Monitoring putative desulfonation genes involved in PFAS degradation by *Gordonia* sp. NB4-1Y using quantitative PCR assays

Honours Proposal

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Introduction

Perfluoroalkyl and polyfluoroalkyl substances, PFAS, are a large group of synthetic compounds characterized by long fluorinated carbon chains, making them incredibly stable and able to easily accumulate in the environment (Bottos et al., 2020). The sulfonated PFAS, 6:2 fluorotelomer (6:2 FTSA) and 6:2 fluorotelomer sulfonamidoalkyl betaine (6:2 FTAB), have been used as ingredients in aqueous film-forming foams (AFFFs) used for hydrocarbon fire suppression (Yang et al., 2022). The prevalence of 6:2 FTSA and 6:2 FTAB in the environment can be attributed to the long-term use of AFFFs in firefighting and firefighting training or from the biotransformation of PFAS precursors (Yang et al., 2022). Due to their toxicity and bioaccumulation potential, researching potential strategies for PFAS degradation is important.

Gordonia sp. strain NB4-1Y, is an aerobic, gram-negative bacterium, originally isolated from vermicompost. NB4-1Y has been previously reported to degrade PFAS into numerous breakdown products (Shaw et al., 2019). Specifically, NB4-1Y has been reported to use 6:2 FTSA and 6:2 FTAB as sulfur sources for growth. Proteins that may be involved in this metabolism have been identified, (Bottos et al., 2020), and, if they are involved, the genes coding for them should be expressed more when NB4-1Y is exposed to PFAS compared to $MgSO_4$.

Previously, six sets of primers and probes were designed and successfully amplified gene regions of interest, coding for a dimethylsulfone monooxygenase (*snfG*), several LLM class flavin-dependent oxidoreductases, and an acyl-CoA dehydrogenase family protein, as confirmed by end-point PCR and Sanger sequencing. These six genes of interest, thought to be involved in PFAS metabolism by NB4-1Y, were selected based on previous transcriptome (Bottos et al., 2020) and proteome (McAmmond et al., unpublished) data. Specifically, dimethylsulfone monooxygenase *sfnG* was selected as a gene target because it may desulfonate 6:2 FTSA. Four LLM class flavin-dependent oxidoreductase genes were selected because these versatile enzymes are known to catalyze a variety of reactions including hydroxylations, epoxidations, and Baeyer–Villiger rearrangements (Maier et al., 2015). One gene coding for an acyl-CoA dehydrogenase family protein was also selected as a target, because there has been some evidence acetyl-CoA adducts could be formed during PFAS metabolism (Mothersole et al., 2024a; Mothersole et al., 2024b). Previous studies have also suspected monooxygenases to be involved in the biotransformation of ether PFAS through oxidation pathways (Jin et al., 2023). Through proteomic analysis, Mendez et al. (2022) observed alkanesulfonate monooxygenase *ssuABCD* upregulation in *Dietzia aurantiaca* sp.

strain J3 when exposed to 6:2 FTSA as a sole sulfur source. Higher expression levels of alkanesulfonate monooxygenase has been observed in *Rhodococcus jostii* RH1 grown with 6:2 FTSA as a sole sulfur source (Yang et al., 2022). It's possible the desulfonation of 6:2 FTSA and 6:2 FTAB by NB4-1Y could be catalyzed by many different enzymes such as an alkane monooxygenase, LLM class flavin oxidoreductase, and dimethylsulfone monooxygenase.

Previously, a method was developed to quantify the expression levels for these genes of interest using reverse transcriptase quantitative digital polymerase chain reaction (dPCR). Although similar methods have been used to study plant and animal systems (Hruz et al., 2011; Zmienko et al. 2015), limited work to date has investigated the use of dPCR to quantify gene expression in bacterial systems. Initial qPCR and dPCR results showed expression levels of genes of interest in NB4-1Y samples grown in 6:2 FTSA were higher compared to $MgSO_4$ and no sulfur source controls, thus supporting the stated hypothesis. In the proposed study the previously developed assays will be used to quantify mRNA levels over time when NB4-1Y is provided with 6:2 FTAB, 6:2 FTSA, $MgSO_4$, or 1-octanesulfonate, a non-fluorinated structural analogue of 6:2 FTSA, as sole sources of sulfur. Quantification of mRNA will be carried out using quantitative PCR (qPCR) and dPCR. If the gene targets are expressed more in samples exposed to PFAS then the hypothesis that these genes of interest contribute to the ability of NB4-1Y to metabolize PFAS will be further supported.

Objectives

The proposed research to be conducted will provide further insight to PFAS metabolism by NB4-1Y through quantification of mRNA levels of putative desulfonation genes over time when NB4-1Y is provided with various sulfur sources. Our project will expand on previous research done to describe the molecular basis of PFAS metabolism by NB4-1Y.

Materials and Methods

Time course experiments will be carried out starting with pure NB4-1Y culture stocks streaked onto nutrient agar plates that will be incubated at 30 °C until isolated colonies grow. Single isolated colonies from the nutrient agar plate will be used to prepare inoculum cultures in autoclaved 50-mL glass tubes containing 20 mL sulfur-free acetate (SFA) medium and incubated on a tissue culture roller drum to 150 rotations per minute at 30 °C. Experimental cultures will be prepared with 200 mL SFA media in autoclaved 500-mL Erlenmeyer flasks. All experimental treatments, 160 μM 6:2 FTSA sterile control, no sulfur source control, 160 μM 6:2 FTSA, 160 μM 6:2 FTAB, and 160 μM $MgSO_4$, will be prepared in triplicate. Experimental cultures will be inoculated with 1 % (v/v) inoculum and incubated in an orbital shaker at 30 °C with 150 rotations per minute. At each time point, optical density at 600 nm (OD_{600nm}) and fluoride release will be measured, and cell pellets will be harvested following the biomass collection and RNA stabilization procedure described in Bottos et al., 2020.

RNA extractions will be performed using NB4-1Y pellets, stored at – 80 °C, obtained from the time course experiment. RNA will be extracted following the RNA extraction protocol outlined in Bottos et al., 2020. To minimize genomic DNA contamination two DNase treatments will be

performed. Qubit High Sensitivity RNA and DNA kits will be used to determine DNA and RNA concentrations after extraction. Absence of DNA contamination will be confirmed by end-point PCR and gel electrophoresis.

To synthesize cDNA from extracted RNA samples, the reverse transcription protocol for SuperScript IV VILO Master Mix with ezDNase enzyme will be followed. A no reverse transcriptase (NRT) control will also be prepared using the SuperScript IV VILO No RT Control mix. Quantification of mRNA over time will be carried out using qPCR and dPCR technologies. Data will be compared using multivariate analysis of variance (MANOVA).

Permits

This project does not require any permits.

Expected Results

This research project would give a better understanding of PFAS metabolism by NB4-1Y by quantifying mRNA, qPCR and dPCR technology using previously designed and validated primers and probes, over time when NB4-1Y is provided with various sulfur sources. Comparisons of these data to transcriptome and proteome data, will inform recommendations made to guide future protein purification and characterization experiments.

Timeline

August 2025 – September 2025

- Literature review
- Order custom assays for qPCR and dPCR assays

September 2025 – October 2025

- Revised thesis proposal
- Time course experiments: measure $OD_{600\,nm}$ and fluoride release over time and harvest cell pellets

October 2025 – January 2026

- Extract RNA from NB4-1Y cell pellets harvested during time course experiment.
- Reverse transcriptase cDNA synthesis
- Quantification of mRNA using qPCR
- Quantification of mRNA using dPCR

January 2026

- Update presentation
- Statistical analysis using multivariate analysis of variance (MANOVA).

February 2026

- Write thesis
- Prepare poster for presentations

March 2026 – April 2026

- Poster presentations: TRU Undergraduate Conference and SUPER Conference

April 2026

- Thesis defence

Budget

Item	Cost
RNA extraction and cleanup	2500.00
RNA and DNA quantification and quality control	250.00
Fluoride quantification	250.00
qPCR and dPCR assay chemistry, plates and chips	5500.00
Culture media	100.00
TOTAL COST	**8600.00**

All expenses for this Honours project will be covered by the NSERC Discovery Grant of Dr. Jonathan Van Hamme.

Literature Sources

Bottos E., AL-shabib E., Shaw D., McAmmond B., Sharma A., Suchan D., Cameron A., Van Hamme J.D. (2020). Transcriptomic response of *Gordonia* sp. strain NB4-1Y when provided with 6:2 fluorotelomer sulfonamidoalkyl betaine or 6:2 fluorotelomer sulfonate as sole sulfur source. *Biodegradation*. 31: 407-422. https://doi.org/10.1007/s10532-020-09917-8.

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Shaw D.M.J., Munoz G., Bottos E.M., Duy S.V., Sauvé S., Liu J., Van Hamme J.D. (2019). Degradation and defluorination of 6:2 fluorotelomer sulfonamidoalkyl betaine and 6:2 fluorotelomer sulfonate by *Gordonia* sp. strain NB4-1Y under sulfur-limiting conditions. *Science of the Total Environment*. 647: 690-698. https://doi.org/10.1016/j.scitotenv.2018.08.012.

Yang S.H., Shi Y., Strynar M., Chu K.H. (2022). Desulfonation and defluorination of 6:2 fluorotelomer sulfonic acid (6:2 FTSA) by *Rhodococcus jostii* RHA1: Carbon and sulfur sources, enzymes, and pathways. *Journal of Hazardous Materials*. 423: Part A. https://doi.org/10.1016/j.jhazmat.2021.127052.

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