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

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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

Strain	Genotype	Source
C5	Virulent wild type	Hormaeche (1979)
C5 ΔrpoS	*rpoS::amp*	Thomsen (2002)
C5 ΔclpP	Δ*clpP*	Thomsen (2002)
ST 4/74	Virulent wild type	Wray & Sojka (1978)
ST 4/74 ΔrpoS	*rpoS::kan*	Knudsen et al. (2012)

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 KH_2PO_4, 0.2 g $CaCl_2$, 0.6 g KCl, 0.2 g lysozyme (Sigma-Aldrich L6876), 0.1 g bile extract (Sigma-Aldrich B8631), 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 (Na_2HPO_4) (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:
Float-A-Lyzer®; cutoff 100 kD, Spectrum® Laboratories, Inc., USA.

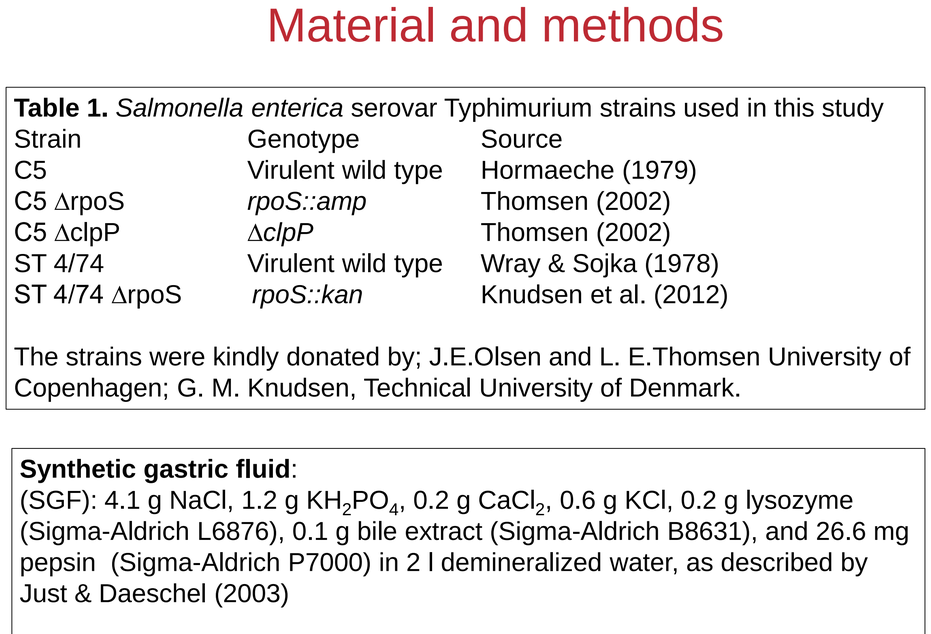

Figure 1. Schematic diagram of the simulated dynamic gastric acid system used in this study and pH-profile employed by the fermentor.

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 prevents pH to drop and this avoids *Salmonella* to adapt . When the buffer collapse the pH drops too fast for the ATR response to be induced.

Results

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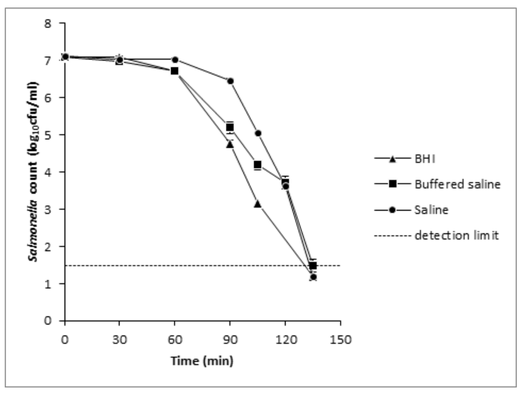

Figure 2. Inactivation of *S*. Typhimurium C5 in BHI, buffered saline and saline in a pH-controlled fermentor modelling gastric acid passage. Experiments were conducted in triplicate and data presented are means of results ± SEM (n=3).

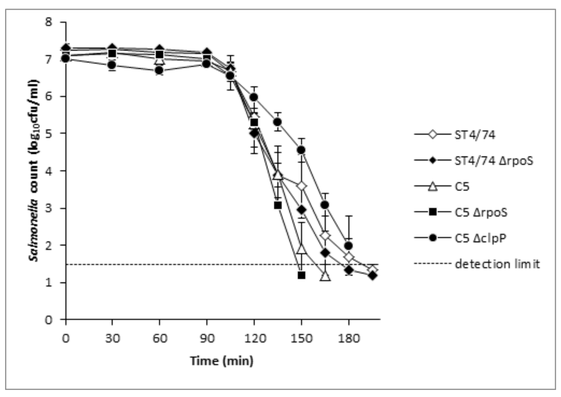

Figure 3. Inactivation during simulated gastric acid passage of *S*. Typhimurium strains C5 and 4/74 wild types and *rpoS* deletion mutants, plus an isogenic C5 *clpP* mutant. Results presented are means from duplicate trials ± SEM (n=2).

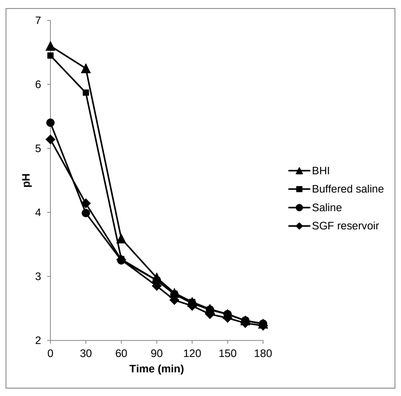

Figure 5. Comparison of the measured pH in buffered and unbuffered media inside the dialysis tubes and in the SGF reservoir (outside dialysis tubes). The pH drop in BHI and buffered saline is app. 60 min delayed in contrast to the pH in saline and the SGF reservoir..

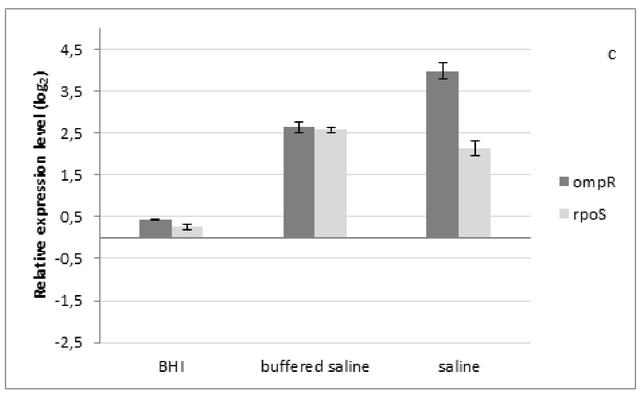

Figure 4. Relative expression (log_2) of *ompR* (dark grey) and *rpoS* (light grey) during gastric acidification with sampling at 30 min (c) versus untreated samples and normalized to reference gene *rpoD*. N=3. Expression in BHI is significantly lower than in saline or buffered saline ($P < 0.001$).

References WATERMAN, S.R. and SMALL, P.L., 1998. Acid-sensitive enteric pathogens are protected from killing under extremely acidic conditions of pH 2.5 when they are inoculated onto certain solid food sources. *Applied and Environmental Microbiology,* **64**(10), pp. 3882-388
LEE, I.S., SLONCZEWSKI, J.L. and FOSTER, J.W., 1994. A low-pH-inducible, stationary-phase acid tolerance response in Salmonella typhimurium. *Journal of Bacteriology,* **176**(5), pp. 1422-1426.