PURIFICATION OF AGARICUS BISPORUS EXTRACELLULAR LACCASE FROM MUSHROOM COMPOST

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SUMMARY

The extra-cellular laccase of the edible mushroom Agaricus bisporus was purified from non-axenic cultures of the fungus on mushroom compost. Quantities of up to 100 mg of pure enzyme were obtained from single purification runs. Purity of the enzyme protein was comparable to that previously obtained from axenic liquid culture supernatants.

INTRODUCTION

Commercial mushroom compost is a bulk low cost solid substrate growth medium which is selective for Agaricus species. When colonised with Agaricus bisporus mycelium, it is a source of many oxidative and hydrolytic extra-cellular enzymes, the most abundant of these being laccase (Wood and Goodenough, 1977). The extracellular laccase of A. bisporus has previously been purified from compost and liquid culture supernatants in milligramme quantities (Wood, 1980a and b). The simplified method we describe here has routinely produced single batches of 50-100 mg pure enzyme from compost extracts. Purity of the final preparation is comparable to that previously obtained from axenic liquid culture.

METHODS

Laccase Extraction

Mushroom compost prepared by the method of Randle (1974) and inoculated with A. bisporus mycelium, strain D649 (Darmycel Ltd., Angmering, UK). Two weeks after inoculation as the laccase activity approached its maximum (Wood and Goodenough, 1977), 3 Kg compost was extracted by steeping for 20 min in 6 ℓ chilled deionised water. The liquor was then decanted, strained through Tygan T975 filter fabric, 1 mm mesh (Fothergill and Harvey, Littleborough, UK) and clarified by centrifugation at 15000 xg using a Sharples ZA continuous centrifuge (Pennwalt Ltd., Camberley, UK).

Purification and Analysis

A slurry of DE 52 DEAE ion exchange cellulose (Whatman Labsales Ltd., Maidstone, UK) was prepared by suspending 100g of solid in 1 ℓ of 0.2M sodium acetate and adjusting the pH to 5.6 with 0.2M acetic acid. This was mixed with the clarified compost extract in the proportions of 30g DE52 solid to 1 ℓ
supernatant. The DE52 was kept in suspension for 20 min by continuous stirring with a paddle stirrer, by which time typically >60% of the enzyme was absorbed. The mixture was filtered through 'Miracloth' (Novabiochem [UK] Ltd., Nottingham, UK) to recover the DE52 which was then resuspended in a small volume of 0.05M sodium acetate buffer pH5.6 and packed into chromatography columns 40 mm dia x 210 mm long. The enzyme was eluted from the columns by flushing with two bed volumes of 0.2M NaCl in 0.05M sodium acetate buffer pH4.0. The fraction of the eluate containing laccase activity was concentrated two-fold by ultra filtration on an Amicon PM10 membrane.

Final purification was by gel filtration chromatography on a column of Sephacryl S-200 with a bed height of 800 mm and 50 mm dia. The eluent was 0.05M sodium acetate buffer pH5.6 pumped at a constant 2 ml/min with a peristaltic pump. The eluate was collected in 10 ml fractions, protein concentration was estimated from measurement of optical density at 280 nm and 260 nm (Warburg and Christian, 1942). Enzyme activity was measured by oxygen electrode using p-phenylenediamine substrate (Wood and Goodenough, 1977). Pure fractions, selected on a specific activity basis (Wood, 1980a) were pooled and concentrated by ultrafiltration.

The final preparation was further assayed for protein by the above method and also the method of Bradford (1976) standardised using bovine serum albumin fraction 5 (Sigma Chemical Co. Ltd., Poole, UK). Enzyme activity was also determined as described above. Sub-samples of 1 ml were pipetted into glass vials and freeze-dried along with buffer only controls for dry-matter determination. Electrophoretic analysis on PAGE gels was performed as described by Wood (1980b).

RESULTS AND DISCUSSION

Data from a typical purification run is summarised in Table 1. The concentration of activity in the crude extract was an order of magnitude lower than that obtained by Wood (1979) from the extraction of 100g compost samples. This may be explained both by the use of a less vigorous extraction procedure and by incomplete colonisation of the compost. However, the concentration was comparable with that found in liquid culture supernatants (Wood, 1980a). The batch adsorption technique facilitated a rapid 16-fold concentration in enzyme activity which allowed the elimination of ammonium sulphate precipitation and reduced the time taken in ultra-filtration. We found it convenient to extract 15 Kg batches of compost, clarify by centrifugation and perform the DE 52 procedure, then to store the enzyme frozen in aliquots of 100-200 ml prior to gel filtration. These quantities were optimal for a single gel-filtration run. If more than an hour was allowed to elapse between adsorption on to DE 52 and elution, recovery would decline. Storage of the enzyme at -20°C for periods of more than a month resulted in losses of activity of up to 86%, though no change in electrophoretic properties was observed. This explains the low specific activity of the enzyme from which the Sephacryl S200 elution profile was obtained (Fig. 1).

The activity peak from the S200 column was collected at an elution volume between 675 ml and 815 ml, 215 ml after the void volume. Peak activity coincided exactly with peak protein concentration (Fig. 1). 5-6 h running time were required to elute the enzyme and a further 10 h elution to clear the column of low molecular weight impurities.

The laccase containing fractions had the characteristic blue-green colour of a