Novel Formulations for Improved Stabilization of Bacterial Viability During Ambient Temperature Storage and Transport

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Abstract

In this report we describe the development of novel, liquid formulations to preserve viable prokaryotic cells without the use of cold-sotrage. The need for this technology is especially poignant for field collection and sample transport where cell death due to temperature extremes presents a significant problem for subsequent bacterial detection and analysis. Here we present data demonstrating the bacterial stabilization properties of lead formulations on three model bacterial strains under lethal temperature conditions and over a range of bacterial starting concentrations. We show that bacterial survival during temperature stress is improved at least two-fold by the presence of these stabilization formulations for both liquid and soil samples. Significantly, we demonstrate that these stabilization formulations do not serve as primary sources of carbon, energy or nitrogen for our test starins and that our formulations protect cell viability without supporting cell division, an important property for transport media in maintaining cell populations in field collection samples.

Materials & Methods

Bacterial strains and growth conditions: Three model BSL-1 bacterial strains were utilized in these studies: *Escherichia coli* DH5α (pUC19), *Staphylococcus epidermidis*, and *Yersinia ruckeri.* Strains were propagated using standard, complex growth media.

Bacterial viability measurements: Bacterial viability was determined by viable plate counts. Cell counts were determined at the start of each experiment and at each time point indicated.

Bacterial growth measurements: Growth measurements (Figure 5 & 6) were conducted in a BioTek Synergy 2 plate reader at the temperatures indicated with continuous shaking and OD_{600} measurements.

Results

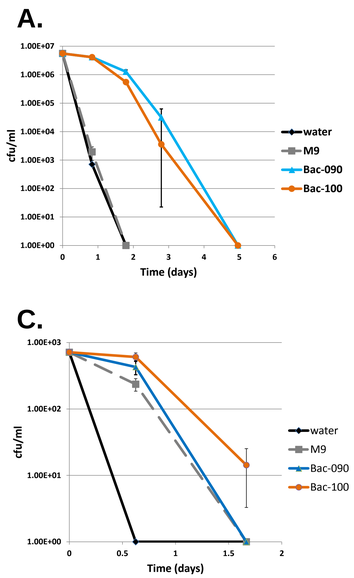


Figure 1. Stabilization of *E. coli* viability at constant, extreme heat (47°C). Stationary phase *E. coli* was spiked into liquid formulations at initial concentrations of 10^7 cfu/ mL **(A)**, 10^5 cfu/ mL **(B)**, and 10^3 cfu/ mL **(C)**. Bacterial survival was measured at the time points indicated by viable plate counts. E. coli survival times are increased in formulations Bac-090 and Bac-100 compared with M9 media and water.

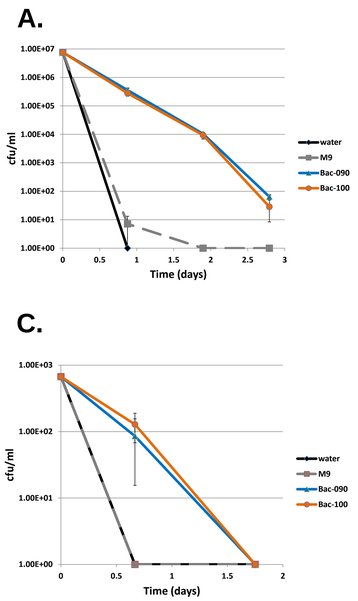


Figure 2. Stabilization of *Staphylococcus epidermidis* viability at constant, extreme heat (47°C). Stationary phase *S. epidermidis* was spiked into liquid formulations at intial concentrations of 10^7 cfu/ mL **(A)**, 10^5 cfu/ mL **(B)**, and 10^3 cfu/ mL **(C)**. Bacterial survival was measured at the time points indicated by viable plate counts. S. epidermidis survival was increased in formulations Bac-090 and Bac-100 compared with M9 media and water.

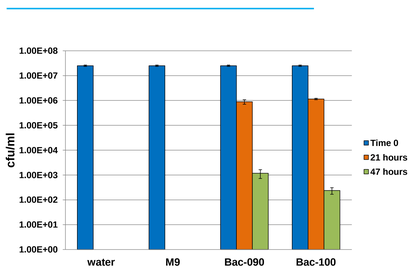
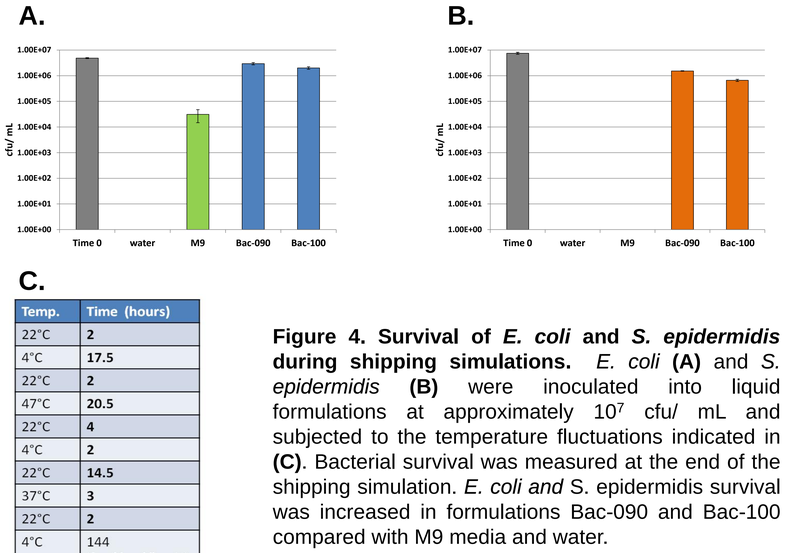


Figure 3. Stabilization of *Yersinia ruckeri* viability at constant extreme heat (42°C). Stationary phase *Y. ruckeri* was spiked into liquid formulations at initial concentrations of 10^7 cfu/ mL. Y. rukeri survival at 42°C was significantly increased by Bac-090 and Bac-100 compared with M9 media and water.



Temp.	Time (hours)
22°C	2
4°C	17.5
22°C	2
47°C	20.5
22°C	4
4°C	2
22°C	14.5
37°C	3
22°C	2
4°C	144 (S. epidermidis only)

Figure 4. Survival of *E. coli* and *S. epidermidis* during shipping simulations. *E. coli* **(A)** and *S. epidermidis* **(B)** were inoculated into liquid formulations at approximately 10^7 cfu/ mL and subjected to the temperature fluctuations indicated in **(C)**. Bacterial survival was measured at the end of the shipping simulation. *E. coli and* S. epidermidis survival was increased in formulations Bac-090 and Bac-100 compared with M9 media and water.

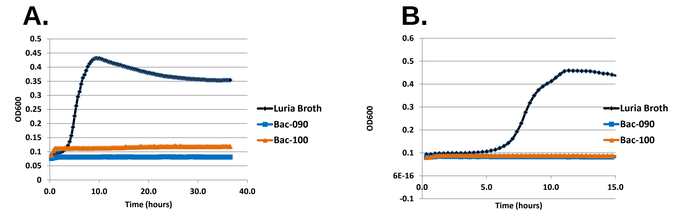


Figure 5. Test for bacterial growth in stabilization formulations. *E. coli* did not grow in either stabilization formulation (Bac-090 and Bac-100) at the optimal growth temperature, 37°C, supporting the conclusion that these formulations increase bacterial survival, not cell growth.

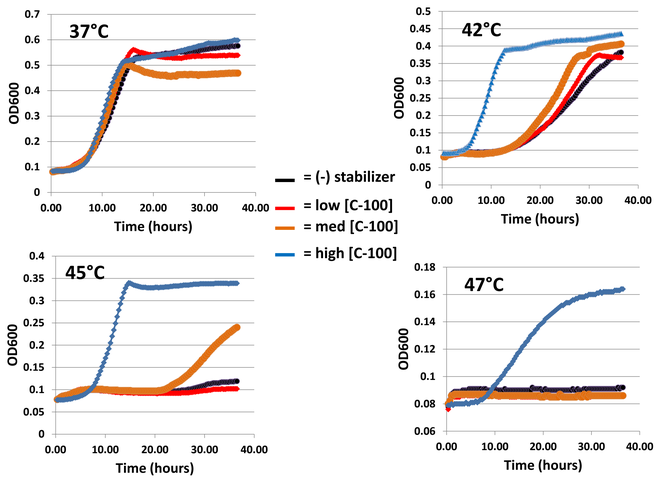


Figure 6. Stabilization compound increases maximum growth temperature. *E. coli* was inoculated into defined growth media in the absence or presence of titrated amounts of the key stabilization compound C-100. Samples were incubated with constant shaking at the temperatures indicated. OD_{600} was measured continuously on a BioTek plate reader. C-100 has no negative effect on growth at 37°C and increases the growth rate at the non-optimal temperature, 42°. C-100 enables growth in a dose-dependent manner at non- permissable high temperatures, 45°C and 47°C

Summary

- Novel formulations Bac-090 and Bac-100 increase the survival of bacteria at lethally elevated temperatures:
  - *Escherichia coli* (Figure 1)
  - *Staphylococcus epidermidis* (Figure 2)
  - Yersinia ruckeri (Figure 3)
- Bac-090 and Bac-100 improve bacterial survival during extreme temperature fluctuations (Figure 4)
- These model bacterial strains do not grow in Bac-090 or Bac-100 (Figure 5)
- Key formulation component, C-100, increases the maximum growth temperature of model bacterial strains (Figure 6)