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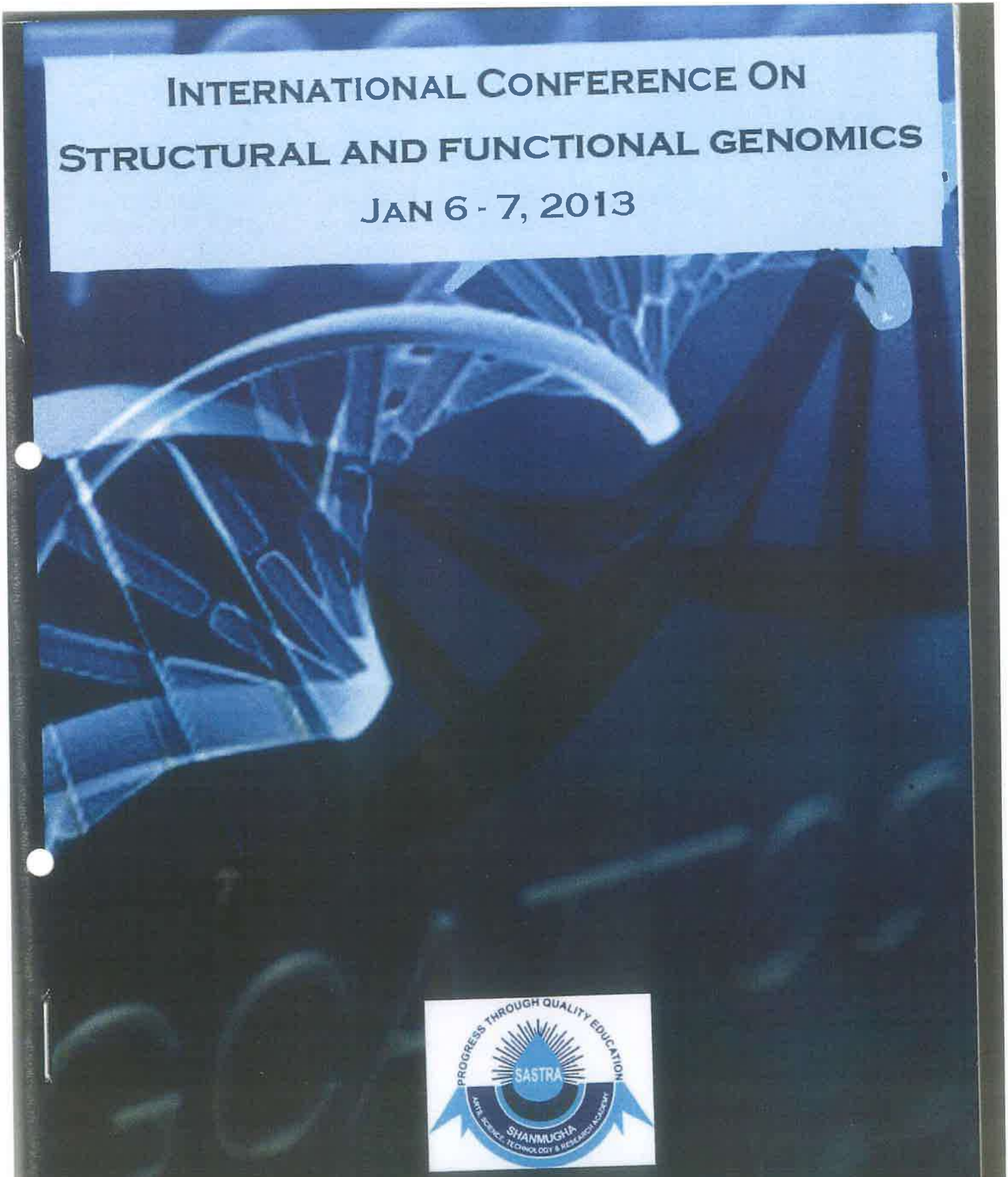

Department of Bioinformatics

SASTRAUNIVERSTY

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Isolation, purification and characterization of secondary structure and kinetic study of lipase from Indian Major Carp, Catla Catla (catla)

PrasidhiTyagi, Neelima Boora and Kameshwar Sharma YVR*

Life Sciences, Sri Venkateswara College, University of Delhi, Delhi-110021. Email: sharmabiochem@gmail.com, prasidhi.tyagi@gmail.com, neelimaboora7@gmail.com

Lipases are ubiquitous enzymes which catalyze the hydrolysis of fats into fatty acids and glycerol at the water-lipid interface and reversing the reaction in non-aqueous media. Lipases occupy a place of prominence among biocatalysts owing to their novel and multifold applications in oleochemistry, organic synthesis, detergent formulation and nutrition. Lipase was extracted and isolated from the alimentary canal and digestive gut of *CatlaCatla*(catla). The tissue was homogenized in a ratio of 1:3 with starting buffer (0.01M TrisHCl, pH 7.2). The crude extract thus obtained was precipitated using ammonium sulphate (20-80%). Excess salt was removed by Dialysis and the resultant dialysate (Desalted Enzyme) was subjected to DEAE-Cellulose column for Ion Exchange Chromatography at a flow rate of 0.5ml/min. Elution was carried out by a step gradient of NaCl (100-800 mM) in the starting buffer. Active fractions were pooled as Purified Fraction (PF) and were used for physical characterization of pH, temperature and effect of calcium on the enzyme activity, structural characterization, molecular characterization and for kinetic studies. The Purified Fraction (PF) showed final specific activity of 1438.72 U/mg. The optimum pH was 7.8 and the optimum temperature was found to be 20^0 C. Melting Temperature (T_m) value was 42^0 C and the activation energy of purified lipase was 34.82 KJ/mol/K. Thermal stability of lipase was found to be at 20^0 C. Lipase activity retained upto 3 h of incubation with 10mM and 20mM of $CaCl_2$ in starting buffer. This shows that Calcium has enhancing property from the denaturation of enzyme. Michaelis-Menten constant, K_M of lipase from Indian major carp, catla for the hydrolysis of pNPP was 6.695 mM. Turnover number (k_{cat}) of lipase (catla) was 0.0022 s^{-1}. Catalytic efficiency (k_{cat}/K_M) of lipase was 0.0003412s^{-1} mM^{-1}. SDS-PAGE of purified lipase PF, revealed a homogenous single band with molecular mass of 32.1 KDa. Secondary structural arrangement of α helices and β strands of purified lipase results 48.51 % and 9.74 %, respectively using Circular Dichroism. Mass Spectrometric studies (MS and MALDI TOF) of purified fraction of lipase were under process for further characterization.