Enviro-coli test: Development of a method for differentiating fecal *E. coli* versus naturally occurring (environmental) *E. coli* in surface water

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Research & Science Note 8

Background

The MRWCC initiated a fecal source tracking project in cooperation with Alberta Agriculture and Rural Development (2012) to characterize sources of fecal *E. coli* bacteria within the Milk River.

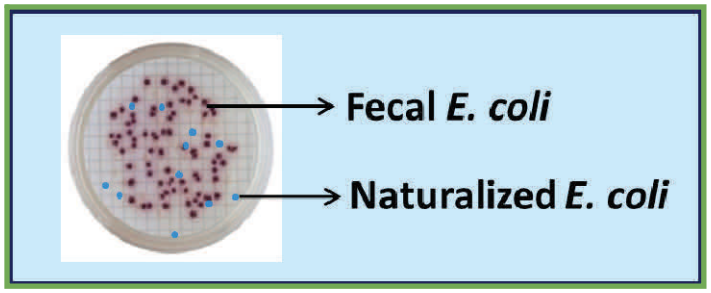
Using microbial source tracking methods *(see: Research and Science Note7)* it was determined that approximately 34% of the *E. coli* in the Milk River was attributed to cattle and cliff swallows. This suggests that multiple host sources contribute to fecal contamination in the Milk River.

Interestingly, approximately 50% of the *E. coli* did not have matching DNA fingerprints to any of the twenty different host sources that were tested. It is likely that a portion of these *E. coli* are from a population of '**naturalized' *E. coli*** that has adapted to the water environment, and can survive long-term and possibly even grow within the turbid conditions of the Milk River.

Naturalized *E. coli* have been known to exist for over a decade. The key concern with these *E. coli* is that they are not an accurate indicator of recent fecal contamination. Their presence in a watershed can lead to the overestimation of fecal contamination, which can in turn impact recreational use. In the Milk River, as temperatures increase and flows reduce in late summer , these naturalized *E. coli* may become more abundant.

Current water quality tests do not differentiate between *E. coli* from human and animal feces and those that have become naturalized in surface water. It is therefore difficult to determine whether *E. coli* levels are high due to actual fecal contamination or whether they may have been overestimated due to the presence of naturalize *E. coli*.

This project will lead to development of a method that will allow us to distinguish between fecal *E. coli* and naturalized *E. coli*. This method has been designated as the 'Enviro-coli' test.

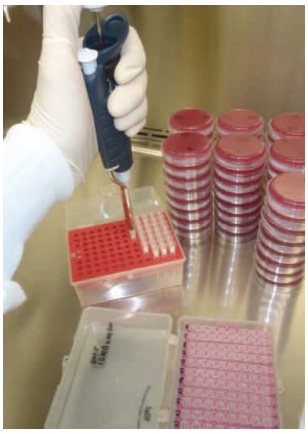


Objective:* develop a practical, rapid, and broadly-applicable test that will discriminate between fecal and naturalized E. coli *in surface water

Project Development

There are three main steps that need to be completed during the development of the Enviro-coli test.

All steps involves significant lab work and analysis.



1) Isolate selection, whole genome sequencing

2) Data analysis, genetic marker (gene) selection

3) Design, optimize, test gene assays on ~400 isolates

***Escherichia coli* (*E. coli*):** A type of fecal indicator bacteria used to quantify fecal contamination

***E. coli* are generally not harmful, but if high levels of these organisms are found in water, it means that other pathogenic bacteria that are harmful to humans may also be present**

Study

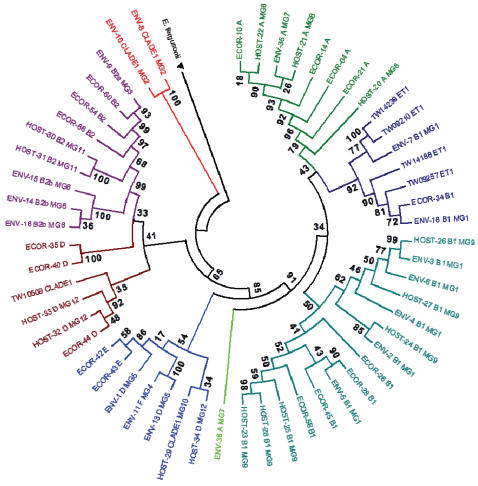
1) Whole Genome Sequencing (WGS) of naturalized and fecal *E. coli*

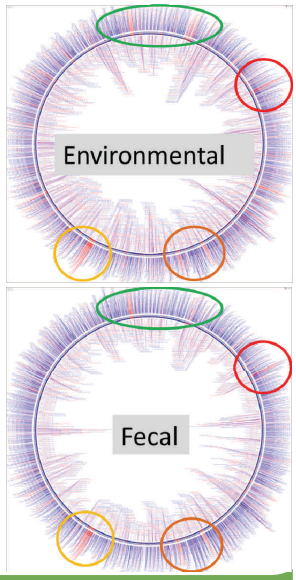
•Genes help determine which environment a particular *E. coli* can survive in.
•Naturalized and fecal bacteria likely have different genes that help them survive in the different places they inhabit.
•WGS is used to identify the types of genes that each *E. coli* has.
•WGS can be used to identify genes that are specific or enriched in fecal *E. coli* versus naturalized *E. coli.*

2) Data analysis, genetic marker (gene) selection

By comparing the genes from naturalized (environmental) *E. coli* and fecal *E. coli*, we can identify which genes are shared between both types of *E. coli* (these are colored blue in the lower figure) and those that are different between the two types of *E. coli* (these are colored red in the lower figure).

This study identified more than 28 different gene families that were different between naturalized and fecal *E. coli*. One of the genes that is more prevalent in naturalized *E. coli* is a gene that helps the *E. coli* acquire iron, which is necessary for growth, but is generally present in low concentrations in river water. On the other hand, fecal *E. coli* tend to have more genes that help them interact with their hosts intestine!





3) Design, optimize, test gene assays on ~400 *E. coli* isolates using PCR

•Only a few fecal *E. coli* and naturalized *E. coli* genomes were sequenced as this is a very costly step.
• Once we identified genes that were different between naturalized *E. coli* and fecal *E. coli* , PCR tests for these genes were developed. The advantage of PCR tests is that they are cheaper, easier, and quicker than Whole Genome Sequencing (WGS).
• The PCR tests were then used to screen ~400 different *E. coli* isolated from various hosts and the Milk River to see if they had the genes.

This step helped confirm that the genes that were identified in step 2 were actually good genetic markers for either fecal *E. coli* or naturalized *E. coli.*

Conclusion

The key concern with naturalized *E. coli* is that they are not an accurate indicator of recent fecal contamination. Their presence in a watershed can lead to an overestimation of fecal contamination, which can in turn impact recreational use. Current water quality tests do not differentiate between *E. coli* from animal feces and those that have become naturalized in the surface water environment. It is therefore difficult to determine whether *E. coli* levels are high due to actual fecal contamination or whether they may have been overestimated due to the presence of naturalized *E. coli*.

Ideally, water quality tests would measure ONLY *E. coli* from fecal sources. The Enviro-coli test will greatly improve the ability to specifically assess fecal contamination in surface water and will be broadly applicable to water quality monitoring.

The potential applications of the test for MRWCC's environmental work related to mitigating impacts of agricultural practices on water quality within the Milk River watershed is significant. In particular, this test will ensure that environmental *E. coli* are not mistaken for fecal contamination, the latter of which may require extensive mitigation strategies to solve. This will allow livestock producers to focus their energy and funding into successful mitigation projects that protect the aquatic environment and thereby strengthen the livestock industry's social license to operate.

Enviro-coli test: practical, RAPID, and broadly-applicable

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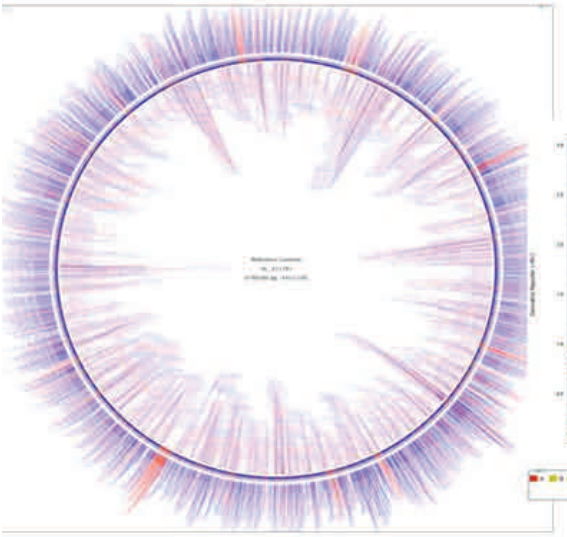
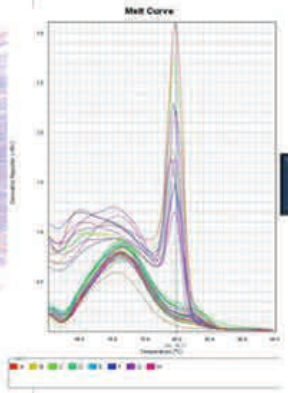


Genome map showing differences between naturalized *E. coli* and fecal *E. coli*

Each colored line ('hair') represents one gene in the *E. coli* genome (there are ~4300 genes represented around the circle). The blue genes are common to both types of *E. coli* while the red genes are present ONLY in the fecal *E. coli* (and missing from the naturalized *E. coli*).



Screening *E. coli* isolates for specific genes using PCR melt-curve analysis

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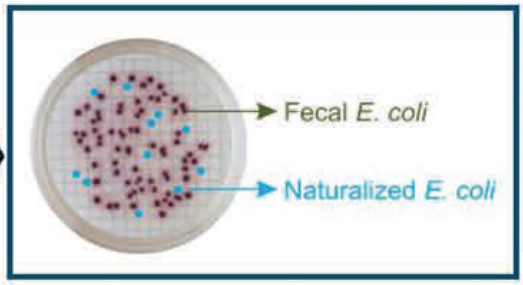

Hypothetical example of the Enviro-coli test.

E. coli from fecal sources appear as red-colored, while those from environmental sources ('naturalized') appear blue-colored.

This is because naturalized *E. coli* have a specific gene that allows them to use certain nutrients which give them the blue-coloration, whereas fecal *E. coli* do not.

Next Steps

Method implementation and validation ★Technology transfer and industry usage ★ Watershed monitoring

Ultimately, the Enviro-coli test will improve the specificity of tests for fecal contamination in surface water

- This science-based approach will ensure more accurate assessment of fecal contamination, while also ensuring accurate assessment of human health risks by identifying only those *E. coli* that are relevant indicators of fecal contamination.
- This test is broadly applicable to water quality monitoring including water quality testing for irrigation water and recreational water
- This test can help inform decisions for BMP implementation and monitoring

The test will be piloted during the summer of 2014 in the Milk River as part of MRWCC's on-going water quality improvement efforts and may also be piloted in the summer of 2014 at select irrigation water sampling sites where water quality concerns (i.e., high *E. coli* levels) have been identified through routine monitoring.

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Our Water ~Our Legacy