Designing a Cell Factory to Produce Novel Microbial Biosurfactants on Cheap Substrate

Philippe JACQUES, Gembloux AgroBio Tech Liege University, Gembloux, Belgium; Magali DELEU, Gembloux AgroBio Tech Liege University, Gembloux, Belgium; Laurence LINS, Gembloux AgroBio Tech Liege University, Gembloux, Belgium; Angelique LEONARD, Liege University, Liege, Belgium; Marnix MEDEMA, Wageningen University, Wageningen, The Netherlands; Tinka MURK, Wageningen University, Wageningen, The Netherlands; Hugo GRAMAJO, University of Rosario, Rosario, Argentina; Harald GROSS, Eberhard Karls University of Tuebingen, Tuebingen, Germany, Arnaud DELECROIX, Lipofabrik Belgium, Liege, Belgium, François COUTTE, Lipofabrik Belgium, Liege, Belgium, John BELK, Dasic International Ltd, Romsey, United Kingdom

Biosurfactants can be defined as surface-active biomolecules produced by microorganisms with a wide-range of applications such as emulsification, detergency, solubilization, lubrication and phase dispersion. In recent years, these biomolecules have attracted wide interest from the scientific community but also from many industrials due to their advantages over chemical surfactants, including higher biodegradability and better environmental compatibility.

In light of all of this, the BestBioSurf project aims at producing novel and eco-friendly biosurfactants in a cost-effective manner through initial pilot validation in laboratory settings, to a larger bio-process scale up. In order to do so, the BestBioSurf project will use the *Bacillus subtilis* bacterial host system as a primary choice for producing these novel biosurfactants. This is mainly due to the strain's long history and well-established experimental proof-of concept use in the biotechnological area of lipopeptide bioengineering.

Precisely, we will introduce a novel and original strategy based on bioinformatics, synthetic biology and metabolic engineering to perform molecular diversification of well established biosurfactants with high efficacy, low toxicity and high biodegradability, and to produce these at high titers. First, toxicity/efficacy assays with existing lipopeptides and molecular modeling of chemical variants of these compounds with target liquids will be used to define the physicochemical properties of the best biosurfactant to produce. Then, genome mining and synthetic biology will

enable the identification and the construction of novel biosynthetic pathway variants towards the target compounds. To reduce their cost price, fatty acid and amino acid metabolism in the producing cells will subsequently be genetically optimized in order to turn very cheap co-substrates provided by a bioethanol industrial producer into high product titers. Finally, the newly produced (novel) surface-active compounds will be checked for their efficacy and ecotoxicity by the end-user study of this project. This end-user is an SME close to the market that could replicate widely the technology through its various activity areas including oil dispersants, innovative chemical cleaning, hygiene, aerospace to rail rolling stock and waterborne pollution. Many other potential applications with high eco-friendly impacts include agriculture (biosurfactants-based biopesticides), medicine (anti-microbial & anti-cancer activity), and cosmetics (emulsification & foaming properties). This wide range of applications opens up a whole new window of opportunities for partnering in the future with many more end-users that could benefit from the BestBioSurf technologies.