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CENTRE OF BIOLOGICAL ENGINEERING

Effect of NaCl on the aggregation behavior of rhamnolipids and implications in their biological activity

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1

INTRODUCTION

- Surfactants;
- Biosurfactants (Rhamnolipids);

2

RESULTS

- Antifungal activity;
- Dynamic Light Scattering;
- Microscopy (Confocal);

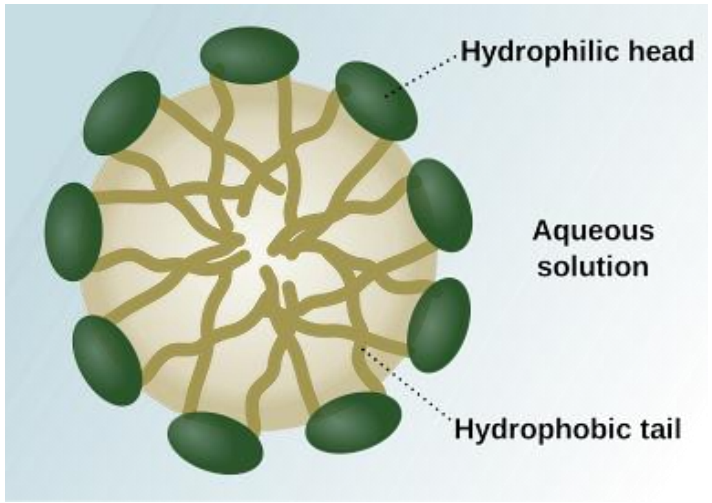
3

CONCLUSIONS

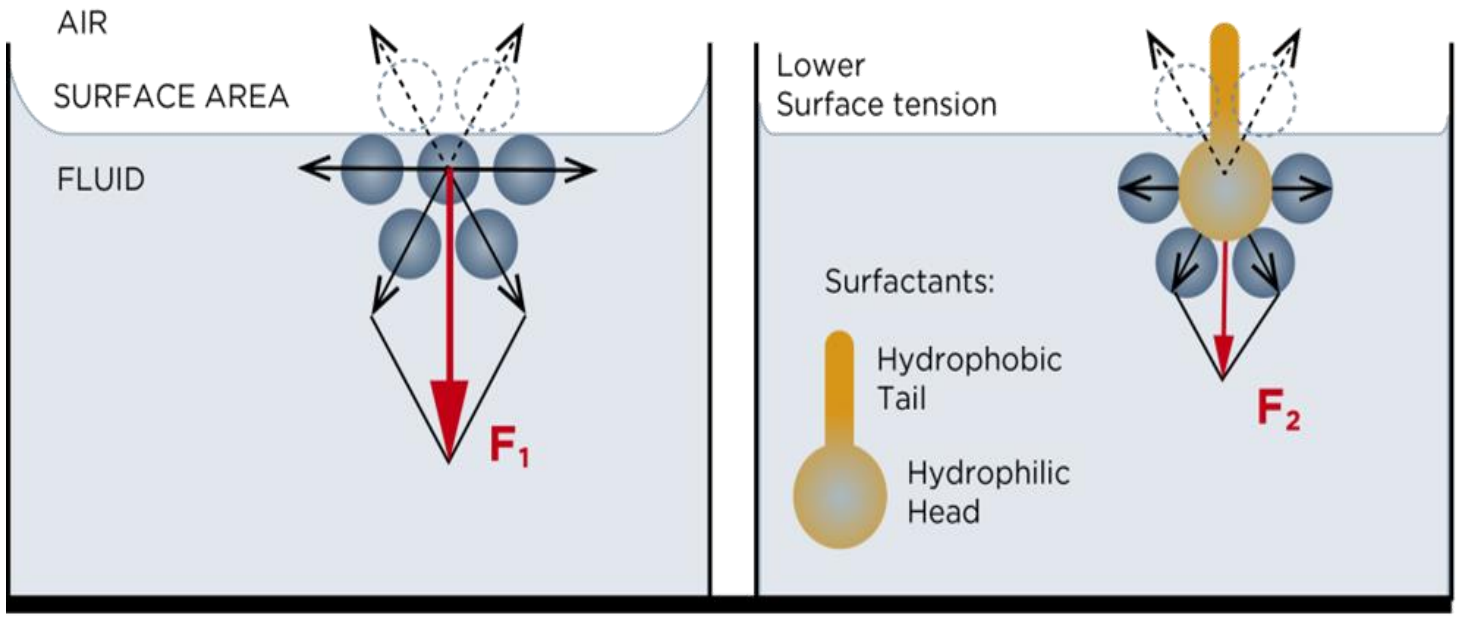
SURFACTANTS

✓ Surfactants are amphiphilic compounds

✓ Contain hydrophilic and hydrophobic groups



✓ Reduce the surface or interfacial tension between two phases with different polarities



BIOSURFACTANTS

- Synthesized by different microorganisms : bacteria, yeasts and filamentous fungi

- Properties: low toxicity
  high biodegradability
  high selectivity
  specific activity at extreme temperatures, pH and salinities

- Can be synthesized from renewable feed-stocks and agro-industrial wastes

BIOSURFACTANTS

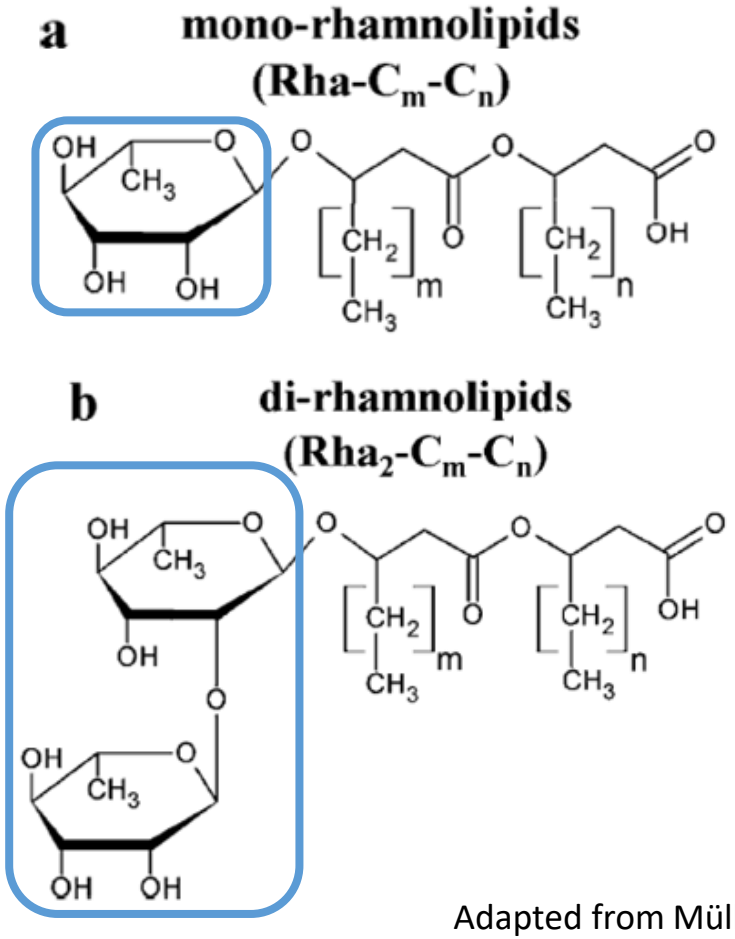
Table 1. Structural classification of biosurfactants. Adapted from Müller *et al.*, 2012

Biosurfactant size	Structural class	Examples
Low-molecular- weight	Glycolipids	Mannosylerythritol-lipids Sophorolipids Rhamnolipids Trehaloselipids
	Lipopeptides/ lipoamino acids	Surfactin Lysin lipids Ornithine lipids
High-molecular- weight	Polymers	Proteins Lipoproteins Polysaccharides Lipopolysaccharides
	Oil/membranes	Glycerolipids Phospholipids Fatty acids

RHAMNOLIPIDS

- **Rhamnolipids**: one (**a**) or two (**b**) rhamnose molecules linked to one or two fatty acid tails of variable length

- They are mainly produced by the Gram-negative bacterium *Pseudomonas aeruginosa*



Adapted from Müller *et al.*, 2012

Production and recovery of rhamnolipids synthesized by *P. aeruginosa* #112

RHAMNOLIPIDS PRODUCTION

Conditions

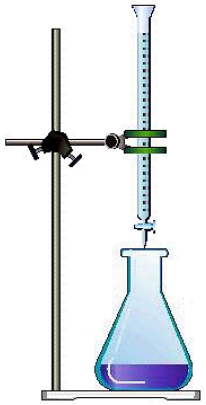
Culture medium : Corn Steep Liquor + Sugarcane molasses (CSLM)

Temperature: 37 ºC

Agitation: 180 rpm

Fermentation Time : 144 h

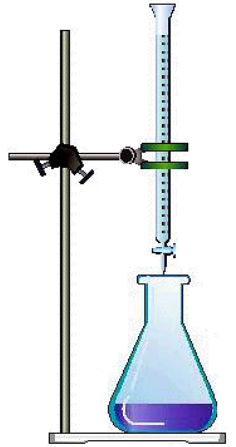
RHAMNOLIPIDS RECOVERY



Adsorption chromatography:

- polystyrene resin Amberlite XAD-2
  - Elution (methanol)

RHAMNOLIPIDS CONGENERS PURIFICATION



Column chromatography:

- silica gel 60
  - Elution (Chloroform: Methanol mixtures with increasing polarity)

Yield = 3194 ± 245 mg/L (cmc = 50 mg/L)

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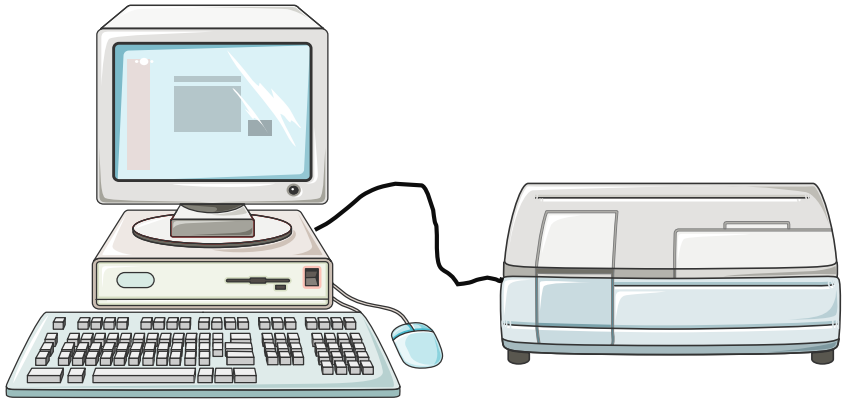
Production and recovery of rhamnolipids synthesized by *P. aeruginosa* #112

✓ Characterization

Table 2. Rhamnolipid congeners produced by *P. aeruginosa* #112 in the culture medium CSLM identified by mass spectrometry.

Rhamnolipid congeners	m/z $[M+Na]^+$	Relative abundance
Mono-Rhamnolipids		
Rha-(C_{10}-C_8)	499.3	11.8 %
Rha-(C_{10}-C_{10})	527.3	100 % *
Rha-(C_{10}-$C_{12:1}$)	553.3	10.4 %
Rha-(C_{10}-C_{12})	555.4	13.8 %
Di-Rhamnolipids		
Rha-Rha-(C_8-C_{10})	645.3	3.8 %
Rha-Rha-(C_{10}-C_{10})	673.3	57.8 %
Rha-Rha-(C_{10}-$C_{12:1}$)	699.3	8.5 %
Rha-Rha-(C_{10}-C_{12})	701.4	14.7 %

* Most abundant ion.



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Study of the biological activity of rhamnolipids against several fungi

Table 3. Growth inhibition percentages obtained with the cell-free supernatant and the crude rhamnolipid (RL) mixture produced by *P. aeruginosa* #112. The assays were performed at 25ºC for 5 days.

		Growth Inhibition (%)		
Strain	**[Rhamnolipids] (g/L)**	**Cell-free supernatant**		**Crude RL**
***Aspergillus niger* MUM 92.13**	3.0	**100.0 ± 0.0**	≠	**20.3 ± 14.7**
	1.5	31.0 ± 2.4		13.0 ± 3.7
	0.75	5.6 ± 8.5		8.1 ± 3.7
	0.375	3.5 ± 3.2		17.1 ± 6.5
***Aspergillus carbonarius* MUM 05.18**	3.0	**100.0 ± 0.0**	≠	**22.6 ± 1.2**
	1.5	**100.0 ± 0.0**		**29.5 ± 8.3**
	0.75	20.4 ± 9.1		26.7 ± 10.3
	0.375	21.7 ± 4.6		24.0 ± 2.1

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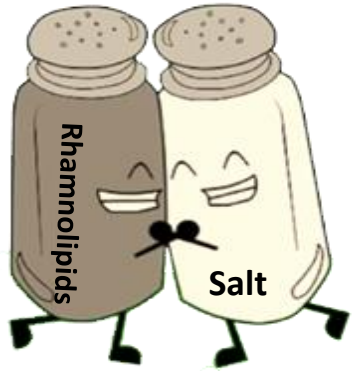
Table 4. Growth inhibition percentages obtained with the cell-free supernatant and the crude rhamnolipid (RL) mixture produced by *P. aeruginosa* #112 at the optimized NaCl concentration. The assays were performed at 25ºC for 5 days.

			Growth Inhibition (%)		
Strain	**[Rhamnolipids] (g/L)**	**NaCl**	**Crude RL + NaCl**		**Cell-free supernatant**
***A. niger* MUM 92.13**	3.0	**0.875 M**	**100.0 ± 0.0**	=	**100.0 ± 0.0**
	1.5		51.8 ± 1.2		31.0 ± 2.4
	0.75		53.2 ± 0.0		5.6 ± 8.5
	0.375		43.3 ± 1.2		3.5 ± 3.2
***A. carbonarius* MUM 05.18**	3.0	**0.375 M**	**100.0 ± 0.0**	=	**100.0 ± 0.0**
	1.5		58.5 ± 3.8		100.0 ± 0.0
	0.75		47.2 ± 3.8		20.4 ± 9.1
	0.375		50.9 ± 6.5		21.7 ± 4.6



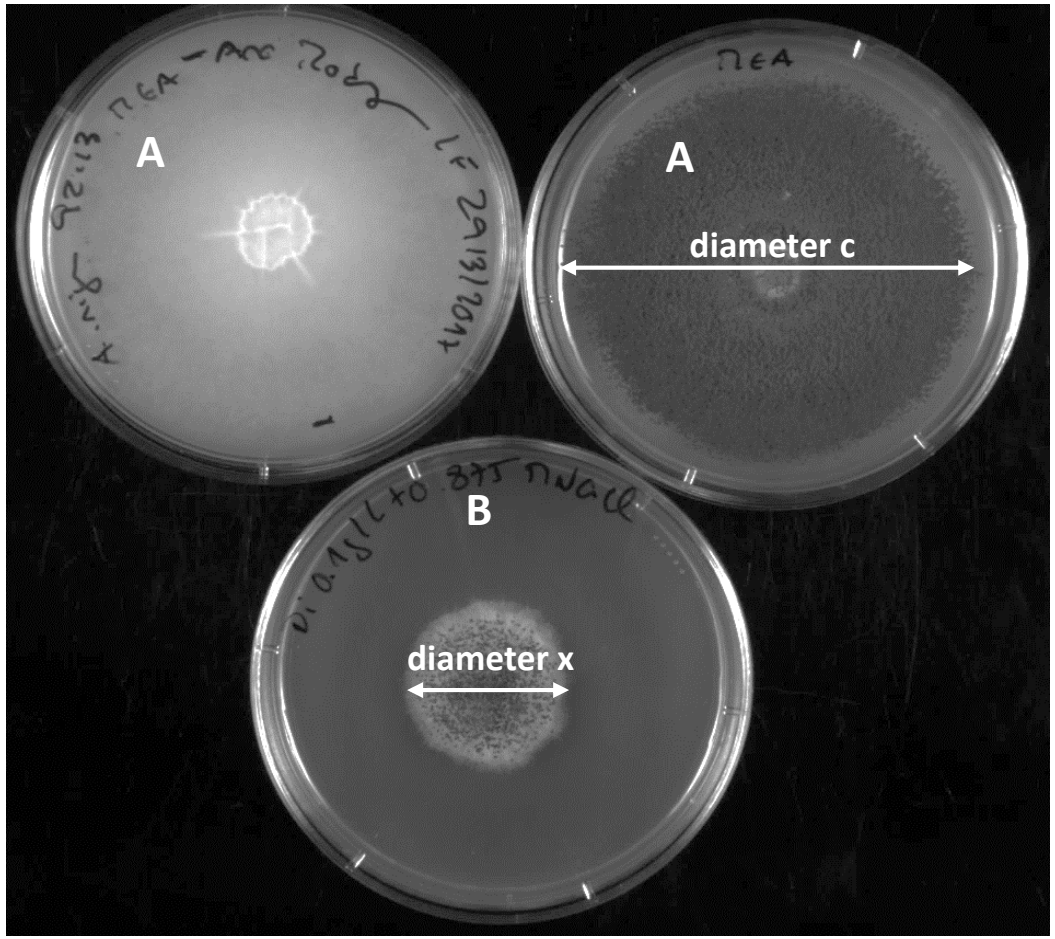
Study of the biological activity of rhamnolipids against several fungi

Table 5. Growth inhibition percentages obtained with the purified mono-rhamnolipid and di-rhamnolipid congeners.

A. niger MUM 92.13				
NaCl	**Di-Rhamnolipid (g/L)**	**Inhibition (%)**	**Mono-Rhamnolipid (g/L)**	**Inhibition (%)**
0.875 M	0.75	100.0 ± 0.0	1.5	41.8 ± 1.4
	0.375	100.0 ± 0.0	0.75	21.2 ± 2.4
	0.2	**100.0 ± 0.0**	-	-
	0.1	**61.9 ± 1.2**	-	-
	0.05	52.4 ± 1.2	-	-

*The assays were performed at 25ºC for 5 days

$$\textbf{\textit{Growth inhibition x}}\ (\%) = \left(1 - \frac{diameter\ x}{diameter\ c}\right) \times 100$$



A: Control

B: 0.1 g $_{\text{Di-Rhamnolipid}}$/L +0.875M NaCl

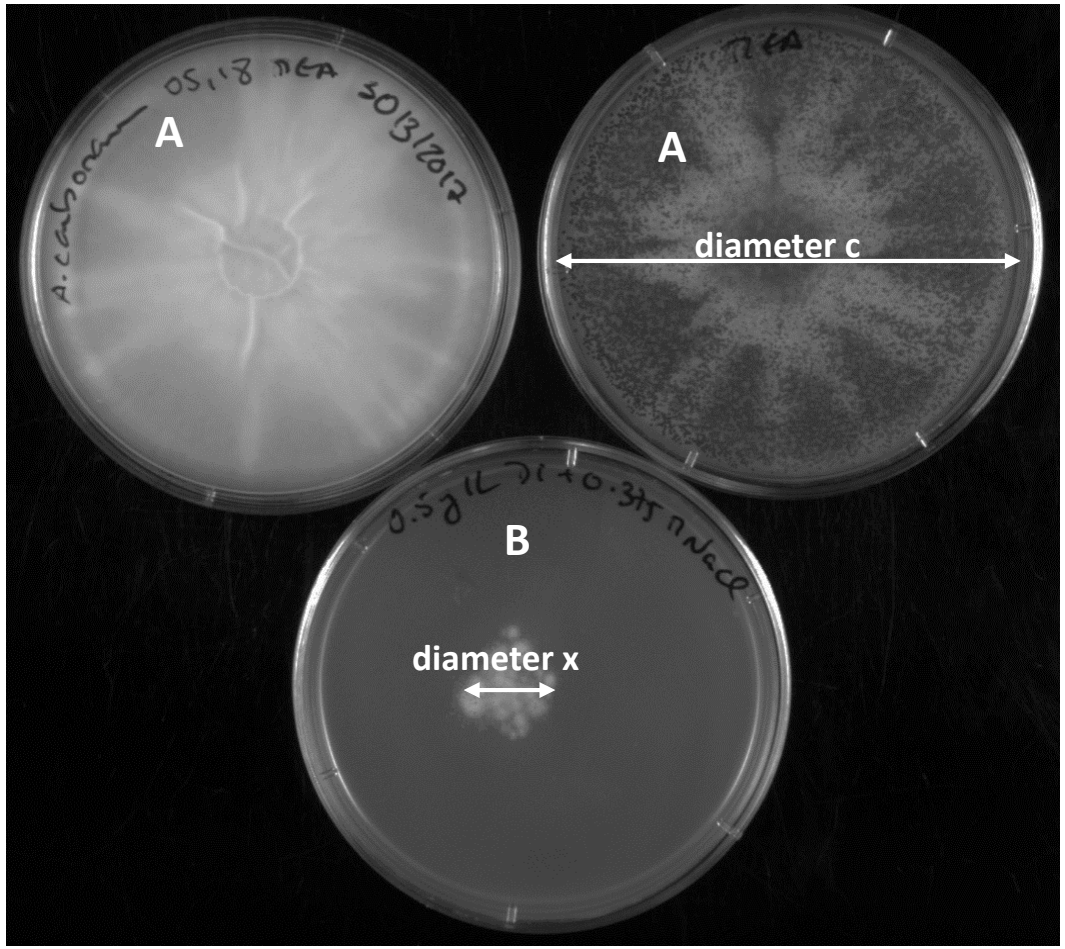
Study of the biological activity of rhamnolipids against several fungi

Table 6. Growth inhibition percentages obtained with the purified mono-rhamnolipid and di-rhamnolipid congeners.

	A. carbonarius MUM 05.18			
NaCl	**Di-Rhamnolipid (g/L)**	**Inhibition (%)**	**Mono-Rhamnolipid (g/L)**	**Inhibition (%)**
	0.75	**100.0 ± 0.0**	1.5	26.4 ± 2.7
	0.6	**80.7 ± 1.2**	0.75	25.2 ± 4.4
0.375 M	0.5	73.8 ± 1.2	-	-
	0.375	72.6 ± 1.3	-	-
	0.05	52.4 ± 1.2	-	-

*The assays were performed at 25ºC for 5 days.

$$\textbf{\textit{Growth inhibition x}}\ (\%) = \left(1 - \frac{diameter\ x}{diameter\ c}\right) \times 100$$



A: Control

B: 0.5 g $_{\text{Di-Rhamnolipid}}$/L +0.375M NaCl

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Dynamic light scattering (DLS)

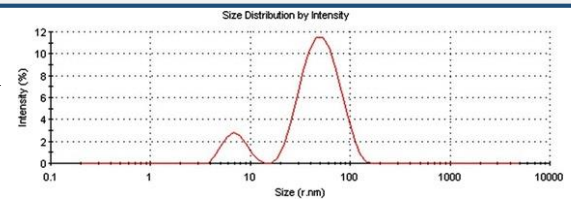

Table 7. Effect of NaCl on the micellar size distribution of the crude rhamnolipid mixture and the mono-rhamnolipid and di-rhamnolipid congeners determined by DLS analysis.

Rhamnolipid	[Rhamnolipids] (g/L)	NaCl (M)	Size (nm)	PDI
Crude (Mixture)	1.5	0.0	302.8 ± 7.4	0.549 ±0.009
		0.375	456.6 ± 42.2	0.596 ± 0.106
		0.875	2343 ± 154.1	0.753 ± 0.190
Mono-Rhamnolipid	1.0	0.0	140.3 ± 2.0	0.263 ± 0.006
		0.375	2212 ± 444.1	0.890 ± 0.107
		0.875	4674 ± 359.8	1.000 ± 0.000
Di-Rhamnolipid	0.5	0.0	133.1 ± 4.9	0.373 ± 0.042
		0.375	> 10 000	-
		0.875	> 10 000	-

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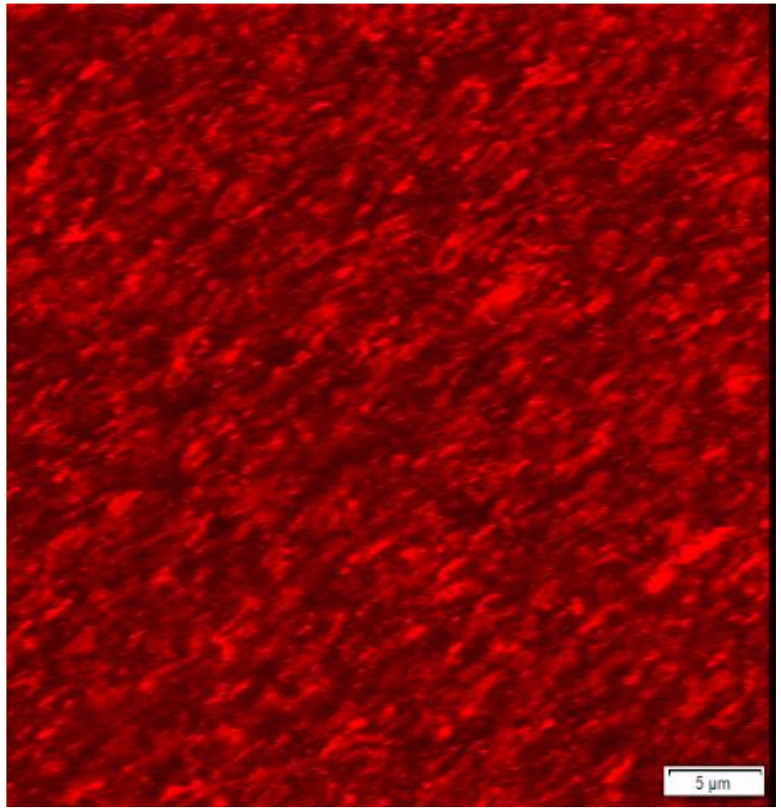
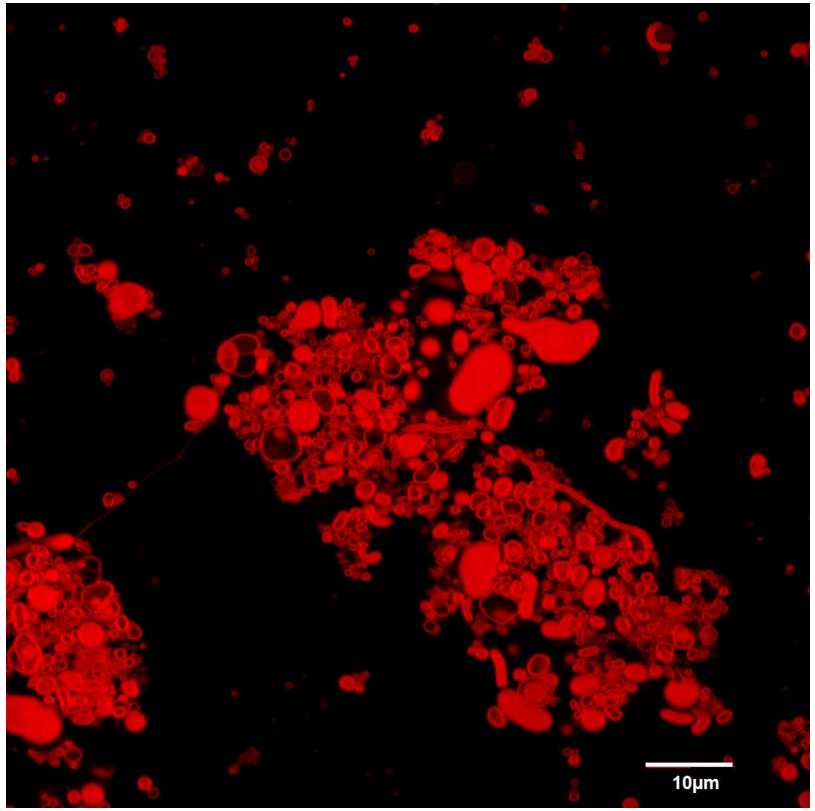
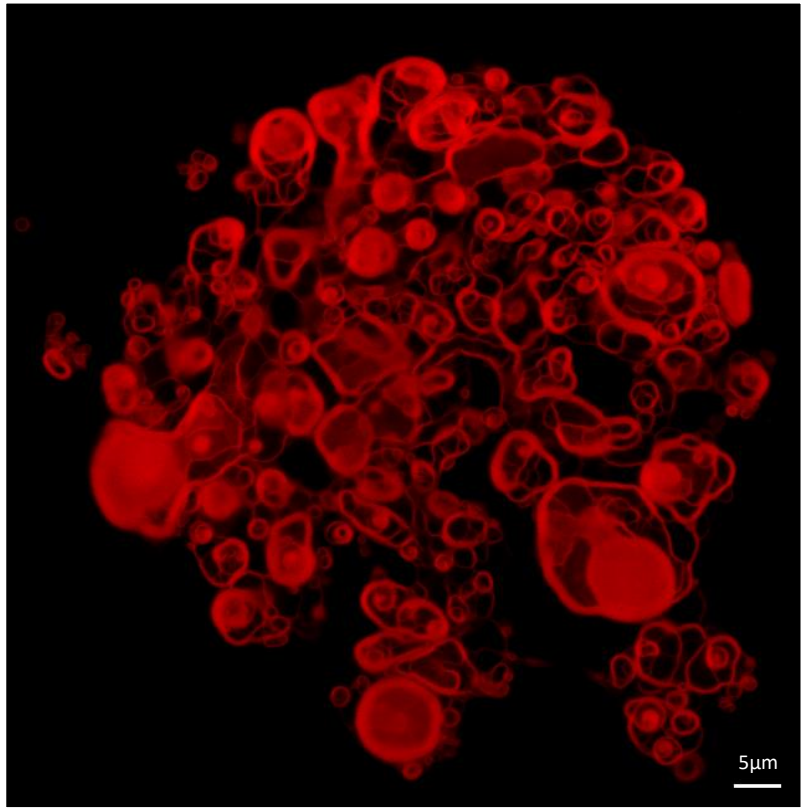


Crude Rhamnolipid (Mixture)





Crude Rhamnolipid (Mixture) + 0.875 M NaCl

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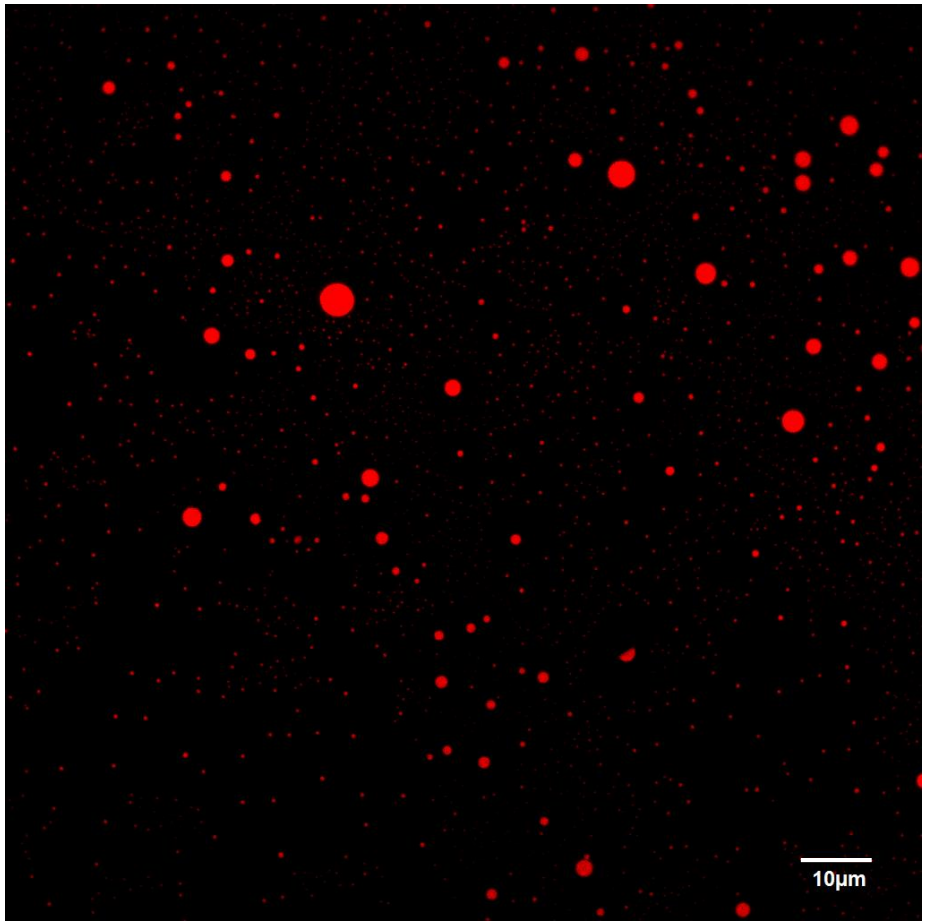
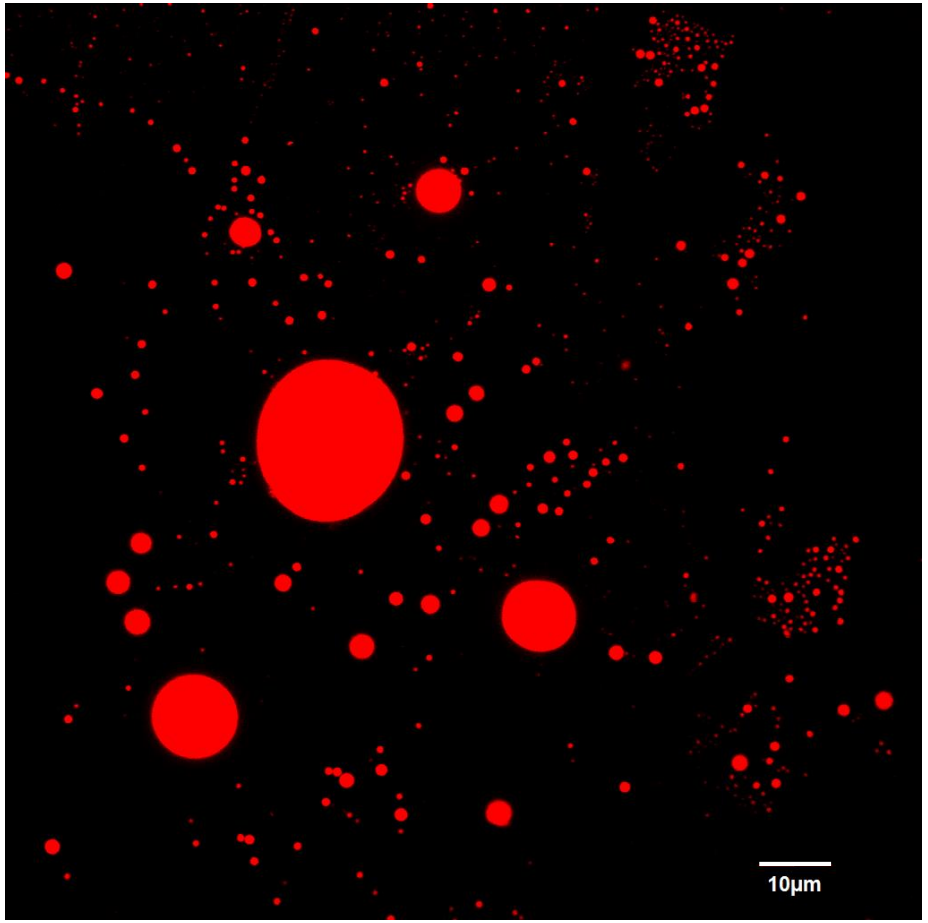


Mono-Rhamnolipid + 0.875 M NaCl



Di-Rhamnolipid + 0.875 M NaCl

CONCLUSIONS

- ✓ The rhamnolipids produced in the culture medium CSLM exhibit antifungal activity against *A. niger* MUM 92.13 and *A. carbonarius* MUM 05.18
- ✓ The antifungal activity is lost during the process of rhamnolipids recovery
- ✓ The addition of NaCl alters the aggregation behavior of the crude rhamnolipids mixture restoring their antifungal activity
- ✓ The di-rhamnolipid congeners are responsible for the antifungal activity
- ✓ The rhamnolipids produced by *P. aeruginosa* #112 are a promising alternative to the chemical fungicides

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