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Heterologous expression and characterization of a novel S-enantioselective lipase TALipB from *Trichosporon asahii* MSR54: Kinetics, conformational stability and homology modeling

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A novel lipase encoding gene, TALipB from *Trichosporon asahii* MSR54 was heterologously expressed in *Escherichia coli* using three vectors, pET22b, pET28a and pEZZ18. Purification was done using respective affinity chromatography as N-hexahistidine fused HLipB, N and C-hexahistidine fused HLipBH and ZZ-fused ZZLipB. Study showed that the enzyme was mid to long chain selective on p-NP esters and S-enantioselective irrespective of tags. Among these, HLipB had lowest activation energy (3.5 Kcal mol^{-1}) and highest catalytic efficiency (254 $mM\text{-}1min^{-1}$) on p-NP caproate followed by HLipBH and ZZLipB. However, ZZLipB demonstrated best pH stability (pH: 6-10), thermostability ($t_{1/2}$: 70° C for 50 min) and stability towards denaturants (GdmCl 500 mM and acrylamide 100 mM). Far-UV CD and fluorescence study showed that N-terminal ZZ-tag conferred stability by altering both secondary and tertiary structure. All the three proteins were thiol activated and structural changes during activation revealed that ZZLipB required higher concentration of BME to attain the similar velocity which indicated the involvement of additional disulfide bonds in its conformational stability. *In silico* analysis revealed that the enzyme had low identity with the available database. However *Candida antarctica* lipase B was identified as closest structural homolog using PHYRE[2]. MULTALIN with CALB predicted the active site residues (Ser137-Asp228-His261) which were confirmed by superimposition and site directed mutagenesis.

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Genetic elements associated with multidrug resistant diarrhoeagenic *E. coli* isolates from children below five years of age

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Integrons are strongly associated with the multidrug resistance seen in Gram-negative Bacilli in the hospital environment. These elements are able to capture and express gene cassettes encoding antibiotic resistance. The main aim of this study was to investigate the distribution of integrons in multidrug resistant diarrheagenic *E. coli* isolates to analyze the possible relationship between the antimicrobial resistance profiles and the integrons and to perform the docking of integron proteins. 80 diarrheagenic *E. coli* strains were isolated from children with diarrhea and examined for the presence of class 1, 2 and 3 integrons by real time PCR. 40 isolates from healthy children were also included as controls. Statistical analysis was used for the comparison of the categorical data. Class 1 integron was identified in most of the isolates while less than 50% isolates harbored class 2 integron and no class 3 integrons were detected in any of the isolate. Integrons were significantly associated with resistance to certain antibiotics including; Cefotaxime (P=0.01), Ceftazidime (P=0.006), Azetronam (P=0.046), Nalidixic acid (P=0.01), Gentamycin (P=0.001), Amikacin (P=0.01) and Piperacillin+tazobactam (P=0.037). Our study demonstrates the importance of integrons for the occurrence and transmission of multidrug resistance. Identical predominant class 1 and 2 integrons in *E. coli* servers' indicate horizontal transfer. This study emphasizes the alarming role of integrons in antibiotic resistance within diarrheagenic *E. coli* strains.

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