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Review: Enantioselective Lipase From*Pseudomonas stutzeri*

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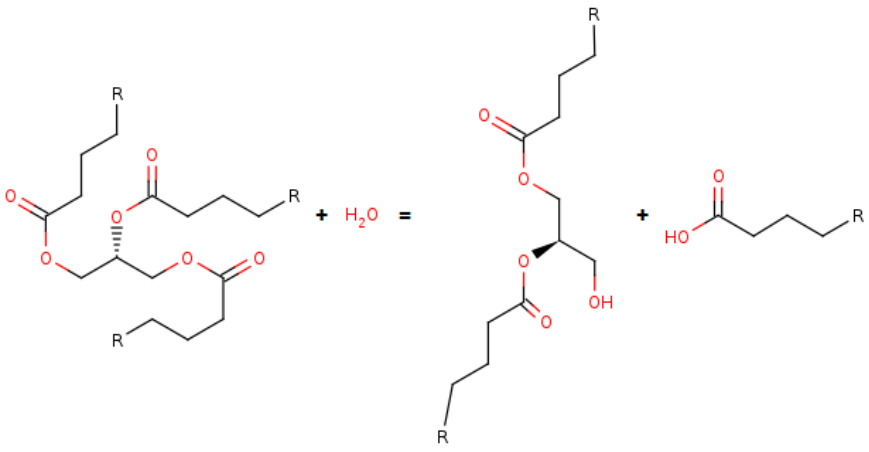
I. Lipase

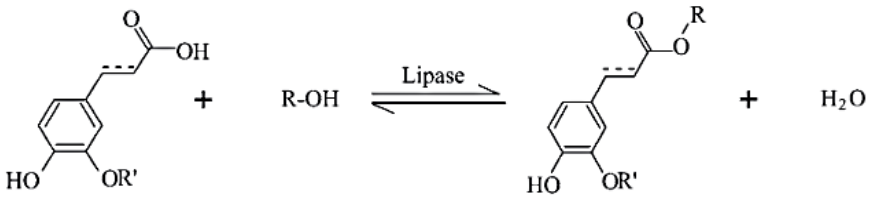
An enzyme called lipase (EC.3.1.1.3) can catalyze esterification and hydrolysis reactions. An ester is created during the esterification reaction when a fatty acid and an alcohol are combined. The opposite reaction is called hydrolysis, and it results in the breakdown of triglycerides of free fatty acids into partial glycerides and glycerol. The three main categories of lipase sources are animals[1], plants[2], and microorganisms[3,4]. Since this microbe can readily proliferate, it can be generated at any time, needs little space, and is not seasonal like plants, there is potential to utilize its lipase. Due to their huge variety of enzymatic characteristics and substrate selectivity, microbial lipases are particularly appealing for industrial applications such as oleochemical[5], dairy industry[6], detergents[7], cosmetics[8].

Serine, aspartate, and histidine are the three active sites of lipase. The active site is inside the lid. This polypeptide lid protects the active side amino acids from proteolysis, preserving the ability of the enzyme to function. There are several types of reactions to lipases, including:

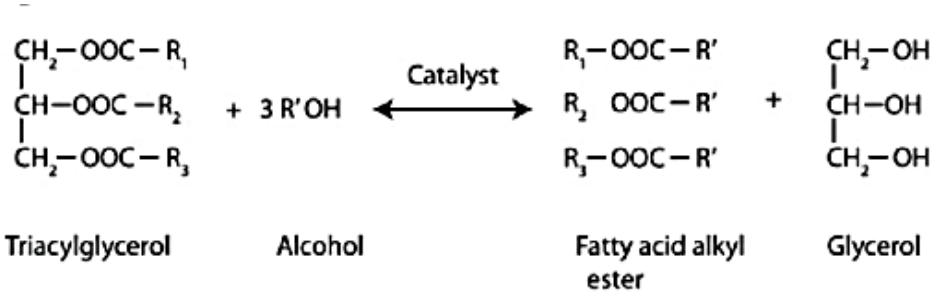
a. Hydrolysis
b. Esterification
c. Transesterification
d. Acetylation
e. Amynolysis



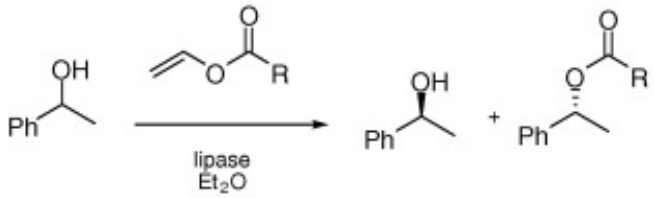
Lipase hydrolysis reaction scheme (https://www.brenda-enzymes.org)



Lipase esterification reaction scheme[9]



Lipase transesterification reaction scheme[10]



Lipase acetylation reaction scheme[11]

Lipase aminolysis reaction scheme[12]

A source of lipase from bacteria and some of their characteristics are just as follows:

Organism	Molecular weight (Da)	Temperature Optimum (^oC)	pH Optimum	Ref
Acinetobacter baylyi	30000	60	8	[13]
Bacillus cereus	42000	33	9	[14]
Burkholderia sp.	35000	40	8	[15]
Pseudomonas aeruginosa PseA	60000	40	8	[16]
Bacillus sp. RSJ-1	37000	50	8	[17]
Staphylococcus warneri	45500		7	[18]

Of the many lipase-producing bacteria, there are exciting things to discuss lipase from *Pseudomonas stutzeri*. *Pseudomonas* is a genus that is relatively abundant in nature. According to recognized enantioselective characteristics, several P*seudomonas stutzeri* can be employed to separate racemic substances. It is acknowledged that *P. stutzeri* belongs to the class of Gamma proteobacteria. The 16S rRNA gene sequences of *P. stutzeri* strains and other phylogenetic markers show that these strains, as well as similar taxa within the genus including *P. mendocina*, *P. alcaligenes*, *P. pseudoalcaligenes*, and *P. balearica*, all belong to the same branch[19].

II. Enantioselective

One method for acquiring both enantiomers is the resolution of racemic substances. Enantiomer is one of a pair of molecules that are nonsuperimposable mirror images. The asymmetric catalysis known as enantioselective catalysis makes use of chiral catalysts, which are frequently chiral coordination complexes. Enantioselective lipase is a type of biocatalysis that carries out chemical transformations using biologically

isolated enzymes. This enantioselective enzyme has many benefits, including strong enantiomeric excess, good reagent specificity, mild conditions, and environmentally friendly. Due to their regio-, stereo-, and chemoselectivity as well as their stability in organic solvents, lipases are frequently used in the resolution of racemic mixtures. Enantiomeric variations can have quite distinct biological effects. Utilizing enzymes for asymmetric synthesis and kinetic resolution to produce pure enantiomers has attracted attention in the past ten years[20].

Enantioselective lipase from *Pseudomonas stutzeri*

Pseudomonas stutzeri lipase has been known to have enantioselective properties towards certain compounds. Therefore, lipase from *Pseudomonas stutzeri* which is enantioselective has been widely used to synthesize compounds.The following are examples of enantioselective Pseudomonas stutzerilipase :

Strain	Type of reaction	Enantioselective	References
*Pseudomonas stutzeri*strain A1501	Hydrolysis	(R,S)-Methylbenzyl butyrate –(R)-1 phenylethanol	[21]
Pseudomonas stutzeri ZS04	Esterification	(R,S)-1-phenyl ethanol --(R)-1-(4-methoxyphenyl)-ethanol	[22]
Pseudomonas stutzeri lipase (PSL)	Aminolysis	Amine , methyl ester	[12]
Pseudomonas stutzeri	Transesterification	(R,S)-1-phenyl-1-propanol-- (R)-acetate	[20]
Pseudomonas stutzeri(Lipase TL@)	Transesterification	(R,S)-benzoin-- (s)-butyrate	[23]

a. ***Pseudomonas stutzeri*strain A1501**

Lipase from Pseudomonas stutzeri strain A1501 was cloned and expressed as a functional protein in E. coli. The results of biochemical characterization indicate that this enzyme is an esterase. The optimum pH of this lipase strain is alkaline with an optimum temperature of 50˚C. The enzyme is capable of separating racemic ester which is very useful for chiral synthesis. The hydrolysis of -methylbenzyl butyrate was used to test stereospecificity and could perform the kinetic resolution of chiral esters since it was R- enantiomer specific with ee-values > 93%[21].

b. Pseudomonas stutzeri ZS04

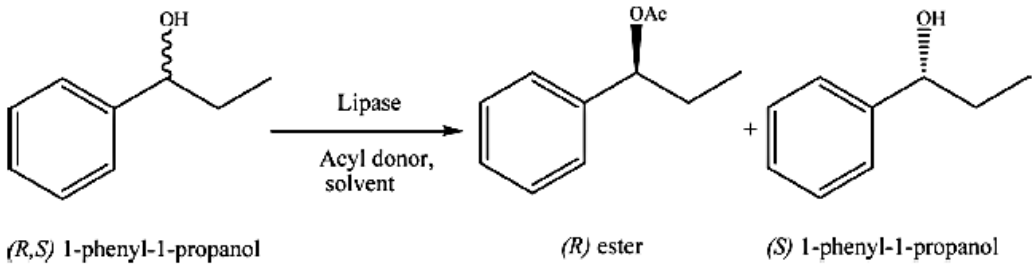
The lipase-producing Pseudomonas stutzeri ZS04 was isolated from oil-contaminated soil samples. The optimal temperature is 50°C , the optimal pH is pH 9.0 and stable in various organic solvents. Ca2+ may considerably increase the thermal stability of lipases, driving promising applications in biocatalysis. This lipase has an esterification reaction pathway and is enantiospecific for (R)-1-(4-methoxyphenyl)-ethanol (MOPE)[22].

c. *Pseudomonas stutzeri lipase* (PSL)

Pseudomonas stutzeri lipase (PSL) is used as a biocatalyst for aminolysis reactions with large substrates. PSL was comparable than the commercial lipase NovozymR 435, which was created from immobilized Candida antarctica lipase B, at aminolyzing a wide range of methyl esters and amines. PSL with secondary amine had a greater yield than NOV435 when acting as a nucleophile[12].

d. Pseudomonas lipase

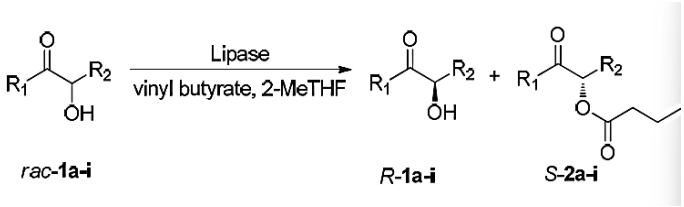
The enantioselective production of 1-phenyl 1-propanol was used as a chiral building block and a synthetic intermediate in the pharmaceutical was investigated. Effects of the enzyme source, the type of acyl donor, and the type of solvent on the kinetic resolution of 1-phenyl 1-propanol were studied. With the transesterification reaction, the kinetic resolution of rac-1-phenyl-1-propanol was accomplished satisfactorily. Secondary alcohols that are enantiomerically pure are crucial synthesis intermediates and chiral auxiliaries. Alcohol (0.5 mmol), vinyl laurate (1 mmol), enzyme (0.1 g), molecular sieve (0.1 g), isooctane (3 mL), temperature (40 oC), and stirring rate is 150 rpm[20].



Transesterification of (R,S)-1-phenyl-1-propanol

e. *Pseudomonas stutzeri*(Lipase TL@)

Commercial TL® lipase (from *Pseudomonas stutzeri*) has been used in the dynamic kinetic resolution (DKR) process of benzoin (secondary alcohol) by in situ racemization modification of the residual substrate through a ruthenium-catalyzed redox process to obtain high conversion and enantiomeric excesses. For the DKR of benzoins in 2-MeTHF, *Pseudomonas stutzeri* immobilized on poly(GMA-co-HDDA) polymer could be employed as a helpful, recoverable, and reusable biocatalyst with high yield and enantioselectivity in all situations[23].



Enantiomeric excess

The excess of one enantiomer over the other in a mixture of enantiomers is known as enantiomeric excess (ee). Enantiomeric excess can be expressed mathematically as follows:

Enantiomeric excess = % of major enantiomer - % of minor enantiomer
% ee = 100(R-S)/(R+S) ,with R= major enantiomer ; S= minor enantiomer of chiral analytes

III. Determination of Enantioselectivity

Several methods, such as the following, can be used to separate the R- and S-enantiomers from a racemic compound:

1. Gas chromatography[24]
   Two techniques are used for chiral separations in gas chromatography:
   (1) a direct approach that uses chiral stationary phases, and
   (2) an indirect approach that requires derivatization using a chiral reagent
2. HPLC with chiral column
   Direct enantioseparation by high-performance liquid chromatography (HPLC) has substantially improved over the past few decades, and a wide variety of chiral stationary phases (CSPs) for HPLC have been produced employing both chiral small molecules and polymers having chiral recognition capabilities[25].

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