Preliminary

Degradability of Natural and Synthetic Polymers by The White Rot Fungus, *Ceriporiopsis subvermispora*[*1]

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Introduction

Use of various kinds of polymers in modern life has caused serious environmental problems due to accumulation of undegradable polymers. White rot fungi, aggressive degraders of lignin, are potential microbes to degrade these waste polymers. For instance, polyethylene and Nylon-66 are reported to be degraded by white rot fungi, *Phanerochaete chrysosporium, Trametes versicolor* and IZU-154[1,2]. Degradation of lignopolystyrene graft copolymers and polyvinyl chloride by *Pleurotus ostreatus, P. chrysosporium* and *T. versicolor* are also reported[3,4].

White rot fungi produce extracellular ligninolytic enzymes, two heme-containing peroxidases, lignin peroxidase (LiP) and manganese peroxidase (MnP). They also produce a copper-containing phenol oxidase, laccase (Lac). In the presence of hydrogen peroxide, LiP oxidizes both phenolic and non-phenolic lignin model compounds, while MnPs oxidize Mn(II) and phenolic substrates[5]. In the presence of lipids, however, MnP oxidizes the lipids to decompose non-phenolic lignin model compounds[6,7] by generating free radicals[8]. Lac oxidises phenolic substrates but it can also degrade recalcitrant non-phenolic lignin models in the presence of enzyme mediators such as 1-hydroxybenztriazole (HBT) and 2, 2′-azinobis(3-ethylbenzthiazoline-6-sulfate) (ABTS)[9,10].

A white rot fungus, *Ceriporiopsis subvermispora* is known to degrade lignin selectively without erosion of wood cell walls. Lipid peroxidation has been proposed as a possible ligninolytic system of this fungus[11] and evidence for the involvement of this system in wood decaying process was reported[12]. The fungal ligninolytic system at a site far from enzymes is attractive for the waste polymer treatments. Therefore, we examined degradation of several natural and synthetic polymers by the selective white rot fungus, *C. subvermispora,* together with *Dichomitus squalens.*

Thirteen polymers, ABS resin, acrylic resin, bakelite resin, chloroprene rubber, natural rubber, polycarbonate, polyethylene, polyethylene terephthalate, polypropylene, polystyrene, polyvinyl chloride, silicone rubber and teflon were cut into 20 mm square sheets, rinsed with distilled water and dried for 24 hours at room temperature. Sea sand (30–50 mesh) which had been conditioned to moisture content 45% was put into 300ml Erlenmeyer flasks. Beech wood chips (10 g) containing 10 ml of a medium composed of glucose (7 mg/ml, Wako Pure Chemicals) and corn steep liquor (7 mg/ml, Nacalai Tesque) was added on the sea sand. Acrylic resin, bakelite resin, chloroprene rubber, natural rubber, polycarbonate, polyethylene resin, polypropylene, polyvinyl chloride, silicone rubber and teflon were placed on the beech wood chips and autocleaved. The other polymers were sterilized with 70% ethanol and placed on the medium aseptically.

C. subvermispora FP90031, and *D. squalens* CBS432.34 were maintained on potato dextrose agar (PDA; Nissui) plates at 4°C. The fungi were inoculated on new PDA plates and precultured for 1 week at 28°C. Five pellets (6 mm in diameter) from the preculture were inoculated onto the wood chip medium. Cultivation was carried out statically at 70% humidity for 250 days. After cultivation for 100, 150, 200 and 250 days, each polymer was recovered, and fungal mycelia covering the polymer surface was carefully removed. The polymers were washed with water and weighed after drying with vacuum pump.

During cultivation of the two fungal strains, we found that addition of natural rubber sheets (NRS) markedly accelerated mycelial growth of *C. subvermispora* after 1 month. The rapid growth of hyphae was not observed in the cultures containing the other polymers. Weight loss of the NRS treated by *C. subvermispora* reached about 35% after cultivation for 250 days. Prolonged cultivation over 250 days deteriorated the NRS to such an extent that accurate weight loss of the rubber sheet was not measurable. On the other hand, weight loss of NRS by *D. squalens* was below 5% after 250 days. Thus, marked differences in the vulcanized rubber sheet degradation were observed between *C. subvermispora* and *D. squalens.* Weight loss of the other polymers by these two fungi were close to that of the control experiments without innoculation. Scanning electron microscopy (JSM 35C; JOEL) clearly demonstrated that the surface of the NRS treated by *C. subvermispora* was intensively damaged.

Waste rubber such as used tire has caused serious waste disposal problems, although a part of the waste tires are recycled as a raw rubber material or converted to energy in

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power plants and cement factories. One particular concern on this problem is unlawful disposal of a huge number of tires, which causes air pollution of SO_x and smoke when they are combusted by autoignition.

Degradation of natural rubber such as latex glove by *Actinomycetes*[13,14], Bacteriomycetes[15,16] and Ascomycetes[15] have been reported. In this study, however, we found that white rot fungi have also great potentials to degrade natural rubber. It should be noted that *C. subvermispora* degraded vulcanized NRS with 1 mm thickness under ligninolytic conditions. Research is in progress to elucidate the degradation mechanisms of the NRS by *C. subvermispora*.

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