



What do cyanide, garlic and marine algae have to do with CF treatment?

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The Australian and New Zealand CF Nurses Conference held in Hobart, Tasmania this August included some presentations that were not what CF nurses usually get to hear at their meetings! Tasmania has the second highest rate of CF in the world after Ireland and perhaps for that reason, it has nurtured and funded some very talented CF researchers. The organisers were provided with the rare opportunity to invite local speakers who are at the absolute laboratory coalface in working toward more effective ways to combat what we, both health professionals and CF families, all dread in our patients – persistent lung infection with *Pseudomonas aeruginosa*.

Associate Professor Sylvia Kirov and research colleague, Dr Louise Roddam gave the conference delegates some very up-to-date insights into *P. aeruginosa* research being done in Tasmania as well as promising research results from other national and international groups.

A/Prof Sylvia Kirov, a medical microbiologist in the Tasmanian School of Medicine, presented some of the ground-breaking work that she and collaborators at the Centre for Marine Bio-Innovation (CMB), University of New South Wales are conducting. The research findings contribute significant new insights into why the growth of organisms in “biofilms” (slime-encased bacterial communities that develop in the CF lung mucus after acquisition of *P. aeruginosa*) ultimately results in intractable infection. For the first time their work has demonstrated that the biofilm developmental cycle of clinical isolates from chronic lung infections, involves an active dispersal mechanism known as “seeding dispersal”. This dispersal process not only leads to the spread of infection to new sites but even more significantly, it is associated with the generation of *variation - different cell types* in the dispersal cells (caused by genetic mutations in the bacteria in maturing biofilms).¹ Some of these variant cells are better able to survive in the lung showing increased nutritional versatility, resistance to antibiotic therapy and increased biofilm-forming ability that helps them to evade host defences. It is likely that such bacterial adaptations are why *P. aeruginosa* cannot be permanently eliminated by existing antimicrobial therapies, however aggressive these may be.

This is where new strategies and marine algae come in!

Ongoing research by A/Prof Kirov and the UNSW group now aims to identify the key adaptations that favour survival in the CF lung and the mechanisms by which they are generated during the dispersal process. These will offer completely new potential therapeutic targets for novel interventions to eliminate organisms.

Marine algae come into the story because of their capacity to prevent the development of biofilms on their fronds by producing signals (called furanones) that mimic, and hence block, the cell to-cell communication signals that bacteria produce to trigger the maturation of their biofilms.² Synthetic furanones based on these algal molecules have subsequently proved successful at preventing biofilm development and increasing the effectiveness of antibiotic therapies in animal models of chronic lung infection.^{3,4}



From left, Associate Professor Sylvia Kirov, Keynote speaker and Associate Professor Claire Wainwright and Dr Louise Roddam.

And what about garlic!



Such biofilm-inhibiting signals have also been found in garlic and other plants.⁵ Clinical trials to test the efficacy of garlic extract in combination therapies with antibiotics are currently underway in UK/Danish collaborative studies⁶. The use of artificial triggers that induce biofilm dispersal and preempt the production of adaptive variations could also provide another approach for dealing with established infections. Nitric oxide (NO) pro-drugs that induce dispersal are being tested in model systems for this role at the CMB.⁷

A/Prof Kirov, in collaboration with Dr David Reid and the Respiratory Research Group at the UTAS School of Medicine and the Menzies Centre, have also shown that removal of iron with iron-binding chemicals (chelators) can hinder *P. aeruginosa* growth and biofilm development. Promising results from *in vitro* model systems show that “robbing” the bacteria of iron in this way may therefore prove another useful adjunct therapy for treating *Pseudomonas* infections in CF.

As for cyanide?

Research being led by Dr Louise Roddam is investigating the changes that occur in the complex bacterial community inhabiting the lung at times of stable disease compared with exacerbations. This research is based on the finding by the UTAS/Menzies Respiratory Group investigators that *P. aeruginosa* produces high amounts of cyanide (a highly toxic entity) in the infected lung during exacerbations.⁸ Furthermore, the group has recently shown that the presence of cyanide impairs the function of the immune system and therefore plays a role in lung damage (unpublished data). Currently, both the level of bacterial infection and the production of several toxic bacterial products are being monitored using molecular methods, in an attempt to understand how changes may contribute to exacerbation. Studies will then focus on finding ways to detect rising levels early and control its production so as to lessen the damaging effects of *Pseudomonas* infection on lung function.



In summary, for those of us dealing with maintaining health in CF patients the research presented at the meeting offered hope of new therapeutic strategies (listed below) that could well ultimately be successful at combating chronic *Pseudomonas* infection.

- Specific targeting (with new drugs) of the mutations that favour *Pseudomonas* persistence and/or the mechanisms by which they are generated
- Adjunct therapies designed to “attack” biofilms and increase antibiotic effectiveness which could include combinations of the following:
 - Chelating agents to reduce iron in the CF lung
 - Biofilm maturation signal inhibitors (furanones)
 - Biofilm dispersal signal triggers (e.g. NO pro-drugs)
- Prevention of bacterial virulence factor (e.g. cyanide) expression to decrease lung damage by:
 - Modified antibiotic therapies and the use of bacterial signal inhibitors, as above.

References

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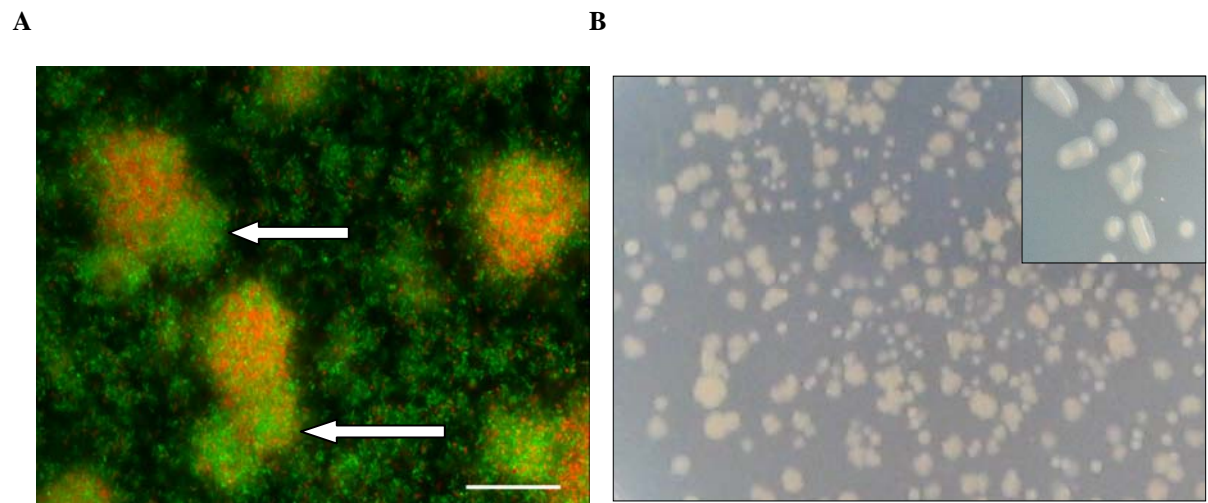


Figure: Biofilm dispersal and variation in a CF strain of *P. aeruginosa*

(A) Seeding dispersal (arrows) from the maturing biofilm microcolonies (7 days post-culture in an *in vitro* flow-cell model system). Mature (dead) biofilm cells are stained red, while active (live) dispersal cells are shown in green.

(B) Variants, evidenced here by different colony types, are generated on dispersal. The inset shows the colony appearance of the initial inoculum strain.

(Adapted from ref 1.)