Investigating the Maillard Reaction in Autoclaved Media and its Effect on *Escherichia coli*

Rachel J. Rhee '15 and Joan L. Slonczewski
Department of Biology, Kenyon College, Summer Science 2013

Abstract

The use of culture media is a chief component in the cultivation of *Escherichia coli* for the purpose of our study. There are two types of media sterilization techniques employed in the lab: filter sterilization and autoclaving. The use of an autoclave to sterilize media under high temperatures however, may result in the formation of unwanted compounds, known as Maillard reaction products (MRPs). MRPs are formed when the amino group of an amino acid reacts with a carbonyl group of a sugar, fusing the two molecules together thus forming compounds that can cause detrimental effects to the cell[4]. This is a concern being addressed in our study because all media base prepared in the lab contain amino-acid-rich components like tryptophan and sugars like yeast extract that may be reduced to harmful products, ultimately inhibiting growth of *E. coli* cultures. In this study, we mimicked MRPs using L-lysine and D-glucose (two MRP precursors used in MRP-rich medium) in order to determine whether the chemical modification of MRPs in media sterilized at high temperatures would have a toxic effect on cell survival under anaerobic conditions at pH 2. Although preliminary research has shown that *E. coli* is capable of surviving more extreme acid challenges when exposed to filtered media than autoclaved media, we found that autoclaving or filter sterilizing exposure LBK media does not affect the ability for *E. coli* to survive in highly acidic conditions.

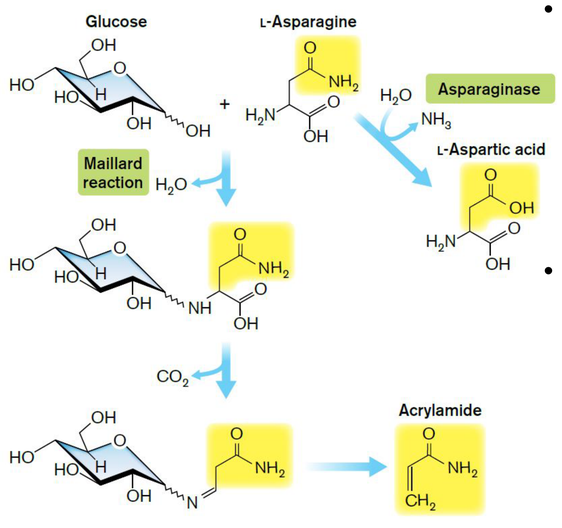
Introduction

Sterilization Techniques

- Two types of media sterilization techniques: filter sterilization and autoclaving (employs high-heat sterilization at 121°C)
- Luria-bertani broth (LBK) is supplemented with amino acids and sugars like tryptophan and yeast extract which may be reduced to harmful products at high temperatures, thus forming unwanted compounds known as Maillard Reaction products (MRPs)
- M63 minimal media includes vital nutrients only necessary for growth thus is unlikely to produce MRPs at higher temperatures

The Maillard Reaction (MR)

- Phenomenon responsible for a plethora of flavors and for the "browning" of meat, bread, beer, and other foods
- At high temperatures, the chemical reaction between amino acid and sugar forms MRPs which can cause detrimental effects to the cell[2]



- In culture medium, amino acids bearing a free amino group such as lysine easily react with reducing sugars during heat treatment[1]
- D-glucose and L-lysine are two MRP precursors that are used in this study to mimic the Maillard reaction in non-heat treated media

Results

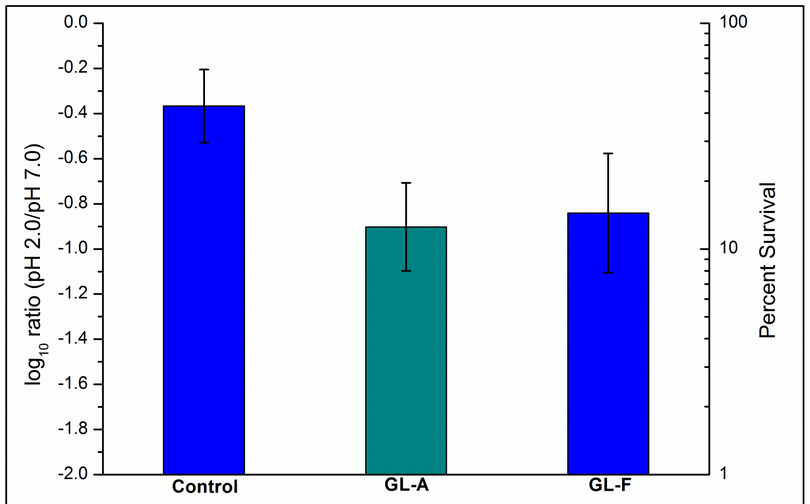


Figure 1. Survival of *E. coli* exposed to autoclaved vs. filter sterilized media treated with glucose and lysine. GL-A media contained LBK with 0.06 M D-glucose and 0.07 M L-lysine autoclaved together. GL-F was the same, but filter sterilized. Control was exposed to filter sterilized LBK at pH 2. All trials were run in anaerobic conditions. Error bars = SEM, n=6.

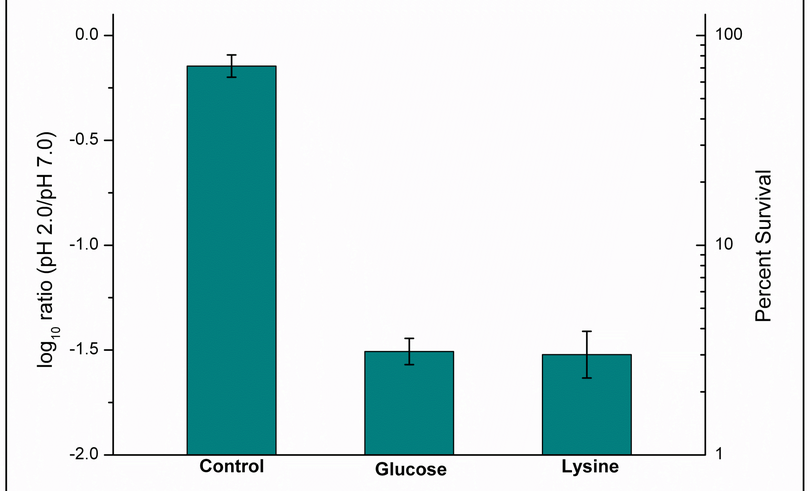


Figure 2. Survival of *E. coli* exposed to LBK media treated with autoclaved glucose or lysine. Exposed cultures were grown in LBK media with 0.06 M D-glucose and 0.07 M L-lysine separately, autoclaved for one hour. Control was exposed to filter sterilized LBK at pH 2. All trials were run in anaerobic conditions. Error bars = SEM, n=6.

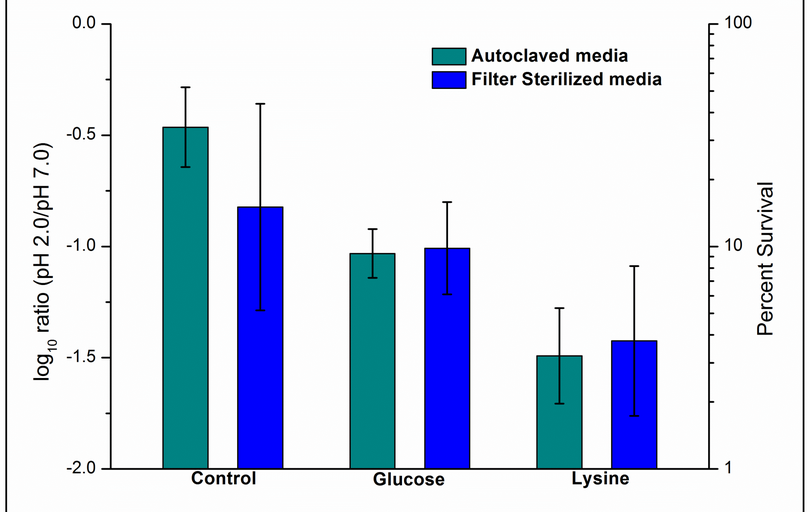


Figure 3. Survival of *E. coli* exposed to autoclaved or filter sterilized LBK media at pH 2 treated with glucose or lysine. Exposed cultures were grown in LBK media with 0.06 M D-glucose or 0.07 M L-lysine and were either autoclaved for one hour or filter sterilized. Control was exposed to filter sterilized LBK at pH 2. All trials were run in anaerobic conditions. Error bars = SEM, n=6.

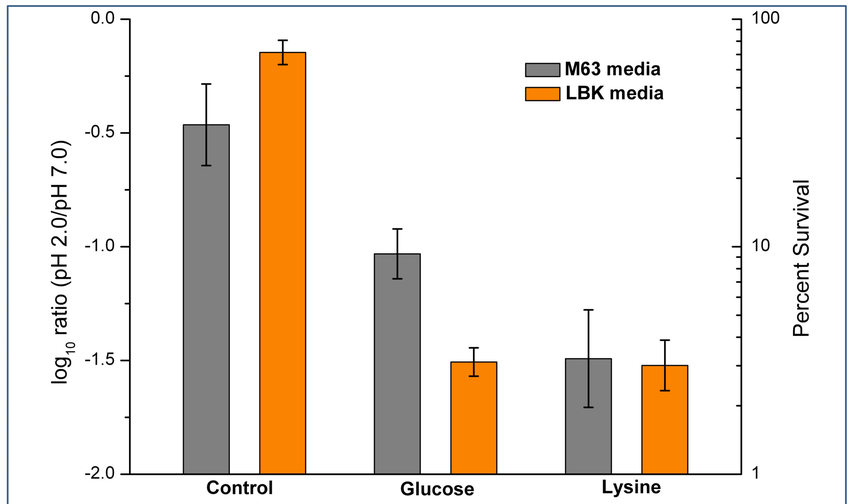


Figure 4. Survival of *E. coli* exposed to autoclaved M63 vs. LBK media treated with glucose or lysine. Exposed cultures were grown in LBK media with 0.06 M D-glucose or 0.07 M L-lysine autoclaved for one hour. Control was exposed to filter sterilized LBK at pH 2. All trials were run in anaerobic conditions. Error bars = SEM, n=6.

Conclusions

1. There was no sign of harmful effects from MRPs. Autoclaving or filter sterilizing media treated with lysine and glucose concurrently had no effect on *E. coli* survival (Fig 1). Therefore, we determined that the inhibitory effects of bacterial survival in acid are due to the induced treatments rather than the chemical formation of MRP.

2. Glucose and Lysine both inhibit *E. coli* survival when autoclaved. Low survival (~2%) of *E. coli* in acidic anaerobic conditions indicate that autoclaving media treated with D-glucose and L-lysine separately had inhibitory effects (Fig 2).

3. Autoclaving or filter sterilizing LBK media with glucose or lysine does not effect the ability for *E. coli* to survive in more extreme acid. If autoclaving LBK media induced toxic MRP, then we would expect a difference in survival of *E. coli* between autoclaved and filter sterilized media. Similar survival rates suggest that glucose and lysine are not responsible for inhibitory effects of MRP (Fig 3).

4. Autoclaving M63 or LBK media with glucose or lysine does not effect the ability for *E. coli* to survive in more extreme acid. M63 media, unlike LBK, does not contain the carbonyl and sugar compounds necessary for the Maillard reaction to occur. Therefore, we expected similar survival rates in autoclaved or filter sterilized M63 media. Similar survival rates in both M63 and LBK media suggest that there is no sign of MR activity in media sterilized at high temperatures with amino acids and sugars (Fig 4). Thus we were unable to successfully mimick the MR

Methods

Anaerobic Extreme Acid Survival Assay: All anaerobic assays took place in the controlled atmosphere chamber (Plas Labs) that contained a gas mixture of 5% CO_2, 10% H_2, and 85% N_2. Acid-adapted cultures of *E. coli* strain W3110 (wild type) were grown to stationary phase in LBK buffered with 100 mM MES to pH 5.5 in 8 mL screw cap tubes rotated end-over-end. Overnight cultures were diluted1:400 in acidic LBK or M63 (filter sterilized or autoclaved) and incubated with rotation at 37 °C. Following a 2 hr. exposure, cultures were then serially diluted to a final dilution of 1:80,000 in pH 7.0 M63, and plated onto LBK agar whereas overnight cultures of the control were diluted 1:400 in M63 pH 7.0. The colony counts of both treatments were compared to determine the percent survival. Percent survival was assessed by subtracting the log number of viable exposure colonies from the log number of control colonies. A phenotype was observed through a decrease in survival.

Acknowledgements

I would like to thank Dr. Joan Slonczewski for all of her help and guidance throughout the course of this project. Also, the completion of this project would not have been possible without the help of Keith Martinez and the Slonczewski lab members- Ian Perrone, Kaitlin Creamer, and Amanda He. Special thanks to Meredith Dunn for all the clean equipment and agar plates. This project was funded by the Kenyon College Summer Science Program.

References

1. Erbersdobler HF, Somoza V. Forty years of Furosine - Forty Years Of Using Maillard Reaction Products As Indicators Of The Nutritional Quality Of Foods.Mol Nutr Food Res. 2007 Apr;51(4):423
2. Norris JR. Design of media: Methods in Microbio. 3rd ed. London: Academic Press Inc; 1970:273.
3. Slonczewski J and Foster J, Microbiology: An Evolving Science 3E, W. W. Norton & Co., 2013.
4. Zhang Q, Ames J, Smith R ,Baynes J, Metz T. A Perspective on the Maillard Reaction and the Analysis of Protein Glycation by Mass Spectrometry: Probing the Pathogenesis of Chronic Disease. J Proteome Res. 2009 February 6; 8(2): 754–769.