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Technology for Production of Lipases

Biotech Consortium India Limited (BCIL) is seeking companies interested in commercializing a technology used for production of Lipase enzyme. The technology has been developed at the Department of Microbiology, University of Delhi South Campus, New Delhi, India, keeping in view the wide application of Lipase enzymes in various industrial applications.

BCIL was incorporated as public limited company in 1990 under the Indian Companies Act 1956. It is promoted by the Department of Biotechnology, Government of India and is financed by several all India financial institutions, venture capital funds and the corporate sector. BCIL has been actively involved in technology transfer, project consultancy, fund syndication, information dissemination, and manpower training & placement related to biotechnology over the last decade and half. BCIL has transferred more than 15 technologies in the last 5 years using its expertise in facilitating licensing agreements that allows a healthy and productive cooperation between the inventor and the licensee.

INTRODUCTION

The chemical methods that are used in different industries for achieving various requirements use very harsh conditions of high temperature, pressure and extreme pH Besides these, the release of effluents during these chemical processes lead to environmental hazards. Therefore, there came the era of enzymes for various industrial processes. Enzymes show numerous advantages over chemical technology as far as their specificity, efficiency and compatibility with environment is concerned. However, their use was limited due to inactivation at extreme condition of temperature and pH. The present technology imparts a step to address this problem by providing highly thermostable Lipase enzymes with wide range of temperature and pH functionality. Apart from these lipases are highly specific for the reactions (chemo-selectivity, stereo-selectivity etc.) they catalyse, which makes them possible to synthesize optically pure compounds that are generally not possible by using conventional chemical methods.

TECHNOLOGY

- **Product:** Two Lipase Enzymes.
  - **Lipase Type-1**
  - **Lipase Type-2**
- **Source:** Indigenous microflora isolated and screened from Indian soils which has been collected from various parts of the country.
- **Properties:** Lipases from these organisms have shown following properties and field of applications as given below:

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Enzyme & Type	Lipase (Type 1)	Alkaline thermostable lipase (Type 2)
Organism	*Aspergillus species*	*Aspergillus species*
Growth Conditions	37 °C and pH 6.2	37 °C, pH 7.0
Production medium	Minimal medium with 2% oil as carbon source	Minimal medium containing 1.5% oil as carbon source
Levels of scale-up	Production scaled up to 20L fermenter capacity	Production scaled upto 20L fermenter capacity
Production yield	14,000 U/L	11000 U/L
Purification strategy	Ultrafiltration, ammonium sulphate and hydrophobic interaction chromatography in 3-4 days and also by aqueous two phase partitioning within 2 hours leading to 60-70% concentration/purification	Ammonium sulphate precipitation followed by hydrophobic interaction on Octyl sepharose CL-4B
Purification fold and yield	45.8 fold purification and 65.1% yield	38% yield with 502U/mg specific lipase activity
Molecular weight	45kDa	27 kDa
Temperature optima	45°C, active in the temperature range 20-90°C	37 °C (Tolerance range = 5°C – 90 °C)
Temperature stability	100% stable at 60 °C for 24 h, 70 °C for 1h, 80 °C for 30min.	100% stable at 70°C.
pH optima	5.5 and 10.0	9.0 (Tolerance range = 6.0 – 12.0)
pH stability	Stable in the pH range 3.0-11.0	pH 6.0 – 12.0 for 24 h
Organic solvent stability	Stable in various organic solvents like propanol, methanol and acetone	
Substrate specificity	1,3 regio-specific. Also hydrolyses methyl esters, p-nitrophenyl esters of fatty acids and triglycerides with high specificity for palmitic acid.	1,3 -regio-specific K_m for p-nitrophenyl laurate is 0.476μM and for p-nitrophenyl palmitate is 2.422 μM
Effect of divalent ions	Stimulated by Ca^{2+} and Mg^{2+} ions. Inhibited by Fe^{2+}, Co^{2+}	

	and Cu^{2+}	
Inhibitors	Inhibited by diethyl *p*-nitrophenyl phosphate and PMSF	
Shelf-life	Stable in lyophilised form at room temperature for more than two years	Stable for more than a year at room temperature in lyophilised form.
Potential applications	Ester synthesis especially in synthesis of monoglycerides, diglycerides, triglycerides and sugar alcohol esters for use as biosurfactants. Unique ability to hydrolyse peracetylated polyphenolsat *ortho* position which can be exploited for synthesis of pharmaceutically important drug intermediates.	Enantioselective synthesis of chromanols (drugs), Cyanohydrins, (insecticides) and amino acid precursors; unique property of chemo- and regio-specific hydrolysis of acetophenones, benzophenones and amides and esters of polyacetoxy aromatic carboxylic acids which can be exploited for synthesis of pharmaceutically important drug intermediates ; peptide synthesis; esterification and transesterification reactions; detergent formulation.
Detergent and Surfacant		Strongly stimulated by taurocholic acid, hexadecyl trimethyl sability ammonium bromide and n-octyl – αand n- octyl-β - D glucopyranosides

- **Scale at which the technology has been developed:**
  This technology has been scaled upto 30L fermenter.

Patents:

- An enzymatic method for preparation of bread. (Application No. 3430/DEL/98).
- Developed a process of preparing improved hair oil from coconut oil by Lipase mediated interesterification. (Application No. 760/DEL/99).
- Process for isolation of optically pur S-enantiomer of cyanohydrin of Meta-phenoxybenzaldehyde via lipase-mediated enatioselective transesterification. (Application No. 1378/Del/99).
- A process for the preparation of lipase from the fungus *Aspergillus carneus* (Application No. 1379/Del/99).
- A process for the preparation of low temperature alkaline lipase from the fungus *Fusarium* sp. (Application No. 1411/Del/99).

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SALIENT FEATURES:

- **Functional flexibility:** Wide functional range in both aqueous and organic solvent system.
- **Highly valuable characteristics:** The following are the important properties of these lipases:

  a) pH and temperature tolerance and stability;

  b) Organic solvent stability;

  c) High substrate specificity;

  d) Stereo-specificity, chemo-specificity and regio-specificity;

  e) Esterification and transesterification ability; and

  f) Use in carrying out reactions in supercritical fluids.

- **Effect on environment:** The processes developed are eco-friendly and will lead to value addition of products where these lipases will replace expensive and harsh chemicals and multi-step processes.
