ERA CoBioTech

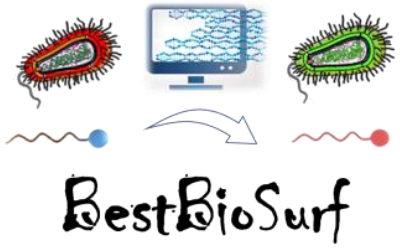
BIOTECH RESEARCH AND INNOVATION HACK 2021

Final seminar of the cofunded projects of ERA CoBioTech

Synthetic Biology for the development of novel, cheap and efficient biosurfactants

Project acronym: BestBioSurf

Name: Philippe Jacques

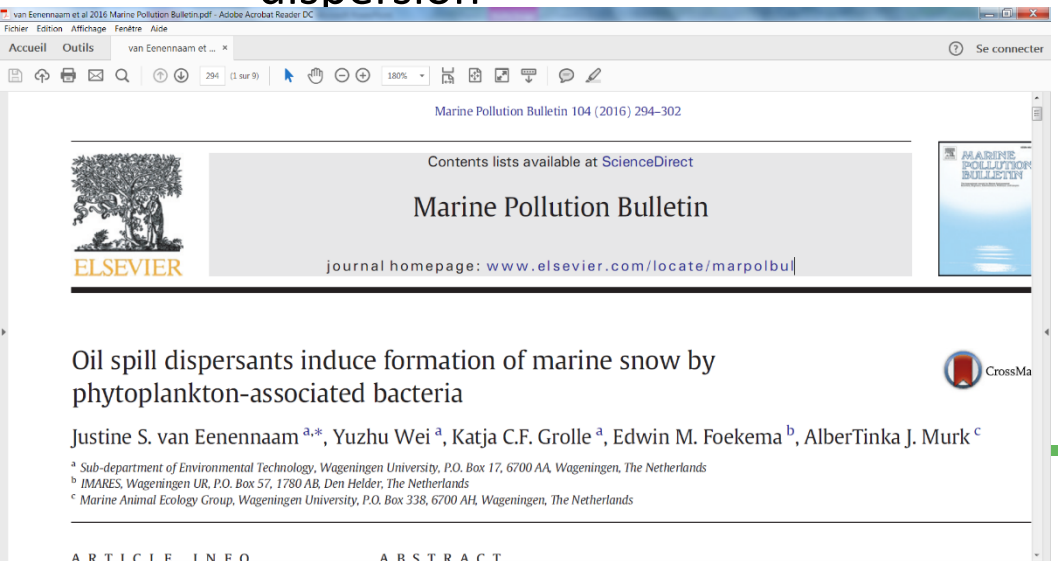


This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant 722361

28.09.2021

Introduction

- Many industrial sectors would like to replace surfactants by biosurfactants
- The challenges are the following ones:
  - The properties
  - The toxicity and ecotoxicity
  - The cost prices
- An example: the problem of ecotoxicity of surfactant used in oil spill dispersion



van Eenennaam et al 2016 Marine Pollution Bulletin.pdf - Adobe Acrobat Reader DC

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Marine Pollution Bulletin 104 (2016) 294–302

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Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

ELSEVIER

Oil spill dispersants induce formation of marine snow by phytoplankton-associated bacteria

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ARTICLE INFO ABSTRACT

Introduction

- BestBioSurf project aims at producing **novel** and **eco-friendly biosurfactants** in a **cost-effective** manner through lab-scale validation to a **bio-process demonstrator** within a real environment.

- Project budget : 1 508 000 € from 2018 to 2021

Project partners

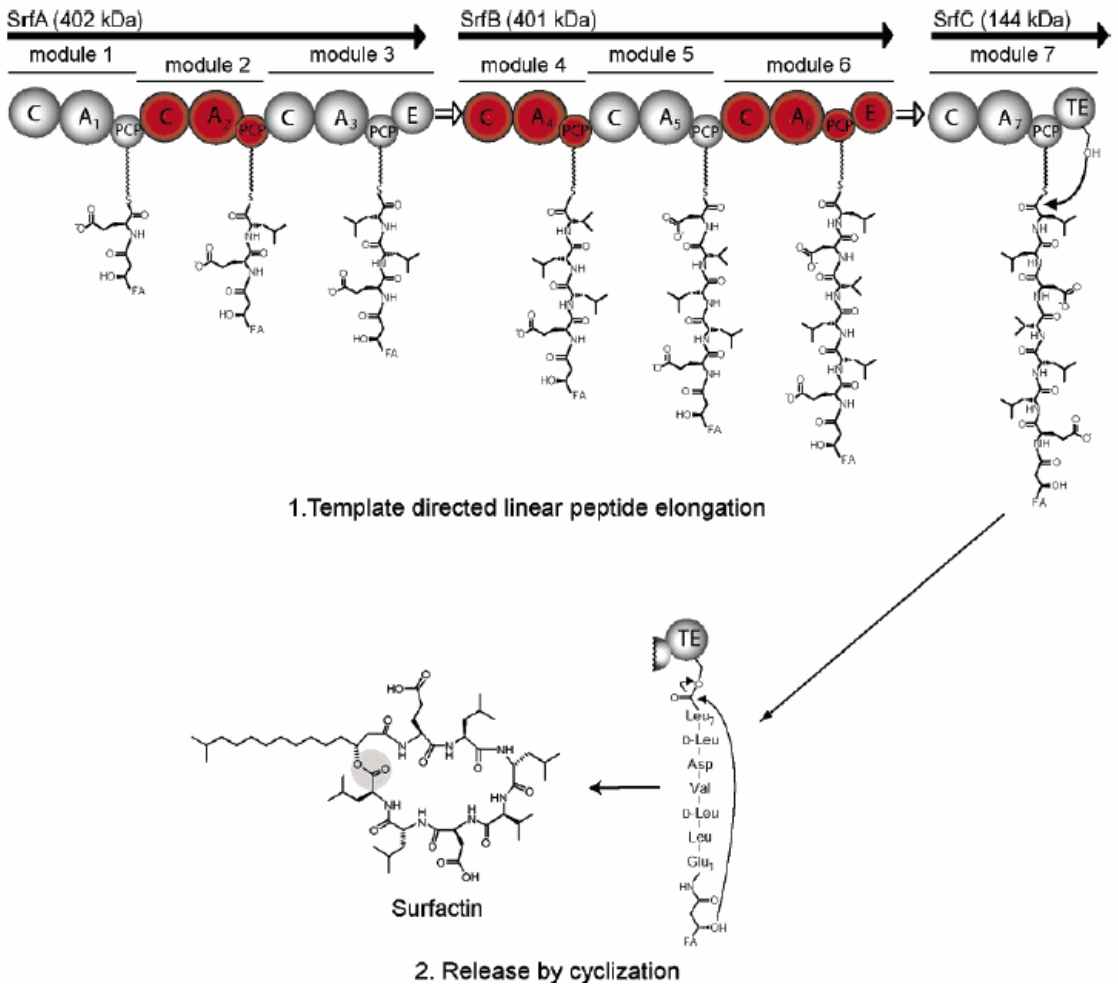
- Microbial Processes and Interactions Team, Biophysics Team and Products, Environment and Processes Team, TERRA Teaching and Research Centre, **Gembloux AgroBioTech-University of Liege,** Belgium – Dr Magali Deleu, Dr Laurence Lins, Prof. Angelique Leonard, Prof Philippe Jacques
- **Lipofabrik Belgium,** Belgium – Mr Arnaud Delecroix
- **BioWanze,** Belgium (Associate partner)

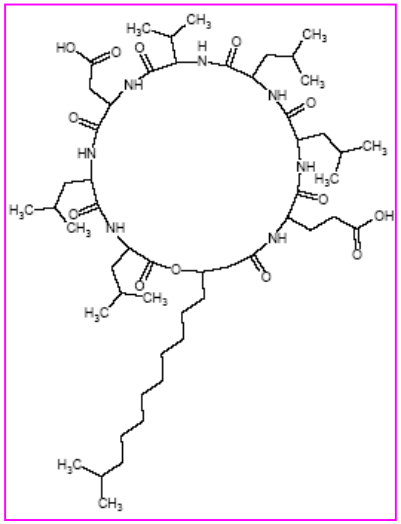
- Bioinformatics Group and Wageningen Marine Research / Marine Animal Ecology Group , **Wageningen University and Research,** The Netherlands – Prof Marnix Medema and Prof Tinka Murk

- Microbiology Department, Institute of Molecular Biology of Rosario, **CONICET-UNR,** Argentina – Prof Hugo Gramajo and Prof. Ana Arabolaza

- Pharmaceutical Biology Department, Pharmaceutical Institute, **Eberhard Karls University of Tuebingen,** Germany – Prof Harald Gross
- **Dasic International Ltd,** United Kingdom – Mr John Belk

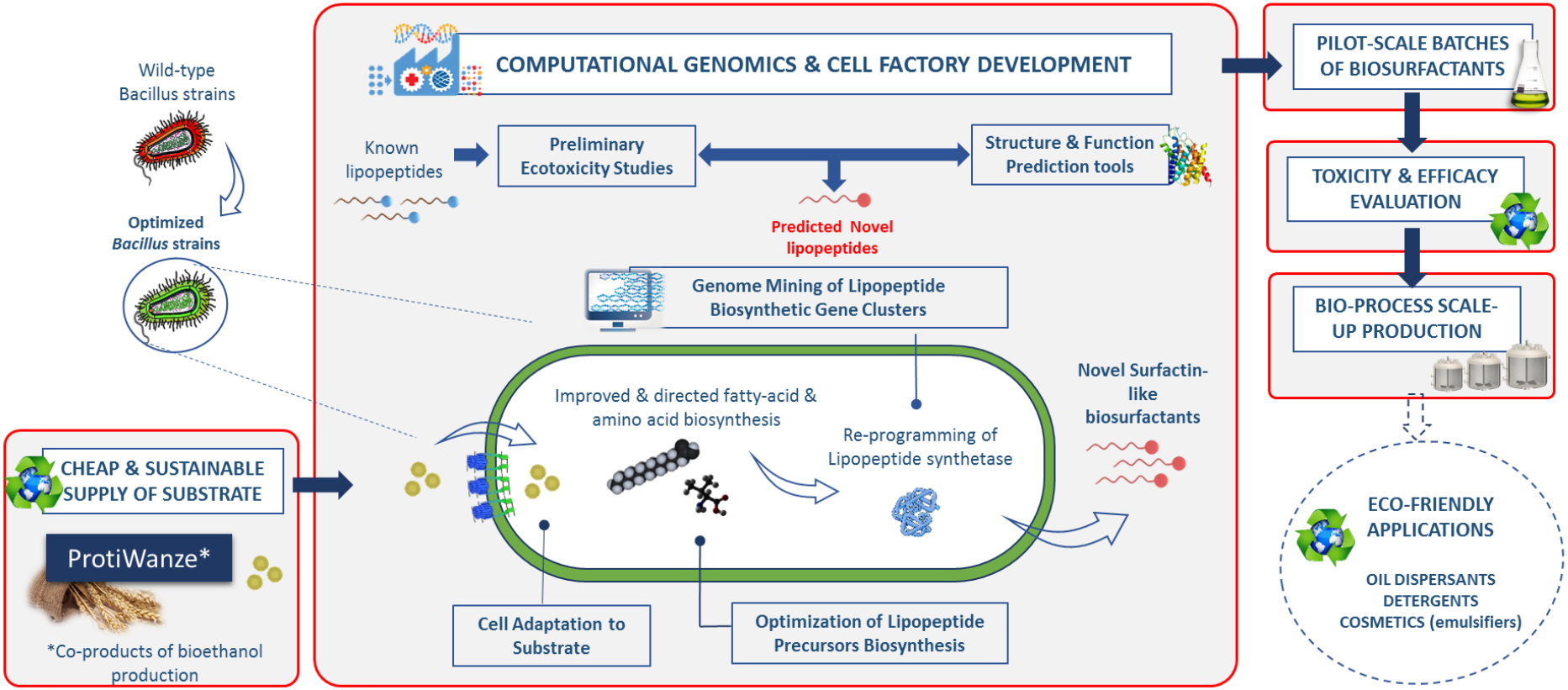
Introduction





Project plan

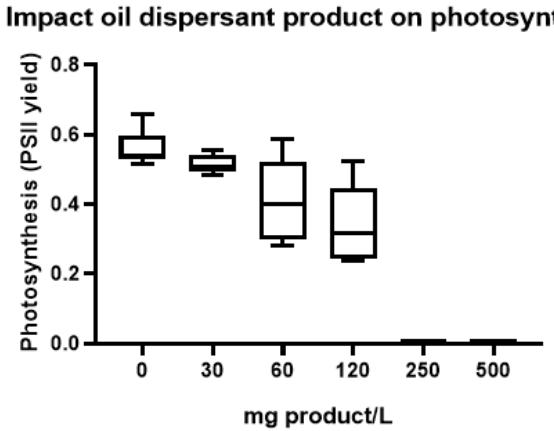
BestBioSurf CONCEPT SCHEME



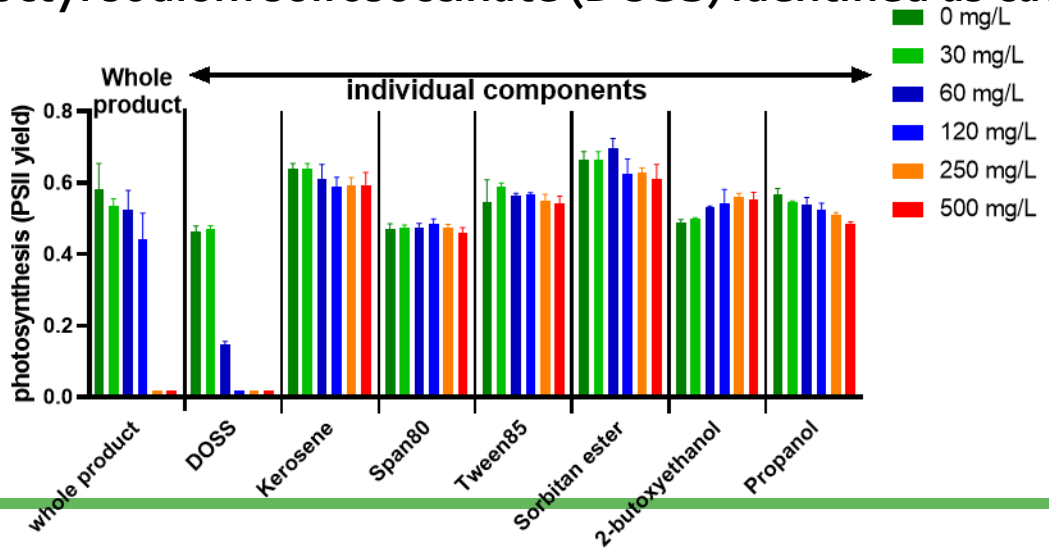
Main results

- To determine which are the compounds responsible for the ecotoxicity of COREXIT surfactant
- To develop a novel and cheap cleaning product based on surfactin
- To identify constraints related to the use of surfactin in some detergent applications
- To screen existing surfactants
- To develop by synthetic biology novel biosurfactants

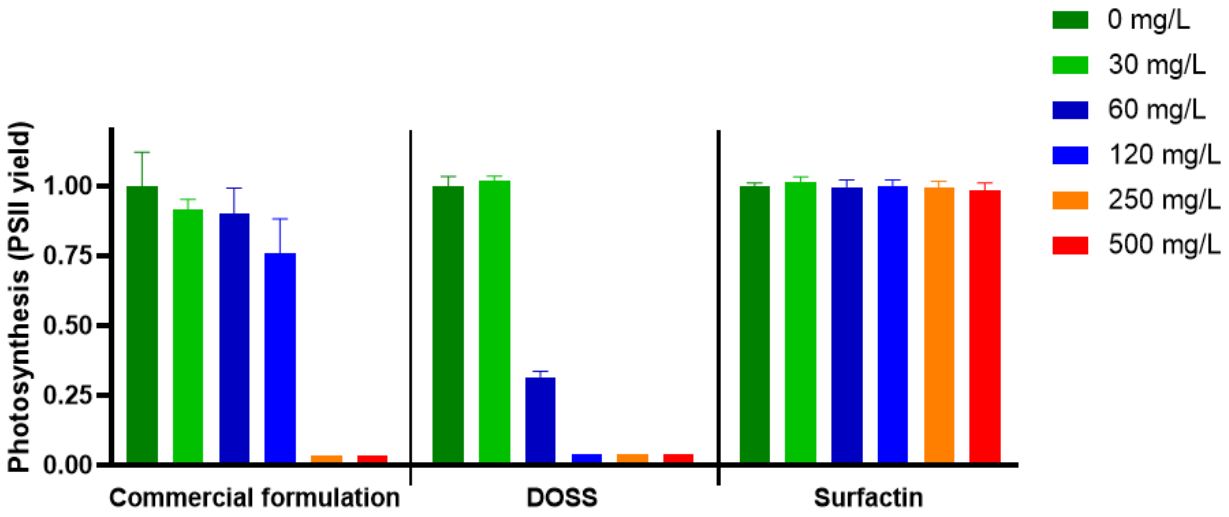
Oil dispersant products toxic to algae



Dioctyl sodium sulfosuccinate (DOSS) identified as cause

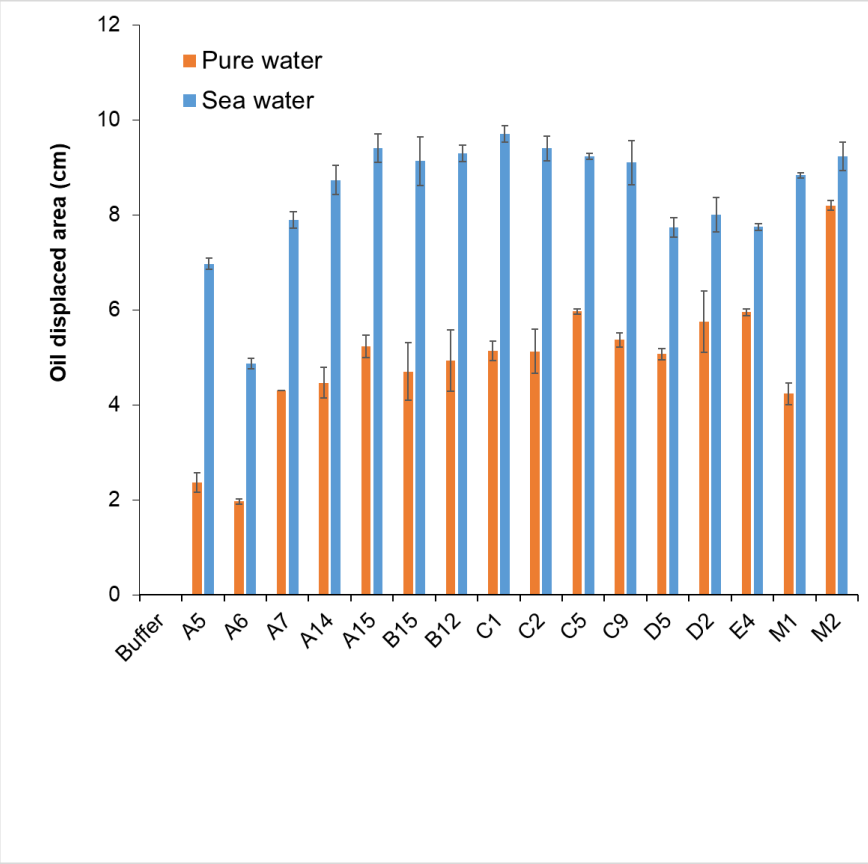


Surfactin did not impact photosynthesis

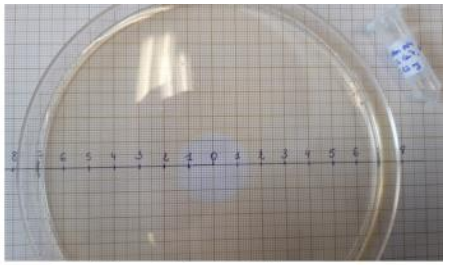


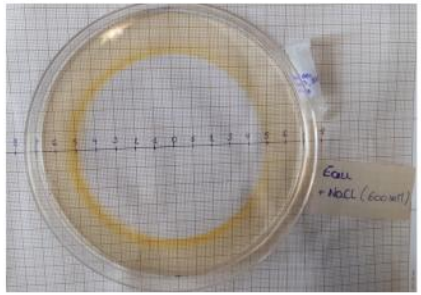
Structure prediction

Eight different surfactin like compounds were modeled by molecular dynamics simulation and structure-function was predicted based on their biosurfactant properties

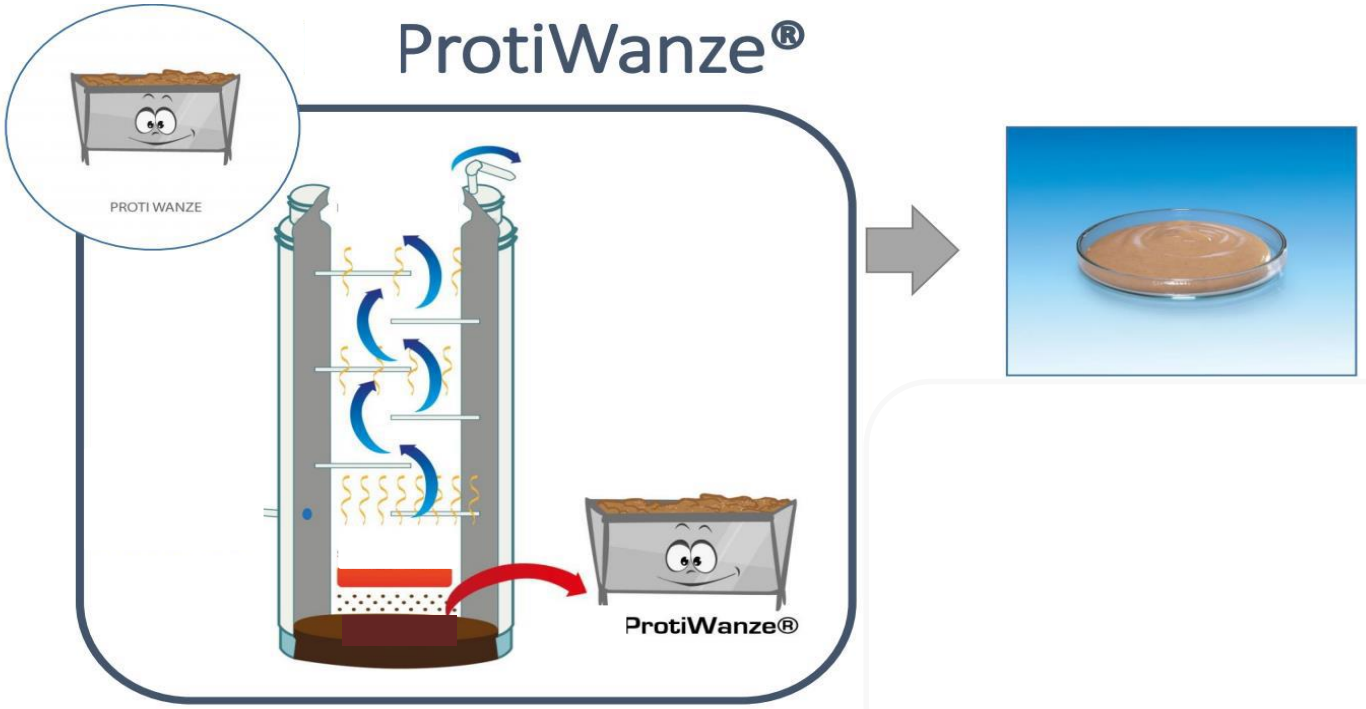


Oil displacement test :

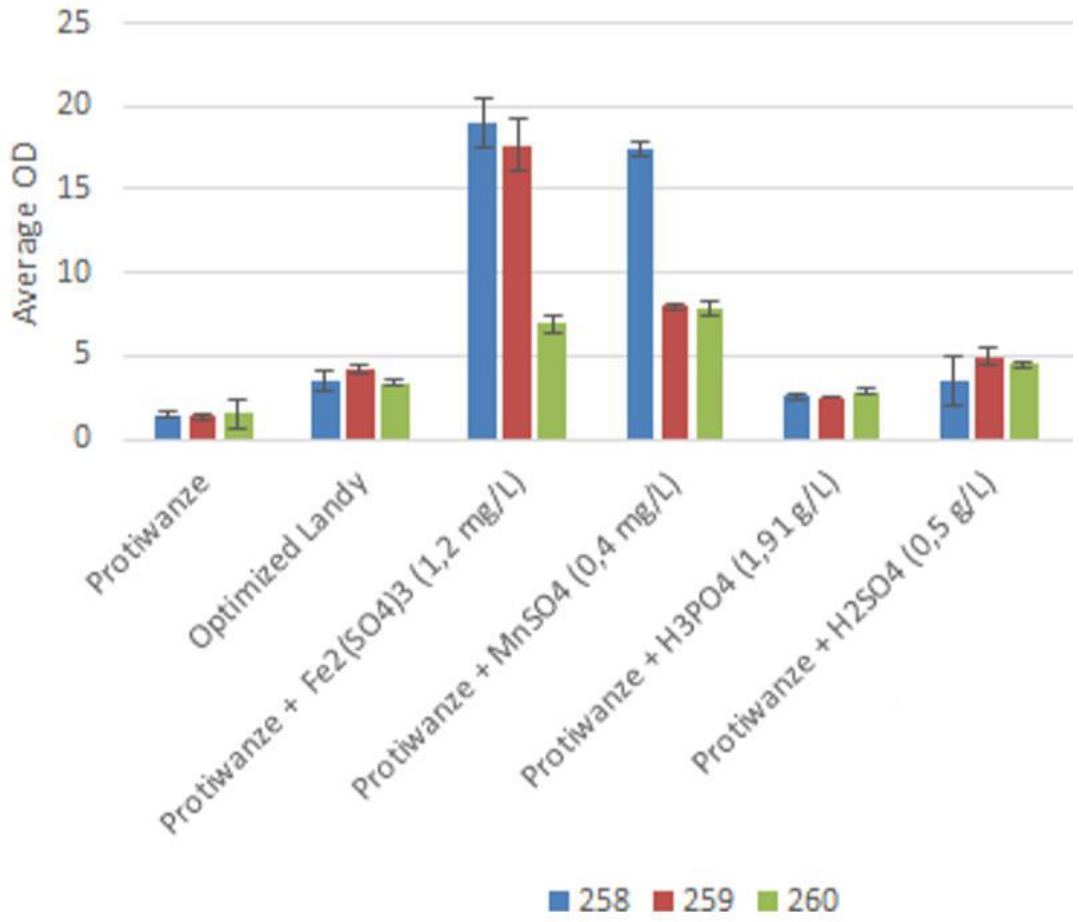




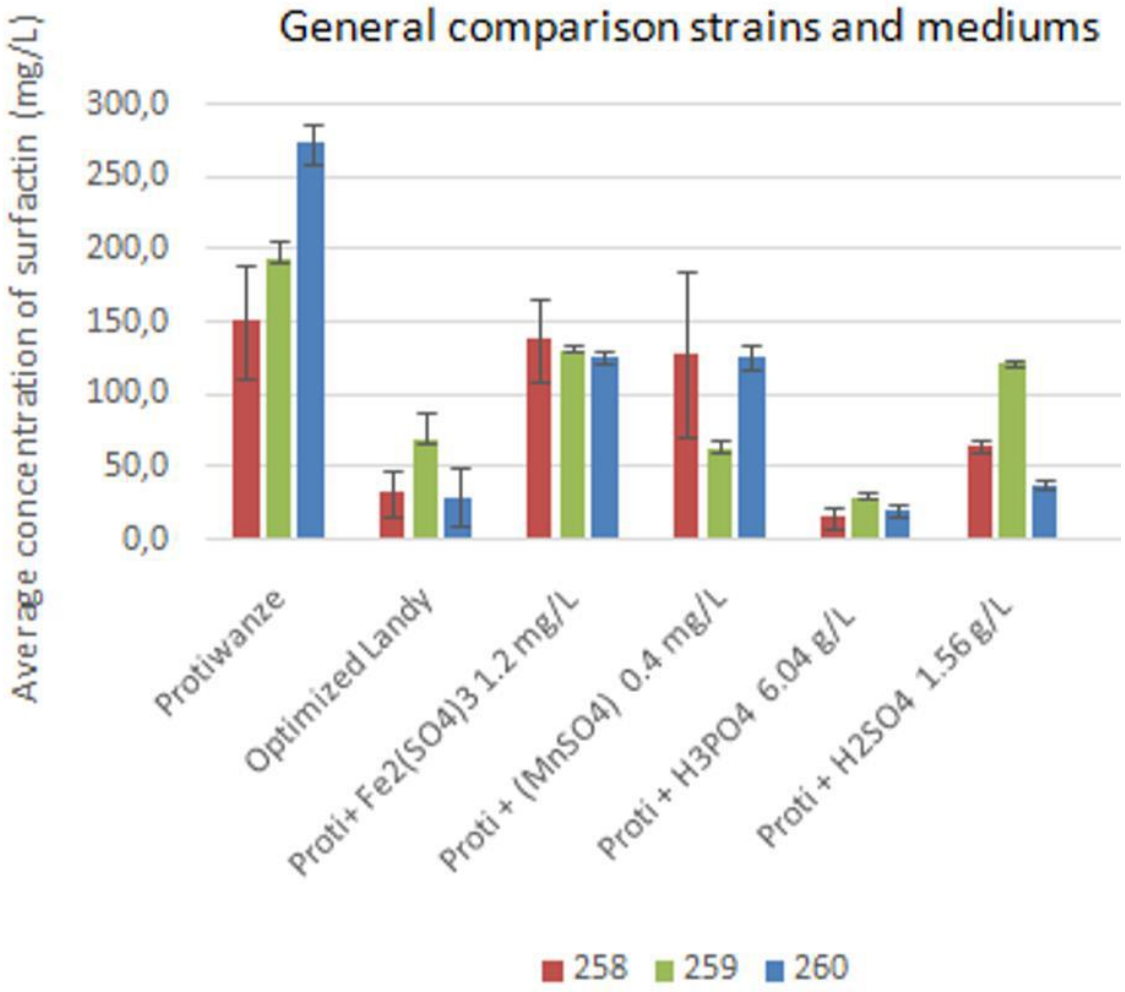
ProtiWanze



Use of ProtiWanze as substrate



Use of ProtiWanze as substrate



Genetic engineering –

improve surfactin production by targeting fatty acid metabolism

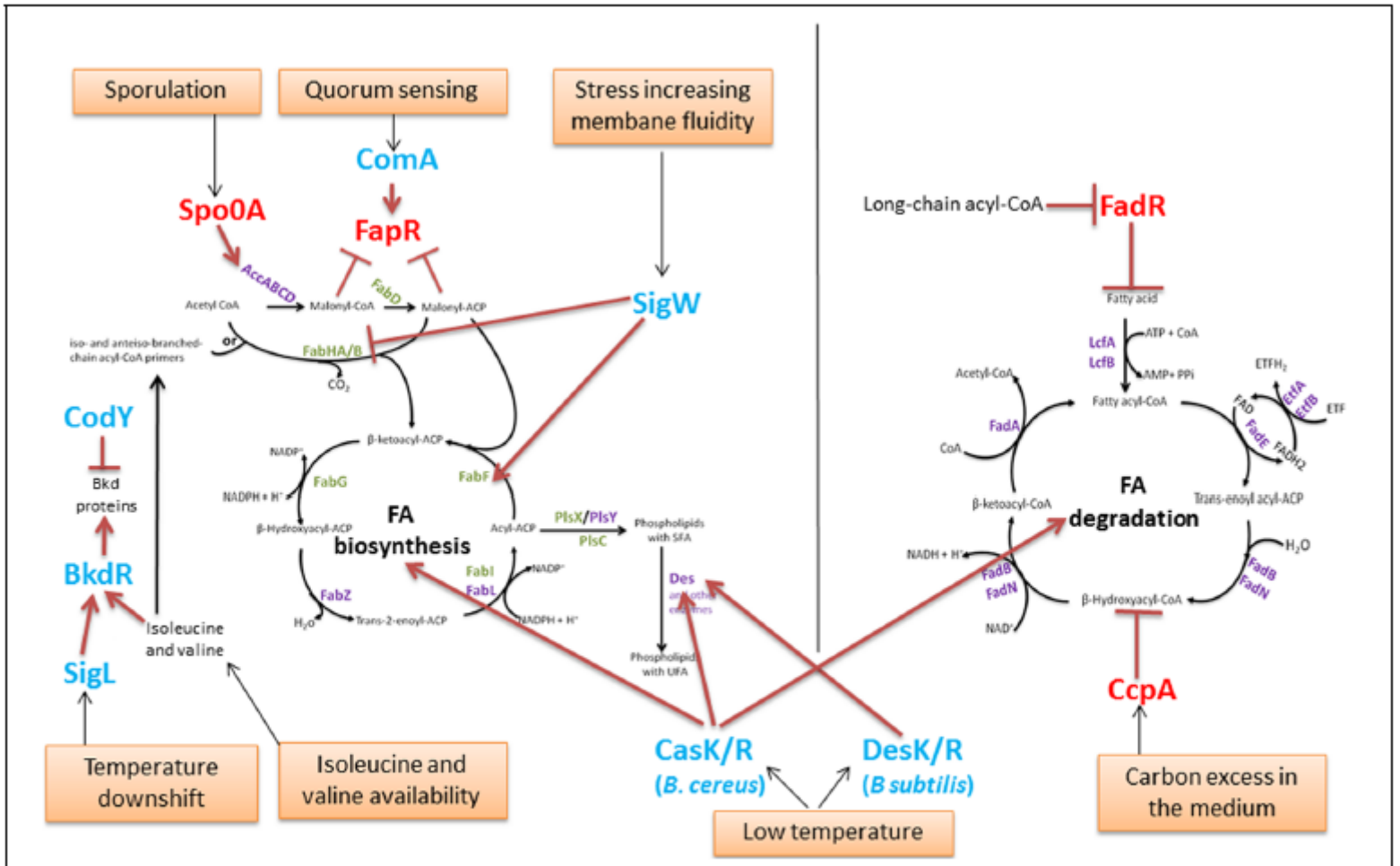
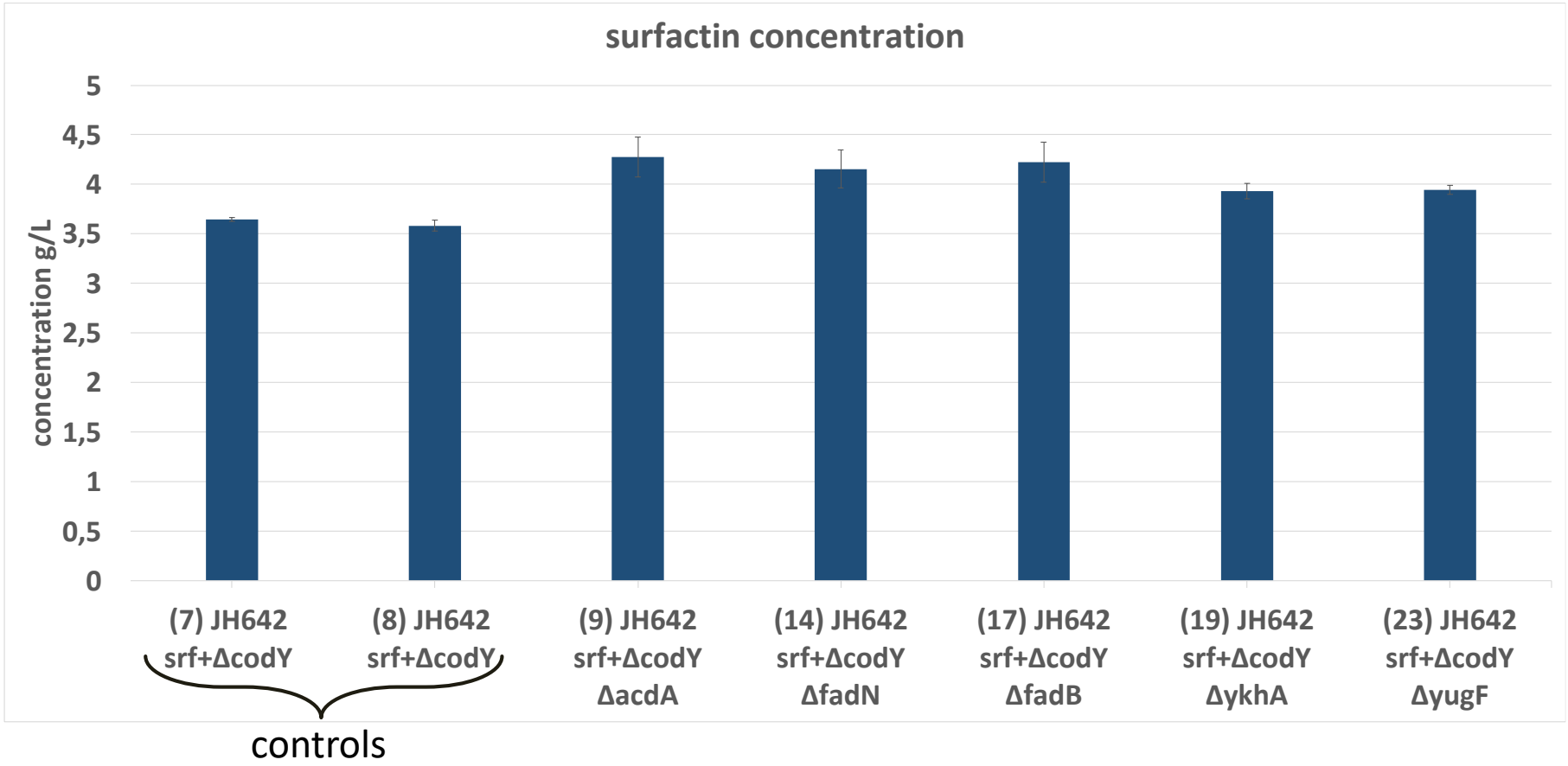


FIGURE 3 | Regulation of the FA pool in *Bacillus*. The enzymes are identified in purple or in green (for those which encoding genes are regulated by the global regulator FapR, as in **Figure 2**). Global regulators of metabolism and sporulation are identified in red. Other regulators are in blue. Red blunt-head line and arrows indicate repression or activation, respectively. Orange boxes represent environmental conditions involved in FA regulation.

Genetic engineering –

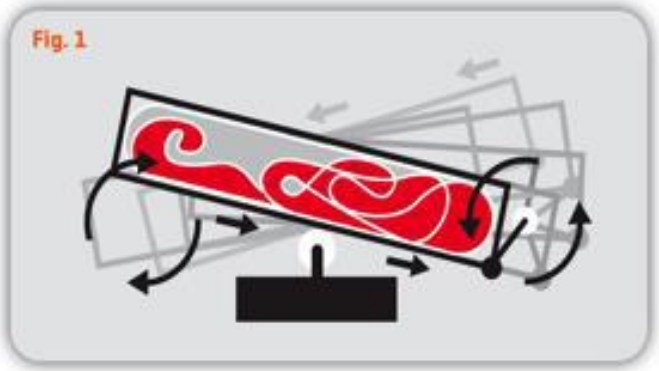
improve surfactin production by targeting fatty acid metabolism



New fermentation process

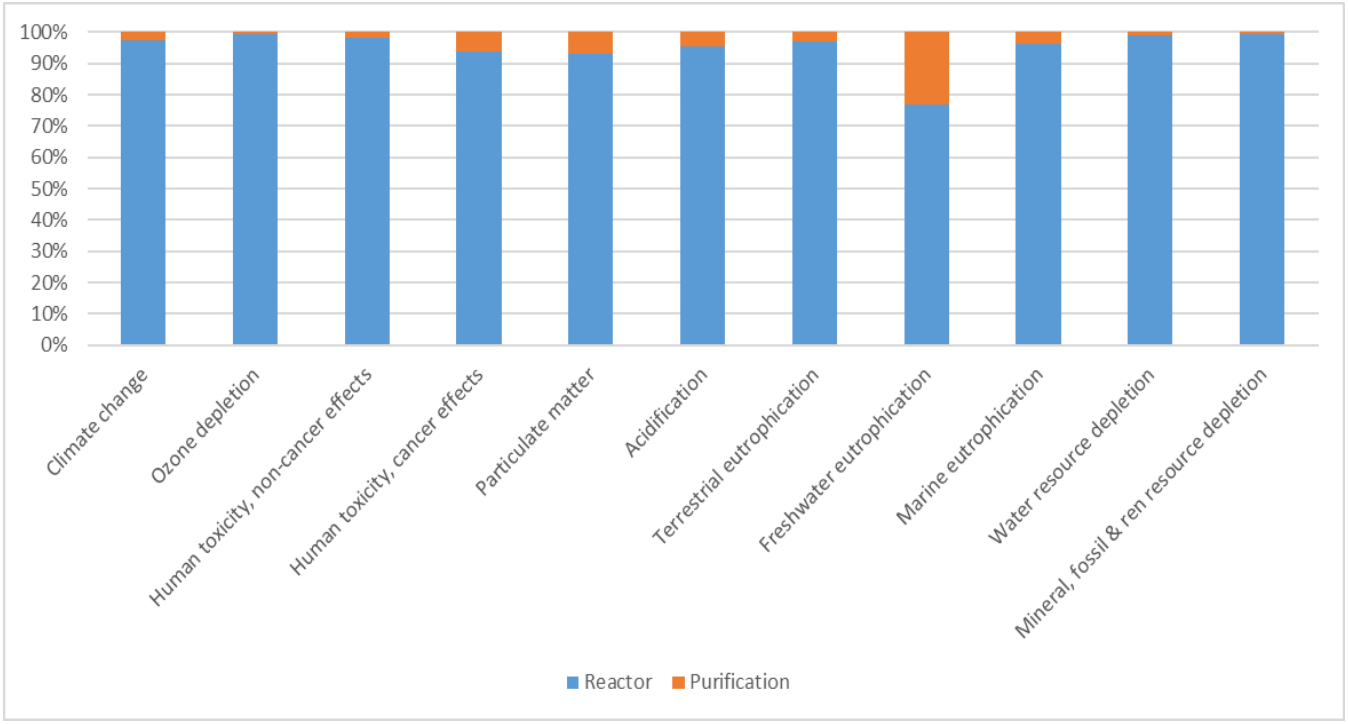
Cell-tainer

Reactor scalable from 2L to 200L.
The agitation is made through a wave like movement and thus **decreases greatly the foam formation** during lipopeptide production.



LCA Surfactin
INDUSTRIAL SCALE

- Results (ILCD recommended methods)



Lab scale culture

↓

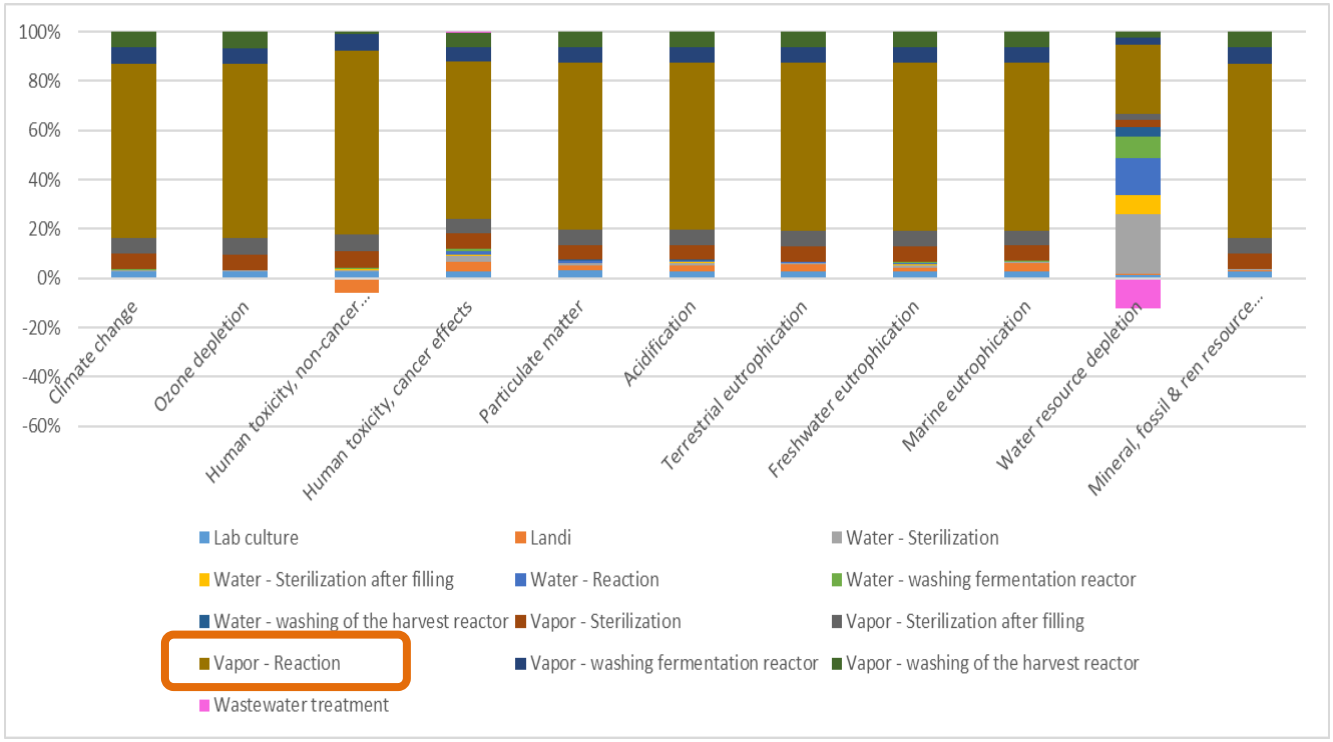
Reactor

↓

Purification

LCA Surfactin INDUSTRIAL SCALE

- Results - reactor



Lab scale culture

↓

Reactor

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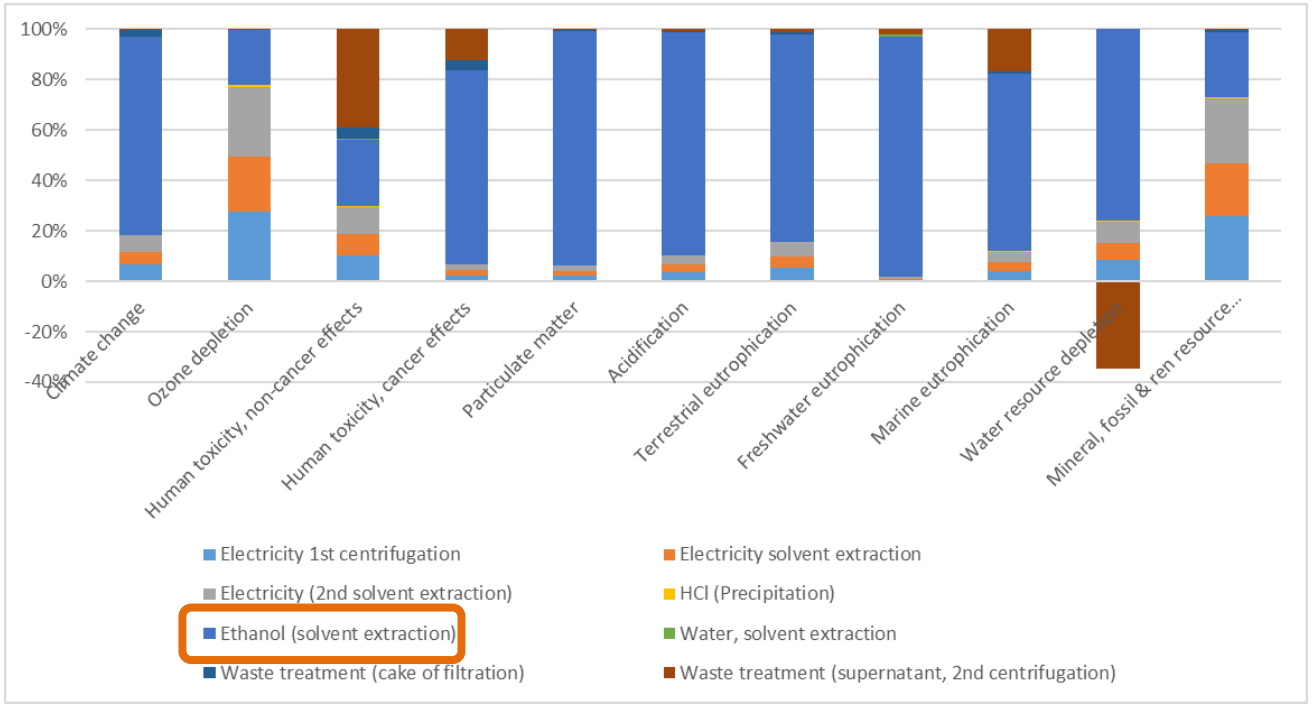
Purification

LCA Surfactin INDUSTRIAL SCALE

- Results - Purification



Lab scale culture

↓

Reactor

↓

Purification

Glass cleaner formulation

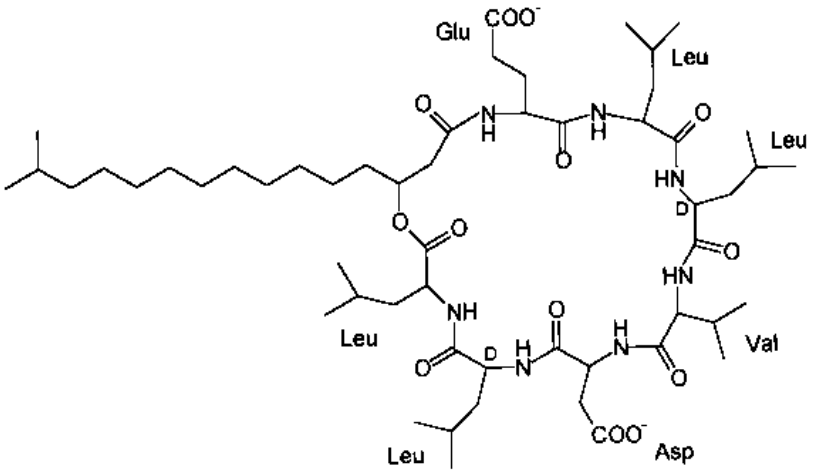
	% weight
Deionised water	94.98
1-methoxypropan-2-ol (DowanolPM)	4.00
1-butoxypropan-2-ol (DowanolPNB)	1.00
Sodium surfactin	0,02

Works well, with minimal streaking but wetting noticeably better with 0.1% sodium surfactin

Constraints

However, with surfactin, two constraints have been identified

- **the two negatives charges interact with Ca^{2+} and Mg^{2+} in the medium which triggers precipitation of the lipopeptides**
- **the ester bond which closes the ring can be easily opened in alkaline pH**



frontiers in Bioengineering and Biotechnology
SYSTEMATIC REVIEW
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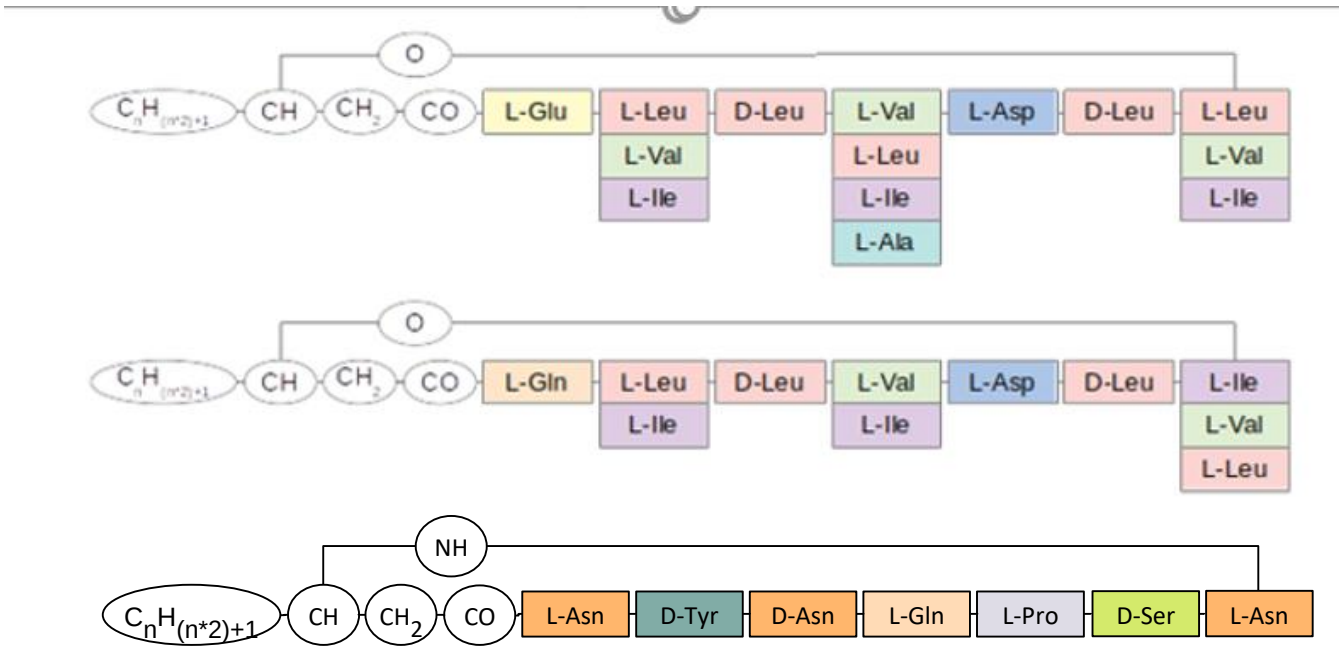
The Surfactin-Like Lipopeptides From *Bacillus* spp.: Natural Biodiversity and Synthetic Biology for a Broader Application Range

Ariane Théatre[1], Carolina Cano-Prieto[2], Marco Bartolini[3], Yoann Laurin[4,5], Magali Deleu[4], Joachim Niehren[6], Tarik Fida[2], Saïcha Gerbinet[7], Mohammad Alanjary[8], Marnix H. Medema[8], Angélique Léonard[7], Laurence Lins[4], Ana Arabolaza[3], Hugo Gramajo[3], Harald Gross[2] and Philippe Jacques[1]

Lipopeptide diversity



Surfactin

Lichenysin

Mycosubtilin

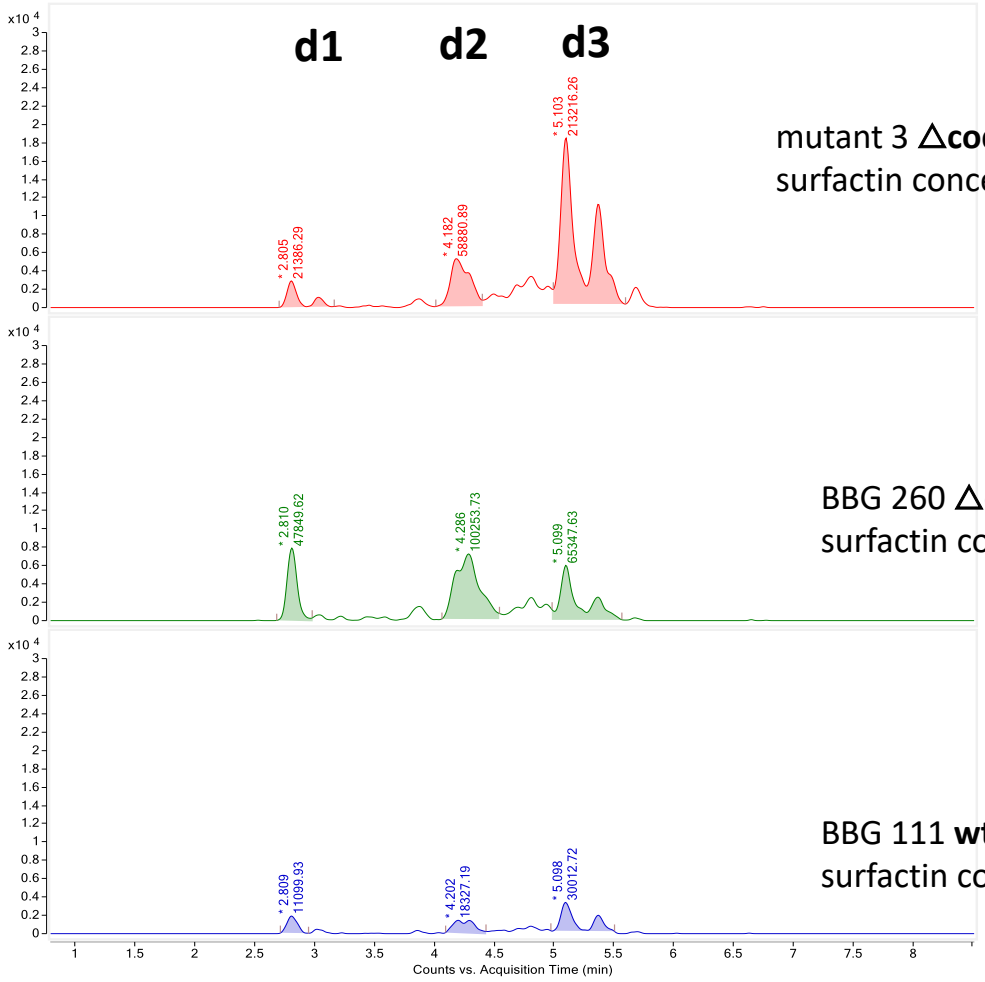
Lipopeptide diversity –

improve surfactin diversity by targeting fatty acid metabolism

D1: **C10**-E-L-L-V-D-L-L

D2: **C11**-E-L-L-V-D-L-V

D3: C12-**E-V-L-V-D-L-V**



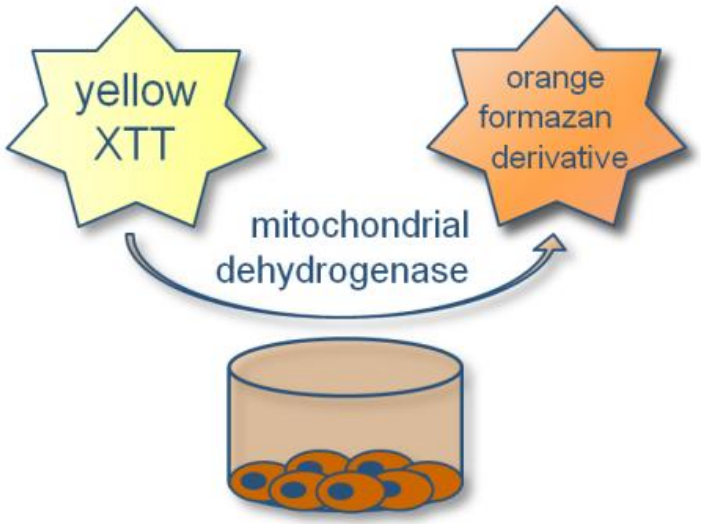
mutant 3 △**codY/ △fapR** (48h OD: 5 – no surfactin concentration)

BBG 260 △**codY** (48h OD 10 – no surfactin concentration)

BBG 111 **wt** (48h OD: 3,39 – no surfactin concentration)

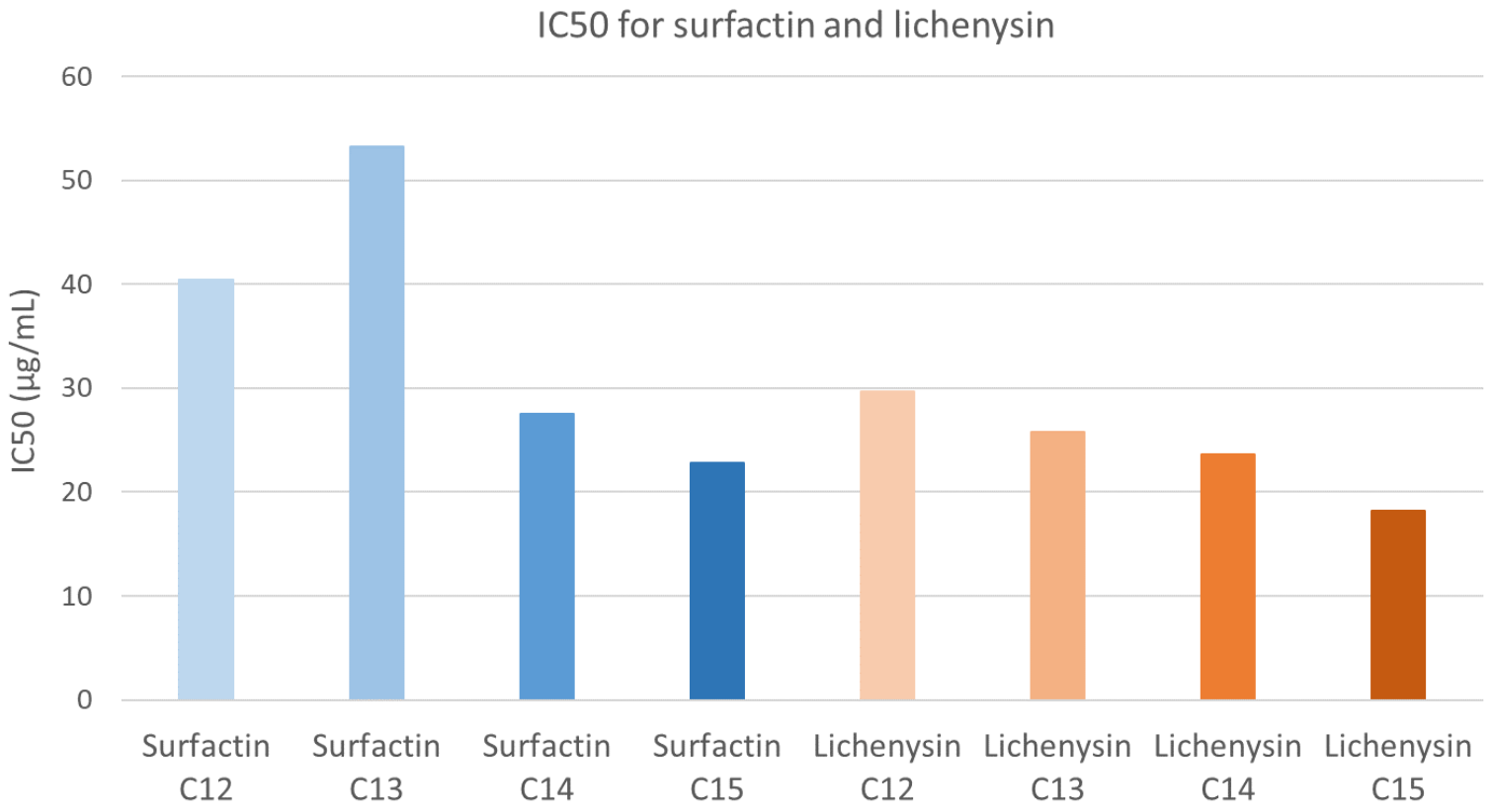
Cytotoxicity assay (XTT assay)

Determination of the number of living cells by measuring their mitochondrial activity.
Mitochondrial dehydrogenase from (living) cells cleave XTT (tetrazolium derivative) which produces soluble orange crystals of formazan that can be measured by spectrophotometry.
Serial dilutions of surfactin and lichenysin were added to Vero cells and the viability of these cells were compared to untreated cells (% of viability).



Cytotoxicity assay (XTT assay)

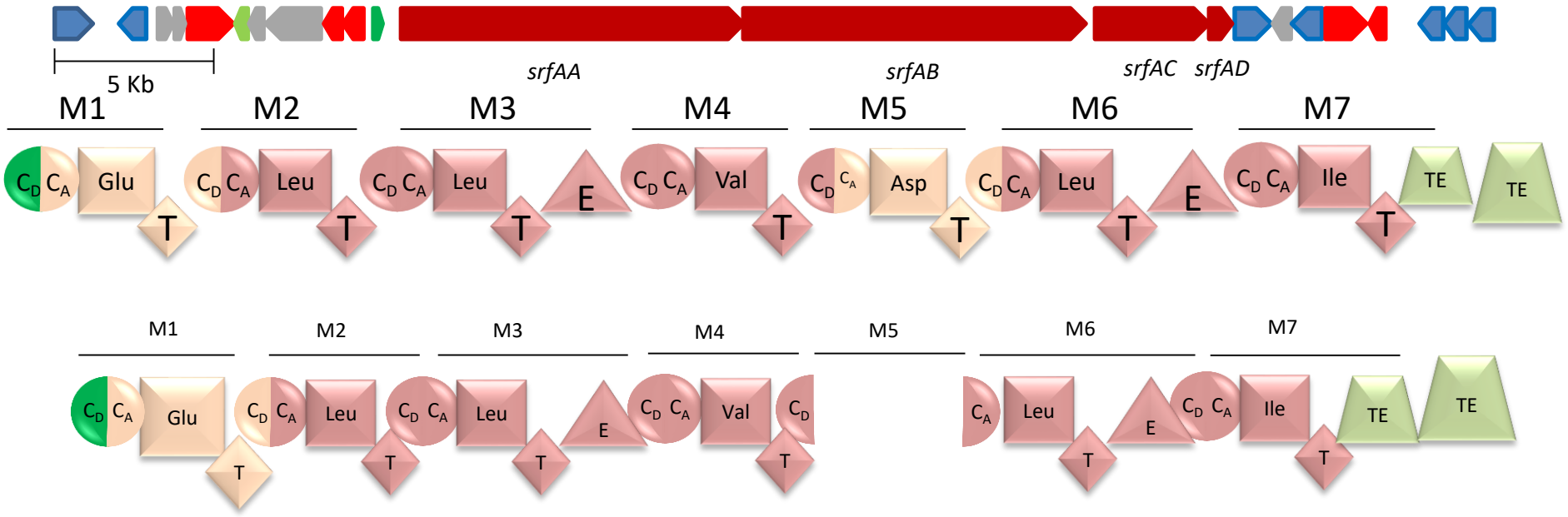
The half maximal inhibitory concentration (IC50) was calculated for surfactin and lichenysin



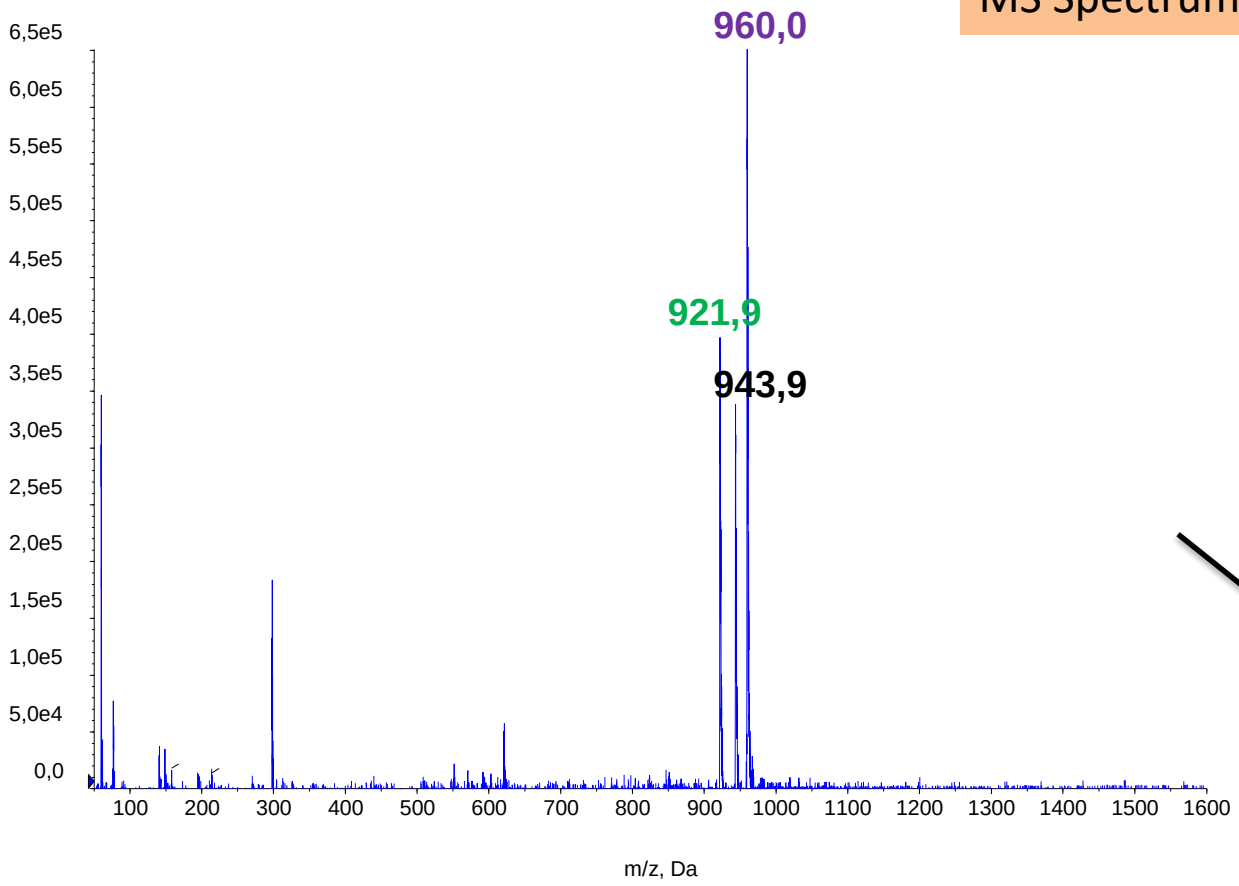
➔ Confirmation that surfactins seem less cytotoxic than lichenysins

Engineering of the enzyme machinery

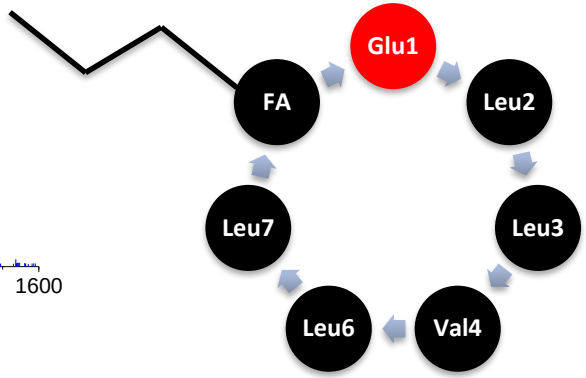
- **Module swapping** => Change in the peptide moiety
- **Module deletions** => Ring contractions
- **Addition of functional domains** (e.g. Knock-in of E-domains) => Stereochemistry



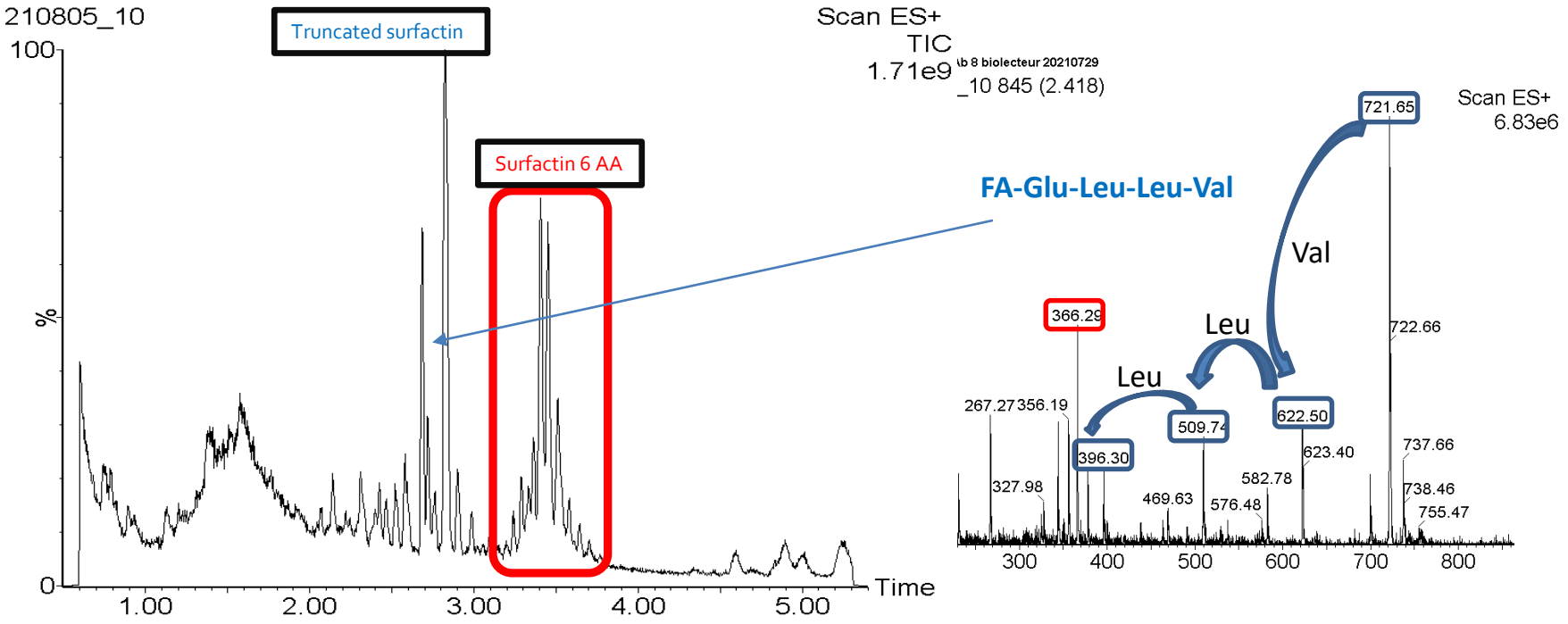
MS Spectrum of peak with r.t. 14.30 min



Surfactin C ΔAsp	
$M+H^+$	921
$M+Na^+$	943
$M+K^+$	960

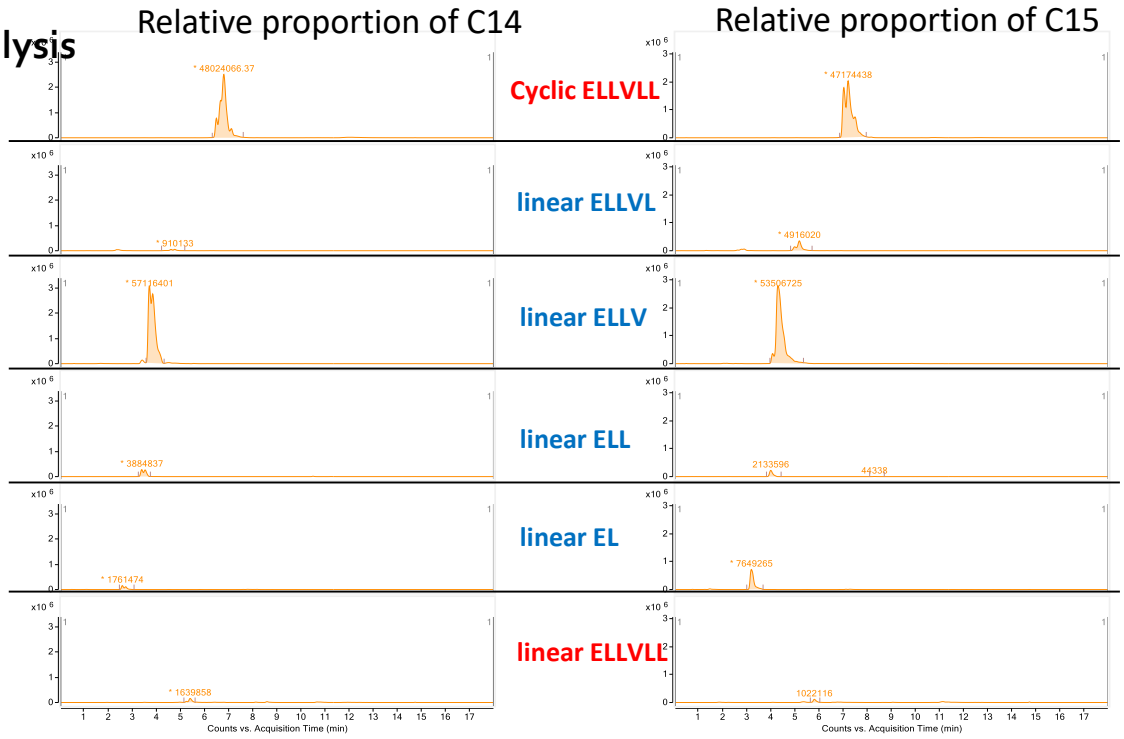


Synthetic biology

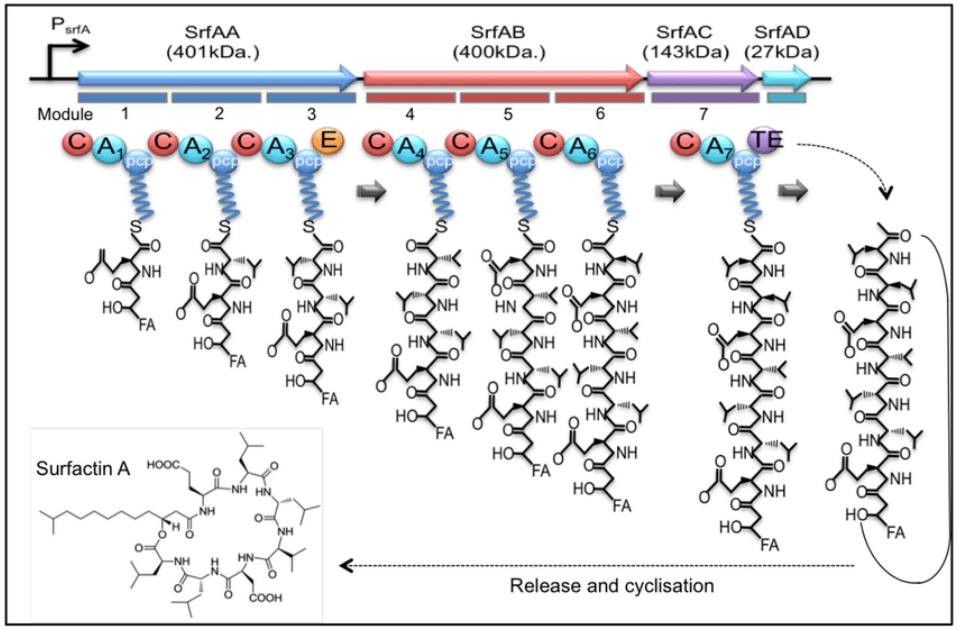


Synthetic biology

Q-tof analysis



Synthetic biology



Harwood et al., 2018

❖ Problem of synthesis ?

Take home message

- Dioctyl sodium sulfosuccinate is responsible for the ecotoxicity of Corexit
- Surfactin does not show ecotoxicity to algae
- Protiwanze is a cheap substrate well adapted to the production of surfactin
- Optimizing the surfactin precursor biosynthesis leads to a strain able to produce more than 5 g/l of surfactin
- The cell tainer is an interesting process to consider for biosurfactant production
- A new glass cleaner formulation was developed with surfactin
- Overproduction of surfactin leads to an increase biodiversity of compounds
- The remove of the fifth module of the surfactin synthetase deeply disturbs the efficiency of the synthetases

Acknowledgement

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- Dr. Saïcha Gerbinet
- Dr. Yoann Laurin

- Pr. Marnix Medema
- Pr. Tinka Murk
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- Pr. Hugo Gramajo
- Dr. Marco Bartolini
- Dr. Ana Arabolaza

- Pr. Harald Groß
- Dr. Carolina Cano-Prieto
- Tarik Fida

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- André Tonneaux

- Arnaud Delacroix
- Dr. Tambi Kar
- Dr. Louis Cossus

THANK YOU FOR YOUR ATTENTION

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