Doctorant dans le cadre d'un projet Européen Horizon 2020 : P4SB (From Plastic waste to Plastic value using *Pseudomonas putida* Synthetic Biology.

Criblage, caractérisation et modification (mutagénèse) d'enzymes de dégradation de polyuréthanes

Le travail se déroulera à l'Université Strasbourg au sein de la Bioteam (voir lien ci-dessous) de l'Institut de Chimie et Procédés pour l'Energie, l'Environnement et la Santé, (ICPEES) sous la tutelle de l'Université de Strasbourg et du CNRS.

The main objective of P4SB is the biotransformation of non-sustainable plastic waste (e.g., polyethylene terephthalate and polyurethane) into sustainable value-added alternative materials such as biodegradable plastic polyhydroxyalkanoates (PHA). With this process, we aim to tackle the Societal Challenges set out by the European Union Horizon 2020 program concerning bio-economy (SC3) and resource efficiency (SC5) in an industry driven, outcome-oriented manner.

The PhD student will use a collection of approx. 65 enzymes among polyurethane (PU) degrading ones, namely esterases, lipases and proteases. First, a screening method for rapid and reliable PU degradation activity evaluation will be designed. The detection method to be set up is a modification of the clearance method used by Russel et al. 2011 [Appl Environ Microbiol, **77**, 6076–6084] for detecting PU-degrading fungi. Here, the reaction medium will be poured in the cupules of 96-wells microtiter plates. This high-throughput format will allow a proper system for PUase measurement optimization, with varying temperatures, different substrates, different pH … Time-dependent kinetics would also be possible with this system. Enzyme drops will be deposited onto the medium, dried and incubated at appropriate temperatures. Timely, the plate will be read thanks to a 96-wells plate reader at an appropriate wavelength (to be determinated).

Then, the enzymes with the best performances will be characterized further at a larger scale. The three most interesting features for industrial-intended enzymes i.e. catalytic activity, protein stability and substrate specificity will be carefully analyzed. Throughout the whole period, bibliographic monitoring will be done in order to identify possibly interesting enzymes. This period will lead to the choice of one or two enzymes among the most promising.

The next task will be the characterization and modification of these enzyme(s), starting by enzyme(s) purification. To achieve this goal, chromatographic methods will be screened depending on the protein properties (affinity, ion-exchange, and/or hydrophobic chromatography). Crystallization experiments will then be performed. When proper crystals have be obtained at small scale, the next step will be to produce labelled proteins in larger quantities. Then, MNR will be performed to resolve the 3D structure(s). The structure features will be then compared with many others by bioinformatics, keeping enzymatic properties in sight. One or several amino-acid residues would probably be identified as responsible for a given (beneficial) property in proteins from databases. This will allow the rational design of mutants of selected PU degrading enzyme(s). Mutations will be performed by single nucleotides exchanges. The resulting mutants will be expressed and their properties evaluated. The mutation process could be repeated to get even better enzymes.

Liens utiles :

P4SB : http://www.rwth-aachen.de/go/id/hwof/?lidx=1 et https://www.facebook.com/pseudomonasputida

Bioteam : http://icpees.unistra.fr/ingenierie-des-polymeres/polymeres-biosources-etou-biodegradables-pour-lenvironnement-et-la-sante/

Profils des candidats :

Biologiste des enzymes : criblage, enzymologie, production, purification, structure.

Compétences :

Enzymologie

Production et purification de protéines

Mutagénèse (Biologie moléculaire)

Bonne maitrise de l'Anglais oral et écrit indispensable

Mots clés :

Biotechnologie, Enzymologie, Structure protéiques, Mutagénèse

Début de la thèse :

Très probablement Octobre 2015

Envoyez CV et motivations à phalip@unistra.fr