Applicazioni biotecnologiche degli enzimi: le lipasi

Le lipasi

- Catalizzano l' idrolisi e la sintesi di acilgliceroli
- Sono stabili in solventi organici
- Non richiedono cofattori
- Hanno bassa specificità di substrato
- Hanno elevata enantioselettività

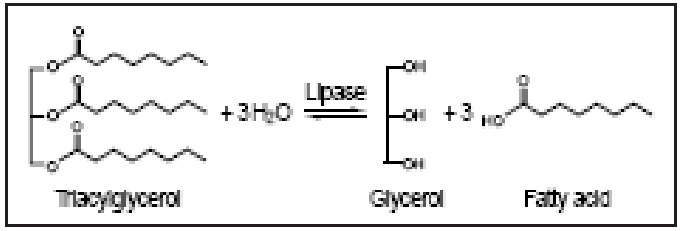


Figure 1

The catalytic action of lipases. A triglyceride can be hydrolysed to form glycerol and fatty acids, or the reverse (synthesis) reaction can combine glycerol and fatty acids to form the triglyceride.

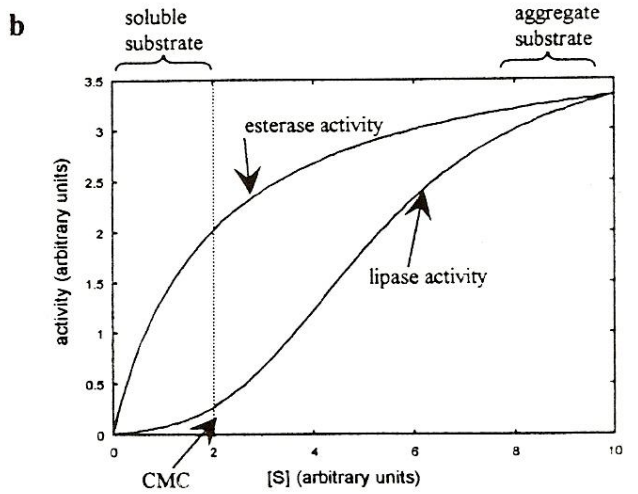
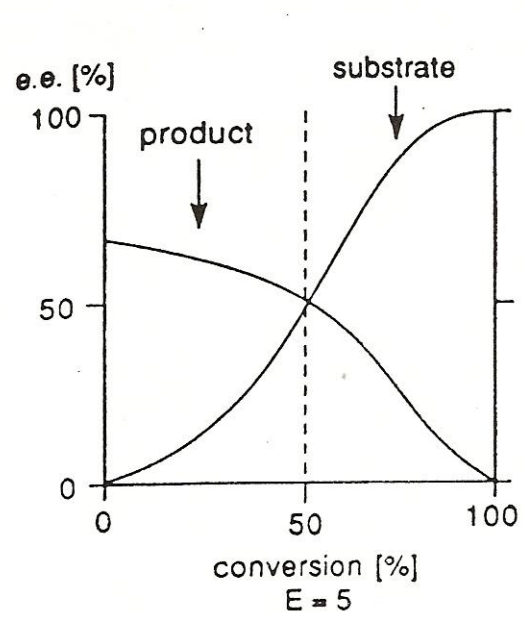
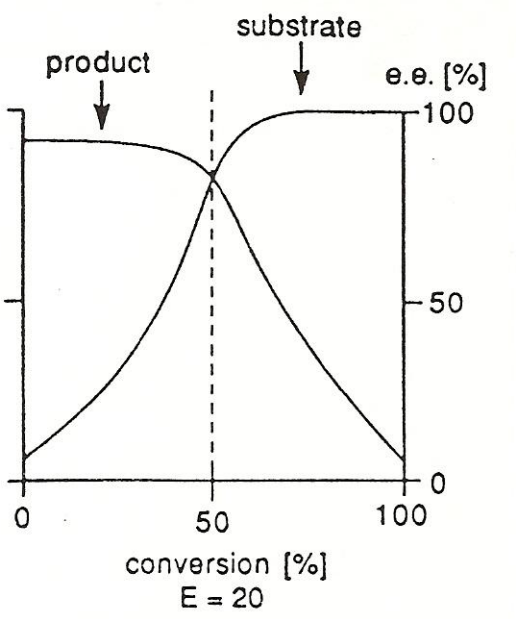


Figure 1 (a) The mechanism of action of lipases by interfacial activation at the oil–water interface. S is the substrate and P is the product. (b) The activity of esterases and lipases in aqueous solution. [S] is the substrate concentration and CMC is the critical micellar concentration of the substrate

Valutazione della purezza chirale di prodotti e substrati per reazioni catalizzate da un enzima enantioselettivo



Enzima con E = 5



Enzima con E = 20

Struttura della lipasi di *Pseudomonas aeruginosa*

Le lipasi catalizzano l' idrolisi di legami estere con un meccanismo simile a quello delle proteasi a serina

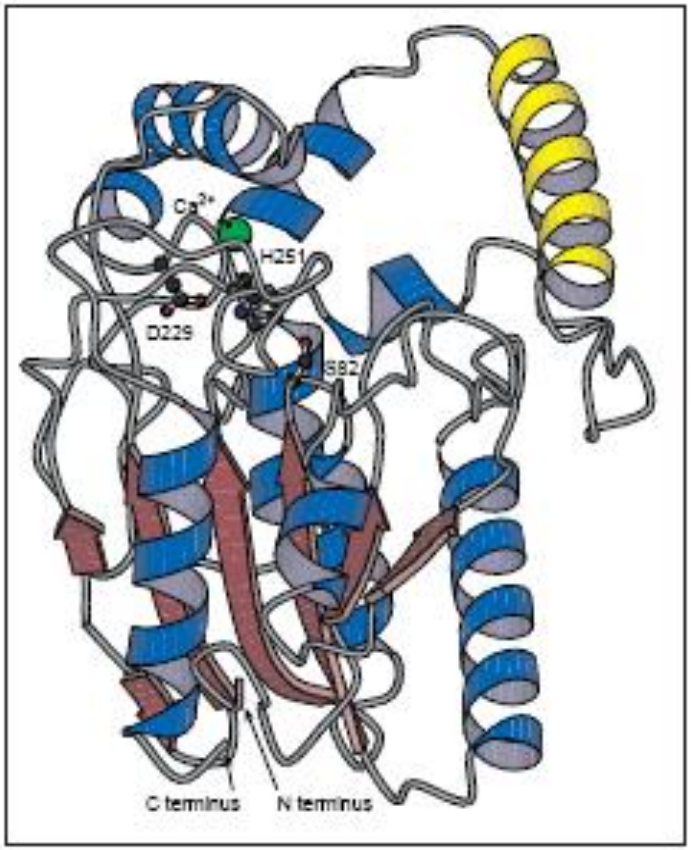


Figure 2

The structure of the lipase from *Pseudomonas aeruginosa* in a model built using the X-ray coordinates determined for *Burkholderia cepacia* lipase[30]. β strands are represented as arrows (dark red) and α helices as coils (blue or yellow); the yellow helix could form a 'lid' over the active site. The active-site residues Ser82, Asp229 and His251 are labelled, and the potential position of a Ca^{2+} ion is indicated by a green ball.

Meccanismo d' azione delle lipasi

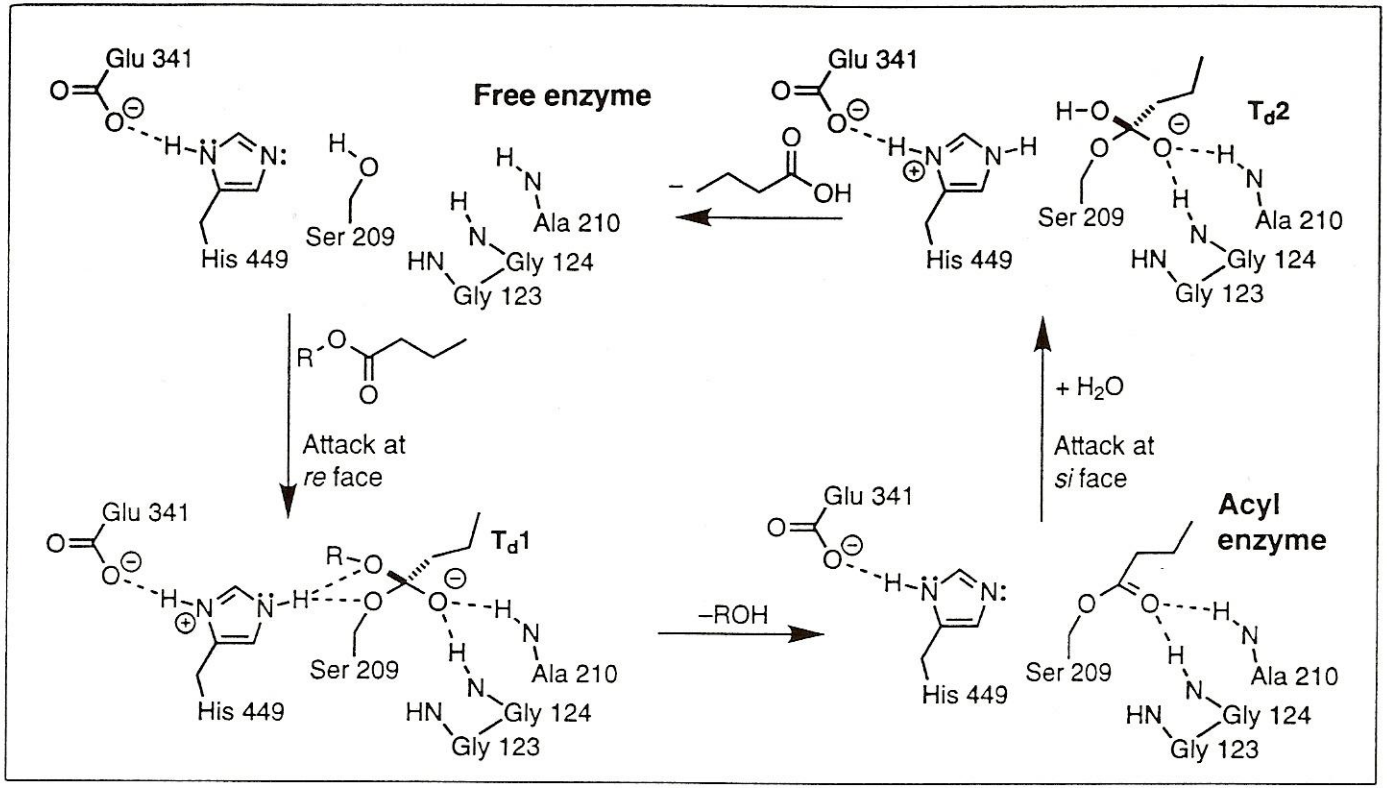


Figure 3

Hydrolysis of a butyrate ester catalyzed by lipase involves an acyl enzyme and two different tetrahedral intermediates. The transition state for the reaction resembles the first tetrahedral intermediate, T_d1, when acylation limits the rate, and resembles the second tetrahedral intermediate, T_d2, when deacylation limits the rate. The amino acid numbering corresponds to the active site of lipase from *Candida rugosa*, CRL. Crystal structures of the transition-state analogs suggest that during the formation of T_d1, Ser209 attacks the ester at the *re* face (from the bottom in the orientation shown); however, during the formation of T_d2, water probably attacks at the *si* face of the acyl enzyme (from the top in the orientation shown).

Basi molecolari dell' enantioselettività delle lipasi

La preferenza per l' isomero R-mentolo della lipasi di *C. rugosa* è dovuta alla formazione di un legame idrogeno tra la His449 e il substrato. Questo legame non si forma con l' isomero S.

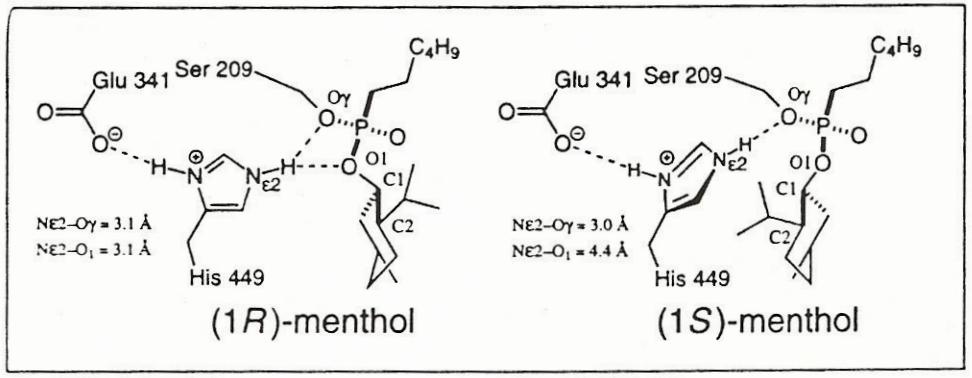


Figure 5

Different interactions between CRL and transition-state analogs containing enantiomeric menthyl groups. For the fast-reacting enantiomer, 1*R*, Nε2 of the catalytic His forms a hydrogen bond to both O_γ of Ser209, which must be deprotonated during the formation of the tetrahedral intermediate, and to O_γ of menthol, which must be protonated during the collapse of tetrahedral intermediate. The slow-reacting enantiomer, 1*S*, distorts the orientation of His so that Nε2 forms a hydrogen bond only to O_γ of Ser209.

Applicazioni biotecnologiche delle lipasi

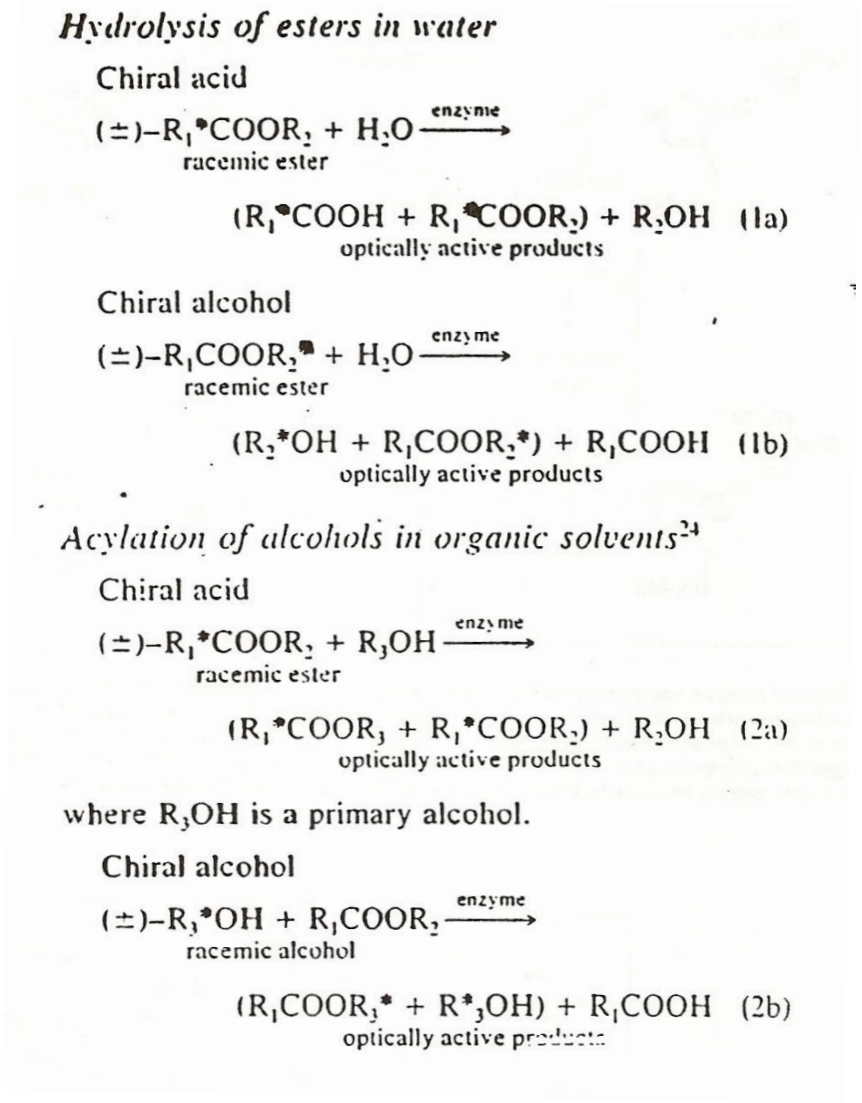
Reazioni di idrolisi

- detersivi: bassa specificità di substrato
  - stabilità ad alte temperature, alle proteasi e alla denaturazione chimica
  - optimum di pH 10-11
- ingredienti nei cibi: produzione acidi grassi poli-insaturi (PUFA)

Reazioni di idrolisi/sintesi

- produzione di biodiesel
- risoluzione cinetica di miscele racemiche
  - ottimizzazione condizioni di reazione (solvente, temperatura, agente acilante)
  - tecniche di immobilizzazione (matrici idrofobiche al silicio R-Si(OCH_3)$_3$ e cristalli reticolati)
  - evoluzione in vitro per aumentare l' enantioselettività
  - risoluzione cinetica dinamica di miscele racemiche per ottenere la conversione completa del substrato

Reazioni catalizzate dalle lipasi



Hydrolysis of esters in water

Chiral acid

$(\pm)$–R_1*$COOR_2$ + H_2O $\xrightarrow{\text{enzyme}}$

racemic ester

(R_1*$COOH$ + R_1*$COOR_2$) + R_2OH (1a)

optically active products

Chiral alcohol

$(\pm)$–R_1COOR_2* + H_2O $\xrightarrow{\text{enzyme}}$

racemic ester

(R_2*OH + R_1COOR_2*) + R_1COOH (1b)

optically active products

Acylation of alcohols in organic solvents[24]

Chiral acid

$(\pm)$–R_1*$COOR_2$ + R_3OH $\xrightarrow{\text{enzyme}}$

racemic ester

(R_1*$COOR_3$ + R_1*$COOR_2$) + R_2OH (2a)

optically active products

where R_3OH is a primary alcohol.

Chiral alcohol

$(\pm)$–R_3*OH + R_1COOR_2 $\xrightarrow{\text{enzyme}}$

racemic alcohol

(R_1COOR_3* + R*$_3OH$) + R_1COOH (2b)

optically active products

Per rendere la reazione di transesterificazione irreversibile si possono utilizzare come gruppi acilanti degli esteri che diano origine a prodotti che non sono più substrato della lipasi.

$R_1COOCH{=}CH_2 + R_2OH$
$\rightarrow R_1COOR_2 + HOCH{=}CH_2$

$HOCH{=}CH_2$ è instabile e si trasforma in CH_3CHO

Acidi grassi poli-insaturi

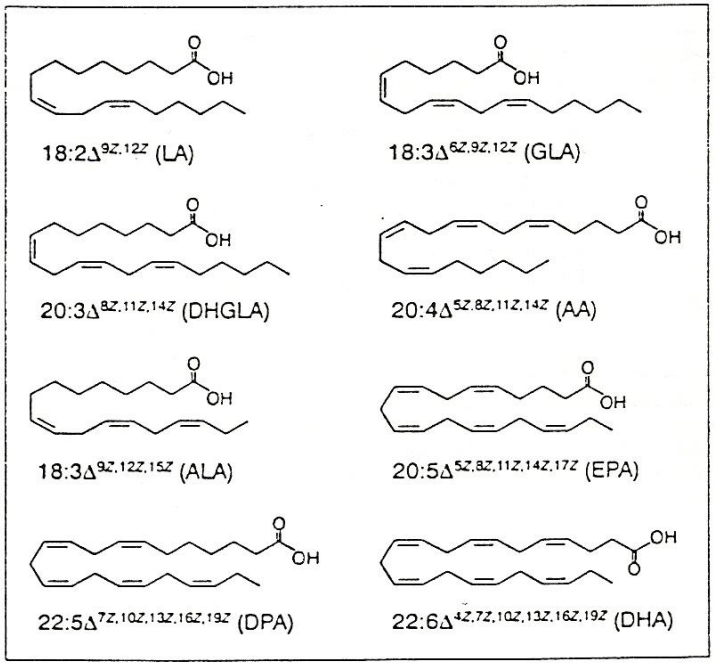


Figure 1
Representative examples of naturally occurring polyunsaturated fatty acids.

Table 2. Physiological effects of ω-3 and ω-6 series eicosanoids

System	ω-6 Eicosanoids	ω-3 Eicosanoids	Biological functions regulated
Cardiovascular	PGI_2, TXA_2	PGI_3, TXA_3	Platelet adhesion and aggregation; fibrinogen; serum lipids; endothelial permeability; vasodilation; vasoconstriction; erythrocyte deformability; blood pressure
Respiratory	PGE_2, $PGF_{2\alpha}$, TXA_2, LTC_4, LTD_4	PGI_3, TXA_3	Bronchodilation; bronchoconstriction; inflammatory responses
Gastrointestinal	PGE_2, PGI_2, $PGF_{2\alpha}$, TXA_2, LTB_4, LTC_4, LTD_4	PGI_3, LTB_5	Gastric secretion; gastric cytoprotection; peristalsis; insulin and pancreatic-amylase
Renal	PGD_2, PGE_2, $PGF_{2\alpha}$, PGH_2, PGI_2, TXA_2, LTB_4	PGE_3, PGI_3, TXA_3, LTB_5	Glomerular permeability and filtration; renal vasodilation; renin secretion; sodium excretion
Immune	5-HPETE, 12-HPETE, 15-HPETE, PGE_2, PGI_2, LTB_4, LTC_4, LTD_4	LTB_5	Vascular permeability; cytokine response; PAF production; leukocyte receptor expression; leukocyte degranulation; macrophage and neutrophil aggregation; chemotaxis; epidermal proliferation

Abbreviations: 5-, 12- and 15-HPETE, 5-, 12- and 15-hydroperoxyeicosatetraenoic acid; LTA_n, LTB_n, LTC_n and LTD_n, leukotriene A_n, B_n, C_n and D_n, respectively; PGE_n and PGF_n, prostaglandin E_n and F_n, respectively; PGI_n, prostacyclin I_n; TXA_n, thromboxane A_n. The numerical subscripts refer to the eicosanoid subclass. Data taken from Refs 3, 6, 46, 52 and 53.

Applicazione di lipasi nella produzione di acidi grassi poli-insaturi

Table 2. Representative industrial applications of lipases to speciality PUFA lipid products.

PUFA product	Lipase catalyst	Substrate	Reaction	Applications	Ref.
FFA concentrates	*Chromobacterium viscosum*, *Pseudomonas fluorescens*	PUFA oils	Hydrolysis	Anticholesterolaemics, etc.	38
FFA concentrates	*Candida* sp.	Fish oils	Hydrolysis	Pharmaceuticals, nutraceuticals	39
DHA concentrate	*Candida* sp., *Penicillium* sp.	Fish oils	Hydrolysis	Pharmaceuticals, nutraceuticals	40
Glycerides	Thermostable lipase	PUFA esters + glycerol	Transesterification	Anti-inflammatories, etc.	41
Monoglycerides	Alkaline lipases	PUFA oils	Hydrolysis	Pharmaceuticals, nutraceuticals	42
sn-2-Diglycerides	(i) Phospholipase A_2 (ii) Phospholipase C	PUFAs + *sn*-2-lysophospholipids	(i) Esterification (ii) Hydrolysis	Anticoagulants, thrombolytics Nutraceuticals	43 43
Triglycerides	*Rhizomucor miehei*, *Rhizomucor javanicus*	PUFAs + triglycerides	Transesterification	Nutraceuticals	44
Triglycerides	Various lipases	PUFA lipids + PUFAs	Transesterification	Pharmaceuticals, nutraceuticals	45
Triglycerides	*Candida antartica*	PUFAs + glycerol	Esterification	Pharmaceuticals, nutraceuticals	46
sn-2-Phospholipids	*Pseudomonas cepacia*, *Humicola lanuginosa*	PUFAs + phospholipids	Transesterification	Anti-inflammatories, etc.	47

Abbreviations: DHA, dicosahexaenoic acid; FFA, free fatty acid; PUFAs, polyunsaturated fatty acids.

Applicazione di lipasi nella produzione di biodiesel

- Il biodiesel è costituito da esteri metilici di acidi grassi (FAME)
- La reazione di transesterificazione di trigliceridi e metanolo produce biodiesel e glicerolo
- Catalisi alcalina (NaOH) vs catalisi enzimatica

Table 4
Comparison of enzymatic technology versus conventional alkaline technology for biodiesel production.

Key issue	Enzymatic process	Alkaline process
Presence of free fatty acid in the starting oil	Free fatty acids are transformed to biodiesel.	Free fatty acids are transformed to soaps.
Water content of starting oil	It is not deleterious for lipase.	Impact on the catalyst by forming soaps. It may hydrolyze the oil and ultimately more soaps are formed.
Biodiesel yield[a]	High, usually around 90%.	High, usually >96%.
Glycerol recovery	Easy, high grade glycerol.	Complex, low grade glycerol.
Catalyst recovery and reusage	Easy or not necessary when operating in a PBR. Reusability not sufficiently studied.	Difficult or not profitable, usually it is neutralized by adding an acid after transesterification. It is partially lost as soaps or in the successive washing steps.
Energy costs	Low, temperature range 20–50 °C.	Medium, temperature range 60–80 °C.
Catalyst cost	High	Low
Environmental impact	Low, waste water treatment not needed.	Medium, alkaline and saline effluents are generated. Wastewater treatment needed.
Process productivity[b]	Low	High

[a] Percentage of starting oil transformed to biodiesel.
[b] Mass of biodiesel produced per volume of reactor and per unit of time.

Applicazione di lipasi nella produzione di biodiesel

- Lipasi purificate o microrganismi che producono elevati livelli dell' enzima (ricombinante o naturale)
- Biocatalizzatore immobilizzato
- Fonti di trigliceridi: oli vegetali commestibili (olio di girasole, soia, palma) e non commestibili, microrganismi ricchi di lipidi (microalghe, batteri o lieviti), oli di scarto (alimentari, lavorazione carta o tabacco)
- Variabili da controllare: temperatura, alcool (inattivazione dell' enzima in eccesso di metanolo), presenza di acqua e solventi, rigenerazione della lipasi, concentrazione di acidi grassi liberi

Applicazione di lipasi nella produzione di biodiesel

Table 5
Examples of research works on enzymatic production of biodiesel by transesterification.

Oil	Enzyme	Acyl-acceptor	Solvent	Yield (%)
Sunflower	Novozym-435	Methanol	No	3
		Methanol	Petroleum ether	79
		Ethanol	No	82
Tallow	Lipozyme IM-60	Primary alcohols	Hexane	94.8–98.5
Soybean	Novozym	Secondary alcohols	Hexane	61.2–83.8
Rapeseed	Lipozyme IM	Methanol	No	19.4
	Lipozyme IM	Ethanol	No	65.5
Soybean	*Rhizopus* oryzae lipase	Methanol	No, water 4–30% by wt.	80–90
Palm	Lipase PS-30	Methanol	No	15
		Ethanol	No	72
Soybean	Novozym-435 preincubated 0.5 h in ethyl oleate	Methanol	No	97
Soybean (crude)	*Candida antarctica* lipase	Methanol	No	93.8
Soybean	Novozym-435	Methyl acetate	No	92
Triolein	Various commercial lipases	Linear and branched alcohols	No	near 100
		Fusel oil-like alcohol mixture		
Soybean	Lipase PS (immobilized)	Methanol	No	67
		Ethanol	No	65
Vegetable oils	*Candida* sp. lipase (immobilized)	Methanol	No	96–93
Frying oils				92
Rapeseed	Lipozyme TL IM	Methanol	*t*-butanol	95
	Novozym-435	Methanol	*t*-butanol	95
Jatropha Sunflower	Novozym-435	2-propanol	Hexane	92.8–93.4
Jatropha Sunflower	Novozym-435	Ethyl acetate	No	91.3
		Ethyl acetate	No	92.7
Microalgae	*Candida* sp. lipase (immobilized)	Methanol	Hexane	98
Cotton	Novozym-435	Methanol	*t*-butanol	97
Vegetable oils	Novozym 435	Methanol	No	near 100
	Lipozyme TL IM	Ethanol		
Microalgae	Various commercial lipases	Long-chain alcohols	Hexane	–
Waste edible oil (2.5% free fatty acids)	Novozym 435	Methanol	No	>90
Acid oil (77.9% free fatty acids)	Novozym 435	Methanol	No	>90
Soybean oil deodorizer distillate (28% free fatty acids)	Novozym 435 Lipozyme TL IM	Methanol	*t*-butanol	around 95%

Applicazione di lipasi immobilizzate nella produzione di biodiesel

Table 1
Biodiesel production with various immobilized lipase (Jegannathan et al., 2008).

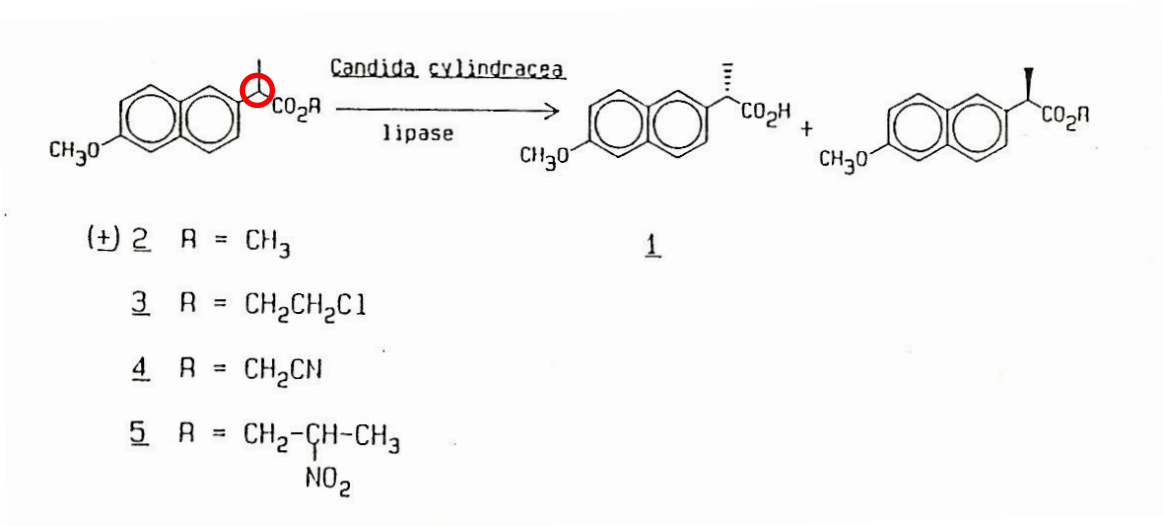
Immobilized method	Carrier used	Lipase origin	Oil	Acyl acceptors	Yield (%)
Adsorption	Acrylic resin	*Candida antartica*	Vegetable oil, waste cooking oil	Methanol, 1-propanol, methyl acetate	>90
Adsorption	Textile membrane	*Candida* sp. 99–125	Lard, waste oil, salad oil	Methanol	>87
Adsorption	Toyonite 200-M, polypropylene	*Pseudomonas fluorescens*	Vegetable oil	Methanol	>87
Adsorption	Celite, Diatomaceous earth	*Pseudomonas cepacia*,	Jatropha oil, vegetable oil	Ethanol, 2-butanol	>98
Adsorption	Anion resin, celite-545	*Porcine pancreatic*, *Rhizomucor Miehei*, *Chromobacterium viscosum*	Sunflower oil, soybean oil, Jatropha oil	Ethanol, methanol	>80
Covalent bond	Silica-PVA styrene-divinylbenzene	*Burkholderia cepacia*, *Thermomyces lanuginosus*	Babassu oil, canola oil	Ethanol, methanol	>97%
Entrapment	Hydrophobic sol–gel support	*Pseudomonas cepacia*, NS44035	Soybean oil, triolein,	Methanol, ethanol	60
Cross-linking	Glutaraldehyde	*Pseudomonas cepacia*	Mahua oil	Ethanol	92

Table 5
Summary of the options to avoid lipase inactivation caused by methanol.

Options	Operating conditions	Yield (%)	Advantages	Disadvantage
Methanol stepwise addition	Three-step or two-step methanol addition	>87	Higher yield is obtained without inactivation to the lipase	The operation is relative complicated in large scale production.
Acyl acceptor alterations	Methyl acetate, acetate ethyl	>90	No inactivation effect occurs and no glycerol is produced.	The reaction rate is low and the acyl acceptor cost is high.
Solvent engineering	With *t*-butanol, 1,4-dioxane, ionic liquid as solvents	>80	Good solvents of methanol and glycerol, so methanol inactivation and glycerol deposit are avoided.	Increment of the solvent recovery cost.

Risoluzione cinetica di miscele racemiche di precursori di farmaci

Il Naproxen (acido metil-2-(6-metossi-2-naftil) propionico) è un farmaco anti-infiammatorio non steroideo che contiene un centro chirale. L' isomero S è 28 volte più attivo dell' isomero R. Può essere ottenuto per idrolisi enantiospecifica della miscela racemica dell' estere precursore della molecola attiva.



(±) 2 R = CH_3

3 R = CH_2CH_2Cl

4 R = CH_2CN

5 R = CH_2-$\underset{NO_2}{CH}$-CH_3

1

La lipasi di *C. cylindracea* (*C. rugosa*) mostra preferenza stereochimica per l' isomero S

TABLE 1. Enantiospecific hydrolysis of (±)-methyl-2-(6-methoxy-2-naphthyl)propionate (2) by microbial lipases.

Lipase Source[1]	Stereochemical Preference	Extent of Conversion (%)	Enantiomeric Excess (%) Ester	Acid	E
Candida cylindracea[1a]	S	39	63	>98	>100
Mucor meihei[1b]	R	18	21	95	51
Rhizopus arrhizus[1c]	R	11	13	97	78
Rhizopus sp.[1d]	R	19	21	92	27
Rhizopus oryzae[1e]	R	11	10	76	8

[1] To one ml of 0.2 M potassium phosphate buffer, pH 8.0, was added 244 mg (1 mmol) of (±)2 and varying amounts of different enzyme preparations. The contents were incubated at 22°C for 120-216 h under gentle stirring. [a] 1 mg of pure enzyme[9] isolated from the Sigma type VII preparation, 216 h; [b] 200 mg of Amano MAP10 powder, 120 h; [c] 10 mg of enzyme of Boehringer-Mannheim, 120 h; [d] 150 mg of powder from Serva, 120 h; [e] 200 mg of Amano FAP powder, 120 h.

[2] E is the ratio of the specificity constants (k_{cat}/K_m) of the two enantiomers.[10]

La velocità di reazione dipende dalla natura del gruppo uscente

TABLE 2. Relative rates of enzymatic hydrolysis.

Compound	Relative rate	Enantiomeric ratio (E)
2	1	>100
3	15	>100
4	6	>100
5	3	81

Nuove tecniche di immobilizzazione delle lipasi

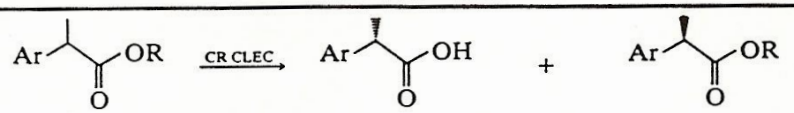
- Intrappolamento in gel di silice modificato con gruppi alchilici $CH_3Si(OCH_3)_3$ e Si $(OCH_3)_4$ per creare un microambiente idrofobico e mantenere attività catalitica

- Cristalli reticolati (cross-linked enzyme crystals) con glutaraldeide. I cristalli reticolati (CLEC) sono insolubili, stabili in acqua e in solventi organici, sono altamente porosi, permettono la diffusione del substrato e possono essere facilmente recuperati al termine della reazione.

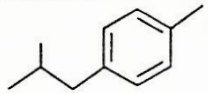
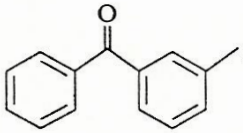
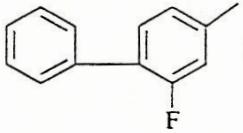
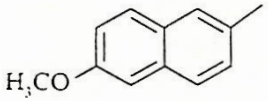
Immobilizzazione della lipasi di *C. rugosa* per la risoluzione di miscele racemiche di esteri di acidi arilpropionici

Metodo di immobilizzazione: cristalli di enzima reticolati con glutaraldeide. La cristallizzazione avviene in 2-metil-2,4-pentandiolo, che permette di mantenere l' attività enzimatica e l' accessibilità del sito attivo.

TABLE I
CR CLEC LIPASE-CATALYZED RESOLUTION OF ARYLPROPIONIC ACID ESTERS



(*R,S*)-α-Arylpropionate ester (*S*)-Arylpropionic acid (*R*)-Arylpropionate ester

Ar	% Enantiomeric excess (% conv.)		*E*	
	CR CLEC	Crude	CR CLEC	Crude
 **1a:** R = H **ibuprofen** **b:** R = Me	94.6 *S*-**1a** (22)[a]	81.9 *S*-**1a** (39.3)[a]	47	17
 **2a:** R = H **ketoprofen** **b:** R = CH_2CH_2Cl	91.1 *S*-**2a** (49.3)[b]	64.5 *R*-**2b** (66)[b]	66	5
 **3a:** R = H **flurbiprofen** **b:** R = CH_2CH_2Cl	94.3 *S*-**3a** (34.4)[c]	61.1 *S*-**3a** (34)[c]	55	6
 **4a:** R = H **naproxen** **b:** R = Me	97.3 *S*-**4a** (39)[d]	76.2 *S*-**4a** (46.8)[d]	>100	12

[a] Reaction buffer 0.1 *M* pH 6 sodium acetate.
[b] Reaction buffer 0.1 *M* pH 5 sodium acetate.
[c] Reaction buffer 0.1 *M* pH 7 sodium phosphate.
[d] Reaction buffer 50% PEG 1000/50% pH 5 ammonium acetate.

Evoluzione in vitro di lipasi per aumentare l' enantioselettività dell' enzima

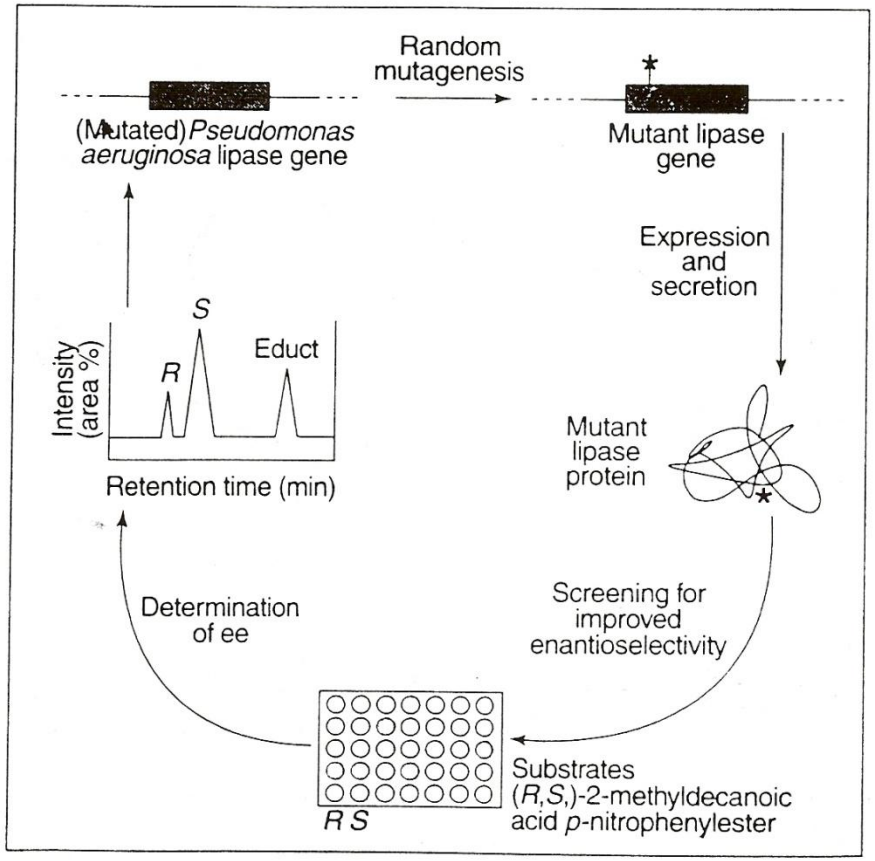


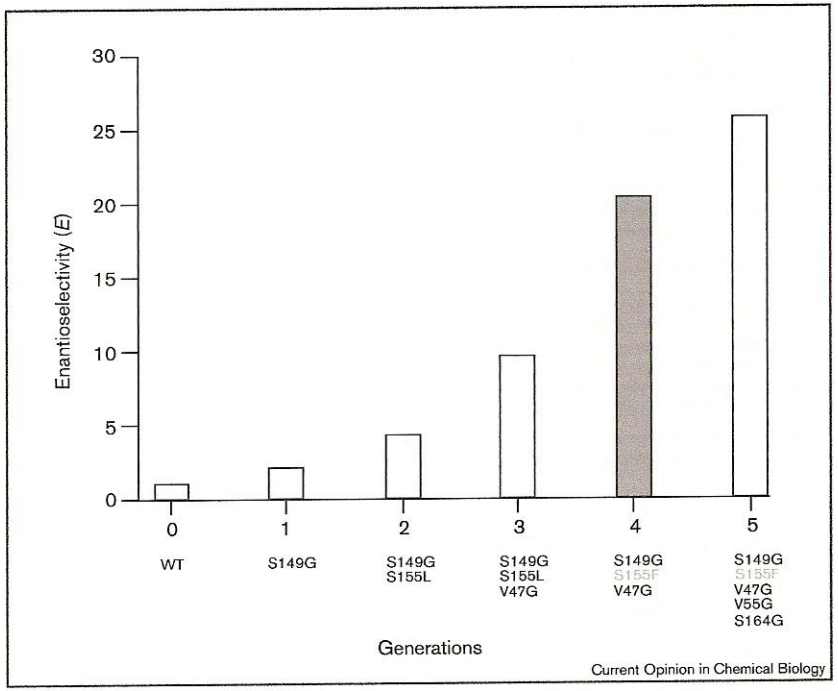
Figure 5

Strategy to create an enantioselective lipase by directed evolution. Intensity (area %) refers to the amount of *R*- and *S*-enantiomer as measured by chiral chromatography.

Evoluzione in vitro di lipasi per aumentare l' enantioselettività dell' enzima

Figure 4

Creation of an enantioselective lipase by directed evolution. The lipase gene from *P. aeruginosa* was subjected to random mutagenesis by ep-PCR. Mutant proteins were identified by UV/Vis spectrophotometry using 2-methyldecanoate *p*-nitrophenylester as the substrate, and mutations leading to improved enantioselectivity (white bars) were identified by DNA-sequencing. The mutations present in each generation are shown along the x-axis in single letter code for amino acids. Subsequent saturation mutagenesis at previously identified amino acid positions lead to a further increase in enantioselectivity for mutant S155F (shaded bar), which proved to be superior over S155L previously identified in the second generation which was generated by ep-PCR. This improvement is highlighted on the x-axis using grey text.



Mutagenesi sito-specifica del sito attivo della lipasi di *Yarrowia lipolitica* per aumentare l' enantioselettività dell' enzima

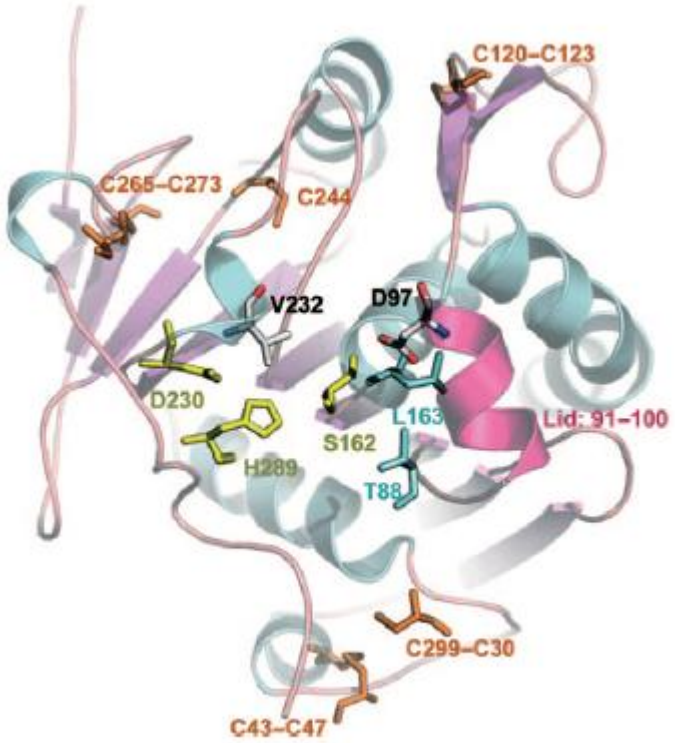


Figure 3. Overall representation of the Lip2p homology model. Hydrogen atoms on amino acid residues have been omitted for clarity purpose.

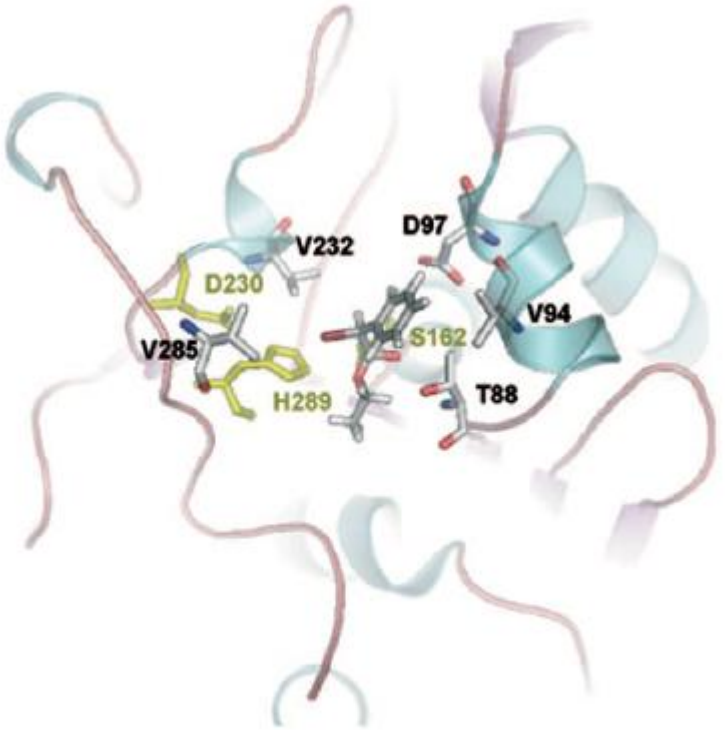


Figure 5. Representation of Lip2p amino acid residues selected for site-directed mutagenesis. The S-2-bromo-phenylacetic acid ethyl ester covalently bound to Ser162 is shown in the active site.

Mutagenesi sito-specifica di lipasi per aumentare l' enantioselettività dell' enzima

Table 1. *p*-Nitrophenol butyrate hydrolysis activity of wild-type Lip2p and its variants.

Enzyme	WT	T88S	T88X[c]	V94A	V94L	V285A	V285L	V232A	V232L	D97A
Initial rate[a,b]	64.0	21.3	0	52.8	42.5	46.0	45.6	47.4	40.3	9.8

[a] µmol of *p*NP liberated per minute and mL of enzyme. [b] Each experiment was carried out in triplicate. [c] X = A, V, L.

Table 2. 2-bromo-phenylacetic acid ethyl ester hydrolysis activity of wild-type Lip2p and its variants.

Enzyme	WT	T88S	V94A	V94L	V285A	V285L	V232A	V232L	D97A
viS[a]	1.71	2.13	1.41	1	0.97	1.3	8.8	0.017	0.010
viR[a]	0.58	1.04	0.39	0.44	0.4	0.4	0.101	0.31	0.34
E value[b]	3(*S*)	2(*S*)	4(*S*)	2(*S*)	2(*S*)	3(*S*)	87(*S*)	18(*R*)	34(*R*)
conversion [%]	54.7 (8 h)						52.9 (8.5 h)		
ee_s[c] [%]	53.5						99.6		
ee_p[d] [%]	43.7						88.7		

[a] µmol of 2-bromo-phenylacetic acid liberated per hour and mL of enzyme. [b] *E* value = viS/viR or viR/viS according to enantiomer preference; viR, viS: initial rates. [c] Substrate enantiomeric excess. [d] Product enantiomeric excess.

Mutagenesi sito-specifica di lipasi per aumentare l' enantioselettività dell' enzima

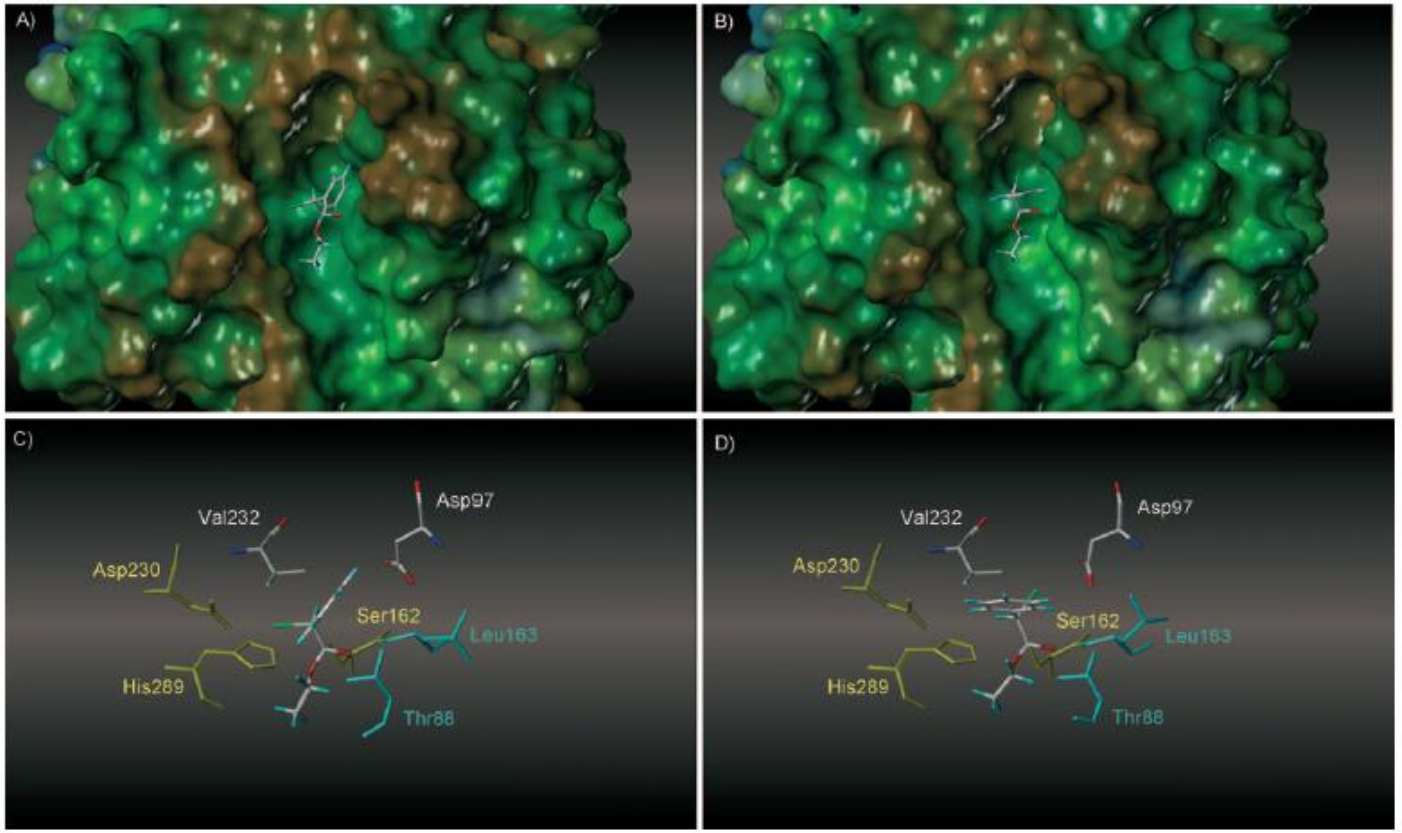


Figure 6. Representation of (*R,S*)-2-bromo-phenylacetic acid ethyl ester enantiomers covalently bound to catalytic Ser162 of Lip2p. A), B) *S* and *R* enantiomers are respectively shown. Lip2p is shown as a Connolly surface mapped with the lipophilic potential, as calculated by the MOLCAD module implemented in SybyL7.3 (Tripos, Saint Louis, USA). The protein surface is colour-coded (brown colour indicates more lipophilic regions whereas blue codes for more polar ones). C), D) Arrangement of the *S* (left) and *R* (right) enantiomers with respect to the catalytic triad (coloured in yellow) as well as the residues forming the oxyanion hole (cyan coloured) and the two key positions (V232 and D97) playing a role on enantio-discrimination.

Mutagenesi sito-specifica di lipasi per aumentare l' enantioselettività dell' enzima

Le dimensioni del residuo in posizione 232 determinano la preferenza della lipasi per un enantiomero rispetto all' altro.
Aminoacidi piccoli: S
Aminoacidi grandi: R

Substrato: esteri dell' acido 2-bromo-fenilacetico, intermedi nella sintesi di farmaci

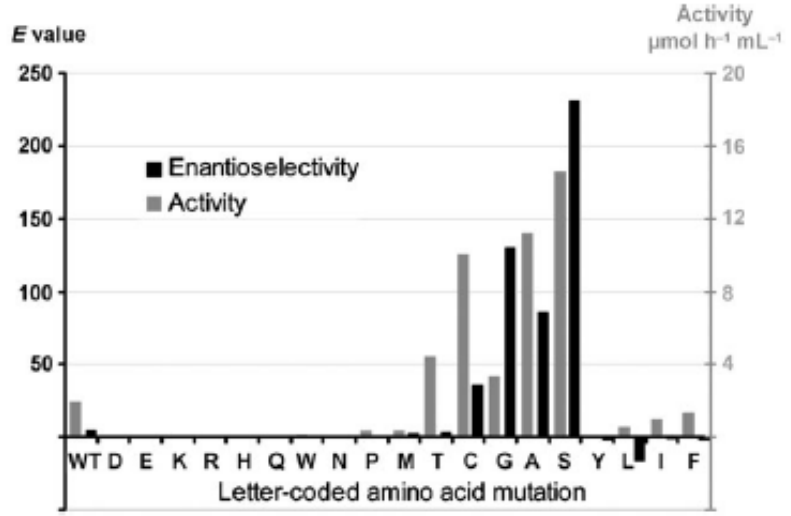


Figure 7. Activity and enantioselectivity of V232 variants in 2-bromo-phenyl-acetic octyl ester racemate hydrolysis reaction. WT: wild-type Lip2p. A positive *E* value corresponds to *S* selectivity, a negative *E* value to *R* selectivity.