

Environmental distribution of genes for type II polyketide biosynthesis pathways

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Introduction:

- Antibiotic resistance is a major healthcare threat
- Discovery of antibiotic natural products, like type II polyketides (eg. tetracycline) may help address this problem
- Little is known about the geographical distribution (biogeography) of the biosynthetic genes that encode type II polyketides
- Using biogeography to guide bioprospecting will increase the rate of new antibiotic discovery.

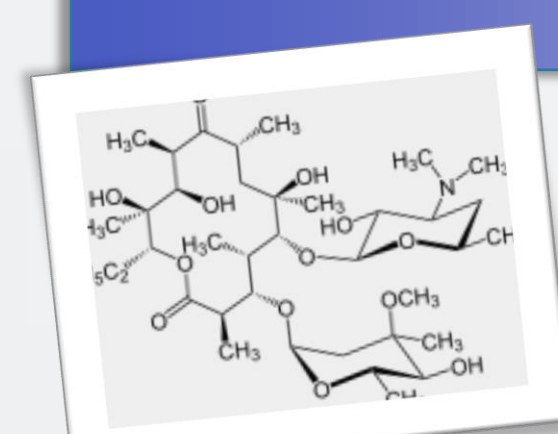


Figure 1. Erythromycin (antibacterial)¹

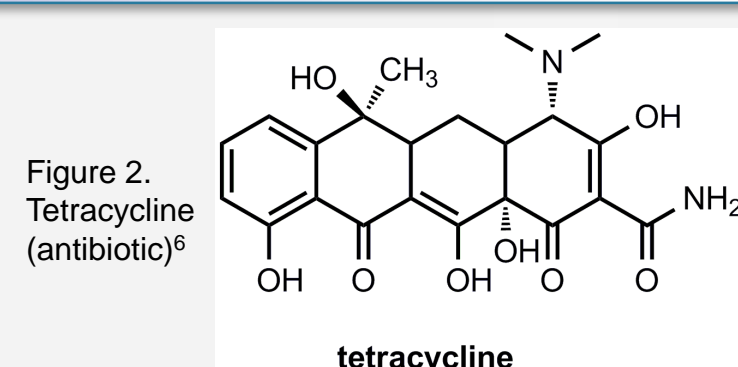


Figure 2. Tetracycline (antibiotic)²

Methodology:

Washing the samples

This experiment compares two extraction methods. The second differed by scrapping the concentrated surface of the soil pellet and rewashing it. Soil was sampled from the Rideau Canal, Ottawa-Gatineau area and a compost bin.

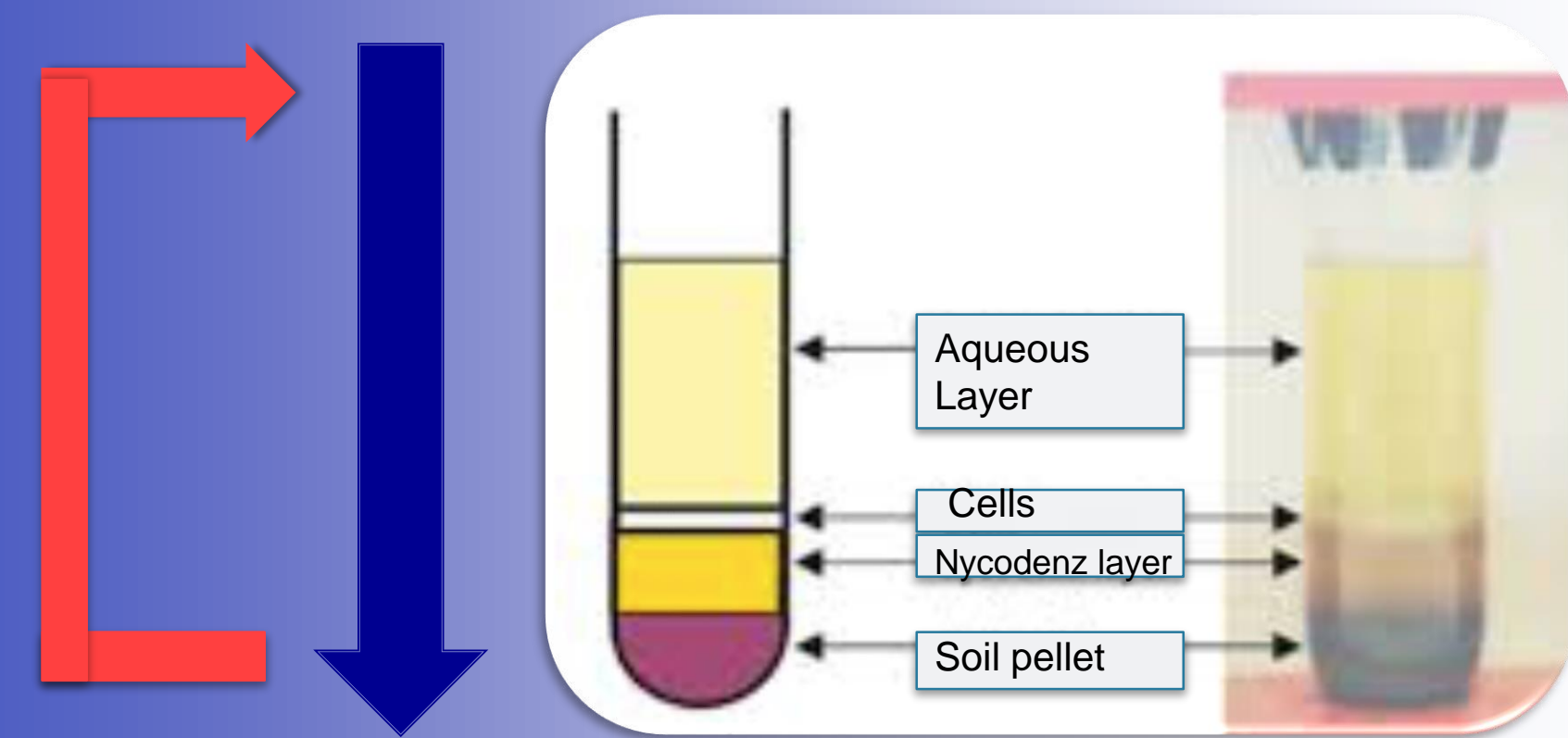


Figure 3. Bacterial extraction tube after centrifugation¹

eDNA Extraction

A lysis DNA extraction was performed.

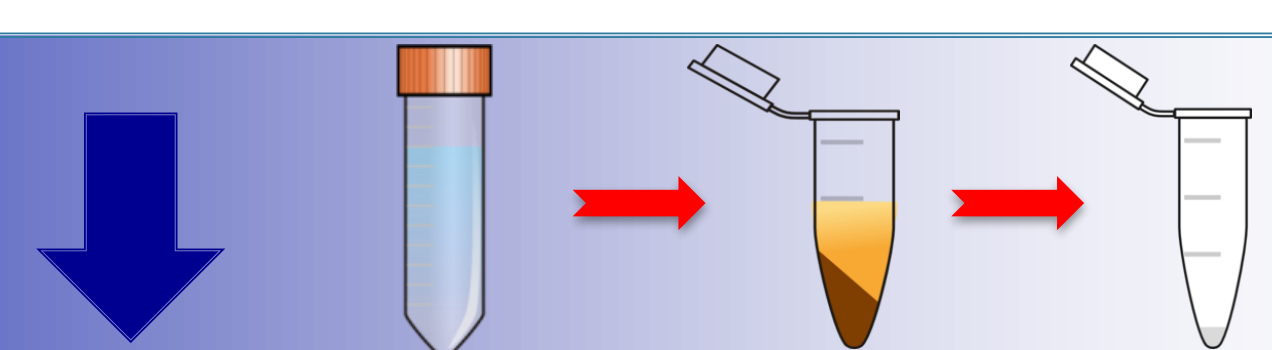


Figure 4. Methodology DNA extraction²

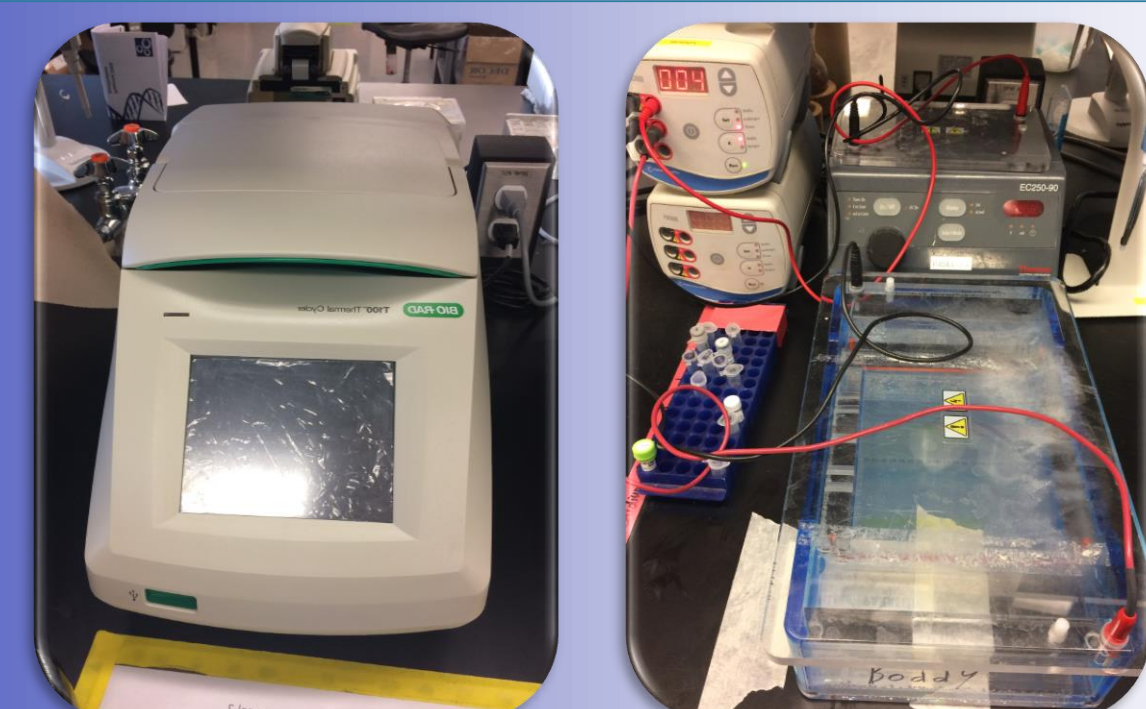
Pulse Field Gel Electrophoresis

A pulse field gel electrophoresis was done to compare the relative size and concentration of eDNA present in all samples. It was also used to compare the two washing methods.



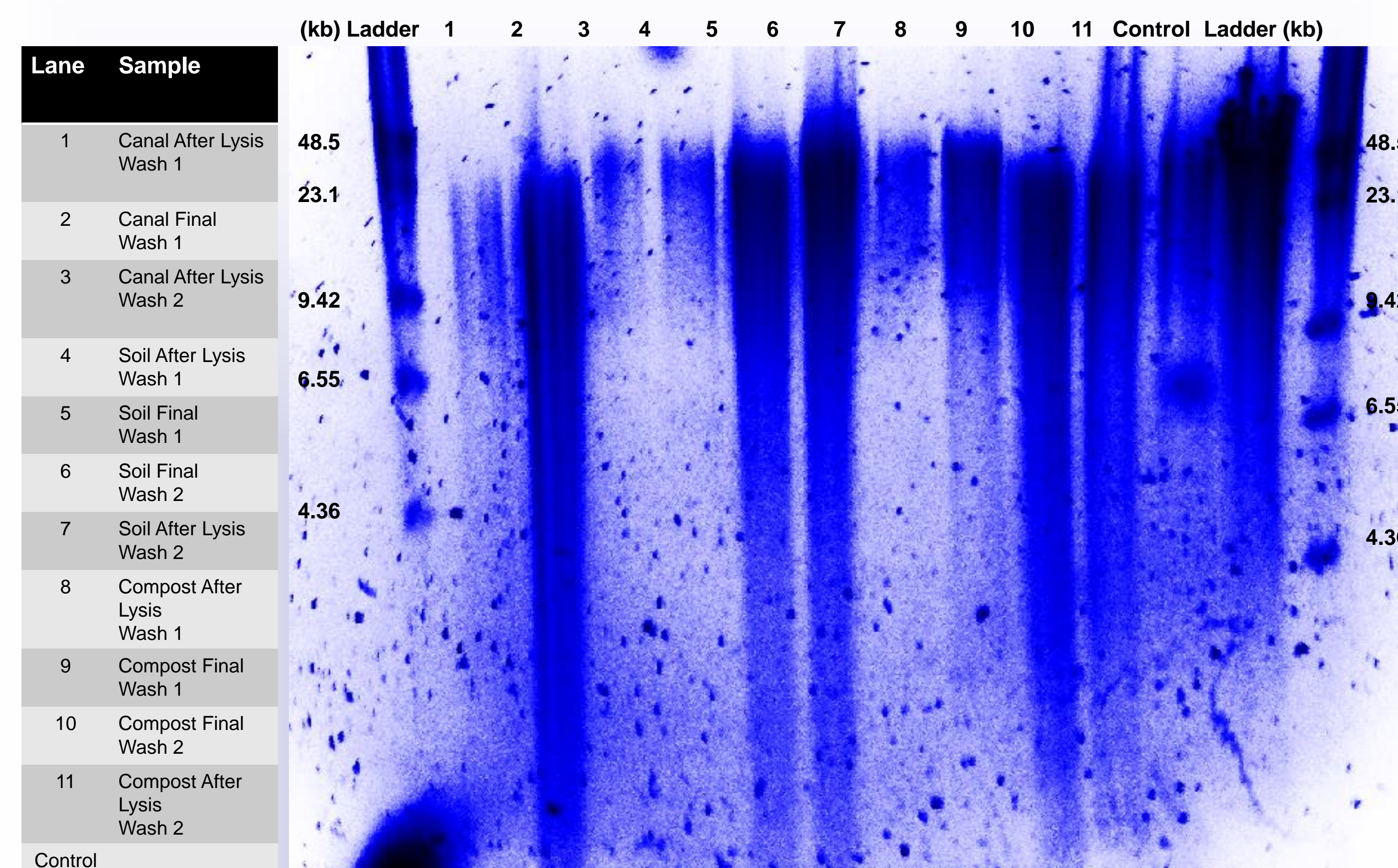
PCR

The eDNA samples were probed with specially designed primers for genes encoding for essential enzymes involved in polyketide pathways. Two PCRs were ran one Mastermix (primers A7R, A3, K1, M6R)¹ and one Type II (primers 540F, 1100R)².



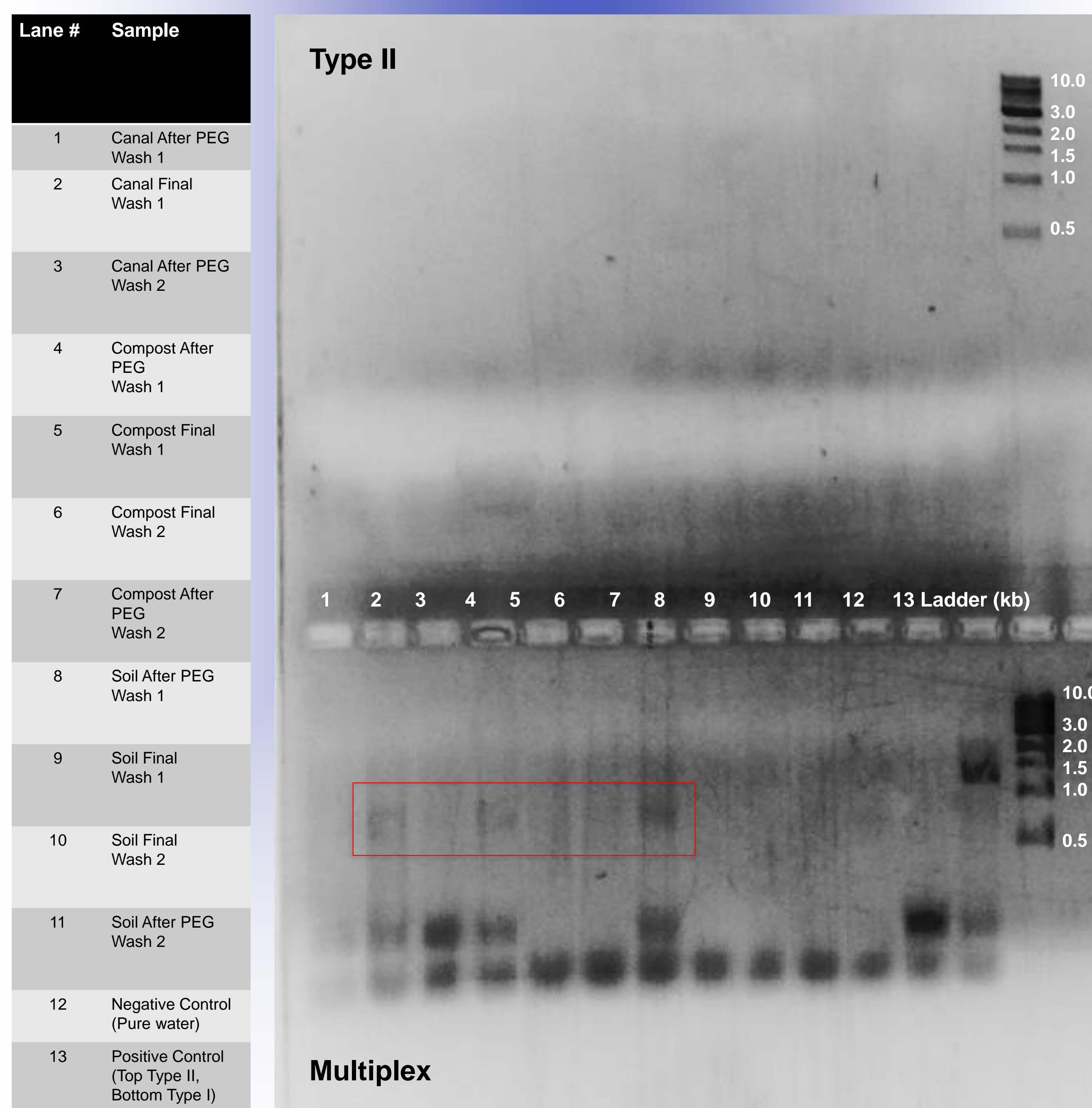
Results:

Results from gel electrophoresis



Results from PCR

The samples were probed by PCR using Multiplex (looking for type I and non-ribosomal peptides) and Type II (type II polyketides) primers. The estimated band size for a positive result for non-ribosomal, type I and type II is, respectfully, around 700-800 bp¹, 1.2 kb¹ and 554 bp².



Discussion:

- Based on the results there is a very slight difference between the sizes and concentrations of the first and second washes.
- Analyzing the results from the PCR (Multiplex) the gel shows bands at around 720 bp in the canal final wash 1 and compost after PEG wash 1 and 2. This is within the range of 700-800 bp, which corresponds to non-ribosomal peptides.
- There are no bands present around 1.2kb for the Multiplex. This means that there was no positive test result for Type I polyketides in any of the samples.
- The PCR results for Type II are inconclusive, this is most likely due to a problem with the execution of the PCR.

Conclusion:

- The results show that the canal and compost soil samples contain the enzymes essential for the biosynthesis of non-ribosomal peptides.
- The next step would be to re-probe the samples with Type II primers, since the first set of data was inconclusive.
- Amplicons generated from the PCR reactions will be sent for next generation sequencing and will be analyzed by community fingerprinting. Promising eDNA samples will be used to generate eDNA libraries for new natural product discovery.

References:

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