ABSTRACT

The present work describes the optimized production, partial purification and application in fructose syrup production of inulinase produced by filamentous fungal cultures. Different fungal cultures were isolated and collected from Lahore and Murree, Pakistan. Screening of these cultures was carried out with specific media i.e., $(NH_4)_2SO_4$, 0.5; KH_2PO_4, 3; $NaNO_3$, 1.5; $MgSO_4$. $7H_2O$, 0.01 and inulin, 3 g/l with pH 7. The best inulinase producing culture was selected for further optimization. The selected *Rhizopus oligosporus* (ROMII) culture was then further optimized for better production of enzyme, for which another screening media i.e., sucrose mineral media was used. Nutritional and physical parameters were optimized for maximum production of inulinase. Maximum inulinase production was obtained with 2.5 g/l glucose (carbon source), 1.5 g/l tryptone (organic nitrogen source) and 2 g/l ammonium phosphate (inorganic nitrogen source). The enzyme exhibited maximum activity at pH 6 and 50°C incubation temperature for 72 h fermentation (158±0.39 U/ml). Kinetic studies were applied on time duration for comparing specific growth rates, volumetric rates, growth and product yield coefficients as well as specific rate constants of the two best strains for optimal production of inulinase. The comparison significantly determined ROMII to be the efficient strain for enzyme production. The partial purification of the enzyme was done with ammonium sulphate followed by dialysis that resulted in 21 % purification of the crude enzyme extract. The molecular weight of inulinase was found to be 86 kDa, determined by SDS poly-acrylamide gel electrophoresis. The thin layer chromatography analysis of the inulinase depicted that inulinase hydrolysis release fructose as the main sugar which showed that fructose syrup can be obtained from inulinase preparation. Thus the results obtained from the present study were highly significant (HS).