ABSTRACT

The present research work is associated with the production, optimization, purification and characterization of lipase from *Pseudomonas putida* NRRL-922. Highest activity of lipase was achieved when 1% olive oil was used as a lipid substrate along with Tween-80 in the submerged fermentation. Different nutrient sources were used in order to optimize the fermentation medium for the production of lipase among which 2% glucose as carbon and 1% peptone as nitrogen source were found to be the best. The time course for production of lipase was 72 hours with optimum pH of 8 and optimum temperature of 30°C. Lipase activity of crude enzyme was 23 U/ml with specific activity of 27.50 U/mg. Lipase was partially purified by ammonium sulphate precipitation at 80% saturation. Purification fold observed was 4.10 having specific activity of 112.82 U/mg. The pH stability of lipase demonstrated that the partially purified enzyme was stable at pH range of 6-8, whereas, thermostability of lipase showed that enzyme was stable up to 40°C. Molecular mass of lipase determined by SDS-PAGE was 45 kDa. Characterization of enzyme revealed that calcium had the most stimulatory effect on lipase activity among the different metal ions used, while, heavy metals like mercury and iron completely inhibited the enzyme activity. EDTA and β-mercaptoethanol inhibited enzyme activity which indicated that lipase is a metal ion dependent enzyme. Organic solvents like acetone and ethanol had a negative effect on lipase activity. The values of Vmax and $Km_{(approx.)}$ were determined which were 17.57 U/ml and 0.98 mM respectively which showed that enzyme has a higher affinity towards its substrate.