Effect of Fatty Acid Supplementation on Bacterial Cell Survival upon Freezing

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Abstract

Sub-zero storage for the preservation of bacterial cells has inherent problems that reduce cell survival rates. Supplementation of E. coli cultures with short chain fatty acids was found to support cell viability during frozen storage. Seven fatty acids, including palmitic, lauric, stearic, dodecanoic, palmitoleic, linoleic and oleic acid, were used for supplementation. Growth studies demonstrated that lauric, oleic and stearic acids improved cell survival rates relative to non-supplemented cell cultures by 94 ± 6.6%, 117.6 ± 8.5%, and 176.5 ± 16.2%, respectively; however, only stearic acid supplemented cultures were determined to significantly increase survival rate compared to non-amended cultures (p<0.028). Supplementation of bacterial cell cultures with stearic acid provides a significant improvement of cell survival upon freezing.

Keywords: Freezing, cell survival, fatty acids, Escherichia coli.

Introduction

Sub-zero storage is one of the most common methods for long-term preservation of bacterial cells (Ray et al. 1971). Unfortunately, inherent problems to cell freezing, such as cell lysis and dehydration due to ice crystal formation and localized increases in salt concentration, reduce cell survival rates (Pegg 1976); this loss of cell viability is observed when cells are stored in higher freezing temperature ranges (-20°C to -40°C) even when supplemented with cryoprotectants such as glycerol or polyethylene glycol (Chung et al. 1989). While ultra-low temperature storage (below -80°C) improves cell viability ultra-low freezers are expensive to purchase and incur high maintenance costs (Singer et al. 2005).

Cells maintain membrane integrity when growing at cold temperatures through homeoviscous adaptation, an adaptation process whereby cell membrane composition is adjusted to contain a higher proportion of triacylglycerols composed of short chain polyunsaturated fatty acids (Marr and Ingraham 1962, Ray et al. 1971). This adaptive process is vital to cell survival as it allows the membrane to
shift to a lower gelling point in an effort to maintain constant membrane fluidity, thereby preventing cell lysis upon freezing (Esfahani et al. 1969).

Hathaway (2012) demonstrated that cell survival after freezing increased by 44% when *Escherichia coli* was grown at 15°C as compared to the same cultures grown at 37°C; however, cell growth slowed dramatically, thereby increasing culturing times from hours to days to reach the same optical density. Other studies have demonstrated that the supplementation of the growth media with short chain fatty acids resulted in their incorporation into the cell membrane (Morrison 1977). The purpose of this research was to determine if supplementation of culture media with short chain fatty acids would improve bacterial cell viability during frozen storage.

**Materials and Methods**

*Escherichia coli* NEB 5α (New England BioLabs) was maintained in LB medium and incubated at 37°C with vigorous shaking (250 rpm). To test the effects of fatty acid supplementation in growth media, 5 mL of Luria Broth (LB) was supplemented with Lauric acid (12:0), Laurolieic acid (12:1), Myristic acid (14:0), Myristoleic acid (14:1), Palmitic acid (16:0), Palmitoleic acid (16:1), Stearic acid (18:0), Oleic acid (18:1), or Linoleic acid (18:2) at a 2% (w/v) final concentration. Media was then inoculated with *E. coli* from a fresh overnight culture at a 1% (v/v) and incubated at 37°C with vigorous shaking until reading an optical density (OD$_{600nm}$) of 0.8; the culture was then dispensed in 1 mL aliquots. Four aliquots of each cell preparation were immediately plated on LB agar in serial dilutions from
10^{-1} to 10^{-7}, incubated for 24 hours, and the resulting colonies enumerated. Four additional aliquots of each treatment were frozen at -20°C for 48 hours, then thawed and plated. The percent survival was determined by dividing the number of colonies arising after freezing by the number of colonies resulting from non-frozen cultures. Averages and standard deviations for each treatment were determined and results were analyzed for significance using a one-way ANOVA with a post-hoc Tukey comparison.

Results

Due to the hydrophobic nature of fatty acids, a slurry was formed at 2% (w/v) concentration. This slurry did not dissipate throughout the growth of the bacteria, wherein the fatty acids remained in an emulsified, suspended state for the duration of the experiment.

Growth studies demonstrated that lauric, oleic and stearic acids improved cell survival rates relative to non-supplemented cell cultures by 94 ± 6.6%, 117.6 ± 8.5%, and 176.5 ± 16.2%, respectively (Figure 1). However, only stearic acid was determined to significantly increase survival rate compared to non-amended cultures (p<0.028).
Figure 1. The interval plot of palmitic, lauric, stearic, dodecanoic, palmitoleic, linoleic, oleic, acids and control group of non-supplemented cell cultures, 95% CI for the means. * = Survival significantly greater (p<0.05) that control.

Discussion:

Supplementation of media with stearic acid demonstrated an increase in cell viability after frozen storage, while supplementation with linoleic, palmitoleic and dodecanoic acid, resulted in little or no improvement of cell viability. Short chain, unsaturated, fatty acids, such as palmitoliec acid (16:1), were expected to be more protective due to their ability to raise the gelling temperature of a membrane, thereby maintaining membrane fluidity upon freezing. Stearic acid (18:0), however, demonstrated the most significant improvement in cell survival despite being a longer chained saturated fatty acid. Each of the fatty acids, being
hydrophobic, formed an emulsion when added to the bacterial growth media and this slurry persisted through to the freezing stage. One possible explanation for the protective ability of stearic acid may related to its role as an external cryoprotectant. Glycerol or sucrose are often added to the cell suspensions as cryoprotectants to prevent ice crystal formation (Chung et al. 1989). If the stearic acid emulsion inhibited ice crystal formation in the bulk fluid it could have acted as a cryoprotectant and served to improve cell viability.

This research suggests that stearic acid supplementation may be able to increase cell viability upon freezing but further testing is needed to elucidate the mechanism by which fatty acid-mediated cryoprotection is achieved. In addition the effectiveness of fatty acid supplementation should be compared with other common cryoprotectants.

**Literature Cited**


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