Abstract

Microbial Source Tracking (MST) is the science of identifying the source of a bacterial species to its host species. This practice is used frequently in applied science to identify sources of contamination in the environment like beaches or lakes, for example. But MST techniques can also be used to study microbial populations dynamics in specific organisms. In this study, a new MST method was developed to investigate the variation of Escherichia coli strains in humans. The new approach uses pyrosequencing to generate DNA fingerprints (or pyrograms) based on the sequences from two polymorphic regions within the ribosomal RNA operons of E. coli. Seven copies of the ribosomal RNA operons are present in the E. coli genome, and each possesses two highly variable regions called Intergenic Transcribed Spacers (ITS). These regions of DNA are non-coding, and thus are able to accumulate nucleotide changes. The pyrosequencing MST method developed by our lab capitalizes on these differences to create patterns of data by simultaneously sequencing seven copies of each ITS region (16S-25S RNA sequence in E. coli). A “fingerprint” between E. coli isolates is determined when the patterns are nearly identical at both ITS 1 and 2. A cluster developed by matching isolates is considered to be a unique strain.

Specifically, this study used a new method to investigate the success of different sampling strategies for obtaining E. coli from humans. Success was determined by the variation of strains detected from each sampling type. Ideally, different types of sampling methods should detect similar proportions of strains. The secondary purpose of this study was to track strain distribution of intestinal E. coli populations over a six-month period in select human subjects. We hypothesized that each individual would host one or two dominant strains over the entire six-month period, but that minor strains would vary.

Introduction

Escherichia coli is a well-studied commensal bacterium inhabiting the mammalian intestinal tract and is an indicator of fecal contamination when found in the environment. Thus, E. coli is commonly selected for Microbial Source Tracking (MST) as it is a bacterial contaminant included to its host species by identifying and differentiating between strains. Current MST methods can be very expensive, in both money and labor, and may have issues in reproducibility when identifying different E. coli strains. In order to address many of the common complaints regarding MST methods, our lab has developed a novel pyrosequencing based method to distinguish between different E. coli strains. The method designates E. coli isolates into strains using the DNA sequences of the seven ribosomal RNA operons. The fingerprints (or pyrograms) that are obtained for comparison inspired the method’s name: Pyroprinting.

This study used a new method to investigate the success of different sampling strategies for obtaining E. coli from humans. Success was determined by the variation of strains detected from each sampling type. Ideally, different types of sampling methods should detect similar proportions of strains. The secondary purpose of this study was to track strain distribution of intestinal E. coli populations over a six-month period in select human subjects. We hypothesized that each individual would host one or two dominant strains over the entire six-month period, but that minor strains would vary.

Methods

Molecular Analysis. Confirmed E. coli isolates were used in colony PCR to amplify both ITS 1 and ITS 2 in the ribosomal RNA operon of the E. coli genome. All seven copies of the RNA operon in E. coli genome were amplified during PCR. Both ITS 1 and ITS 2 were pyrosequenced using pyrosequencers. Normally during pyrosequencing, a sequence is determined by the heights and order of peaks of light produced when nucleotides dispersed in a particular order are incorporated to an extending strand of DNA. The amount of light produced is proportional to the number of nucleotides added in a row.

E. coli Sampling & Confirmation. E. coli were sampled from three individuals once a month for six months. The individuals are two females and one male, ranging in age from 20-25. Four swabbing techniques were used to obtain intestinal E. coli. Anal swabs were collected before, immediately after, and several hours later after defecation. Lastly, collected feces were sampled with a sterile swab post homogenization. Incubated swabs were streaked onto agar plates for single isolated colonies. From these initial plates, 15 single colonies from each swab-technique were selected for a series of metabolic tests to confirm E. coli.

A pattern of peaks is produced based on the 7 different sequences; these are the pyrograms that are used for comparison in this method. Each E. coli isolate pyrogram is based on the results of two pyrograms: one for each ITS region. Unique E. coli pyrograms are considered to represent unique E. coli strains.

Results

Swab Technique Distribution of E. coli Isolates among Detected Strains

<table>
<thead>
<tr>
<th>Swab Technique</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal swab</td>
<td></td>
<td>100</td>
<td></td>
<td>90%</td>
</tr>
<tr>
<td>Anus swab</td>
<td>20</td>
<td>100</td>
<td></td>
<td>80%</td>
</tr>
<tr>
<td>Combined swab</td>
<td>15</td>
<td>50</td>
<td></td>
<td>90%</td>
</tr>
</tbody>
</table>

Temporal Distribution of Detected E. coli Strains

<table>
<thead>
<tr>
<th>Month</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>100</td>
<td></td>
<td></td>
<td>90%</td>
</tr>
<tr>
<td>Feb</td>
<td>20</td>
<td>100</td>
<td></td>
<td>80%</td>
</tr>
<tr>
<td>Mar</td>
<td>15</td>
<td>50</td>
<td></td>
<td>90%</td>
</tr>
</tbody>
</table>

Conclusions

- Sampling technique may influence the strains that are detected by pyroproting.
- Could be influenced by strain diversity (see Person A v. B & C)
- These results could pose a question for other MST/detection methods
- Human hosts are variable in the amount diversity and change within

A Future Direction

- Larger study regarding sampling method
- Conduct study including other hosts
- Investigate E. coli strains sampled from a larger human population

Acknowledgments

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