

# The provenance of microorganisms adapted to extreme salinity, extreme temperature, and toxic metals within the Montney shale formation.

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## Abstract

### Introduction

Shale oil reservoirs are hypothesized to be sterile due to the extremely high temperature, pressure and salinity within these formations (Evans et al. 2018). High concentrations of toxic metals also pose challenges that demand specific microbial adaptations (Boyd and Barkay 2012, White and Gadd 1998, Ben Fekih et al. 2018). While some microorganisms are introduced into and are selected for within shale formations during hydraulic fracturing, the possibility that certain microorganisms are pre-existing inhabitants of these formations is less clear.

Here, we followed the microbial diversity of input and output fluids injected into a Montney formation shale reservoir to assess the distribution and transport of microbial populations during hydraulic fracturing. Enrichment cultures distinguished various metabolisms in the microbial populations found in different sample types, and adaptations allowing them to colonize such niches.

### Material and methods

Fracturing fluid, drilling muds (3302 m, 3350 m and 3400 m depths), shale cuttings (rinsed from the drillings muds), shale core plugs and produced water samples (12-month period) were sampled from a Montney shale oil reservoir. Microbial community compositions were analyzed by amplicon sequencing. Metal content was analyzed by inductively coupled plasma-mass spectrophotometry. High salinity enrichments at 90°C of the drilling muds or rinsed shale samples were set up in triplicate and amended with glucose and guar gum (a mannose/galactose-based polymer used during hydraulic fracturing). Sugars were measured through spectrophotometric assays. Metagenomic analyses were performed to assess microbial gene content.

## Results/Discussion

- Provenance of microorganisms from the Montney shale formation

Input fluids (fracturing fluid, drilling muds) were revealed to be the likely source of most of the microbial diversity. However, some microorganisms were only detected in the subsurface samples. ASVs affiliated with *Aurantimonas*, *Caminicella*, BRH-c8a (Family *Desulfallas*) and *Geotoga* exhibited occurrence patterns consistent with being derived from subsurface shale formations. *Geotoga* has only ever been reported from oil reservoirs (Semenova et al. 2020). Analysis of produced water revealed ASVs from these groups increasing in abundance during hydraulic fracturing operations, suggesting selective pressure from oil reservoir conditions (e.g., toxic metal presence, input of saline water, temperature and pressure fluctuations). Incubations set up from drilling muds showed a preference for glucose while incubations of the rinsed shale cuttings showed a microbial preference for guar gum (i.e., mannose production; Fig. 0), reinforcing the presence of different populations being derived from surface and subsurface samples.

- Adaptations for life in Montney shale

When considering adaptations of microorganisms for environmental conditions found in oil reservoirs, it is relevant to note the presence of toxic metals such as arsenic, cadmium and mercury. Levels of all three metals were found to vary over time within the 28-day shale microbial enrichments and 12-month produced water time course analyses (Suppl. material 1). Metagenomics revealed various genes for the internalization and metabolism of all three metals within the shale microbiome (i.e., arsenate reductases, arsenite transporters, metallothioneins, mercuric reductases).

In conclusion, the results of this study suggest that shale reservoirs thus might not be sterile environments, and host microorganisms are able to contend with major perturbations.

## Keywords

Hydraulic fracturing, shale, Montney formation, cadmium, arsenic, mercury, produced water, extremophile

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## Conflicts of interest

The authors have declared that no competing interests exist.

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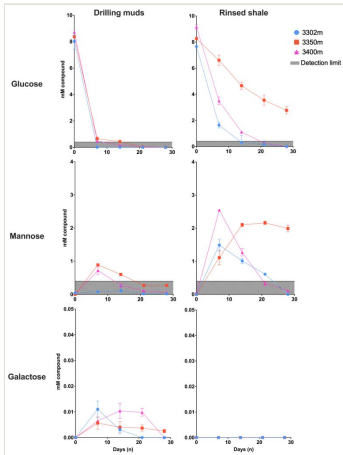


Figure 1.

Levels of glucose, mannose and galactose in enrichments of drillings muds and rinsed shale (3302 m, 3350 m, 3400 m depth) amended with glucose and guar gum and incubated at 90°C for 28 days. Mannose and galactose are produced via hydrolysis of guar gum.

## Supplementary material

### **Suppl. material 1: Table S1: Measurements of arsenic, cadmium and mercury within various Montney formation samples.**

**Authors:** Gabrielle Scheffer

**Data type:** Concentrations of toxic metals within the Montney shale formation

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