Internal and external stressors to the MELiSSA loop

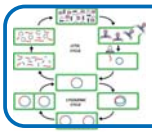
Tom Sassen[1,2], Jennifer Mahony[2], Rob Van Houdt[1], Felice Mastroleo[1], Douwe van Sinderen[2,3]
[1] *Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium*
[2] *Department of Microbiology and* [3] *Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland*
E-mail: 116221225@umail.ucc.ie

Introduction

The ESA research program 'Micro Ecological Life Support System Alternative' (acronym MELiSSA) targets the development of a 5-compartments artificial ecosystem. The bacteria-based reactors are under threat by stress factors (e.g. radiation, pH), which might influence the productivity of the loop through genetic drift or even activate dormant prophages in the genome. Additionally, if bacteriophages with a host range that includes *Rhodospirillum rubrum* S1H exist in the highly biodiverse waste streams and the mixed culture fermentation of the first compartment, they might invade the consecutive *R. rubrum* based bioreactor and impact its performance (*Jensen EC et al.* (1998)).

Viruses can destroy cultures relatively quickly and without warning, therefore it's vital to ensure whether or not they are present, and if they are active.

Materials and methods



Prophage threat investigation

Compare genomic sequences of *Arthrospira* sp. PCC8005 and *Rhodospirillum rubrum* S1H with known prophages by PHASTER (Arndt et al.2016)

"Completeness" score increases for every viral protein with a known function, if region bigger than 30kb and includes at least 40 genes.
<60: Incomplete.
60-90: Questionably complete
>90: Likely intact

Exposure in vitro to DNA stress to induce potential prophages into entering a lytic cycle

Analysis by flow cytometry using a BD Accuri C6 with Syto9 staining (final concentration 0.05 mM) and DNA extraction, positive control *Lactococcus lactis* NZ4000+TP9, negative control *Lactococcus lactis* UCS%9.

Bacteriophage threat investigation

To quantify risk of *R. rubrum*- or *Arthrospira*- infecting bacteriophages in wastewater, several dissimilar sources of waste water were sceened with an enrichment assay:
Overnight incubation after addition of 10 mL sample, filtered with a 0.45µm filter, to 10 mL of exponentially growing *R. rubrum* culture and filtering again

Sources of bacteriophages were samples from Compartment I reactor in Leuven, AQS Environmental solutions in Ireland, RWIZ in the Netherlands and Aquafin in Belgium

Presence/absence of bacteriophages was scored using spot assays on *R. rubrum* S1H, *E. coli* BL21 and *Arthrospira sp.* PCC8005

Results

Genomic analysis

Genomic analysis of *R. rubrum* revealed no intact prophages (the completeness scores were <100). Genomic analysis of *Arthrospira* revealed a questionably intact phage with a completeness score of 80 in the region of 2209450-2219147bp. However, this region doesn't contain enough other viral genes necessary for phage activity and is smaller than the typical 30 kb genome size of prophages (Zhou, Liang, Lynch, Dennis, & Wishart, 2011) and is therefore unlikely that the region contains a truly active phage.

Shake flask induction

After determining sublethal concentrations of mitomycin C, cultures were grown over a range of mitomycin C.

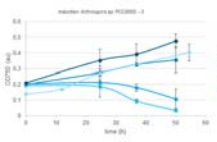


Figure 1: Growth profile of Arthrospira sp. PCC8005 growing in shakeflasks in the presence of 0, 0.05, 0.125, 0.25 and 0.5 µg/ml of mitomycin.

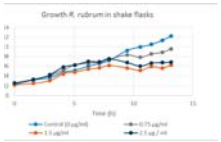


Figure 2: Growth profile of R. rubrum growing in shakeflasks in the presence of 0, 0.75, 1.5, and 2.5 µg/ml of mitomycin

Flow cytometry analysis

The *R. rubrum* lysates were analyzed by flow cytometry, and induced particles would show as a peak in the M1 range (Oliveira 2017). *Arthrospira* sp. PCC8005 samples will be tested in future work.

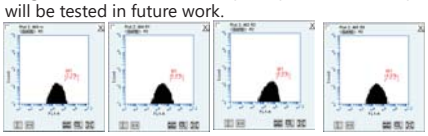


Figure 3: Histograms of relative fluorescence. From left to right, cultures were grown in the presence of 0, 0.75, 1.5 and 2.5 µg/ml mitomycin. Phage particles would cause a distinct peak at region M1 (Oliveira, J. et al.(2017))

DNA extraction

Phage DNA extraction of *R. rubrum*, *Arthrospira* and negative control lysates yielded concentrations below the detection limit (2 ng/µl DNA). The positive control yielded 44 ng/µl DNA, with an OD260/280 ratio of 1.93.

Enrichment and spot assay

No bacteriophages with lytic activity against either *R. rubrum* S1H or *Arthrospira* sp. PCC8005 were found, as expected most samples did contain baceteriophages capable of infecting *E. coli* BL21.

Source	*R. rubrum* lysis	*Arthrospira* lysis	*E. coli* lysis
MELiSSA compartment I (Leuven)	0	0	0
Wastewater plants Ireland (N=5)	0	0	3
Wastewater plants Netherlands (N=3)	0	0	3
Wastewater plants Belgium (N=10)	0	0	7

Discussion

Exposure of *R. rubrum* S1H and *Arthrospira* sp. PCC8005 to mitomycin C did not produce viral particles as detected by flow cytometry, confirming the results of the *in silico* genomic analyses.

DNA extraction revealed no viral DNA from the lysates of *R. rubrum* S1H and *Arthrospira* sp. PCC8005.

As expected since *E. coli* is prevalent in wastewater facilities, wastewater contained a lot of *E. coli* bacteriophages, however no bacteriophages with activity against *R. rubrum* were found. In the sample of Compartment I, no bacteriophages against either *E. coli* or *R. rubrum* were found, possibly due to growth conditions which differ from conventional waste water treatment plants and extremophile species being dominant in Compartment I.

Conclusion

A broad range of mitomycin C concentrations were tested in shake flasks, none of which produced viral particles. Analysis of the genome *in silico* also did not reveal likely intact prophages. Therefore, prophage presence in *R. rubrum* S1H and *Arthrospira* sp. PCC8005 is highly unlikely. Initial evidence suggests that the risk of compartment I-II cross infection with viruses is low. The influence of other environmental stressors such as temperature or light shocks will also be investigated.

Acknowledgements: This work is funded by the MELiSSA Foundation

References: *Jensen EC et al.* (1998): " Prevalence of Broad-Host-Range Lytic Bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*", *Corina P. D. Brussaard* (Mar. 2004), "Optimization of Procedures for Counting Viruses by Flow Cytometry", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, p. 1506–1513, *Arndt, D. et al. (2016):* PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res., *Tyson, G. W. and J. F. Banfield* (2007): "Rapidly evolving CRISPRs implicated in acquired resistance of microorganisms to viruses." Environ Microbiol 10(1): 200-207. *Oliveira, J. et al.*(2017): "Detecting *Lactococcus lactis* Prophages by Mitomycin C-Mediated Induction Coupled to Flow Cytometry Analysis". Zhou, Y., Liang, Y., Lynch, K. H., Dennis, J. J., & Wishart, D. S. (2011). PHAST: A Fast Phage Search Tool. Nucleic Acids Res, 39(Web Server issue), W347-W352. doi: 10.1093/nar/gkr485