Presence of Sodium Monoflurophosphate and Fluoride in Streptococcus mutans Cells

AUTHORS

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AFFILIATIONS

We're also proud of the institutions that we are with and support our research. We appreciate Colgate Palmolive Company's support.

INTRODUCTION

Dental caries (cavities) cause the breakdown of teeth. The bacterium streptococcus mutans is the main cause of this phenomenon. S. Mutans can metabolize sucrose to create lactic acid and create biofilm. Biofilm allows bacteria to remain attached to teeth all while the bacteria causes degradation of the tooth enamel. Cavities are formed because of teeth's enamel being weakened from the exposure to acid produced by bacteria. Toothpaste treatments use sodium fluoride and monofluorophosphate to reduce cavities. There are a lot of questions about how each of the reagents interact biologically with the bacteria of the mouth. Earlier studies suggest that although both MFP and NaF influence the ability of S. mutans to metabolize sucrose and generate weak organic acids, however; the mechanisms and methods may differ.

OBJECTIVE

In this lab, we stimulate the environment of the mouth and treat the bacteria with different concentrations of MFP and NaF and quantitatively measure as the pH changes. Furthermore, we obtain samples and run chemical analyses, using the Ion Chromatography machine and the NMR as well. The project will address the key questions surrounding the mechanistic differences and response between NaF and MFP, which are as follows:

- Is MFP more effective than NaF at inhibiting lactic acid production at higher pH?
- What is the difference in response between the two mechanisms (NaF does not enter the cell whereas MFP does)?
- What are the levels of cellular penetration of the active agents into the bacteria by performing F-19 NMR studies.

METHODOLOGY

Streptococcus mutans (SM7 or SM6) grow best in anaerobic conditions in a 37° incubator. Colonies of SM7 grow best on an agar brain heart infusion (BHI) plate. We begin our week by streaking out the SM7 on the agar plate and growing for a little over 48 hours. After the streaking process, we begin our first overnight culture process by preparing tubes with 5mL of BHI and obtaining several colonies on our streak plate and placing them in the tube. We grow this culture for a little over 24 hours. We pour the first over night into LAPTg, a broth of yeast extract, peptone, tryptone, tween and glucose for our final overnight culture. We allow the bacteria to grow in the LAPTg flask for at least 12 hours on a stir plate to avoid bacterial clumping from glucose.

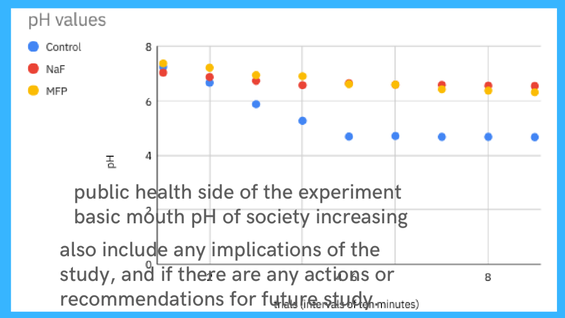
We use the autoclaves to sterilize the media to reduce the risk of contamination. For our artificial saliva buffer, we use sodium chloride, sodium monophosphate, sodium diphosphate, and deionized water. After mixing the materials, we will pH the buffer to 7 and will add diluted KOH. We autoclave the buffer and allow it to cool before adding calcium chloride and magnesium chloride, to avoid formation of precipitates. When we run out of our reagents, we will use stock MFP and NaF and dilute it in nanopure water, found in the chem lab, to our desired concentrations.

Preparing to run the pH drop begins approximately 15 hours after our 2nd overnight of LAPTg. The process begins by centrifuging the bacteria to separate the highly nutrient broths from the bacteria. After separating, we rinse with the artificial saliva buffer using a micropipette. We return it back to the centrifuge. While the bacteria are being washed, we prepare the artificial saliva with a carbon source of sucrose or glucose. We add MFP to one flask, NaF to the other and the other is our control. As soon as the bacteria have been rinsed, we commence the resuspension process. We add a 30 mL of artificial saliva and then divide the bacteria amongst the 3 flasks. We begin our pH drop as soon as the bacteria is added to the flask. We measure the pH every 10 minutes and obtain a sample every 30 minutes. Depending on the results we will come back and measure the pH every hour to observe more data points.

We obtain 1mL of samples, centrifuge and freeze them. After the run, we thaw them out and filter them out using 25 mm syringe filters. After filtering out, another student runs them through the ion chromatography machine and the NMR.

RESULTS/FINDINGS

The graph below supports our theory that MFP works better at higher pH's and NaF inhibits acid production at lower pH's.



ANALYSIS

In the early stages of our experiment, we were unable to identify the proper growth period of the bacteria. As a possible solution, we decided to use LAPTG as the growth media for both of the overnight processes. The LAPTG had different carbon sources than the original BHI media that was used prior. In order to support our decision, we Quantitatively conducted optical density test which calculated how much growth the Streptcoccus Mutans had within the LAPTG media. Our weekly average optical density was around 0.8 - 1.0, indicating effective growth media. Through our pH curve of our control, MFP, and NaF, we found that the MFP was found to be more effective at higher pHs, seen by the initial data points. As the pH becomes more acidic, NaF was found to be more effective. However, Americans' dental care has increased greatly, the average pH of the mouth environment has increased as well, making MPF a more effective Flouride to inhibit lactic acid production.

CONCLUSION

Our original hypothesis suggested that MFP would operate more efficiently at higher pH values, considering MFP experiences a degradation in its form that renders it less and less effective as time passes. This is of interest under a public health framework of view, due to the projected idea that are dental care has overall improved over the years. This is only projected due to the fact that it is an assumption based on the relative inaccessibility of census data related to teeth from a US standpoint, let alone that of the global environment.

Furthering a discussion about public health application is important for conceptualizing where we are going with this experiment. We are currently hoping to work with Colgate as was done before, to a capacity that there is interest in a solution based on MFP. Moving forward with our research, we plan to use diluted KOH and transfer the experiment from a pH drop to a controlled pH drop. This will allow us to move into the space of evaluating how much acid is actually produced and how it is being stored in cells or released into solution for measurement. We will start the experiment by controlling the pH of the bacterial suspension until it is around 7.4. After this point, we will run the drop for the same duration (2 hours), ensuring that every 10 minutes we assess each treatment flask, and potentially add KOH if it drops below 6.00. In this way, we will be able to monitor MFP at its most optimal state of efficiency in a forgiving environment that eliminates the disproportionate advance that NaF has over MFP under current experimental conditions.

RELATED LITERATURE