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Secretion of Membrane-Associated Laccase in Liquid Culture of *Coprinus congregatus*

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*Coprinus congregatus*의 세포막 연관 laccase의 세포외 분비

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ABSTRACT: The hyphal tip laccase of *Coprinus congregatus* which is a membrane-associated enzyme and shows different banding patterns on PAGE analysis when compared with the enzyme of liquid culture (Choi *et al.*, 1987) has been successfully secreted to culture medium in liquid shake culture by lowering the pH of medium to 4.0. When the fungus is cultivated in YpSs(pH 4.0) liquid, only the hyphal tip laccase is found in the medium after 6 hr incubation and there is no liquid-type enzyme when examined by PAGE analysis.

KEY WORDS *Coprinus congregatus*, **enzyme secretion, liquid culture**

Coprinus congregatus Fries has several different phenoloxidases during its growth and development (Ross, 1985; Choi *et al.*, 1987). Since all are associated with the cell membrane (Choi *et al.*, 1987; Choi and Ross, 1988, 1990), it has been difficult to isolate large amount of enzymes. Furthermore, the laccase of the hyphal tip grown on a agar medium has totally different electrophoretic mobility from that of liquid shake culture when compare by the native PAGE analysis (Choi *et al.*, 1987). Choi and Ross (1988) used a low-temperature-liquifying medium which was solidified with Pluronic Polyol F-127 (Gardener and Jones, 1984; polyol) in order to get protoplast which had the same laccase as the hyphal tip enzyme. Even though the polyol medium gave a positive result for the localization of the hyphal tip phenoloxidases (Choi and Ross, 1990), the biochemical studies of the enzymes had been stopped for long period.

It has been well known that simple alterations of a culture medium like the change in pH, temperature and nutrient concentrations can induce the metabolic changes. Ross (1985) reported that the laccase activity was decreased by the changes of the glucose concentration or the medium volume. We have changed the medium pH to examine whether there is any alteration in the laccase or not.

C. congregatus dilkaryon (cc13×cc6) was grown on YpSs liquid medium for 5 days and then macerated by waring blendor. This was inoculated to YpSs liquid medium which had different pH (3.0~7.0) and incubated in shaking incubator at 25℃. On day one and two, the enzyme activity in the culture filtrate was determined spectrophotometrically by using o-tolidine as a substrate (Ross, 1982). Only in the pH 4.0 and pH 4.5 media, there were significant amount of the enzyme activity (Table 1). In case of pH 4.5, even though the enzyme was detected, the induction rate was slower than the case of pH 4.0 (Fig. 1). As shown in the Figure 1, the enzyme was found at 6 hr after inoculation and started to decrease after day 2. We compared the extracellular laccase with the hyphal tip enzyme and with the normal liquid shake culture enzyme (pH 7.0), both of which were membrane associated ones (Choi *et al.*, 1987) by the native, non-denaturing PAGE analysis. The extracellular laccase showed identical mobility with the hyphal tip laccase as in the Figure 2.

Table 1. *Laccase activities of different pH media. Determination of protein concentrations was followed by the Lowry method (Lowry et al., 1951). Mean value of 3 replicates. (Specific activity: units/mg protein)*

Day	pH 3.0	pH 3.5	pH 4.0	pH 4.5	pH 5.0	pH 6.0	pH 7.0
1	0	0	40.2	34.9	0	0	0
2	0	0	127.4	131.6	0	0	0

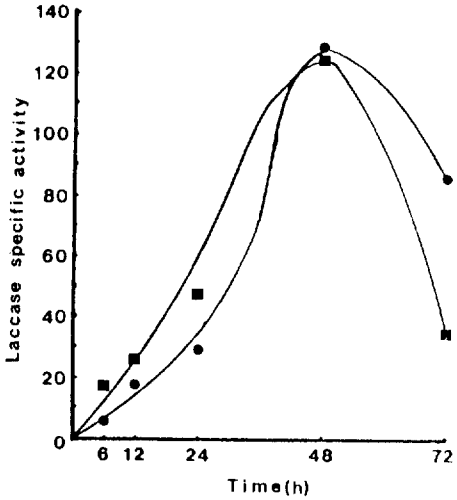

Fig. 1. *Production of laccase at the time indicated from two different cultures. Details are same as Table 1.*
square : laccase from pH 4.0 culture.
circle : laccase from pH 4.5 culture..

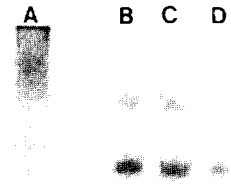

Fig. 2. *Native, non-denaturing PAGE analysis of the laccases from hyphal tip and liquid cultures.*
A : Laccase of the mycelial pellets from liquid culture (pH 7.0).
B : Laccese from liquid culture superntant (pH 4.0).
C : Laccese from liquid culture superntant (pH 4.5).
D : Laccese of the hyphal tips from agar culture.

The result postulates two important points. First, the role of hyphal tip enzyme in the development has been postulated that it is involved in the fixation step of light signal (Ross, 1985). In this study, the same enzyme is induced and secreted so rapidly in liquid by low pH medium. Cory (1967) has reported that a mushroom tyrosinase (one of phenoloxidases) exhibits a pronounced effect on the permeability of membrane: glucose uptake is inhibited 28% to 44% wherease glycine uptake is stimulated to 50%. It can be postulated that the enzyme works as a protection mechanism for the high concentration of proton as reported by Molitoris (1978). Secondly, the liquid-type laccase which is formed in an old culture (usually older than 6 days) is not found in liquid medium even though the medium pH is changed to 4.0 after the culture (pH 7.0) showed good enzyme activity. This tells that the extracellular laccase in the low pH medium is not secreted liquid-type laccase but *de novo* induced gene product. We do not know whether the laccase gene does respond to the proton or there is any regulatory system which reponds to proton and regulates the synthesis of a new enzyme. This system gives good research topic about the signal transduction at the molecular level.

적 요

한천배지상에서 자란 *C. congregatus*의 균사 끝 부분에 존재하는 세포막에 연관된 laccase는 버섯생성에 필요한 '빛' 자극의 전달에 관여하는 것으로 판단되며 액체 진탕배양시 균사체에 나타나는 laccase와는 native, non-denaturing PAGE로 비교분석할 때 전혀 다른 양상을 보이므로 효소의 대량 확보가 어려워 동 효소의 생화학적 특징 및 생리학적 기능을 규명하지 못하였다. 액체진탕배양액의 pH를 4.0으로 낮춤으로써 효소가 배양액으로 짧은시간에 대량 생성분비됨을 확인하였고, 전기영동방법으로 분석한 결과 한천배지상의 균사 끝 부분에만 존재하는 효소와 동일함을 확인하였다.

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