The Effect of pH upon the Growth of *Streptococcus salivarius*

Rohit Kottomtharayil

Abstract

S. mutans is a detriment to the oral microbiome leading to tooth decay and cavities. However, *S. salivarius* can produce alkaline to restrict the growth of *S. mutans*. Thus, to optimize the growth of *S. salivarius*, the present study analyzes the favorable pH level standards for reproduction of *S. salivarius*. It was hypothesized that environments with alkaline pH levels will disrupt a *Streptococcus salivarius* cells' activity and hydrogen bonding. The hypothesis was not supported by the results. The independent variables were the pH levels and time, and the dependent variable was the growth of *S. salivarius*. Bacteria colonies grew in agar plate environments of pH 5, 6, 7, 8 and 10. Environments with pH 5, pH 6, and pH 10 were incapable of fostering bacteria colonies, while pH7 and pH8 colonies could.

Introduction

Oral streptococcal infections are bacterias that are commonly found in individuals directly after birth and are essential in the formation of oral microbiota. This oral microbiota is essential in protecting the human body from the colonization of extrinsic microbial bacteria to secure health (Arweiler and Netuschil). Oral infections relating to *Streptococcus* are one of the utmost essential research areas, as 75% of oral species are found within this genus (Deo and Deshmukh). These species produce adhesive molecules which can colonize different tissues and form acids as byproducts of fermentation. However, not all streptococcus species have similar effects. *Streptococcus mutans* is a highly acidic strain that can often be linked with the forming of dental caries (Abranches et al.). Less acidic-tolerant species such as *Streptococcus salivarius* however can produce larger amounts of alkaline and also restrict the growth of *Streptococcus mutans* (Abranches et al.).

Hence, theoretically, increasing the growth of *Streptococcus salivarius* would counter the negative effects of *Streptococcus mutans*. Thus, the question is, how would environments of different pH levels change the growth of *Streptococcus salivarius*? For background knowledge, there has been research performed relating to the understanding of *Streptococcus* and its growth. Streptococcus salivarius is capable of restricting the growth of *S. mutans* through the generation of hydrogen peroxide (Stašková et al.). However, other streptococcus variants can restrict the growth of *S. mutans* in other manners, such as *Streptococcus* A12. This strain produces a challisin-like protease that interferes with the function of *S. mutans* ComDE two-component system, which regulates the transcription of molecules which exhibit antimicrobial behavior (Abranches et al.).

To further understand the growth, the following research will explore and document how pH affects the rate of growth of *Streptococcus salivarius*. If the environment for the growth of *S. salivarius* is optimized, then the growth of *Streptococcus mutans* will be proportionally inhibited. As *S. mutans* is responsible for developing dental caries, restricting the growth of it when presented with an underdeveloped cavity is key to preventing further complications (Poorni et al.). Once the ideal pH levels for *Streptococcus salivarius* are acknowledged and identified, further research can be done to execute this in the medical field to prevent the formation of

cavities. However, research would also have to be done to ensure that these pH levels would be suitable for the systems of humans and would not enact more detriment than benefit.

Furthermore, the research would apply and contribute to the broader understanding of *Streptococcus* as it will provide further insight into the relationship between pH and oral species. *S. mutans* is commonly considered a highly acidic species, while *S. salivarius* is of lower acidity. However, the exact pH levels under which it would thrive is not precisely distinguished. Once it is documented, this could help the field of *Streptococcus* as more information would be presented upon the relationship between pH and the species. Overall, this research would be important for the prevention of caries and other oral-related reactions that may present a danger to systemic health. This would benefit individuals developing caries or exposed to oral streptococcus bacteria.

Based on the fundamentals of organic biology, as pH decreases, the levels of acidity within the given environment increases. As an environment's acidity increases, the concentration of hydrogen ions will also increase, meaning that hydrogen bonding will not be disrupted and may be increased. Thus, the study predicts lower pH values will allow for greater growth of the bacteria as a higher pH’s alkaline environment will deter the reproduction of the *Streptococcus salivarius*.

However, this is not applicable to increased levels of alkalinity, as alkaline solutions are rich in hydroxide ions. These negatively charged ions can remove hydrogen ions from the hydrogen bonds, which can cause major detriment to the pairing of bases within DNA molecules of the sample. Hence, based on this understanding, environments with radical alkalinity levels will disrupt a *Streptococcus salivarius* cell's activity and hydrogen bonding.

Furthermore, this scientific explanation leads to the statistical prediction that lower pH values will allow for greater growth of the bacteria as a higher pH’s alkaline environment will deter the reproduction of the *Streptococcus salivarius*. This will be tested by placing *S. salivarius* specimens into environments with varying pH levels and measuring their effects on the growth rate. Hence, the independent variables will be the varying environments: acidic environment (6pH), Neutral environment (7pH), Basic environment(8pH), Highly Acidic Environment (5pH), Highly Basic Environment (10pH). These variables will, in turn, work with the dependent variable: the reproduction rate of *Streptococcus salivarius*. Overall, this controlled experiment will detail the relationship between pH and the growth of the specified sample.

Materials

For the process of preparing the base pH solutions, it will be necessary to acquire 5x Carolina Biological Pyrex Bottle, Square, Glass, 250mL; Karter Scientific 100mL glass graduated cylinder; Carolina Biological Colorless, 500mL, pH 5.0 Buffer solution for pH Testing; Carolina Biological Colorless, 500mL, pH 6.0 Buffer solution for pH Testing; Carolina Biological Colorless, 500mL, pH 7.0 Buffer solution for pH Testing; Carolina Biological Colorless, 500mL, pH 8.0 Buffer solution for pH Testing; Carolina Biological Colorless, 500mL, pH 10.0 Buffer solution for pH Testing. Once the bottles of Ph solutions have been prepared, adding the agar and LB will require a Bonvoisin Lab Scale High Precision Electronic Analytical Balance, Carolina Biological Luria Broth Agar Base Dehydrated Medium 500g, Carolina Biological Weighing Boats, Plastic, 3 5/16 x ¾", Carolina Biological Agar Powder 500g and a Scientific Labware Stainless Steel 7" Length Lab Spatula. Finally, for the autoclave process, the required materials are AmazonBasics Aluminum Foil, Amazon Autoclave Sterilization Tape (0.5" Wide), and Fisherbrand SterilElite Tabletop Autoclave.

To prepare the petri dishes, it is necessary to have 25 Polystyrene 60mm petri dishes, Carolina Biological *Streptococcus salivarius*, MicroKwik Culture Vial, Eowpower 10mL disposable plastic graduated transfer pipettes, 25 Carolina Biological Disposable Inoculating Loops, Clorox Bleach, Quincy Lab Model 10-140 Incubator, and Edvotek 10L Digital Shaking Water Bath. During the entire experiment, it is essential to maintain a neutral atmosphere within the facility while conducting any actions. For instance, there must be a neutral temperature of seventy degrees Fahrenheit (plus or minus ten degrees), as any temperature outside of this range may disrupt the growth of bacteria and alter the results. Furthermore, any excessive humidity may disrupt the process of the experiment. Moreover, both bacterial samples must receive similar amounts of sunlight as differences in sunlight may alter results. The data presented in this research paper was collected at the High Technology High School, Lincroft, NJ laboratory. This laboratory meets the necessities for the experiment, including the required equipment, such as the incubator or autoclave.

Methods

The first step was to prepare the five Pyrex 250mL glass bottles by labeling each with one of the five pH levels. Then, 100mL of the pH5 buffer solution was measured using the 100mL glass graduated cylinder and poured into its corresponding labeled bottle. This process was repeated four times for each pH buffer solution. Once this was completed, the analytical balance was zeroed with one styrofoam container, and 2.5 grams of LB powder were measured using the stainless steel spatula. The LB powder was poured into one of the glass bottles, and this process was repeated four times until each bottle had the LB powder. A new styrofoam container was used, and the stainless steel spatula was sterilized each time. Afterward, the analytical balance was zeroed with one styrofoam container, and 2.5 grams of agar powder were measured using the stainless steel spatula. The agar powder was poured into one of the glass bottles, and this process was repeated four times until each bottle had the agar powder. Again, a new styrofoam container was used, and the stainless steel spatula was sterilized each time. Finally, the cap of each bottle was closed, and the bottle was gently shaken until the powder settled in the buffer solution. Each bottle was then ready for the autoclaving process to remove any unwanted particles or bacteria. The cap of each bottle was slightly loosened, and it was covered in aluminum foil. The aluminum foil was taped using the autoclave sterilization tape, which would tell the scientist if the solution was sterilized and if the process was effective. At this point, the five bottles were allowed to rest for twenty-four hours.

Once the twenty-four-hour rest period had finished, the laboratory was returned to, and each bottle was placed in the autoclave machine. While the bottles were being sterilized, one two-inch strip of masking tape was placed on the lid of the petri dishes. Then, each of the 25 petri dishes was labeled according to their pH and sample (Ex. pH5 Sample 1). There were five samples for each of the five pH levels. After the autoclaving process was done, it was ensured that each bottle's autoclave tape had black streaks along the top, ensuring the process was effective. Once ensured, the cap was removed, and each solution was poured into the appropriate petri dish, until approximately half the dish was full. This process was repeated until each dish had its corresponding solution. Afterward, each of the petri dishes was allowed to rest at room temperature for twenty-four hours.

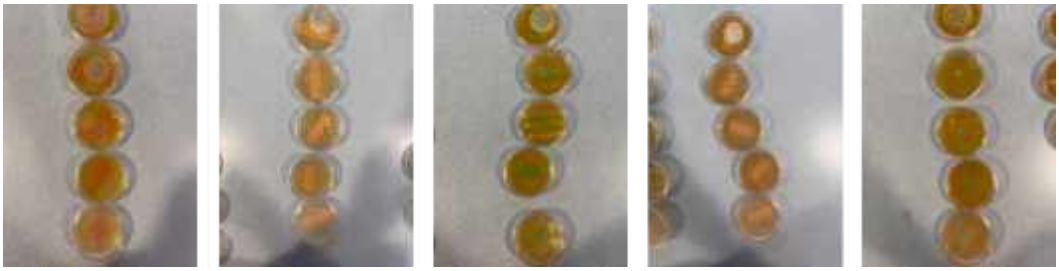

Figure 1

This graphic depicts the twenty-five agar plates and how they should appear after being poured and properly labeled. They are organized in their respective categories as shown in order: pH5, pH6, pH7, pH8, pH10.

During the twenty-four-hour rest period for the petri dishes, the scientist can utilize the time to reactivate the *S. salivarius* culture. According to the instructions provided by Carolina Biological, the first step is to remove the culture vial from the bag and to properly identify it by writing the culture name on the vial and test tube. Once this is done, remove the metal band and gray butyl stopper from the vial. Using the disposable pipette, remove 1.0mL of rehydration medium from the test tube and add it to the lyophilized culture in the vial. Mix gently with the sterile pipette. Then remove all the rehydrated culture from the vial, and transfer it back into the tube containing the remaining rehydration medium. After this, incubate the vial at 37℃ for 48 hours.

After the forty-eight hours rest period, it is time to streak the petri dishes. Take the vial from the water bath and place it adjacent to the agar plates. Carefully opening the vial, take one inoculating loop, and dip it into the reactivated culture. Swirling it around the vial, take it out ensuring there are no residual drops. Open an agar plate and swipe in a horizontal manner, holding the inoculating loop at an angle.

Due to the small size of the agar plate, there is no necessity to divide the agar plate into separate sections to streak. Hence, the streaking process should look like the diagram that follows.

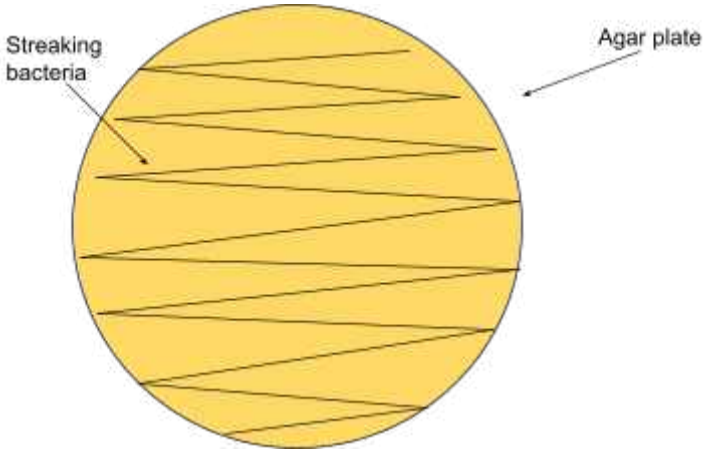

Figure 2

This figure visually describes the streaking pattern that should be performed upon each of the twenty-five agar plates using the bacteria and the inoculating loop.

Once the entire agar plate has been streaked with the bacteria, place the loop in a waste container. Repeat this process twenty-four more times until all the plates have been streaked. At this point, the agar plates are ready for analysis. When the plates are not being analyzed, safely store the dishes in an incubator with the lids securely closed.

Quantitative Results

This study analyzed the effects of different pH levels on the reproduction rate of *S. salivarius*.

Table 1: Growth of *S. salivarius* exposed to differing pH Environments, Raw Data Table

Independent Variables: Time, differing pH environments (5,6,7,8,10)
Dependent Variable: Growth of *S. Salivarius*

Sample	Count	Total Area	Average Size	%Area	Average
12/3/2022 PH5 S1	0	0	N/A	0	0
12/3/2022 PH5 S2	0	0	N/A	0	0
12/3/2022 PH5 S3	0	0	N/A	0	0
12/3/2022 PH5 S4	0	0	N/A	0	0
12/3/2022 PH5 S5	0	0	N/A	0	0
12/3/2022 PH6 S1	0	0	N/A	0	0
12/3/2022 PH6 S2	0	0	N/A	0	0
12/3/2022 PH6 S3	0	0	N/A	0	0
12/3/2022 PH6 S4	0	0	N/A	0	0
12/3/2022 PH6 S5	0	0	N/A	0	0
12/3/2022 PH7 S1	654	120537	184.307	2.768	194.194
12/3/2022 PH7 S2	194	71103	180.464	1.307	212.134
12/3/2022 PH7 S3	276	20310	73.587	0.441	201.532
12/3/2022 PH7 S4	353	153606	435.144	3.033	206.065
12/3/2022 PH7 S5	266	44728	168.15	0.997	202.89
12/3/2022 PH8 S1	467	150424	322.107	3.15	204.557

12/3/2022 PH8 S2	495	445489	298.227	3.657	210.685
12/3/2022 PH8 S3	694	75307	108.512	1.874	205.114
12/3/2022 PH8 S4	331	25753	77.804	0.569	208.892
12/3/2022 PH8 S5	276	62830	227.645	1.322	207.265
12/3/2022 PH10 S1	0	0	N/A	0	0
12/3/2022 PH10 S2	0	0	N/A	0	0
12/3/2022 PH10 S3	0	0	N/A	0	0
12/3/2022 PH10 S4	0	0	N/A	0	0
12/3/2022 PH10 S5	0	0	N/A	0	0
Sample	**Count**	**Total Area**	**Average Size**	**%Area**	**Average**
12/5/2022 PH5 S1	0	0	N/A	0	0
12/5/2022 PH5 S2	0	0	N/A	0	0
12/5/2022 PH5 S3	0	0	N/A	0	0
12/5/2022 PH5 S4	0	0	N/A	0	0
12/5/2022 PH5 S5	0	0	N/A	0	0
12/5/2022 PH6 S1	0	0	N/A	0	0
12/5/2022 PH6 S2	0	0	N/A	0	0
12/5/2022 PH6 S3	0	0	N/A	0	0
12/5/2022 PH6 S4	0	0	N/A	0	0
12/5/2022 PH6 S5	0	0	N/A	0	0
12/5/2022 PH7 S1	1066	212381	199.232	3.928	178.688
12/5/2022 PH7 S2	200	105147	525.735	1.489	175.139
12/5/2022 PH7 S3	708	41823	59.072	0.629	214.343

12/5/2022 PH7 S4	860	225175	261.381	3.819	175.863
12/5/2022 PH7 S5	535	103390	193.252	1.67	193.212
12/5/2022 PH8 S1	740	215931	78.807	3.462	182.447
12/5/2022 PH8 S2	947	232899	95.177	4.012	178.632
12/5/2022 PH8 S3	1593	153412	96.304	2.291	191.42
12/5/2022 PH8 S4	915	35975	39.317	0.688	213.351
12/5/2022 PH8 S5	1141	79691	69.843	1.434	201.526
12/5/2022 PH10 S1	0	0	N/A	0	0
12/5/2022 PH10 S2	0	0	N/A	0	0
12/5/2022 PH10 S3	0	0	N/A	0	0
12/5/2022 PH10 S4	0	0	N/A	0	0
12/5/2022 PH10 S5	0	0	N/A	0	0
Sample	**Count**	**Total Area**	**Average Size**	**%Area**	**Average**
12/7/2022 PH5 S1	0	0	N/A	0	0
12/7/2022 PH5 S2	0	0	N/A	0	0
12/7/2022 PH5 S3	0	0	N/A	0	0
12/7/2022 PH5 S4	0	0	N/A	0	0
12/7/2022 PH5 S5	0	0	N/A	0	0
12/7/2022 PH6 S1	0	0	N/A	0	0
12/7/2022 PH6 S2	0	0	N/A	0	0
12/7/2022 PH6 S3	0	0	N/A	0	0
12/7/2022 PH6 S4	0	0	N/A	0	0
12/7/2022 PH6 S5	0	0	N/A	0	0

12/7/2022 PH7 S1	1006	341637	339.599	5.3	187.651
12/7/2022 PH7 S2	1961	504826	309.47	12.807	255
12/7/2022 PH7 S3	1279	106869	223.109	1.326	188.547
12/7/2022 PH7 S4	1664	621333	169.578	7.633	151.627
12/7/2022 PH7 S5	1475	70884	286.418	10.506	145.558
12/7/2022 PH8 S1	1642	627197	109.443	9.747	146.195
12/7/2022 PH8 S2	1385	1243413	367.33	18.773	142.112
12/7/2022 PH8 S3	2156	1102795	265.53	15.707	138.339
12/7/2022 PH8 S4	1242	345420	55.338	4.767	156.084
12/7/2022 PH8 S5	1235	270637	219.139	4.538	182.218
12/7/2022 PH10 S1	0	0	N/A	0	0
12/7/2022 PH10 S2	0	0	N/A	0	0
12/7/2022 PH10 S3	0	0	N/A	0	0
12/7/2022 PH10 S4	0	0	N/A	0	0
12/7/2022 PH10 S5	0	0	N/A	0	0
Sample	**Count**	**Total Area**	**Average Size**	**%Area**	**Average**
12/9/2022 PH6 S1	0	0	N/A	0	0
12/9/2022 PH6 S2	0	0	N/A	0	0
12/9/2022 PH6 S3	0	0	N/A	0	0
12/9/2022 PH6 S4	0	0	N/A	0	0
12/9/2022 PH6 S5	0	0	N/A	0	0
12/9/2022 PH6 S1	0	0	N/A	0	0
12/9/2022 PH6 S2	0	0	N/A	0	0

12/9/2022 PH6 S3	0	0	N/A	0	0
12/9/2022 PH6 S4	0	0	N/A	0	0
12/9/2022 PH6 S5	0	0	N/A	0	0
12/9/2022 PH7 S1	1962	328893	167.631	8.495	134.075
12/9/2022 PH7 S2	2310	614131	265.858	14.519	138.015
12/9/2022 PH7 S3	2819	595345	211.19	11.009	136.417
12/9/2022 PH7 S4	2252	40231	206.107	8.41	135.697
12/9/2022 PH7 S5	2865	557913	229.149	10.706	145.738
12/9/2022 PH8 S1	2452	47451	193.946	9.529	138.372
12/9/2022 PH8 S2	1691	758821	448.741	18.883	132.542
12/9/2022 PH8 S3	2381	62783	454.296	15.348	138.643
12/9/2022 PH8 S4	1881	237431	269.502	5.053	153.541
12/9/2022 PH8 S5	1283	22422	174.764	4.851	151.217
12/9/2022 PH10 S1	0	0	N/A	0	0
12/9/2022 PH10 S2	0	0	N/A	0	0
12/9/2022 PH10 S3	0	0	N/A	0	0
12/9/2022 PH10 S4	0	0	N/A	0	0
12/9/2022 PH10 S5	0	0	N/A	0	0
Sample	**Count**	**Total Area**	**Average Size**	**%Area**	**Average**
12/12/2022 PH6 S1	0	0	N/A	0	0
12/12/2022 PH6 S2	0	0	N/A	0	0
12/12/2022 PH6 S3	0	0	N/A	0	0
12/12/2022 PH6 S4	0	0	N/A	0	0

12/12/2022 PH6 S5	0	0	N/A	0	0
12/12/2022 PH6 S1	0	0	N/A	0	0
12/12/2022 PH6 S2	0	0	N/A	0	0
12/12/2022 PH6 S3	0	0	N/A	0	0
12/12/2022 PH6 S4	0	0	N/A	0	0
12/12/2022 PH6 S5	0	0	N/A	0	0
12/12/2022 PH7 S1	4900	758863	154.87	18.85	162.243
12/12/2022 PH7 S2	8114	632708	77.9	20.245	158.768
12/12/2022 PH7 S3	5245	496717	94.703	13.758	178.241
12/12/2022 PH7 S4	5046	656783	130.159	18.091	158.594
12/12/2022 PH7 S5	5155	619797	120.232	18.633	201.404
12/12/2022 PH8 S1	5541	465955	84.092	14.23	168.056
12/12/2022 PH8 S2	2637	892592	338.488	22.292	152.759
12/12/2022 PH8 S3	3365	909132	270.173	21.92	148.438
12/12/2022 PH8 S4	2906	436484	229.005	11.037	165.556
12/12/2022 PH8 S5	2980	448335	150.448	12.055	169.237
12/12/2022 PH10 S1	0	0	N/A	0	0
12/12/2022 PH10 S2	0	0	N/A	0	0
12/12/2022 PH10 S3	0	0	N/A	0	0
12/12/2022 PH10 S4	0	0	N/A	0	0
12/12/2022 PH10 S5	0	0	N/A	0	0
Sample	**Count**	**Total Area**	**Average Size**	**%Area**	**Average**
12/14/2022 PH6 S1	0	0	N/A	0	0

12/12/2022 PH6 S2	0	0	N/A	0	0
12/12/2022 PH6 S3	0	0	N/A	0	0
12/12/2022 PH6 S4	0	0	N/A	0	0
12/12/2022 PH6 S5	0	0	N/A	0	0
12/14/2022 PH6 S1	0	0	N/A	0	0
12/14/2022 PH6 S2	0	0	N/A	0	0
12/14/2022 PH6 S3	0	0	N/A	0	0
12/14/2022 PH6 S4	0	0	N/A	0	0
12/14/2022 PH6 S5	0	0	N/A	0	0
12/14/2022 PH7 S1	4900	758863	154.87	18.85	162.243
12/14/2022 PH7 S2	8114	632708	77.9	20.245	158.768
12/14/2022 PH7 S3	5245	496717	94.703	13.758	178.241
12/14/2022 PH7 S4	5046	656783	130.159	18.091	158.594
12/14/2022 PH7 S5	5155	619797	120.232	18.633	201.404
12/14/2022 PH8 S1	5541	465955	84.092	14.23	168.056
12/14/2022 PH8 S2	2637	892592	338.488	22.292	152.759
12/14/2022 PH8 S3	3365	909132	270.173	21.92	148.438
12/14/2022 PH8 S4	2906	436484	229.005	11.037	165.556
12/14/2022 PH8 S5	2980	448335	150.448	12.055	169.237
12/14/2022 PH10 S1	0	0	N/A	0	0
12/14/2022 PH10 S2	0	0	N/A	0	0
12/14/2022 PH10 S3	0	0	N/A	0	0
12/14/2022 PH10 S4	0	0	N/A	0	0
12/14/2022 PH10 S5	0	0	N/A	0	0

Sample	Count	Total Area	Average Size	%Area	Average
12/16/2022 PH6 S1	0	0	N/A	0	0
12/16/2022 PH6 S2	0	0	N/A	0	0
12/16/2022 PH6 S3	0	0	N/A	0	0
12/16/2022 PH6 S4	0	0	N/A	0	0
12/16/2022 PH6 S5	0	0	N/A	0	0
12/16/2022 PH6 S1	0	0	N/A	0	0
12/16/2022 PH6 S2	0	0	N/A	0	0
12/16/2022 PH6 S3	0	0	N/A	0	0
12/16/2022 PH6 S4	0	0	N/A	0	0
12/16/2022 PH6 S5	0	0	N/A	0	0
12/16/2022 PH7 S1	4900	758863	154.87	18.85	162.243
12/16/2022 PH7 S2	8114	632708	77.9	20.245	158.768
12/16/2022 PH7 S3	5245	496717	94.703	13.758	178.241
12/16/2022 PH7 S4	5046	656783	130.159	18.091	158.594
12/16/2022 PH7 S5	5155	619797	120.232	18.633	201.404
12/16/2022 PH8 S1	5541	465955	84.092	14.23	168.056
12/16/2022 PH8 S2	2637	892592	338.488	22.292	152.759
12/16/2022 PH8 S3	3365	909132	270.173	21.92	148.438
12/16/2022 PH8 S4	2906	436484	229.005	11.037	165.556
12/16/2022 PH8 S5	2980	448335	150.448	12.055	169.237
12/16/2022 PH10 S1	0	0	N/A	0	0
12/16/2022 PH10 S2	0	0	N/A	0	0

12/16/2022 PH10 S3	0	0	N/A	0	0
12/16/2022 PH10 S4	0	0	N/A	0	0
12/16/2022 PH10 S5	0	0	N/A	0	0
Sample	**Count**	**Total Area**	**Average Size**	**%Area**	**Average**
12/20/2022 PH6 S1	0	0	N/A	0	0
12/20/2022 PH6 S2	0	0	N/A	0	0
12/20/2022 PH6 S3	0	0	N/A	0	0
12/20/2022 PH6 S4	0	0	N/A	0	0
12/20/2022 PH6 S5	0	0	N/A	0	0
12/20/2022 PH6 S1	0	0	N/A	0	0
12/20/2022 PH6 S2	0	0	N/A	0	0
12/20/2022 PH6 S3	0	0	N/A	0	0
12/20/2022 PH6 S4	0	0	N/A	0	0
12/20/2022 PH6 S5	0	0	N/A	0	0
12/20/2022 PH7 S1	4900	758863	154.87	18.85	162.243
12/20/2022 PH7 S2	8114	632708	77.9	20.245	158.768
12/20/2022 PH7 S3	5245	496717	94.703	13.758	178.241
12/20/2022 PH7 S4	5046	656783	130.159	18.091	158.594
12/20/2022 PH7 S5	5155	619797	120.232	18.633	201.404
12/20/2022 PH8 S1	5541	465955	84.092	14.23	168.056
12/20/2022 PH8 S2	2637	892592	338.488	22.292	152.759
12/20/2022 PH8 S3	3365	909132	270.173	21.92	148.438
12/20/2022 PH8 S4	2906	436484	229.005	11.037	165.556

12/20/2022 PH8 S5	2980	448335	150.448	12.055	169.237
12/20/2022 PH10 S1	0	0	N/A	0	0
12/20/2022 PH10 S2	0	0	N/A	0	0
12/20/2022 PH10 S3	0	0	N/A	0	0
12/20/2022 PH10 S4	0	0	N/A	0	0
12/20/2022 PH10 S5	0	0	N/A	0	0
Sample	**Count**	**Total Area**	**Average Size**	**%Area**	**Average**
12/21/2022 PH6 S1	0	0	N/A	0	0
12/21/2022 PH6 S2	0	0	N/A	0	0
12/21/2022 PH6 S3	0	0	N/A	0	0
12/21/2022 PH6 S4	0	0	N/A	0	0
12/21/2022 PH6 S5	0	0	N/A	0	0
12/21/2022 PH6 S1	0	0	N/A	0	0
12/21/2022 PH6 S2	0	0	N/A	0	0
12/21/2022 PH6 S3	0	0	N/A	0	0
12/21/2022 PH6 S4	0	0	N/A	0	0
12/21/2022 PH6 S5	0	0	N/A	0	0
12/21/2022 PH7 S1	4900	758863	154.87	18.85	162.243
12/21/2022 PH7 S2	8114	632708	77.9	20.245	158.768
12/21/2022 PH7 S3	5245	496717	94.703	13.758	178.241
12/21/2022 PH7 S4	5046	656783	130.159	18.091	158.594
12/21/2022 PH7 S5	5155	619797	120.232	18.633	201.404
12/21/2022 PH8 S1	5541	465955	84.092	14.23	168.056

12/21/2022 PH8 S2	2637	892592	338.488	22.292	152.759
12/21/2022 PH8 S3	3365	909132	270.173	21.92	148.438
12/21/2022 PH8 S4	2906	436484	229.005	11.037	165.556
12/21/2022 PH8 S5	2980	448335	150.448	12.055	169.237
12/21/2022 PH10 S1	0	0	N/A	0	0
12/21/2022 PH10 S2	0	0	N/A	0	0
12/21/2022 PH10 S3	0	0	N/A	0	0
12/21/2022 PH10 S4	0	0	N/A	0	0
12/21/2022 PH10 S5	0	0	N/A	0	0

Table 1: Growth of *S. salivarius* exposed to differing pH Environments, Raw Data Table

This table exhibits the raw data collected from days 12/3/2022 to 12/21/2022 by analyzing images of the bacterial agar plates using software ImageJ. The software describes the count of bacterial particles, total area of the agar plate, average size of each particle, percentage of the area the bacteria covers and the average of the bacteria.

Table 2: Count of Bacterial Particles of *S. Salivarius* exposed to pH7 and pH8, Summative Data Table

	COUNT						
	Measures of Central Tendency		Measures of Variation				n
Samples	Mean	Median	Range	IQR	Variance	St. Dev	# of samples
12/3/2022 PH7	348.6	276	460	230	32320.8	179.7798654	5
12/3/2022 PH8	452.6	467	418	209	26563.3	162.9825144	5
12/5/2022 PH7	673.8	708	866	433	108353.2	329.1704726	5
12/5/2022 PH8	1067.2	947	853	426.5	106646.2	326.5672978	5
12/7/2022 PH7	1477	1475	955	477.5	132568.5	364.0995743	5
12/7/2022 PH8	1532	1385	921	460.5	148848.5	385.8088905	5
12/9/2022 PH7	2441.6	2310	903	451.5	151245.3	388.9026871	5
12/9/2022 PH8	1937.6	1881	1169	584.5	238431.8	488.294788	5
12/12/2022 PH7	5692	5155	3214	1607	1849710.5	1360.040624	5
12/12/2022 PH8	3485.8	2980	2904	1452	1387725	1178.0176	5

					.7	99	
12/14/2022 PH7	5692	5155	3214	1607	1849710.5	1360.040624	5
12/14/2022 PH8	3485.8	2980	2904	1452	1387725.7	1178.017699	5
12/16/2022 PH7	5692	5155	3214	1607	1849710.5	1360.040624	5
12/16/2022 PH8	3485.8	2980	2904	1452	1678050.7	1295.395963	5
12/20/2022 PH7	5692	5155	3214	1607	1849710.5	1360.040624	5
12/20/2022 PH8	3485.8	2980	2904	1452	1387725.7	1178.017699	5
12/21/2022 PH7	5692	5155	3214	1607	1849710.5	1360.040624	5
12/21/2022 PH8	3485.8	2980	2904	1452	1387725.7	1178.017699	5

Table 2: Count of Bacterial Particles of *S. Salivarius* exposed to pH7 and pH8, Summative Data Table

This table shows the mean, median, range, interquartile range, variance, standard deviation and number of trials for the count of bacterial particles of *S. salivarius*.

Table 3: ANOVA Test of Average Count of Bacterial Particles of *S. Salivarius* exposed to pH7 and pH8, Statistical Data Table	
p-value	**Alpha**
0.007647	0.05

Table 3: ANOVA Test of Count of Bacterial Particles of *S. Salivarius* exposed to pH7 and pH8, Statistical Data Table

This table shows the results of the ANOVA test between each of the average counts of bacterial particles. The value of 0.05 was used as the alpha to determine the statistical significance and the calculated p-value proved to be less than the alpha value. A single-tailed

Table 4: T-Test Comparison of Average Count of Bacterial Particles of *S. Salivarius* exposed to pH7 and pH8, Statistical Data Table	
T-Test Comparison (pH)	**p-value**
pH7 (Control) - pH5	0.058007
pH7 (Control) - pH6	0.058007
pH7 (Control) - pH8	0.702351
pH7 (Control) - pH10	0.058007

Table 4: T-Test Comparison of Average Count of Bacterial Particles of *S. Salivarius* exposed to pH7 and pH8, Statistical Data Table

In this table, each pH value was compared to the control group pH7 using a t-test. None of the given p-values proved to be statistically significant.

Table 5: Percent Area of Bacterial Particles compared to Area of Agar Plate of *S. Salivarius* exposed to pH7 and pH8, Summative Data Table

	%AREA						
	Measures of Central Tendency		Measures of Variation				n
Samples	Mean	Median	Range	IQR	Variance	St. Dev	# of samples
12/3/2022 PH7	1.7092	1.307	2.592	1.296	1.2877072	1.134771871	5
12/3/2022 PH8	2.1144	1.874	3.088	1.544	1.6315083	1.277305093	5
12/5/2022 PH7	2.307	1.67	3.299	1.6495	2.2010905	1.483607259	5
12/5/2022 PH8	2.3774	2.291	3.324	1.662	1.8999538	1.378388117	5
12/7/2022 PH7	7.5144	7.633	11.481	5.7405	20.0438033	4.477030634	5
12/7/2022 PH8	10.7064	9.747	14.235	7.1175	41.0805288	6.409409396	5
12/9/2022 PH7	10.6278	10.706	6.109	3.0545	6.1900847	2.487988083	5
12/9/2022 PH8	10.7328	9.529	14.032	7.016	39.0076662	6.245611755	5
12/12/2022 PH7	17.9154	18.633	6.487	3.2435	6.0325683	2.456128722	5

12/12/2022 PH8	16.3068	14.23	11.255	5.6275	29.3730817	5.419693875	5
12/14/2022 PH7	17.9154	18.633	6.487	3.2435	6.0325683	2.456128722	5
12/14/2022 PH8	16.3068	14.23	11.255	5.6275	29.3730817	5.419693875	5
12/16/2022 PH7	17.9154	18.633	6.487	3.2435	6.0325683	2.456128722	5
12/16/2022 PH8	16.3068	14.23	11.255	5.6275	8.1425223	2.853510522	5
12/20/2022 PH7	17.9154	18.633	6.487	3.2435	6.0325683	2.456128722	5
12/20/2022 PH8	16.3068	14.23	11.255	5.6275	29.3730817	5.419693875	5
12/21/2022 PH7	17.9154	18.633	6.487	3.2435	6.0325683	2.456128722	5
12/21/2022 PH8	16.3068	14.23	11.255	5.6275	29.3730817	5.419693875	5

Table 5: Percent Area of Bacterial Particles compared to Area of Agar Plate of *S. Salivarius* exposed to pH7 and pH8, Summative Data Table

This table shows the mean, median, range, interquartile range, variance, standard deviation and number of trials for the percent area of bacterial particles to area of agar plate of *S. salivarius* when exposed to both environments of pH7 and pH8.

.

Table 6: ANOVA Test of Average Percent of Bacterial Particles Compared to Area of Agar Plate of *S. Salivarius* exposed to pH7 and pH8, Statistical Data Table

p-value	Alpha
0.002	0.05

Table 6: ANOVA Test of Count of Bacterial Particles of *S. Salivarius* exposed to pH7 and pH8, Statistical Data Table

This table shows the results of the ANOVA test between each of the average percent of bacterial particles. The value of 0.05 was used as the alpha to determine the statistical significance and the calculated p-value proved to be less than the alpha value.

Table 7: T-Test Comparison of Average Percent of Bacterial Particles Compared to Area of Agar Plate of *S. Salivarius* exposed to pH7 and pH8, Statistical Data Table	
T-Test Comparison (pH)	**p-value**
pH7 (Control) - pH5	**0.027463**
pH7 (Control) - pH6	**0.027463**
pH7 (Control) - pH8	0.917332
pH7 (Control) - pH10	**0.027463**

Table 7: T-Test Comparison of Average Count of Bacterial Particles of *S. Salivarius* exposed to pH7 and pH8, Statistical Data Table

In this table, each pH value was compared to the control group pH7 using a t-test. Note: bolded font indicates statistical significance.

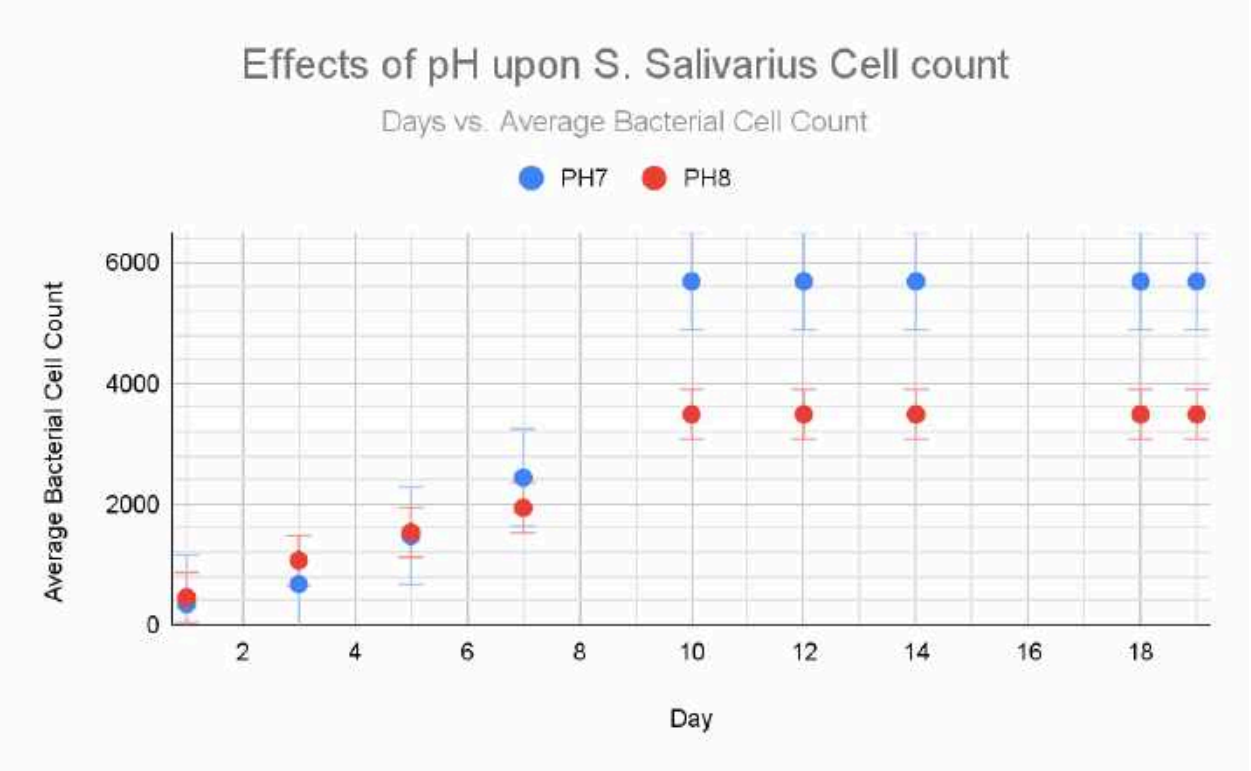

Figure 3: Effects of pH upon S. Salivarius Bacterial Cell Count

This figure shows the scatter plot correlation between the days passed and the average bacterial cell count. At day 10, the agar plates went through desiccation, halting the pH7 plates at 5692 bacterial cells and the pH8 plates at 3485.8 bacterial cells. Each pH7 data point has a standard deviation of 805 and each pH8 data point has a standard deviation error of 414. The data for this graph comes from Table 2.

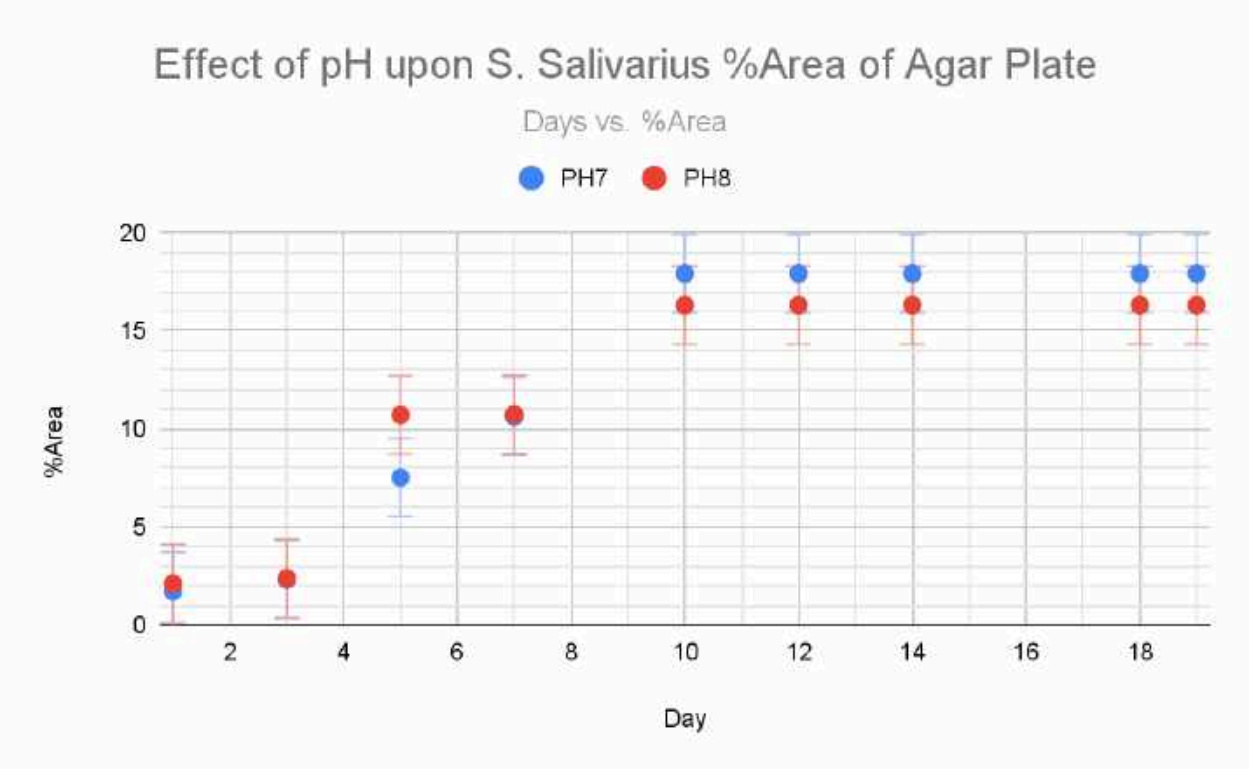

Figure 4: Effects of pH upon S. Salivarius %Area of Agar Plate

This figure shows the scatter plot correlation between the days passed and the average percent area the bacterial cells account for of the agar plate. At day 10, the agar plates went through desiccation, halting the pH7 plates at 17.9154% and the pH8 plates at 16.3068%. Each pH7 data point has a standard deviation of 1.944 and each pH8 data point has a standard deviation error of 1.88. The data for this graph comes from Table 5.

Qualitative Results

The following images are only of the first bacteria colony of each of the pH buffers. If no bacteria colonies grew in the pH buffer, images are not included besides the first date or last date of data collection.

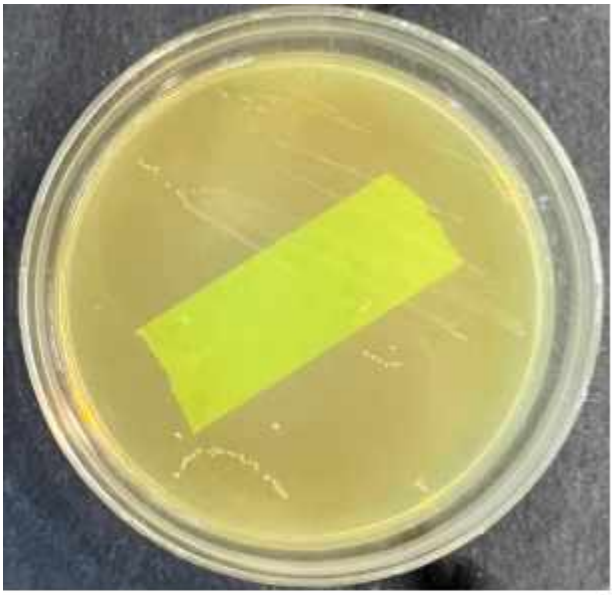

Figure 5
This is an image of pH7 Bacteria Colony #1 on 12/03/2022. As can be seen, the bacteria colonies are smaller and thinner as it is still going through the reproduction phase.

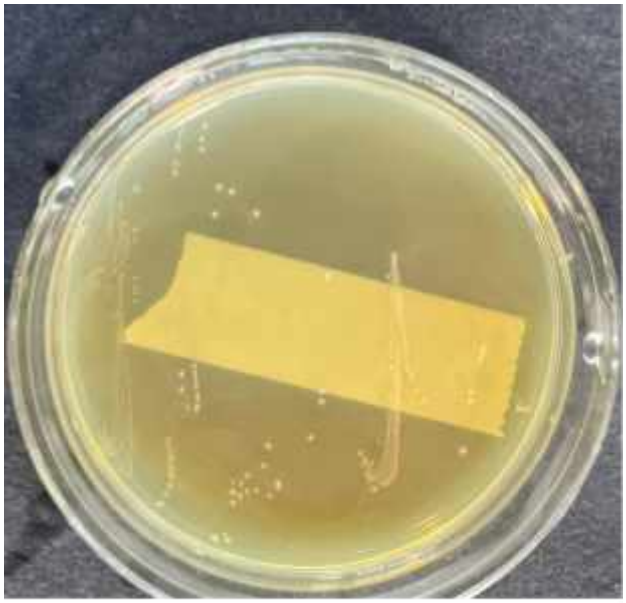

Figure 6
This is an image of pH* Bacteria Colony #1 on 12/03/2022. As can be seen, the bacteria colonies are smaller and thinner as it is still going through the reproduction phase.

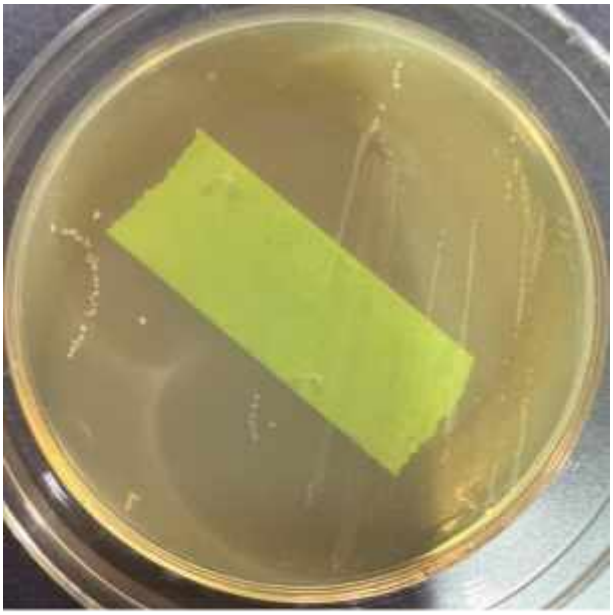

Figure 10
This is an image of pH7 Bacteria Colony #1 on 12/05/2022. As can be seen, the bacteria colonies are growing, yet thin as it is still going through the reproduction phase.

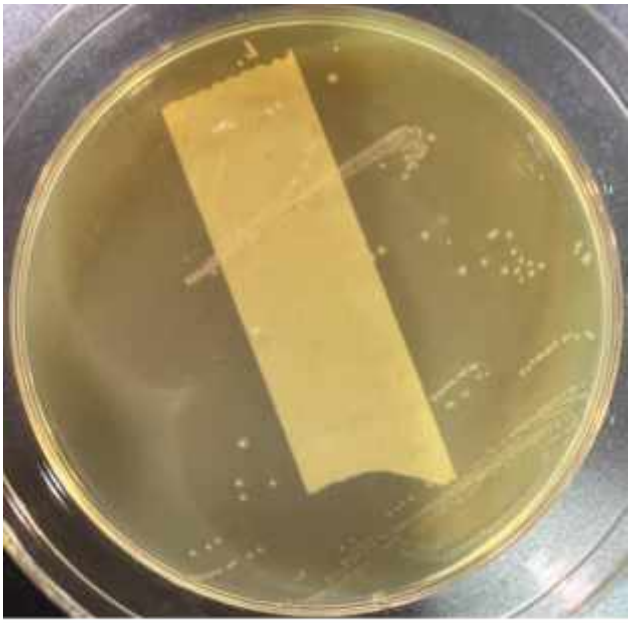

Figure 11
This is an image of pH8 Bacteria Colony #1 on 12/03/2022. As can be seen, the bacteria colonies are growing, yet thin as it is still going through the reproduction phase.

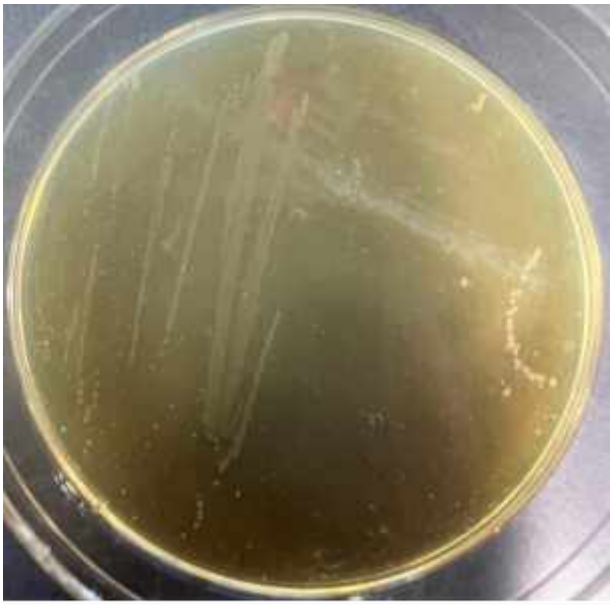

Figure 12
This is an image of pH7 Bacteria Colony #1 on 12/07/2022. As can be seen, the bacteria colonies are growing and smaller colonies grow over the plate as it is still going through the reproduction phase.

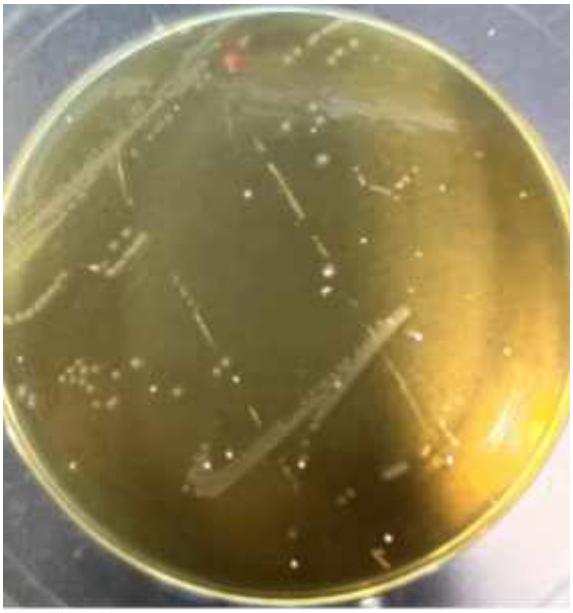

Figure 13
This is an image of pH8 Bacteria Colony #1 on 12/07/2022. As can be seen, the bacteria colonies are growing and smaller colonies grow over the plate as it is still going through the reproduction phase.

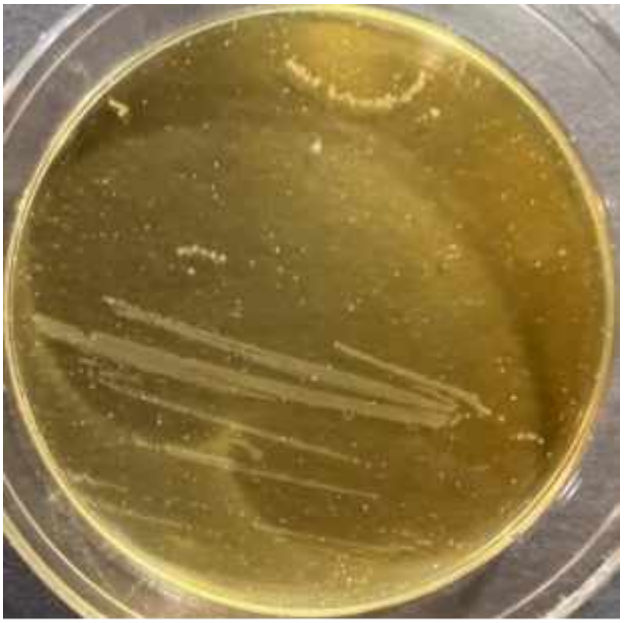

Figure 14
This is an image of pH7 Bacteria Colony #1 on 12/09/2022. As can be seen, the bacteria colonies are growing and forming more clumps as it is still goes through the final stages of the reproduction phase.

Figure 15
This is an image of pH8 Bacteria Colony #1 on 12/09/2022. As can be seen, the bacteria colonies are growing and forming more clumps as it is still goes through the final stages of the reproduction phase.

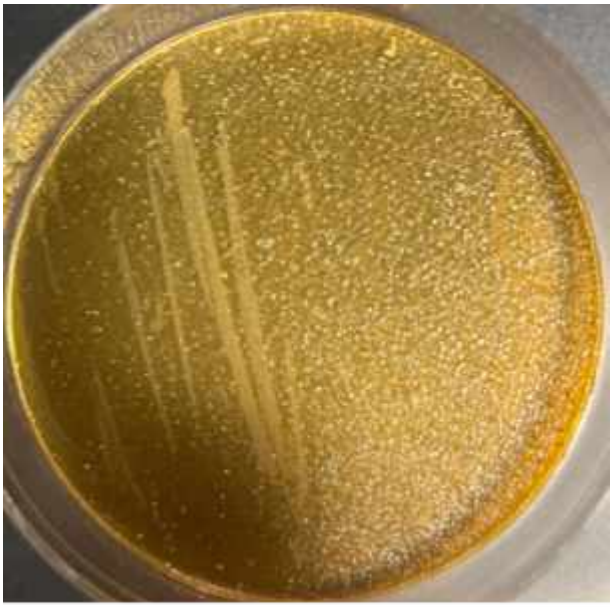

Figure 16
This is an image of pH7 Bacteria Colony #1 on 12/12/2022. As can be seen, the bacteria colonies have finished growing and the agar plate has gone through desiccation. This will prevent any further reproduction between the colonies. Agar plate looks as shown for rest of data collection.

Figure 17
This is an image of pH8 Bacteria Colony #1 on 12/12/2022. As can be seen, the bacteria colonies have finished growing and the agar plate has gone through desiccation. This will prevent any further reproduction between the colonies. Agar plate looks as shown for rest of data collection.

Figure 18
This is an image of pH5 Bacteria Colony #1 on 12/21/2022. As can be seen, no bacteria colonies grew in the acidic environment. After the point of dessication, the agar plates dried up and gave it a crisp, textured look.

Figure 19
This is an image of pH6 Bacteria Colony #1 on 12/21/2022. As can be seen, no bacteria colonies grew in the acidic environment. After the point of dessication, the agar plates dried up and gave it a crisp, textured look.

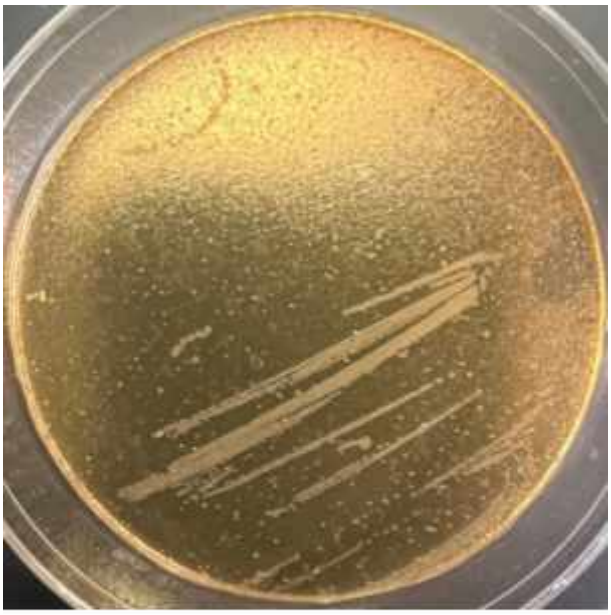

Figure 20
This is an image of pH7 Bacteria Colony #1 on 12/21/2022, the final day of data collection. As can be seen, the bacteria colonies have finished growing and the agar plate has gone through desiccation. Bacteria colonies begin to fade, due to lacking the proper resources.

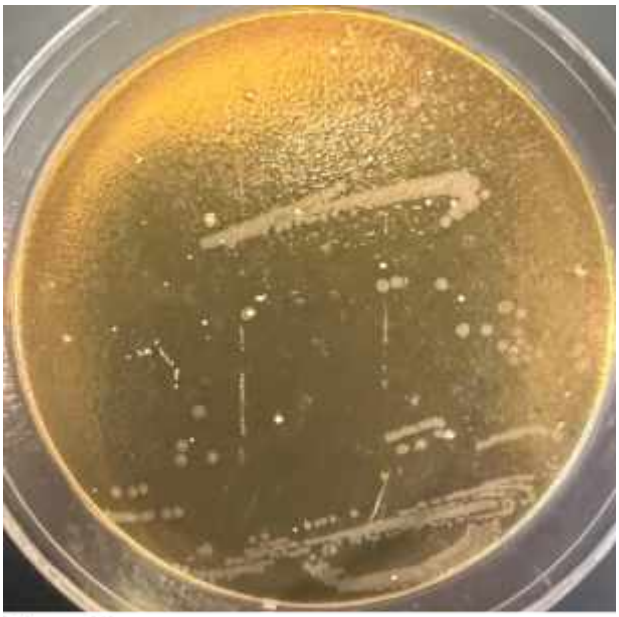

Figure 21
This is an image of pH8 Bacteria Colony #1 on 12/21/2022, the final day of data collection. As can be seen, the bacteria colonies have finished growing and the agar plate has gone through desiccation. Bacteria colonies begin to fade, due to lacking the proper resources.

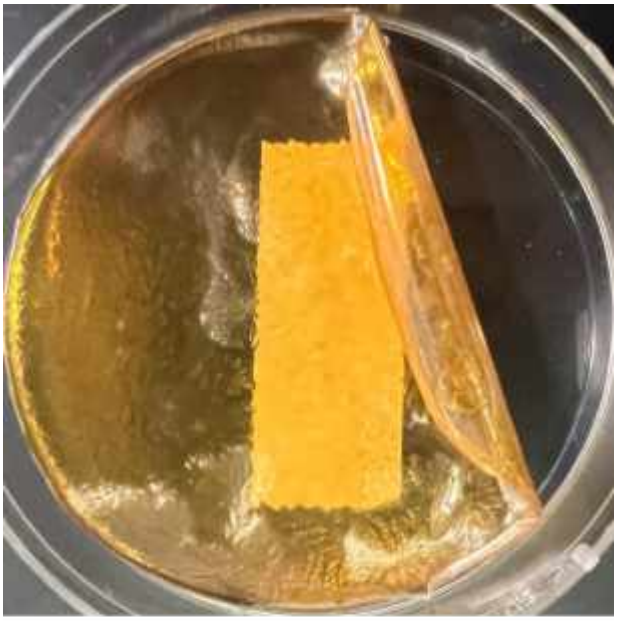

Figure 22
This is an image of pH10 Bacteria Colony #1 on 12/21/2022, the final day of data collection. As can be seen, no bacteria colonies have grown and the desiccation deformed the shape of the agar plate.

Discussion

Based on the results of the present study, *S. salivarius* has proved to be a standard bacteria which tends to gravitate towards environments of more neutral pH standards. Once the data collection phase of the experimentation began, bacteria colonies only began to form in environments of pH7 and pH8. The agar plates consisting of more radical pH buffers displayed macroscopically blank results (See Figure 5, 6, 7). At the initiation of data collection (12/03/22), data was collected by taking pictures of the agar plates with visible bacteria and analyzing it using ImageJ, an image processing program developed by the National Institutes of Health. This software provided the necessary information to analyze, such as the count of bacterial particles, total area, average size, %area, and mean (See Table 1). Table 2 analyzes the measures of central tendencies and measures of variation for the count of bacterial particles per day. As can be seen, by the mean, there are greater counts of bacterial particles daily for pH7 and pH8. However, the count stops on 12/12/2022 due to the agar plates going through the process of desiccation, the natural removal of moisture from the agar plates. This prevents any bacteria from reproducing, however, will not immediately kill the existing bacteria colonies. Nevertheless, no bacteria were able to grow past the desiccation point. This can be seen in Figure 3, the Effects of pH upon *S. salivarius* Bacterial Cell Count. This figure analyzes the correlation between the days passed and the average bacterial cell count. Although for the first seven days of data collection, the bacteria colonies proved to grow larger at a steady rate, they leveled out at a higher value due to desiccation. This higher value was attained as the ImageJ software viewed the new texture of the agar plate to be bacterial particles, giving a higher bacterial count. Finally, the bacterial cell count was statistically analyzed in Table 3 and Table 4. Table 3 analyzes the statistical significance of the collected data for bacterial count particles by analyzing the p-value of the data using a single-tailed t-test. A two-tailed single-paired t-test was used to analyze if the data's mean would be greater than or less than the control pH7. The given p-value was 0.007647 compared to the alpha 0.05, signifying the data was statistically significant. Table 4 analyzes each experimental group the control (pH7) by performing t-test comparisons of the collected data. However, none of the derived p-values were statistically significant. Hence, the data was not sufficient enough to reject the null hypothesis.

In a similar manner, the results analyze the %Area data found in the raw data table. As shown in summative data table 5 which analyzes the measures of central tendency and

measures of variation, the %Area of the agar plate, which the bacterial particles account for also proportionally increases until 12/12/2022. This can be seen by analyzing the measures of central tendency, which increase at each data point. Furthermore, this is also visualized in Figure 4, Effects of pH upon *S. salivarius* %Area of Agar plate. The scatter plot draws a correlation between days passed and the average percent area the bacterial cells account for of the agar plate. The bacteria followed an exponential growth pattern until Day 10, where it leveled out at a greater %Area. Finally, the %Area data were statistically analyzed in Table 6 and Table 7. Table 6, ANOVA Test of Average Percent of Bacterial Particles Compared to Area of Agar Plate of *S. Salivarius* exposed to pH7 and pH8, analyzes the statistical significance of the %Area Data by comparing the p-value to the alpha. The given p-value was 0.002, hence the data was statistically significant. Table 7 analyzes the statistical significance of each experimental group to the control group by using a two-tailed single-paired t-test. Although pH8 data was not statistically significant, the data for pH5, pH6, and pH10 was statistically significant compared to the control group (pH7).

The study tested the hypothesis that environments with radical levels of pH will disrupt *Streptococcus salivarius* cells' activity and hydrogen bonding. As can be seen from the data, both pH7 and pH8 appeared to be at an equal point on the final day of data collection before desiccation, factoring in standard deviation error bars (See Figures 3 and 4). Hence, there was no statistically significant difference between the data collected for the two groups.

Nevertheless, there was a statistically significant difference in the %area data from pH7 to the non-response agar plates. However, environments of lower pH, such as pH5 and pH6, also disrupted the reproduction of the agar plates, as shown by Table 1. This presents data contrary to the hypothesis, as it was believed the hydrogen-ion-rich environment would nourish the hydrogen bonding of the bacteria. Thus, it can be assumed that *S. salivarius*'s growth is optimized in both environments of pH7 and pH8. Overall, the data does not support the hypothesis that environments with radical levels of alkalinity will not support the bacteria's growth, as radical levels of acidity did not support the bacteria's growth either.

The scientific reasoning behind the given results in the study relate to the basic fundamentals of pH (Olson). Environments with more radical levels of pH will disrupt a bacteria's reproduction as it modifies the ionization of the amino-acid functional groups and disrupts hydrogen bonding (Jin and Kirk). However, the inaccurate prediction made in the research

hypothesis was that the hydrogen-ions found in the acid environments would result in optimized hydrogen bonding. This prediction was made as self-ionization of bacterial particles occurs when there is the collision of two H_2O molecules resulting in dissociation—$H_2O \rightarrow H^+ + OH^-$ (Tristram et al.) This self-ionization allows for greater hydrogen bonding due to more hydrogen ions being available (Gerdt et al.). Based on these principles applicable to bacteria, the prediction was made that the hydrogen ions in the acid pH will allow for greater hydrogen bonding. However, in this experiment, it was shown that the hydrogen-ion did not induce greater hydrogen bonding between the *S. salivarius,* and rather environments of neutral pH are ideal for the bacteria.

Finally, there were variables beyond control during experimentation. For instance, the desiccation of the agar plates was unexpected and uncontrollable—most agar plates are prone to desiccation as limited moisture and dry air leads to the inevitable drying out of the agar plate (Hassel). Hence, data collection was halted early due to the desiccation's negative effect upon bacteria growth. More variables that may have influenced results include the buffers addressed in the methods. Each pH buffer was used to simulate the environments instead of using a pH meter due to the agar potentially damaging a pH meter. However, each pH buffer may not have been 100% accurate and may have influenced results to a certain extent. Finally, a variable that may have affected the experimental parameters is the ImageJ software. Although it has highly accurate precision when identifying the particles, there is still a source of uncertainty deriving from the analysis of the images.

Future Studies

In the future, the hypothesis tested in the present study should be retested by furthering the results of this study to see if the effects *S. salivarius* holds on *S. mutans* changes after growing in different pH levels. As discussed in the results, the bacteria grows most effectively in pH7 and pH8 (See Table 1). However, the bacteria growth in different pH levels may alter the cell's activity and hydrogen bonding. The different properties of the bacteria may alter how it counteracts the negative effects of *S. mutans* on the oral microbiome (Garcia et al.). This can be done by placing the two bacteria samples in an environment to replicate the oral microbiome and analyze the bacteria population of *S. mutans*. Expected results would include a greater detriment to *S. mutans* according to research which states that modifying the environmental pH can drive bacterial interactions (Ratzke and Gore). Overall, the present study adds to the research field of cavities and the oral microbiome as it shows how to optimize the growth of *S. salivarius* to prioritize the detriment it holds on *S. mutans*.

Acknowledgments

Special thanks to Mr. Craig Queenan for his continuous support and guidance through my research process. He was able to answer several of my questions with his expertise and helped with revising my methods and materials. Thank you to Dr. Dina Ellsworth for her expertise on using the pH buffers to simulate environments of different pH levels and for helping with the overall setup of my experiment. Thank you to the Monmouth County Board of Commissioners and MCVSD Board of Education for their support of the research program and MCVSD. Finally, thank you to the HTHS faculty and Principal Teresa Hough for the support of the research program.

Works Cited

Abranches, J., et al. “Biology of Oral Streptococci.” *Microbiology Spectrum*, vol. 6, no. 5, 5 Oct. 2018, www.ncbi.nlm.nih.gov/pmc/articles/PMC6287261/, 10.1128/microbiolspec.gpp3-0042-2018.

Annotation: This paper helped in understanding how streptococcus strains function, especially *S. mutans*. Furthermore, it aided in understanding the relationship between oral bacteria and the oral microbiota.

Arweiler, Nicole B., and Lutz Netuschil. “The Oral Microbiota.” *Springer Link*, Springer International Publishing, 2016, link.springer.com/chapter/10.1007%2F978-3-319-31248-4_4.

Annotation: The abstract of this paper provided a vague understanding of the oral microbiota before diving further into the complexities of how it functions coupled with oral bacterial species.

Deo, Priya Nimish, and Revati Deshmukh. “Oral Microbiome: Unveiling the Fundamentals.” *Journal of Oral and Maxillofacial Pathology : JOMFP*, vol. 23, no. 1, 2019, pp. 122–128, www.ncbi.nlm.nih.gov/pmc/articles/PMC6503789/, 10.4103/jomfp.JOMFP_304_18.

Annotation: This gave insight into the types of viruses found within the oral microbiota and also provided a vague understanding of how it functions.

Garcia, S.S., et al. “Targeting Of Streptococcus Mutans Biofilms by a Novel Small Molecule Prevents Dental Caries and Preserves the Oral Microbiome.” *Journal of Dental Research*, vol. 96, no. 7, 10 Mar. 2017, pp. 807–814, 10.1177/0022034517698096. Accessed 1 Dec. 2020.

Annotation: This source gave a proper understanding of how *S. mutans* can be affected by other molecules to prevent cavities and help preserve the oral microbiome. This information carried on to applying the knowledge to *S. salivarius* in future studies.

Gerdt, Joseph P., et al. “Unraveling the Contributions of Hydrogen-Bonding Interactions to the Activity of Native and Non-Native Ligands in the Quorum-Sensing Receptor LasR.” *Organic & Biomolecular Chemistry*, vol. 13, no. 5, 2015, pp. 1453–1462, www.ncbi.nlm.nih.gov/pmc/articles/PMC4303524/, 10.1039/c4ob02252a. Accessed 10 Jan. 2023.

Annotation: When identifying the reasoning behind the results, this source helped in discussing the science behind it. Self-ionization and hydrogen bonding is a big part of how pH affects bacteria reproduction.

Hassel, Tammy. “EJPPS | Article| Agar Desiccation – the Causes and How to Address Them.” *EJPPS*, www.ejpps.online/agar-desiccation-the-causes-and-how#:~:text=Solid%20culture%20media%2C%20or%20agar.

Annotation: Due to the desiccation of the agar plates on 12/12/2022, this source helped identify the cause for it and the science behind it.

Jin, Qusheng, and Matthew F. Kirk. “PH as a Primary Control in Environmental Microbiology: 1. Thermodynamic Perspective.” *Frontiers in Environmental Science*, vol. 6, 1 May 2018, 10.3389/fenvs.2018.00021.

Annotation: This was another source that helped identify how pH affects bacteria reproduction. Essentially, the premise of the research article was how temperature can affect pH’s control of the environment and its interactions with amino acids.

Olson, Eric R. “Influence of PH on Bacterial Gene Expression.” *Molecular Microbiology*, vol. 8, no. 1, Apr. 1993, pp. 5–14, 10.1111/j.1365-2958.1993.tb01198.x.

Annotation: Gene expression is a big part of cellular reproduction and this articles dives into pH’s impact on bacterial gene expression which helped enlighten me on certain aspects.

Poorni, Saravanan, et al. “Probiotic Streptococcus Strains in Caries Prevention: A Systematic Review.” *Journal of Conservative Dentistry*, vol. 22, no. 2, 2019, p. 123, 10.4103/jcd.jcd_505_18. Accessed 5 Sept. 2021.

Annotation: This research paper covered similar ideas to what I wish to understand. It covers how streptococcus strains can work against *S. mutans* to prevent the formation of caries.

Ratzke, Christoph, and Jeff Gore. “Modifying and Reacting to the Environmental PH Can Drive Bacterial Interactions.” *PLOS Biology*, vol. 16, no. 3, 14 Mar. 2018, p. e2004248, www.ncbi.nlm.nih.gov/pmc/articles/PMC5868856/, 10.1371/journal.pbio.2004248.

Annotation: This research paper assisted with the future studies portion of the paper as it described how environmental pH can affect bacterial interactions.

Stašková, Andrea, et al. “Antimicrobial and Antibiofilm Activity of the Probiotic Strain Streptococcus Salivarius K12 against Oral Potential Pathogens.” *Antibiotics*, vol. 10, no. 7, 29 June 2021, p. 793, 10.3390/antibiotics10070793. Accessed 2 Dec. 2021.

Annotation: This covers more about the lower acidity of streptococcus salivarius and how it works against pathogens within the oral microbiota which may threaten systematic health.

Tristram, H., et al. “Review of Biochemistry of Bacterial Growth.” *Science Progress (1933-)*, vol. 57, no. 225, 1969, pp. 122–125, www.jstor.org/stable/43423756. Accessed 11 Jan. 2023.

Annotation: This helped analyze the science behind self-ionization and what happens to the H_2O particles in the process and how this impacts bacterial growth.

“What Are the Effects of an Alkaline PH on the Structure of DNA?” *Sciencing*, 2011, sciencing.com/effects-alkaline-ph-structure-dna-12030337.html.

Annotation: This was simply to help understand with the formation of the hypothesis and statistical prediction how a more basic environment would affect the hydrogen bonding of DNA.