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bringing Microbiologists together
NSW-ACT Branch

Syntrophy

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From the editor

By Jim Manos

Greetings from the Chair,

We hope everyone is well and the strains caused by COVID-19 are more bearable as we hopefully have been through the worst of it! The branch had its 60th Annual General Meeting on August 20 (for the first time ever by Zoom), and we have a new President-elect Maurizio Labbate from UTS, who will take over at the next AGM. Congratulations Maurizio! Tim Newsome was returned for another term as Secretary he is doing such as fantastic job, congratulations Tim! We also have a new committee member, Anukriti Mathur from ANU, thank you Anukriti for joining us! I would also like to thank the continuing members for their support of the Branch work, and our retiring members for their contributions to the branch.

The AGM was followed with an exciting and well attended webcast seminar by Dr Amy Cain from Macquarie University entitled "Examining antibiotic resistance in hospital ESKAPE pathogens". We also got to hear about Amy's work at the Wellcome centre in Malawi, which was an interesting eye-opener in that it demonstrated the difficulties faced by hospitals in poorer nations.

This issue has an interesting article by Joachim Larsen from Brett Neilan's lab at Newcastle University on polyketide synthase, the enzyme involved in making polyketides. We also have the third of our winning articles from UTS by Maximilian Bordignon on the menace of chlamydia that is devasting our koala populations.

We will soon bring you more exciting seminars by Zoom, so please stay tuned and stay safe!

Jim

For the last 2 issues of Syntrophy and this one, we have published winning articles produced by second year microbiology students (in the subject General Microbiology) from the University of Technology Sydney.

Courtesy of the Australian Society for Microbiology NSW-ACT Branch, winning articles were given certificates and a $100 JB Hi-Fi voucher for their efforts. Students were asked to write a 2-page magazine-style article on a contemporary microbiology topic which in 2020 included human coronaviruses and koala chlamydia.

This assessment is designed to enhance students' communication of science to society. They were given journalistic freedom to identify an interesting angle of the topic and to write and present their article in a way that engages with a broad audience in the style of a popular science magazine (e.g. Scientific American).

In this issue of Syntrophy, on pages 5 to 7 we present the article written by one of the winners of the koala chlamydia topic.

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Focus Article

The biological function and evolution of type III polyketide synthases

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Polyketides are a group of natural products produced by plants, fungi and bacteria, including cyanobacteria. They are synthesised by specialised enzymes called polyketide synthases (PKSs), which are classified depending on their structural organisation. Polyketide synthesis occurs by synthesising a chain of alternating carbonyl and methylene groups via the condensation of malonyl-CoA, methylmalonyl-CoA or ethylmalonyl-CoA units onto a substrate molecule. Type III PKSs are of particular interest because of their simple reaction mechanism, having only a single active site, while still being able to use a wide range of substrates to produce a wide range of products 1.

Type III PKSs and their products have mainly been investigated in plants, particularly through characterisation of chalcone synthase and stilbene synthase, which carry out the first step in flavonoid (pigment) biosynthesis. In contrast, only a few type III PKSs have been characterised from bacteria. For example, in Mycobacterium, these enzymes synthesise methylated polyketides from short-chain aliphatic fatty acids and both malonyl- and methylmalonyl-CoA. These methylated polyketides are important for biofilm formation2. Likewise, in Azotobacter vinelandii two type III PKSs synthesise phenolic lipids from long-chain aliphatic fatty acids and malonyl-CoA (Figure 1a). Disruption of the type III PKS gene cluster in A. vinelandii disrupted cyst coat formation3. Other type III PKSs have been identified in bacterial genomes, however, in most cases their associated biosynthesis pathways and products are unknown.

Being particularly rich in polyketides, it is surprising that only two type III PKS products have been isolated from cyanobacteria ('blue-green algae'), namely the (7.7)paracyclophanes and the hierridins4,5 (Figure 1b-c). Interestingly, the former is produced by a hybrid cluster using a combined type I/III PKS system, only identified in cyanobacteria. The native biological function of these compounds is still unknown, however, they have been demonstrated to have cytotoxic and anti-plasmodial activities, respectively4,5.

To better understand the evolution and function of type III PKSs, we carried out a phylogenetic analysis based on enzymes from plants, fungi, and bacteria (including cyanobacteria) (Figure 2). The phylogeny showed that cyanobacterial type III PKSs underwent two major changes during their evolution – one giving rise to hybrid gene clusters ((7.7)paracyclophane-like clusters), closely related to other bacterial sequences, and the other giving rise to single type III PKS clusters (hierridin-like clusters).

Interestingly, the plant and fungal type III PKS sequences partition with the hierridin-like type III cyanobacterial PKSs, suggesting that plants acquired the type III PKS from cyanobacteria during the evolution of the plant kingdom. This aligns with vertical evolution being the main driver for the evolution of type III PKS. However, the phylogenetic distribution of a few sequences from non-related microorganisms suggested that horizontal gene transfer may have also played a role in shaping the evolution of these enzymes.

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About the lead author

Joachim Steen Larsen did his Bachelor's and Master's degrees in Biology-Biotechnology at the University of Copenhagen, Denmark. He initially studied alkaloid biosynthesis in plants, then later specialised in glycosylation pathways from plants, yeast and humans. He relocated to Prof. Brett Neilan's lab at the University of Newcastle (UoN) in 2018 and commenced research on the biological functions and evolution of type III PKSs in cyanobacteria. Joachim is the president of the Higher Degree by Research Society at UoN and a member of the International Genetically Engineered Machine (iGEM) advisory team.

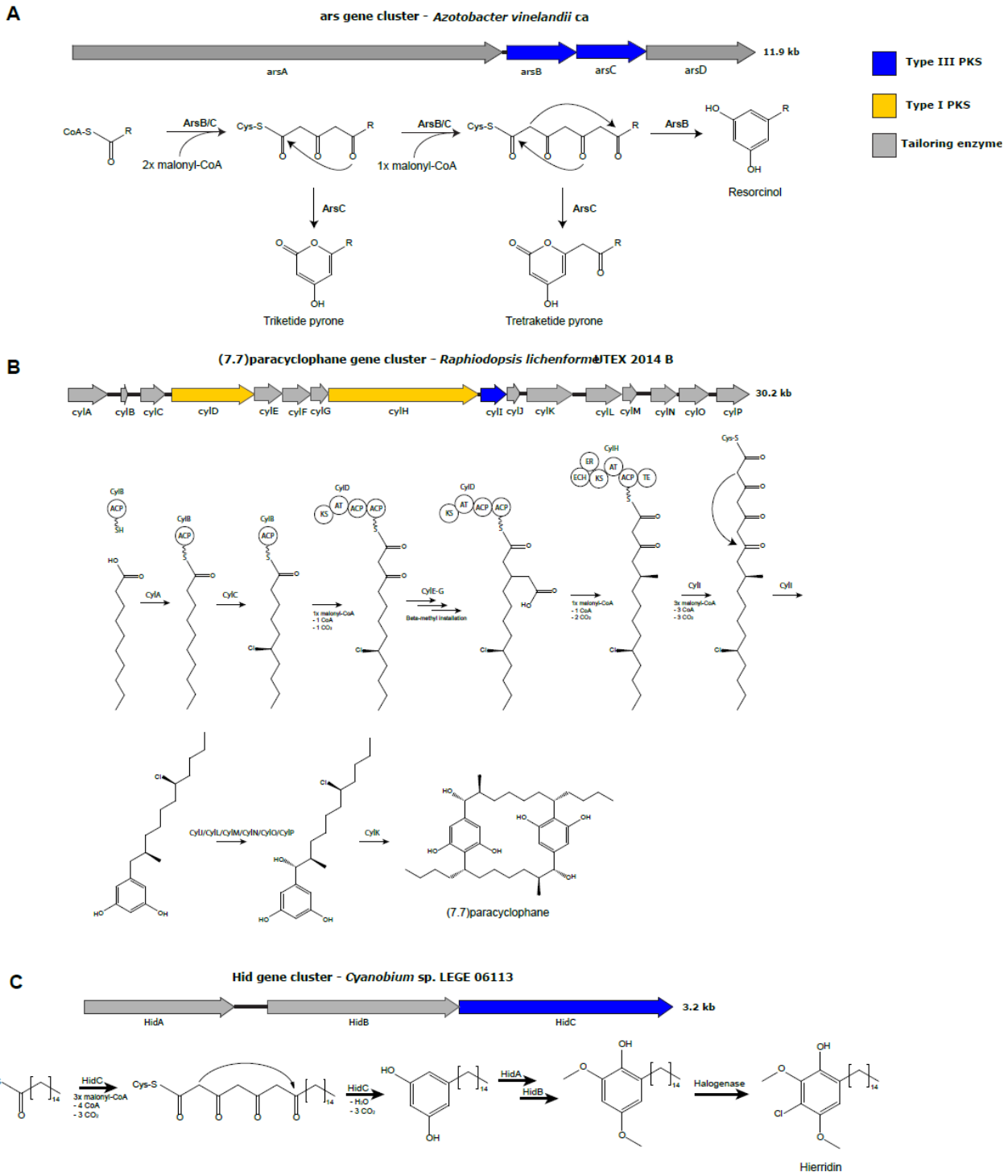

Figure 1: Overview of three type III PKS gene clusters and pathways. Blue indicates type III PKS genes, yellow indicates type I PKS genes, grey indicates tailoring enzyme genes. The size of the cluster is shown at the right of the gene cluster.

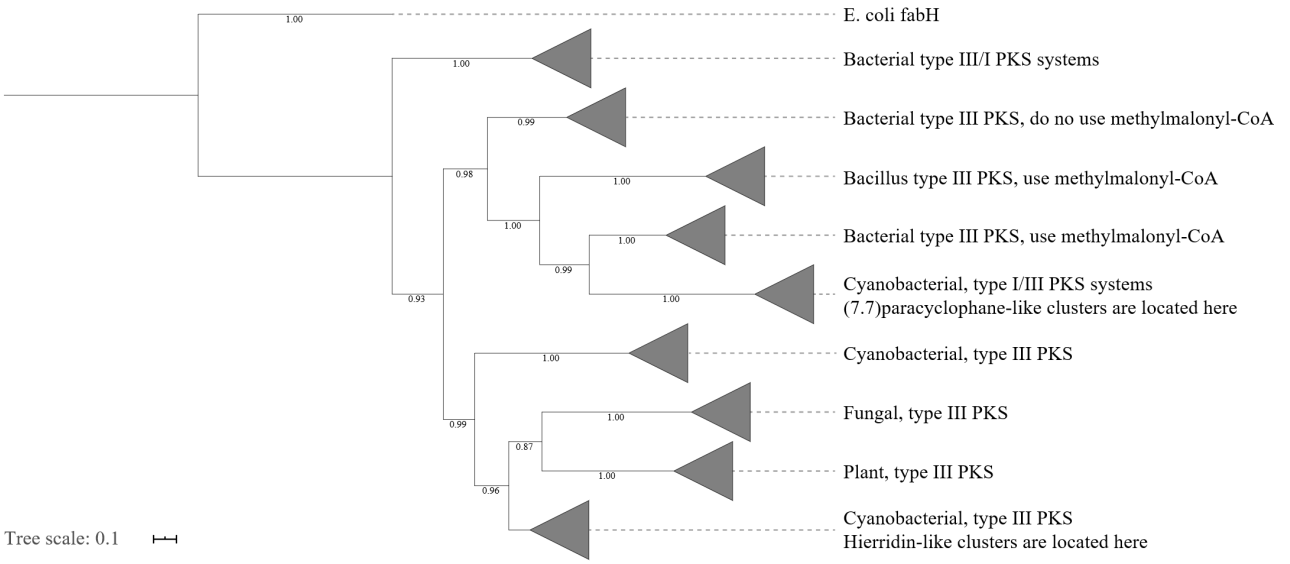

Figure 2: Phylogenetic tree of type III PKS amino acid sequences from bacteria, fungi, and plants. The outgroup is the fatty acid fabH from E. coli. The clades have been collapsed to show the overall topology of the tree, with a short description of the clade to the right. A total number of 89 cyanobacterial, 26 other bacterial, 17 plant and 3 fungal sequences were used. The tree was made in mrbayes using the LG+I+G model as predicted by prottest to be the best model. Bootstrap values above 0.7 are shown on the branches. The tree scale corresponds to the number of substitutions pr. site.

Maximilian Bordignon

The Invisible Menace Emptying Koala Populations

Our furry friends are fighting a losing battle against chlamydia. Is there anything we can do to save them?

Every Australian loves koalas. They're small, round, and so fluffy you always end up wishing you could cuddle one. In fact, we adore koalas so much that, at Australia Zoo, the opportunity to pat one costs $50 per person!

Unfortunately, however, these incredible animals are fading away. In 2012, the NSW, ACT and Queensland populations were all listed as 'vulnerable' – just one level away from 'endangered' [Australian Government Department of Agriculture, Water and the Environment 2013]. One of Queensland's most densely populated areas, the Koala Coast, saw an 80% decline from 1996 to 2014 [Rhodes et al. 2015]. Koalas are undeniably disappearing.

The causes? Well, they're a little complicated…

What has gone wrong?

There are two major sources of decline. Firstly, the loss of habitat from urbanisation forces koalas into dangerous, man-made environments, such as roads or backyards containing potentially predatory dogs [Australian Government Department of Agriculture, Water and the Environment 2013].

Secondly. The impact of chlamydia has been significant. Chlamydial infections vary widely in their prevalence across Australia; for examples, the infection rate in South Australian koalas is reported at 88%, while a recent survey of Queensland found a 45% rate [Gonzalez-Astudillo et al. 2017]. Kangaroo Island, on the other hand, is chlamydia free [Jelocnik et al. 2019].

It is undeniable that the most effective restoration of koalas will be achieved by responding to both of these issues. Habitat loss is currently being addressed through conservation programs and policies. So how do we combat chlamydia?

Chlamydia: A Fierce Foe

The mechanisms that enable chlamydia to spread so expansively throughout koala populations are yet to be fully understood. We do know this much, however: chlamydia is transmitted between koalas sexually, as well as from mother to joey through the consumption of pap, a nutrient-rich form of faeces. Chlamydia is ruthless, infecting koalas of all ages and sexes. It reduces population sizes by causing death,

infertility, and blindness (which often results in an early death by predation or starvation) [Gonzalez-Astudillo et al. 2017].

Furthermore, Koala Retrovirus (KoRV) has been identified as immunosuppressive and linked to an increased risk of chlamydial infection [Robbins et al. 2019]. Differences in predominant site of infection, as well as KoRV prevalence, between populations could help account for the vast variability in chlamydia infection rates.

Not too unlike Covid-19, it has been shown that chlamydia can be carried asymptomatically. In koalas, chlamydia can be asymptomatically carried for longer than 6 months. [Robbins et al. 2019]. This makes it near-impossible to rid a population of all its chlamydia with our current technology.

Finally, to put the icing on the cake, the only treatment we have to fight this thing, antibiotics, is now known to be harmful to the koala it's used on [**Figure 1**]. Treatment with antibiotics tends to kill not only the chlamydia bacteria, but also many vital gut micro-organisms upon whose metabolism koalas have evolved to rely on. Without these microbes, the abundant toxins in Eucalyptus leaves cannot be digested. [Dahlhausen et al. 2018].

After reading all these factors, you'd be forgiven for feeling hopeless. This is going to be a tough bug to squash, but it's not all gloomy.

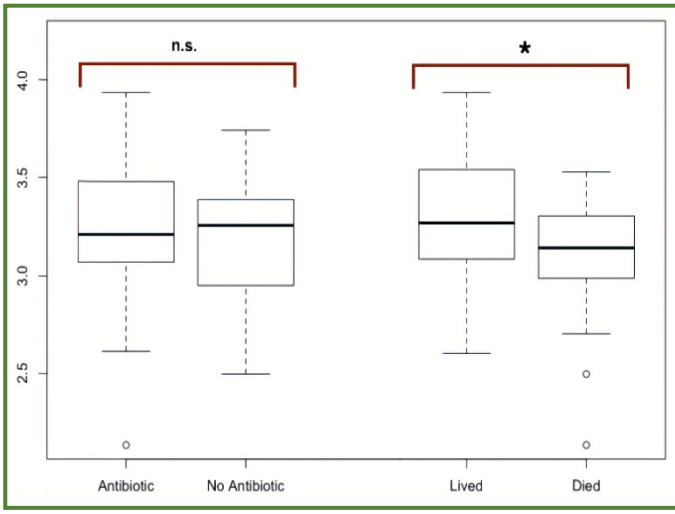

Figure 1. Boxplots of diversity of koala gut microbiota. They show that:
- Antibiotic use decreases microbiota diversity.
- Decreased diversity significantly correlates to higher mortality rate.

[Dahlhausen et al. 2018].

A Glimmer of Hope

As more people learn of the challenges koalas face, koala hysteria has escalated. The most infamous example is a 2019 announcement by the Australian Koala Foundation that Australian koalas are "functionally extinct." Sydney University wildlife ecologist, Assoc. Prof. Matthew Crowther, was interviewed to quell the resulting panic. "Some populations are doing well and even increasing in size," He says, "Hence it is alarmist and adds nothing to the conversation to say koalas are 'functionally extinct'" [Blake 2019]

Crowther has reason to be hopeful. Development of a *C. Pecorum* vaccine has made extensive progress in recent years. One such candidate synthetically mimics a *C. Pecorum* membrane protein, offering safe, long-lasting protection from multiple strains of the pathogen in koalas [Nyari 2018]. If this vaccine, or a similarly effective one, can be successfully implemented in koala populations we would finally have a shot (forgive the pun) of fighting back against the bacteria. It's important to keep in mind however, this treatment is preventative, thus a combination with current (or chlamydia-specific) antibiotics will be most efficient in exterminating *C. Pecorum*.

Ultimately, koalas are facing severe threats, but the world in which Blinky Bill is a memoir is not as sure as it seems. ●

The Two Species

Koalas are affected by two bacterial *Chlamydia* species: *C. pecorum* and *C. pneumoniae*.

C. pecorum is considered significantly more pathogenic than *C. pnuemoniae*. Its symptoms include conjunctivitis (which can cause blindness), cystitis, rhinitis/pneumonia, and ovarian bursitis or testicular orchitis (which both lead to infertility).

C. pnuemoniae is mostly asymptomatic but can cause conjunctivitis.

[Gonzalez-Astudillo et al. 2017]

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<u>Decorative Images</u> (I.e. Images I didn't use to convey/ summarise information)

Koala title banner:
https://www.morrisanimalfoundation.org/article/last-chlamydia-free-koala-population-may-safeguard-future-species