Activated sludge foaming: can phage therapy provide a control strategy?

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Foaming in activated sludge systems is a global problem leading to environmental, cosmetic and operational problems. Proliferation of filamentous hydrophobic bacteria (including the Mycolata) are responsible for the stabilisation of foams. Currently no reliable methods exist to control these. Reducing the levels of the filamentous bacteria with bacteriophages below the threshold supporting foaming is an attractive approach to control their impact. We have isolated 88 bacteriophages that target members of the foaming Mycolata. These double stranded DNA phages have been characterised and are currently being assessed for their performance as antifoam agents.

The activated sludge process

The activated sludge process is a robust and proven system for treating domestic and industrial wastewater and is used globally[1]. It relies on a specialised community of microbes organised into structures called flocs, which metabolise organic nutrients and remove inorganic nitrogen and phosphorus compounds so that the treated effluent can be discharged safely into a receiving body of water without leading to eutrophication from growth of toxic *Cyanobacteria*[1].

These systems are no longer considered as wastewater water disposal systems, but as valuable sources of purified water for reuse and useful chemicals. Despite their popularity most suffer from the problem of foaming where a brown foam layer develops on the reactor surface and leaves in the treated effluent[2].

What is foaming?

Foaming, which increases plant operating costs, reduces effluent quality and acts as a source of opportunistic human pathogens, is a flotation event, requiring three components; air bubbles, surfactants and hydrophobic particles (bacterial cells), which act to stabilise it (Figure 1). With only air bubbles and surfactants, an unstable foam forms, and is often seen in start-up, where abundances of hydrophobic bacteria are below the threshold supporting foam formation[3]. With insufficient levels of surfactants, air bubbles collapse and a greasy surface layer, a scum, forms, consisting of hydrophobic bacteria. There are no reliable control measures to deal with an already established foam, but any proposed strategy should target the hydrophobic bacterial cells, since control of the other two is impractical.

It is now clear that a diverse range of bacteria are responsible for foaming episodes[4–8]. Theoretically, any sufficiently hydrophobic cell can stabilise this foam, but surveys suggest that the unbranched actinobacterial filamentous organism '*Microthrix parvicella*' and the right angled branching mycolic acid producing filaments placed in the Mycolata (include members of the genus *Gordonia*, *Nocardia, Rhodococcus, Tsukamurella, Skermania* and related members)[5,7] are the main culprits (Figure 2). Being strongly hydrophobic, these organisms escape the plant bulk liquid to the air liquid interface, often carrying biomass or sludge with them, and there attach to the liquid air films of the bubbles, preventing liquid drainage from them and hence stabilise them.

Foaming control

The conventional way to deal with existing foams is commonly non-selective using bactericidal chemicals, where organisms other than those causing foaming are likely to be harmed. Others include changing aeration rates, or reducing sludge ages hoping that foaming organisms, assumed to be slow growing, are washed out. Unfortunately other desirable bacteria are also lost. All

Figure 1. Foam covering the surface of an aeration basin and walkways.

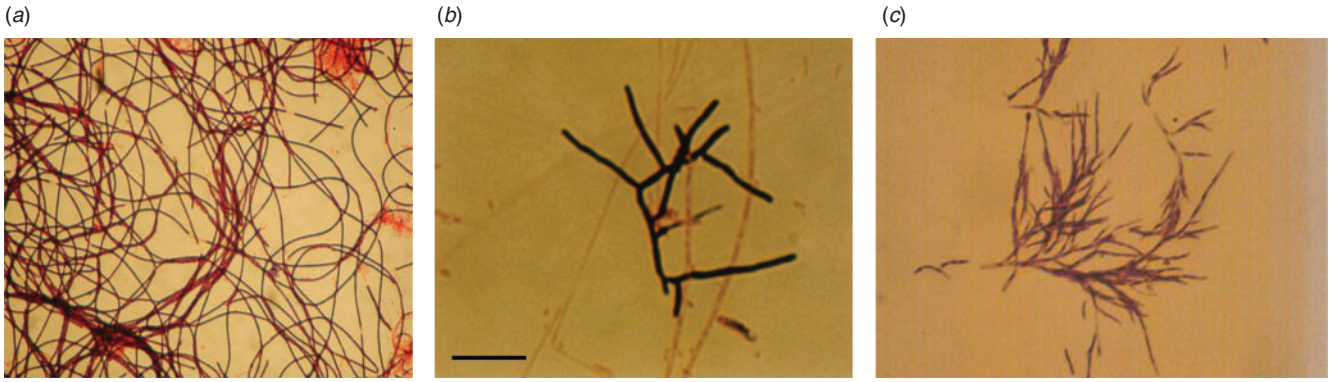

Figure 2. Gram stain of (a) '*Microthrix parvicella*', (b) *Gordonia amarae* like organism (GALO) and (c) *Skermania piniformis. Gordonia amarae* like organism (GALO) and *Skermania piniformis* are right angled branching mycolic acid producing filaments and belong to the Mycolata.

reflect our inadequate understanding of the microbial ecology of foams.

What is needed is a specific control strategy, which is environmentally safe and importantly only removes the nuisance organisms[9,10]. Bacteriophages (or phages), viruses that specifically target only their bacterial hosts, and are naturally occurring and self-dosing, seem especially attractive. They are used clinically to treat infectious bacterial diseases, where the causative organism is antibiotic resistant[11]. As they infect their hosts, they replicate and upon lysis, release often hundreds of new phages that then infect other host cells.

The general experience has been that wherever bacteria are present, phages able to lyse them will also be present. Consequently, phages lytic for members of the foaming Mycolata should be plentiful in activated sludge. Thomas and colleagues[9], demonstrated that phages, some polyvalent, are isolated readily from activated sludge plants, capable of killing their Mycolata hosts under laboratory conditions. What we know of phage/host population dynamics suggest that their presence would not lead to the total loss of their bacterial hosts. Such outcomes would be disastrous, since Mycolata play important roles in metabolising recalcitrant xenobiotics there. Strategically the aim is to reduce Mycolata numbers below individual threshold levels needed for stable foam formation. This requires identifying which are the causative organisms. FISH probing provides the tools to screen foam samples, and while their true level of biodiversity, is not known, FISH data suggest a limited number of foaming bacteria are common in plants.

What have we achieved?

The advent of Next Generation DNA Sequencing (NGS) has revolutionised our understanding of phage genomics and allowed us to screen those attractive for phage therapy, avoiding those possessing virulence or toxin genes. We have isolated 88 double stranded DNA phages that seem suitable for further study. These include phages against foaming *Gordonia*[12,13], *Rhodococcus*[14–16], *Nocardia*[17], *Skermania*[18] and *Tsukamurella*[19,20]. While most are monovalent, polyvalent phages are clearly more attractive, since most foams contain more than one Mycolata member. Not surprisingly, sequencing reveals that all are highly novel at the DNA level, but share the same genomic arrangements. They have all been screened against foaming Mycolata hosts using a simple foaming apparatus[21]. Almost always their foaming abilities were reduced to the point where no foam was detected as previously described[13,22].

Where next?

The next step is to scale up the system. Before this is warranted, it is important to determine their host specificities and burst sizes *in situ*, their persistence times in full scale plants, the host cell threshold values for foam production and how much inoculum is required. Equally, the location for introducing the phages into the system is likely to be important. These parameters are plant specific, and so will need to be determined on an individual basis. Whether these phages are involved in gene transfer between host cells (transduction), and whether they acquire, as a consequence, antibiotic resistant genes and hence pose a possible threat upon release into the environment, will need investigation. In addition, the possibility of the bacterial strains developing phage resistance will need to be investigated and one possible solution would be to add multiple phages for the same host as multiple mutations is less likely.

References

1. Seviour, R. and Nielsen, P.H. (2010) Microbial ecology of activated sludge. IWA publishing.
2. Jenkins, D. *et al*. (2003) Manual on the causes and control of activated sludge bulking, foaming, and other solids separation problems. CRC Press.
3. Petrovski, S. *et al*. (2011) An examination of the mechanism for stable foam formation in activated sludge systems. *Water Res.* **45**, 2146–2154.
4. Soddell, J. (1998) Foaming. In *The microbiology of activated sludge*. pp. 161–202, Kluwer Academic Publishers, Dordrecht.
5. de los Reyes III (2010) Foaming. In *The Microbial Ecology of Activated Sludge*. pp. 215–258. IWA Publishing, London.
6. Seviour, R.J. *et al*. (2008) Ecophysiology of the Actinobacteria in activated sludge systems. *Antonie van Leeuwenhoek* **94**, 21–33. doi:10.1007/s10482-008-9226-2
7. Nielsen, P.H. *et al*. (2009) Identity and physiology of filamentous bacteria in activated sludge. *FEMS Microbiol. Rev.* **33**, 969–998.
8. Kragelund, C. *et al*. (2007) Ecophysiology of mycolic acid-containing Actinobacteria (Mycolata) in activated sludge foams. *FEMS Microbiol. Ecol.* **61**, 174–184. doi:10.1111/j.1574-6941.2007.00324.x
9. Thomas, J.A. *et al*. (2002) Fighting foam with phages? *Water Sci. Technol.* **46**, 511–518. doi:10.2166/wst.2002.0527
10. Liu, M. *et al*. (2015) Bacteriophages of wastewater foaming-associated filamentous Gordonia reduce host levels in raw activated sludge. *Sci. Rep.* **5**, 13754. doi:10.1038/srep13754
11. Abedon, S.T. *et al*. (2017) Phage therapy:past, present and future. *Front. Microbiol.* **8**, 981. doi:10.3389/fmicb.2017.00981
12. Dyson, Z.A. *et al*. (2015) Lysis to kill: evaluation of the lytic abilities, and genomics of nine bacteriophages infective for *Gordonia* spp. and their potential use in activated sludge foam biocontrol. *PLoS One* **10**, e0134512. doi:10.1371/journal.pone.0134512
13. Petrovski, S. *et al*. (2011) Characterization of the genome of the polyvalent lytic bacteriophage GTE2, which has potential for biocontrol of *Gordonia*-, *Rhodococcus*-, and *Nocardia*-stabilized foams in activated sludge plants. *Appl. Environ. Microbiol.* **77**, 3923–3929. doi:10.1128/AEM.00025-11
14. Petrovski, S. *et al*. (2012) Small but sufficient: the *Rhodococcus* phage RRH1 has the smallest known Siphoviridae genome at 14.2 kilobases. *J. Virol.* **86**, 358–363. doi:10.1128/JVI.05460-11
15. Petrovski, S. *et al*. (2013) Genome sequence and characterization of a *Rhodococcus equi* phage REQ1. *Virus Genes* **46**, 588–590. doi:10.1007/s11262-013-0887-1
16. Petrovski, S. *et al*. (2013) Characterization and whole genome sequences of the *Rhodococcus* bacteriophages RGL3 and RER2. *Arch. Virol.* **158**, 601–609. doi:10.1007/s00705-012-1530-5
17. Petrovski, S. *et al*. (2014) Genome sequence of the *Nocardia* bacteriophage NBR1. *Arch. Virol.* **159**, 167–173. doi:10.1007/s00705-013-1799-z
18. Dyson, Z.A. *et al*. (2016) Isolation and characterization of bacteriophage SPI1, which infects the activated-sludge-foaming bacterium *Skermania piniformis*. *Arch. Virol.* **161**, 149–158. doi:10.1007/s00705-015-2631-8
19. Dyson, Z.A. *et al*. (2015) Three of a Kind: Genetically Similar *Tsukamurella* Phages TIN2, TIN3, and TIN4. *Appl. Environ. Microbiol.* **81**, 6767–6772. doi:10.1128/AEM.01145-15
20. Petrovski, S. *et al*. (2011) Genome sequence and characterization of the *Tsukamurella* bacteriophage TPA2. *Appl. Environ. Microbiol.* **77**, 1389–1398. doi:10.1128/AEM.01938-10
21. Blackall, L.L. and Marshall, K.C. (1989) The mechanism of stabilization of actinomycete foams and the prevention of foaming under laboratory conditions. *J. Ind. Microbiol.* **4**, 181–187. doi:10.1007/BF01574075
22. Petrovski, S. *et al*. (2011) Prevention of *Gordonia* and *Nocardia* stabilized foam formation by using bacteriophage GTE7. *Appl. Environ. Microbiol.* **77**, 7864–7867. doi:10.1128/AEM.05692-11

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