PRODUCTION, IMMOBILIZATION AND SYNTHESIS OF PHARMACOLOGICAL DERIVATIVES OF LIPASE B FROM CANDIDA ANTARCTICA IN PICHIA PASTORIS

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Lipase B from Candida antarctica (CALB) is widely used because of its excellent enantioselectivity. Producing this recombinant lipase in Pichia pastoris has advantages since it can be cultured in simple media and can reach high cell densities. This capability is especially important when using a constitutive promoter for lipase production, as here. The P_{PGK} promoter is similar to the well-known P_{GAP} promoter and also circumvents the need for inducing production with methanol, which is a hazard when used on a large scale and would increase the downstream production costs, which could be prohibitive for pharmaceutical products.

This study tested two main fermentation strategies: continuous and fed-batch. In both cultures, different specific growth rates occurred (0.05, 0.10, 0.15 and 0.18 h^{-1}), and process parameters (q_P, q_S, Y_{X/S}, Y_{P/X}, Y_{P/S}) were evaluated in order to properly compare them. The highest specific production rate achieved with a continuous culture was 57.71 U/gX.h with \mu = 0.15 h^{-1} and 16 U/gX.h with \mu = 0.14 h^{-1} for a fed-batch culture. Productivity decreased dramatically near the \mu_{max} (0.18 h^{-1}) for P. pastoris (57.6% lower). The best strategy for production was calculated over a three-month period. In both cases, the enzyme is secreted to the supernatant and purification is needed to ensure that only LIPB participates in further reactions. The immobilization process is ideal because purification and concentration is achieved in only one step, reusability is made possible, and in certain cases, stability and efficiency are boosted. Hydrophobic core-shell polymeric supports synthesized by a combined suspension and emulsion polymerization process have shown good potential for lipase immobilization procedures and were used in this study, compared to traditional supports such as Accurel, in order to determine their efficiency.

After the enzyme was immobilized, the reactions included the resolution of (±)-1,3,5-O-benzyl-myoinositol (DL-1) via acylation using vinyl acetate in hexane, and resolution of (±)-1,2-O- isopropylidene-3,6-di-O-benzyl-myoinositol (DL-2) via acylation using vinyl acetate (solvent-free system). The support used directly affected the reaction, but trends were observed. In general, the recombinant lipase produced (LIPB) had higher resolutions than the commercial lipase (CALB, Novozym 435). In the resolution of DL-1 and DL-2 via transesterification (using different media), LIPB immobilized in Accurel or PS-co-DVB/PS-co-DVB showed more activity per enzyme molecule than CALB immobilized in similar supports, while when immobilized in PMMA-co-DVB/PMMA-co-DVB the activities of the two enzymes were similar. The recombinant LIPB immobilized on PS-co-DVB proved to be the most efficient in the enantioselective resolution of both racemic derivatives, DL-1 and DL-2. The productivity for DL-2 resolution was 50% higher than the commercial Novozym 435, and the new derivative was operationally more stable than Novozym 435. The products obtained had a high level of purity (ee of 99% for both derivatives). Both products of the enantio-selective reaction, L-2 and L-5, obtained from the racemic derivatives (DL-1 and DL-2, respectively), are intermediates from different pharmacological pathways involved in the synthesis of building blocks for drugs that inhibit the etiological agent of Chagas disease, Trypanosoma cruzi.