Cantaurus, Vol. 23, 10-12, May 2015 © McPherson College Department of Natural Science

Lipase related growth in E.coli K-12

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ABSTRACT

The purpose of this study was to determine any amount of growth from *Escherichia coli* K-12 while using the carbon source of squalene. *E. coli* K-12 is a gram-negative bacterium that was grown in spirit blue agar at the conditions of 37ºC over a period of 4 days (96) hours. After about every 24 hours new plates were made and a count of colonies were totaled. The squalene was placed into the agar before making the plates so that squalene was used in all the plates including the blanks. Over this time the bacteria *E. coli* K-12 had grown using squalene but only the first 36 hours were valid enough to use.

Keywords: *Carbon source, E.coli, Lipase reagent, Spirit Blue agar, Staphylococcus Epidermis, Squalene.*

INTRODUCTION

A lipase reaction is the breakdown of fatty acids. Lipases are commonly known from those of enzymes that break down fat molecules. Fats, also called lipids, they exist in many forms. And different kinds of fats require different lipases to break them down. Your body uses lipases to digest fat and it also depends on lipases to help move cholesterol in the body. Fatty acids are found in oil that is a part of soil, humans, and animals.

Escherichia coli (*E. coli*) are a large diverse group of bacteria. *E. coli* belongs to the taxonomic family known as *Enterobacteriaceae*. *E. coli* is commonly found in the intestines of people and animals. The majority of the strains of which are not dangerous. *E. coli* bacteria are essential to the healthy functioning of human and animal digestive systems. *E. coli* is Gram negative facultative, rod-shaped bacteria that grows in anaerobic conditions and is better off in an environment that is stabilized around 37°C and with a pH7. Living in a facultative anaerobic condition means the bacteria is able to grow with or without oxygen. E.coli can sense the changes of temperature, pH levels, certain chemicals and osmolarity (Moder, 2008). More severe strains of the bacteria can cause life threatening diseases such as respiratory illnesses and pneumonia. These strains can be transmitted through contaminated food and water or via contact with the infected person or animal.

Squalene is a natural chemical carbon compound originally developed for viable purposes. This 30-carbon organic compound has can be produced from shark liver oil, although plant sources and humans are used as well. Squalene's primary purpose is to help in the synthesis of all plant and animal sterols, including cholesterol, hormones and vitamin D in the human body. Some have come to believe that squalene may be a chemo preventive substance that protects people from cancer (Das, Baruchel).

The strain *E. coli* K-12 in the human intestine has been shown to grow poorly. History shows K-12 has safe commercial use, and is not known to have any negative effects on plants or microorganisms (Biotechnology Program, 2012). In order to better understand the properties of E.coli K-12 in our world and bodies we can study its source of carbon. Even though it has extremely simple cell structure, with only one chromosomal DNA and a plasmid, it can perform complicated metabolism to maintain its cell growth and cell division (Escherichia Coli – MicrobeWiki, 2015). Due to the high presences of squalene in human intestines, and also with *E. coli* K-12 we can use squalene as a carbon source.

MATERIALS AND METHODS

The materials squalene ≥98% liquid and Polysorbate 80 were from Sigma-Aldrich. *E. coli* K-12, *Staphylococcus epidermis* (37°C) and *Bacillis subtilis* (30°C) came from Carolina Biological Supply Company. The spirit blue agar formula and M9 minimal salts 5x all came from McPherson College.

The protocol used was based on the spirit blue agar lipase reagent. This is a reagent for detecting lipase producing microorganisms. By preparing 0.05ml of Polysorbate 80 with 20ml of warm purified water and adding 5ml of squalene makes the lipase reagent. The spirit blue agar uses two controls: *Staphylococcus aureus* and *Staphylococcus epidermidis*. I only replaced *Staphylococcus aureus* with bacillus subtilis and used *Staphylococcus epidermidis* as my control. By the time I was ready to start the *Bacillus subtilis* wasn't viable to use anymore. So the formula for making spirit blue agar contains 35g. This includes Pancreatic Digest of Casein (10g), Yeast Extract (5g), Agar (20g), and Spirit Blue (.15g). Pancreatic Digest of Casein is a protein, the yeast extract is a b-complex vitamin that contains everything in a living yeast cell, the agar is a solidifying agent, and the spirit blue consists of carbon, nitrogen.

Before making the agar and plates, the turbidity was measured; which measures water clarity of how much the material suspended in water decreases the passage of light through the water (5.5 Turbidity, 2015). While only having 10ml of squalene I knew I

wasn't going to need more than 10 plates. 4 plates total for the *E. coli* K-12; 2 for *E. coli* 1 and 2 for *E. coli* 2 which is the same thing I just double up on *E. coli* plates. And 2 plates for the *Staphylococcus epidermidis*, 2 plates for a 3 way split between E.coli K-12, *Staphylococcus epidermis* and the blank as shown in Figure 1b. I then reduced the 35g down to 7g: 10 × 20 = 200ml 35g → 1000ml 200/1000 × 35 = 7g and by that reducing the spirit blue agar formula by a factor of 5.

So with that, measure out 7g of the spirit blue agar. Add 200ml of water to the agar as it stirs and heats on a hot plate, until fully dissolved. Autoclave at 121°C for 15minutes, then cool at 55° C. In the hood, mix the lipase reagent into the agar waiting about 15-20 minutes to become a solid. This was done shortly after the agar was taken out of the agar. After the agar plates are made, the plates were inoculated by a broth and then streaking them with *E. coli* K-12 and the *Staphylococcus epidermidis*. Incubate at 35 ± 2°C because *E. coli* K-12 grows best under those conditions and also checking every 24 hours while making new plates each day. After each 24 hours I also tried counting as much of the colony forming units to the best that I could.

RESULTS

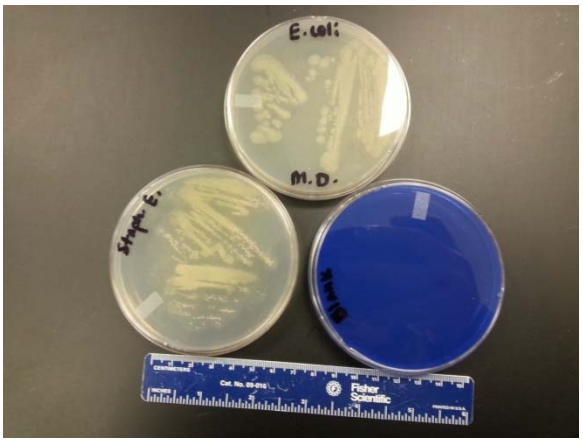

Figure1a. Individual plates of *E. coli*, *Staphylococcus epidermis* and also the blank. There was a tremendous amount of growth for the staph and *E. coli* because the agar completely changed colors and is no longer blue like the blank.

Figure1b. The change in growth also but instead of individual plates all three were put on one plate.

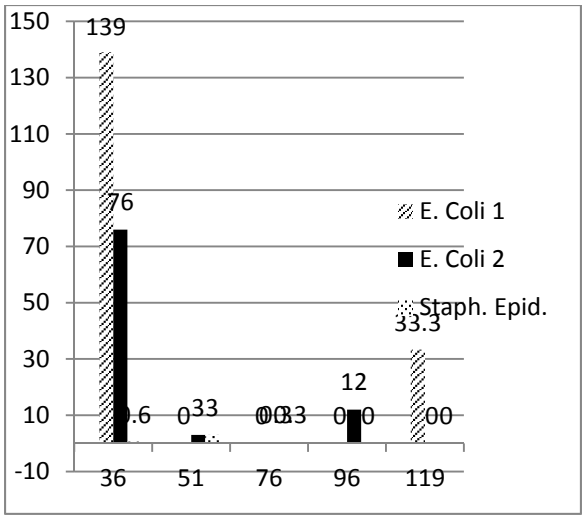

Figure 2. A great amount of growth up to 36 hours but after that there is not a dramatic amount of growth happening to know the difference of what's causing the growth.

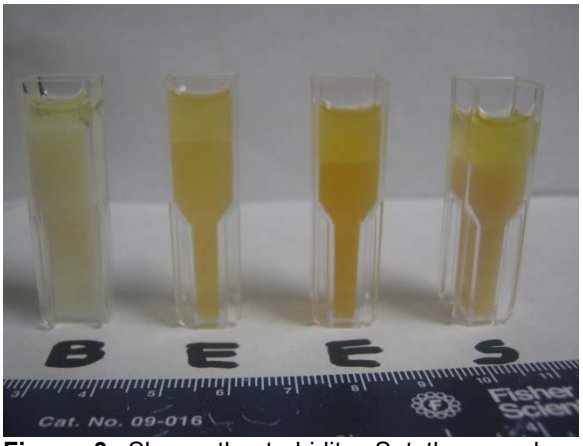

Figure 3. Shows the turbidity. Set the wavelength based off of the color each one was when tested for absorbance. A wavelength of 420nm is used when the solution is clear, 540nm when the solution is light yellow, and 600-625nm is used for yellow to brown solutions. Under these circumstances 540nm was used.

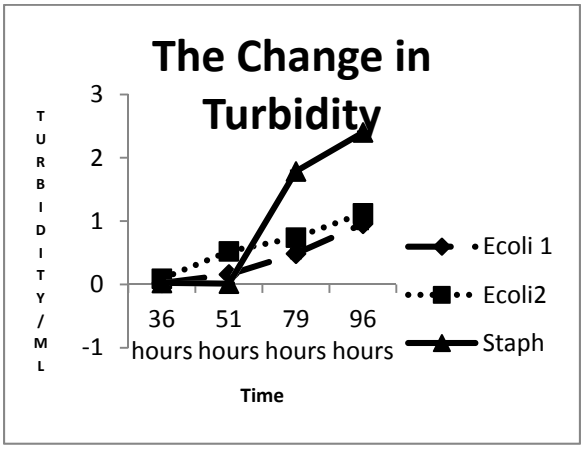

Figure 4. Change in the turbidity over every 24 hours. Both E.coli 1 and 2 were the same there is just two to have more examples. So that would explain why they were relatively the same. However, the staph had a rapid change after 51 hours.

DISCUSSION

During the experiment there were some complications. I messed up with pouring the plates and had to start over. It was all about learning and messing up to try again and to know what not to do the next time around. My predicted results were very similar with the results that I ended up getting. In conclusion, the *E. coli* K-12 did in fact grow with squalene being one of its carbon sources even though it wasn't the only one present.

Originally I questioned whether or not squalene could be *E. coli* K-12's sole carbon source but using the spirit blue agar I knew that squalene then wouldn't be the only carbon source *E. coli* K-12 was getting. I think something I would change however would be the amount of trials and the number of plates I had for each so that way maybe the number colony forming units could have been more accurate. It would at least give me more of something to go off of instead of just the first 36 hours; which were the best results.

ACKNOWLEDGEMENTS

First off, I couldn't have done any of this without the help of my family supporting me and reminding me that I can get through this.
And lastly, to the Natural Science Department; thank you for all your help over the years.

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