ABSTRACT

PhD Thesis

PURIFICATION AND CHARACTERIZATION OF LIPASE FROM THERMOPHILIC *Anoxybacillus flavithermus* HBB 134

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In this study, 201 thermophilic bacteria that were isolated from natural hot springs in and around Aydin and registered in Adnan Menderes University Department of Biology culture stocks were used. It was determined that 43 of these bacteria exhibited lipolytic activity and 22 of them exhibited lipase activity. These 22 lipase positive isolates were grown in LB broth medium and the quantitative lipase activities were determined. HBB 134 was chosen as the best lipase producing isolate with the activity of 19,925 U/mL. According to 16S rRNA sequences, it was found that the isolate showed maximum similarity (% 99) with *Anoxybacillus flavithermus*. The best enzyme production from HBB 134 was determined in medium including % 0,5 olive oil as carbon source and % 0,5 pepton as nitrogen source, pH 6,50 and 45 °C. When the isolate HBB 134 was grown in optimum culture conditions it was determined that production of the lipase started at the beginning of the logarithmic growth phase and it reached maximum level in the middle (12 hour) of the logarithmic phase. It was determined that most of the enzyme activity was intracellular. The lipase from HBB 134 was purified 7,4 fold using ammonium sulphate precipitation, dialysis, hydrophobic interaction chromatography and gel filtration chromatography. Molecular weight of the enzyme was found to be about 64 kDa by SDS-PAGE method. The enzyme showed maximum activity at pH 9,00 and 50 °C. It was determined that the enzyme was stable for 24 hour between pH 6,00-11,00 and at 25, 40 and 50 °C it retained %100, 92 and 85 of the original activity respectively. It was found that the Km and Vmax of the enzyme were 83,47 μM and 500 U/mg respectively. Glycerol, sorbitol and mannitol enhanced the enzyme thermostability. The enzyme was found to be highly stable against acetone (% 10), ethyl acetate (% 10) and diethylether (% 10, 50). The enzyme activity was inhibited in the presence of NBS (triptophane inhibitor) and PMSF (serine inhibitor). Hg^{2+}, Fe^{3+}, Pb^{2+}, Al^{3+} and Zn^{2+} were strongly inhibited the enzyme while Li^{+}, Na^{+}, K^{+} and NH_4^{+} were slightly activated. At least % 60 of the enzyme activity and stability were retained against sodium deoxycholate, sodium taurocholate, n-octyl-β-D-glucopyranoside and CHAPS. The enzyme activity was elevated about % 34 in the presence of % 1 Triton X-100. The lipase showed a broad range of substrate

specificity. The maximum enzyme activity was determined when the Span 80 and p-nitrophenyl caprylate was used as real and synthetic substrates, respectively. The lipase of HBB 134 cleaved triolein at only 3-position releasing 1,2-diolein and oleic acid.

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Keywords
Lipase, *Anoxybacillus*, lipase production, thermostable enzyme, characterization