BIOTRANS
LA ROCHELLE 2023

N°1238 / PC
TOPIC(s) : Industrial biocatalysis / Enzyme production, immobilization

Enzymatic synthesis of novel amino acid-based biosurfactants from microbial oil

AUTHORS

CHEVALOT Isabelle / LRGP-UL, 1 RUE GRANDVILLE, NANCY
Dimitris KARAYANNIS / DEPARTMENT OF FOOD SCIENCE AND HUMAN NUTRITION, AGRICULTURAL UNIVERSITY OF ATHENS, IERA ODOS 75, ATHENS
Emma BOULANGER / UNIVERSITÉ DE LORRAINE, CNRS, LRGP, 1 RUE DE GRANDVILLE, NANCY
Seraphim PAPANIKOLAOU / DEPARTMENT OF FOOD SCIENCE AND HUMAN NUTRITION, AGRICULTURAL UNIVERSITY OF ATHENS, IERA ODOS 75, ATHENS
Isabelle CHEVALOT / UNIVERSITÉ DE LORRAINE, CNRS, LRGP, 1 RUE DE GRANDVILLE, NANCY
Corresponding author : Isabelle CHEVALOT / Isabelle.Chevalot@univ-lorraine.fr

PURPOSE OF THE ABSTRACT

Biosurfactants, a class of green and sustainable surfactants are naturally synthetized from microorganisms (bacteria, fungi, yeast and microalgae) or they can be generated from the association of a polar amino acid (hydrophilic moiety) and a non- polar long-chain compound (hydrophobic moiety) from renewable sources. To date, amino acid-based surfactants are industrially produced via a chemical route, called the Schotten- Baumann reaction, which are being related with major environmental drawback (e.g., salted wastes, acyl chlorides, organic solvents etc). These amino acid-based surfactants have low degree of toxicity, low haemolytic activity and are easily biodegradable. They are widely applied in the pharmaceutical, food and cosmetic industries due to their excellent emulsifying and antimicrobial activities.

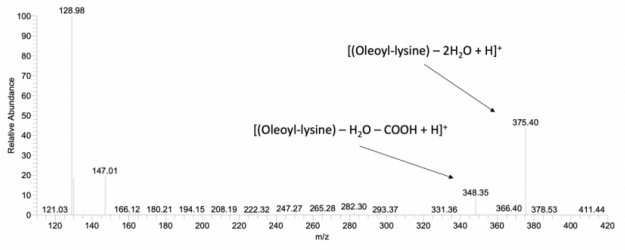
The present work is divided into two main objectives, in order biosurfactants be synthetised. The first was the production of microbial lipids (MLs) from oleaginous yeast cultivated on biodiesel-derived glycerol and the second was the enzymatic synthesis of high-value biosurfactant-type molecules, with MLs implicated as acyl donors (ADs). These novel biosurfactants were produced through the enzymatic N-acylation of lysine from MLs, catalyzed by aminoacylases produced from Streptomyces ambofaciens ATCC 23877 under aqueous conditions [1] and by Candida antarctica lipase B (CALB) under non-aqueous conditions [2]. The N-acylation reaction was evaluated and characterized based on regioselectivity, productivity and the substrate specificity of the enzyme towards the major fatty acids (FAs) found in MLs (e.g., linoleic acid).

The oleaginous yeast Cryptococcus curvatus ATCC 20509 accumulated lipids up to 50% w/w in dry cell weight (DCW) (MLsmax = 6.1 g/L) after the optimization of the bioprocess according to the nature of the nitrogen source and the initial concentration of glycerol (Glyc0) employed in the medium. Aminoacylases revealed excellent activity towards the synthesis of acyl-lysine only when free fatty acids (FAs) were used, and the rare regioselectivity in the α-amino group, which has a great impact on the preservation of the functional side chains of any amino acids or peptides. CALB was able to catalyse the N- acylation of lysine by direct or trans-esterification. Unlike aminoacylases, the regioselectivity of CALB was oriented towards the ε-position of lysine. The substrate specificity of both enzymes was studied, and a new parameter was defined, viz. Specificity factor (Sf), which expresses the relative substrate specificity of an enzyme towards a FA present in a FA mixture (oil). HPLC-MS2 analysis revealed that aminoacylases have Sf in favor of palmitic acid, while CALB have Sf towards linoleic acid. In addition, a successful di-acylation of lysine exploiting the different regioselectivities of both enzymes is presented.

To the best of our knowledge, this is the first time that a microbial oil has been successfully used as AD for biosurfactant synthesis [3] and that double N-acylation of lysine in the α and ε positions using two enzymes has been reported. This bio-refinery approach illustrates the concept of a state-of-the-art combination of biocatalysis

and yeast culture technology.

FIGURES



	C16:0 (Sf)	C18:1 (Sf)	C18:2 (Sf)
CALB			
C. curvatus oil	0.25	1.47	2.84
Iso-mix	0.61	1.03	1.36
Aminoacylases			
H-C. curvatus oil	2.19	0.75	0.75
TM-C. curvatus	2.00	0.85	0.84
Iso-mix	1.98	0.71	0.32

FIGURE 1

HPLC-MS2 spectrum of the product

Fragmentation of the alpha-oleoyl-lysine, parent ion (m/z = 410), synthesised by aminoacylases.

FIGURE 2

Specificity factor of CALB and aminoacylases towards the 3 major fatty acids of oils and tailor-made solutions.

H-C. curvatus oil: Hydrolysed Cryptococcus curvatus oil; TM-C. curvatus: Tailor-made solution of the major fatty acids of Cryptococcus curvatus oil; Iso-mix: Tailor made solution containing 1/3 palmitic acid, 1/3 oleic acid and 1/3 palmitic acid.

KEYWORDS

biosurfactants | microbial lipids | aminoacylases | N-acylation

BIBLIOGRAPHY

1. Bourkaib, M.C.; Delaunay, S.; Framboisier, X.; Ho□tel, L.; Aigle, B.; Humeau, C.; Guiavarc'h, Y.; Chevalot, I. N-acylation of L- amino acids in aqueous media: Evaluation of the catalytic performances of Streptomyces ambofaciens aminoacylases. Enzyme Microb. Technol. 2020, 137, 109536.

2. Dettori, L.; Jelsch, C.; Guiavarc'h, Y.; Delaunay, S.; Framboisier, X.; Chevalot, I.; Humeau, C. Molecular rules for selectivity in lipase-catalysed acylation of lysine. Process Biochem. 2018, 74, 50-60.

3. Karayannis, D.; Papanikolaou, S.; Vatistas, C.; Paris, C.; Chevalot, I. Yeast Lipid Produced through Glycerol Conversions and Its Use for Enzymatic Synthesis of Amino Acid-Based Biosurfactants. Int. J. Mol. Sci. 2023, 24, 714.