PRODUCTION OF EXTRACELLULAR LIPASES BY SOME FUNGAL ISOLATES AND THEIR APPLICATIONS AS BIOCATALYST

By

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B.Sc. Agric. Sc. (Plant Pathology), Ain Shams University, 1995
M.Sc. Agric. Sc. (Agric. Microbiology), Ain Shams University, 2011

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ABSTRACT

Ahmed Mohamed El-Sayed El-Sayed: Production of Extracellular Lipases by Some Fungal Isolates and their Applications as Biocatalyst. Unpublished Ph.D. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, 2016.

One hundred cultures of fungal isolates were isolated from various sources i.e., rhizosphere soil of corn and sunflower, raw milk and household butter by serial dilutions agar plate technique. Out of one hundred fungal isolates, only thirty five were found to produce extracellular lipases on Rhodamine-B agar medium. The most efficient extracellular lipases producing fungal isolates were identified on the basis of morphological characteristics as ***Aspergillus niger***, ***Aspergillus terreus*** and ***Fusarium solani***. Five different culturing media were investigated for the maximum production of extracellular lipases in shake flasks as a batch culture. Among all the tested media, ML3 medium exhibited the highest extracellular lipases activity. The production rate of extracellular lipases was studied and the maximum activity was secured after 72-h of incubation period by all the selected fungal isolates. The addition of different nutritional requirements and the effect of some environmental factors were investigated for improving extracellular lipases production. The maximum extracellular lipases activity (20.17U/mL) was obtained by ***Fusarium solani*** which was grown on the medium supplemented with (g/L): sesame oil (30), peptone (5.8), K_2HPO_4 (2), $MgSO_4.7H_2O$ (0.6), $CaCl_2$ (0.5), $FeSO_4.7H_2O$ (0.05), $MnSO_4.H_2O$ (0.025), $ZnCl_2$ (0.05), $CoCl_2.6H_2O$ (0.03), pH 7.5 incubated at 30°C, 3% v/v inoculum size and fermentation period 72-h. Optimization of different nutritional requirements and some environmental factors for extracellular lipases production by ***Fusarium solani*** and their interactions was carried out by using Response Surface Methodology (RSM). Fifteen parameters of both nutritional requirements and environmental factors were investigated by using Plackett–Burman (PB) experimental design to figure out the significant

parameters that affect extracellular lipases production. Based on the results of Plackett–Burman (PB) design, seven parameters proved to be the most significant variables and employed by using Central Composite Design (CCD) to estimate the optimum level of each parameter along with their interactions on extracellular lipases production. The optimum concentrations of seven significant parameters for maximum extracellular lipases activity (18.89U/ mL) were obtained with (%w/v): 2.0 fish waste-frying oil, 0.58 peptone, 0.06 $MgSO_4.7H_2O$, 0.05 $CaCl_2$, pH 7.5, agitation speed 100 rpm and incubation period 72-h. The produced extracellular lipases were purified by acetone precipitation, ion exchange and gel filtration chromatography technique which resulted 3.81 fold of purification. SDS-PAGE investigation yielded a single band of 95.27 kDa. The optimum pH and temperature for maximum activity of extracellular lipases were 8.5 and 35°C, respectively. Ca^{2+} and Mg^{2+} enhanced extracellular lipases activity while, metallic ions (Mn^{2+}, Fe^{2+} and K^{1+}) did not enhance. The K_m and V_{max} values were determined by hydrolysis of *p*-nitrophenyl palmetate and found to be 0.63µM and 29.4µM/min, respectively. The extracellular lipases exhibited stability in the presence of organic solvents when mixed with dimethyl sulfoxide (DMSO), methanol, acetonitrile, ethanol, acetone, isopropanol, and ethylacetate while hexane and butanol caused loss of activity. A novel and simple method was developed by preparing chitosan-coated magnetic iron oxide (Fe_3O_4) nanoparticles. By using glutaraldehyde as coupling agent, the lipases were successfully immobilized onto magnetic iron oxide-chitosan beads. The results showed that 94.27% of immobilization efficiency which was achieved on the synthesized immobilization matrix. The immobilized lipases were successfully applied for the esterification reaction of sugar esters which was confirmed by TLC and HPLC-MS.

Keywords: Fungi, Extracellular Lipases production, Nutritional requirements, environmental factors, Response surface methodology, Bioreactor, purification, characterization, Immobilization, Biocatalyst.

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