Abstract

Therapeutic proteins are of great interest in the pharmaceutical industry due to their remarkable effects in treating different diseases. Human epidermal growth factor is biologically active molecule that plays an important role in dermal wound healing andtissue damage repair, regulating cell growth and inducing cell proliferation. The methylotrophic yeast *Pichia pastoris*, has been successfully used for the expression of Epidermal Growth Factor (EGF) extracellularly. Expression of hEGF in *Pichia pastoris* was optimized for its shake flask fermentation conditions (i.e. temperature, pH, methanol concentration, incubation time, inoculum size). The best medium for optimal conditions was BMGY (Buffered Glycerol complex media) and BMMY (Buffered-Methanol complex media)with a pH range of 6.0. An initial culture OD_{600} of 2 during induction phase, 28°C temperature, 0.5% Methanol induction, for 96 hour incubation period was found to be optimal for extracellular expression of hEGF-M2. Slight changes in pH and temperature have a large effect on hEGF production. The protein hEGF was partially purified by ammonium sulfate precipitation, at various concentrations (10 to 60%). Ammonium Sulfate (AS) at a concentration of 50% showed maximum purification of hEGF, while concentrations of ammonium sulfate (AS) above or below50% showed minimal purification. Further purification of the protein was performed by ion metal exchange chromatography (IMAC). The highest amount of hEGF was obtained with an average yield of 0.298 mg/ml through IMAC (Ion metal affinity chromatography). The protein concentration of mutant hEGF-M2 obtained after purification was 5 folds as high as compared to wild type GS115. Molecular characterization was also performed by SDS-PAGE and Western blot analysis.