A study of the inhibition of biofilm formation on multiple surfaces by a biosurfactant containing four discrete lipopeptides.

Shadi Khonsari, Alejandra Gonzalez Baez, Ha Nguyen Leonardo Pantoja Munoz, Diane Purchase

Department of Natural Sciences, Faculty of Science and Technology, Middlesex University, London, United Kingdom

Background

Figure 1 - Showing the Biofilm formation cycle on surfaces; attachment (1), growth (2) and dispersal (3) [1]

Biosurfactants are nontoxic, surface-active compounds synthesized by a wide variety of microorganisms. They are molecules that have both hydrophobic and hydrophilic domains and are capable of lowering the surface tension and the interfacial tension of the growth medium [2]. Biosurfactants have been found to be involved in various processes. For instrance: in bioremediation, as antimicrobial, antifungal and biocontrol agents, and as emulsifiers. Another major property is their anti-adhesive and anti-biofilm formation capability; lipopeptides have been reported as an important group of biosurfactants able to successfully disrupt bacterial biofilms [3].

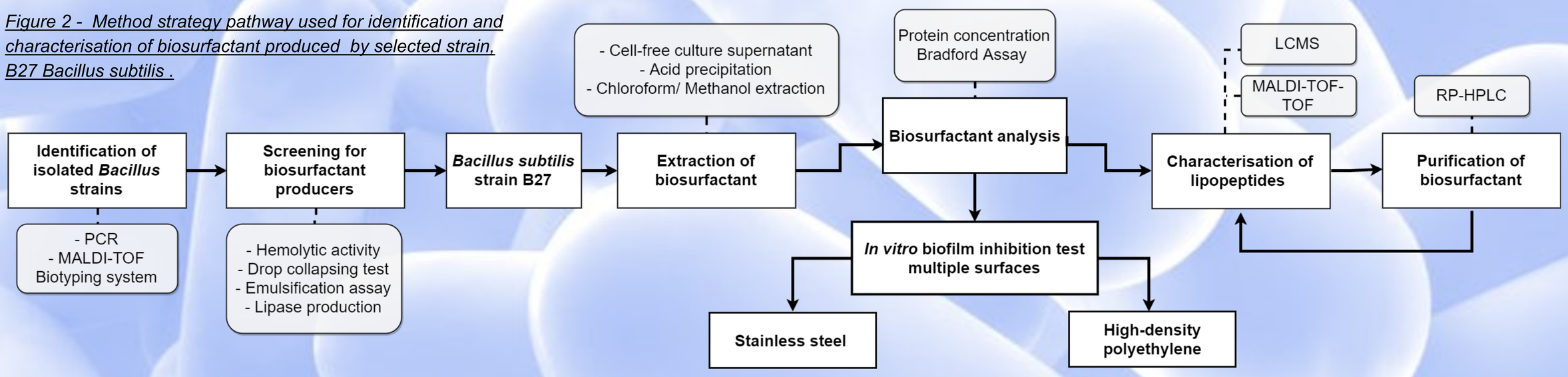
In this study a number of different *Bacillus* strains were identified using PCR and Matrix MALDI-MS Biotyping and their ability to produce lipopeptides examined. Inhibition of biofilm formation was assayed on stainless steel and high-density polyethylene surfaces against a range of gram positive and gram negative bacteria.

Methods

The *Bacillus* strains were isolated from primary effluent (Deepham Sewage Treatment Facilities, Edmonton, UK) and poultry/animal waste (A.K. Wood Poultry Farm, Fold Farm Partners and Leamon Pig Farm Ltd, UK) [4].

Figure 2 shows the pathway leading to identification and characterisation of the biosurfactant produced by the selected strain B27. Biofilm inhibition tests were also performed.

Figure 2 - Method strategy pathway used for identification and characterisation of biosurfactant produced by selected strain, B27 Bacillus subtilis .



Results and Discussion

The crude biosurfactant demonstrated anti-biofilm properties against different bacteria including MRSA on both stainless steel and plastic surfaces. In most cases, the crude biosurfactant performed as efficiently as a commercially-available purified biosurfactant (Surfactin) and was more effective against *Enterococcus faecalis*, *Pseudomonas fluorescens* and MRSA (Table 1).

The extract produced by strain B27 was analysed using MALDI-TOF-TOF and was found to contain not only one but ***four*** lipopetides: surfactin [m/z 1016–1074], fengycin [m/z 1447–1491], subtilomycin [m/z 3230] and subtilosin-A [m/z 3400] (Figure 3).

Figure 3 - Characterisation of lipopeptides produced by Bacillus subtilis.

Table 1a. 1b - Crude biosurfactant inhibition on Stainless steel and HD-Polytehylene surfaces; 100% biofilm inhibition, < 100% biofilm inhibition, No biofilm inhibition, NA There was no biofilm formation. Surfactin was used as positive control.

1a.

BACTERIA \ SURFACE	STAINLESS STEEL Control	1:10 Biosurfactant	1:100 Biosurfactant
E. faecalis			
P. fluorescens			
MRSA			
S. mutans			
E. coli			
K. pneumonia			
S. typhimurium			
S. aureus			
S. pyogenes			
E. aerogenes			
P. aeruginosa			
P. mirabilis			
M. luteus			
L. monocytogenes			

1b.

BACTERIA \ SURFACE	HD-POLYETHYLENE Control	1:10 Biosurfactant	1:100 Biosurfactant
P. fluorescens			
S. mutans			
P. mirabilis			
MRSA			
L. monocytogenes			
M. luteus			
S. pyogenes			
E. aerogenes			
E. faecalis			
P. aeruginosa			
S. aureus			
S. typhimurium			
E. coli	*NA*	*NA*	*NA*
K. pneumoniae	*NA*	*NA*	*NA*

Conclusion

Bacillus subtilis is a promising strain with ability of producing a number of lipopeptides able to inhibit microbial biofilms.

The biosurfactant extracted from strain B27 was highly effective against *E. faecalis*, *P. fluoroscens*, MRSA and *S. mutans*.

Biofilm inhibition was more successful on stainless steel surface compared to HD-Polyethylene.

Subtilomycin production is very uncommon and has been hardly reported.

Future Work

- Further investigation of biosurfactant effects on bacterial biofilm as a bactericidal or inhibitor agent .
- Purification and analysis of lipopeptides in order to identify their specific role in biofilm disruption.
- Investigation of possible synergy effects of the different lipopeptides.

References

1. Stoodley P, Dirckx P. (2003) Biofilm formation in 3 steps. [Online] Available at: https://www.biofilm.montana.edu/resources/images/ multicellularextracellular/biofilm-formation-3-steps.html [Accessed 15 June 2016]
2. Cameotra SS et al. (2010) Synthesis of biosurfactants and their advantages to microorganisms and mankind. AdvExp Med Biol.672:261-80.
3. Banat et al. (2014). Microbial biofilms: biosurfactants as anti-biofilm agents. Appl Microbiol Biotechnol. 98(24):9915–9929.
4. Okoroma EA et al. (2012). Identification and characterisation of a *Bacillus licheniformis* strain with profound keratinase activity for degradation of melanised feather. *International Biodeterioration & Biodegradation* 74, 54-60. DOI: 10.1016/j.