Title:

Quantitative PCR assay development for monitoring genes suspected of contributing to PFAS degradation by *Gordonia* sp. strain NB4-1Y

Introduction and Hypotheses:

This project is a continuation of previous research to investigate the ability of *Gordonia* sp. strain NB4-1Y to degrade environmentally concerning perfluoroalkyl and polyfluoroalkyl substances, PFAS. The end goal is to describe the molecular basis of PFAS metabolism in NB4-1Y, ultimately characterizing individual proteins responsible for generating the numerous breakdown products identified to date (Shaw et al., 2019). For this project, six genes of interest have been selected as targets for reverse transcriptase quantitative digital polymerase chain reaction (dPCR) assay development. These selections are based on published transcriptome data (Bottos et al., 2020) and recently acquired proteome data (McAmmond et al., unpublished). 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 the versatile enzymes are known to catalyze a variety of reactions including hydroxylations, epoxidations, and Baeyer–Villiger rearrangements (Bottos et al., 2020). One gene coding for a acyl-CoA dehydrogenase family protein is also a target, because there has been some evidence acetyl-CoA adducts could be formed during PFAS metabolism (Bottos et al., 2020). Once validated, the assays will be used to quantify mRNA levels over time when NB4-1Y is provided with PFAS, MgSO4 and octanesulfonate as sole sources of sulfur. 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 supported.

Methods:

Using the most recent, and complete, version of the NB4-1Y genome (Ony et al. 2024, unpublished, https://www.ncbi.nlm.nih.gov/nuccore/CP132196.1/), forward and reverse primer pairs and dPCR probes will be designed using Geneious Prime (Dotmatics, 2024) and Primer Express (Thermo Fisher Scientific, 2019) software for the six genes of interest. Once designed, the primer pairs will be tested and validated using a combination of end-point PCR to optimize PCR conditions and Sanger sequencing to confirm PCR amplicon identity. If the primers amplify the correct gene regions, a time course experiment with NB4-1Y provided with PFAS (6:2 fluorotelomer sulfonic acid; 6:2 FTSA), $MgSO_4$ or octanesulfonate will be conducted. For this. mRNA will be extracted and purified prior to using reverse transcriptase to generate cDNA

which will be used for dPCR quantification. Experiments will be carried out in triplicate or better and data will be compared using multivariate analysis of variance (MANOVA). Results will be compared to the previously obtained transcriptome and proteome data, and recommendations will be made to guide future protein purification and characterization experiments.

Timeline:

Work on this project will begin January 2025 and will be continued throughout the semester. The first week will be spent reading literature on dPCR and primer design to determine the characteristics needed to make successful and gene specific primers. One week will be spent designing the primers and probes for the targets of interest. Primer testing using end-point PCR and Sanger sequencing will take two weeks. Primers and probes will be ordered around the end of January and 2-3 weeks will be spent carrying out NB4-1Y time course experiments on the various sulfur sources in order to obtain cell pellets for mRNA extraction. RNA extraction and purification will take about 1-2 weeks to complete. The next two weeks will be spent converting the RNA to cDNA and executing dPCR. The last few weeks of March will be spent analyzing data, writing a report, and preparing a poster to be presented at the TRU Undergraduate Research and Innovation Conference. This work, along with a bioinformatic summary of the NB4-1Y full genome, will be presented at the 2025 Annual Meeting of the Canadian Society of Microbiologists in Montreal.

References:

Bottos E, AL-shabib E, Shaw D, McAmmond B, Sharma A, Suchan D, Cameron A, Van Hamme J. 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.

Dotmatics. (2024) *Geneious Prime* (Version 2024.0) [Computer program]. Available at: https://www.geneious.com/updates (accessed Oct. 13, 2024).

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.

Thermo Fisher Scientific. (2019) *Primer Express* (Version 3.0.1) [Computer program]. Available at:https://www.thermofisher.com/ca/en/home/technical-resources/software-downloads/primer-express-software-download.html (accessed Oct. 13, 2024).