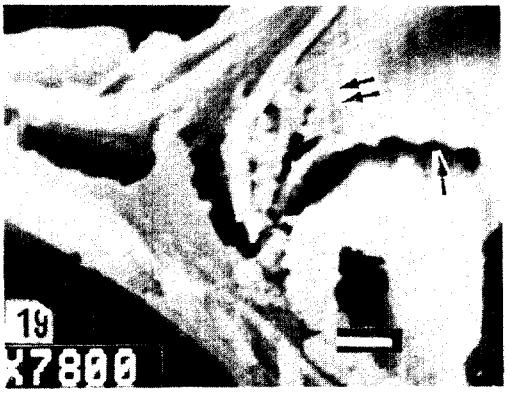


Fig. 15. Degraduation of cell corners by hyphae. Note the adjacent cell wall and middle lamella without any appreciable degradation and the irregular erosion of cell wall (arrow) (x 4,800). Fig. 16. Preferential attack of cell corners by hyphae and hyphal sheath (arrow) (x 4,000). Fig. 17. Degraded cell corners. Note the adjacent cell walls without any noticeable degradation (arrow) (x 600). Fig. 18. Formation of cavities along the microfibril angle and fissures (x 2,000). Fig. 19. Degradation of cell corner with irregulary degraded surface (arrow). Note the ball-like unidentified substances (double arrows) (x 7,800). Fig. 20. Perforations and degradation in transition layers between S_1 and S_2. Note the small cavities in middle lamella and serrate cell wall surface (x 5,400).

depolymerizing agents and also serve as cources of support and nutrition[19,20,33]. Further studies are also needed for the clarification of various degradation patterns caused by *L. edodes* in oakwood.

5. CONCLUSION

The microscopic study on the oakwood cultivated with Shiitake mushroom has shown that degradation occurs first in fibre cells, while vessels and axial parenchyma cells remain intact to fungal attack even at the advanced stage of cultivation.

The most conspicuous change in structure of the oak wood was the thinning of secondary walls from the lumen outward toward the middle lamella and the cell wall separation between the S_1 and middle lamella. Another feature of degradation was the formation of cavities within the secondary walls and the considerable susceptibility of cell corners to fungal attack in comparision with the middle lamella. The fungal system of *L. edodes* responsible for degradation of oakwood created the microscopic characteristics of white- and soft-rot fungi.

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