Not so Cryptic Anymore: Genetic and Functional Differences of *Escherichia marmotae* from *E. coli*

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Background

Escherichia marmotae is an emergent member of the *Escherichia* cryptic clades that were first isolated from animal feces and environmental waters*. *E. marmotae* recently gained scientific prominence due to its association with human infections including sepsis, pyelonephritis and spondylodiscitis*. Due to its phenotypic resemblance to *E. coli* but distinct genome, with a 10% pairwise difference from *E. coli* of its whole genome sequence (Fig. 1), the significance of *E. marmotae* as a pathogen may be underestimated. Given its potential as a human pathogen, sensitive, specific, and rapid identification techniques for *E. marmotae* are urgently needed to understand its functional capabilities, pathogenicity, and prevalence.

*references at http://www.ramlabwsu.org/emarmotae.html

Objectives

To understand the genetic, functional and virulence-related diversity of *E. marmotae* and to develop rapid identification methods to distinguish *E. marmotae* from *E.coli*.

Materials and Methods (summarized in Figure 2)

E. marmotae were whole-genome sequenced by Illumina short-read technology. We used bioinformatic tools such as Abricate to identify virulence and antibiotic-resistance genes. PanX and Roary were used for Pangenome analysis to identify unique genes among *E. marmotae*. We used the SNIPPY tool to find genetic alterations that could potentially mediate functional differentiation and enable discovery of unique phenotypic traits.

We examined functional diversity in *E. marmotae*, focusing on motility, biofilm formation and growth, in comparison to *E. coli.* Species-specific PCR primers & probes were designed and tested.

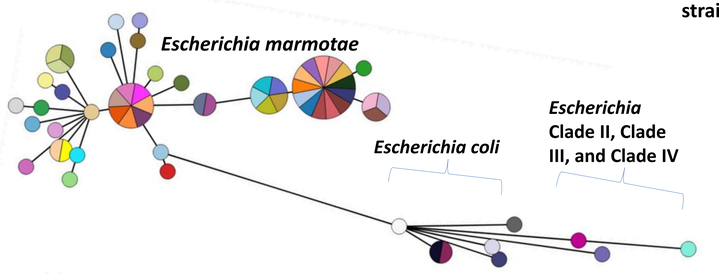

Figure 1: GrapeTree View showing the phylogenetic relationships among the *Escherichia* cryptic clades and *Escherichia coli*. The Average Nucleotide Identity based on whole genome sequence analysis shows that *E. marmotae* is 90% similar to *E. coli.*

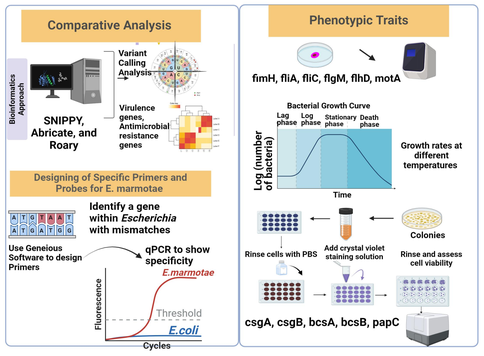

Figure 2: Graphic summary of methods (see Materials & Methods text)

RESULTS

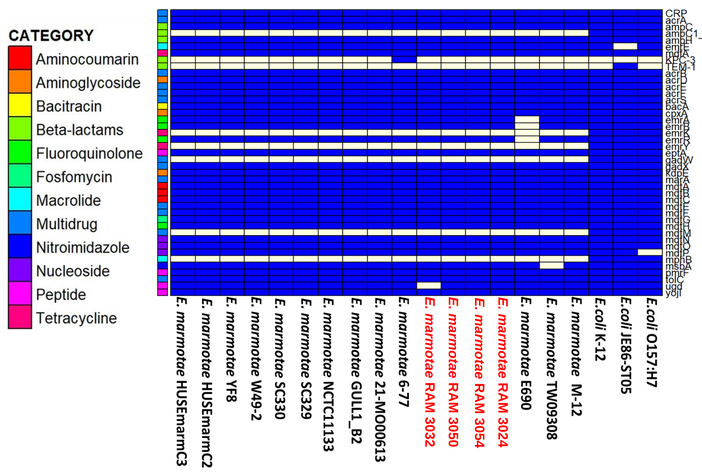

Figure 3. Heatmap of antibiotic resistance genes present in *E. marmotae* using Resfinder, Comprehensive Antibiotic Resistance Database (CARD Databases). Blue indicates presence and white the absence of the gene. Sequences are from GenBank and from RamLab (red labels) isolates (Walk et al., 2009).

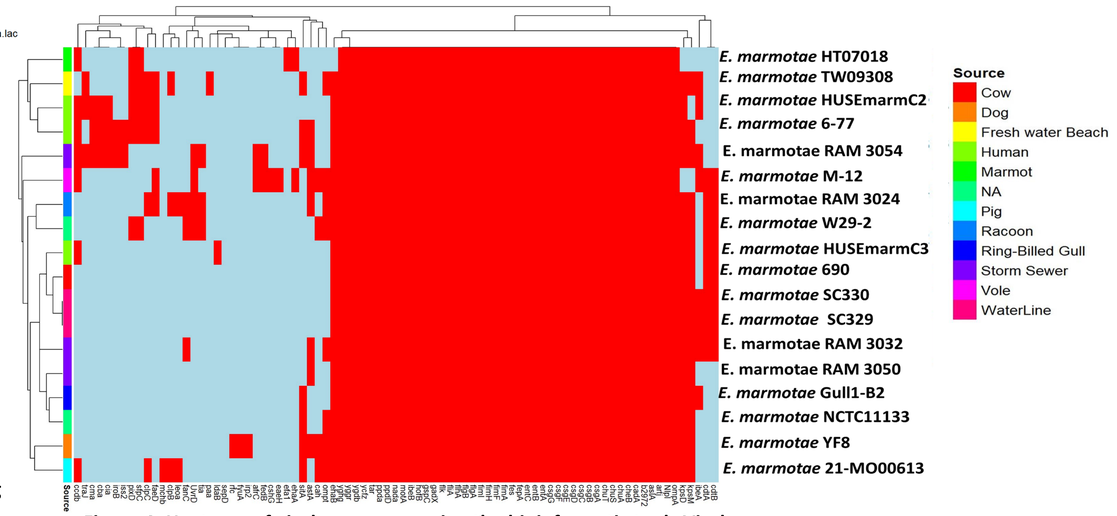

Figure 4: Heatmap of virulence genes using the bioinformatic tools Virulence Finder and *E.coli* Virulence Factor (Ecoli-VF). The red color shows pres*ence* and the light blue color shows absence of the gene .

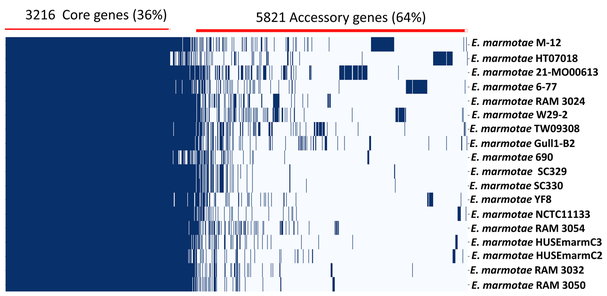

Figure 5: Pan-genome heatmap showing core genes and accessory genes, compared among 18 *Escherichia marmotae* strains. The blue bar represents the presence of a gene.

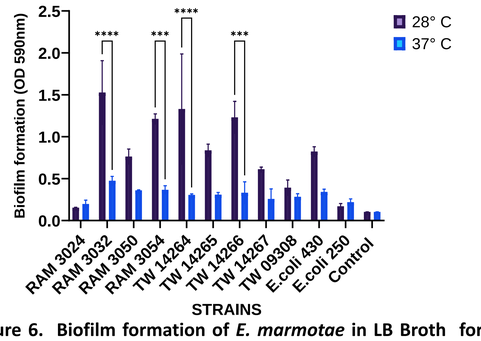

Figure 6. Biofilm formation of *E. marmotae* in LB Broth for 48hr at 28°C and 37°C. The biofilm formation was determined by crystal violet assay. The vertical bars represent the standard deviation of the mean. ***P<0.001, ****P<0.0001.

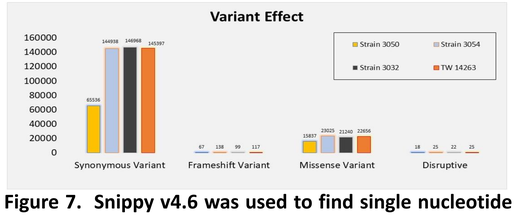

Figure 7. Snippy v4.6 was used to find single nucleotide polymorphisms in *E. marmotae*, using *E.coli* K-12 and *E.coli* J86-ST05 as a reference genome for WGS alignment. The functional effect of SNPs was investigated by SNPEff v4.3T, via Snippy.

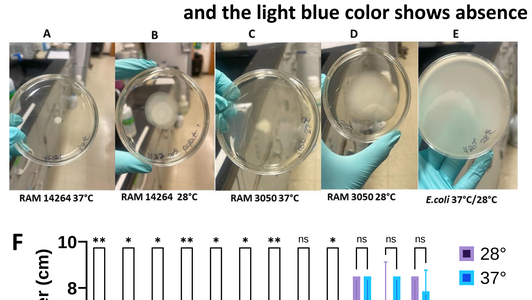

Figure 8. Motility determined by swimming zone diameters after 24 hr at different temperatures. (A & C) Representative swimming zones of *E. marmotae* at 37°C. (B & D) Representative swimming zones of *E. marmotae* at 28°C. (E) Swimming zone of an *E.coli* strain spreads over the entire plate at both 28°C and 37°C (representative result for both temperatures. (F) Swimming zone diameters of *E. marmotae* and *E. coli* strains after 24 hr at 28°C and 37°C. The vertical bars represent the standard deviation. *P<0.01, **P<0.001, ***P<0.0001.

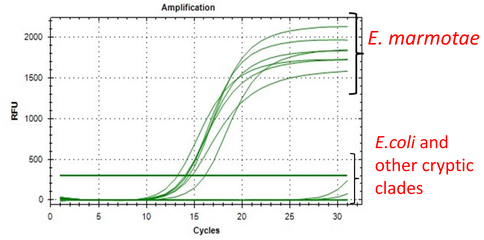

Figure 9. Species-specific primers and Taqman probes, designed to target the *uidA* gene, successfully differentiated *E. marmotae* from other *Escherichia* species and cryptic clades.

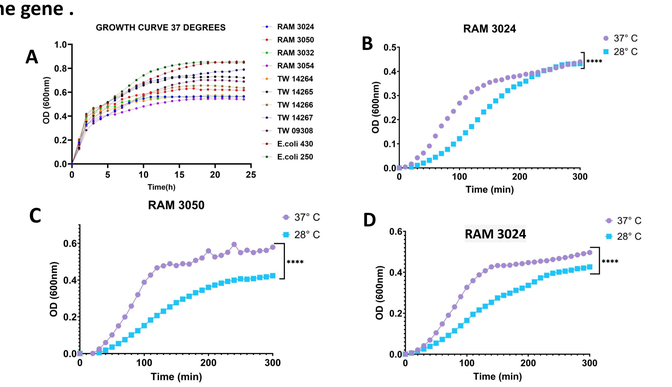

Figure 10. Growth curve of *E. marmotae* in LB broth at 37 °C and 28 °C over the period of 24 hours (A) and 5 hours (B, C, D). *E. marmotae* exhibited significantly less growth at 37°C compared to 28°C after 5 hours (P < 0.0001). The decrease in spreading on motility plates is not due to a decrease in growth at higher temperatures.

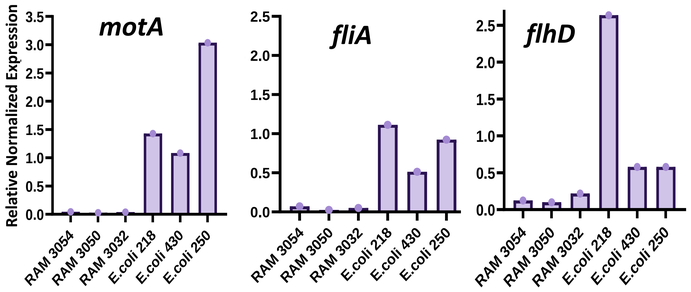

Figure 11. qRT-PCR relative expression levels of motility genes *motA, fliA, flhD* in strains of *E. marmotae* and *E.coli*. The strains were cultured in LB Broth at 28 °C and 37 °C for 18hr. *adk* gene was used as a reference for the calculation of relative expression levels. The normalized expression was calculated by using $2^{-\Delta ct}$ method.

Discussion and Future Direction

E. marmotae differs from *E. coli* in motility and biofilm formation at 28°C and 37°C. Gene expression of key motility genes *motA*, *fliC*, and *flhD* is much lower. These phenotypic and expression differences make *E. marmotae* "not so cryptic" anymore.

E. marmotae has demonstrated the ability to adhere to HEP-2 cells (Liu et al., 2019, Zhurilov et al., 2023) and possesses virulence genes common to pathogenic *E.coli.* Using a species-specific PCR (Fig. 9) and MALDI-TOF MS (not shown) we plan to determine its prevalence in *Escherichia* infections currently misclassified as *E. coli.* We will also explore pathogenicity mechanisms in animal (zebrafish) and cell line models.