Influence of Enterococcal Surface Protein (esp) on the Transport of Enterococcus faecium within Saturated Quartz Sands

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Contaminated GW

- 44% of US population uses GW as primary drinking water source
- Most gw has little to no treatment
- 52% of waterborne disease outbreaks (1971-2006) resulted from GW
- Majority of GW related disease is from microbial contamination
- Fecal matter is the major cause of microbial contamination

Outbreaks of Waterborne Diseases

FIG. 5. Percentages of deficiencies (n = 801) in 780 outbreaks associated with drinking water, 1971 to 2006. The untreated groundwater deficiency category includes one outbreak involving treated groundwater where the treatment provided was not expected to remove the chemical contaminants.

Common *indicator* microorganisms for *fecal contamination*

(not necessarily pathogenic, but are plentiful, and detection is cheap, easy, reliable)

- *Escherichia coli*, (E. coli)

  coliphage (E. coli-eating virus)

**Enterococci (gram positive)**

- Not much studied in gw, but sw indicator since 2004
- Possible human pathogen
- Enterococcal surface protein (esp) may enhance formation of biofilms, facilitating cell attachment to abiotic surfaces
# Source Tracking Using *esp*?

## TABLE 1. Detection of the *esp* Gene Sequence in Composite DNA Samples Extracted from Enterococci Isolated from Domestic Sewage, Birds, and Livestock

<table>
<thead>
<tr>
<th>host/source</th>
<th>sample type</th>
<th>no. of samples analyzed</th>
<th>PCR results</th>
</tr>
</thead>
<tbody>
<tr>
<td>human/sewage</td>
<td>primary influent</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>human/sewage</td>
<td>secondary effluent</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>human/sewage</td>
<td>filter effluent</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>human</td>
<td>septic tank</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>poultry</td>
<td>feces/lagoon</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>swine</td>
<td>feces/lagoon</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>dairy cattle</td>
<td>feces/lagoon</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>beef cattle</td>
<td>feces</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Canada goose</td>
<td>feces</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>seagull</td>
<td>feces</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>pelican</td>
<td>feces</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>wild birds</td>
<td>feces</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>total human</td>
<td></td>
<td>65</td>
<td><strong>63</strong></td>
</tr>
<tr>
<td>total animal</td>
<td></td>
<td>102</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* Total *Enterococcus* levels were \(<1 \times 10^2\) CFU and showed atypical colony morphology. 

*b* Statistically significantly different as shown by chi square analysis \(p < 0.001\).

Source: Scott et al., 2005
Protection of public health requires:

1. Fast, reliable detection of groundwater microbial contamination;

2. Identification of the contamination sources.
Research Objectives

1. Will esp affect the transport of *E. faecium* within quartz sands?
   - Wild type: *E. faecium* E1162
   - *esp*-negative mutant: *E. faecium* E1162Δesp

2. If the answer to question (1) is Yes, what are the underlying mechanisms?

Strains (provided by Dr. Rob J. L. Willems and Dr. Toni L. Poole):
Methods

Assessment of transport behavior

Laboratory column transport experiments performed using quartz sands.

\[ C_0 = 4 \times 10^7 \text{ cells/ml} \]

1, 2.5, 5, 20, 50 mM ionic strength solutions, NaCl buffered with NaHCO₃ to pH 7.2
Bacteria Transport Behavior

With \textit{esp} (human)  
Without \textit{esp} (non-human)

\begin{figure}
\centering
\includegraphics[width=\textwidth]{bacteria_transport_behavior.png}
\caption{Graphs showing the transport behavior of bacteria with and without \textit{esp} under different conditions.}
\end{figure}
Why is the bacteria with esp more attracted to the sand?

• xDLVO says the interaction is based on 3 factors
  (Extended Dejerguin Landau Verwey Overbeek theory)

\[ \Phi^{\text{LW}} - \text{Van der Waals attractive forces} \quad \text{electrostatic} \]
\[ f(\text{Hamaker constant, radius, distance}) \]

\[ \Phi^{\text{EDL}} - \text{Electrostatic Double Layer} \quad f(\text{dist, radius, surf potential}) \]

\[ \Phi^{\text{AB}} - \text{Hydrophobicity} \quad (\text{Lewis acid base parameters; the } x \text{ in xDLVO}) \]
\[ f(\text{radius, hydrophobicity interaction free energy}) \]

\[ \Phi^{\text{Total}} = \Phi^{\text{LW}} + \Phi^{\text{EDL}} + \Phi^{\text{AB}} \]

Changes with separation distance
DLVO: electrostatic forces

- Solve for each force at different distances,
- combine to get overall force
- Need to characterize cells to calculate forces
  - Radius
  - Zeta potential
  - Contact angle
Cell Characterization

Cell Size: (measured from calibrated photo)

E1162 (human): $1.29(\pm0.12) \, \mu m$
E1162Δesp: $1.30(\pm0.12) \, \mu m$

No significant difference in size due to esp

$$\Phi^{EDL} = \pi \varepsilon_0 \varepsilon_w a_b \left\{ 2 \psi_b \psi_s \ln \left[ \frac{1 + \exp(-\kappa h)}{1 - \exp(-\kappa h)} \right] + (\psi_b^2 + \psi_s^2) \ln[1 - \exp(-2\kappa h)] \right\}$$

$$\Phi^{LW} = -\frac{A d_b}{6h}$$

$$\Phi^{AB} = 2\pi a_r \lambda_w \Delta G_{h0}^{AB} \exp \left( \frac{h_0 - h}{\lambda_w} \right)$$
Cell Characterization

Zeta Potential: used for surface potential

- Both sand and bacteria negatively charged, so EDL repulsive
- Bacterial strains not significantly different
  - [edl not the factor]
- At low mM (<10) no significant changes, but the increase (less negative) at high mM is significant
# Cell Characterization

**Contact angle:** Water, Glycerol and diiodomethane

<table>
<thead>
<tr>
<th>Properties</th>
<th>E1162</th>
<th>E1162Δesp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact angle (°) (n≥4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>21.2 (± 4.1)</td>
<td>16.1 (± 1.6)</td>
</tr>
<tr>
<td>Glycerol</td>
<td>24.3 (± 1.6)</td>
<td>32.5 (± 4.6)</td>
</tr>
<tr>
<td>Diiodomethane</td>
<td>48.2 (± 2.5)</td>
<td>44.2 (± 0.2)</td>
</tr>
</tbody>
</table>

Solve for the values of

\[
\gamma_i^L (1 + \cos \theta) = 2 \sqrt{\gamma_i^{LW} \gamma_i^{LW}} + 2 \sqrt{\gamma_i^+ \gamma^-} + 2 \sqrt{\gamma_i^- \gamma^+}
\]

Used to calculate the values of Hamaker constant (A), hydrophobic interaction energy constant, \( \Delta G_{AB}^{AB} \), cell hydrophobicity parameter \( \Delta G_{iwi} \).
### Cell Characterization: calculated results

<table>
<thead>
<tr>
<th>Properties</th>
<th>E1162</th>
<th>E1162Δesp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface tension components</td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ\textsuperscript{LW}</td>
<td>35.3</td>
<td>37.4</td>
</tr>
<tr>
<td>γ\textsuperscript{+} electron accepting</td>
<td>3.1</td>
<td>1.4</td>
</tr>
<tr>
<td>γ\textsuperscript{-}</td>
<td>44.7</td>
<td>53.5</td>
</tr>
<tr>
<td>A (10^-21 J) from vdW</td>
<td>4.2</td>
<td>4.8</td>
</tr>
<tr>
<td>ΔG\textsuperscript{AB}_{RO} (mJ/m^2)</td>
<td>*repulsive</td>
<td>24.1*</td>
</tr>
<tr>
<td>ΔG\textsuperscript{AB}_{tw} (mJ/m^2)</td>
<td>*hydrophylic</td>
<td>18.3*</td>
</tr>
</tbody>
</table>

Note: Microbial Adhesion To Hydrocarbons (MATH) test also indicated hydrophylic results.
Extended DLVO calculation

\[ \Phi^{\text{Total}} = \Phi^{\text{LW}} + \Phi^{\text{EDL}} + \Phi^{\text{AB}} \]

Energy barrier without esp is higher, which explains less bacterial attachment to sand.
Conclusions

1. *esp* enhances the attachment of *E. faecium* to quartz sands.

2. *esp* led to alterations in cell surface properties and the effects of *esp* on the transport behavior of *E. faecium* can be explained by the XDLVO theory.
Environmental Implications

1. Sewage-derived \textit{E. faecium} may have lower mobility within sand and gravel aquifers.

2. The effects of \textit{esp} on the transport of \textit{E. faecium} complicates its effectiveness as a groundwater microbial source tracking tool.
Acknowledgement

Dr. Rob J. L. Willems of University Medical Center Utrecht, Utrecht, The Netherlands.

Dr. Toni L. Poole of USDA-ARS Southern Plains Area Research Center.

Lixia Wang and Dr. Jin Li of UWM.

American Water Resources Association - Wisconsin Section
Van der Waals attractive forces

Act at close distances

- London dispersion forces (induced attractions)
- Dipole-dipole attractions (permanent attractions)

\[
\Phi_{LW} = \frac{-A a_b}{6h}
\]

- \(A\) = Hamaker constant
  - \(f\) (interfacial tension parameters, \textit{bacteria}, water, sand) [contact angle]
- \(a_b\) = \textit{bacterial radius}
- \(h\) = separation distance bacterium to sand (varies to create graph)
Electrostatic Double Layer forces

\[ \Phi_{\text{EDL}} = \pi \varepsilon_0 \varepsilon_w \left( \psi_b \psi_s \ln \frac{1 + \exp(-\kappa h)}{1 - \exp(-\kappa h)} + (\psi_b^2 + \psi_s^2) \ln [1 - \exp(-2\kappa h)] \right) \]

f(radius, surface potential, Debye length [T, ionic strength, e charge])
Hydrophobicity

– Lewis acid base parameters;
– the x in xDLVO

\[ f(\text{radius, hydrophobicity interaction free energy}) \]

\[ \Phi^{AB} = 2\pi a_b \lambda_w \Delta G_{h_0}^{AB} \exp\left(\frac{h_0 - h}{\lambda_w}\right) \]

» Radius, contact angle measurement
Transport Behavior

$k_d$: First-order deposition rate coefficient

\[ k_d = -\frac{\nu}{\varepsilon L} \ln \left( \frac{C}{C_0} \right) \]

- $\varepsilon = \text{porosity} = 0.37$
- $L = \text{column length} = 15 \text{ cm}$
- $\nu = \text{specific discharge} = 0.31 \frac{\text{cm}}{\text{min}}$
- $\left( \frac{C}{C_0} \right) = \text{normalized breakthrough concentration}$
Transport Behavior with esp (human)

RECOVERY

• 1 mM ~ 90+%  
• 2.5 mM ~ 75%  
• 5 mM ~ 50%  
• 20 and 50 mM <5%
Transport Behavior with no esp (non-human)

**Recovery**

- 1 mM ~ 100+%
- 2.5 mM ~ 90%
- 5 mM ~ 75%
- 20 mM ~ 20%
- 50 mM ~ 5%
Source Tracking Using *esp*?

<table>
<thead>
<tr>
<th>source</th>
<th>number of samples</th>
<th>number of positives$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>esp</em>$_{fm}$ Gene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sewage influent</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td>pit toilets</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>septic trucks</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>cats</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>deer</td>
<td>4</td>
<td>0</td>
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<tr>
<td>dogs</td>
<td>43</td>
<td>9</td>
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<tr>
<td>geese</td>
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<tr>
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<td>0</td>
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<td>raccoons</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>songbirds</td>
<td>55</td>
<td>0</td>
</tr>
</tbody>
</table>

Source: Whitman et al., 2007