Name(s)
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Project Number
J2009

Project Title
Using Next-Gen Sequencing to Compare Levels of Bacteria in Bagged Salads

Abstract

Objectives/Goals
The purpose of this project was to analyze types of bacteria present in bagged salads and determine if any bacteria were pathogenic and whether bacterial composition and quantities changed over time. I believed that older packages of lettuce would show more evidence of cell deterioration, which would mean that they might contain a higher concentration of bacteria.

Methods/Materials
I tested "Herb Salad Mix" and "Butter Lettuce." I tested samples with five different expiration dates to simulate how long the lettuce might be stored in the refrigerator. I poured 30 mL of DPBS into each bag to suspend the bacteria, then poured the bacterial suspension into a 50 mL tube. I genetically sequenced each sample to find the bacterial composition, and also grew bacteria from each sample group for a total of 20 different agar plates to get a sense of the quantity of bacteria. The final five plates were inoculated with 50 microliters of bacterial suspension diluted at 1:500.

Results
In the agar plates, I saw no significant difference in the number of bacteria in each sample. When I sequenced the samples, I found many different types of bacteria. In the Herb Salad Mix, the majority of the bacteria encountered were from the genuses Shewanella, Pseudomonas, and Flavobacterium, in addition to small quantities of many other types of bacteria. In the Butter Lettuce, the bacteria were even more diverse and once again represented in small percentages, except for the Pseudomonas, Nostocales, and Bacillales. In both types of bagged salads it was consistently observed that the Pseudomonas bacteria essentially took over, and showed significant increases over time.

Conclusions/Discussion
Although there did not appear to be any significant difference in the overall numbers of bacteria in these salads over time, the composition of the bacteria showed a significant change over time. The Pseudomonas seemed to dominate over the other bacteria. Very few other bacteria showed increases in population. In future experiments it might be interesting to introduce a pathogen, such as E. coli, to see how it affects the bacterial composition and bacterial growth patterns. Because some strains of bacteria observed, such as Pseudomonas, may be pathogenic to humans, I would recommend that packages of bagged salads be consumed as soon as possible. I would also recommend the lettuce be washed before consumption to reduce risk of infection.

Summary Statement
I analyzed samples from bagged salads with different expiration dates to compare how the composition and quantities of bacteria present in the bagged salads changed over time.

Help Received
I would like to thank my parents for driving me to the lab and to the store to obtain my materials. I would also like to thank Tanya Biorac, Loni Pickle, Yutao Fu, Mark Andersen, and Joydeep Goswami for supervising me while I performed the sequencing process at Thermo Fisher Scientific.