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Detection of Extracellular Enzyme Activities in *Ganoderma neo-japonicum*

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The ability of *Ganoderma* to produce extracellular enzymes, including β-glucosidase, cellulase, avicelase, pectinase, xylanase, protease, amylase, and ligninase was tested in chromogenic media. β-glucosidase showed the highest activity, among the eight tested enzymes. In particular, *Ganoderma neo-japonicum* showed significantly stronger activity for β-glucosidase than that of the other enzymes. Two *Ganoderma lucidum* isolates showed moderate activity for avicelase; however, *Ganoderma neo-japonicum* showed the strongest activity. Moderate ligninase activity was only observed in *Ganoderma neo-japonicum*. In contrast, pectinase, amylase, protease, and cellulase were not present in *Ganoderma*. The results show that the degree of activity of the tested enzymes varied depending on the *Ganoderma* species tested.

KEYWORDS : Chromogenic media, Extracellular enzymes, *Ganoderma neo-japonicum*

Numerous studies have isolated and purified high-quality enzymes from fungi [1]. Mushrooms, like all other fungi, lack chlorophyll and are nongreen organisms. Thus, they cannot covert solar energy by photosynthesis to organic matter as in green plants, but they do produce an extensive array of enzymes that degrade lignocellulosic materials for growth and fruiting [2]. The genus *Ganoderma* is a fungi famous as a tonic in traditional Asian medicines because of its characteristics and biological activities and is also a very important genus economically [3]. *Ganoderma* species grow on wood and are classified as wood-decaying fungi. Wood-decaying fungi degrade cellulose, hemicellulose, and lignin, the wood cell wall substrate, by secreting cellulase, hemicellulase, and ligninase [4, 5].

Hong *et al*. [6] extracted a cellulase from *Pleurotus sajor-caju,* and Hashimoto [7] isolated a carboxymethyl cellulase from *Pholiota nameko*. Ro [8] and Min *et al*. [9] produced and purified a protease from *Fomes fomentarius* (Fr.) Kicky and *Pleurotus cornucopiae*, respectively. Additionally, laccase was produced from *Flammulina velutipes* by Lee and Suh [10]. These results suggest that *Ganoderma* may have a variety of extracellular enzymes such as cellulases, ligninases, and proteases. However, little information on the activity of extracellular enzymes in *Ganoderma* has been reported. Therefore, we chose *Ganoderma neo-japonicum* and *Ganoderma lucidum* to test for enzyme activity. We used a method based on a dye coupled to a polysaccharide to detect the extracellular enzymes secreted from the fungi [11, 12].

Three *Ganoderma* species were prepared from the Korean Agricultural Culture Collection (Suwon, Korea) and from our laboratory. All cultures were precultured on potato dextrose agar (Difco, Franklin Lakes, NJ, USA) at 25°C for 5 days, and the precultures were transferred to chromogenic media [13]. A 0.1% yeast nitrogen base (Difco) as a nitrogen source and 1.5% agar powder were used as the basic media substrate. Congo Red (0.5%; Sigma, St. Louis, MO, USA) was used as the chromogenic dye, media pH was adjusted to 7.0, and the incubation temperature was controlled at 25°C. One of the following substrates was included as a carbon source in the basic media: 0.5% CM-cellulose (Sigma) for CM-cellulase, Avicel (Fluka, Arklow, Ireland) for avicelase, D-cellobiose (Sigma) for β-glucosidase, xylan from brichwood (Sigma) for xylanase, polygalacturonic acid (MP Biomedical, Vannes, France) for pectinase, lignin (Sigma) for ligninase, starch from potato (Duchefa, Haarlem, The Netherlands) for amylase, and skim milk (Fluka) for protease. To correctly identify extracellular enzyme activity, the cellulolytic fungi *Trichoderma* was used as a positive control, and *Saccharomyces* was used as a negative control.

After a 5 day incubation at 25°C, the activity of each enzyme was estimated by observing the clear zone formed around the fungal colony resulting from the reaction between

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Table 1. Detection of extracellular enzyme activities in *Ganoderma* species using the chromogenic reaction

Species	β-glucosidase	Cellulase	Avicelase	Pectinase	Xylanase	Protease	Amylase	Ligninase
G. neo-japonicum (GBGN-01)	S	W	S	W	M	W	W	M
G. lucidum (ASI 7039)	M	M	M	W	W	W	W	W
G. lucidum (GBGL-01)	M	W	M	W	W	W	W	W
Saccharomyces	N	N	N	N	N	N	N	N
Trichoderma	S	S	S	S	S	S	W	W

S, strong activity group (1.0~1.5 cm); W, weak activity group (0.1~0.5 cm); M, moderate activity group (0.5~1.0 cm); N, no activity group (< 0.1 cm).

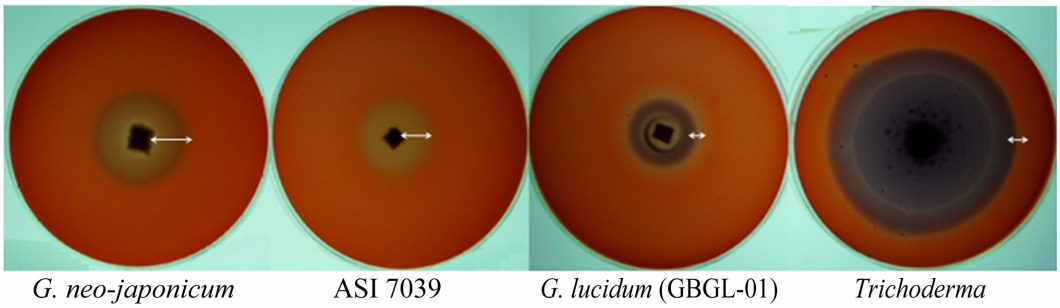

Fig. 1. Examples of β-glucosidase activity on chromogenic media by different *Ganoderma* species. Bars indicate clear zones.

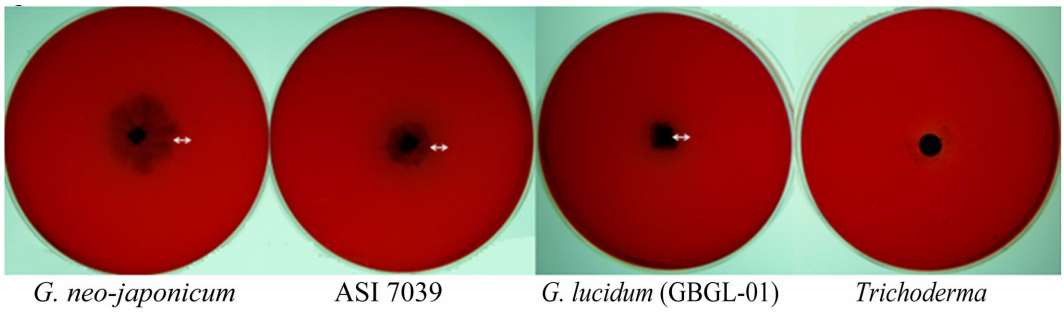

Fig. 2. Examples of ligninase activity formed on chromogenic media by different *Ganoderma* species. Bars indicates clear zones.

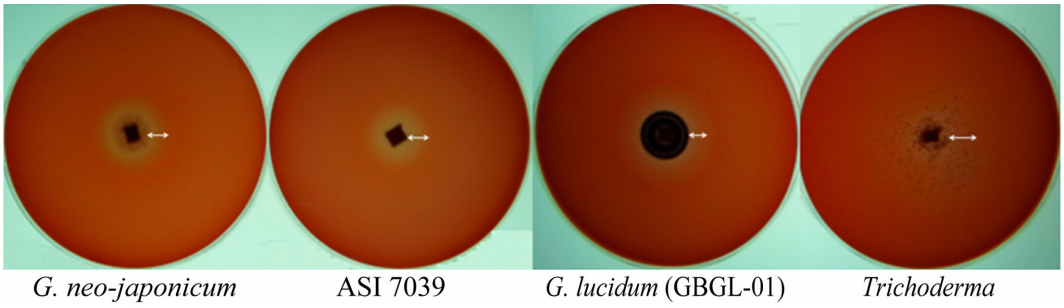

Fig. 3. Examples of avicelase activity formed on chromogenic media by different *Ganoderma* species. Bars indicates clear zone.

the enzymes secreted by the fungi and the chromogenic substrates. The clear zone was measured from the end of the fungal colony to the end of the color change zone and was divided into the following four groups: no activity group (N, 0~0.1 cm), weak activity group (W, 0.1~0.5 cm), moderate activity group (M, 0.5~1.0 cm), and a strong activity group (S, 1.0~1.5 cm). These tests were repeated three times.

The results of the extracellular enzyme activity testing for *G. neo-japonicum* and *G. lucidum* are shown in Table 1. Among the eight tested enzymes, β-glucosidase had more activity than that of the other enzymes. In particular, *G. neo-japonicum* showed significantly strong activity. Fig. 1 shows several examples of β-glucosidase detection on chromogenic media. Only *G. neo-japonicum* showed activity in the lignase assay (Fig. 2). Strong activity for avice-

lase was only observed in *G. neo-japonicum*, whereas the other species showed moderate activity (Fig. 3). In contrast, pectinase, amylase, and cellulose tended to show weak activity. *G. neo-japonicum* had moderate xylanase activity, and *G. lucidum* ASI 7039 showed moderate cellulase activity.

When we compared the *Ganoderma* species for β-glucosidase and avicelase activity, only *G. neo-japonicum* had strong β-glucosidase activity and a strong ability to produce avicelase. However, the *Ganoderma* species showed moderate activity. In conclusion, we tested *G. neo-japonicum* and two *G. lucidum* isolates for eight extracellular enzyme activities using chromogenic media. There appeared to be differences in the production of these enzymes among the tested *Ganoderma* species. In most cases, they showed high activity for enzymes related to degrading cellulose such as β-glucosidase and avicelase. Studies of the biological, chemical, pharmacological, and clinical applications of *Ganoderma* have been extensively reported, but there is limited information on industrial applications such as the use of extracellular enzyme activity. Thus, these data will be useful to apply high-quality enzymes derived from an edible mushroom such as *G. neo-japonicum* to industries, including food processing, brewery, biofuels, and bioremediation.

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