Microbial Growth and Assessing Mutagenic Properties in Plastic Bottles

By: Eliza Chevalier, Eliza Doncaster, and Steven Lamonde

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Introduction

It is often debated whether plastic has adverse effects on humans and considering that a large portion of our food is packaged in plastic, it is important to understand our biological response to its chemical compounds. In research conducted by Ohnishi et. al (2013), the growth of microorganisms and microbial contamination in various beverages were studied. This research was completed by investigating microorganisms that contaminated bottles and analyzing the growth of the microorganisms in the beverages at room temperature. They found a variety of foodborne pathogens which suggests that the microbial community of a plastic bottle could be harmful to humans. In our study, we plan to compare their research to the microbial community found in stainless steel and glass bottles to see if this is something that is specific to plastic bottles or if these pathogens are found in any bottle. Therefore, through a culturing study, growth study, and an Ames assay, we plan to assess the microbial communities of plastic bottles compared to other bottle types and their potential for mutagenic chemical compounds.

Hypothesis

Plastic bottles promote more diverse microbial growth and have a higher potential for mutagenic chemical compounds than stainless steel or glass bottles (Mortelmans and Zeige 2000).

Methods and Materials

The materials needed for Growth study, Culture Study and the Ames assay are listed below.

- The appropriate bacteria (has yet to be decided)
- Media mix (according to which bacteria is found in culture study)
- (2) Micropipette set (1000, 200, 100, 20)
- (2) Pipette tips (1000, 200, 100, 20)
- 5ml glass pipette tips
- (2)Pipette aids
- Incubated shaker (holding 12)
- (2 sleeve) Petri dishes
- Media + Agar mixture (dependent on chosen bacteria)
• (2) Bunsen burners
• Autoclave usage
• Biohazard bags
• (2) Biohazard buckets
• Parafilm
• Inoculations loops
• Biophotometer
• Flask Stoppers
• Flasks (11 250ml flasks, 2 125ml flasks, 1 2L flask, 2 1L flask)
• Ames assay kit
• Refrigerator
• Kim wipes
• (500 or less if rewashed) Cuvettes
• 10% bleach solution
• Vesphene
• Rubber gloves (small/ 2 boxes)
• Lab coats (two size medium)
• Safety glasses
• Autoclave tape
• 50ml conical tubes (1 package)
• 15ml conical tubes (1 package)
• (4 each) plastic, stainless steel and glass bottles
• Materials for classifying bacterial identity (method has yet to be determined)

Note: The molecular lab would be the ideal workspace considering that it is the only lab that is sterile enough for this experiment and it already contains all of the equipment we need such as the biophotometer, shaker, etc.
**Culture Study**

In order to assess the microbial community of the three different types of bottles, as well as determine which bacteria will be used in the growth study, we first must conduct a culture study. To simulate reusable water bottles, we will leave one of each type of bottle on the bench in the Molecular Lab at room temperature for 48 hours with 250 mL of water. After 48 hours we will culture bacteria from the bottles and assess the diversity of the microbial community of the three bottles. Bacteria will be cultured according to methods determined by the Connecticut Valley Biological Supply Company (in Molecular Lab Manual).

**Growth Study**

We plan to grow the bacteria determined by the culture study in plastic, glass, and stainless steel bottles containing media specific to that bacteria as recommended by the American Tissue Culture Collection (ATCC). There will be three bottles of each type and the bottles will be placed on a shaker at room temperature and 250 rpm for three days in order to determine the differences in the bacterial growth curve in each type of bottle.

**Ames Assay**

We would also measure the mutagenic potential for chemical compounds using an Ames assay, or a biological test that measures chemical substances that can produce genetic damages (Mortelmans and Zeige 2000). Methods will be determined by the instructions included in the Ames Assay kit.
References
