29ème colloque - Edition spéciale Jeunes Chercheurs

Club Biocatalyse en Synthèse Organique

29éme CBS Club Biocatalyse en Synthèse Organique

Edition 2022 - Visioconférence

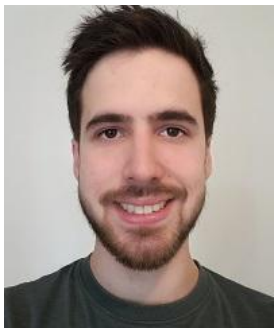
Comité d'organisation du 29ème colloque du CBSO

Célestin BOURGERY
Doctorant 2ème année, URD ABI - AgroParisTech

Cédric GASTALDI
Doctorant 3ème année, Université Clermont-Auvergne

Laurine DUCROT
Doctorante 3ème année, Genoscope, Université Paris-Saclay

Chloé LELIEVRE
Post-doctorante, Institut de Recherches Servier



Juan RODRIGUEZ MOSHEIM
Doctorant 2ème année, UMRt BioEcoAgro/RealCat, Université de Lille

Bienvenue au 29ième colloque
du Club Biocatalyse en Synthèse Organique !

Le 29ème colloque du CBSO est une édition spéciale organisée par des doctorants et post-doctorants, cette édition souhaite mettre à l'honneur les jeunes chercheurs. La majorité des présentations orales sera donc assurée par des doctorants ou post-doctorants ponctuées d'interventions de conférenciers invités.

Un nouveau format, une nouvelle organisation

Cette 29ème édition, qui se déroule en visioconférence sur son intégralité, est organisée par une équipe temporaire, composée de doctorants et post-doctorants français. Le but est de rassembler et mettre en valeur les jeunes chercheurs autour des thématiques du CBSO.

Comme à son habitude, le colloque rassemblera une centaine de participants autour des thèmes de la Biocatalyse et des Biotechnologies Blanches. Son objectif est de créer des rapprochements interdisciplinaires entre chimistes et biologistes issus des recherches académique ou industrielle dans les domaines de la biochimie, de la biologie, de la chimie fine, du génie des procédés ou de la biologie synthétique. Les thématiques abordées pourront porter sur l'ingénierie des biocatalyseurs, la métagénomique, les techniques de criblage, les réactions enzymatiques en cascade ou encore les bioprocédés.

Le Club Biocatalyse en Synthèse Organique (CBSO)

Le **Club Biocatalyse en Synthèse Organique (CBSO)** est un réseau français d'une vingtaine de laboratoires et d'industries qui a été créée en 1982. Il est régi par une association loi 1901 et a pour vocation d'œuvrer à une meilleure visibilité de la Biocatalyse en assurant les missions suivantes :

- **Favoriser l'interdisciplinarité**

Le CBSO est un réseau de compétences pluridisciplinaires en chimie organique et analytique, microbiologie, ingénierie protéique et métabolique, génie des procédés, bio-informatique, modélisation moléculaire.

- **Lever les verrous scientifiques et technologiques**

Les laboratoires du CBSO développent des technologies innovantes, répondant aux principes de la chimie Verte, centrées sur les enzymes utilisées comme biocatalyseurs, pour la synthèse de molécules organiques.

- **Promouvoir les collaborations entre acteurs privés et publics**

Le CBSO organise régulièrement depuis sa création des colloques afin d'échanger les avancées récentes dans le domaine de la biocatalyse et de créer des partenariats entre acteurs académiques et industriels.

Année	Edition	Lieu	Année	Edition	Lieu
1982	1ère	Marseille	1998	15ème	Nouan-le-Fuzelier
1984	2ème	Clermont-Ferrand	1999	16ème	Batz-sur- Mer
1985	3ème	Marseille	2000	17ème	Nîmes
1986	4ème	Gif-sur-Yvette	2001	18ème	La Londe-les-Maures
1987	5ème	Bordeaux	2002	19ème	Aillon-le-Jeune
1988	6ème	Montpellier	2004	20ème	Obernay
1989	7ème	Toulouse	2006	21ème	Ax-les thermes
1990	8ème	Paris	2008	22ème	Bussang
1991	9ème	Grasse	2010	23ème	Royat
1992	10ème	Saint Agnan	2012	24ème	Evry
1993	11ème	Mèze	2014	25ème	Marseille
1994	12ème	Batz-sur-Mer	2016	26ème	Evian-Les Bains
1995	13ème	Lalonde-les-Maures	2018	27ème	Guidel
1996	14ème	La Grande-Motte	2021	28ème	Lège-Cap ferret

Bureau

Présidente : Laurence Hecquet, ICCF, Université Clermont Auvergne
Trésorière : Véronique Alphand, ISM2, Université Aix-Marseille
Secrétaire : Didier Buisson, Museum National d'Histoire Naturelle (MNHN), Paris

Membres du conseil d'administration

Véronique de Bérardinis, Genoscope-CEA, Evry
Bastien Doumèche, ICBMS, Université Lyon 1
Sandrine Morel, TBI, Toulouse
Sandrine Moreau, Servier, Orléans
Sébastien Roy, Sanofi, Vitry/seine
Rob Ter Hall, Clariant, Toulouse

Pour être membre du CBSO, aucune cotisation n'est demandée mais il est nécessaire d'assister régulièrement au colloque (une absence à deux colloques consécutifs ne permet plus de figurer dans la mailing list).

Partenaires

Institut de Recherches Servier

Servier est un groupe pharmaceutique international et indépendant gouverné par une Fondation. S'appuyant sur un chiffre d'affaires de 4,7 milliards d'euros en 2020 réalisé dans 150 pays, Servier emploie 22 500 personnes dans le monde. Sa croissance repose sur un engagement constant dans les maladies cardiovasculaires et du métabolisme, l'oncologie, les neurosciences et les maladies immuno-inflammatoires.

Servier est le deuxième groupe pharmaceutique français avec son siège, deux sites de production (98 % des principes actifs des médicaments princeps du Groupe sont produits en France), deux centres de R&D et un Centre International de Recherche Thérapeutique implantés dans l'Hexagone. Le futur Institut de R&D Servier, au cœur du pôle d'innovation de Paris-Saclay, témoigne de la détermination du Groupe à participer au rayonnement de la recherche française dans le monde.

Centre Européen de Biotechnologies et Bioéconomie (CEBB)

Situé au cœur de la bioraffinerie de Bazancourt-Pomacle, soutenu par les collectivités territoriales, le Centre Européen de Biotechnologie et de Bioéconomie (CEBB) réunit les expertises scientifiques et techniques de quatre chaires et unités d'AgroParisTech, de CentraleSupélec, de NEOMA Business School et de l'URCA pour la valorisation des biomasses et des coproduits des industries agro-alimentaires.

Pour ce faire, le CEBB dispose de nombreuses compétences en prétraitement de la biomasse (caractérisation, fractionnement, FMS/FML et extraction/purification), en transformation de la biomasse (chimie verte, biotechnologies et génie des procédés), en analytique et caractérisation, et en modélisation, instrumentation, simulation. Son approche holistique des problématiques lui permet aussi l'étude de la contribution de la bioéconomie à la transition écologique.

Concernant les thématiques abordées par le CBSO, peuvent être plus spécifiquement mentionnées les expertises suivantes :

- Exploration de la diversité microbienne pour des souches microbiennes connues ou nouvelles : physiologie microbienne, cultures microbiennes, fermentations, omiques et bioinformatique (transcriptomique, génomique, métagénomique, protéomique, métabolomique)

- Exploration de la diversité enzymatique : biologie moléculaire (clonage, expression hétérologue), caractérisation d'enzymes, développement de procédés biocatalytiques (cellulases, hémicellulases, enzymes ligninolytiques, (per)oxydases, estérases, pectinases)

- Exploration de la diversité fongique : physiologie, cultures, fermentations (FMS)

- Couplage procédés biocatalytiques et électrochimie, couplage procédés biocatalytiques et extraction continue (ISPR)

- Fermentation (éthanolique)

- Bioproduction de métabolites secondaires et molécules d'intérêt (e.g., synthons, pigments)

- Fonctionnalisation-modulation enzymatique pour la production de biomolécules et de polymères : glycosylation, acylation, dimérisation/oligomérisation, hydrogénases, oxydo-réductases, transaminases (polyphénols, amines, esters de sucres, esters phénoliques, alkyl glycosides)

- Polymérisation et dégradation enzymatique de polymères/matériaux biosourcés

- Design de bioréacteurs et photo-bioréacteur, optimisation de bioprocédés, étude métabolique de population, contrôle et instrumentation de bioprocédés, jumeau numérique en bioprocéde

Institut Charles Viollette

La chaire industrielle Charles Viollette est portée par l'UMRt BioEcoAgro. Elle a pour objectif la valorisation de coproduits d'origine végétale par des outils de biotechnologies industrielles (fermentation et biocatalyse enzymatique) pour produire des molécules bioactives pour des applications en nutrition, santé, bioénergie et chimie.
La chaire s'appuie sur les compétences fortes de l'UMRt 1158 BioEcoAgro et de l'INAF dans le cadre du Laboratoire International Associé sur les Antimicrobiens Naturels (LIAAN), et associe également les partenaires industriels APEF, Leroux, PremierTech, Eurabiotech, VFBioscience, Gecco et Extractis. Le projet est soutenu par la Métropole Européenne de Lille et l'I-Site et labellisé par les pôles Bioeconomy For Change et ClubsterNSL.

Genopole

Biocluster français dédié à la recherche en génétique et aux biotechnologies appliquées à la santé et à l'environnement, Genopole rassemble 77 entreprises de biotechnologies, 19 laboratoires de recherche, 25 plateformes technologiques, ainsi que des formations universitaires (université d'Évry, Paris Saclay) (Chiffres fin décembre 2021). Son objectif : créer et soutenir des entreprises de biotechnologie et le transfert de technologies vers le secteur industriel, favoriser le développement de la recherche dans les sciences de la vie, développer des enseignements de haut niveau dans ces domaines. Genopole est principalement soutenu par l'État, la Région Ile-de-France, le Département de l'Essonne, l'agglomération Grand Paris Sud, la Ville d'Évry-Courcouronnes et l'AFM-Téléthon.

Evotec

Evotec est une société des sciences de la vie dotée d'un modèle commercial unique qui remplit sa mission de découvrir et de développer des traitements hautement efficaces et de les mettre à la disposition de tous les patients.

La plateforme multimodalité de la Société comprend une combinaison unique de technologies, de données et de sciences innovantes pour la découverte, le développement et la production de produits pharmaceutiques de premier ordre. Evotec exploite cette «Data-driven R&D Autobahn to Cures» pour des projets propriétaires et au sein d'un réseau de partenaires comprenant tous les Top 20 Pharma et plus de 800 sociétés de biotechnologie, des institutions universitaires, ainsi que d'autres acteurs de la santé.

Evotec a des activités stratégiques dans un large éventail de domaines thérapeutiques actuellement sous-desservis, notamment la neurologie et l'oncologie, ainsi que les maladies métaboliques et infectieuses. Dans ces domaines d'expertise, Evotec vise à créer le premier pipeline mondial en copropriété de thérapies innovantes et a à ce jour établi un portefeuille de plus de 200 projets de R&D propriétaires et en copropriété, de la découverte précoce au développement clinique.

Evotec opère dans le monde entier avec plus de 4 200 employés hautement qualifiés. Les 15 sites de la Société offrent des technologies et des services hautement synergiques et fonctionnent comme des pôles d'excellence complémentaires.

Conférenciers invités

Pour cette 29ème édition, nous aurons le plaisir d'accueillir deux conférenciers invités.

Nous avons le plaisir de vous annoncer la participation du **Dr. Caroline Emilie Paul** ainsi que du **Pr. Damien Debecker** , qui nous présenteront leurs travaux lors de conférences plénières. Vous pourrez également les retrouver lors d'une table ronde, un moment privilégié pour en apprendre plus sur leurs parcours et leurs visions de la recherche.

Caroline E. Paul a obtenu sa licence et son master en chimie biologique à l'Université de Toronto avec le professeur M. Nitz, puis un doctorat en 2013 à l'Université d'Oviedo avec les professeurs V. Gotor-Fernández et I. Lavandera dans le cadre du projet européen BIOTRAINS.

Après un travail postdoctoral en tant que boursière Marie Curie avec le Prof. F. Hollmann (TU Delft), elle a obtenu une bourse NWO VENI et a poursuivi ses intérêts de recherche sur les cofacteurs biomimétiques pour les oxydoréductases à l'Université de Wageningen.

Depuis octobre 2018, elle est professeure adjointe en biocatalyse au département de biotechnologie de TU Delft.

Après des études de bioingénierie, **Damien Debecker** a obtenu un doctorat en catalyse hétérogène à l'Université catholique de Louvain avec le professeur Eric Gaigneaux.

Après un premier travail postdoctoral en chimie des matériaux avec le Prof. Clément Sanchez (Collège de France, Paris), et un second en biocatalyse avec le Prof. Nicholas Turner (Université de Manchester), il s'est installé à l'Université de Louvain où il a fondé son propre groupe de recherche, se focalisant à la fois sur la chimie inorganique et des matériaux, la caractérisation de l'état solide et sur la biocatalyse. Son objectif étant d'utiliser des catalyseurs hétérogènes (et des enzymes immobilisées) pour réaliser des réactions chimiques et concevoir des processus chimiques de manière plus écologique.

Abstracts

Oraux

Regioselective enzymatic glucosylation of flavonoids

M. Demonceaux, M. Goux, C. Solleux, J. Hendrickx, B. Offman, C. Miral*

*Unité en Sciences Biologiques et Biotechnologiques, Nantes Université, 44322 France *marie.demonceaux@univ-nantes.com*

Key words: antioxidants, flavonoids, regioselectivity, sucrose phosphorylases, biocatalysis, modeling

Flavonoids are the most quantitative secondary metabolites in plants. As members of the polyphenols family, they are characterized by a tricyclic structure in C6-C3-C6. They are powerful antioxidant molecules that present multiple biological benefits due to their ability to modulate cellular signaling pathways. Nevertheless, their low aqueous solubility prevents them from being well-absorbed by the human body. During the last steps of their biosynthesis in plants, flavonoids are functionalized and, in most cases, they are β-glucosylated, which increases their biodisponibility in living organisms.

Extraction of glucosylated flavonoids from plants yields in complex mixtures of molecules hardly purifiable. On the other hand, chemical synthesis also presents drawbacks as it requires man purification steps to control the regioselectivity of the reaction. Thus, a solution is to get inspiration from Nature itself using enzymes to functionalize the flavonoids. Enzymatic systems are known to be efficient, specific, and eco-friendly. Very few researchers are studying this topic around the world, yet it is a scientific and economic valuable vector. Having control on enzymatic coupling reaction regioselectivity remains a methodologic challenge for scientists.

Sucrose phosphorylases (SPs) are enzymes from the Glycosyle Hydrolase family GH13.18 (EC 2.4.1.7). They catalyze in vivo reversible phosphorolysis of sucrose in α-D-glucose-1-phosphate and D-fructose via a glucosyl-enzyme intermediate. It has been shown that SP from Bifidobacterium adolescentis (BaSP) could synthesize rare disaccharides [1, 2], but also α-glucosylated phenolic compounds using other acceptors than phosphate [3]. Innovant non-natural new molecules, α-glucosylated flavonoids, could thus be synthesized thanks to SPs. Moreover, another advantage of those enzymes is their use of sucrose as a substrate, a cheap, non-activated stable, and natural glucose donor.

By mutating the amino acid glutamine in position 345 in phenylalanine (mutant Q345F), penetration of flavonoids in the enzyme active site has been made possible [4]. Working with resveratrol (a stilbene), a new molecule called resveratrol-3-α-D-glucoside was obtained. Three α-glucosylated products were also synthesized from two flavonoids, catechin and quercetin.

During my thesis, the mutant Q345F was produced and all of the glucosylated components previously mentioned were successfully synthesized. In a second phase, four other mutants of BaSP were produced and reaction kinetics were monitored using innovant analytic methods. As a result, we observed a modification of the reaction regioselectivity depending on the mutations, and these differences were studied by molecular docking in bioinformatic modeling. This original combination of theoretical and experimental complementary approaches is an asset in the understanding of specific regioselectivity of these enzymes for the flavonoids glucosylation.

References

1. M. Kraus, J. Görl, M. Timm, J. Seibel; Chemical Communications, 2016, vol. 52, n. 25, pp. 4625-4627.
2. J. Franceus, S. Dhaene, H. Decadt, J. Vandepitte, J. Caroen, J. Van Der Eycken, K. Beerens, T. Desmet; Chemical Communications, 2019, vol. 55, n. 31, pp. 4531-4533.
3. M. Kraus, C. Grimm, J. Seibel; Chemical Communications, 2017, vol. 53, n. 90, pp. 12181-12184.
4. M. Kraus, C. Grimm, J. Seibel; ChemBioChem, 2016, vol. 17, n. 1, pp. 33-36.

Kinetic study of the synthesis of butyl levulinate by Novozym® 435 under continuous flow

Cordier A.[1,2] Legros J.[1], Held C.[3], Leveneur S.[2]*
[1]Lab COBRA, CNRS, University of Rouen, 76000 Rouen, France
[2]Normandie Univ, INSA Rouen, UNIROUEN, LSPC, EA4704, 76000 Rouen, France 3 TU Dortmund University, EMIL-FIGGE-STR. 70, Dortmund, Deutschland

Key words: Novozyme 435, continuous flow microreactor, predictive model

Thanks to its ketone and carboxylic acid group, levulinic acid is a good candidate for the synthesis of non-petroleum product particularly into biodiesel through levulinate esters [1] and gamma-valerolactone [2]. For an efficient and greener synthesis, we aim to develop intensified miniaturized flow reactor, with recyclable supported biocatalyst. Indeed, flow systems allow a better temperature control and limit the concentration gradient, with consequently a better selectivity and productivity [3]. As catalyst, Novozym 435 was chosen since it is a well-known supported Cal-B used for the synthesis or hydrolysis of amide and ester [4]. Herein, we now report the development of a predictive model for the synthesis of levulinate esters by Novozym 435 in a microreactor under continuous flow.

The kinetic study of levulinates ester synthesis were performed with Novozym 435 placed in an Omnifit column which allow to carry out the reaction under continuous flow (figure 1). Sample were analyzed by GC-FID and Athena Visual Studio® software, using a Bayesian framework [5], is used for the modelling.

The comparison between different kinetic models shows that a Ping-Pong Bi-Bi mechanism [6] with alcohol inhibition is the most relevant. Figure 2 shows the fit of the model with one equivalent of BuOH at 50°C.

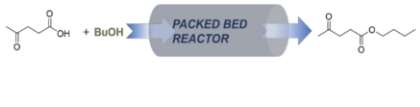


Figure 1. *Biocatalytic butyl levulinate synthesis into a microfluidic system*

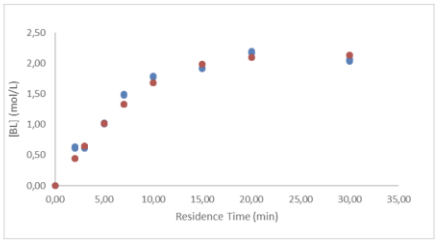


Figure 2. *Fit of model to the experimental concentration of microfluidic system BL at 50°C and [LA]inlet=5.16 mol/L. (red dot: simulated; blue dot: experimental)*

Thanks to this study we have been able to describe a biocatalytic mechanism and a predictive model by developing an original method, assessed by Bayesian inference, for the synthesis of a biobased product with a continuous flow microreactor.[7]

References

1. F. D. Pileidis; MM. Titirici, ChemSusChem, 2016, 9, 562.
2. J. Delgado; S.N. Vasquez Salcedo; G. Bronzetti; C. Moreno; M. Mignot; J. Legros; C. Held; H. Grénman; S. Leveneur, Chemical Engineering Journal, 2022, 3, 430.
3. B.M, Plutschack; B. Pieber; K. Gilmore; P.H. Seeberger, Chemical Reviews 2017, 117, 11796.
4. C. Ortiz; M. Lujan Ferreira; O. Barbosa; J.C.S dos Santos; R.C. Rodrigues; A. Berenguer-Murcia; L.E. Briand; R. Fernandez-Lafuente, Catalysis Science & Technology, 2019, 9, 2380.
5. W. E. Stewart; M. Caracotsios, “Athena Visual Studio”, 2010.
6. M. N. Varma; G. Madras, Journal of Chemical Technology and Biotechnology, 2008, 83, 1135.
7. A. Cordier, M. Klinksiek, C. Held, J. Legros, S. Leveneur ; manuscript in progress.

The terpene mini-path, cell factory

Létitia Leydet[1], Katia Duquesne[1,5], Gilles Iacazio[1,5]

iSm2 - BiosCiences, UMR 7313, Aix-Marseille Université case 341 – St Jérôme – Av. Normandie Niemen - 13013 Marseille

Key words: terpenoid, biocatalysis, in vivo

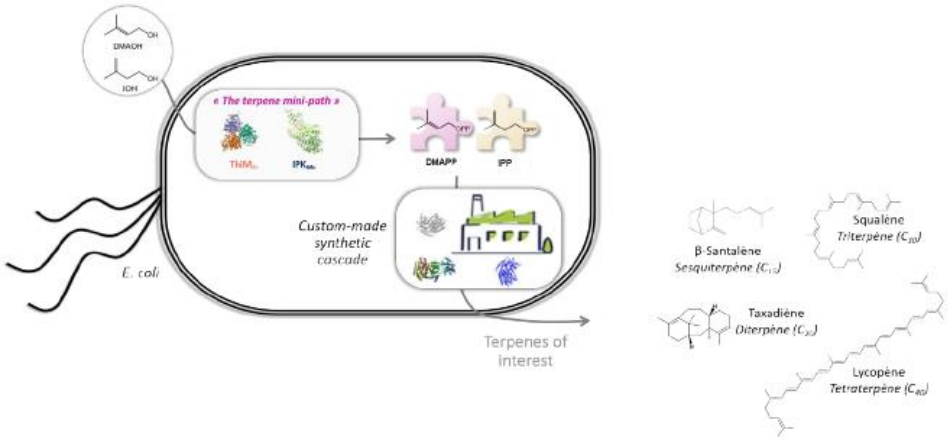


Figure 1: *In vivo access to terpenes*

To date, terpenoids form the most abundant and diversified class of natural products with more than 80,000 compounds [2] whose structural, biological and physicochemical properties hold the attention of the scientific community [3-4]. As access to these terpenoids is limited, we have developed a new *in vitro* production pathway, the terpene "mini-path"[5] (TMP), carried out from two alcohols with five carbon atoms. This synthesis is in order to give different terpene compounds like santalene (aroma), squalene (after hydrogenation generates squalane used in cosmetics), taxadiene (biosynthetic precursor of taxol, a well-known anti-cancer drug in the medical field) or lycopene (an antioxidant and food coloring). For this, four enzymes are needed: two enzymes making it possible to obtain diphosphates (DMAPP and IPP) *via* the TMP, the universal precursors of all terpene synthases, one prenyl transferase and finally the enzyme allowing cyclization (Figure 1). Today, by combining bioinformatic, biochemical and molecular biology approaches, we have developed this concept *in vivo*, by combining two plasmids including two genes necessary for this mini-pathway. This *in vivo* system would then make it possible to dispense with all of the enzyme purification steps, necessary when using the mini-route *in vitro*, and at the same time, to simplify production while reducing i) the costs , ii)time to implement, and thus make it an industrializable process.

References

1. Couillaud J, Leydet L, Duquesne K, Iacazio G, *The Terpene Mini-Path, a New Promising Alternative for Terpenoids Bio- Production.* Genes, (2021), *Volume12(Issue12)*.
2. Köksal M, Jin Y, Coates RM, Croteau R, Christianson D W. *Taxadiene Synthase Structure and Evolution of Modular Architecture in Terpene Biosynthesis*. Nature (2011), 469 (7328), 116–120.
3. Bohlmann J, Keeling CI. *Terpenoid biomaterials*. Plant J. (2008) 54(4):656-69.
4. Paramasivan K, Mutturi S. *Progress in Terpene Synthesis Strategies through Engineering of Saccharomyces cerevisiae*. Crit. Rev. Biotechnol. (2017) 37(8):974-989.
5. Couillaud J, Rico J, Rubini A, Hamrouni T, Courvoisier-Dezord E, Petit JL, Mariage A, Darii E, Duquesne K, de Berardinis V, Iacazio G. *Simplified in Vitro and in Vivo Bioaccess to Prenylated Compounds*. ACS Omega. (2019) 4(4), 7838–7849.

Enhancing non-canonical amino acid incorporation towards enzyme engineering upgrading

Emeline Vernhes, Nuria Ramos, Donna-Joe Bigot, Virginie Ramillon-Delvolve, Cédric Y. Montanier, Gilles Truan, Sébastien Nouaille, Régis Fauré

Toulouse Biotechnology Institute (TBI), Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France

Key words: Non-canonical amino acid, genetic code expansion, enzyme engineering, artificial enzyme

Standard enzyme engineering strategies relies on one or several amino acid permutations among the 20 amino acids (AA). The natural AA repertoire displays only limited chemical functions, which further restrain potentialities of engineering tailored proteins. To circumvent such limitation, systems have been developed over the last decades to incorporate into proteins non-canonical amino acids (ncAAs) with non-naturally encountered chemical functions [1]. The applications of ncAAs use are multiple, including protein labeling [2], protein immobilization, and in-depth redesign of enzyme active sites, thus opening avenues for new catalytic opportunities [3,4]. Despite the tremendous potential of ncAAs, their use is still limited because of technical constraints. The main bottleneck consists in the poor incorporation efficiency, which can be dependent or at least related to the ncAA itself, the incorporation position and the target protein. Optimizing the incorporation system is required to overcome these limitations to efficiently produce proteins with ncAA incorporation in a more versatile way and at high production yields.

Genetic code expansion is based on the reassignment of a nonsense codon to an ncAA by introducing an orthogonal amino-acyl tRNA synthetase (aaRS)/tRNA pair. In E. coli, the pEVOL system is the historical and most widely used [5]. The pUltra system allows improved incorporation efficiencies in some conditions and can be combined with the pEVOL system for the incorporation of two different ncAAs [6]. While these systems have proven their value, the incorporation efficiency remains highly variable.

To go further in improvement and provide to the community a more efficient tool for ncAA incorporation, the pINS system has been developed. We focused on the expression levels of both the tRNA and the aaRS. We have demonstrated that the incorporation efficiency was increased for the three different tested ncAAs, either at the surface or surrounding the catalytic site. In addition, the incorporation position bias observed with standard systems was suppressed. The pINS system allows satisfying incorporation efficiencies with reduced ncAAs concentrations. Finally, the overall production level was increased up to 4-fold compared to pEVOL. The pINS system, making ncAA incorporation more efficient, more reliable and cheaper, should facilitate the use of ncAA in many areas of enzyme engineering.

References

1. F. Agostini; J.-S. Völler; B. Koksch; C. G. Acevedo-Rocha; V. Kubyshkin; N. Budisa *Angew. Chem. Int. Ed.*, **2017**, *56*, 9680–9703.
2. P. Laxman; S. Ansari; K. Gaus; J. Goyette *Frontiers in Chemistry*, **2021**, *9*.
3. A. D. Pagar; M. D. Patil; D. T. Flood; T. H. Yoo; P. E. Dawson; H. Yun *Chem. Rev.*, **2021**, *121*, 6173–6245.
4. I. Drienovská; G. Roelfes Nat Catal, **2020**, *3*, 193–202.
5. T. S. Young; I. Ahmad; J. A. Yin; P. G. Schultz *Journal of Molecular Biology*, **2010**, *395*, 361–374.
6. A. Chatterjee; S. B. Sun; J. L. Furman; H. Xiao; P. G. Schultz *Biochemistry*, **2013**, *52*, 1828–1837.

Deciphering the biosynthesis of the hybrid PKS-NRPS derived metabolites pyrrocidines produced by the maize endophytic fungi *Sarocladium zeae*.

Youwei Chen, Didier Buisson and StéphaneMann

Chimie des Produits Naturels Fongiques, MCAM UMR7245 MNHN-CNRS - Muséum national d'Histoire naturelle 63, rue Buffon 75005 Paris

Keywords: biosynthesis, PKS-NRPS, secondary metabolites, fungi.

The fungi *Sarocladium zeae* is a maize endophyte and recognized as having a protective effect on its host plant against microbial pathogens. This bioactivity is likely due to the production of two secondary metabolites, the pyrrocidines A and B. Indeed, these compounds exhibit antifungal and antibiotic activities [1], as well as cytotoxicity [2]. Preliminary works based on isotopic incorporation suggested that a **hybrid polyketide synthase - non ribosomal peptide synthetase** (PKS-NRPS) is involved in the biosynthetic pathway of these complex polycyclic structures [4][5].

PKS-NRPS gene cluster 47:

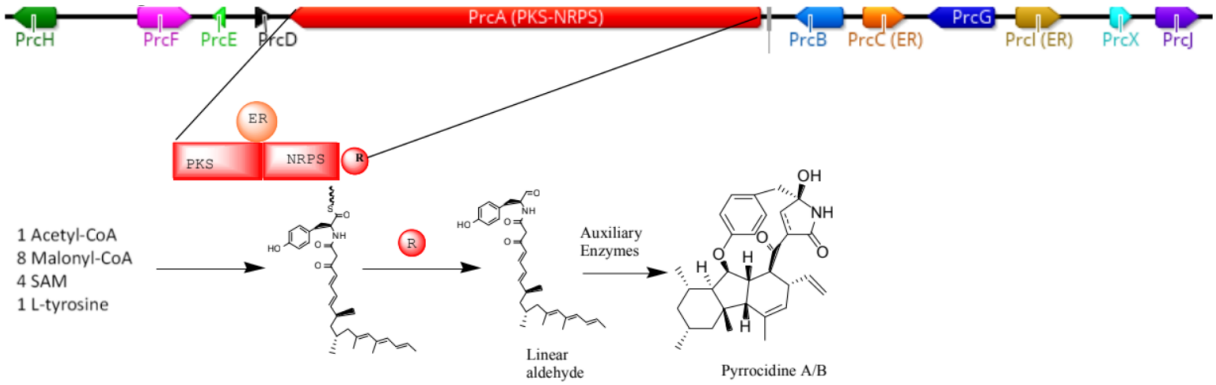


Figure: biosynthetic gene cluster at the origin of pyrrocidines and biosynthetic pathway involving a PKS-NRPS

The study of the biosynthetic pathway and its mode of regulation constitutes a prerequisite to the understanding of the ecological role of pyrrocidines in the tri-partite interaction involving the endophyte, the host plant and the pathogen. It is also a key step for the discovery of 1) enzymes with new activities involved in the building of these molecules and 2) bioactive molecules by microbial engineering (via combinatorial biosynthesis or mutasynthesis) as well as by genome mining.

The genome of *Sarocladium zeae* was sequenced in our group and bioinformatic analysis allowed us to identify a putative gene cluster for the biosynthesis of pyrrocidines. In order to gain insights into the biosynthetic pathway, several critical genes of the cluster were inactivated in the fungi. This gives rise to the accumulation of new metabolites which were isolated and characterized by NMR and mass spectrometry. The formation of these compounds allowed us to propose a biosynthetic pathway for the pyrrocidine family molecules. The latest results will be presented.

References

1. D.T. Wicklow, S.M. Poling, *Phytopathology* 99 (2009) 109- 115.
2. S. Uesugi, N. Fujisawa, J. Yoshida, M. Watanabe, S. Dan, T. Yamori, Y. Shiono, K.-I. Kimura, *J. Antibiot.* (Tokyo) (2015).
3. H. Oikawa, *J. Org. Chem.* 68 (2003) 3552- 3557.
4. A. Ear, S. Amand, F. Blanchard, A. Blond, L. Dubost, D. Buisson, B. Nay, *Org. Biomol. Chem.* 13 (2015) 3662- 3666.

Characterization of AA9 LPMOs from fungal saprotrophs holding a conserved module of unknown function

Jean-Lou Reyre[1,2], Sacha Grisel[1], Mireille Haon1, Antoine Margeot2, Simon Arragain2, Jean-Guy Berrin1 and Bastien Bissaro1

[1]INRAE, Aix Marseille University, UMR1163 Biodiversité et Biotechnologie Fongiques, 13009 Marseille, France.

[2]IFP Energies nouvelles, 1 et 4 avenue de Bois-Préau, 92852 Rueil-Malmaison, France

Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent oxidoreductases able to catalyze the oxidative cleavage of various polymers1. Their ability to target recalcitrant parts of cellulose, the major component of plant cell walls (PCW), has settled LPMOs as essential enzymes, both in the toolset of fungal wood decayers (especially white-rot Polyporales) and for biorefinery applications. Genome sequencing campaigns and transcriptomic studies have highlighted the diversity of secreted enzymes during biomass decay, but their biochemical characterization is still lagging behind. Based on the transcriptomic analyses of six Polyporales grown on cellulosic substrates2, we identified peculiar LPMOs holding a conserved C-term domain of unknown function. We selected and heterologously produced six of these LPMOs in Pichia pastoris and carried out detailed enzymatic characterization. While a strict C1-oxidizing activity could be observed on cellulose, the rather low amount of detected oxidized products, coupled with a low substrate binding and the lack of synergy with canonical enzymatic partners were unexpected observations. Further in silico analyses also revealed unusual features questioning the involvement of these enzymes in the degradation of PCW.

References

1. Vandhana, T. M.; Reyre, J.-L.; Sushmaa, D.; Berrin, J.-G.; Bissaro, B.; Madhuprakash, J. On the expansion of biological functions of lytic polysaccharide monooxygenases. The New phytologist 2022, 233, 2380–2396.
2. Hage, H.; Miyauchi, S.; Virágh, M.; Drula, E.; Min, B.; Chaduli, D.; Navarro, D.; Favel, A.; Norest, M.; Lesage-Meessen, L.; Bálint, B.; Merényi, Z.; Eugenio, L. de; Morin, E.; Martínez, A. T.; Baldrian, P.; Štursová, M.; Martínez, M. J.; Novotny, C.; Magnuson, J. K.; Spatafora, J. W.; Maurice, S.; Pangilinan, J.; Andreopoulos, W.; LaButti, K.; Hundley, H.; Na, H.; Kuo, A.; Barry, K.; Lipzen, A.; Henrissat, B.; Riley, R.; Ahrendt, S.; Nagy, L. G.; Grigoriev, I. V.; Martin, F.; Rosso, M.-N. Gene family expansions and transcriptome signatures uncover fungal adaptations to wood decay. Environmental Microbiology 2021, 23 (10), 5716–5732.

Synergistic approach of pulsed electric fields and immobilized biocatalysts for obtaining biologically active peptides from agrifood proteins

Hélorie Clouzot

[1] University of Lille, [2]Laval University

Key words: agrifood proteins, enzymatic hydrolysis, immobilized biocatalyst, pulsed electric fields, bioactive peptide

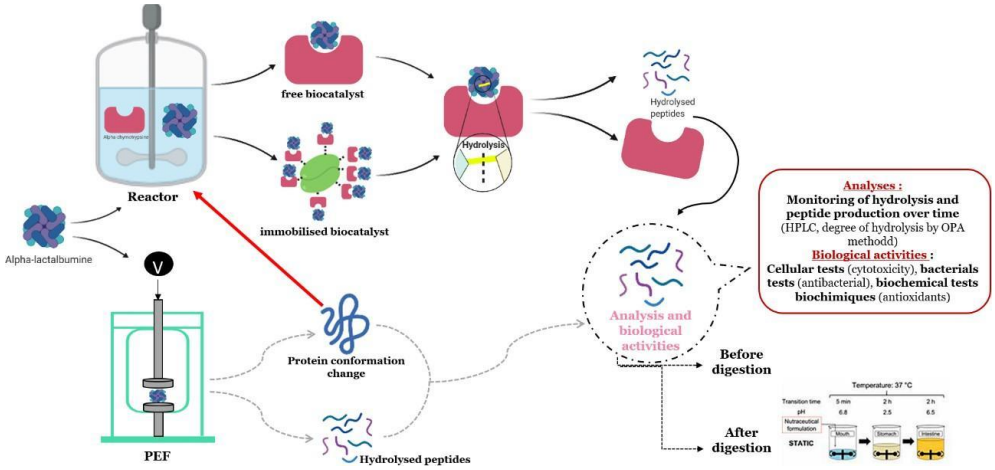


Figure . *Strategy for improving the production of biologically active peptides following enzymatic hydrolysis*

The food and biotechnology industries are constrained by the environmental burden of a rapidly growing world population. They must adapt to meet the growing demand for ever higher quality food and nutraceutical products. In 2019, 881 million tonnes of milk (cow, buffalo, goat, and other mammals) were produced worldwide, and this figure continues to grow each year, resulting in a major increase in whey production. Its high concentration of lactose and nitrogenous matter makes it a major environmental pollutant but also an invaluable resource to produce bioactive peptides. The present study addresses a synergistic and innovative approach to meet the demand for whey valorisation, while reducing the impact on our environment. This approach consists of combining the immobilization of an enzyme with an emerging eco-efficient green technology, pulsed electric fields, to obtain biologically active peptides from a whey protein, alpha-lactalbumin (α-lac). The enzymatic hydrolysis of this protein is difficult due to its compact globular structure. The use of pulsed electric fields would make it possible to pre-treat α-lac and improve its proteolysis by reducing the enzymatic activity of immobilized chymotrypsin. The first results show chymotrypsin could be immobilized to the extent of 99,6 % on a porous support, alumina.

References

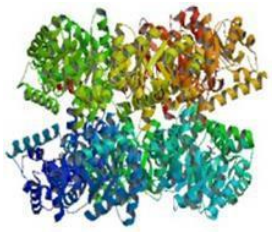
1. S. Mikhaylin, et *al*., *ACS Sustainable Chemistry & Engineering « High Voltage Electrical Treatments To Improve the Protein Susceptibility to Enzymatic Hydrolysis »*, **2017**, vol. 5, p. 11706-14.
2. G W. Smithers, International Dairy Journal “Whey-ing up the options – Yesterday, today and tomorrow”, 2015, vol 48, p 2-14.

Study of new fructose-6-phosphate aldolases from extremophilic organisms

Léo Paulat[1], Christine Guérard-Hélaine[1], Marielle Lemaire[1], Virgil Hélaine[1], Lionel Nauton[1], Cédric Gastaldi[1], Jean-Louis Petit[2] and Véronique de Berardinis[2]

[1]ICCF - UMR 6296, Campus Universitaire des Cézeaux, 24 avenue des Landais, 63178 AUBIERE Cedex, France

[2]Genoscope – UMR 8030, 2 rue Gaston Crémieux, 91057 Evry-Courcouronnes



FSA : Decameric structure

Biocatalysis is a tool to access new products of interest in a greener and more eco-friendly way. It allows us to respond to many current issues related to the 12 principles of green chemistry. In this context, Aldolases are C-C bond forming enzymes of particular interest for synthetic applications. Indeed, the aldol reaction allows to generate up to 2 asymmetric centers, providing chiral adducts. Depending on the aldolases used, the stereochemistry of these asymmetric centers can be controlled[1].

Fructose-6-phosphate aldolase (FSA) belonging to class I aldolases, was discovered in *E. coli* by Shürmann and Sprenger[2] in the 2000s. It was demonstrated as the first aldolase able to use hydroxyacetone[3], as nucleophile substrate, and furthermore particularly robust, efficient and versatile towards other nucleophiles such as dihydroxyacetone, hydroxybutanone[4] and glycolaldehyde[5]. These discoveries were the basis for mutagenesis [1] work or for the search from biodiversity for new aldolases presenting for example different stereochemistry. In the framework of our collaboration with the Génoscope (Evry), two FSA from acidophilic organisms are studied, one from, *Acidobacteria*

Bacterium (A0A399XV01) and one from *Acidiplasma Aeolicum* (A0A0Q0RVA3). These catalysts have revealed atypical properties. Molecular modeling and their kinetic constant determination helped their characterization. These results will be presented here, as well as some synthetic applications.

References

1. Hélaine, V.; Gastaldi, C.; Lemaire, M.; Clapés, P.; Guérard-Hélaine, C. *ACS Catal.* **2022**, *12* (1), 733–761.
2. Schürmann, M.; Sprenger, G. A. *Journal of Biological Chemistry* **2001**, *276* (14), 11055–11061.
3. Schürmann, M.; Schürmann, M.; Sprenger, G. A. *Journal of Molecular Catalysis B: Enzymatic* **2002**, *19–20*, 247–252.
4. Sugiyama, M.; Hong, Z.; Liang, P.-H.; Dean, S. M.; Whalen, L. J.; Greenberg, W. A.; Wong, C.-H. *J. Am. Chem. Soc.* **2007**, *129* (47), 14811–14817.
5. Garrabou, X.; Castillo, J. A.; Guérard-Hélaine, C.; Parella, T.; Joglar, J.; Lemaire, M.; Clapés, P. *Angew. Chem. Int. Ed.* **2009**, *48* (30), 5521–5525.

CoA ligase for amide and lactam synthesis

Océane Touré,[1] Nikolas Capra,[2] Chloé Lelièvre,[1] Aurélie Fossey,[1] Carine Vergne-Vaxelaire,[1] Andy-Mark Thunnissen,[2] Dick Janssen,[2] Anne Zaparucha[1]

[1]Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, 2 rue Gaston Crémieux 91000 Evry-Courcouronnes, France

[2]Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijienborgh 4 9747 AG Groningen, the Netherlands

Key words: amide, lactam, CoA ligase, protein engineering

CoA ligases are ATP-dependent enzymes that catalyze the formation of acyl-CoA thioester in two-step reaction: 1) activation of the carboxylic acid function to generate the acyl-AMP intermediate in presence of ATP and 2) thioesterification with HSCoA to obtain the acyl-CoA thioester.

In previous study, we employed thermophilic CoA ligases to obtain amide compounds in a diverted synthesis reaction coupled to an enzyme-catalyzed ATP-regeneration system, without any substrate activation by coenzyme A, an expensive and poorly available

substrate.[1] Indeed, the use of thermophilic enzymes permitted the reactions to take place at high temperature, which promotes the uncatalyzed intramolecular attack of the amine.

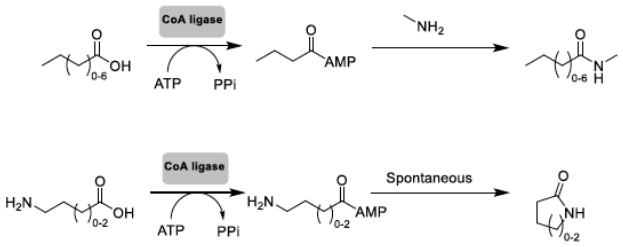


Figure. *Chemoenzymatic synthesis of N-methyl amides and lactams*

The study was then extended to the formation of lactams from 5- to 7-membered rings. In collaboration with researchers from the University of Groningen, the structure of the most promising enzyme, MsACL from *Metallosphera sedula* (UniProt KB ID A4YDT1) was resolved. Structure-guided approach was used to design mutants, which have been produced and characterized. We herein present the results of this study.

References

1. Lelièvre, C. M.; Balandras, M.; Petit, J.-L.; Vergne-Vaxelaire, C.; Zaparucha, A., ATP Regeneration System in Chemoenzymatic Amide Bond Formation with Thermophilic CoA Ligase. *ChemCatChem* **2020,** *12* (4), 1184-1189.

Membrane-immobilized transaminase for intensified chiral amine production

Hippolyte Meersseman[1], Tom Leyssens[1], Patricia Luis[1], Francesca Paradisi[2], Damien P. Debecker[1]*

[1] UCLouvain, [2] University of Bern

Key words: transaminase, enzyme immobilization, chiral amines

Chiral amines are essential building blocks to manufacture high-value active pharmaceutical ingredients. Their synthesis often involves non-green processes requiring expensive homogeneous catalysts and high-energy inputs[1]. Hence, amine transaminases (ATAs) have gained considerable attention lately, as their use in transaminations provide greener routes to produce chiral amines with excellent enantioselectivity. Industrial applications of ATAs remain however restricted to batch processes in which ATAs are employed as non-reusable homogenous biocatalysts[2]. To overcome this limitation, more versatile heterogenized biocatalysts able to operate in continuous flow have been designed[3]. Importantly, most targeted transaminations are strongly limited by unfavourable thermodynamics. One strategy to shift the equilibrium towards high chiral amine yields is the set-up of integrated reaction-purification processes. In this context, coupling transamination with membrane technology in an enzyme membrane reactor is of particular interest, as it could allow the selective removal of products during operation. Enzymes onto polymeric membranes has already been reported, e.g. with lipase or glucose oxidase but not transaminase. As proof of concept, we present the immobilization of an ATA from Halomonas elongata (HeWT) onto polyacrylonitrile-based membranes (PAN) and its application to catalyze a model transamination (Fig.1). To this end, the PAN surface was chemically modified and then coated with polyethyleneimine (PEI) to host the enzyme. Eventually, glutaraldehyde (GA) was used to cross-link the loosely attached HeWT and thus prevent their leaching from the membrane's surface. The obtained immobilized biocatalyst successfully performed subsequent catalytic cycles and showed negligible HeWT leaching. The decisive parameters impacting the enzyme specific activity were then assessed. As a result, we aim to demonstrate the use of a novel immobilized transaminase support material well-suited for upcoming operations, that pave the way to intensified biocatalytic processes for chiral amines production.

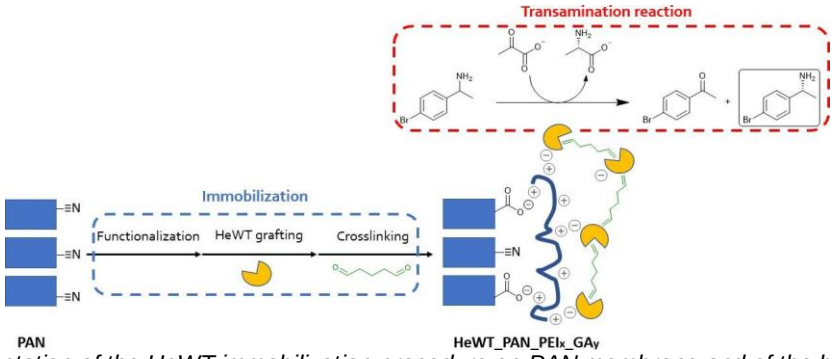


Figure 1. *Representation of the HeWT immobilization procedure on PAN membrane and of the batch model kinetic resolution of 4-bromo-α-methylbenzylamine considered herein.*

References

1. T.C. Nugent, M. El-Shazly, *Advanced Synthesis & Catalysis*, **2010**, *352*, 753– 819.
2. D.M. Patil, G. Grogan, A. Bommarius, H. Yun, *Catalysts*, **2018**, *8*, 254.
3. L. van den Biggelaar, P. Soumillion, D.P. Debecker, *RSC Adv.,* **2019**, *9*, 18538.

Des électrodes à base de papier comme outil pour la caractérisation des enzymes ligninolytiques

*Fall Issa [1], Abdellaoui Sofiene [1], Rémond Caroline [1], Rakotoarivonina Harivony[1], Doumèche Bastien [2], Ochs Marjorie [2]**

[1]Université de Reims Champagne Ardenne, INRAE, FARE, UMR A 614, Chaire AFERE, 51097, Reims, France.

[2]Université Lyon, Université Claude Bernard Lyon 1, CNRS, INSA, CPE-Lyon, UMR 5246, ICBMS (Institut de Chimie et Biochimie Moléculaires et Supramoléculaires), F-69622, Villeurbanne, France.

Key words: lignine, ligninase, électrodes papiers

La lignine est un hétéropolymère hydrophobe et amorphe, composée majoritairement d'unités phénols propanoides. Elle est considérée comme la plus importante source naturelle de composés aromatiques, et est le deuxième polymère le plus abondant dans la nature après la cellulose. Dans les industries de pâtes à papiers, la lignine représente actuellement le principal déchet et plus de 50 millions de tonnes de lignines sont produites par an (1) . La plupart de cette lignine est brûlée pour produire de la chaleur et de l'électricité au sein des bioraffineries.

La valorisation des lignines en molécules d'intérêt nécessite une étape de fractionnement qui peut être catalysée par des enzymes ligninolytiques, notamment des oxydoréductases issues de champignons et de bactéries (2) . Ces enzymes sont principalement des laccases, des peroxydases, des dioxygénases etc. et agissent en synergie avec des radicaux libres également produits par les microorganismes. L'utilisation de ces enzymes pour la bioraffinerie est limitée par leur faible efficacité et leur stabilité. De plus, l'identification de biocatalyseurs robustes présentant des activités ligninolytiques représente un verrou technologique. En effet, les méthodes spectrophotométriques ou chromatographiques classiquement utilisées pour la détection des activités ligninolytiques ne sont pas totalement adaptées à un dosage à haut débit dans des milieux opaques et hétérogènes contenant des lignines. Ceci complexifie la découverte de biocatalyseurs efficaces sur substrat solide.

Dans ce travail, le développement d'un nouvel outil utilisant des électrodes à base de papier pour la détection d'activités ligninolytiques est décrit. Cette stratégie consiste à déposer de la lignine sur la face opposée du papier où les électrodes sont sérigraphiées. Les résultats présentent son application pour la mise en évidence du potentiel ligninolytique d'une oxydoréductase isolée chez la souche bactérienne Thermobacillus xylanilyticus.

References

1. Bajwa, *et al*., 2019. A concise review of current lignin production, applications, products and their environmental impact. Industrial Crops and Products 139:111526.
2. Becker, *et al*., 2019. A field of dreams: Lignin valorization into chemicals, materials, fuels, and health- care products. Biotechnology advances 37:107360.

Computational design of the cofactor specificity of GAPN enzyme for cell-free biocatalysis

Delphine Dessaux[1], Sam Mallinson[2], Yannick Bomble[2], Sophie Barbe[1]

[1] TBI, Université de Toulouse, CNRS, INRAE, INSA, ANITI, Toulouse, France., [2] Biosciences Center, National Renewable Energy Laboratory, Golden, CO, USA.

Keywords: Enzyme engineering, Computational Protein Design, cofactor specificity, cell-free biocatalysis

The use of enzymes for the sustainable and environmentally friendly production of biochemicals is continuously expanding and allows to bypass some of the drawbacks of the chemical production. However, these bioprocesses currently rely mainly on microbial fermentation, whose effectiveness may be limited. An appealing alternative is the use of *in vitro* multi-enzymatic pathways for cell-free bioproduction that may enable higher productivity while reducing production costs.

Furthermore, the non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN, **Fig.1**) catalyzes the transformation of glyceraldehyde-3-phosphate into 3-phosphoglycerate (**Fig. 2**) and can hence be used as a shortcut into the glycolysis pathway. It is thus a crucial enzyme that can be involved in the regulation of ATP concentration in cell-free biocatalysis processes. However, GAPN is strictly dependent on the $NADP^+$ cofactor [1], which prevents its use for NAD^+ cofactor-dependent pathways or those based on the use of more stable and less expensive biomimetic cofactors. Therefore, we aimed to engineer this enzyme to be active with the NAD^+ cofactor.

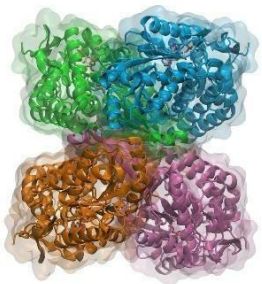


Figure 1. *Tetrameric structure of the GAPN enzyme.*

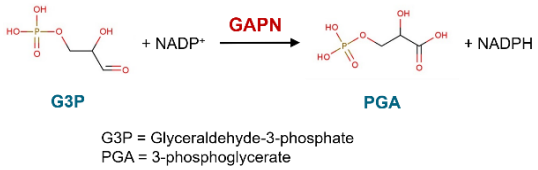


Figure 2. *Enzymatic reaction catalyzed by GAPN.*

In order to alter the cofactor specificity of GAPN, we developed and implemented a new rational enzyme engineering strategy based on computational protein design (CPD) methods [2-3]. These CPD approaches rely on artificial intelligence algorithms to predict mutant sequences for the design target while simultaneously considering several conformational states of the enzyme representing different steps along the catalysis reaction. We combined these CPD methods with all-atom molecular dynamics simulations and binding free energy calculations, and ranked the GAPN mutants according to cofactor binding free energy and geometric features required for catalysis. This overall rational design strategy led to the prediction and the selection of mutants for experimental testing. As a result, although the wild-type enzyme was not able to use the NAD^+, we succeeded in generating several mutants exhibiting an activity with this cofactor, reaching up to about 75% of the activity of the wild-type enzyme with $NADP^+$.

References

1. D.A., Boyd; D.G., Cvitkovitch; I.R., Hamilton. *J Bacteriol*, **1995**, *177*(10), 2622–2627.
2. J., Vucinic; D., Simoncini; M., Ruffini; S., Barbe; T., Schiex. *Bioinformatics*, **2020**, *36*(1), 122-130.
3. S., Traoré; D., Allouche; I., André; S., de Givry; G., Katsirelos; T., Schiex; S., Barbe. *Bioinformatics*, **2013**, *29*(17), 2129–2136.

Glycosyltransferases domains « Mix and Match » : tailor-made biocatalysts for glycosides enzymatic biosynthesis.

Damien Bretagne, Richard Daniellou[1], Pierre Lafite[2]*

[1] AgroParisTech, [2]Orleans University

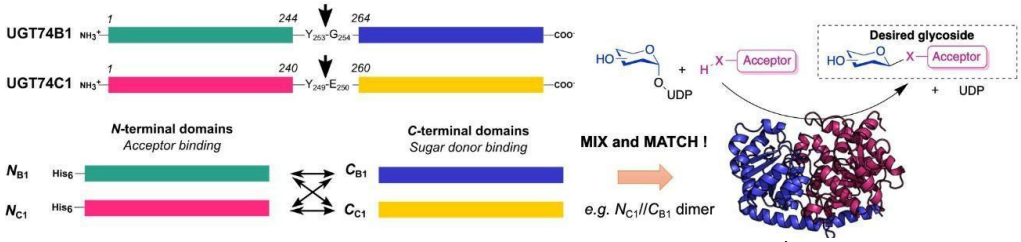
Key words: young, researchers, tailored glycosyltransferases, glycosides, chimerization, modelization, enzymatic engineering

Enzyme engineering in glycosides synthesis has been proficiently used to improve rate, selectivity, and even drive the enzymatic mechanism towards new reactions (transglycosidase, thioglycoligase)[1].

However, when the structure of the desired glycosidic product has to be modified (sugar and/or aglycone moieties, nature of the glycosidic bond), it often requires the identification of a new enzyme, which then needs to be subsequently engineered to afford the desired biocatalyst.

Herein, we aimed at providing a modular approach to generate tailored biocatalysts, based on the structure of the targeted glycoside. We based this methodology on the peculiar structures of Glycosyltransferases from GT-B family, that are composed of two separated domains facing each other; each of them binding selectively either the nucleotide-sugar donor (C-ter) or acceptor (N-ter)[2]. Relying on seminal works that proved the feasibility to swap domains and generate active chimeras[3], we aimed at offering a complete modular approach (Mix-and-Match), based on the co-expression of desired separated domains in expression host, in vivo dimeric chimera reconstitution, purification and use in glycoside synthesis.

In this context, 2 GT-B from *A. thaliana* were chosen as a proof-of-concept model - UGT74B1 and UGT74C1, both



involved in the rare S-glycosylation reaction in glucosinolate biosynthesis pathway[4]. The 4 truncated genes coding for N-ter and C-ter domains of each parental enzyme were cloned and mixed to afford the 4 potential co-expressed dimeric enzymes. If the dimerization of domains proved not to be straightforward, even between separated domains from the same parental enzyme, we identified and isolated the first active chimeric GT-B expressed as a dimer. Kinetic analyses towards donor and acceptor were compared with those of the parental enzymes, and revealed a modified two-substrates mechanism. Moreover, the chimer exhibited broader substrate selectivity, when compared to wtUGT74B1 and wtUGT74C1, which could be attributed to a flexible interface between the two domains, as demonstrated by structural biology, domain-domain docking and molecular dynamics analyses.

Figure 1 . *« Mix and Match » :tailor-made biocatalysts*

Chimeric GT-B can now be considered as an attractive modular approach to generate tailored biocatalysts, as the nature of the donor and acceptor can be defined by the chosen domains to assemble. Yet, if exemplification of this methodology is currently under investigations, the extension of the substrate promiscuity of the chimeric GT-B dimer described in this study paves the way towards a new biocatalyst engineering approach for glycosides synthesis.

References

1. S.M, Hancock; *Curr. Opin. Chem. Biol.*, **2006**, *10*, 509–519.
2. L.L, Lairson; Annu. Rev. Biochem., **2008**, *77*, 521–555.
3. M.M, Palcic; Curr. Opin. Chem. Biol., **2011**, *15*, 226–233.
4. S., Marroun; *Org. Biomol. Chem.*, **2016**, *14*, 6252–6261. L., Lafite; *Mol. Catal.*, **2019**, *479*, 110631.

Flash Comm & Posters

Development of an enzymatic process for the synthesis of alkyl hydroxycinnamates

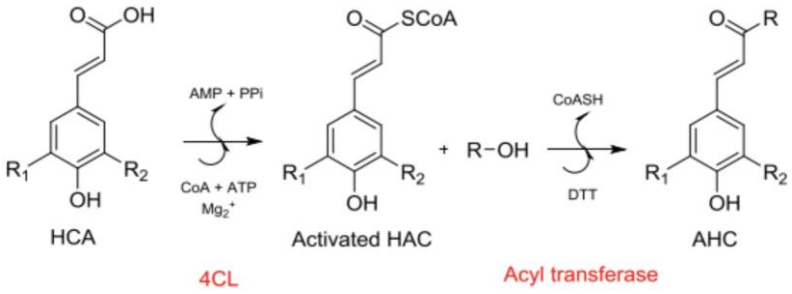
Horiya Nassiba HAM, Nabila IMATOUKENE, Louis MOUTERDE, Michel LOPEZ

URD ABI AgroParisTech, Pomacle, France,

Key words: Alkyl hydroxycinnamates (AHCs), p-hydroxycinnamic acid (HCA), 4CL, Acyl transferase, enzymatic process.

Alkyl hydroxycinnamates (AHCs) are hydroxycinnamic acid (HCA) esters aliphatic derivatives1found in plants associated with suberin and cutin2. The presence of the aliphatic moiety on AHCs modulates their hydrophilic/lipophilic balance and improves their integration in oil-based formulations and their biological activities compared to their phenolic acid parents3. These features open a broad panel of applications for these molecules as antioxidants, antimicrobials, and UV protectants among others1. Lipases are usually used to graft aliphatic moieties on phenolic acids. However, it has been reported that lipase's activity is inhibited in the case of p-hydroxycinnamic acids due to the simultaneous presence of the p-hydroxyl and the double bound near the carboxylic function conjugated to the aromatic cycle on the phenolic acid4. Our work seeks to develop an enzymatic process for the synthesis of AHCs by miming their biosynthetic pathway in plants.

Figure 1. Reactions implicated in the enzymatic synthesis of AHCs in plants.



Two recombinant enzymes have been expressed in E. coli competent cells, the first one is a CoA ligase (4CL) implicated in the activation of p-HCAs and the second one is an acyltransferase responsible for the transfer of the acyl moiety of the activated phenolic acid on an aliphatic moiety (fatty alcohol or fatty acid) (Figure 1). The enzymatic activity of the purified 4CL has been tested on two HCAs, coumaric acid and ferulic acid, HPLC results showed their successful activation by 4CL. We also aim to optimize the different quantities of 4CL, CoA and ATP used for HCAs activation to minimize it as much as possible to make the process economically viable.

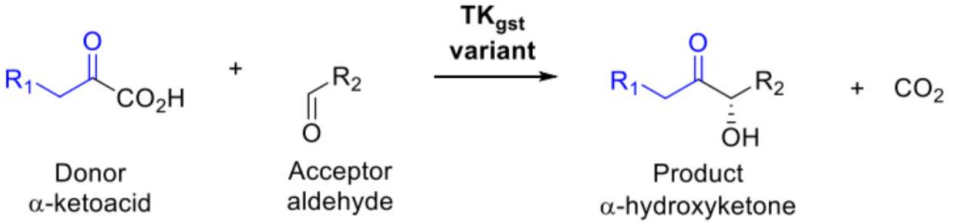
References

1. First name initials, Last name; J.S. Smith Journal title, year, volume, page numbers. [1] D. A., Grajales-Hernández; M. A., Armendáriz-Ruiz; F. L., Gallego, and J. C., Mateos-Díaz, Applied Microbiology and Biotechnology, 2021, 105(10), 3901-3917.
2. F., Domergue and D. K. Kosma, Plants, 6(3), 25-41.
3. Y. G., Shi; Y.J., Zhu; S. Y., Shao; R. R., Zhang; Y., Wu; C. M., Zhu; X.
4. P., Villeneuve, Biotechnology Advances,2007, 25(6), 515-536.

Evolved transketolase from Geobacillus stearothermophilus for new α-hydroxyketones synthesis

G. Arbia, N. Ocal, A. Lagarde, F. Charmantray, L. Hecquet

Université Clermont Auvergne, Institut de Chimie de Clermont-Ferrand, CNRS UMR 6296, Aubière, France 2



Geobacillus stearothermophilus thermostable transketolase enzyme (TKgst) catalyzes, in one step a stereoselective and irreversible carboligation reaction using α-ketoacid, hydroxypyruvate (Li-HPA) as donor substrate and an aldehyde as acceptor to generate various α-hydroxyketones valuable in food and pharmaceutical areas [1-4]. Our current goal consists in TKgst engineering by mutagenesis to broaden the substrate specificity towards pyruvate analogs and polyhydroxylated aldehydes with different configurations and carbon chain length. We will present some recent results showing the high synthetic potential of TKgst variants for obtaining new α-hydroxyketones.

References

1. J. Abdoul-Zabar, I. Sorel, V. Hélaine, F. Charmantray, T. Devamani, D. Yi, V. de Berardinis, D. Louis, P. Marlière, W.-D. Fessner, L. Hecquet, Advanced Synthesis & Catalysis, 2013, 355, 116–128.
2. T. Saravanan, S. Junker, M. Kickstein, S. Hein, M.-K. Link, J. Ranglack, S. Witt, M. Lorillière, L. Hecquet, W.-D. Fessner, Angewandte Chemie International Edition, 2017, 56, 5358–5362.
3. M. Lorillière, R. Dumoulin, M. L'enfant, A. Rambourdin, V. Thery, L. Nauton, W.-D. Fessner, F. Charmantray, L. Hecquet, ACS Catalysis, 2019, 9, 4754–4763.
4. H. Casajus, A. Lagarde, M. Leremboure, T. De Dios Miguel, L. Nauton, V. Thery, W-D Fessner, N. Duguet, F. Charmantray, L. Hecquet. ChemCatChem, 2020, 12, 5772-5779.

Eco-friendly synthesis of some amidoprofens catalyzed by Candida antarctica lipase: Optimization of some parameters.

*Guenifi Nour Elhouda[*1], Kilani Samira[1], Merabet-Khelassi Mounia[2], Sifi Karima[1], Zeror Saoussen[2]*

*[1]Laboratory of systems and advanced materials (LSAM), Badji Mokhtar Annaba University . [2]Ecocompatible Asymmetric Catalysis Laboratory (LCAE), Badji Mokhtar Annaba University . *Nour-elhouda.guenifi@uni-annaba.org*

Key words: Iburofen, Amides, Biotransformation, Candida Antarctica lipase, Sustainable chemistry.

Ibuprofen is considered as one of the most important non-steroidal anti-inflammatory drugs (NSAIDs) despite its side effects mostly on the gastro-intestinal mucosa [1]. The modification of these traditional NSAIDs using an eco-friendly bioprocess for the purpose of reducing some of their undesirable effects but also improving their efficiency still important. For this purpose, the amidation constitute an interesting issue, especially, since amides were considered high valuable synthetic intermediates used in the manufacture of several pharmacological and biological active products [2]. Biocatalysis fits well with the "Green chemistry" principles which are considered as one of the toolkit of white biotechnology especially for the prodrugs developments [3]. Previously, the direct enzymatic amidation of some racemic profens was described using the molecular sieves 4Å as water-controlling environment [4].

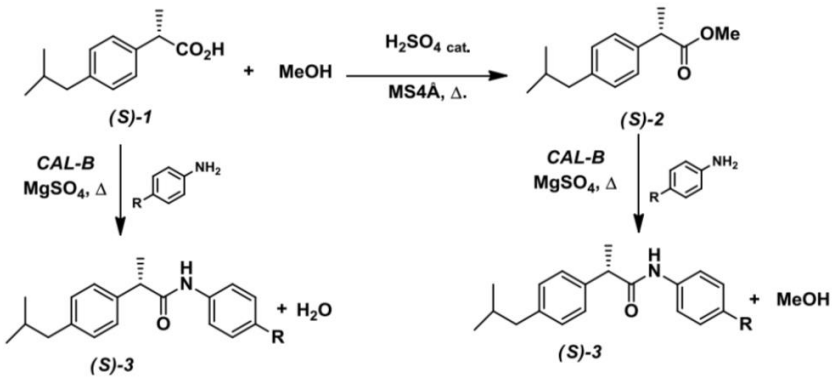


Figure 1 . Enzymatic amidation pathways of Ibuprofen.

Herein, we present our investigation concerning the amidation of (S)-Ibuprofen catalyzed by the Candida antarctica lipase immobilized on acrylic resin (CAL-B), as biodegradable and recyclable catalyst. Two eco-compatible pathways are considered; the first one by the direct condensation of the carboxylic acid and aromatic amines using the MgSO4 as water-controlling environment, and the second one after activation of the carboxylic acid into the corresponding ester in the presence of MgSO4 to avoid the competitive parallel hydrolysis of the starting material (Figure 1). The obtained amides are recovered with good to excellent isolated yields and that in function of the amine structure.

References

1. Proshkina, Ekaterina, et al. "Geroprotective and radioprotective activity of quercetin,(-)- epicatechin, and ibuprofen in Drosophila melanogaster." Frontiers in pharmacology 7 (2016): 505.
2. Greenberg A, Breneman CM, Liebman JF. The amide linkage: structural significance in chemistry, biochemistry and materials science. Wiley, New York. 2000.
3. Reetz, Manfred T. "Biocatalysis in organic chemistry and biotechnology: past, present, and future." Journal of the American Chemical Society 135.34 (2013): 12480-12496.
4. N, Benamara ; M, Merabet-Khelassi ; L, Aribi-Zouioueche, O, Riant Chem. Pap. 2021, 75, 4045– 4053.

Enzymatic scaffolding around a protein ring

Alexis Boutilliat[1], Marc Quinternet[2], Alexandre Kriznik[2-3], Marie-Eve Chagot[3], Florent Canonico[1]

[1]SAYENS, 2 rue Jean Zay 54519 Vandœuvre-lès-Nancy ; [2]UMS IBSLor, University of Lorraine ; [3]UMR IMOPA University of Lorraine

Key words: scaffold, enzymes, biocatalyst, peroxyredoxin

Peroxiredoxin (PRX) is an ubiquitous protein physiologically involved in the redox machinery of the cell, many of them have a natural decameric ring structure [1]. In this work, this structural property has been exploited to create a scaffold structure using a non-catalytic variant of the thermally stable PRX coming from the thermophilic organism Pyrococcus furiosus (PfuPRX). More precisely, the N and C ends of the PRX monomer could be used to graft proteins such as enzymes on a robust scaffold resistant to thermal denaturation. The overall scaffold is either obtained by genetically fused protein sequences or by using specific protein adapter pairs. The scaffolded enzymes were produced in E. coli system and purified by simple chromatographic methods (metal affinity and gel filtration). This new scaffold technology [2] allows the creation of soluble and large catalytic objects (0.5 to 1 MDa and about 20 nm) and has potential advantages such as improved stabilization, solubilization, production rate and activity of the scaffolded enzyme. Our method was successfully applied to several enzymes such as proteases (3c, TEV), cellulases (CelCD) [3] and plastic degrading enzymes (LCC PETase) [4]. In all cases, the activity of the scaffolded enzyme was maintained.

The diversity of the PRX family offers a wide range of biophysical properties, allowing tailor-made scaffolds. It could also allow the co-localization of two or more complementary enzymatic activities scaffolded on the same ring structure.

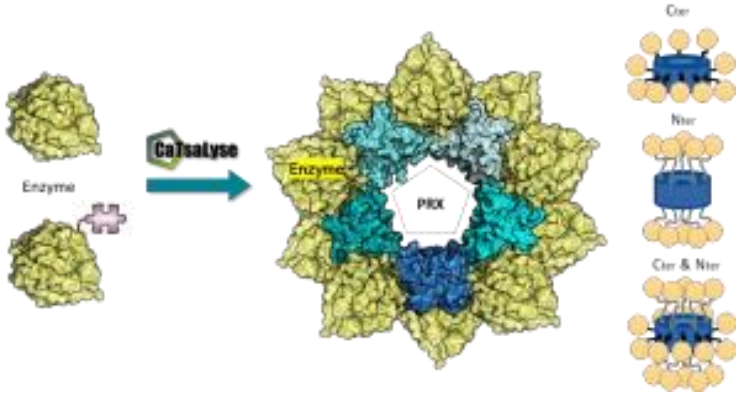


Figure1: Schematic representation of the scaffolding strategy. Direct genetic fusion or protein adaptor allow the grafting of enzyme on the different positions of PRX ring.

References

1. Perkins, Arden, et al. « Peroxiredoxins: Guardians Against Oxidative Stress and Modulators of Peroxide Signaling ». Trends in biochemical sciences, 2015, vol. 40, no 8, p. 435- 45.
2. Marc Quinternet, Alexandre Kriznik, Marie-Eve Chagot; EU patent registration number 20306539.6-1118, 2022 to be published
3. Gao, Dongfang, et al. « Identification of a Heterologous Cellulase and Its N-Terminus That Can Guide Recombinant Proteins out of Escherichia Coli ». Microbial Cell Factories, 2015, vol. 14, no 1, p. 49.
4. Tournier, V., et al. « An Engineered PET Depolymerase to Break down and Recycle Plastic Bottles ». Nature, 2020, vol. 580, no 7802, p. 216- 19.

Synthesis of 5-Hydroxymethylfurfural from D-glucose by hybrid catalysis : from “batch” to continuous process

Amandine Lorthios, Elena-Florentina Grosu, Nabil Ait Radi[1], Jean-Sébastien Girardon[2], Rénato Froidevaux

UMR Transfrontalière BioEcoAgro N°1158 – Institut Charles Viollette, Villeneuve d’Ascq, France

Unité de Catalyse et de Chimie du Solide – UMR CNRS 8181, Villeneuve d’Ascq, France

Key words: 5-HMF, D-glucose, hybrid catalysis, processes configuration

5-Hydroxymethylfurfural (5-HMF) is a platform molecule of great economic interest, it is the product of the dehydration of 6-carbon sugars such as D-fructose and D-glucose. As D-glucose is more available and less expensive than D-fructose, it is interesting to use it as a substrate. However, D-glucose has a lower selectivity and dehydration yields of 5-HMF than D-fructose. This problem can be overcome by using hybrid catalysis, combining enzymatic and mineral catalysis. Xylose isomerase allows the first isomerisation step, the second reaction consists of dehydration by exploiting the acidic properties of tosylic acid. The conditions of the two catalysis are diametrically opposed, so it is a question of making concessions on both sides in order to be able to move from “batch” to continuous flow. Previously, the development of an “H-reactor” demonstrated that it was possible to combine the two catalysts in a 2P1S hybrid reactor [1]. This reactor had two aqueous phases called donor and receiving, and an organic phase to transport the D-fructose from the donor phase where isomerisation took place to the receiving phase containing the acidic resin [1]. For continuous flow, a modular system from the company Ehrfeld made it possible to obtain some optimal parameters (temperatures, flow rates, residence times and pH) for the individual reactions. Our strategy was to combine the two catalysts in cascade and to a 1P1S hybrid process. The first step was to produce a xylose isomerase issue from Streptomyces Sp. Sk more resistant at acidic pH and to immobilize it on metallic supports (Figure 1-b). Then, to optimise the conditions of dehydration of D-fructose with heterogeneous tosylic acid in batch and in continuous flow. Finally, to implement the combination of immobilized glucose isomerase and heterogeneous tosylic acid in the continuous flow reactor (Figure 2).

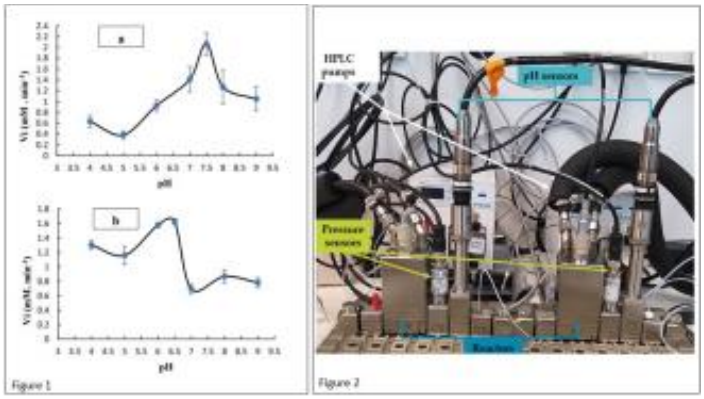


Figure 1: *Initial velocity (mM.min^{-1}) versus pH for Sweetzyme (A) and xylose isomerase from Streptomyces sp.SK (B).* ***Figure 2:*** *Modular system from the company Ehrfeld comprising two reactors, two HPLC pumps, two pressure sensors and two pH sensors.*

References

1. A, Gimbernat; M, Guehl; M, Capron; N, Lopes Ferreira; R, Froidevaux; J.S, Girardon; P, Dhulster; D, Delcroix; F, Dumeignil ChemCatChem, 2017, 9, 2080-2084.

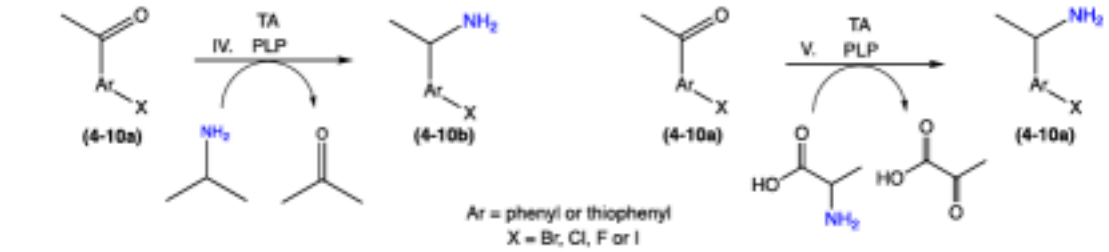
Biocatalyzed synthesis of chiral amine scaffolds for oncology projects.

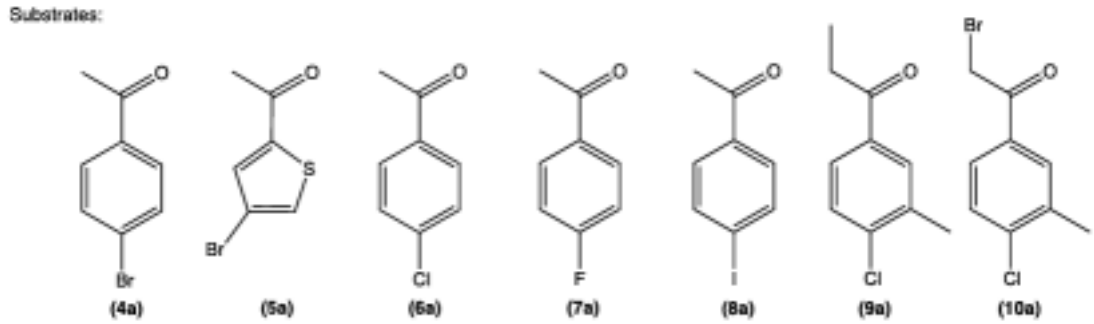
Katie Burke[1], Chloé Lelievre[2].

[1,2]Institut de recherches Servier (IdRS), 125 Chemin de Ronde, 78290 Croissy-Sur-Seine, France

Key words: Biocatlysis, Transaminases, Green Chemistry, Medicinal Chemistry

Servier have a need for chiral amine scaffolds for oncology projects. A greener approach to these chemically possible pathways is to use biocatalysts. Transaminases (TAs) and 7 halogenated substrates were employed to fulfill the need of the research projects. Chiral amines synthesis catalyzed by TAs was achieved. Enzymatic stability tests were carried out for TA-1024-AL and ATA-025, and optimization conditions for ATA-025 were performed to improve the conversion of 2-acetyl-4-bromothiophene (5a) to 1-(4-bromothiophen-2-yl)ethanamine (5b) from 11% to 63%. A 50 mg-scale reaction using substrate (5a) and ATA-025 was carried out to afford a 66% yield. These results are promising to obtain a library of aromatic halogenated chiral amines for MedChem projects.





Enzymatic Synthesis of *(R)*-Linalyl acetate: study of some parameters.

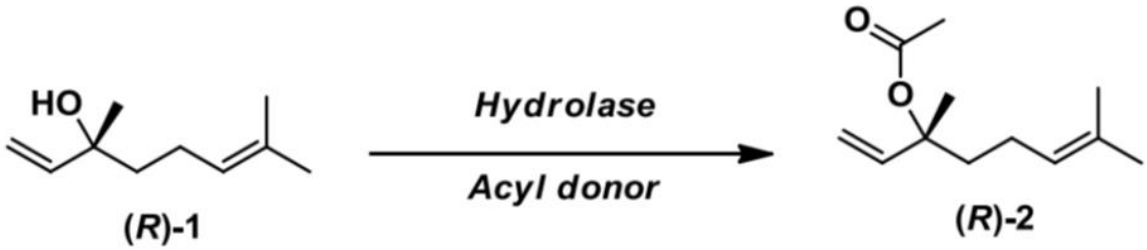
Chaibrassou Naouel[*1], *Kilani-Morakchi Samira*[1], *Zeror Saoussen* [2], *Sifi Karima*[1], *Merabet-Khelassi Mounia* [2]

[1]*Laboratory of systems and advanced materials (LSAM), Badji Mokhtar Annaba University.* [2]*Ecocompatible Asymmetric Catalysis Laboratory (LCAE), Badji Mokhtar Annaba University.* * *chaibrassou.naouel@gmail.com*

Key words: Hydrolases, (R)-Linalool, (R)-Linalyl acetate, Acylation, Eco-friendly conditions.

Linalool, a tertiary allylic terpene alcohol, is found in the essential oils of several plants, such as Brazilian rosewood and Chinese Ho leaf oils, and is also produced industrially from α-pinene [1]. Linalool and their acetate are the principal components of many essential oils known to possess several biological activities such as Lavender and Lavandin. For the industrial applications the linalyl acetate is highly used as food additive owing its flavor, high stability and low toxicity, whilst linalool is used for non-food purpose, such as a biocide [2].

Several synthetic methods drive to the linalyl acetate using harmful reagents and causing



environment pollution. To circumvent those disadvantages and according to the increase demand for greener, environment-friendly processes, biocatalysis seems the ideal solutions affording this valuable acetate [3]. Furthermore, enzymatic methods are labeled as natural in accordance with the United States and European Legislations, thereby satisfying the consumer trend towards natural products in various industries [4]. It is important to highlight that the acylation of this tertiary alcohol using enzyme catalysis represent a challenging task [5].

Figure 1 . *Enzymatic Acylation of (R)-Linalool.*

In the present study, we examine several parameters having a crucial influence on the biocatalytic acylation of the *(R)*-Linalool, such as: Enzyme nature, acyl donor, organic solvent and temperature. The outcomes of the enzymatic reaction are monitored by GC and the structure and the purity of acetate furnished are checked by the usual spectroscopic analyses.

References

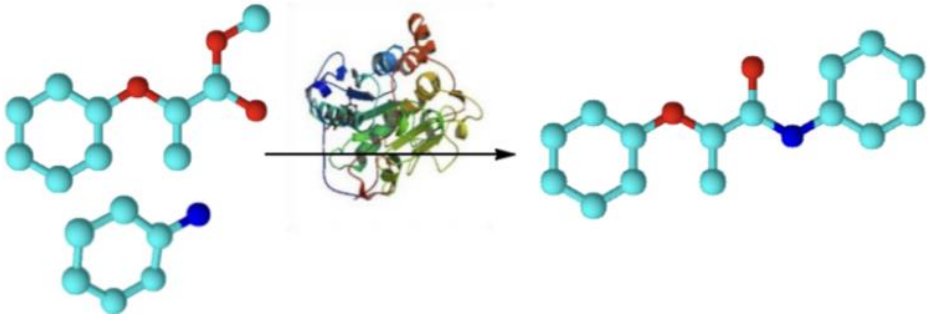
1. A, Martin ; V, Silva ; L, Perez ; J, García- Serna ; MJ, Cocero, *Chem. Eng.Technol*, **2007,** *30*(6), 726-731.
2. VV, Costa; KA, da Silva Rocha; LC, Oliveira; EF, Kozhevnikova; IV, Kozhevnikov, EV, Gusevskaya, *RSC advances*, **2016**, 6(49), 43217-43222.
3. RA. Sheldon, Green Biocatalysis, In: Biocatalysis and green chemistry, RN. Patel, Eds Wiley online library, **2011**, Chap1, 1–15.
4. AB, Vanin; T, Orlando; SP, Piazza; BM, Puton; RL, Cansian; D, Oliveira; N, Paroul, *Appl. Biochem. Biotechnol.* **2014**, 174, 1286–1298.
5. R, Kourist ; P, Dominguez de Maria ; UT, Bornscheuer, *ChemBioChem*., **2008**, 9(4), 491-498.

CAL-B catalyzed aminolysis of methyl 2-phenoxypropanoate as an eco efficient target to valuable anilide herbicides.

Benlaribi Rim[*1], *Merabet-Khelassi Mounia*[2], *Sifi Karima*[1], *Kilani-Morakchi Samira*[1], *Zeror Saoussen*[2]

[1]*Laboratory of Systems and Advanced Materials (LASMA), Badji Mokhtar Annaba University.* [2]*Ecocompatible Asymmetric Catalysis Laboratory (LCAE), Badji Mokhtar Annaba University.* **rim.benlaribi@gmail.com*

Key words: CAL-B, Aminolysis, Biotransformation, Phenoxypropionic acid, Anilide herbicides, Sustainable chemistry.



Herbicides represent closely 48% of pesticides consumption worldwide in protecting crops from weeds competition. The 2-aryloxy-substituted carboxylic acids and their derivatives are very interest because of high practical value of compounds of this class. Those compounds mimic the action of plant growth hormones (auxins) and act as systemic herbicides. They are widely used to control annual grasses and some broadleaf weeds. In addition, Anilides constitute an important class of amides owing by their several biological activities, such as anti-mycobacterial, antifungal, larvicidal, turbeculostatic, insecticidal and mainly herbicidal activities. Anilide herbicides are used to control weeds on hard surfaces, such as roads, railway tracks, paths, forestry, and crops [1].

Figure 1. *Enzymatic aminolysis of methyl-2-phenoxypropanoate.*

Because of the importance of the amide compounds and in the continuity of our previous investigations [2], we have envisaged the synthesis of a new 2-phenoxy propionamides by ester aminolysis. For that, we used a green chemistry processes based on biotechnological methods respectful of the environment [3]. The *candida antarctica lipase* immobilized on acrylic resin (*CAL-B*)

was selected as robust recyclable and biodegradable catalyst for the enzymatic aminolyis of methyl 2-phenoxypropanote using substituted anilines as nitrogen sources.

The obtained 2-phenoxy propionamides were recovered with good isolated chemical yields and their structures were confirmed by usual spectroscopic analyses.

References

1. KR, Rakhimol; S, Thomas; T, Volova; K, Jayachandran. Controlled release of pesticides for sustainable agriculture. Springer International Publishing. (Eds.). (**2020**).
2. N, Benamara ; M, Merabet-Khelassi ; L, Aribi-Zouioueche, O, Riant *Chem. Pap.* **2021**, 75, 4045–4053.
3. RA. Sheldon, *Green Biocatalysis, In: Biocatalysis and green chemistry*, RN. Patel, Eds Wiley online library, **2011**, Chap1, 1–15.

Sustainable process to valorize end-life poly (L-lactic acid) with enzymes

Alaric Catard, Isabelle Chevalot and Sandrine Hoppe

[1] Laboratoire Réactions et Génie des Procédés, University of Lorraine, CNRS, F-54000 Nancy, France
**alaric.catard@univ-lorraine.fr*

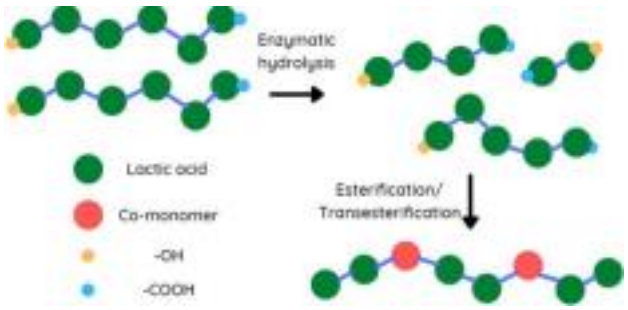
Key words: polylactic acid, green solvent, dimethyl carbonate, diethyl carbonate, enzymes, lipase B Candida antarctica, Proteinase K

General context: Currently, the vast majority of the plastics used are oil-based and it raises several issues: pollution from oil industries, oil resources depletion… Last decades have seen the emergence of biobased plastics, such as polylactic acid (PLA) to decrease the environmental impacts of plastics. At the end of its life, PLA may be chemically recycled by total depolymerization into its monomers and repolymerization of them enabling « infinite recycling ». However, this way may not represent the most efficient due to the energy required to produce a polymer from monomers. Consequently, researches are still needed to develop a sustainable and efficient way to valorize end-life PLA.

Objectives: This work will focus on partial hydrolysis of PLLA (poly L-lactic acid) with two kinds of enzymes as a first step: a protease (proteinase K) and a lipase (CALB). The activity of these enzymes is different according to the isomers of PLA: proteases are efficient against PLLA unlike lipases which are efficient against PDLA (poly D-lactic acid) (1). But, some authors reported that lipases can depolymerize PLLA in organic solvents (2). However, these solvents are harmful for human health and environment. We aim to use “green solvents” with a low toxicity and good degradability, to dissolve PLLA in order to make it more accessible to enzymes. Oligomers are preferred product of hydrolysis compared to lactic acid, because it’s possible to obtain high molecular weight PLA from oligomers and it needs higher amount of energy to repolymerize PLA from lactic acid than oligomers (3,4). As a second step, different enzymes from various microorganisms will be tested in order to obtain better hydrolysis rates. Finally, as a third step, we aim to develop a method to copolymerize it with enzymes in order to improve its properties like toughness or biodegradability.

Figure 1. *Summary scheme of the objectives of this work.*

Method: The methodology is divided into different parts : 1 - Solubility test of PLLA in green solvents : dimethyl carbonate (DMC) and diethyl carbonate (DEC) . 2 - Test of hydrolysis of PLLA by enzymes in solvents : Several parameters are investigated to understand their impact on hydrolysis : temperature, crystallinity, pH… 3 - Screening of other enzymes which are able to hydrolyze PLLA and try to obtain better hydrolysis performance than those



obtained with CALB and proteinase K. 4 - Finding of molecules to co-polymerize with PLLA by enzymatic transesterification to obtain better properties.

References:

1. Kawai F, Nakadai K, Nishioka E, Nakajima H, Ohara H, Masaki K, et al. Different enantioselectivity of two types of poly(lactic acid) depolymerases toward poly(l-lactic acid) and poly(d-lactic acid). Polymer Degradation and Stability. 2011;96(7):1342- 8.
2. Takahashi Y, Okajima S, Toshima K, Matsumura S. Lipase-Catalyzed Transformation of Poly(lactic acid) into Cyclic Oligomers. Macromol Biosci. 2004;4(3):346- 53.
3. Van Wouwe P, Dusselier M, Vanleeuw E, Sels B. Lactide Synthesis and Chirality Control for Polylactic acid Production. ChemSusChem. 2016;9(9):907- 21.
4. Tsuneizumi Y, Kuwahara M, Okamoto K, Matsumura S. Chemical recycling of poly(lactic acid)-based polymer blends using environmentally benign catalysts. Polymer Degradation and Stability. 2010;95(8):1387- 93.