Accumulation of phages in drinking water biofilms: a way to elude chlorine?

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Accumulation of F-specific RNA bacteriophages MS2, GA and Qβ on surfaces colonised with drinking water biofilms

Δ Phages showing differences in their surface properties
Δ Different water velocities in laminar conditions

Efficiency of a cleaning protocol based on high level chlorination

Δ On phages in suspension in water
Δ On phages accumulated in the biofilm

Context and Objectives

Accidental contamination with virus

Detection
3 hours (75 rpm) (T0)
GA
GA
Q
105 eq PFU/cm2

Drinking water biofilm characteristics

> 95% showing intact membranes
≈ whatever the wall shear rate

Materials & Methods

Two-month-old water drinking biofilms

Materials & Methods

Rotating disc reactor

HDPE coupons (d: 18 mm)

Drinking water (1 Lh)

35 L
19 ± 1°C
pH 8.2 ± 0.2

Water velocity equivalent to 0.6, 0.8 and 0.9 m/s in a 20 cm-diameter pipe

F-specific RNA bacteriophages MS2, GA and Qβ

• Structural similarities with enteric pathogenic viruses
Size: 20-30 nm
Non-enveloped capsid and RNA genome

• Detection methods
- Specific detection of viral genome by real time RT-PCR (Ogorzaly and Gantzer, 2006)
- Titration by culture method

• Surface properties recently studied (*Langlet et al., 2008)

<table>
<thead>
<tr>
<th>Isoelectric point (Michen and Graule, 2010)</th>
<th>MS2</th>
<th>Qβ</th>
<th>GA</th>
<th>Norwalk virus</th>
<th>Hepatitis A virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2 - 3.9</td>
<td>1.9 - 5.3</td>
<td>2.1 - 2.6</td>
<td>5.5 - 6.0</td>
<td>2.8 - 5.8</td>
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Negative charge at pH 6.5° (electrophoretic mobility)

MS2 = Qβ < GA

Hydrophobicity*

MS2 < Qβ < GA

Phages / Virus

P-values

Mann-Whitney

Phage concentration on surfaces: MS2 < Qβ < GA
(P-values < 0.0001, Mann Whitney)

Adhesion profile of phages may be related to their hydrophobicity.

Accumulation of MS2, GA and Q6 phages on HDPE coupons colonised with drinking water biofilm

Drinking water biofilm characteristics

1.7x10³ cells/cm² (Live/Dead BacLight kit)
whatever the wall shear rate
= 0.1% cultivable (15 d)
> 95% showing intact membranes

Experimental conditions

Two-month-old drinking water biofilms (pH 8.2 ± 0.2, 19 ± 1°C)

10⁵ PFU/mL of each phage spiked in the reactor

• Adsorption step: 3 hours (75 rpm) (T0)

• Chlorination step: 4 mg/L of sodium hypochlorite (HOCl)

Protective effect of drinking water biofilm against chlorination

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Protective effect of drinking water biofilm against chlorination

• Rapid inactivation of phages in water by chlorine

• Protective effect of the biofilm against chlorination (4 mg/L) for adsorbed phages

Water: 5 log₁₀ reduction in less than 30 min
Surfaces: 2.5 log₁₀ reduction in 60 min

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