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## ► To cite this version:

Lara Wehbe, Rémi Bascou, Erwann Guénin, Alla Nesterenko. Valorization of silk biomass for the development of amphiphilic lipopeptides. 10th International Conference on Engineering for Waste and Biomass Valorisation - WasteEng2024, Aug 2024, Sendai, Japan. hal-04765629

## HAL Id: hal-04765629 https://hal.science/hal-04765629v1

Submitted on 4 Nov 2024

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### VALORIZATION OF SILK BIOMASS FOR THE DEVELOPMENT OF AMPHIPHILIC LIPOPEPTIDES

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#### 1. Keywords

Silk biomass, silk protein, extraction lipopeptides, mechanochemistry, surface tension

#### 2. Highlights

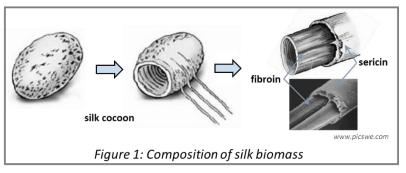
- Extraction and hydrolysis of silk proteins were performed using eco-friendly processes
- Silk lipopeptides were produced by mechanochemistry under solventless conditions
- Amphiphilic silk lipopeptides showed interesting surface activity
- Structure/properties relations of lipopeptides were studied

#### 3. Purpose

Silk is defined as non-food biomass produced by cultured silkworms. The traditional use of silk is predominantly the textile industry for its luster and mechanical properties. World silk cocoons production is about 800 000 tons per year [1]. In recent years, silk-related materials have provided many opportunities for new applications, particularly in the biotechnological and biomedical fields, because of their biocompatibility, biodegradability, non-toxicity and mechanical property. Silks are composed of two kinds of proteins: a fibrous one, named fibroin, that forms the thread core and represents 70-80% of the silk cocoon, and a glue-like one, named sericin, that surrounds the fibroin fibers to link them together (Figure 1) [2]. Silk sericin is an industrial waste from textile industry actually

not valorized and release in the environment more than 50 000 tons per year.

The development of lipopeptide surfactants from silk biomass, which is not in competing for food sector, represents a major challenge on a global scale, since natural-based surfactants are produced from



agricultural biomass such as sugars, starches, vegetable proteins and oils. However, these high-quality fractions of biomass are in direct competition with food and feed industries, which gives rise to ethical, environmental and political concerns due to the rapid growth of the world population [3].

The main objective of this work is the valorization of silk proteins as a non-food biomass for the development of amphiphilic molecules. The first step of the work is the separation of two silk proteins fractions using green and ecological processes. Then, silk proteins were successively modified by enzymatic hydrolysis and acylation reaction to produce lipopeptides. The originality is to develop an alternative method to the classic synthesis of lipopeptides by using mechanochemistry. Finally, the surface adsorption properties of silk lipopeptide surfactants with variable structure were studied in order to investigate on structure/property relationships.

#### 4. Materials and methods

The first step of silk lipopeptide production involves the separation of two silk protein fractions: fibroin and sericin by alkaline method and high temperature high pressure method (HTHP) respectively [4,5]. Then, fibroin and sericin peptides were obtained by enzymatic hydrolysis. The grafting of the hydrophobic chain onto the silk peptides was carried out using the classic reaction of Schotten-Baumann [6] and then a novel alternative approach of acylation by mechanochemistry was successfully developed (Figure 2).

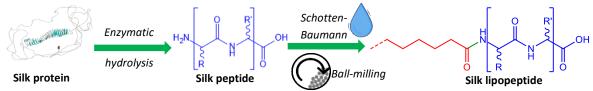


Figure 2: Valorization of silk biomass to produce lipopeptide surfactants

The acylation rate (ratio of grafted amine functions) and the degree of hydrolysis was estimated by spectrophotometry. The adsorption properties of the peptides and lipopeptide mixtures with different structure was then studied by surface tension measurements using the pendant drop method.

#### 5. Results and discussion

The obtained results show that the two silk protein fractions can be efficiently separated with a yield of 80% for sericin extraction with HTHP method and 90% for fibroin extraction with alkaline method. Then silk protein fractions are hydrolysed using Alcalase® enzyme with a degree of hydrolysis varying between 20 and 40%. The grafting of a fatty chain on silk sericin developed using conventional method in solution gives an acylation rate from 27 to 42% [6]. Moreover, the results of solvent-free grafting of fatty acid on silk peptides by mechanochemistry show very promising results with comparable rate of functionalization. The produced lipopeptides show good surface activity and ability to reduce the air/water surface tension. We noted that fibroin peptides and lipopeptides show better surface activity than those of sericin because of the hydrophobic nature of fibroin. Finally, the structure of the hydrophilic and hydrophobic parts of lipopeptides have a key role in the determination of the surface tension.

#### 6. Conclusions and perspectives

The original way for the non-food biomass valorization to produce a new class of silk protein-based surfactants was studied. The amphiphilic molecules obtained with a new eco-friendly synthesis can be considered as a good potential candidates for the replacement of petroleum-based in different applications, especially as mild surfactants in cosmetics, household detergents, toiletry and pharmaceutical products for topical treatment. Thereafter, functional properties of lipopeptides, such as emulsifying and foaming capacities as well as biological effects will be studied in view of enlarging potential applications of lipopeptides. Also, the enzymatic hydrolysis of silk proteins by mechanochemistry will be investigated allowing the synthesis of lipopeptides in one pot two step thus limiting the impact of the synthesis.

#### 7. References

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