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Enzyme Activities of the Fruit Body of *Ramaria botrytis* DGUM 29001

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The fruit body of *Ramaria botrytis* DGUM 29001 was used to determine enzyme activities of fruit body. The specific activity of laccase was the highest (6.5 unit/mg · protein) and that of α-amylase and xylanase was relatively high. However, little or no enzyme activity of β-glucosidase, CMCase, exo-β-1,4-glucanase, chitinase, lipase and protease was found.

KEYWORDS: Enzyme activity, Fruit body, *Ramaria botrytis*

Ramaria botrytis is an ectomycorrhizal fungus in the broadleaf forest, which widely distributes out mostly around the plateaus and mountains of the Eastern Asia, Europe, and North America (Lee, 1988; Kim *et al.*, 1989; Park *et al.*, 1997). It is distinguished by its size and white to creamy, vinaceous tipped, branches which fade in age. Especially, the vinaceous-tipped coral fruit body has been regarded as an excellent edible mushroom with fruit scent and chicken breast meat taste. So far, there has been a few information of the Genus *Ramaria*, e.g., tasty constituents and minerals of *R. botrytis* (Seoh *et al.*, 1974; Pyo *et al.*, 1975), anticancer activity of *R. formosa* against sarcoma 180 implanted in mice (Yoo *et al.*, 1982), and immunomodulating activity and anticancer activity of *R. botyritis* extract (Kim *et al.*, 1995; Kim *et al.*, 1999). However, no information has been available, so far, upon the culture condition and physiological characteristics of *R. botrytis*. In this study, various enzyme activities of the fruit body of *R. botrytis* DGUM 29001 were investigated to compare with those of the mycelia and to further use for artificial production of this mushroom.

The fruit body of *R. botrytis* (Fig. 1) used was collected from Mt. Namsan, Gyeongju during the period from September to October 1999. The fruit body and spores were identified by the taxanomy keys of mushrooms published by Lee (1988), Kim *et al.* (1989) and Park *et al.* (1997) and named as *Ramaria botrytis* DGUM 29001. The fruit body of *R. botrytis* was observed to be a coralloided having intricately branched from a large fleshy base and the stipe was observed to be white and tapered downward. The spores were observed to be elliptically elongated with longitudinally striated and pale yellowish-brown.

For determination of enzyme activities, the fruit body was washed with distilled water for 3 times and homogenized for 6 times of 20 sec using a homogenizer (Braun Co., Model MR-500-MCA). And then, the unbroken debris of the fruit body were completely broken using a French press (Fred S. Carver Inc.) under the pressure of 15,000 psi. The homogenate then was centrifuged (Vision Co.) at 5000 rpm for 20 min and the supernatant was used as a crude enzyme solution.

Fig. 1. Morphological features of the fruit body of *Ramaria botrytis* DGUM 29001 from Mt. Namsan, Gyeongju.

The activities of α-amylase, β-glucosidase, CMCase, and exo-β-1,4-glucanase were determined by the methods of Uriyo and Eigel (1999), Tokao (1985), Kanda (1976), and Kim (1993), respectively. The amount of reducing sugar produced was determined by DNS method (Miller, 1959) with D-glucose as a standard. The activity of xylanase was determined by using the modified method of Kim (1995). The reducing sugar was detected by DNS method (Miller, 1959) with D-xylose as a standard. One unit of each enzyme was defined as the amount of enzyme that produced 1 μmol of reducing sugar in 1 min. For determination of chitinase activity, colloidal chitin was

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Table 1. The enzyme activities of fruit body of *Ramaria botrytis* DGUM 29001

Enzyme	Activity (μmol/ml/min)	Conc. of protein (mg protein/ml)	Specific activity (unit/mg · protein)
α-Amylase	13.6	2.23	6.10
β-Glucosidase	0.06	2.23	0.03
Xylanase	0.36	2.23	0.16
CMCase[a]	0.04	2.23	0.02
Exo-β-1,4-glucanase	0.03	2.23	0.01
Chitinnase	0	2.23	0
Lipasse	0.003	2.23	0.001
Protease	0.09	2.23	0.04
Laccase	14.5	2.23	6.50

[a]CMC; carboxy methyl cellulose.

prepared by using the method of Hsu and Lockwood (1975). Chitinase activity was determined by using the method of Jeong and Lee (1995). The reducing sugar was detected by DNS method (Miller, 1959) using a N-acetyl glucosamine (NAG) as a standard. One unit was defined as the amount of enzyme that produced 1 μmol of NAG in 1 min. Protease activity was determined by using the method of Braun and Schmitz (1980). One unit was defined as the amount of increase $ABS_{420\,nm}$×3.33. The activity of lipase was determined by using the method of Yang (1997). One unit was defined as the amount of enzyme that produced 1 μmol of *p*-nitrophenol in 1 min. Laccase activity was determined by using the method of Leonowicz and Grzywnowicz (1981). One unit was defined as the amount of enzyme catalyzing the oxidation of 1 μmol of syringaldazine to its quinone form per min at 25°C in 0.2 M sodium acetate buffer (pH 5.4), using a molar absorptivity of 65,000 for product. Protein concentration was determined using the method of Bradford (Bradford, 1976) with bovine serum albumin as a standard.

Among the extracellular enzyme activities tested (Table 1), laccase activity was the highest (6.52 unit/mg · protein). With respect to lignin degradation, many white-rot fungi produce extracellular laccase including *Phanerochaete chrysosporium* (Srinivasan *et al.*, 1995). Some ascomycetous fungi have been reported to degrade lignin, and certain of these produce laccase alone without either LiP or MnP activities (Barbosa *et al.*, 1996; Raghukumar *et al.*, 1994). Unfortunately, it is still unknown why this mushroom, known as an ectomyccorrhizal fungus, possess laccase activity. The activity of α-amylase was relatively high (6.1 unit/mg · protein). However, compared with that of *Tricholoma matutake* (Lee *et al.*, 1998), it was relatively lower. Xylan is the major hemicellulose component of plant cell wall, the enzyme activity of fruit body of xylanase was 0.16 unit/mg ·protein (Table 1). Little enzyme activities of β-glucosidase, CMCase and exo-β-1,4-glucanase of the fruit body were shown, e.g., 0.03, 0.02 and 0.01 unit/mg · protein, respectively (Table 1), compared with those of *Ganoderma lucidum* (Do and Kim, 1986), *Pleurotus ostreatus* (Hiroi and Ekriksson, 1970), *P. sajor-caju* (Madan and Bisaria, 1983) and *Irpex lacteus* (Kanda *et al.*, 1976). Little or no enzyme activity of lipase, protease and chitinase was found. Except for laccase activity, these results might conclude indirectly that *R. botrytis* is an ectomycorrhizal symbiont or parasite. These results will be helpful to determine the media composition for further cultivation of the mycelia isolated from the fruit body of *R. botrytis* DGUM 29001.

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