

2017

The Effect of Common Household Spices on Streptococcus Mutans

Lauren Lau

Follow this and additional works at: <http://scholarworks.seattleu.edu/suurj>

Recommended Citation

Lauren Lau (2017) "The Effect of Common Household Spices on Streptococcus Mutans," *SUURJ: Seattle University Undergraduate Research Journal*: Vol. 1 , Article 11.

Available at: <http://scholarworks.seattleu.edu/suurj/vol1/iss1/11>

This Short Communications is brought to you for free and open access by ScholarWorks @ SeattleU. It has been accepted for inclusion in SUURJ: Seattle University Undergraduate Research Journal by an authorized editor of ScholarWorks @ SeattleU.



The Effect of Common Household Spices on Streptococcus Mutans

Lauren Lau, Biology

Faculty Mentor: Dan Smith, PhD

Faculty Content Editor: Dan Smith, PhD

Student Editor: Julia Borello

Introduction

The most common infectious disease to affect humans is dental caries, affecting 97% of the population at some point in a given lifespan (Berg 2006). *Streptococcus mutans* are bacteria found in the mouth that are primarily responsible for dental caries formation. These bacteria adhere to the surface of teeth and synthesize extracellular polysaccharides from the enzyme glucosyltransferase (Ren et al. 2016). Glucosyltransferases use sucrose to synthesize these polysaccharides, creating a biofilm on the surface of teeth (Devulapalle and Mooser 2001). When the concentration of a chemical signal produced by these primary colonizers is of high enough concentration, other bacteria are signaled to colonize on the biofilm and can thrive on the tooth surface as well (Loesche 1986). Eventually the tooth demineralizes due to organic acids produced by these bacteria and starts to decay, leading to dental caries (Ren et al. 2016).

Different cuisines around the world call for the use of different spices, such as cumin and coriander. Some of these spices are believed to have antibacterial effects as well. However, little research has been done on the effects of spices and dental caries formation by *S. mutans*. Previous research has found the essential oil of turmeric and cocoa polyphenols to inhibit the biofilm formation and growth of *S. mutans* (Lee et al. 2011; Percival et al. 2006). Researchers found that the essential oil of *Curcuma longa*, commonly known as turmeric, inhibits the growth of *S. mutans* as well as their adherence to surfaces (Lee et al. 2011). It was also found that turmeric and cocoa inhibited biofilm formation of these bacteria by inhibiting their ability to produce organic acids (Lee et al. 2011; Percival et al. 2006). This project investigates whether the turmeric and cocoa data could be replicated with the use of store-bought spices rather than extraction of these spices. In addition, I examine what effects other spices, such as cinnamon and ginger, may have on the growth and biofilm formation of *S. mutans*.

Materials and Methods

Preparation of Cells: A wild type strain of *S. mutans* was grown in tryptic soy broth containing soybean-casein digest medium (TSB) fortified with a final concentration of 2% sucrose. Agar was added to a final concentration of 1.5%

for solid media plates. Cultures grown on plates were incubated in an oxygen-deprived candle jar at 30°C for five days. An isolated colony of *S. mutans* was transferred to a conical tube containing 10 mL TSB with 2% sucrose, and placed in an oxygen-deprived candle jar at 30°C to grow overnight.

Spice Preparation: Several scoops of each spice were added to test tubes and autoclaved for 15 minutes. Spice stock mixtures were prepared in Eppendorf tubes with 0.02 g of spice mixed in 1 mL of sterile water. Two stock mixtures were prepared for each spice.

Inhibition of Growth Detection: 200 µL of TSB with 2% sucrose were added to each well of a 96 well plate. Serial dilutions of spices were prepared in the wells by adding 200 µL of spice stock mixture to the first well in each row, mixing by repeated pipetting, and pipetting 200µl into the subsequent well creating dilutions from 1:2 to 1:4096, giving final spice concentrations between 10 and 0.005 mg/ml. No-spice controls were treated identically using water in place of the spice stock. 10 µL of overnight *S. mutans* culture were added to the wells diluted with water and with the spice mixture. Controls for the spices did not contain any *S. mutans* culture. Plates were incubated for three days in the candle jar at 30°C. Bacterial growth was detected by using a plate reader detecting 490 nm (Figure 1.)

Inhibition of Biofilm Formation: Plates for biofilm formation were prepared exactly as described above for growth detection. After three days in the candle jar, cells were removed from each well by aspirating the growth medium. Leftover material in the wells was fixed with 100 µL of 10% formaldehyde and incubated at room temperature overnight.

Formaldehyde was removed the next day and 100 µL of 1% crystal violet was added to each well and incubated for 30 minutes at room temperature. Excess crystal violet was then removed and wells were rinsed with water three times. Plates were left to dry overnight. 250 µL of isopropanol was added to each well to solubilize the crystal violet and cell material. The amount of crystal violet in each well was determined by using a plate reader detecting 490 nm (Figure 2.)

Data Analysis: Data was collected and processed in Microsoft Office Excel. In order to remove interference, wells treated exactly as the experimental but without cells were subtracted from the experimentals to record only cell growth or biofilm formation. T-tests were conducted between the wells diluted with water and the wells diluted with the spice mixtures to obtain p-values.

Results

Some Spices Inhibit Growth of S. Mutans: Ginger, cocoa, and cinnamon all seemed to inhibit the growth of *S. mutans*, while turmeric and cardamom seemed to have no effect. Ginger significantly inhibited growth at a minimum inhibitory concentration (MIC) of 2,500 mg/mL ($p=0.03$). Cocoa significantly inhibited growth with a MIC of 78.1 mg/mL ($p<0.001$). Cinnamon significantly inhibited growth with a MIC of 5,000 mg/mL ($p=0.01$). Figure 1 summarizes the growth inhibition of each of the spices tested.

Some Spices Inhibit Biofilm Formation: Cocoa and cinnamon inhibited the biofilm formation of *S. mutans* while ginger, turmeric, and cardamom had no effect. Cocoa significantly inhibited biofilm formation at a MIC of 2,500 mg/mL ($p<0.01$). Cinnamon significantly inhibited biofilm formation at a MIC of 5,000 mg/mL ($p<0.01$). Figure 2 summarizes the effect on biofilm formation by each of the spices.

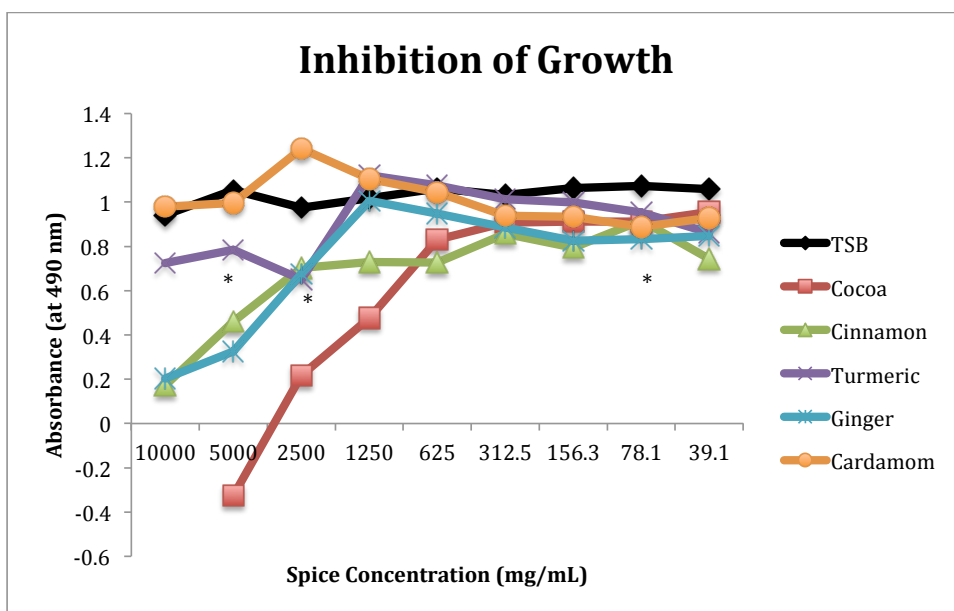


Figure 1 Average absorbance readings at 490 nm of *S. mutans* at varying spice concentrations. A t-test was conducted to determine significance. *Cocoa, cinnamon, and ginger significantly inhibited growth of *S. mutans* at varying concentrations.

Discussion

Although previous research has discovered the use of essential oils of turmeric and cocoa polyphenols to have an inhibitory effect on the biofilm formation and growth of *S. mutans*, it appears that less-concentrated,

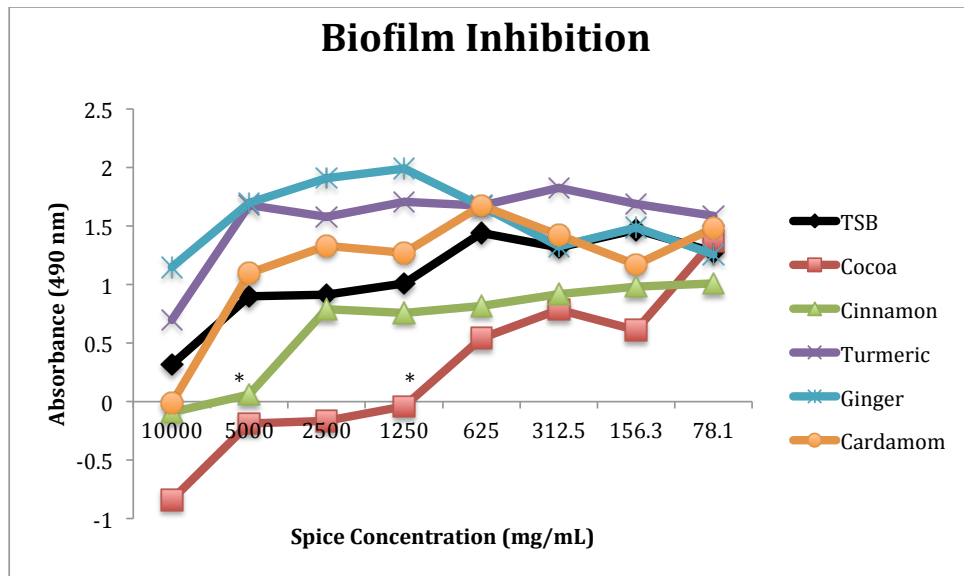


Figure 2 Average absorbance readings at 490 nm of biofilm formation at varying spice concentrations. A t-test was conducted for significance. *Cocoa and cinnamon significantly inhibited biofilm formation of *S. mutans* at varying concentrations.

common household spices can have the same effects. Using powdered forms of cocoa, ginger, and cinnamon from the grocery store inhibited the growth and biofilm formation of these organisms as well.

Ginger seemed to inhibit only the growth of *S. mutans* at a minimum inhibitory concentration of 2,500 mg/mL and have no effect on the biofilm formation of these organisms. Cocoa and cinnamon both inhibited growth at minimum inhibitory concentrations of 78.1 mg/mL and 5,000 mg/mL respectively. Cocoa and cinnamon also inhibited biofilm formation at minimum concentrations of 2,500 mg/mL and 5,000 mg/mL. This decrease in biofilm formation of *S. mutans* by cocoa and cinnamon could be due to inhibition of acid production and reduced adherence.

Despite previous findings indicating inhibitory effects of turmeric, the current study did not confirm these results. This contradiction could be due to concentration differences between the studies as well as variation in starting materia such as raw powdered form versus isolated extracts. Cardamom also seemed to have no effect on growth or biofilm formation. On average, a typical serving size in a recipe containing ginger powder has about 0.25 of a teaspoon of the spice. This equates to a concentration about 1,800 times smaller than the concentration we were studying. Similarly, a serving size containing cinnamon has between 0.25 of a teaspoon and 0.04 of a teaspoon, which is 31,000 to 3,500 times less concentrated than our studies. The concentration of cocoa in our studies is a little closer to the average serving size of cocoa powder, being seven to 11 times greater.

Previous studies had tested concentrated extracts and oils of a few of these spices and found them to inhibit the growth and biofilm formation of *S. mutans*. However, it is surprising to see a similar effect from the use of common household spices on these organisms. Despite serving sizes having a smaller concentration of these spices than our study concentrations, our research was done in vitro. It is possible the presence of other ingredients in recipes have an additive effect on the growth of *S. mutans* in vivo.

These findings suggest that an increase in the consumption of cocoa, cinnamon, and ginger powder could decrease dental caries formation. Future analysis could rely on an established mouse caries model with carefully monitored consumption of spices to determine the validity of this argument. Cooking with these spices more often could potentially supplement dental care in preventing dental caries formation.

References

Berg JH. 2006. The Marketplace for New Caries Management Products: Dental Caries Detection and Caries Management by Risk Assessment. *BMC Oral Health*, 6:S6.

Devulapalle KS and Mooser G. 2001. Glucosyltransferase inactivation reduces dental caries. *Journal of Dental Research*, 80: 466-469.

Lee KH, et al. 2011. Essential Oil of *Curcuma long* Inhibits *Streptococcus mutans*. Biofilm Formation. *Journal of Food Science*, 76: 226-230.

Loesche WJ 1986. Role of *Streptococcus mutans* in human dental decay. *Microbiological Reviews*, 50: 353-380.

Percival R, et al. 2006. The effect of cocoa polyphenols on the growth, metabolism, and biofilm formation by *Streptococcus mutans* and *Streptococcus sanguinis*. *European Journal of Oral Sciences*, 114: 343-348.

Ren Z, et al. 2016. Molecule Targeting Glucosyltransferase Inhibits *Streptococcus mutans* Biofilm Formation and Virulence. *Antimicrobial Agents and Chemotherapy*, 60: 126-135.