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Certificate

This is to certify that Dr / Mr / Mrs. / Miss Kameshwar Sharma YVR, Sri Venkateswara College Delhi

has PARTICIPATED/PRESENTED PAPER in the International Symposium on Genomics in Aquaculture organized by the Central Institute of Freshwater Aquaculture in collaboration with Association of Aquaculturists during 22-23 January, 2013 at Bhubaneswar, India

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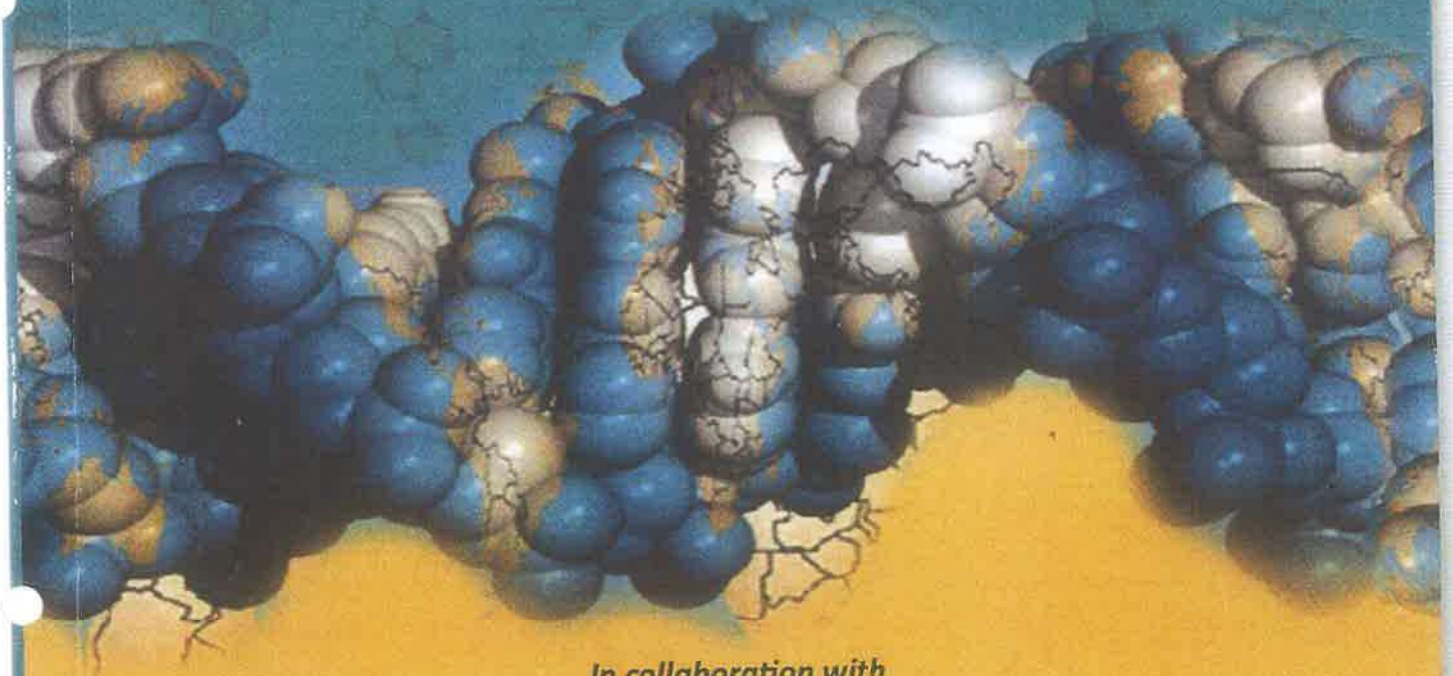
Lead Lectures & Abstracts



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PURIFICATION AND CHARACTERIZATION OF SECONDARY STRUCTURE AND KINETIC STUDY OF LIPASE FROM INDIAN MAJOR CARP, *CATLA CATLA* (CATLA)

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Lipases play an important role in lipid metabolism and energy homeostasis as fatty acids mostly stored as Triacylglycerides (TAG) are the major endogenous source of energy. Lipases are widely used in medicinal and industrial applications owing to their substrate specificity such as fatty acid, alcohol, region and stereo specificity. Lipase was extracted and isolated from the alimentary canal and digestive gut of *Catla Catla* (catla). The tissue was homogenized in a ratio of 1:3 with starting buffer (0.01M Tris HCl, 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 0.5 ml/min. Elution was carried out by a step gradient of NaCl (100-800 mM) in the starting buffer. Active fractions were pooled as purified sample (PF) and were used for physical characterization of pH, temperature and effect of calcium on the enzyme activity.

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 °C. Melting temperature (T_m) value was 42 °C and the activation energy of purified lipase was 34.82 KJ/mol/K. Thermal stability of lipase was found to be at 20 °C. Lipase activity retained upto 3 h of incubation with 10 and 20 mM 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 0.0003412 s^{-1} mM^{-1}. SDS-PAGE of purified lipase PF, revealed a homogenous single band with molecular mass of 56.0 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 are under process for further characterization.