Optimisation of production of extracellular non-haem peroxidases by Thermomonospora fusca BD25 in aerobic bio-reactor conditions

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Peroxidases are involved in redox reactions [1-2] and in particular the redox reaction of haem-containing horseradish peroxidase has been exploited in a diagnostic procedure such as serum cholesterol determination [3]. The current chemical techniques for the determination of serum cholesterol is subjected to various interference [4-6]. The use of biological catalysts such as horseradish peroxidase has substantially improved the results [7]. However, as horseradish peroxidase contains labile haem, it is subjected to denaturation by temperature and could give rise to falsely low results. The problem can be circumvented by using a thermostable peroxidase enzyme such as Thermomonospora fusca BD25 peroxidase [9]. However, the availability of this peroxidases is limited, since the enzyme is expressed at a very low level by T. fusca BD25 and detection in the extra-cellular supernatant is often difficult [10]. The problem can be overcome by optimisation of the growth conditions of T. fusca BD25 [11]. However, in-house optimisation could not allow precise monitoring of the growth parameters such as dissolved oxygen, temperature and pH. In this respect, the use of an automated bio-reactor would be helpful to resolve this problem. In this paper we describe the optimum bio-reactor conditions for the production of extra-cellular non-haem peroxidases.

Stock cultures of T. fusca BD25 were maintained as a suspension of spores and hyphal fragments in 20 % (v/v) glycerol at -20 °C and routinely cultured on L-agar plates or slants [12] with subsequent incubation at 45 °C for 48-72 hours or until sporulation had occurred. For peroxidase production in an automated bio-reactor suspensions, 50 ml (48 h old) liquid cultures of T. fusca BD25 was inoculated directly into a sterile production medium (1 L) based on that described by Ramachandra et al. [13]. The medium contained 8.0 g oat spelt xylan (Sigma); 6.0 g yeast extract (Oxoid); 0.1 g (NH4)2 SO4; 0.3 g NaCl; 0.1 g MgSO4?7H2O; 0.02 g CaCO3; 500 µl antifoam (204); 100 mM potassium phosphate buffer and 1 ml of trace element solution per litre of distilled-water, final pH of 7.5. The trace element solution contained 1.0 g FeSO4?7H2O; 0.9 g ZnSO4?7H2O; 0.2 g MnSO4?7H2O per litre of distilled-water. Inoculated cultures were incubated at 50 °C at 250 rpm with dissolved oxygen of either 50 % (v/v) or 5 % (v/v). The growth parameters during the exponential growth phase were carefully monitored and any deviations from the setting values automatically corrected.

The results of optimisation of the production of extra-cellular non-haem T. fusca BD25 peroxidases using an automated bio-reactor are shown in Figure 1. It should be noted from the graph that maximum peroxidase production (0.1 Uml-1) occurred after 36 h of incubation at 50 % (v/v) dissolved oxygen at a pH of 8.41; temperature 50 °C and agitation 250 rpm.

The level of enzyme production, however, falls rapidly after 42 h of incubation, reaching a minimum level at 60 h. The peroxidase level obtained from the bio-reactor experiment corresponds approximately to an increase of three fold with respect to in-house production level. Thus, the use of automated bio-reactor has reduced the time of production of this enzyme and significantly increased the expression level.

**Figure 1: Optimisation of production of non-haem T.fusca BD25 peroxidases in a bio-reactor.** Notice that the maximum peroxidase activity is noted after 36 h in 50 % (v/v) dissolved oxygen.

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**REFERENCES**