The effect of multi parameter nutritional regime on *Escherichia coli* strains

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Abstract

Escherichia coli (E.coli) is a gram-negative, rod shaped bacterium that is commonly found in the lower intestine of warm-blooded animals it is part of the feces of the animal. Fecal-oral transmition is the major route through which pathogenic types of the bacteria cause disease. *E.coli* are able to survive for a limited amount of time outside the body approximately 2 weeks (E.P.A., 2005). *Escherichia coli* are capable of growing in environments ranging from very dilute aqueous solutions of nutrients to media containing molar concentrations of salts or non-electrolyte solutes. This is one of the reasons why *E. coli* can grow in different types of media and environments. *E.coli* strains differ among warm-blooded animals, because of their diet and, possibly, environmental factor in the gut of the animal. This research is focused in the examination of *E.coli* and the different strains in the animal gut and comparing the growth rate of the different strains. I hypothesize that the growth rate will vary among *E. coli* species, based on their growth medium, which will be modeled after animals diet (T. Record, 2009). We used a genetic method called Ribotyping. This method requires selective cultivation of indicator bacteria from feces samples. We cultivated the bacteria and the identification of *E.coli* isolates were confirmed with MacConkey agar with MUG method and with enterotube II (BD Diagnostic Systems, Heidelberg, Germany). We measured bacteria growth using a spectrophotometer. Each sample was measured six times, and carnivore and herbivores were compared. The results showed there is a difference between the growth of the bacteria between animals: dogs bacteria grew better on carnivorous diet than in herbivorous, cows bacteria grew at the same rate.

Introduction

E.coli is a major component of the feces of warm-blooded animals, primarily mammals and birds. Fecal-oral transmition is the major route through which pathogenic types of the bacterium cause disease. Cells are able to survive for a limited amount of time outside the body approximately 2 weeks (EPA, 2005), which makes them ideal indicator organisms to test environmental samples for fecal contamination. The bacterium can also be grown easily in a laboratory, and has been intensively investigated for over 70 years. *E. coli* is one of the most widely studied prokaryotic model organisms (G.Tortora, B. Funke, 2009). Escherichia coli is capable of growing in environments ranging from very dilute aqueous solutions of nutrients to media containing molar concentrations of salts or non-electrolyte solutes. Growth in environments with such a wide range of osmolarities poses significant challenges for cells. To meet these challenges, *E. coli* adjusts a wide range of cytoplasmic solution variables, including the cytoplasmic amounts both of water and of charged and uncharged solutes. This is one of the reasons why *E. coli* can grow in different types of media and environments. The purpose of this research is to see the variability of *E.coli* growth on different diets. *E. coli* strains differ among warm-blooded animals, because of their diet and, possibly, environmental factor in the gut of the animal. I hypothesize that the growth rate will vary among *E. coli* species, based on their growth medium which will be model after animals diet.

Methodology

- The genotypic method that we used is called Ribotyping.
- This method requires selective cultivation of indicator bacteria from fecal samples.
- Ribotyping is a version of restriction fragment polymorphism (RFLP)-Southern hybridization analysis that has found wide application in the subtyping of a variety of Gram-negative and Gram-positive bacteria Ribotyping is based on the detection of genetic differences in the genomic sequences (EPA 2005).
- We cultured *E. coli* in different types of media, to see how the different nutrients (diet) affect the growth of the bacteria.
- The process to cultivate the bacteria started collecting the feces.
- A little amount of the sample was incubated for 12-24 hours in a LB broth media.
- Afterwards we placed some of the bacteria in a plate with Mac Conkey agar, followed by isolation of 10 of the colonies, and placing them in separate tubes with LB broth, incubated again for 12-24 hours.
- We made another plate striking the bacteria around the plate to isolate it, and incubate for 12-24 hours.
- We confirmed if the colonies were *E.coli* with enterotube II (BD Diagnostic Systems, Heidelberg, Germany).
- If they were, we placed the colony in other plate and then in different tubes with brain heart infusion (carnivorous) and Schaelder (herbivorous) broth and we made a plate with LB agar to ribotype the bacteria (EPA, 2005).
- Afterwards we measured the growth of the bacteria using a spectrophotometer. Each sample was measured six times, and carnivore and herbivores were compared.

(a) 

(b) 

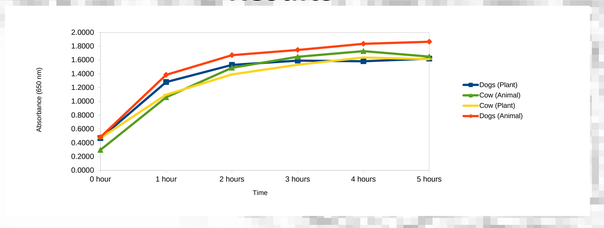
(c) 

(e) 

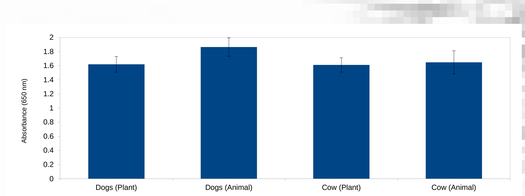
(d) 

Figure 1: Clockwise from the upper left: (a) Ribotype printer, (b) Samples preparation,(c) carnivore medium for *E. coli* growth,(d) collecting samples at UVM farm (e) Herbivore medium for *E. coli*

Results



Graph 1. Comparison of the absorbance between animals and diets. The results were analyzed with SPSS, and there is not a significance for the type of E. coli with a result of
.070, and there was not significance for the different diet with a result of .259



Graph 2. Histogram comparing the growth of the bacteria between diets, and animals, which shows smalls difference between the dog bacteria, but there is no difference between the cow bacteria growth.

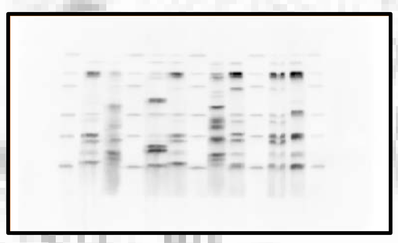


Figure 2. Electrophoresis Gel, of the different animal bacteria.

Discussion

The results showed that there is no relationship between the diet of the animal and the growth of the bacteria. This might because the *E.coli* can grow on different media, and can get used to different environments. There is a small difference between the growth of the dogs bacteria and the diet. But that is not of statistical significance(p= .070). The cows bacteria did not show any difference so it is not affected by the diet.
My conclusions are that the bacteria adapted to the media and grew normally, I suggest that in future investigations the analysis has to be done quickly, so in that way the bacteria does not have the time to adapt to the new diet, and might show a difference in growth.

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