Efficient media for high production of microbial lipase from *Bacillus Subtilis* (BSK-L) using response surface methodology for enantiopure synthesis of drug molecules

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Key message. Lipases are a multipurpose enzyme that holds a significant position in industrial applications due to its ability to catalyse a large number of reactions such as hydrolysis, esterification, interesterification, transesterification which makes it a potential candidate. It is also used for the separation of chiral drugs from the racemic mixture and this property of lipase is considered very important in pharmaceutical industries for the synthesis of enantiopure bioactive molecules. Assuming the tremendous importance of lipases, as stereoselective biocatalysts, in pharmaceuticals and various other commercial applications, industrial enzymologists have been forced to search for those microorganisms which are able to produce novel biocatalysts at reasonably high yield. In the present study microbial lipase was isolated from the water sample of pond at Katra, Jammu and Kashmir (India). This enzyme has shown wide specificity and higher enantioselectivity, which make it pharmaceutical important enzyme. To make it economical for industrial application, it was produced on cheap nutrient media using Response Surface Methodology and got maximum production. It was used for resolution of chiral drugs and the significant results obtained during the course of work shall have potential towards pharmaceutical industries.

Keywords: microbial lipase, enantioselectivity, hydrolysis and response surface methodology

Relevance and purpose of work: Microbial lipases are known to depict diverse properties and substrate specificity, making them a potential source for various industrial applications. Several microbial lipases are produced commercially but their high cost and poor stability seem to be a major factor in their successful commercial applications. A crucial factor of industrial prices of these enzymes is the culture media composition that is constantly under review by researchers. In the present study, maximum lipase production by *Bacillus Subtilis* (BSK-L), was achieved using response surface methodology (RSM). This strain was collected from the water sample of Pond at Katra, J&K (India). The work is very relevant based on the review of literature and background of the area of research. The methodologies have been utilized accordingly and work has been done carefully towards achieving the objectives.

Methods: Culture medium parameters such as low and high cost carbon & nitrogen sources, substrates, temperature, pH, salts, metal ions and incubation time were evaluated. The production of lipase was optimized through RSM based on central composite design. Process optimization involved one variable at time (OVAT), wherein olive oil was used as an inducer and as a carbon source.

Results: Olive oil and peptone were found to be the best carbon and nitrogen sources respectively, whereas, MnSO_4 and temperature observed to be most significant factors for production of lipase from BSK-L. Optimized media components obtained from RSM produced satisfactory result of 7500U/g cell biomass and 12-fold increases in lipase production was achieved than that of conventionally optimized conditions (627 U/g cell biomass). The lipase produced from indigenous strain (BSK-L) was used for resolution of enantiomers of butyl-ketoprofen via hydrolysis.

Conclusion: In this investigation, significant enhancement in the production of microbial lipases was obtained using statistical tools. The isolated enzyme used for racemic resolution of ketoprofen butyl esters which exhibited better enantiomeric excess of 70% ee compared to commercially available *Candida antarctica* lipase (63% ee). This potential of lipase can be exploited in pharmaceutical industry.