

목재부후균 (Wood Rot Fungi)

1. 백색부후균에 의한 유기용매 리그닌의 생물학적 변환 - 균 배양 및 기질 투입 (1주차 실험)

2. 백색부후균에 의한 유기용매 리그닌의 생물학적 변환- 유기용매 리그닌 구조 변화 분석 및 효소 활성 측정

■ Incubation

- White rot fungi (백색부후균)
 - *Phanerochaete chrysosporium* (PCH)
 - *Crustoderma flavesescens* (CRF)
 - *Abortiporus biennis* (ABB)
- Medium : Kirk's medium (SSC medium, nitrogen-limited)
- Incubation : 28°C, Organosolv lignin 접종 후, 10일 배양

■ Biomodification of Organosolv lignin

- ① 원심분리로 상등액과 리그닌+Fungal residue 분리
- ② 0.5 M NaOH와 Lignin+fungal residue의 반응을 통해 0.5 M NaOH에 리그닌만 용해시킴
- ③ 2M HCl을 이용하여 pH2 이하로 적정
- ④ 원심분리 후, 고형분 획득 ▶ 동결건조
- ⑤ FT-IR을 이용해 리그닌 관능기 변화 분석

■ Analysis of enzyme activity

● Laccase

- 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was used as substrate for the determination of laccase activity in extracellular culture media. The reaction mixture for laccase was prepared 0.85 mL of 0.2 M lactate buffer (pH 4.5) with 50 μ L of ABTS (0.08 g/L) and 100 μ L of culture medium to make total volume of 1 mL. The absorbance of that mixture at 420 nm wavelength was determined after 3 min of incubation at room temperature, and the enzyme activity was expressed using the equation below ($\epsilon_{420} = 36,000 \text{ M}^{-1}\text{cm}^{-1}$).

● Manganese-dependent Peroxidase (MnP)

- MnP assay was performed by using ABTS as substrate for measuring enzyme activity. The reaction mixture for MnP was prepared 0.8 mL of 0.2 M lactate buffer (pH 4.5) with 50 μ L of ABTS, 33 μ L of MnSO_4 , 100 μ L of culture medium, and 17 μ L of H_2O_2 . The absorbance at 420 nm wavelength was measured after 30 min. The enzyme activity was expressed using the equation below ($\epsilon_{420} = 36,000 \text{ M}^{-1}\text{cm}^{-1}$).

$$\text{Enzyme activity (unit / mL)} = \frac{\Delta\text{Absorbance} \times 10^6 \times \text{Total volume(L)}}{\Delta\text{Time} \times \epsilon_{420} \times \text{Sample volume(mL)}}$$

■ Report

- FT-IR의 각 파장대별로 나타내는 관능기 분석
 - UV-Vis spectrophotometer의 원리, 사용 분야 등 전반적인 것을 조사할 것.
 - 세 가지 fungi의 효소 활성 결과와 FT-IR의 결과를 종합하여 여러 가지 방향에서 비교추론해볼 것.
 - Laccase, Manganese-dependent peroxidase (MnP)가 어떠한 기작으로 기질에 작용하는지 조사해올 것.
- ※ 반드시 reference 추가할 것