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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* 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.

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% $^w/_v$ saline added 0.1% $^w/_v$ peptone and $1^w/_v$% 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 BHI (incubated at 37 °C, 23 ± 1h).
- Reduction was calculated using Equation 1 and plotted against time for modelling starting from day 1. (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%$^w/_v$ sucrose in PSP-G.

Equation 1: $$\text{Reduction [CFU/ml]} = \log\left(\frac{N_t}{N_0}\right)$$

Equation 2: $$\%\ \text{Reduction} = \frac{mean\ (Reduction\ day\ 13)_{osmoprotectant} - mean\ (Reduction\ day\ 13)_{control}}{mean\ (Reduction\ day\ 13)_{control}} \times 100$$

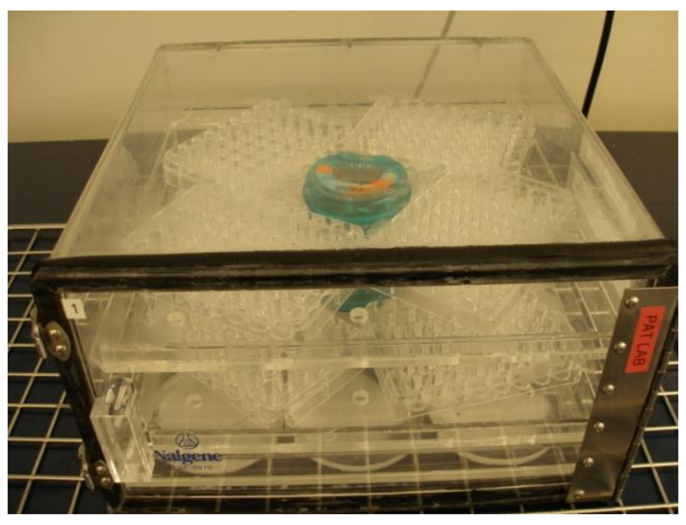

Figure 1: Cells desiccated in 48 well plates in closed chambers at 22°C, 43% RH.

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 reduction and p-value from the one way ANOVA test (both strains are marked with red boxes)

S. Typhimurium strains	Strains selected for testing with osmoprotectants	Origin	Reduction day 14 [CFU/ml] mean ±std. dev	One way ANOVA p-values
***S.* Typhimurium 364/87**	**Lowest reduction**	Chocolate	**-3.18±0.6**	-
S. Typhimurium U292		Pig	-3.50±0.8	0.54
S. Typhimurium 4/74		Bovine	-6.25±2.0[a]	0.01
S. Typhimurium 224/87		Clinical	-5.75±3.2	0.12
S. Typhimurium DT12		Clinical	-3.79±1.3	0.40
S. Typhimurium U292		Clinical	-8.87±1.5[a]	<0.00
***S.* Typhimurium C5**	**Highest reduction**	Mouse	**-9.94±0.7**[b]	<0.00

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

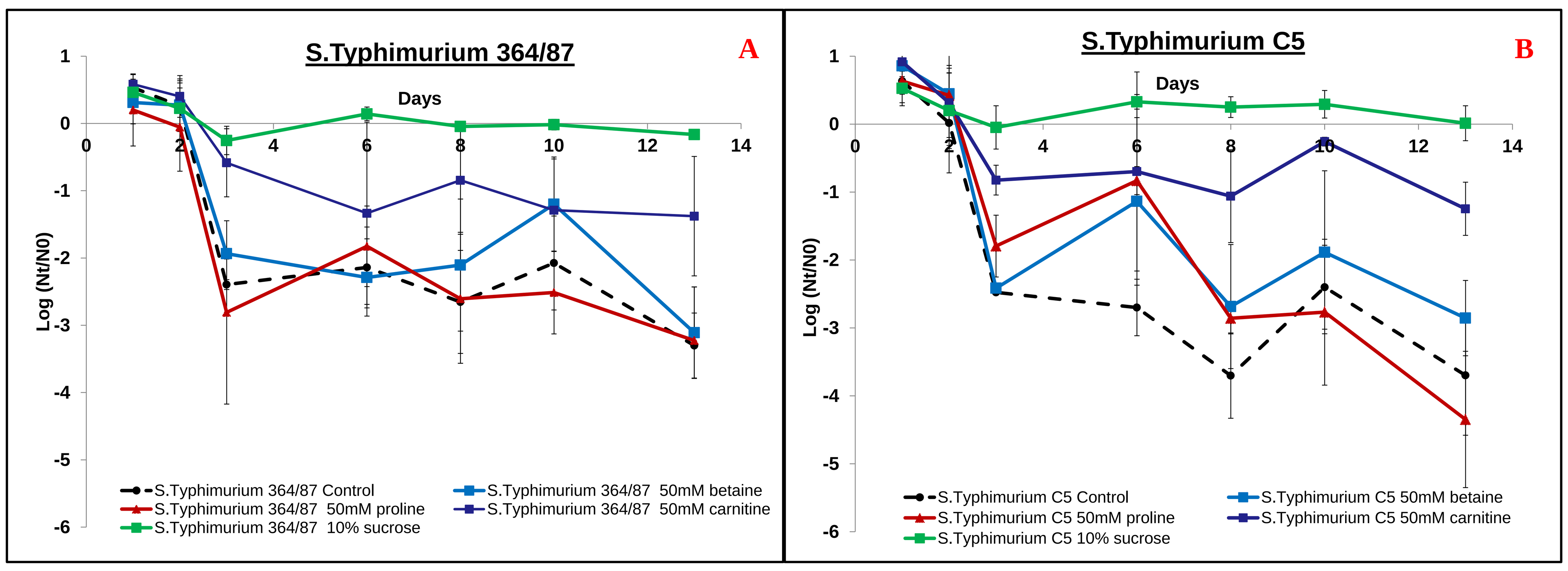

Figure 2: Reduction kinetics for *S.*Typhimurium 364/87 and *S.*Typhimurium C5 desiccated for 13 days at 22°C, 43%RH (mean values of four replicates and standard deviation shown with bars for each sampling point). A and B: *S.*Typhimurium 364/87 and *S.*Typhimurium C5 desiccated in PSP-G alone, or added 50mM betaine, proline, carnitine or 10%$^w/_v$ sucrose, respectively.

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 at 22°C, 43%RH. The cells were desiccated in PSP-G alone or added 1mM betaine, 50mM betaine, proline, carnitine or 10%$^w/_v$ sucrose, respectively. Significant positive impact on reduction was seen with carnitine and sucrose when performing One way ANOVA test (marked with red box) compared to the control.

Set-up	1mM betaine	50mM betaine	50mM proline	50mM carnitine	10%$^w/_v$ sucrose
S.Typhimurium 364/87	3%	-18%	-5%	-55%[b,d]	-92%[a]
S.Typhimurium C5	22%	-6%	28%	-58%[c]	-94%[c]

[a] Significant (p <0.01) for all three trial.
[b] Significant (p <0.05) for all three trials.
[c] Significant (p <0.01) for one trial out of two trials as data is missing for one trial.
[d] Statistical analysed performed with t-test as only one endpoint value for control is available.

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)

Conclusion

- Desiccation tolerance differs within the same serovar.
- The presence of 10% $^w/_v$ 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**.**

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