Influence of osmoprotectants on survival of Salmonella Typhimurium strains during desiccation
Christensen, Nanna; van der Berg, Franciscus Winfried J; Risbo, Jens; Knøchel, Susanne

Publication date:
2012

Document version
Peer reviewed version

Citation for published version (APA):
Influence of osmoprotectants on survival of *Salmonella* Typhimurium strains during desiccation

Nanna Christensen*, Frans v.d. Berg*, Jens Risbo* and Susanne Knochel*  
*Department of Food Science, University of Copenhagen, Rolighedvej 36, 1958 Frederiksberg C, Denmark

**Background**

Outbreaks caused by *Salmonella* associated with low moisture products are commonly reported, and *Salmonella* are known to survive desiccation well. The environment incl. food contains naturally occurring osmoprotectants, which may increase the survival of *Salmonella* cells in low moisture environments.

**Method**

- 20 μl of 10^6 CFU/ml cell solution pr. well were desiccated in 0.9% saline added 0.1% te, peptone and 1%, glucose (PSP-G) in 48 well plates in closed chambers at 22°C, 43% RH (Figure 1).
- Sampling was performed after resuscitation for 30 min in PSP and plating of appropriate dilutions on BH (incubated at 37°C, 23 ± 1 h).
- Reduction was calculated using Equation 1 and plotted against time for modelling with technical replicates (Figure 2). Day 0 to day 1 was not included due to initial growth.
- Testing with osmoprotectants was performed with 1mM betaine, 50mM betaine, proline and carnitine, and 10%, sucrose in PSP-G.

\[
\text{Reduction} = \log \left( \frac{N_{w}}{N_{d}} \right)
\]

where 
\[
N_{w} = \text{number of cells in the well solution}
\]
\[
N_{d} = \text{number of cells in the desiccated sample}
\]

Equation 1: Reduction [CFU/ml] = log \left( \frac{N_{w}}{N_{d}} \right)

Equation 2: % Reduction = \left( \frac{\text{mean (Reduction day 14) - (mean (Reduction day 14)control)}}{\text{mean (Reduction day 14)control}} \right) \times 100

**Table 1:** Reduction of seven *S. Typhimurium* strains after desiccation for 14 days at 22°C and 43% RH calculated using equation 1. The reduction on day 14 is shown as mean of five technical replicates ± standard deviation. One way ANOVA test was performed to identify strains with significant higher reduction on day 14 compared with *S. Typhimurium* 364/87, as this strain was chosen as the one with the lowest reduction. *S. Typhimurium* C5 was chosen as the strain with the highest reduction based on the reduction and p-value from the one way ANOVA test (both strains are marked with red boxes).

<table>
<thead>
<tr>
<th><em>S. Typhimurium</em> strains</th>
<th>Strains selected for testing with osmoprotectants</th>
<th>Origin</th>
<th>Reduction day 14 [CFU/ml]</th>
<th>One way ANOVA p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. Typhimurium</em> 364/87</td>
<td>Lowest reduction</td>
<td>Chocolate</td>
<td>-3.18±0.6</td>
<td></td>
</tr>
<tr>
<td><em>S. Typhimurium</em> 1292</td>
<td>Pig</td>
<td></td>
<td>-3.50±0.8</td>
<td>0.54</td>
</tr>
<tr>
<td><em>S. Typhimurium</em> 4/74</td>
<td>Boilie</td>
<td></td>
<td>-6.55±0.0</td>
<td>0.01</td>
</tr>
<tr>
<td><em>S. Typhimurium</em> 224/87</td>
<td>Clinical</td>
<td></td>
<td>-5.75±1.2</td>
<td>0.12</td>
</tr>
<tr>
<td><em>S. Typhimurium</em> 0/12</td>
<td>Clinical</td>
<td></td>
<td>-3.79±1.3</td>
<td>0.40</td>
</tr>
<tr>
<td><em>S. Typhimurium</em> 1292</td>
<td>Clinical</td>
<td></td>
<td>-8.67±1.5</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td><em>S. Typhimurium</em> C5</td>
<td>Highest reduction to Mouse</td>
<td></td>
<td>-3.94±1.7</td>
<td>&lt;0.00</td>
</tr>
</tbody>
</table>

**Results**

**Discussion**

- *S. Typhimurium* strains tested varied in desiccation tolerance based on reduction (Table 1). *S. Typhimurium* 364/87 and *S. Typhimurium* C5 (marked with red) represented highest and lowest desiccation tolerance.
- Previous studies have demonstrated a positive effect of betaine, proline and carnitine on survival of *Salmonella* subjected to NaCl stress (Cairney et al. 1985a; Cairney et al. 1985b; Gutierrez & Csonka 1995). Here, desiccation tolerance increased for both strains investigated (364/87 and C5) with carnitine and, in particular, with sucrose (Figure 2A+B) while limited effect was seen for proline and betaine.
- Hardly any reduction was seen within the period with sucrose (Figure 2A+B).
- Sucrose and carnitine had a significant (p<0.05) positive effect on desiccation tolerance based on reduction on day 13 (Table 2).

**Table 2:** % reduction in relation to the control on day 13 as calculated using equation 2, are shown for *S. Typhimurium* 364/87 and *S. Typhimurium* C5 desiccated for 13 days at 22°C, 43% RH (mean values of four replicates and standard deviation shown with bars for each sampling point). A and B: *S. Typhimurium* 364/87 and *S. Typhimurium* C5 desiccated in PSP-G alone, or added 50mM betaine, proline, and carnitine respectively.

<table>
<thead>
<tr>
<th><em>S. Typhimurium</em> 364/87</th>
<th>1mM betaine</th>
<th>50mM betaine</th>
<th>50mM proline</th>
<th>50mM carnitine</th>
<th>10% sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. Typhimurium</em> C5</td>
<td>22%</td>
<td>-6%</td>
<td>28%</td>
<td>-58%</td>
<td>-92%</td>
</tr>
</tbody>
</table>

**Conclusion**

- Desiccation tolerance differs within the same serovar.
- The presence of 10% sucrose and 50mM carnitine, respectively, increased the desiccation tolerance in that order for *S. Typhimurium* 364/87 and *S. Typhimurium* C5, with a very marked effect of sucrose.

**References**