MICROBES, DRUG DISCOVERY & AUSTRALIAN STINGLESS BEES

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Introduction. The discovery of antibiotics and antifungal agents with novel modes of action is essential for the continued treatment of infectious diseases.
An understanding of the complex interactions between microbes and insects has revealed intimate chemical interactions that may provide the novel chemistry that we seek. We have focused our research on the co-evolution of microbes and stingless bees on the Australian continent and hypothesize that these complex relationships may result in complex and novel bioactive chemistry.
Australian stingless bees and their associated microbes are an unexplored niche in the quest for new bioactive compounds.
The objective of this work was to isolate microbes associated with 3 species of stingless native bee and to determine if *in vitro* culturing of these microbes may biosynthesize bioactive compounds with novel modes of action.

Methods. Stingless bees *Autroplebia australis, Tetragonula carbonaria* and *Tetragonula hockingsi* were aseptically collected from Brisbane, QLD, Australia.
Diversity: Bee gut bacterial diversity was determined by 454 tagged pyrosequencing of the 16s rDNA gene from total gut DNA. [1]
Isolation: Bacterial and fungal isolates were derived from bee gut and dissected body surfaces and cultured using nutrient rich and poor media plus organism specific media
De-replication: Enrichment of isolate pool was achieved via genetic methods targeting: Poly-Ketide Synthase (PKS) genes, Non-Ribosomal Peptide Sythetase (NRPS) genes, 16s rDNA and 18s rDNA genes. [2]
Bioactivity: Target organisms were grown in 2L liquid culture and extracted with ethyl acetate. 100 µg crude extracts were assayed using disk diffusion bioassays against *S. aureus*, *C. albicans* & *P. aeruginosa.*

Results. The *T. carbonaria* gut was dominated by the bacterial genus *Paralactobacillus* and *Sachribacter*.
The initial isolation experiment yielded 35 different micro-organisms. Four of those isolates demonstrated antimicrobial activity

Table 1. Proportionate abundance of bee gut bacteria, Genus level, by 454 tagged pyrosequencing of 16s rDNA genes Pooled results from 13 individual *T. carbonaria* bees sampled from 2 different hives,

Genus	% of total gut bacteria (n=13)
Paralactobacillus	35.9
Saccharibacter	24.8
Lactobacillus	10.5
Nicoletella	7.1
Stenoxybacter	6.4
Zymobacter	6.1
Neoasaia	4.6
Kozakia	1.5
Acinetobacter	1.3
Other	1.8

Isolates with activity
1) *Citrobacter sp*., isolated from *A. australis*. Active against *S. aureus* & *C. albicans*. PKS & NRPS positive. Potato dextrose growth media
2) *Klebsiella sp*., isolated from *T. carbonaria.* Active against *S. aureus* & *C. albicans*. PKS positive. De Man, Rogosa & Sharp growth media.
3) *Serratia sp*., isolated from *T. carbonaria.* Active against *S. aureus*. Nutrient broth growth media.
4) *Bacillus sp*., isolated from *T. carbonaria.* Active against *S. aureus*, *C. albicans* & *P. aeruginosa.* De Man, Rogosa & Sharp growth media.

Conclusions. Isolation and screening of stingless bee associated micro-organisms revealed 4 candidates that exhibited antimicrobial activity. On going work includes, purification and structure elucidation of active compounds and characterization of biosynthesis mechanisms.

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