Industrial applications of biocatalysts

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SOLVAY, Research and Innovation Center Lyon

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ENZINOV: Enzymes Innovations Industries
27-28 October 2014

Solvay - Historical strength in fast-growing regions

Employees Sites R&I center

EUROPE
33% sales
15,800 49 8

ASIA PACIFIC & REST OF THE WORLD
31% sales
6,000 28 4

NORTH AMERICA
25% sales
3,700 33 2

LATIN AMERICA
11% sales
3,900 7 1

90% of sales in businesses among the top 3 global leaders

A balanced portfolio of activities, directed at growth regions

A diversified offer serving numerous end markets

Solvay way - a culture of sustainability,

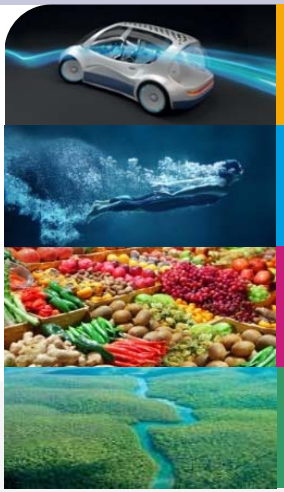
€9.9 bn NET SALES

117 IND SITES 29400 employees in 55 countries

15 MAJOR R&I 1950 employees

252 new patents in 2013

280 m€ R&I effort



Automotive & Aeronautics **17%**

Consumer Goods & Healthcare **25%**

Agro, Feed & Food **12%**

Energy & Environment **11%**

Building & Construction **10%**

Electrical & Electronics **6%**

Industrial Applications **19%**

% of Group net sales (2013)

R&I - Opening up Innovation

Academic partnerships a link between fundamental and applied research

Exploratory partnerships with start-ups and venture capital funds

Partnership with market key players (customers, suppliers)

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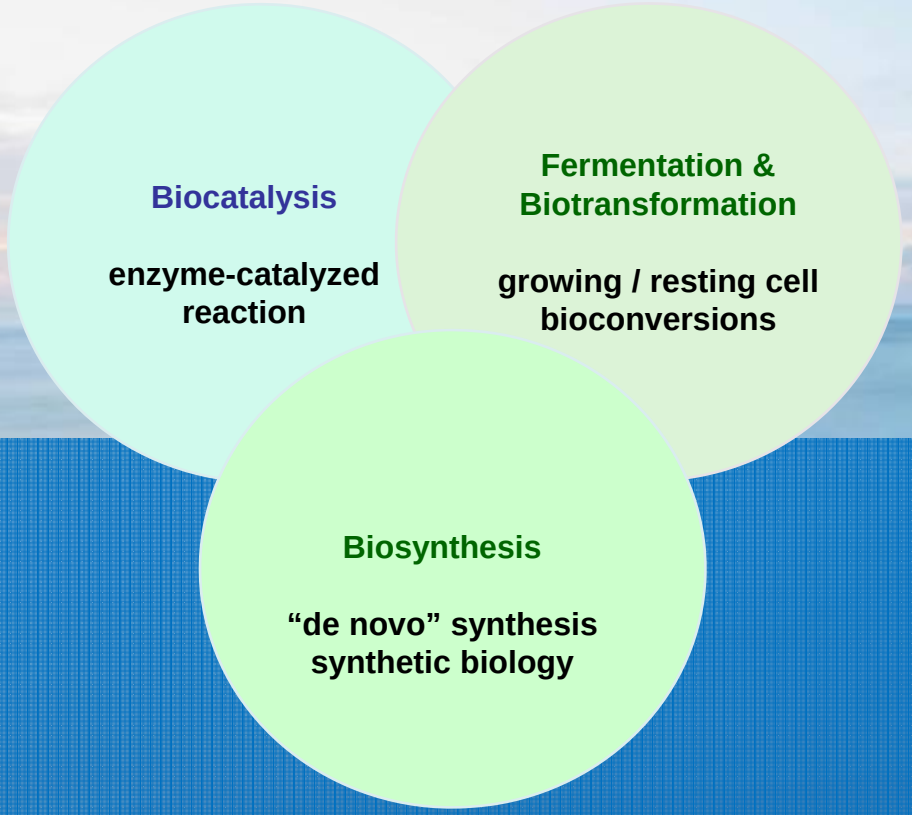
2. Biotechnologies for an Industrial

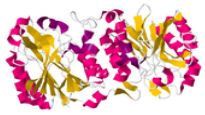
Use of living organisms or substances obtained from living material to make products of value from renewable carbon sources using eco-friendly bioprocesses.

- living organism - essentially of microbial origin
- substances obtained from living material – enzymes
- products of value - chemicals, materials and biofuels



Advantages of using biocatalysts

- Catalyze a wide variety of organic reactions
- Very efficient catalysts
- Chiral catalysts → selectivity
- Act under mild conditions
- Environmentally “friendly”
- Can be easily prepared by fermentation
- etc. etc.



Pyrococcus abyssi nirtilase

Bacteria (*Bacillus*)

Fungi (*Penicillium*)

Yeasts (*Saccharomyces*)

A Strain or an Enzyme ?

Enzyme is preferred when:	Strain is preferred when:
- commercially available or easy to produce - one or two-step reaction - no need for cofactors - good stability - label “natural” (e.g. flavors)	- multi-step reactions - whole pathway design - enzyme not commercially available - label “natural” (e.g. flavors)

Bio-process block

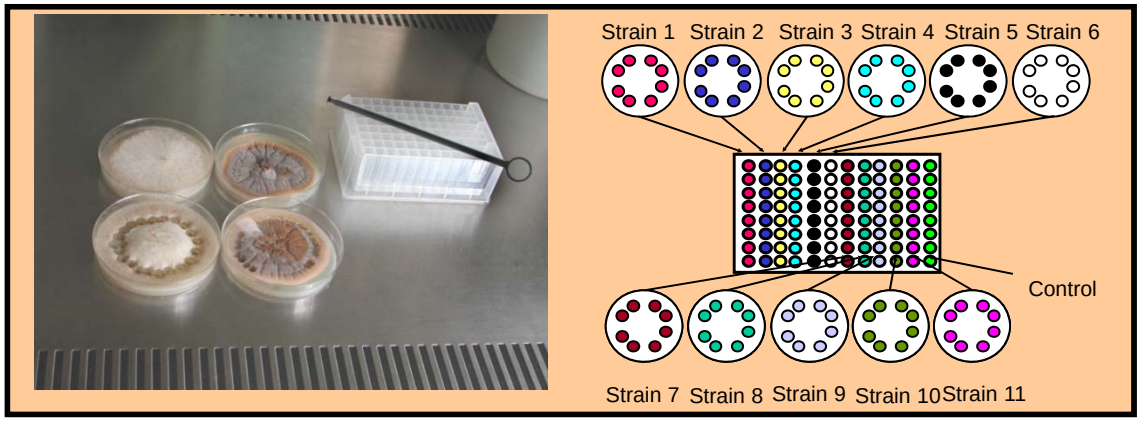
Enzyme / Strain / Library (ex. metagenomic…)
screening
genetic constructions, engineering …

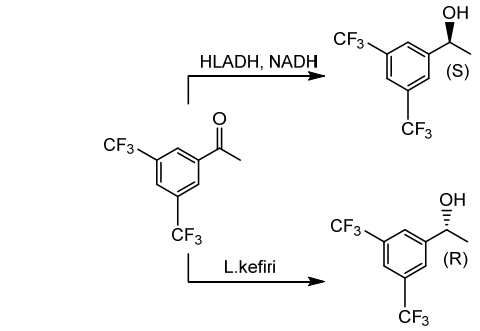
Process for
Biotransformation / fermentation
Biocatalysis (bio-chemistry)

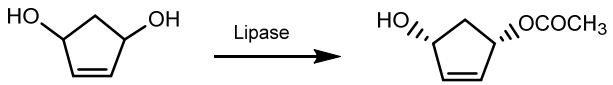
Process for product recovery (DSP)

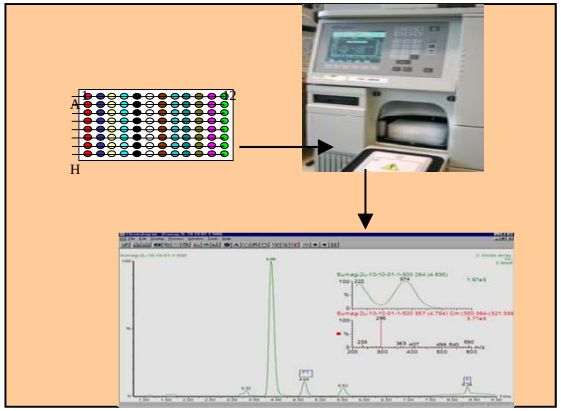
Enzymatic & microbial screening

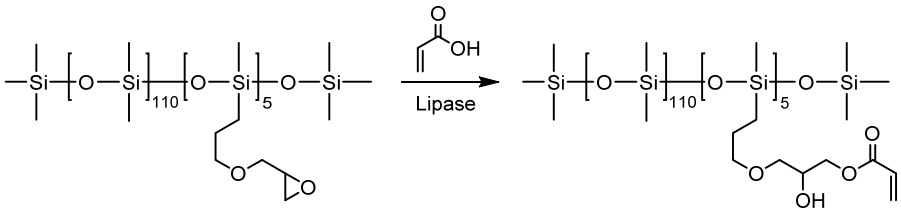
Screening of enzymes, wt & recombinant strains

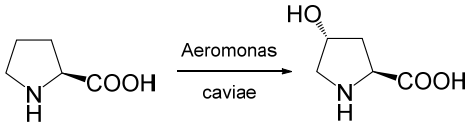


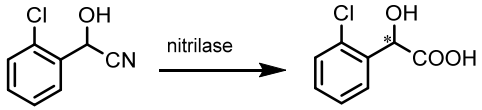


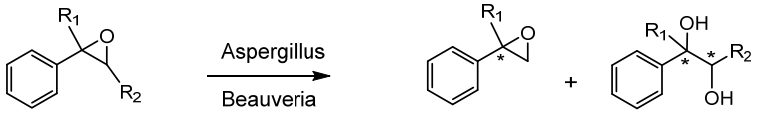




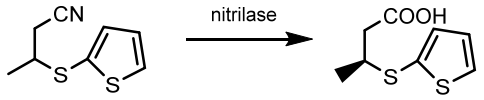






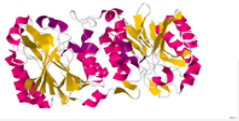


R_1 = H, CH_3
R_2 = H, $COOC_2H_5$



150 YEARS | SOLVAY asking more from chemistry®

Enzymatic & microbial screening



Pyrococcus abyssi nirtilase

Bacteria (*Bacillus*)

Fungi (*Penicillium*)

Yeasts (*Saccharomyces*)

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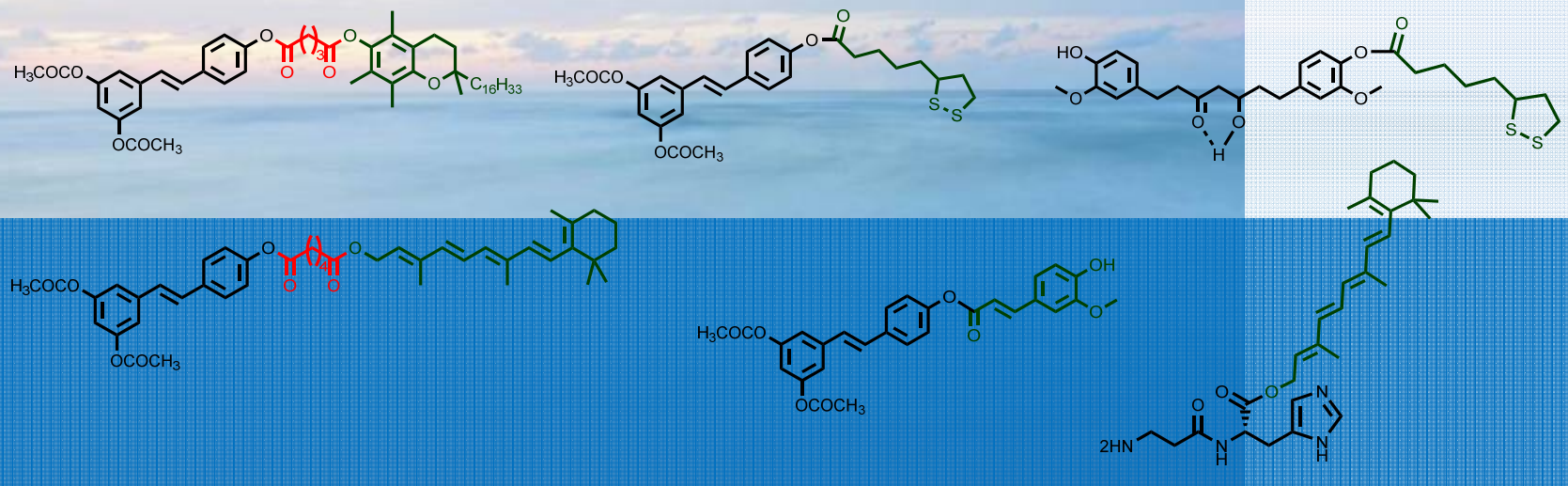
Biocatalysis in the synthesis of antioxidant derivatives for dermo-cosmetic applications and their controlled release by skin enzymes

Our experiences & examples

Antioxidants and anti-aging compounds lack solubility and stability in cosmetic preparations.

Aim:

- synthesis of new & original molecules combination of vitamins and anti-oxidans: Resveratrol, Luteolin, Tetrahydrocurcumin, Lipoic acid, Vitamins (A & E), Ferulic acid, L-Carnosine
- stable precursors of bioactive compounds
- their controlled release by the action of extracellular skin enzymes

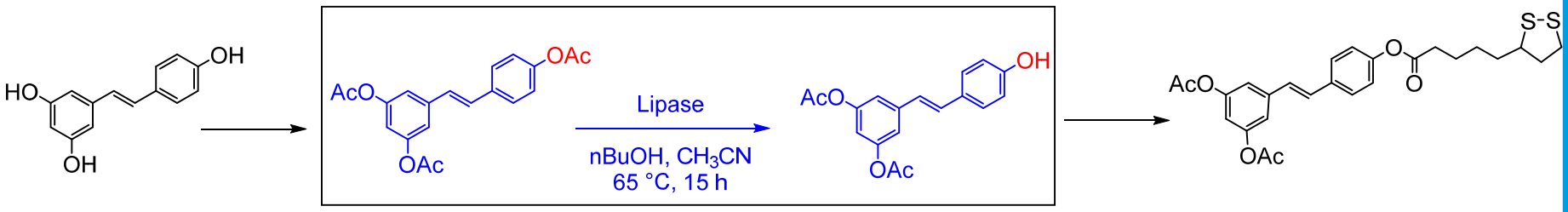


Solvay (Rhodia) - Chanel partnership

WO2006/134282; International Journal of Cosmetic Science, 2008, 30, 195

Resveratrol-lipoate derivative

Key step - selective de-acetylation of resveratrol triacetate



Enzymatic step is required to selectively remove an acetate group from Res(Ac)$_3$ precursor

Substrate concentration:	200 g/l
Enzyme load:	1 % w/w
Productivity:	15 g/l/h
Isolated yield:	95 %
Selectivity:	99 % di-Acetate (1% mono-Acetate)

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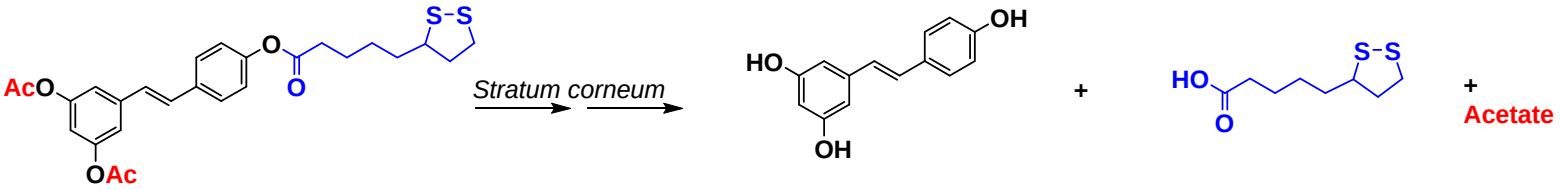
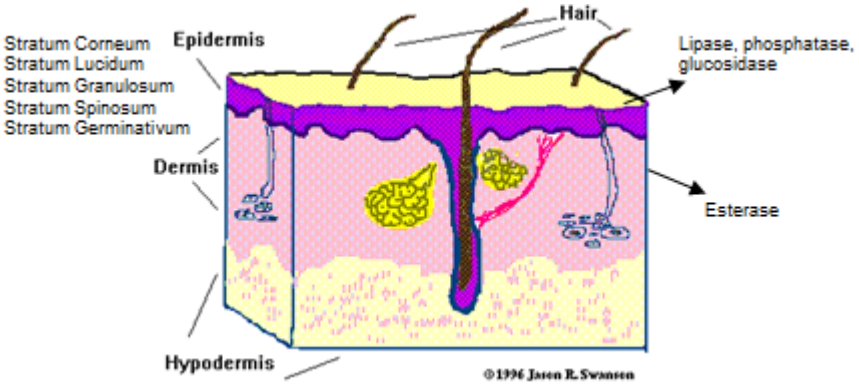
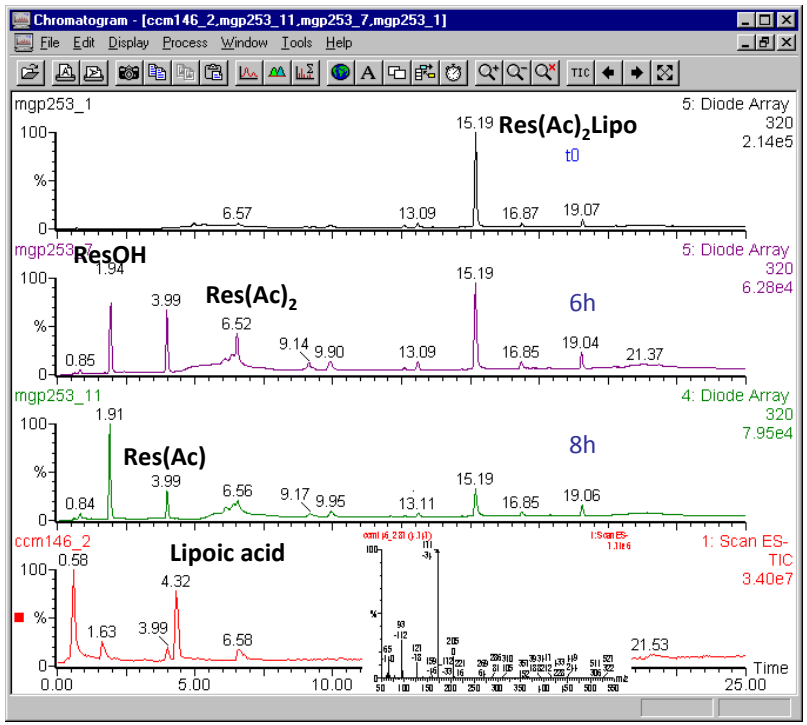
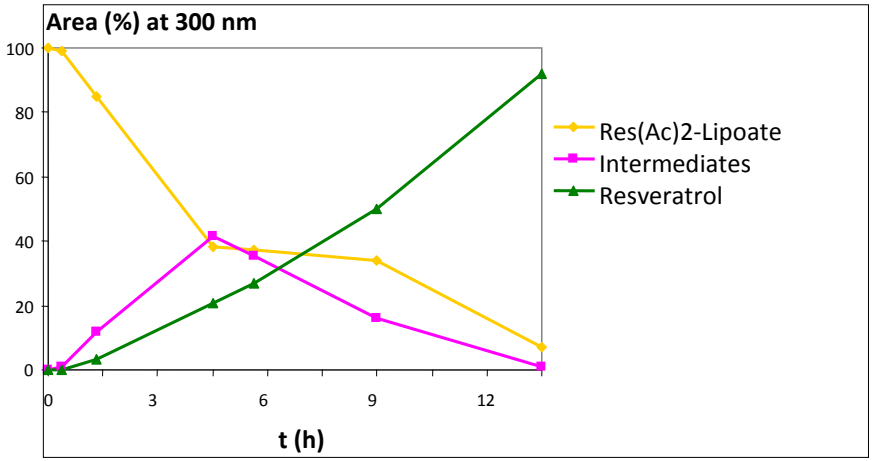
In vitro hydrolysis with skin enzymes



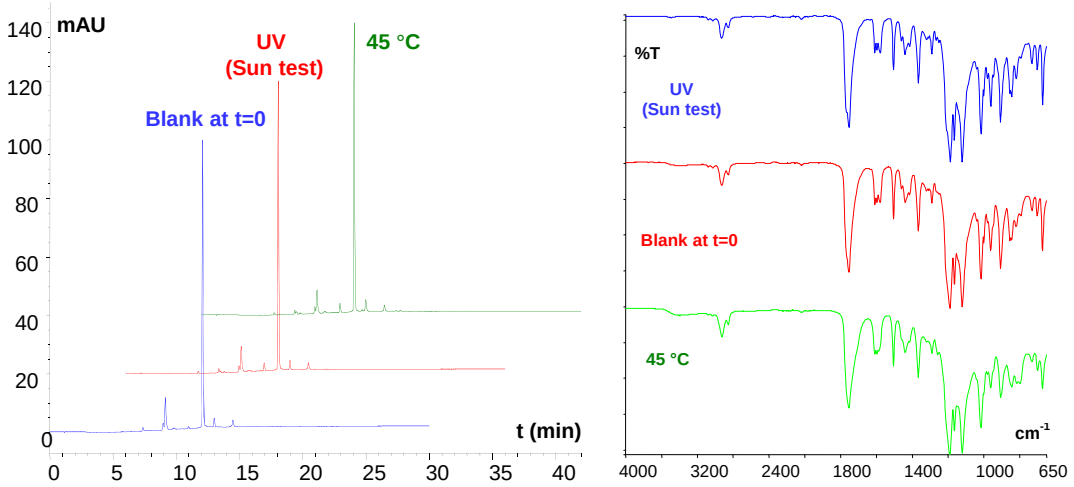






Toxicology

Local Lymph Node Assay (LLNA)	Negative
Ames	Negative
Acute oral toxicity	Non classified (LD_{50} > 2000 mg/kg)
Skin irritation	Non irritant
Eye irritation	Irritant



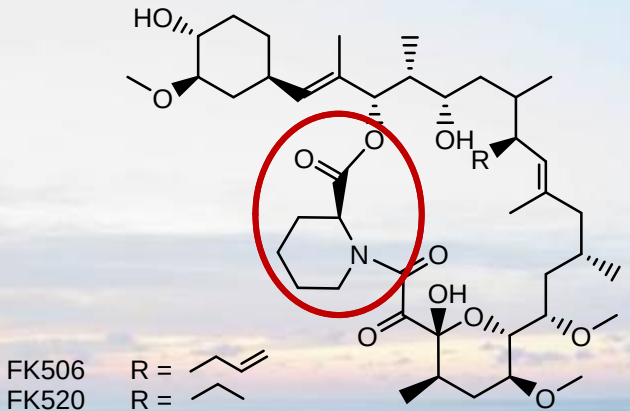
Stability & sun test

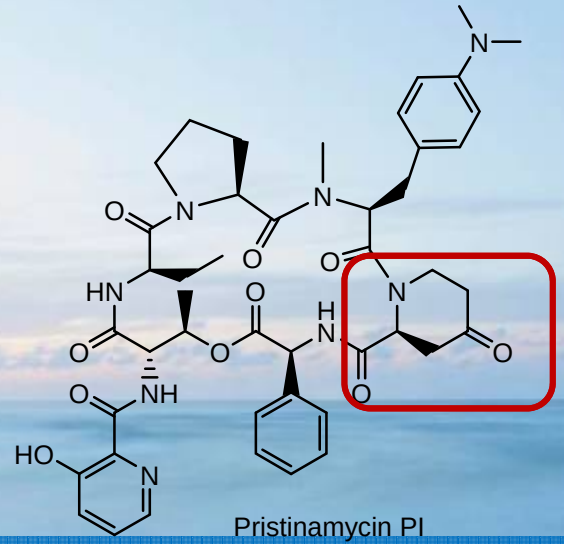
- ✓ **New original molecules, stable and non-toxic precursors of antioxidants**
- ✓ **Efficient and easy method for preparation of a human *Stratum corneum* enzymes**
- ✓ **POC of *in vitro* hydrolysis of different resveratrol precursors with skin enzymes**

Fermentation / biotransformation in the synthesis of L-pipecolic acid from L-Lysine using *Streptomyces pristinaespiralis* L-Lysine CycloDeaminase (LCD)

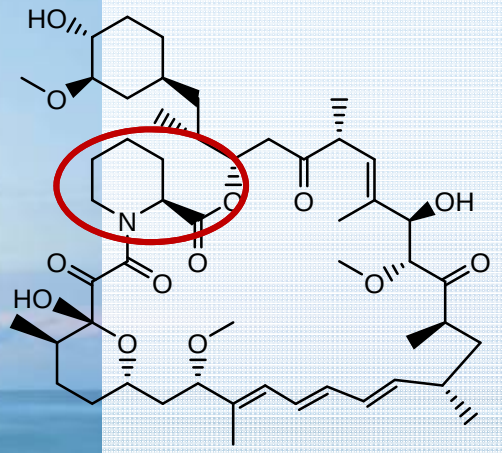
Our experiences & examples

Polyketides from *Streptomyces spp*



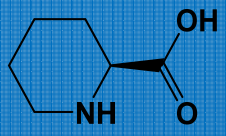


Pristinamycin PI



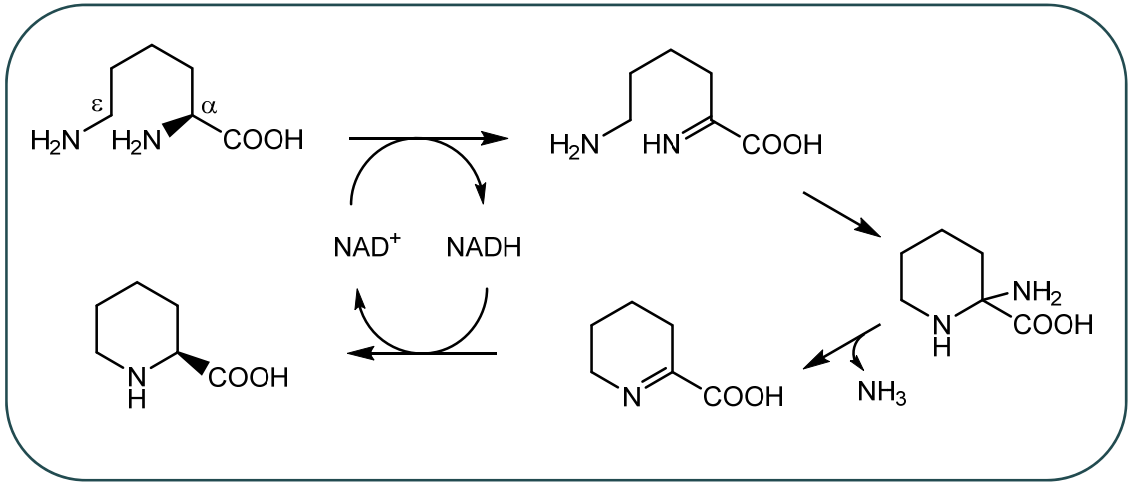
Rapamycin

L-pipecolic acid

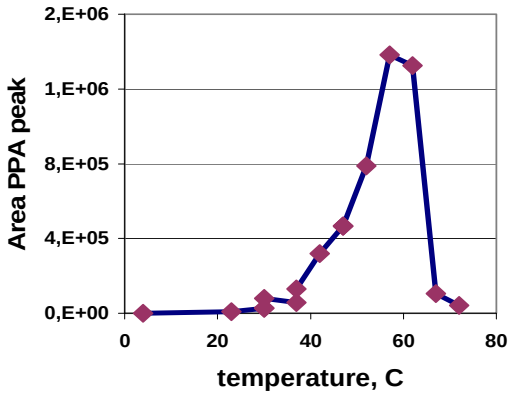


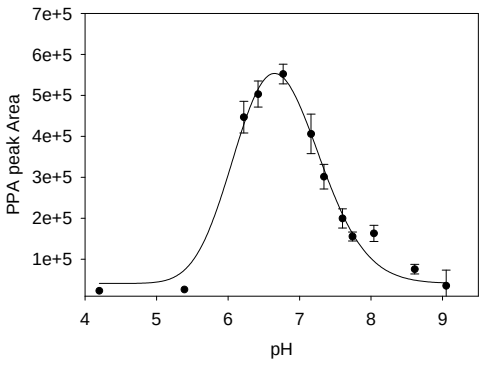
Solvay (Rhodia) – Evologic (Virtual genome) collaboration

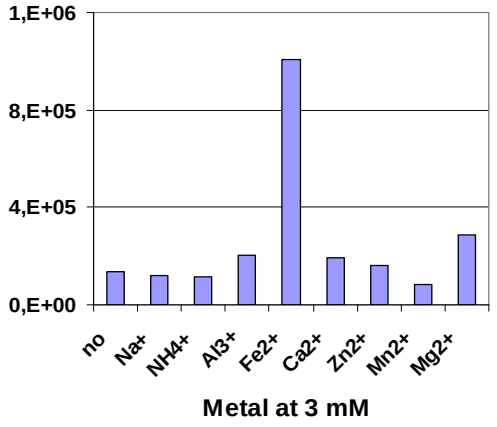
Synthesis of L-pipecolic acid from L-Lysine using Lysine Cyclo Deaminase



- Identification & characterization of *Streptomyces pristinaespirales* wt LCD
- *pipA* gene cloned & expressed as native and His-tag LCD in *E.coli*
- Characterization of recombinant LCD enzyme
- Random mutagenesis to increase LCD performances







Biochimie vol. 89 issue 5 May, 2007. p. 591-604

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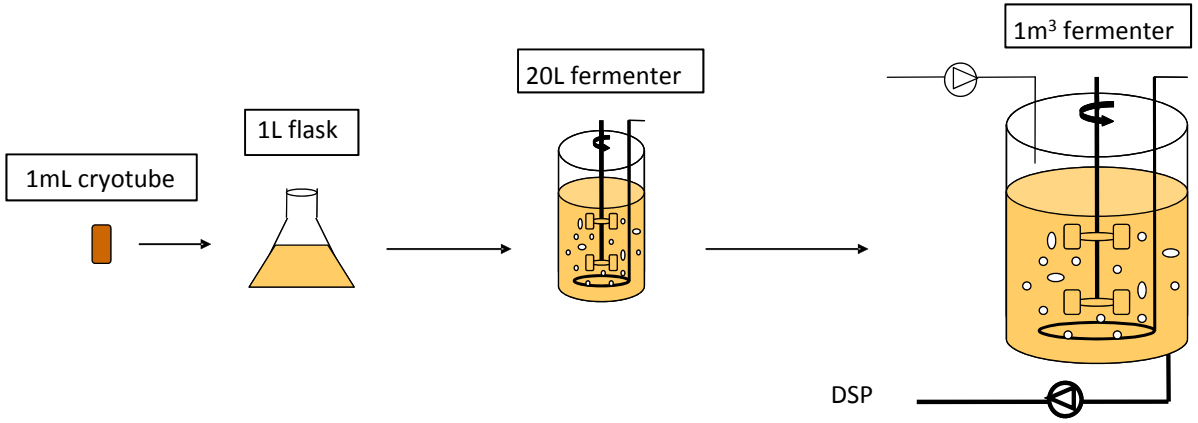
Synthesis of L-pipecolic acid from L-Lysine using Lysine Cyclo Deaminase

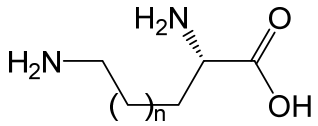


Steps:

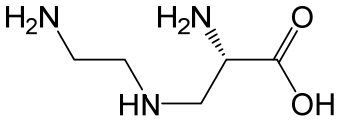
- Growth of recombinant *E.coli* on C source & biomass production (culture seed in a 1l flask, 20L inoculum fermenter, 1m^3 production fermenter)
- Induction of enzyme production
- Cell permeabilisation, lysine addition and biocatalysis
- Pipecolic acid isolation and purification

Substrate (L-Lys) concentration:	120	g/l
Productivity:	5	g/l/h
Isolated yield:	75	%
Selectivity:	100	%

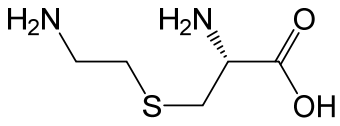
- **Highly efficient & selective conversion of L-Lys → L-pipecolic**
- **Random mutagenesis of LCD toward novel substrates using XL1-Red mutator strain**
- **Colorimetric test for a rapid screening of mutants**
  - ✓ C5 and C6 amino acid substrates
  - ✓ 4-position can be C, O, N, S, C-OH



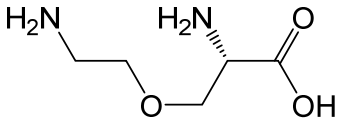
n=0, L-Ornitine
n=1, L-Lysine



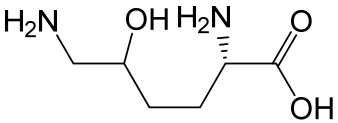
4-Aza-Lysine



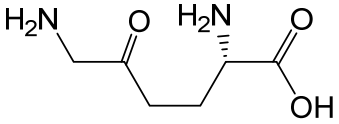
4-Thia-Lysine



4-Oxa-Lysine



5-Hydroxy-Lysine



5-Keto-Lysine

4. Conclusions

The use of enzymes has been expanded by advances in genetic engineering, “omics”, synthetic biology, improved stabilization and immobilization techniques and by understanding of structure-function relationships…

Industrial applications are today in pharmaceutical chemistry & healthcare industry, food and consumer products, biopolymers, bioremediation…

There is an enormous potential among the microbial world with almost no reaction that can not be catalyzed by an enzyme !
Enzymes can do what chemists have always been dreamed of and have not been able to realize.
You just have to imagine !

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Acknowledgments

Team

Claude Bensoussan
Fabien Barbirato
Véronique Bastiani
Marie-Claude Bontoux
Florence Foray
Mirjana Gelo-Pujic
Cédric Marion
Erwan Vermel
Virginie Neugnot-Roux (postdoctoral fellow)
Georgia Tsotsou (postdoctoral fellow)
Interns

Financial support

Corporate Research
Rhodia Organics
Marie Currie Fellowship

Thanks for your attention

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