

# **ENGINEERING OF LIPASES FOR NON- AQUEOUS SOLVENTS**

By

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DEPARTMENT OF BIOCHEMICAL ENGINEERING AND  
BIOTECHNOLOGY

Submitted

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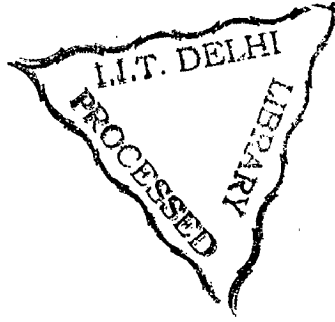
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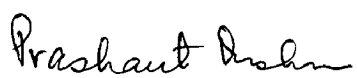
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*Dedicated to my  
Parents  
to Whom I am Indebted  
for  
What I am*



## CERTIFICATE

This is to certify that the thesis entitled “**ENGINEERING OF LIPASES FOR NON-AQUEOUS SOLVENTS**” being submitted by **Ms. Bhawna Madan** to the Indian Institute of Technology, Delhi for the award of the degree of ‘**DOCTOR OF PHILOSOPHY**’, is a record of the bonafide research work carried out by her, which has been prepared under my supervision in conformity with the rules and regulations of the “Indian Institute of Technology, Delhi”. The research reports and the results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.



**Dr. Prashant Mishra**

**Associate Professor**

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*Bhawna*  
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## ABSTRACT

Lipases (triacylglycerol ester hydrolases, E.C. 3.1.1.3) catalyze the hydrolysis of carboxylic acid esters in aqueous media, whereas, in non-aqueous media they catalyze the esterification and transesterification reactions. Owing to the properties like wide substrate specificity, enantio- and regioselectivity they have vast industrial applications in organic synthesis, in detergent formulations and in the food and pharmaceutical industries. However, the catalytic efficiency of enzymes is 2-6 orders of magnitude lower in organic solvents than in aqueous solutions which hamper the practical applications of lipases in synthetic reactions. Amongst bacterial lipases *Pseudomonas sp.* lipases have attracted major attention and have been studied in greater details. In this work the organic-solvent tolerance of *P. aeruginosa* B2264 has been enhanced by chemical mutagenesis. The lipase from the best mutant "*P. aeruginosa* BML-207" has shown 3.8 fold increased interesterification activity compared with the wild type. The lipase from *P. aeruginosa* BML-207 has been purified and characterized. The major bottleneck of overexpression of *Pseudomonas* lipase in *E. coli* is that they require an assistant protein, a lipase specific chaperone to fold into an active conformation. In this study, the *lipA* gene, a structural gene encoding for protein of molecular mass 48 kDa and *lipB* gene encoding for a lipase specific chaperone with molecular mass of 35 kDa of *P. aeruginosa* B2264 were co-expressed in heterologous host *E. coli* BL21 (DE3) to obtain *in vivo* expression of functional lipase. The recombinant lipase was expressed with histidine tag at its N-terminus and was purified to homogeneity using nickel affinity chromatography and was characterized.

*Bacillus* has also been employed for the production of industrial enzymes and is known to produce lipases *albeit* at low quantity but have applications in synthetic reactions of practical utility. Moreover, *Bacillus* lipases do not require the assistance of chaperone for folding. In view of the potential of *B. licheniformis* to be employed for commercial production of lipase, the lipase gene from thermophilic bacterial isolate *B. licheniformis* RSP-09 was cloned, overexpressed in *E. coli* and purified to homogeneity using single step his-tag chromatography. The enzyme was characterized and its organic solvent tolerance was tested. The thermostability of *Bacillus licheniformis* RSP-09 lipase have been further improved following two rounds of directed evolution. The best variants after second round of directed evolution have been purified and characterized. The variants show increased thermostability with no loss of catalytic function. The temperature at which 50% of the initial enzymatic activity is lost after 60 min ( $T_{50}^{60}$ ) of the most stable variant, eP-231-51 has been increased by 8°C over the wild type. In addition, the thermostable variants have shown resistance to hydrophobic organic solvents. The role of mutations which have led to increased stability have been postulated by molecular dynamics simulations.

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