Influence of osmoprotectants on survival of Salmonella Typhimurium strains during desiccation

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Influence of osmoprotectants on survival of Salmonella Typhimurium strains during desiccation

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Background
Outbreaks caused by Salmonella associated with low moisture products are commonly reported, and Salmonella is 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.

Aim
To investigate the desiccation tolerance of S.Typhimurium strains of different origin, and the osmoprotective effect of extracellular added betaine, proline, carnitine, and sucrose.

Method

- 20μl of 10^6 CFU/ml cell solution pr. well were desiccated in 0.9% NaCl (%RH 30, 1958 Frederiksberg C, Denmark)
- Desiccation tolerance was measured by calculating the log (Nt/N0) of S.Typhimurium 364/87, S.Typhimurium U292, S.Typhimurium C5, and S.Typhimurium 4/7.
- Reduction was calculated using Equation 1 and plotted against time for modelling the survival kinetics of S.Typhimurium 364/87 and S.Typhimurium C5 in PSP-G.

\[ \text{Log (Nt/N0)} = -kt \]

\[ \% \text{Reduction} = \frac{\text{mean (Reduction day 14 osmoprotectant)} \times 100}{\text{mean (Reduction day 14 control)}} \]

- Significant (p <0.05) for all three trials.
- Significant (p <0.05) for one trial out of two trials as data is missing for one trial.
- Statistical analysis performed using t-test as only one endpoint value for control is available.

Results

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 p-value from the one way ANOVA test (both strains are marked with red box).

<table>
<thead>
<tr>
<th>S. Typhimurium 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>S. Typhimurium 364/87</td>
<td>Lowest reduction</td>
<td>Chocolate</td>
<td>-3.18±0.6</td>
<td></td>
</tr>
<tr>
<td>S. Typhimurium U292</td>
<td>Pig</td>
<td></td>
<td>-3.50±0.8</td>
<td>0.54</td>
</tr>
<tr>
<td>S. Typhimurium 6/7</td>
<td>Boilie</td>
<td></td>
<td>-3.55±2.0</td>
<td>0.01</td>
</tr>
<tr>
<td>S. Typhimurium 224/87</td>
<td>Clinical</td>
<td></td>
<td>-5.75±3.2</td>
<td>0.12</td>
</tr>
<tr>
<td>S. Typhimurium DT2</td>
<td>Clinical</td>
<td></td>
<td>-3.79±1.3</td>
<td>0.40</td>
</tr>
<tr>
<td>S. Typhimurium U252</td>
<td>Clinical</td>
<td></td>
<td>-8.67±1.5</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>S. Typhimurium C5</td>
<td>Highest reduction</td>
<td>Mouse</td>
<td>-5.94±0.7</td>
<td>&lt;0.00</td>
</tr>
</tbody>
</table>

Values are significant (p<0.05) from S. Typhimurium 364/87 in a one way ANOVA test based on the p-values.

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 14 days at 22°C, 43%RH (mean values of four replicates and standard deviation shown with bars for each sampling point).

<table>
<thead>
<tr>
<th>S. Typhimurium 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>3%</td>
<td>-18%</td>
<td>-5%</td>
<td>-55%</td>
<td>-92%</td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>-6%</td>
<td>28%</td>
<td>-58%</td>
<td>-94%</td>
<td></td>
</tr>
</tbody>
</table>

| S. Typhimurium C5     | 22%         | 6%           | 28%          | 58%           | 94%         |

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 & Csnoka 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).

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