Optimization of laccase production by white rot fungus, *Trametes sp.*

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Introduction

Laccase(-diphenol: dioxygen oxioreductase; EC 1.10.3.2) is one of the few enzymes that has been the subject of continuous study. Because of the capability of catalyzing the oxidation of phenol, laccase is receiving increased attention in potential biotechnological applications. Production of laccase is affected by many typical fermentation factors such as medium composition, carbon and nitrogen ratio, pH, temperature, etc. Low concentrations of several laccases are produced constitutively on wood and in submerged fungal culture, while higher concentration are produced by addition of inducers. Several attempts have been made to find inducers of laccase synthesis by different fungi. In this context, our work has been focused on the optimal conditions for laccase production by *Trametes sp.*, a kind of white-rot fungi.

Materials and Methods

In the liquid media, mycelial discs of about 1cm diameter were cut from the zone of the mycelium on malt agar plates and transferred to 300ml Erlenmeyer flasks each containing 100ml of YMPG liquid mediu. And mycelium was grown for 5days at 28■ under 200rpm of agitation. After 5days, the mycelium was then ground by a homogenizer for 30s to break up and to make the pellets homogeneous. For suspended cultures, 10ml of the homogenized mycelial pellets were used to inoculate 300ml flask containing 100ml of laccase-production medium consisting of 20g glucose, 4g ammonium tartrate, 2g KH_2PO_4, 0.5g $MgSO_4$, 0.1g $CaCl_2$, 0.0001g Thiamine HCl, 0.0001g Biotin, and 10ml mineral stock solution per liter of demineralized water. The medium was buffered in pH 4.5 by succinic acid. Incubation was carried out at 28■ under 200rpm of agitation. The initial concentration of *Trametes sp.* was adjusted to about 0.3g DCW/L. After 24hr-cultivation, the inducers were added to the medium. For

determination of laccase activity, samples of the culture liquid were taken daily. Also, repeated batch cultures using free cell with shake-flask were tested.

Results and Discussion

In order to reach a high laccase activity, the optimum conditions for production of laccase produced by *Trametes sp.*, a kind of white rot fungi, were investigated. Among various carbon sources and nitrogen sources, glucose and ammonium tartrate showed the highest potential for laccase production. The optimum concentration for laccase production was 20g/L glucose and 4g/L ammonium tartrate. When 1.0mM of 2,5-xylidine together with 1.0mM of ABTS was added as inducer to the medium after 24hr-cultivation, the production of laccase was 3.6 times higher than that in the absence of the inducer. The optimum pH and temperature conditions for laccase production were pH 4.5 and 28[redacted], respectively. Under these conditions, the experiment was made to compare laccase activity using immobilized cell and free cell with shake-flask batch culture on a time culture of 10days. The highest level of laccase activity was observed between the fourth and the sixth days of growth. The total laccase activity using immobilized cell in shake-flask batch culture was 7,800U/L, whereas the laccase activity using free cell peaked at about 14,600U/L. Also, it was possible to produce laccase with high activity for at least eight successive repeated batch cultures using free cell with shake-flask fermentation. The use of eight successive repeated batch cultures made possible an increase in the laccase activity from about 12,000U/L to 19,600U/L on the culture of 45days.

References

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