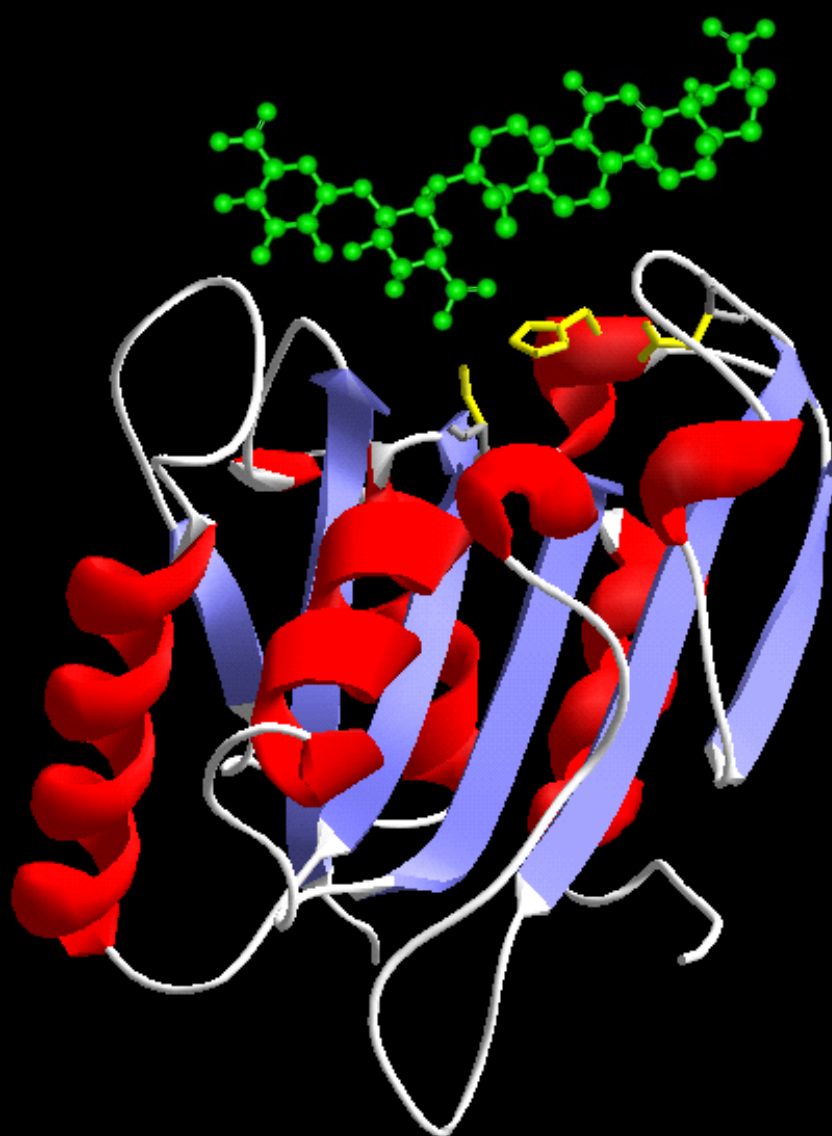


MICROBIAL LIPASES WITH INTEREST IN BIOTECHNOLOGY AND INFECTIOUS DISEASES: ISOLATION, CHARACTERIZATION AND INHIBITION BY NATURAL SUBSTANCES



Cristian Ruiz Rueda

PhD Thesis

Universitat de Barcelona

Facultat de Biologia

Departament de Microbiologia



Microbial lipases with interest in biotechnology and infectious diseases: isolation, characterization and inhibition by natural substances

Lipasas microbianas con interés en biotecnología y en enfermedades infecciosas:
aislamiento, caracterización e inhibición por sustancias naturales

Cristian Ruiz Rueda

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PhD program: Environmental microbiology and biotechnology (2000–2001).

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Dr. Pilar Díaz Lucea, professor from the Department of Microbiology of the Faculty of Biology of the Universitat de Barcelona, certifies that the research work “**Microbial lipases with interest in biotechnology and infectious diseases: isolation, characterization and inhibition by natural substances**” presented as PhD Thesis by Cristian Ruiz Rueda has been performed under her direction in the mentioned department, and that satisfies the necessary requirements to compete for the PhD degree by the Universitat de Barcelona. And for the record, she signs this certificate dated May 10th 2005.

La Dra. Pilar Díaz Lucea, profesora titular del Departamento de Microbiología de la Facultad de Biología de la Universidad de Barcelona, certifica que el trabajo de investigación “**Microbial lipases with interest in biotechnology and infectious diseases: isolation, characterization and inhibition by natural substances**” presentado como Tesis Doctoral por Cristian Ruiz Rueda se ha realizado bajo su dirección en dicho departamento, y que reúne los requisitos necesarios para optar al grado de Doctor por la Universidad de Barcelona. Y para que así conste, firma la presente certificación con fecha 10 de mayo de 2005.

Pilar Díaz Lucea

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“Only unknown things make the soul bigger”

Michel Eyquem de Montaigne

“Sólo ante lo desconocido el alma se agranda”

Michel Eyquem de Montaigne

To my parents, my sister and Laura

A mis padres, a mi hermana y a Laura

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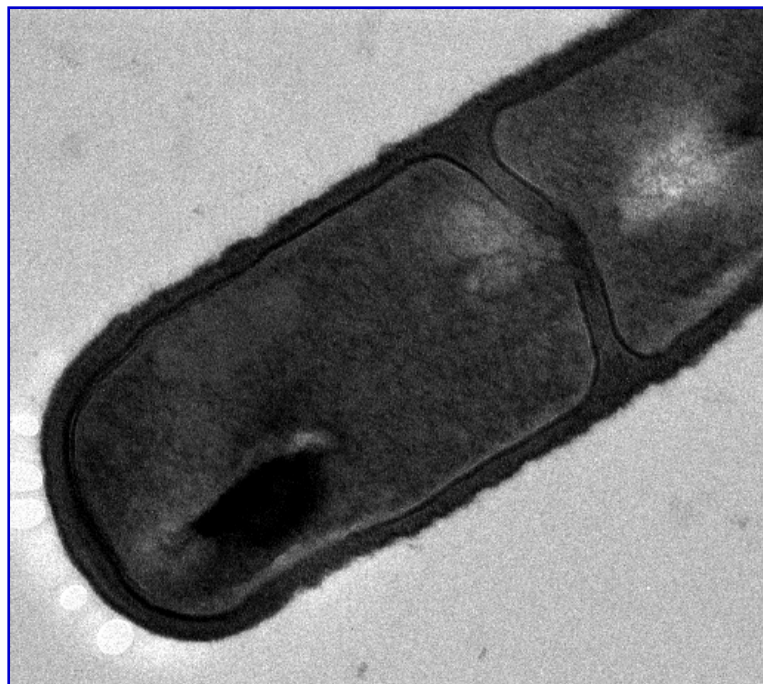


Figure A.1 *Bacillus* sp. CR-179.

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ABBREVIATIONS



Figure A.2 Isolate CR-273.

1-9

16S rDNA: 16S ribosomal DNA

3-HF: 3-hydroxyflavone

5-HF: 5-hydroxyflavone

A

$A_{405\text{ nm}}$: Absorbance at $\lambda = 505\text{ nm}$

$A_{600\text{ nm}}$: Absorbance at $\lambda = 600\text{ nm}$

AA: Amino acid

ABC: ATP-binding cassette

AG(s): Acylglycerol(s)

Ap: Ampicillin

APS: Ammonium persulphate

ATP: Adenosine triphosphate

B

B.: *Bacillus*

BCIP: 5-bromo-3-chloro-4-indolyl phosphate

BHL: *N*-butyryl-homoserine lactone

BMLipA: *Bacillus megaterium* CECT370 LipA carboxylesterase.

BmlipA: *Bacillus megaterium* CECT370 LipA gene

BP6LipA: *Bacillus* sp. BP-6 LipA carboxylesterase

BP6lipA: *Bacillus* sp. BP-6 LipA gene

BP7LipA: *Bacillus* sp. BP-7 LipA carboxylesterase

BP7lipA: *Bacillus* sp. BP-7 LipA gene

BP7EstA1: *Bacillus* sp. BP-7 EstA1 carboxylesterase

BP7estA1: *Bacillus* sp. BP-7 EstA1 gene

BP23EstA: *Paenibacillus* sp. BP-23 EstA carboxylesterase

BP23estA: *Paenibacillus* sp. BP-23 EstA gene

BSlipA: *Bacillus subtilis* MB216 LipA gene

BSLipA: *Bacillus subtilis* MB216 LipA lipase.

BW: Backward

C

C.: *Candida*

cagA: *H. pylori* cytotoxin associated gene

CagA: *H. pylori* cytotoxin associated protein

CE(s): Carboxylesterase(s)

CECT: Spanish Type Culture Collection

Cm: Chloramphenicol

CMC: Critical micelle concentration

CoA: Coenzyme A

CRH: Corticotrophin releasing hormone

CRL(s): *Candida rugosa* lipase(s)

D

DAG(s): Diacylglycerol(s)

ddNTPs: Dideoxynucleotide triphosphates

DNA: Deoxyribonucleic acid

dNTPs: Deoxynucleotide triphosphates

Dsb: Disulfide bond formation

E-F

E.: *Escherichia*

EDTA: Ethylenediamine-N,N,N',N'-tetraacetic acid

EGME: Ethyleneglycol monomethylether

E.g.: *Exempli gratia* (as example)

EstV: *Helicobacter pylori* EstV carboxylesterase

estV: *Helicobacter pylori* EstV gene

FA(s): Fatty acid(s)

FPLC: Fast protein liquid chromatography

FW: Forward

G-H

GA: Glycyrrhizic acid

Gb.: *Geobacillus*

GehA: Glycerol-ester hydrolase A (*Propionibacterium acnes* lipase).

gehA: Glycerol-ester hydrolase A gene (*Propionibacterium acnes* lipase gene).

GRAS: Generally regarded as safe

H.: *Helicobacter*

HLA: Human leucocyte antigen

HPLC: High Pressure Liquid Chromatography

HSL(s): Hormone-sensitive lipase(s)

HSPs: Heat shock proteins

I

IC₁₆: Concentration yielding a lipase inhibition of 16%

IC₅₀: Concentration yielding a lipase inhibition of 50%

i.e.: *id est* (that is)

IEF: Isoelectric focusing

IFN- γ : Interferon-gamma

IL: Interleukin

IPCR: Inverse polymerase chain reaction

IPTG: Isopropyl- β -D-thiogalactopyranoside

J-K-L

k_{cat}^{app} : Apparent catalytic constant

Km: Kanamycin

K_M^{app} : Apparent Michaelis-Menten constant

LB: Luria-Bertani broth

Lif(s): Lipase intermolecular foldase(s)

LPL: Lipoprotein lipase

LPS(s): Lipopolysaccharide(s)

M

MAG(s): Monoacylglycerol(s)

MALT: Mucosa associated lymphoid tissue

MFP: Membrane fusion protein

MHC II: Major histocompatibility complex II

MIC: Minimal inhibitory concentration

MUF: Methylumbelliferone

MUFA(s): Monounsaturated fatty acid(s)

MUF-butyrate: Methylumbelliferyl butyrate

MUF-oleate: Methylumbelliferyl oleate

MW: Molecular weight

N-O

NAI: *N*-Acetylimidazole

NBS: *N*-Bromosuccinimide

NBT: Nitroblue tetrazolium chloride

NMR: Nuclear magnetic resonance

NTA-Ni: Nitrilotriacetic-nickel acid

O/N: Overnight

OMP: Outer membrane protein

ORF(s): Open reading frame(s)

P

P.: *Propionibacterium*

PAGE: Polyacrylamide gel electrophoresis

PAF: Platelet-activating factor

Pb.: *Paenibacillus*

PBS: Phosphate Buffered Saline

PCR: Polymerase chain reaction

PGA: Polygalacturonic acid

PGE₂: Prostaglandin E₂

PHA: Polyhydroxyalkanoate

PHMB: *p*-Hydroxymercuribenzoic acid

pI: Isoelectric point

PldA: *H. pylori* phospholipase A2

pldA: *H. pylori* phospholipase A2 gene

PMSF: Phenylmethylsulfonyl fluoride

p-NP: *p*-Nitrophenyl

p-NPL: *p*-Nitrophenyl laurate

p-NPs: *p*-Nitrophenyl esters of fatty acids

Ps.: *Pseudomonas*

PUFA(s): Polyunsaturated fatty acid(s)

PVDF: β-Polyvinylidene difluoride

Q-R

□S: *Quillaja* saponin

RCM: Reinforced Clostridial Agar

RNA: Ribonucleic acid

RT: Room temperature

S

S.: *Staphylococcus*

SAPHO: Synovitis, acne, pustulosis, hyperostosis, and osteitis

SEM: Scanning electron microscopy

SDS: Sodium dodecyl sulphate

SFA(s): Saturated fatty acid(s)

sn: stereospecific number

SP: Substance P (neuropeptide)

T

TAG(s): Triacylglycerol(s)

Tat: Twin arginine translocation

Tc: Tetracycline

TEM: Transmission electron microscopy

TEMED: N,N,N',N'-
Tetramethylethylenediamine

TL(s): “True” lipase(s)

Tm¹: Theoretical melting temperature (at 50 mM Na⁺) of each primer.

Tm²: Melting temperature at which the amplification reaction was performed.

TNF- α : Tumour necrosis factor alpha.

Tris: Tris(hydroxymethyl)amino methane

U-V-W-X-Y-Z

UV: Ultraviolet

VacA: *H. pylori* vacuolating cytotoxin

vacA: *H. pylori* vacuolating cytotoxin gene

VLDL: Very low density lipoproteins

V_{\max}^{app} : Apparent maximal velocity

X-gal: 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside

β

β -N: β -naphthol

β -NL: β -naphthyl laurate