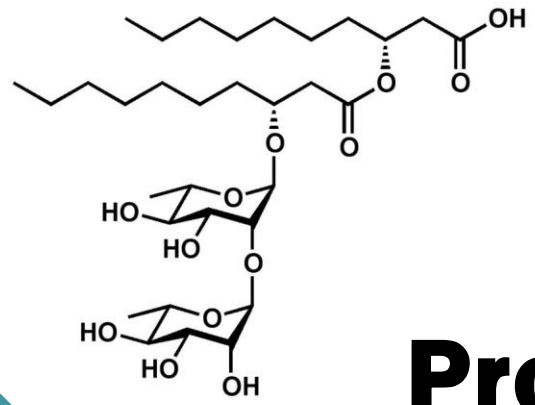


EBIC Environmental Biotechnology Innovation Centre

HERIOT WATT UNIVERSITY

Production of rhamnolipids in *Pseudomonas putida*

Tatiana Spatola Rossi

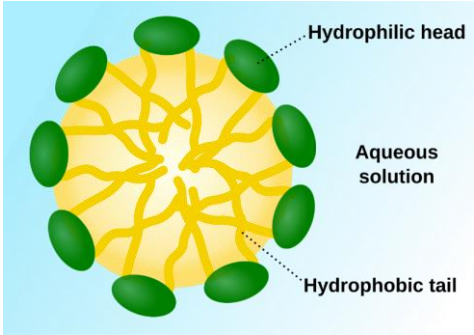
Gutierrez lab, Heriot-Watt University, Edinburgh, UK

FEMS summer school for postdocs

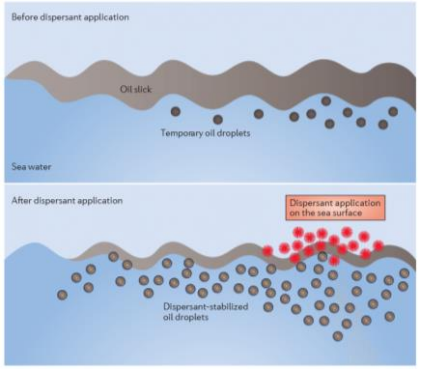
Ohrid, North Macedonia, September 2025

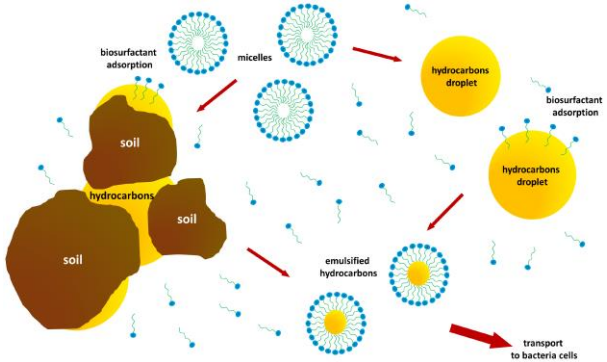
Federation of European Microbiological Societies

Applications of surfactants



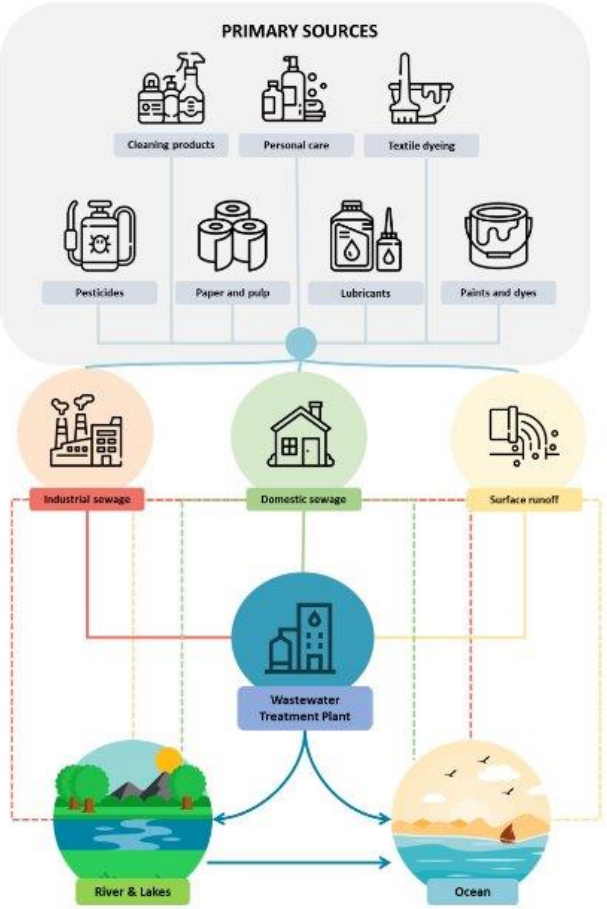
- Surfactants = "surface active agents", amphiphilic molecules
- Decrease surface tension or interfacial tension between two liquids, a liquid and a gas, or a liquid and a solid
- Used as emulsifiers, foaming agents, wetting agents, antistatic additives, dispersants
- Industries: detergents and cleaning products, paints, inks, petroleum and oil, plastics, resins, textiles, agricultural, food, cosmetics, pharmaceutical
- Marine oil spills (e.g. Deepwater Horizon oil spill, used up to 7 million litres of Corexit sprayed on sea surface and injected near the seafloor)
- Remediation of contaminated soils: enhancement of soil washing, bioremediation and phytoremediation of persistent organic pollutants and heavy metals





Environmental impacts of SCSs

- Synthetic chemical surfactants (SCSs) made by organo-chemical synthesis using fossil fuel-based precursors
- Global surfactant usage is estimated to be over 15 million tons annually
- Partly or slowly biodegradable
- Environmental damage (e.g. accumulation of phosphates in aquaticsystems, eutrophication, toxicity to marine life, may accumulate inthe food chain)
- Severe effects of linear dodecylbenzene sulfonates (LAS) and 4-nonylphenol (4-NP) on aquatic species

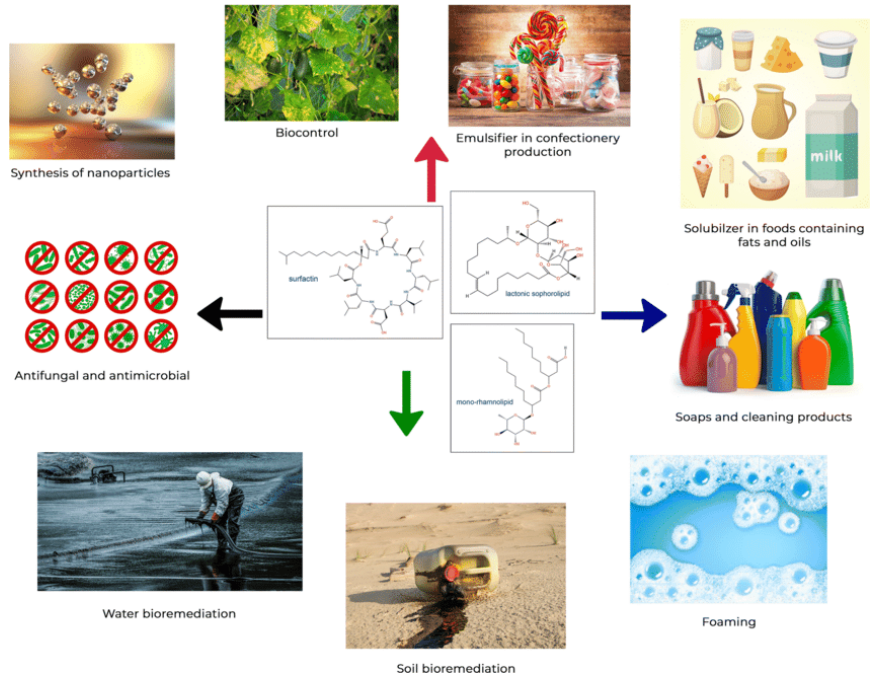


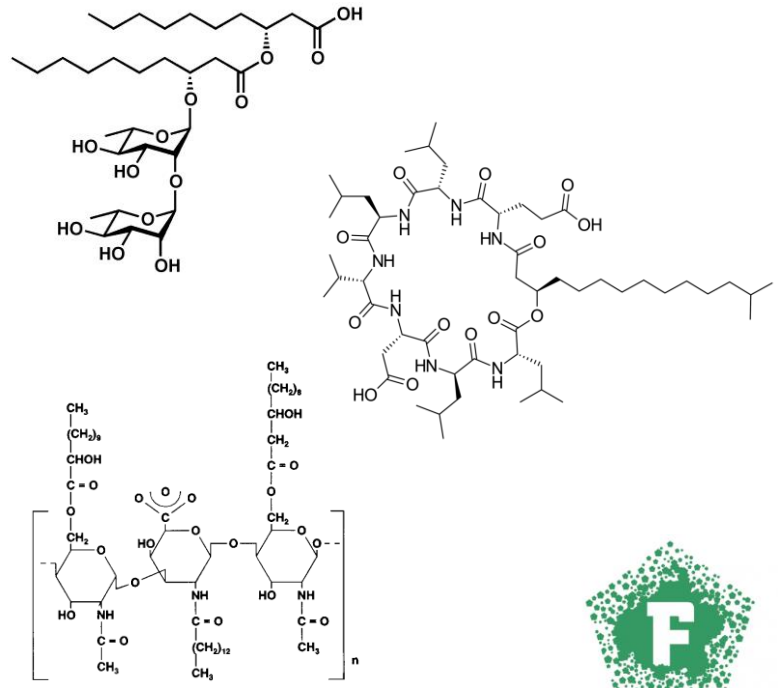
Fung et al., 2023

Biosurfactants

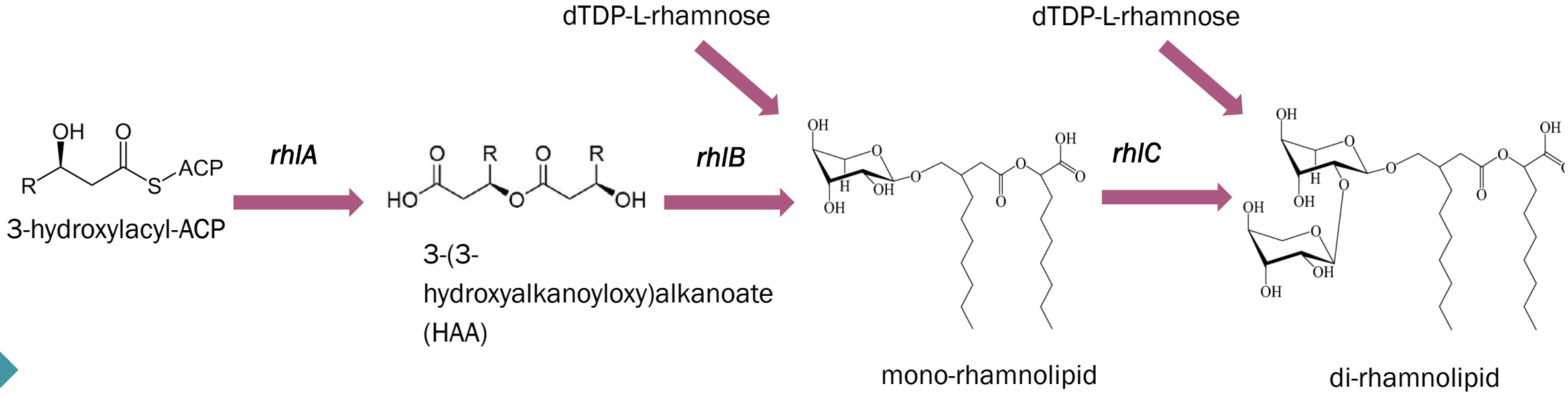
- Produced by some microorganisms (eg bacteria from the genus *Pseudomonas, Rhodococcus*, *Bacillus*; yeasts)
- Biobased, biodegradable, non-toxic
- Stable under extreme environmental conditions
- Classification:

LMW surfactants	Glycolipids	Rhamnolipids	*Pseudomonas aeruginosa*
	Lipopeptides	Surfactin	*Bacillus subtilis*
HMW emulsifiers/ polymeric surfactants		Emulsan	*Acinetobacter calcoaceticus*
		Liposan	*Candida lipolytica*



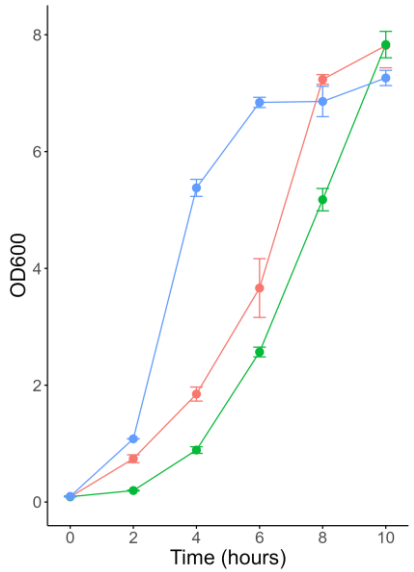


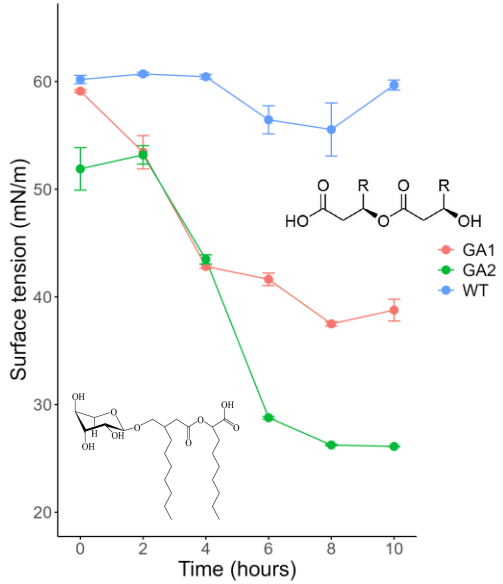
Biosynthesis of RL in *P. aeruginosa*



3 genes:
rhlAB = rhamnosyltransferase 1
rhlC = rhamnosyltransferase 2

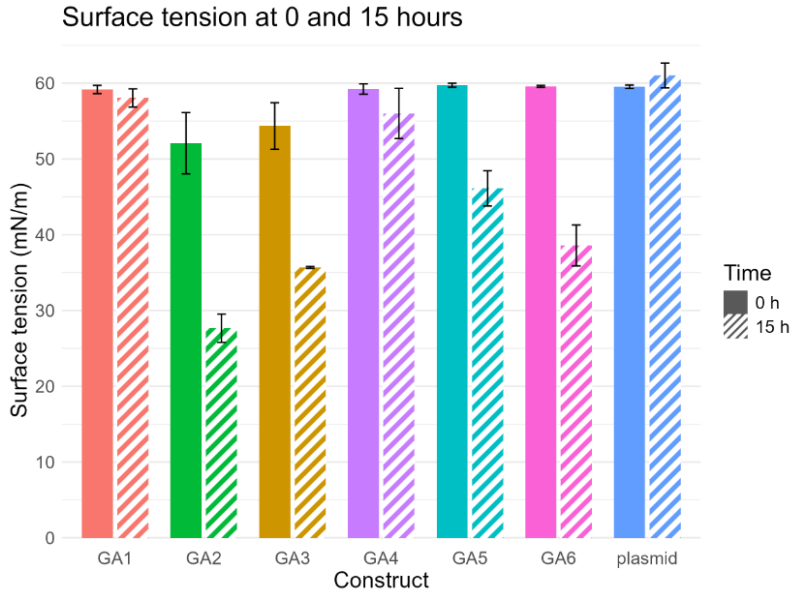
Recombinant expression in *P. putida*



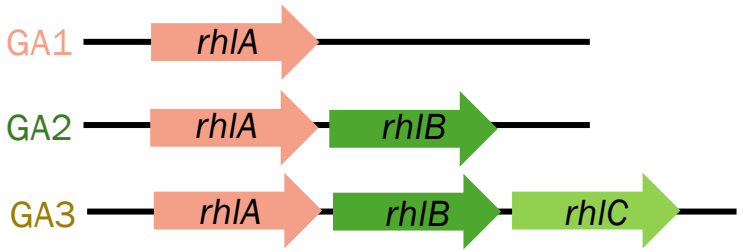


WT
GA1 (rhlA PAO1)
GA2 (rhlAB PAO1)

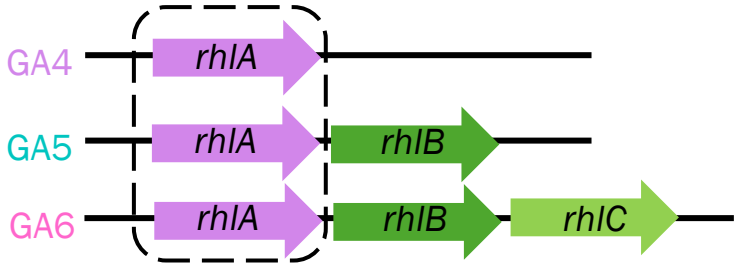
Final surface tension
rhlA: 38.8 ± 1.01
rhlAB: 26.1 ± 0.02



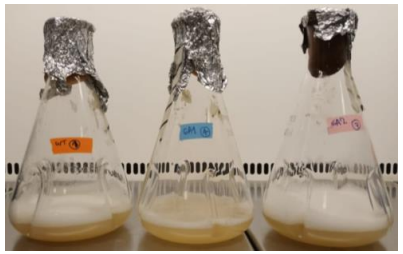
Genes from *P. aeruginosa*



rhlA from *Halomonas* sp.



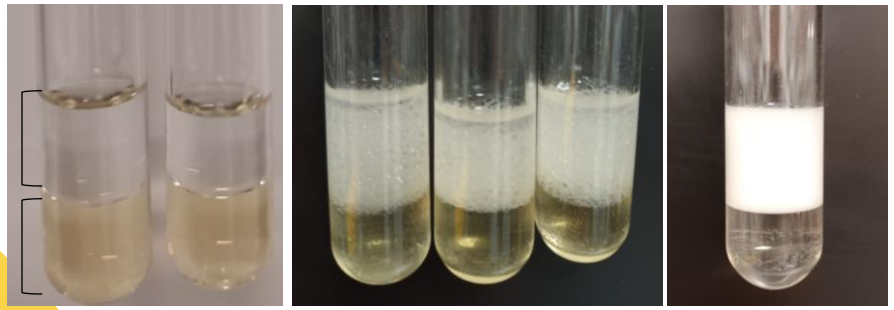
Emulsification assay



Cell-free supernatant

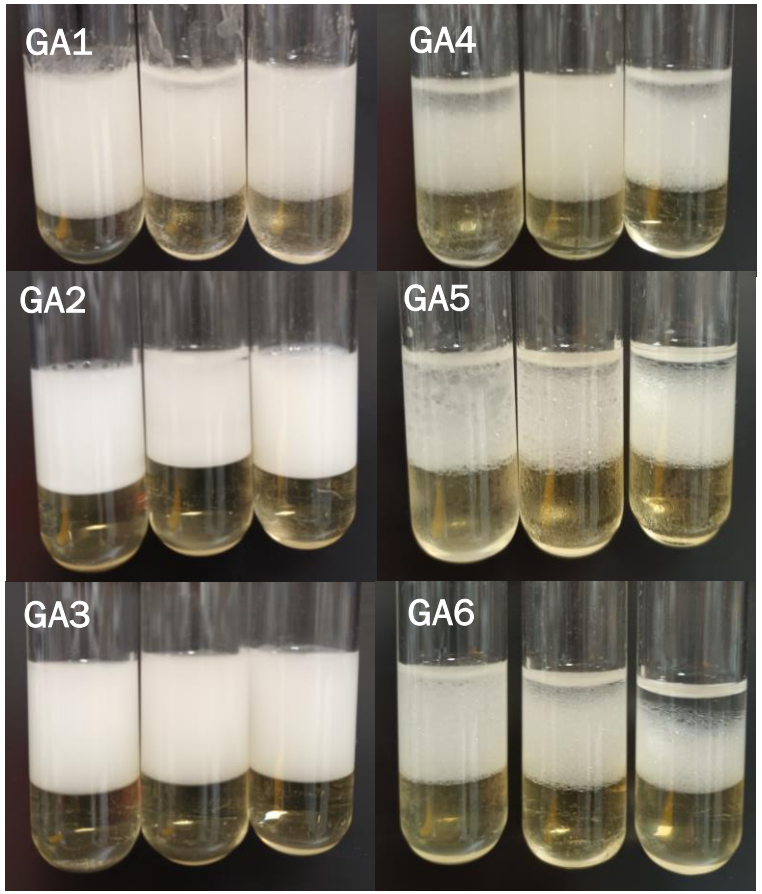
1:1 (v/v) with *n*-hexadecane, Vortex at high speed, Leave to stand

	Initial	Plasmid	Di-RL (10 mg/mL)
n-hexadecane			
supernatant			

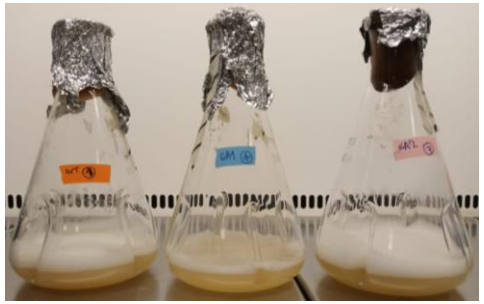


RhlA ≠ RhlA → mutagenesis, novel homologs

	P. aeruginosa	*Halomonas* sp.
HAA	GA1	GA4
Mono-RL	GA2	GA5
Di-RL	GA3	GA6

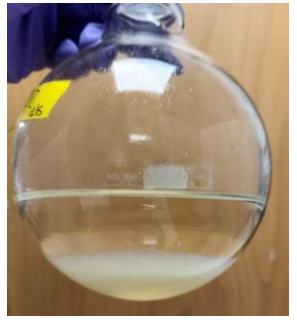


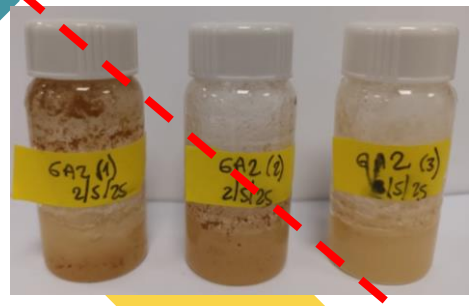
Rhamnolipid extraction

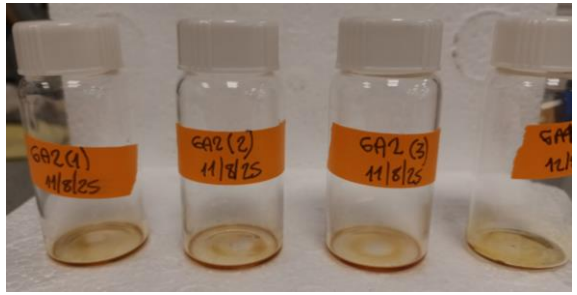


Acidified cell-free supernatant

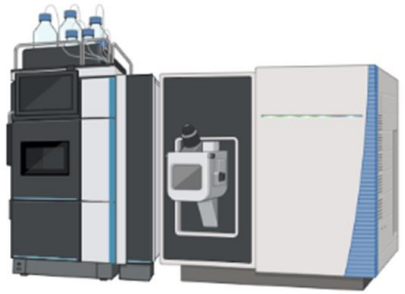
Liquid-liquid extraction 1:1 (v/v) with ethyl acetate



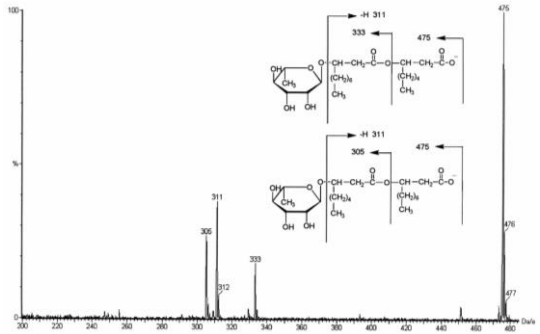




Yields (g/L): 0.72 0.70 0.74 0.36

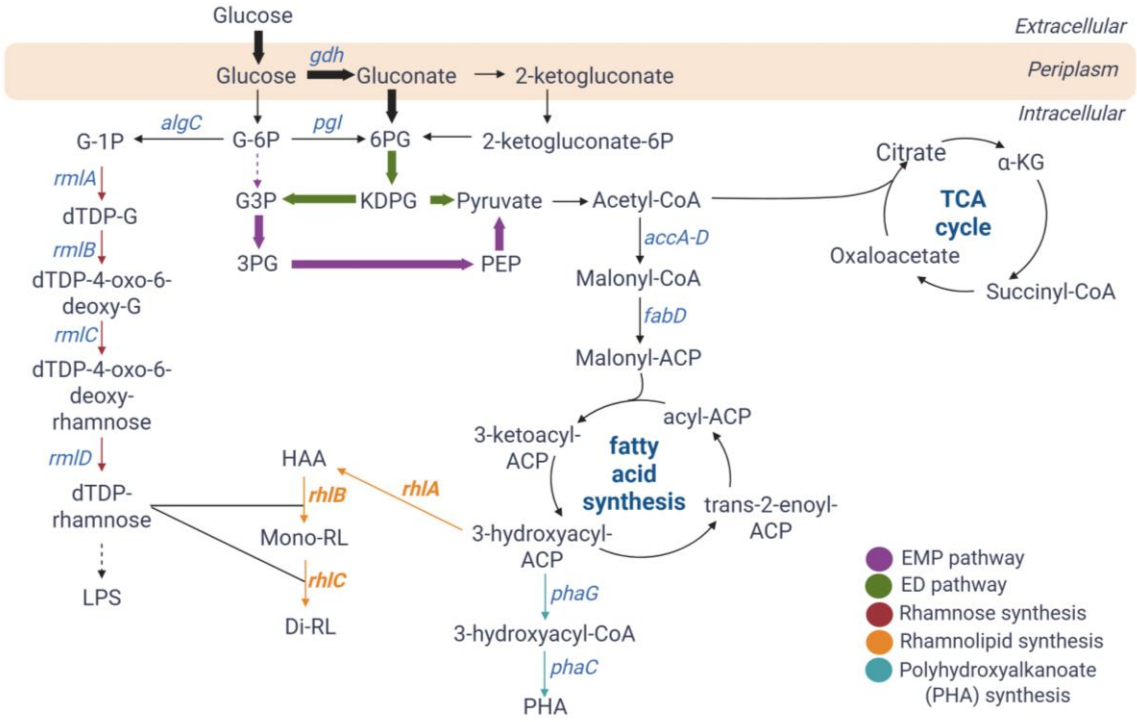


Liquid Chromatography-Mass Spectrometry



Ongoing and future work

- Investigation of novel *rhlA*, *rhlB*, and *rhlC* homologues from the marine environment
- Characterisation of biosurfactants produced by HPLC-MS, critical micelle concentration (CMC), yields and emulsifying properties
- Genomic insertion of the *rhlA*, *rhlB*, and *rhlC* genes
- Metabolic engineering to enhance rhamnolipid yields in recombinant strains
- Up-scaling to a 7L bioreactor
- Testing microbial biosurfactants produced for bioremediation applications



The city of Edinburgh

Edinburgh castle

Arthur's seat

Scott monument

Heriot-Watt University

Gutierrez lab
Prof. Tony Gutierrez

Acknowledgement:
Karen Fung

Thank you!

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