Structural elucidation and bioactivities of natural products synthesised by Antarctic desert *Actinobacteria*

Nicole Benaud

I would like to express my gratitude to the Board of Antarctic Science Ltd for awarding me an Antarctic Science Bursary in 2020. The funding has enabled me to extend the research from my PhD thesis, which aimed to discover novel bioactive natural products from Antarctic soil bacteria.

Microbial natural products (NP) are biosynthesised through secondary metabolite pathways and remain at the forefront of drug discovery for important therapeutics such as antimicrobials, antitumour agents, immunosuppressants and statins [1, 2]. For my PhD work I employed novel culturing methods to isolate NP-producing Antarctic bacteria, followed by long-read whole genome sequencing to enable genome mining for unusual biosynthetic gene clusters (BGCs). Two cold-adapted, slow-growing Actinobacterial isolates, *Kribbella* sp. SPB151 and *Streptomyces* sp. NBSH44 [3, 4], were particularly interesting because they both harboured BGCs with low similarity to known genomes (6 - 40% similarity), suggesting the encoding of new chemical entities (Fig. 1A) [5, 6].

The aims of the Antarctic Bursary-funded extension project were to conduct large scale cultivations of *Kribbella* sp. SPB151 and *Streptomyces* sp. NBSH44, followed by compound extraction, fractionation, structural elucidation and bioactivity assays, incorporating both antimicrobial resistant pathogen strains, as well as tumour cell lines to determine cancer therapeutic potential. The COVID 19 pandemic unfortunately created significant delays to planned lab work, equipment access and supply delays for consumables for the project. Work is still ongoing for the project, and current results have been very encouraging.

A common challenge in NP discovery is that many BGCs are transcriptionally silent or are expressed in insufficient quantities under standard lab conditions [7]. For this project, growth optimisation experiments were performed, with *Kribbella* sp. SPB151 and *Streptomyces* sp. NBSH44 tested on 10 different media under a range of pH and temperature conditions. For *Kribbella* sp. SPB151, the secretion of a diffused, brown-pigmented metabolite was initiated on PM01 media [8], at pH 7 and 20°C incubation. Further examination of the *Kribbella* sp. SPB151 genomic data led to the hypothesis that supplementation with glutamic acid may improve production of the target metabolites, as it was predicted as a precursor amino acid for adenylation domains of two of the target NRPS BGCs [9, 10].

Results

Following a small size fermentation (75 mL), *Kribbella* sp. SPB151 was found to produce 10.3 mg of crude extract when grown in media supplemented with glutamic acid, which was 2.7 times greater than the crude extract recovered from the PM01 media alone (Fig.1 B).

Crude extracts were then analysed using C18 liquid chromatography tandem mass spectrometry (LC-MS/MS). By comparing differences between the *Kribbella* sp. SPB151 extracts and their media controls (Fig. 1C-F), we detected a high molecular weight compound eluting at approximately 4.1 min which was detected uniquely in the *Kribbella* sp. SPB151 extract from the fermentation grown with supplemented glutamic acid (pink arrow, Fig. 1F), and this was also the most relatively abundant eluent in the sample.

The mass spectra for this compound had a predicted molecular weight of 1272.4 Da based on the most abundant ion with 637.21 m/z, representing the doubly charged $(M+2H)2^{+}$ ion (Fig. 2). Interestingly, there were no known matching compounds in the available databases for this spectrum.

Ongoing work

Larger fermentation extracts (4L) will be fractionated with a goal to purify the target compound. Cytotoxic screening of the *Kribbella* sp. SPB151 extracts using a brine shrimp lethality assay have been optimised and early results suggest the crude extract has cytotoxic activity. Fractions will be reanalysed by LC-MS/MS and the fractions screened for cytotoxicity using the brine shrimp lethality assay. This will be followed by NMR for structural elucidation, and MTT cytotoxicity/cell viability assays.

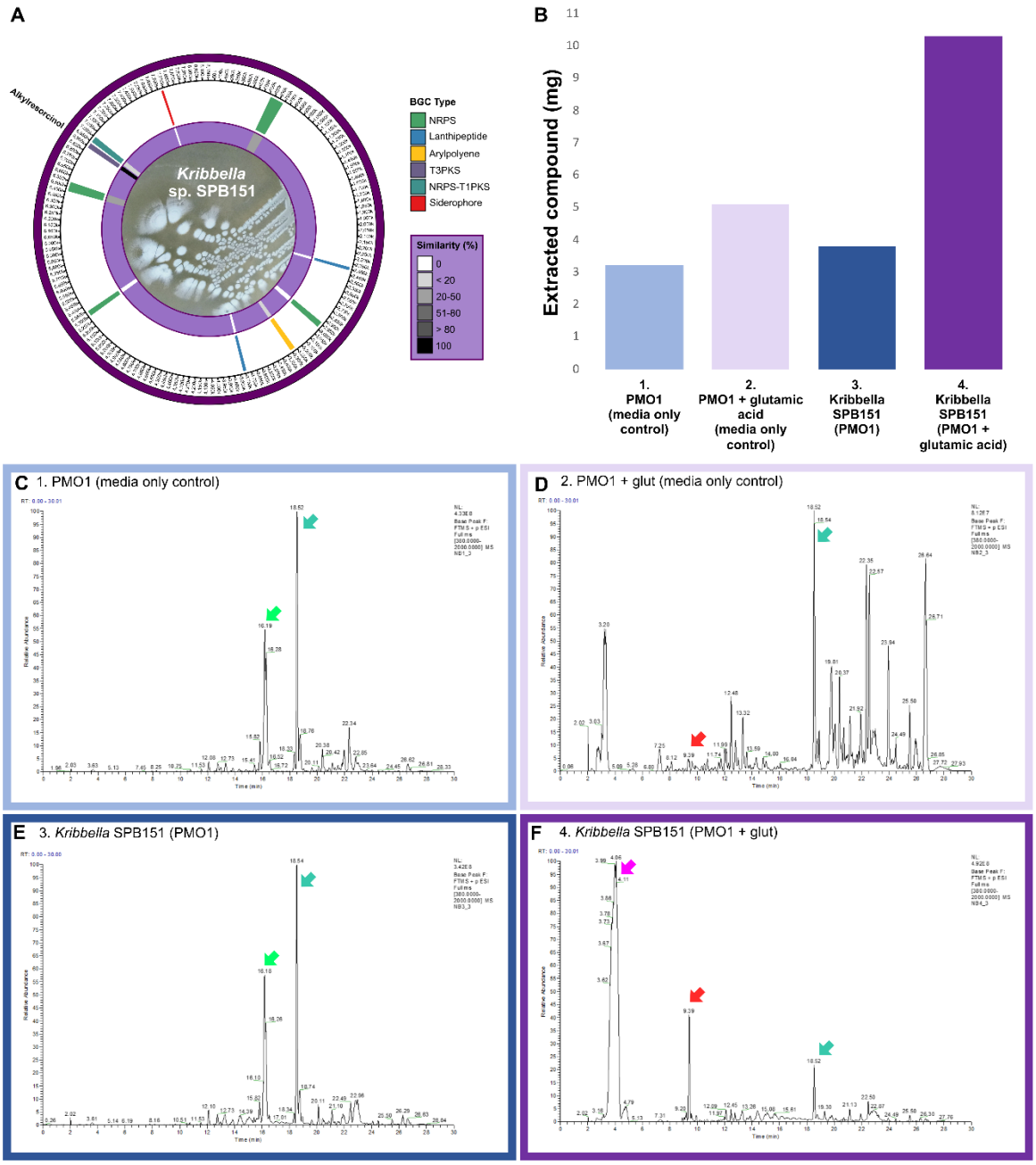

Figure 1. Solvent extractions from 75 mL broth cultures of *Kribbella* sp. SPB151 and two media controls, and chromatogram results from high mass positive mode LC-MS/MS analysis. (A) Circular representation of the *Kribbella* sp. SPB151 genome, showing detected biosynthetic gene clusters types, their location and similarity to known clusters. **(B)** Crude solvent extract yield for (1) Control PM01 media, (2) Control PM01 media supplemented with glutamic acid, (3) *Kribbella* sp. SPB151 fermented in PM01 media, and (4) *Kribbella* sp. SPB151 fermented in PM01 media supplemented with glutamic acid. **(C)** Chromatogram for Control PM01 media extract. **(D).** Chromatogram for Control PM01 media supplemented with glutamic acid. **(E)** Chromatogram for *Kribbella* sp. SPB151 fermented in PM01 media. **(F)** Chromatogram for *Kribbella* sp. SPB151 fermented in PM01 media supplemented with glutamic acid.

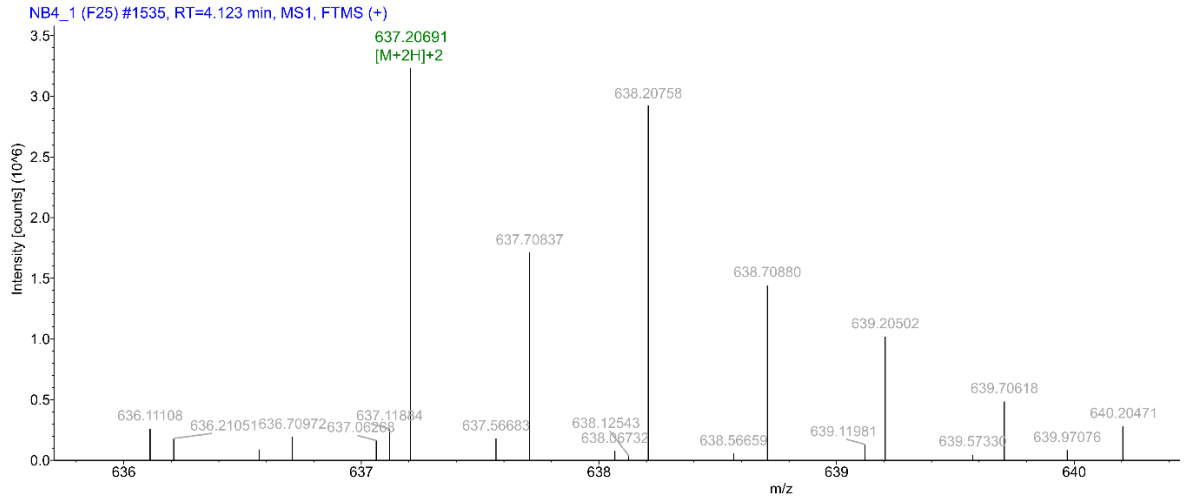

Figure 2. Mass spectra for the most abundant compound present in the *Kribbella* sp. SPB151 solvent extract fermented in PM01 media supplemented glutamic acid, eluting at 4.1 min. The most abundant ion was 637.21 m/z, representing the doubly charged ion $(M+2H)2^{+}$, with no known compound predicted from the available databases.

References

1. Bérdy, J., *Bioactive microbial metabolites: A personal view.* Journal of Antibiotics, 2005. 58(1): p. 1-26.
2. Donadio, S., P. Monciardini, and M. Sosio, *Polyketide synthases and nonribosomal peptide synthetases: The emerging view from bacterial genomics.* Natural Product Reports, 2007. 24(5): p. 1073-1079.
3. Benaud, N., et al., *Antarctic desert soil bacteria exhibit high novel natural product potential, evaluated through long-read genome sequencing and comparative genomics.* Environmental microbiology, 2020.
4. Pudasaini, S., et al., *Microbial Diversity of Browning Peninsula, Eastern Antarctica Revealed Using Molecular and Cultivation Methods.* Frontiers in Microbiology, 2017. 8(591).
5. Blin, K., et al., *antiSMASH 4.0-improvements in chemistry prediction and gene cluster boundary identification.* Nucleic Acids Research, 2017. 45(W1): p. W36-W41.
6. Challis, G.L., *Genome mining for novel natural product discovery.* Journal of medicinal chemistry, 2008. 51(9): p. 2618.
7. Ren, H., B. Wang, and H. Zhao, *Breaking the silence: new strategies for discovering novel natural products.* Current opinion in biotechnology., 2017. 48: p. 21-27.
8. Sivakala, K.K., et al., *Desert Environments Facilitate Unique Evolution of Biosynthetic Potential in Streptomyces.* Molecules : a journal of synthetic chemistry and natural product chemistry., 2021. 26(3): p. 588.
9. Kim, S.H. and B.-G. Kim, *NAD+-specific glutamate dehydrogenase (EC.1.4.1.2) in Streptomyces coelicolor; in vivo characterization and the implication for nutrient-dependent secondary metabolism.* Applied microbiology and biotechnology., 2016. 100(12): p. 5527-5536.
10. Walker, M.C. and W.A. van der Donk, *The many roles of glutamate in metabolism.* Journal of industrial microbiology & biotechnology., 2016. 43(2-3): p. 419-430.