

Master of Science Thesis

The University of Western Ontario



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February 8, 2021

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Chapter 1

Introduction

Antimicrobial resistance (AMR) is the natural phenomenon by which microorganisms acquire defences against harmful chemicals within their environment. All microorganisms have the potential to acquire AMR under various environments, including terrestrial and aquatic ecosystems, agri-food, hospitals, and the human body. In the context of an infection, resistant microorganisms limit available treatment options. Acquired antimicrobial resistance (AMR) is estimated to have caused 5,400 Canadian fatalities in 2015 — a number which is expected to climb to 13,700 deaths per year by 2050, while disproportionally affecting populations that are at a greater risk of acquiring infections (Council of Canadian Academies, [2019](#)).

AMR is acquired in response to any chemical that imposes a selective pressure on the microorganism. AMR is a huge burden to global healthcare systems, economies, and societies. As Buongermino Pereira et al. ([2020](#)) has said...

Biosolids are produced from the separation of wastewater into water and solids, followed by the treatment of the solids portion to reduce pathogens and odour. These biosolids may be treated using a combination of chemical, biological, or physical processes. The purpose of applying biosolids to agricultural soil is to improve the soil quality and fertility: Soil that is more fertile requires less inorganic fertilizer, which reduces the risk of runoff into adjacent water sources, and soil that has more organic matter has increased moisture retention and *better structure*. In Canada, biosolids are suitable for the growth of crops which require... such as cereals, hay, field corn, and soybeans (<http://www.omafra.gov.on.ca/english/nm/nasm/info/brochure.htm#7>). Currently, most wastewater treatment plants do not have technologies to remove micropollutants (such as antibiotics) from influent.

Chapter 2

Methods

2.0.1 Class 1 integron gene cassettes

2.0.1.1 DNA isolation from soil microplots

Total genomic DNA was isolated from 2019 Day 30 post-application soil microplots using the DNeasy PowerSoil Kit (Qiagen) with an input of 250 mg of dry soil. The DNA was eluted in 100 μ L of 10 mM Tris-HCl, and Nanodrop readings were taken to assess quality. The isolated DNA was stored at -20°C until it was used as template for gene cassette PCR.

2.0.1.2 Gene cassette PCR

Class 1 integron gene cassettes were amplified using primers and thermocycling conditions described by Stokes et al. (2001), except with 33 and 34 bp oligonucleotides ligated onto the 5' ends (Figure XX). The purpose of these oligonucleotides was to provide more distance between the 5' end of the PCR product (lost during library preparation) and the desired gene cassette sequence. DNA from each soil microplot was used as template for five technical replicates of 25 μ L reactions (125 μ L total). PCR product was analyzed by gel electrophoresis and visualized under UV light. Technical replicates were pooled together and PCR product was purified using the GenepHlow PCR Cleanup Kit (Geneaid). Cleaned PCR product was quantified using the Qubit dsDNA HS Assay Kit (Fisher Scientific). PCR and cleanup were repeated as described above using the same template DNA for a total of two technical replicates for each soil microplot.

2.0.1.3 Library preparation

The Nextera XT DNA Library Preparation Kit (Illumina) was used to prepare the library for sequencing by following the manufacturer's protocol. The indexed PCR product was quantified using the Qubit dsDNA HS Assay Kit and sized using the High Sensitivity DNA Kit on a Bioanalyzer 2100 (Agilent). Individual libraries were diluted to 10 nM, and 15 μ L of each diluted library was pooled into a single library for 2 x 125 bp sequencing at Sick Kid's Hospital (Toronto, ON) on a HiSeq 2500 (Illumina).

2.0.1.4 Data processing

Adapters were trimmed using CUTADAPT (v2.10) and the sequence quality was assessed using FastQC (v0.11.9) and MultiQC (v1.8) (Martin, 2011; Andrews, 2010; Ewels et al., 2016). The adapter-trimmed reads were assembled into contigs using MEGAHIT (v1.2.9) with default options (D. Li et al., 2015). Contigs that didn't contain the terminal 9 bp of each primer sequence were removed using BBduk with options

copyundefined=t rcomp=f mm=f k=9. After filtering out contigs that didn't contain the terminal primer sequences, the metagenomic assemblies of each sample were merged into a single assembly, and the open reading frames (**ORFs**) of the remaining contigs were called using Prokka (Bushnell, 2020; Seemann, 2014). The ORFs were concatenated into a non-redundant protein database at 97% identity using CD-HIT (vX.X) and then reverse translated back to nucleotides (W. Li and Godzik, 2006; Fu et al., 2012). To obtain fold-coverages, the adapter-trimmed reads of each sample were mapped back onto the concatenated nucleotide database of ORFs using BMap (vX.X) (Bushnell, 2020). ORFs were then annotated using CARD-RGI with options `--low_quality --include_loose --clean -t protein (vX.X)` for ARGs and eggNOG-mapper (v2.0) for functional categories (Alcock et al., 2019; Huerta-Cepas, Forslund, et al., 2017; Huerta-Cepas, Szklarczyk, et al., 2019). CARD annotations with a percentage identity less than the mean percentage identity of all ARG hits, along with hits of less than 20% coverage to the subject ARG sequence were discarded.

2.0.1.5 Compositional data analysis and statistics

The fold-coverages of technical replicates were combined. A non-zero count of 0.1 was added to the resulting feature table prior to centred log-ratio (**CLR**) transformation, although we acknowledge that a non-zero count could lead to **x, y, and z errors**. The CLR transformation was performed using the base 2 logarithm, thereby producing relative abundances where the difference between two values is the fold-change between them. The Kruskal-Wallis test was used to determine if there were differences between the median relative abundances of class 1 integron gene cassettes ORFs between treatment groups ($P < 0.05$), followed by Dunn's post-hoc test for multiple comparisons ($P < 0.05$) with Bonferroni correction.

Chapter 3

Results

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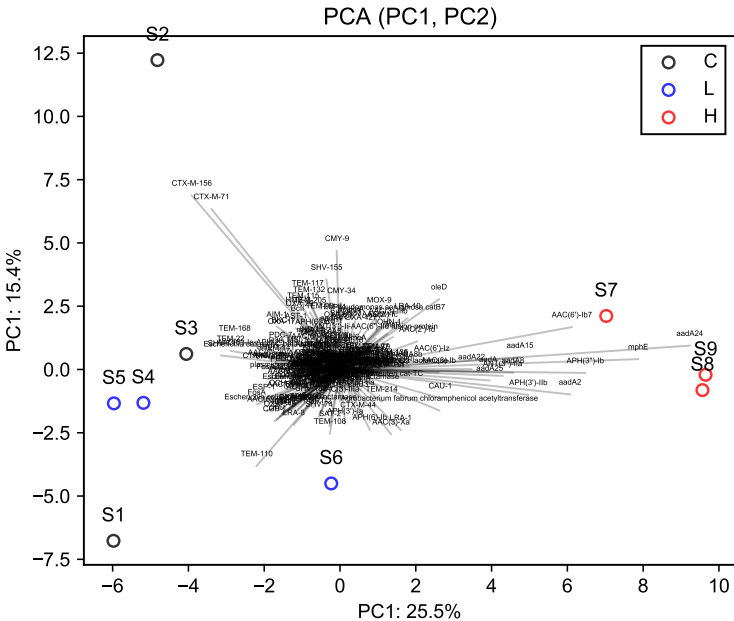


Figure 3.1 PCA ordination biplot of antibiotic target replacement mechanisms in soil unexposed (0 mg/kg) and exposed (0.1, 10 mg/kg) to macrolide antibiotics over ten years. A pseudocount of 0.1 was added to the fold-coverage table and fold-coverages were centered log-ratio (**CLR**) transformed to obtain relative abundances centered on the geometric mean of each sample.

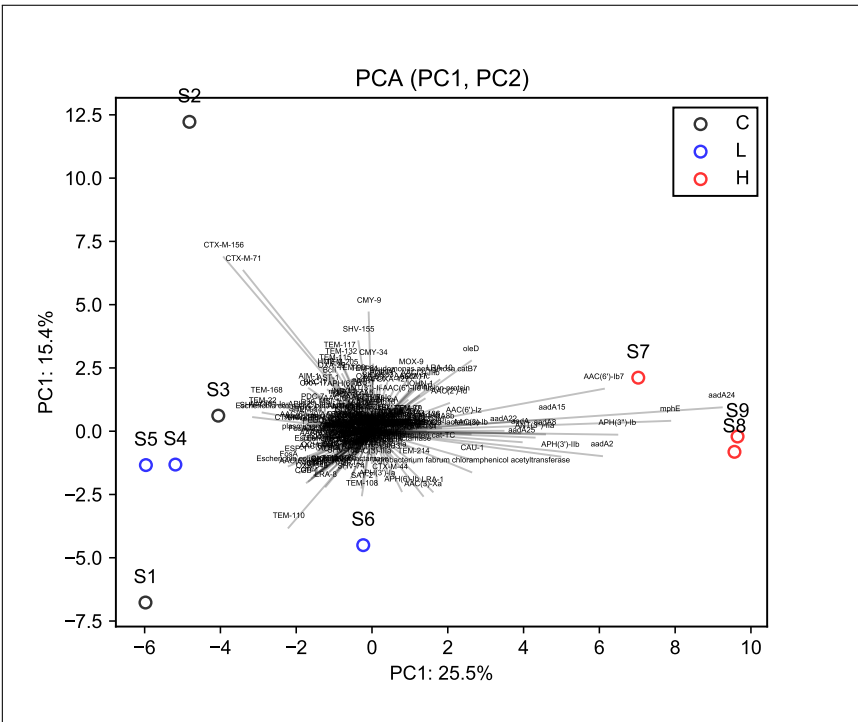


Figure 3.2 Same as 3.1 but with a boxed border.

Chapter 4

Discussion

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Chapter 5

Conclusion

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