The marine environment: a promising source of microorganisms for the production of biosurfactants.

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Marine organisms have developed strategies to cope with specialised environmental conditions. One common strategy is the production of biosurfactant compounds to access specific nutrients, promote biofilm development and as act defence against pathogenic microorganisms. The MARISURF consortium phenotypically screened over 600 marine bacterial strains for biosurfactant production, resulting in the identification of 7 strains of interest. **Here we present the characterisation of biosurfactants produced by one of these marine strains: *Marinobacter* sp. MCTG107b. We also present an investigation of the antibiofilm properties of biosurfactants produced by a second strain, *Halomonas* sp. TGOS10, against known human pathogens.**

Two marine bacterial strains, TGOS-10 and MCTG107b were investigated for biosurfactant production. BLASTn analysis of partial 16S rRNA gene sequences amplified from these strains showed >99% similarity to the genus *Halomonas* and *Marinobacter*, respectively. Strains TGOS-10 and MCTG107b significantly reduced the surface tension of the culture supernatant to 29.0 mNm^{-1} and 31.0 mNm^{-1} respectively, Fig 1.

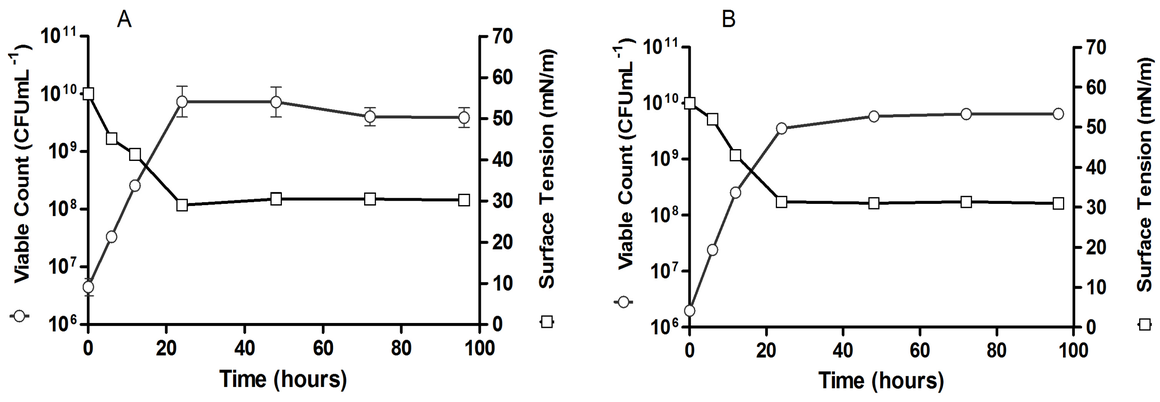

Fig. 1 CFU and surface tension reduction kinetics of *Halomonas* TGOS-10 (**A**) and *Marinobacter* sp. MCTG107b (**B**). During growth in Zobell marine media using 1% (v/v) rapeseed oil as a carbon source in a 5 L bioreactor. Surface tension (□) was seen to reduce to a stable value within the first 24 h of growth and corresponded with the strain reaching the stationary growth phase, as measured by viable cell counts (○).

Characterisation of the biosurfactants produced by *Marinobacter* sp. MCTG107b using both HPLC-MS and tandem MS showed a mixture of 14 different rhamnolipid congeners, Fig 2 & Table 1.

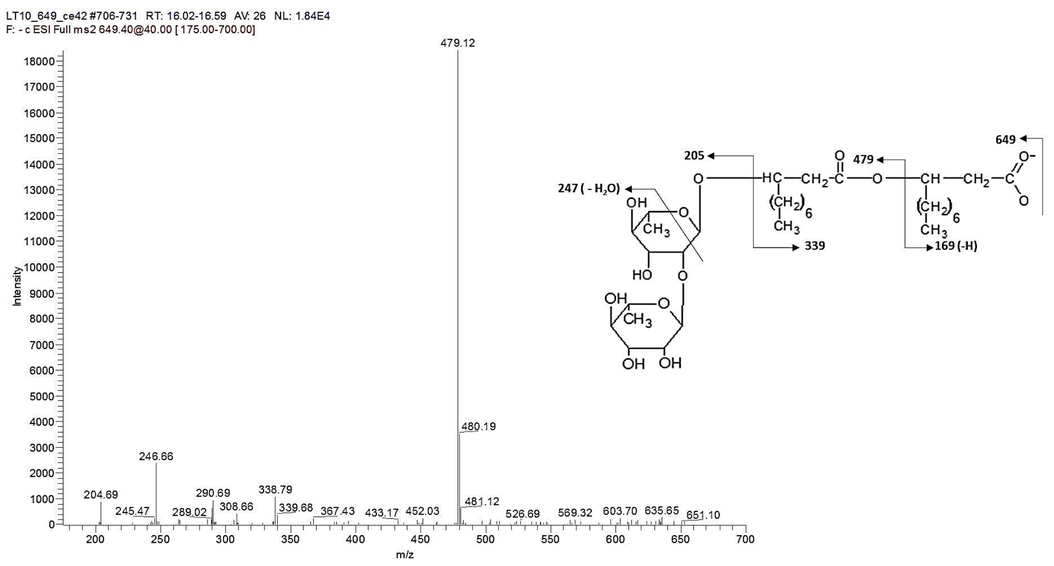

Fig. 2 HPLC-MS-MS profile of daughter products resulting from the fragmentation of a molecular ion, m/z of 651.73, observed in a HPLC-MS analysis to be the predominant compound from *Marinobacter* sp. MCTG107b. The observed products corresponded to the predicted mol. weights of the fragmentation of di-rhamnolipid Rha-Rha-C10-C10.

RT min	*m/z* value	Compound	Mw (Da)	Molecular Form	Relative %
Mono-rhamnolipid congeners					
14.8	387.22	Rha-$C_{14:2}$	386.48	$C_{20}H_{34}O_7$	3.18
21.5	533.46	Rha-C_{10}-C_{12} / Rha-C_{12}-C_{10}	532.71	$C_{28}H_{52}O_9$	0.22
24.2	503.47	Rha-C_{10}-$C_{10:1}$	502.64	$C_{26}H_{46}O_9$	0.27
26.9	561.52	Rha-C_{12}-C_{12}/ Rha-C_{10}-C_{14}	560.76	$C_{30}H_{56}O_9$	0.94
Subtotal					4.61
Di-rhamnolipid congeners					
4.6	453.27	Rha-Rha-C_8	452.49	$C_{20}H_{36}O_{11}$	1.95
12.7	480.39	Rha-Rha-C_{10}	480.55	$C_{22}H_{40}O_{11}$	5.13
22.1	537.45	Rha-Rha-C_{14}	536.65	$C_{26}H_{48}O_{11}$	0.21
31.0	649.71	Rha-Rha-C_{10}-$C_{10:1}$ / Rha-Rha-$C_{10:1}$-C_{10}	648.74	$C_{32}H_{56}O_{13}$	2.85
32.1	651.73	Rha-Rha-C_{10}-C_{10}	650.79	$C_{34}H_{58}O_{13}$	52.45
32.8	677.77	Rha-Rha-C_{10}-$C_{12:1}$	676.83	$C_{33}H_{60}O_{13}$	1.06
33.0	665.77	Rha-Rha-C_{10}-C_{10}-CH_3	664.82	$C_{42}H_{60}O_{13}$	23.07
34.5	803.54	Decenoyl-Rha-Rha-C_{10}-$C_{10:1}$	801.01	$C_{35}H_{72}O_{11}$	0.40
35.1	679.78	Rha-Rha-C_{10}-C_{12} / Rha-Rha-C_{12}-C_{10}	678.84	$C_{35}H_{64}O_{13}$	5.01
37.2	693.90	Rha-Rha-C_{10}-C_{12}-CH_3 / Rha-Rha-C_{12}-C_{10}-CH_3	692.80	$C_{35}H_{64}O_{13}$	3.26
Subtotal					95.39

Table. 1 List of rhamnolipid congeners synthesised by *Marinobacter* sp. MCTG107b. Rhamnolipid congeners were identified via HPLC-MS in SPE purified extracts from cell-free culture supernatant samples obtained after 96 h growth in a 5 L bioreactor.

The antibiofilm properties of biosurfactants derived from *Halomonas* sp. TGOS-10 against pathogenic microorganisms were investigated. The growth of *P. aeruginosa* PAO1 was inhibited by 45% and growth of *S. aureus* ATCC 9144 was inhibited by 100% during co-incubation for 24h with biosurfactant at a concentration of 1.0 $mgmL^{-1}$, Fig 3.

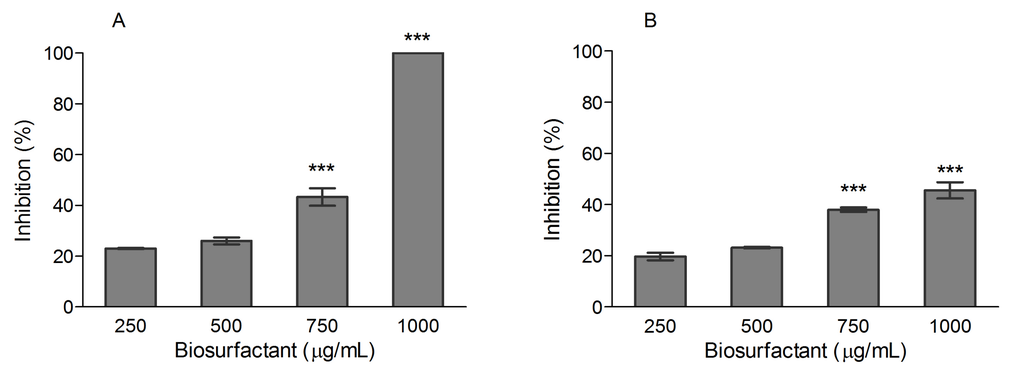

Fig. 3 Percentage inhibition of biofilms formed of *S. aureus* ATCC 9144 (**A**) and *P. aeruginosa* PAO1 (**B**) during co-incubation with biosurfactant-TGOS-10. *** represents a significant level of inhibition compared to biofilms cultures in the absence of biosurfactant (n = 3)

Biosurfactant-TGOS-10 effectively inhibited biofilms of *S. aureus*. Scanning electron microscopy revealed potential damage to the the EPS matrix of the biofilm, Fig 4. Due to the increasing problem of inhibiting biofilm associated pathogenic bacteria with conventional antibiotics, marine biosurfactants can be a promising alternative or additive for treating these infections.

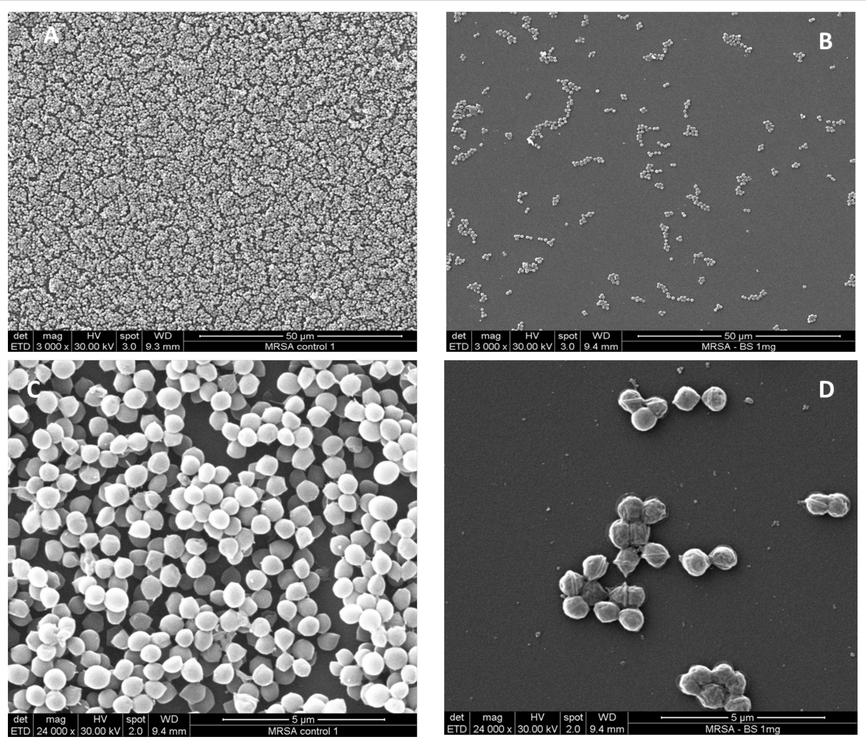

Fig. 4 Images of *S. aureus* biofilm grown in the absence and presence of 1.0 $mgmL^{-1}$ biosurfactant-TGOS-10 observed at 3000x (**A,B**) and 24000x (**C,D**).

Ongoing work and Conclusions

- Marine-derived biosurfactants are promising compounds for product development due to their, surface-activity, non-toxicity and anti-microbial properties combined with sustainable production.
- The biosurfactant produced by *Marinobacter* sp. MCTG107b was phenotypically and structurally characterized as rhamnolipids with varying congeners.
- The biosurfactant produced by *Halomonas* sp. TGOS-10 showed antibiofilm activity against pathogenic microorganisms.
- The chemical characterisation of TGOS-10 derived biosurfactant (HPLC-MS/NMR) is ongoing.
- *Marinobacter* sp. MCTG 107b was shown to be avirulent in a *Galleria mellonella* model, *Halomonas* sp. TGOS-10 is yet to be tested, however reports of *Halomonas* infectious disease in humans are rare.

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