Buffer capacity of food components influences the acid tolerance response in Salmonella Typhimurium during simulated gastric passage

Aabo, Søren; Buschhardt, Tasja; Hansen, Tina Beck; Birk, Tina; Henriksen, Sidsel

Publication date: 2014

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
Buffer capacity of food components influences the acid tolerance response in *Salmonella* Typhimurium during simulated gastric passage

AABO, Søren Aabo1, BUSCHHARDT, Tasja1, HANSEN, Tina Beck1, BIRK, Tina1 and HENRIKSEN, Sidsel Division of Food Microbiology, National Food Institute, Technical University of Denmark, Denmark1; Moerkhoj Bygade 19, 2860 Soeborg, Denmark

Background and introduction

Successful intestinal *Salmonella* infection depends on survival of the acidic barrier of the stomach. The buffer capacity of ground beef has previously been shown to enhance survival of *Salmonella Typhimurium* on ground beef in a low pH environment (Waterman & Small 1998). We hypothesise that high buffer capacity of food also would protect against gastric acid killing. *Salmonella* possesses two independently regulated stationary phase acid tolerance systems. The global stress response regulator RpoS induces a pH-independent acid tolerance as part of the general stress response upon entry into stationary phase. The second is the acid-induced Acid Tolerance Response (ATR) system key regulated by OmpR (Lee et al., 1994). We have studied the survival of *Salmonella Typhimurium* and its expression of ATR genes in environments with different buffer capacities. A computer-controlled fermentor (Figure 1) mimicking the dynamic pH during gastric passage has been developed. Bacteria and food model were contained in a dialysis tube.

Material and methods

Table 1. *Salmonella enterica* serovar Typhimurium strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5</td>
<td>Virulent wild type</td>
<td>Hormaeche (1979)</td>
</tr>
<tr>
<td>C5 -rpoS</td>
<td>rpoS::kan</td>
<td>Thomsen (2002)</td>
</tr>
<tr>
<td>C5 -clpP</td>
<td>clpP::kan</td>
<td>Thomsen (2002)</td>
</tr>
<tr>
<td>ST 4/74</td>
<td>Virulent wild type</td>
<td>Wray &amp; Sojka (1978)</td>
</tr>
<tr>
<td>ST 4/74 -rpoS</td>
<td>rpoS::kan</td>
<td>Knudsen et al. (2012)</td>
</tr>
</tbody>
</table>

The strains were kindly donated by J.E. Olsen and L. E. Thomsen University of Copenhagen; G. M. Knudsen, Technical University of Denmark.

Synthetic gastric fluid: (SGF): 4.1 g NaCl, 1.2 g KH2PO4, 0.2 g CaCl2, 0.6 g KCl, 0.2 g lysozyme (Sigma-Aldrich L6876), 0.1 g bile extract (Sigma-Aldrich B8831), and 26.6 mg pepsin (Sigma-Aldrich P7000) in 2 l demineralized water, as described by Just & Daeschel (2003).

Food models (lab. Media):
- Brain Heart Infusion Broth (BHI) (pH 7.4).
- Saline 0.9% (w/v) NaCl buffered with disodium hydrogen phosphate (Na2HPO4) (2.5 g/l) as in BHI and adjusted to pH 7.4.
- Saline 0.9% (w/v) NaCl adjusted to 7.4 with 0.4 M HCl

Dialysis tubes: Fical-A Lyzer®, cutoff 100 kD, Spectrum® Laboratories, Inc., USA.

Figure 1. Schematic diagram of the simulated dynamic gastric acid system used in this study and pH-profile employed by the fermentor.

Objective

To study the impact of buffer capacity of lab media on the survival and acid tolerance response of *Salmonella Typhimurium* in a gastric acid model.

Conclusion

- In contrary to hypothesis less buffered media was found to provide higher protection of *Salmonella*, compared to media with high buffer capacity (Figure 2).
- We suggest this to be associated with the ability of *Salmonella Typhimurium* to mount a stationary phase acid tolerance response (ATR) in the less buffered media.
- Expression of *rpoS* and *ompR* encoding two major stationary phase ATR regulators were found to increase by app. four-fold (*ompR*) and app. three-fold (*rpoS*) in saline (low buffer capacity), (Figure 5).
- The relative expression of these genes, were significantly lower in Brain Heart Infusion Broth having a higher buffer capacity.
- The inactivation of *Salmonella Typhimurium* rpoS deletion mutants supported that the mounting of the acid tolerance response is responsible for the higher survival.
- We hypothesise that a high buffer capacity can prevent pH drop and this avoids *Salmonella* to adapt. When the buffer collapse the pH drops too fast for the ATR response to be induced.

Results

![Figure 2](image2.png)

**Figure 2.** Inactivation of *S. Typhimurium* C5 in BHI, buffered saline and saline in a pH-controlled fermentor modulating gastric acid passage. Experiments were conducted in triplicate and data presented are means of results ± SEM (n=3).

![Figure 3](image3.png)

**Figure 3.** Inactivation during simulated gastric acid passage of *S. Typhimurium* strains C5 and 4/74 wild type and rpoS deletion mutants, plus an isogenic C5 clpP mutant. Results presented are means from duplicate trials ± SEM (n=2).

![Figure 4](image4.png)

**Figure 4.** Relative expression (log2) of *ompR* (dark grey) and *rpoS* (light grey) during gastric acidification with sampling at 30 min (c) versus untreated samples and normalized to reference gene *rpoD*. N=3, Expression in BHI is significantly lower than in saline or buffered saline (*P < 0.001)*.

![Figure 5](image5.png)

**Figure 5.** Comparison of the measured pH in buffered and unbuffered media inside the dialysis tubes and in the SGF reservoir (outside dialysis tube). The pH drop in BHI and buffered saline is app. 60 min delayed in contrast to the pH in saline and the SGF reservoir.