Effect of high hydrostatic pressure (HHP) on inactivation of *Vibrio* spp.

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Vibrio spp.

- Gram (-)
- Halophilic bacteria
- Widely occur in marine & estuarine environments
Vibrio spp. – the most common bacterial pathogens in seafood associated with human infections

Vibrio infections:
- Foodborne diseases (V. parahaemolyticus, V. cholerae, V. vulnificus)
- Wound infections & septicaemia (V. vulnificus, V. alginolyticus)
- Commonly reported in The US, Asia (Japan, China, Taiwan)
- Sporadic cases in EU
**Mussels**
- The most important bivalves in Europe, mainly cultured in North East Atlantic
- > 34% of the overall EU aquaculture production (Eurostat, 2017)

**Problems**
- Mussels – filter feeders → accumulate pathogens (*Vibrio*) during filtration
- Concentration up to 100-fold the amount in surrounding water (Butt et al., 2004)
- Prevalence of *Vibrio* spp. in retail mussels: 41% (Vu et al., 2018)
Effective processing methods - HHP:
- Inactivate spoilage & pathogenic microorganisms
- Fruit juices, meat, meat products, shellfish

Application of HHP in inactivation *V. parahaemolyticus* & *V. vulnificus* in oysters & clams (*Kural & Chen, 2008; Mootian et al., 2013*)
Objectives

- To determine the effect of HHP on inactivation of *Vibrio* spp. in pure culture & mussel homogenates
## Bacterial strains & preparation of overnight culture

<table>
<thead>
<tr>
<th><strong>Vibrio</strong> spp.</th>
<th>Strain</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. alginolyticus</em></td>
<td>ATCC 17749</td>
<td>Clinical strain</td>
</tr>
<tr>
<td><em>V. cholerae</em></td>
<td>NCTC 4711</td>
<td>Clinical strain</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>RIMD 2210633</td>
<td>Clinical strain</td>
</tr>
<tr>
<td><em>V. vulnificus</em></td>
<td>V57/10</td>
<td>Clinical strain</td>
</tr>
</tbody>
</table>

**Preparation of Overnight Culture**

- **Medium**: APW, 2% NaCl
- **Incubation**: 37°C/24h

**Drop plating**

- Dilutions: $10^{-1}$ to $10^{-6}$

**Initial counts**: 8.0-8.5 log$_{10}$ (CFU/ml)
HHP treatment of *Vibrio* spp. in pure culture

Overnight *Vibrio* culture
APW, 2%NaCl

Initial counts:
8.0-8.5 log$_{10}$ (CFU/ml)

1.8 ml cryo-tubes

High pressure treatment at 25°C
(5 repetitions each condition)

<table>
<thead>
<tr>
<th></th>
<th>1 min</th>
<th>3 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 MPa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>350 MPa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>450 MPa</td>
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</table>

Drop plating
37°C/72h

TCBS
HHP treatment of *Vibrio* spp. in pure culture

Note: Groups with different letters are significantly different ($P < 0.05$)

Log reductions of *Vibrio* = $\log_{10} \left( \frac{N_0}{N} \right)$

($N_0$ = initial count of *Vibrio*, $N$ = number of cell surviving after HHP treatment)

Data are mean values ± SD

- 350 & 450 MPa for 1 & 3 min completely inactivated *Vibrio* spp. to non-detectable levels (except for *V. parahaemolyticus*)

- *V. vulnificus* - the most susceptible *Vibrio* sp. to HHP
**HHP treatment of Vibrio spp. in mussel homogenates**

- **Artificial inoculation** (10^7-10^8 CFU/g)
- **Overnight Vibrio culture** APW, 2%NaCl
- **Heat treated mussel homogenates**
  - Total aerobic counts
  - Vibrio spp.
- **1.8 ml cryo-tubes**
- **Matrix-adaptation** (8°C overnight)
- **High pressure treatment at 25°C** (5 repetitions each condition)
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<td>450 MPa</td>
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</table>
- **Initial counts of Vibrio spp. in mussels homogenates:**
  - 7.5-7.9 log_{10} (CFU/g)
  - (drop plating on TCBS)

- **Heat treated mussel homogenates**
  - **Total aerobic counts**
  - **Vibrio spp.**
  - **Matrix-adaptation** (8°C overnight)
  - **TSA 1%NaCl**
  - **TCBS**

- **APW, 2%NaCl**
- **Drop plating**
- **37°C/72h**
HHP treatment of *Vibrio* spp. in mussel homogenates

**TCBS agar**

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>Log reductions (CTU g⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td><strong>V. alginolyticus</strong></td>
</tr>
<tr>
<td>250</td>
<td>a</td>
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<td>350</td>
<td>b</td>
</tr>
<tr>
<td>450</td>
<td>c</td>
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**TSA agar**

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<table>
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<tr>
<th>Treatment time</th>
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<tbody>
<tr>
<td>● 1 min</td>
</tr>
<tr>
<td>▼ 3 min</td>
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Note: Groups with different letters are significantly different (*P* < 0.05)

Log reductions of *Vibrio* = \( \log_{10} \left( \frac{N_0}{N} \right) \)

(N₀ = initial count of *Vibrio*, N = number of cell surviving after HHP treatment)

Data are mean values ± SD

- □ The recovery of *Vibrio* spp. in TSA was significantly higher than in TCBS (particularly at 250 MPa)
- □ *V. alginolyticus* – the most resistant at 250 MPa for 1 min
- □ *V. parahaemolyticus* - the most resistant at 350 MPa for 1 min
- □ *V. vulnificus* - the most susceptible *Vibrio* sp. to HHP
To achieve > 5 log reduction of *Vibrio* spp.

- 350-450 MPa for ≥1 min at 25°C for *V. alginolyticus* & *V. cholerae*
- 250 MPa for ≥3 min or 350-450 MPa for ≥1 min for *V. vulnificus*
- 350 MPa for ≥3 min or 450 MPa ≥1 min for *V. parahaemolyticus*
Acknowledgment
Thank you for your attention!

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