Recent Advances in Quantification of Molecular Markers

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New Water Quality Standards Based on Molecular Markers

In October 2012, the US Environmental Protection Agency will issue revised water quality criteria, including the use of molecular methods, PCR and quantitative PCR (qPCR), to estimate water quality (http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/update.cfm).

– Rapid
– Inexpensive
– Sensitive
– Quantitative ??

• Must be reliably quantitative if we are to set specific water quality standards for molecular markers.
In the lab, Quantitative PCR (qPCR) is reliably quantitative.

How quantitative is it with environmental samples?
What is required for quantitative source tracking?

1) **Hosts comparable:**

Assumption: all members of a population or a species always have indicator bacteria and molecular markers, and they have them in the same proportions, throughout their range.
What is required for quantitative source tracking?

2) **Markers comparable:**

Assumptions:

– Once they reach the environment, all markers have the same or similar survival and persistence.
– Markers are transported and stored in the environment in the same way.
What is required for quantitative source tracking?

3) **Water samples comparable:**

Assumption: Marker DNA or RNA can always be extracted from all samples, and equivalent amounts/proportions are always recovered from different kinds of samples.
What is required for quantitative source tracking?

4) Analyses are reproducible and error can be both measured and controlled
1. Hosts comparable: Geographic distribution of 3 avian markers

Legend

Gu = Gull
Go = Goose
Du = Duck
Ch = Chicken

Marker 1
Marker 2
Marker 3

Conclusion: Marker distributions vary geographically
1. **Hosts comparable**: Ratios in fecal mixtures, *E. coli* versus Enterococci

<table>
<thead>
<tr>
<th>Sample</th>
<th><strong>E. coli</strong></th>
<th><strong>Enterococci</strong></th>
<th>Field, 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Human 96%: Gull 4%</td>
<td>Human 18%: Gull 82%</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Dog 86%: Cattle 14%</td>
<td>Dog 54%: Cattle 46%</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Human 58%: Gull 42%</td>
<td>Human 1%: Gull 99%</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Cattle 90%: Gull 10%</td>
<td>Cattle 32%: Gull 68%</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Sewage 58%: Dog 42%</td>
<td>Sewage 4%: Dog 96%</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Human 44%: Gull 56%</td>
<td>Human 4%: Gull 96%</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion**: Different host species vary widely in their proportions of different bacteria
Survival of molecular source tracking markers in dark vs. light, marine vs. freshwater mesocosms

- Lag phase and persistence longer in marine water; persistence longer in the dark
- Some markers showed significant differences in decay rates

**Conclusion:** *All markers NOT the same.*

- different standards for marine and freshwater may be needed
- ratios between markers not useful, as they change over time
3. Water samples comparable:

- Sample interference: features of a water sample that affect
  - nucleic acid extraction
  - PCR
- Could include
  - chemical characteristics
  - physical characteristics
Mean AF504 and HF183 amplification curves with 0, 5, and 10 ng humic acids per reaction. Curves are averages of 16 amplification reactions per humic acid concentration.
Sensitive Detection of Sample Interference
Green et al., 2012. Water Research

1) Develop a **control assay** that (when spiked into samples) can:
   a) estimate recovery of nucleic acids
   b) estimate inhibition of qPCR

2) Borrow a method of statistical analysis called “**Kinetic Outlier Detection**” from cell biology (Tichopad et al. 2010)

3) By adapting this method, we can separately measure DNA recovery and PCR inhibition from environmental water samples
Kinetic Outlier Detection is highly sensitive to qPCR inhibition
Estimation of inhibition and recovery with a single control

Control assay targets mutated region of E.coli

Green & Field, Water Research, 2012
How much difference does this make in a real analysis?

Example: Source tracking in Samish River watershed (Washington)

- Waterways contaminated with fecal coliforms
- 69 water samples from a variety of sites
- Analyzed by qPCR for human, ruminant, dog and horse *Bacteroides* markers, and 2 avian markers (gull and general avian) that are not based on *Bacteroides*
FROM WATER SAMPLE TO RESULTS

1. Filter, store filters in freezer
2. Extract DNA from filters
3. Quantitative PCR
4. Analyze results
Results:

• Extraction recoveries of spiked controls ranged from 4.5% to 65%
  • without correction for recovery, results would have been badly skewed (wrong).
To make quantitative source-tracking more effective, we must:

- Combine source-tracking assays with reliable methods to detect PCR inhibition and poor extraction recovery.

- This is needed in order to compare samples both to each other, and to a national standard.
Thanks to:


Go Beavs!