The Antimicrobial Effect of Coconut Oil and its Fatty Acids on Oral Microorganisms Compared to Chlorhexidine Mouth Rinse: An in vitro Study

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Abstract

Aim: Due to the antiseptic characteristic of its main ingredients (monolauric and monocapric fatty acids), coconut oil poses a promising natural antibacterial alternative to chemically-based mouth rinses to fight cariogenic oral microorganisms. Several studies have shown the antimicrobial activity of its fatty acids against different oral biofilm. Although there is some research on coconut oil’s antibacterial efficacy, more research is needed to investigate its efficacy against the main cariogenic bacteria (*Streptococcus mutans, Lactobacillus fermentum, and Streptococcus mitis*) in comparison with its pure fatty acids and a commercially available mouth rinse, chlorhexidine. Therefore, the aim of this study was to investigate the antimicrobial effect of coconut oil in comparison to monolauric acid, monocapric acid, and chlorhexidine on selected oral microorganisms (*Streptococcus mutans, Lactobacillus fermentum, Streptococcus mitis*).

Methods: The antimicrobial activity of coconut oil, the fatty acids and chlorhexidine was examined using two methods: serial dilution and plate counting method and agar-well diffusion method. Colony forming units were calculated by counting the colonies on the plates after incubation at 37°C for 24 to 48 hours. The inhibition zone was determined by measuring the diameter around each well after incubating with each solution for 24 hours at 37°C. Incubations were performed in aerobic conditions.

Results: Results of this *in vitro* study showed that coconut oil, monocapric acid, and monolauric acid have appreciable antimicrobial activity against three types of strains of oral microorganisms associated with oral infections; *S. mutans, Lactobacillus fermentum*, and *S. mitis*. Using one-way ANOVA, the results from the serial dilution method were statistically significant for *S. mutans* (p-value <0.001), *Lactobacillus fermentum* (p-value
<0.001) and *S. mitis* (p-value=0.005). Similarly, the results from the agar-well diffusion method were statistically significant for *S. mutans* (p-value=0.003), *Lactobacillus fermentum* (p-value <0.001) and *S. mitis* (p-value <0.001).

**Conclusion:** Coconut oil has potential to be used as an antimicrobial agent in mouth rinses to reduce cariogenic microorganisms in the oral cavity. Future studies should be specifically designed to examine the potential biological role of coconut oil and its fatty acids in the characteristics of oral biofilm formation and biofilm dynamics.
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The Antimicrobial Effect of Coconut Oil and its Fatty Acids on Oral Microorganisms Compared to Chlorhexidine Mouth Rinse: An in Vitro Study
The human oral cavity is a host for a large number of indigenous bacterial species, called oral flora.\(^1\) While in equilibrium, the oral flora actively help in protecting the human mouth from exogenous pathogens; however, when the balance is disrupted, bacteria can cause infectious diseases, such as dental caries,\(^2,3\) and periodontal diseases.\(^3,4\)

Dental caries is the single most common chronic disease of childhood, with prevalence five times greater than asthma.\(^3\) It is reported that dental care is the most common unmet health care need among children in the United States.\(^2\) Treatment for children with caries can be costly, and might require prolonged restorative procedures under general anesthesia. Nonetheless, children who experience dental caries remain at a higher risk for new infections regardless of any restorative therapy efforts.\(^2\) Individuals are recommended to use fluoride-based toothpaste daily to help prevent dental caries. As a second line of defense, fluoride-based and antimicrobial mouth rinses are highly recommended.\(^3\)

Consequently, it is important to develop strategies to prevent and treat dental caries by reducing the count of cariogenic bacteria in the oral cavity as well as disrupting the pathobiology of these aggressive infectious diseases.

Almost 260 species of oral bacteria have been cultivated from humans, and actual number has been estimated at nearly 500 common species.\(^2,5\) Importantly, many research efforts have attempted to link certain pathogens to oral diseases.\(^2,4,6-9\)

Considerable evidence associates \textit{Streptococcus mutans} with dental caries,\(^1,2,6\) and several laboratory studies have shown that strains of this species can produce lactic acid, which causes dental caries.\(^2\) Moreover, various \textit{Lactobacillus} species have also been linked to
dental caries as important secondary pathogens. In addition, Lactobacillus present in the oral cavity contributes to the progress of carious lesions. On the other hand, Streptococcus mitis is the predominant species in the oral cavity of healthy individuals. Table 1 summarizes the above microorganisms with their relevant characteristics.

Research shows that reducing or eliminating the suspected microbial pathogens in the oral cavity can prevent dental infections. Many oral hygiene approaches have been introduced to control the presence of the above microorganisms in the oral cavity including chemotherapeutic mouth rinses, essential oil mouth rinses, and oil-pulling oral therapy.

Chemotherapeutic agents including antimicrobial mouth rinses are commonly used as a supplement to tooth brushing and flossing to reduce or eliminate pathogens which cause caries and plaque. Mouth rinses provide an advantage in oral hygiene because of their liquid form that targets the oral cavity as a whole and their quick antiseptic action. As a broad spectrum antimicrobial, chlorhexidine mouth rinse has been demonstrated in many studies to be effective in reducing oral bacterial viability, inhibiting plaque regrowth, and preventing gingivitis. However, these agents are not without side effects. As extensively listed in the Oral Health in America report, other studies have associated chlorhexidine use with teeth staining, alterations in taste, and potential hypersensitivity reactions. Alternatively, essential oils based antiseptic mouth rinses were proposed and have demonstrated comparable efficacy to chlorhexidine.
As an alternative to chemically-based mouth rinses, several studies have been conducted on the antimicrobial efficacy of oil pulling (mouth rinse) on the bacterial or fungal colonization in the mouth using different types of natural edible oils such as coconut oil, sunflower oil, sesame oil, etc. in dental hygiene settings.\textsuperscript{17-20} Notably, the use of coconut oil in oral hygiene has gained popularity in recent years.\textsuperscript{21,22}

Coconut oil is extracted from the kernel or meat of ripe coconuts. Grown in tropical regions, it has been among the primary sources of dietary fat for decades.\textsuperscript{23} Coconut oil contains important medium chain fatty acids, some of which are well-known for their antimicrobial and antiviral activity against enveloped viruses, certain bacteria, and the yeast \textit{Candida albicans}.\textsuperscript{24} The most significant medium chain fatty acids present at high concentration in coconut oil are lauric acid and capric acid, at about 48.5% and 5.8% by weight, respectively.\textsuperscript{23,25} Lauric acid is among the highest antimicrobial medium chain fatty acids. When lauric acid is esterified to glycerol making monolaurin, its antimicrobial effect is increased. Several studies showed that monolaurin is mainly active \textit{in vitro} against Gram-positive pathogenic bacteria.\textsuperscript{26} Similarly, capric acid possesses bactericidal activity as well as its monoglycerol derivative monocaprin. It is also known that monocaprin is active against Gram-positive bacteria and enveloped viruses.\textsuperscript{24} Although the exact antimicrobial mechanism of coconut oil is still unclear, some studies had hypothesized that medium chain fatty acids (monolauric and monocapric) had the capacity to alter bacterial cell walls, penetrate and disrupt cell membranes, inhibit enzymes involved in energy production and nutrient transfer, leading to the death of the bacteria.\textsuperscript{27} Both monolaurin and monocaprin are approved by the US Food and Drug Administration (FDA) as non-toxic compounds and used as generally recognized as safe
(GRAS) food additive. However, coconut oil and its fatty acids are insoluble in water and therefore must be dissolved in appropriate medium before their application.

Few studies have been carried out in the literature testing the role of coconut products in dental health. Bidkar et al. showed that the water extract of coconut shell ash had an *in vitro* inhibitory effect against oral microflora. Likewise, 100% concentration of coconut oil showed an inhibitory effect on some species of *Candida* when compared to fluconazole. A study by Thaweboon et al. found an effective antimicrobial activity of coconut oil against *S. mutans* and *C. albicans* compared to other edible oils when used as oil-pulling therapy. Besides, a double-blind clinical trial using virgin coconut oil found significant antibacterial effectiveness against *Staphylococcus aureus* collected from human atopic dermatitis skins. Another study that investigated the potential use of monocaprin acid, a coconut capric acid derivative, as a denture disinfectant reported a significant antimicrobial activity against *Candida* when applied as a topical agent. In addition, monolauric acid has significant *in vitro* broad-spectrum sensitivity against gram-positive and gram negative bacterial isolates from superficial human skin infections as reported by Carpo et al. In contrast, a study carried out by Taheri et al. on commercially available coconut flour showed no appreciable antimicrobial effect on oral microorganisms. However, as the literature provides no data on the effect of different concentrations of coconut oil on the microorganisms or on how effective coconut oil compared to other antimicrobial mouth rinses, further studies are needed.

In this study, the antimicrobial effects of coconut oil, chlorhexidine mouth rinse, monolauric acid, and monocapric acid on the viability and survival of selected oral microorganisms were investigated. *Streptococcus mutans* and *Lactobacillus*
microorganisms were selected because they are associated with caries and periodontal
diseases. In addition, *Streptococcus mitis* was selected due to its positive role on the
reduction of dental diseases, and thus, the study will also investigate the effect of these
solutions on favorable bacteria.

**Specific Aims and Hypothesis**

The aim of the study was to investigate the antimicrobial effect of coconut oil and its
essential fatty acids (monolauric acid and monocapric acid) in comparison to a
commercially available chlorhexidine mouth rinse on selected oral microorganisms
(*Streptococcus mutans*, *Lactobacillus fermentum*, and *Streptococcus mitis*). This was
accomplished by analyzing the growth of the selected microorganisms after their
inoculation in the various tested solutions.

We hypothesized that coconut oil would have inhibitory effect against oral
microorganisms and would have antimicrobial properties comparable to those of the
chlorhexidine mouth rinse.
Research Design:

This project was a prospective laboratory study investigating the antimicrobial effect of coconut oil and its two main fatty acids ingredients; monocapric and monolauric acids, on oral microorganisms in comparison to chlorhexidine mouth rinse. The study was conducted at the microbiology lab facility at Tufts University School of Dental Medicine. The study was approved by Tufts University Health Sciences Campus Institutional Review Board and the Biosafety Office at Tufts University.

Materials and Preparation:

The antimicrobial activity of coconut oil, the fatty acids and chlorhexidine was examined using two methods: serial dilution and plate counting method and agar-well diffusion method. Colony forming units were calculated by counting the colonies on the plates after incubating at 37°C for 24 to 48 hours. The inhibition zone was determined by measuring the diameter around each well after incubating with each solution for 24 hours at 37°C. Incubations were performed in aerobic conditions.

Strains:

Three bacterial strains were used; *Streptococcus mutans*, (ATCC® 25175™), *Lactobacillus fermentum* (ATCC® 11976™) and *Streptococcus parasanguinis*, which is deposited as *Streptococcus mitis* (ATCC® 903™). All strains were purchased from American Type Culture Collection (ATCC, Manassas, VA).
Media:

Media for microbial culturing were obtained from Becton Dickinson. Each bacterial strain has its specific preferred media. Brain Heart Infusion Broth (powder-BD 237500), and Brain Heart Infusion Agar (powder-BD 211065) were used for *Streptococcus mutans*; Lactobacilli MRS Broth (powder-BD 288130), Lactobacilli MRS Agar (powder-BD 288210) for *Lactobacillus fermentum*; Tryptic Soy Broth (powder-BD 211825), Tryptic Soy Agar (powder-BD 236950), and de-fibrinated Sheep Blood (liquid-B11947) for *Streptococcus mitis*.

Solutions:

Four different solutions were tested: Coconut oil (semi-solid form, 46949 Supelco, Sigma-Aldrich Co. LLC, St. Louis, MO), Chlorhexidine gluconate antiseptic mouthwash (liquid form, Ecolab, Lotherton Way, Garforth, Leeds, LS25 2JY), monolauric acid (powder form, M1765, Sigma-Aldrich Co. LLC, St. Louis, MO) and monocapric acid (powder form, M2140, Sigma-Aldrich Co. LLC, St. Louis, MO). As control groups, we used 95% and 100% ethanol.

Preparation:

Media:

Culture media were prepared following the manufacturers’ instructions (Appendix C). All media were prepared by mixing the right amount of the suitable powder with 1000 ml distilled water and then autoclaved at 121°C for 15 minutes. To prepare the agar, the autoclaved solution was cooled down to 55°C and then poured into petri dishes (about 10ml per plate). Plates were stored upside down along with the broth at 2-8°C.
**Bacterial cultures:**

Bacterial cultures for all strains were prepared following the manufacturers’ instructions (Appendix D). After opening each one of the bacterial vials in the hood, the entire pellet was rehydrated with 1ml of the recommended broth and the whole aliquot was then transferred to a single tube with 5ml of broth and mixed well. Several drops of the suspension were used to inoculate agar plates. All tubes and plates were incubated aerobically at 37°C for 24 to 48 hours. Consequently, cultures for each bacterium were examined for growth and viability after the recommended bacterial growth period was reached. A single colony from each plate was picked and transferred to a tube of 5ml of the suitable broth and incubated for another 24 hours at 37°C to prepare a fresh broth culture (primary culture). The remaining plates were stored at 2-8°C. The broth cultures were then spun to generate a pellet that was frozen in glycerol for future use.

Serial dilutions were performed for each of the primary cultures to get the optimal bacterial concentration that showed countable colonies after testing and plating the aliquot. For a reference, the optical density of each bacterial culture that was used was: 0.220, 0.117, and 0.118 for *S. mutans*, *Lactobacillus fermentum*, and *S. mitis*, respectively. At these optical densities, cultures consistently yielded countable colonies, and therefore were used throughout the study.

**Stock Solutions:**

Stock solutions of monolauric and monocapric acids were prepared by dissolving 100mg of the acid powder in 1ml of 95% ethanol to get a stock solution with a concentration of 100mg/ml that was stored at -20°C. Subsequently, several concentrations were prepared for the fatty acids (2, 4, 8, 12.5, 16, 25, 50, 100µg/ml) to select the minimum inhibitory
concentration. Coconut oil was also prepared by adding 100µl of the semi-liquid oil to 1ml 100% ethanol, since 95% ethanol was not enough to dissolve the oil to a homogeneous solution. Two concentrations of coconut oil were tested for inhibition (100µg/ml and 200µg/ml). To make 100µg/ml of coconut oil for example, ten-microliters (10µl) of the stock solution were added to 1000µl of broth. To prepare the 50µg/ml concentration, five microliters (5µl) of the stock were added to 1000µl of broth. The same procedure was followed to prepare each concentration using the correct volume of stock. Each experiment was started by testing the lower concentrations first and when no effect on all strains was observed, only 100µg/ml and 200µg/ml concentrations were used. In addition, the experiments were carried out in triplicate, and the mean values were used in the statistical analysis.

**Preliminary Experiments:**

Prior to implementing the serial dilution and agar-well diffusion methods, the antimicrobial effect of coconut oil was explored using various concentrations and time periods. First, serial dilution of bacterial stock was carried out to produce different concentrations in order to select the best dilution point for colony counts. Optical density measurement was used to aid the selection. Four serial dilutions were done by adding 1ml of primary culture to 5ml of media broth for (1:5) ratio mixture. Then 1ml of that mixture was added to a fresh 5ml of media broth for (1:25) ratio mixture, and this dilution was repeated similarly for (1:125) and (1:625) ratio mixtures. The optical density was measured using a spectrophotometer.

In order to select the best dilution for all the experiments, all four mixtures were plated and incubated for 24 and 48 hours. After performing colony counting, it was found
that the dilution ratios that produced countable colonies were corresponding to the following optical densities: 0.220 (1:5), 0.117 (1:25), and 0.118 (1:25) for *S. mutans*, *Lactobacillus fermentum*, and *S. mitis*, respectively. Table 2 lists the optical density readings for all dilution ratios used above. Consequently, these dilution ratios were used to investigate the antimicrobial effect of coconut oil on all the tested microorganisms using three time points (5, 10 and 20 minutes). Two concentrations of coconut oil (100µg/ml and 200µg/ml), chlorhexidine and 100% ethanol were tested to determine the optimal incubation time periods. For each microorganism, three ELISA plates were used for each tested solution, one plate per time point. After plating and colony counting, a difference between the three time periods was not observed. Thus, the 5 minute time point was selected for all the experiments.

For selecting the proper concentration for monolauric and monocapric acids, the agar-well diffusion method was used for each microorganism for the selected dilution ratios. Only 100µg/ml concentration for both acids showed measurable inhibition zones.

**Serial Dilution and Plate Counting Method:**

To achieve the total viable bacterial count of each condition, an ELISA plate was prepared for each bacterium with 100µl of each solution (coconut oil, monolauric acid, monocapric acid, chlorhexidine, 95% and 100% ethanol, or broth as control) that were dispensed into 8 wells. Next, for each solution, another seven wells, labeled 1-7, were filled with 180µl of the appropriate broth to conduct the dilution process. The timer was started once a 100µl of the bacterial stock was added to the tested solutions and pipetted up and down several times. Different time periods (5, 10, and 20 minutes) were tested in order to select the optimal one for the experiment, and the five-minute time period was
selected for this study. After 5 minutes, the dilution process was initiated by transferring 20µl of the bacterial-compound mixture into the first well to make $10^1$ dilution followed by pipetting up and down several times. Then 20µl were removed from this mix and added to the next well to make $10^2$ dilution followed by pipetting up and down several times. The dilution process was continued in each of the following wells so that the last well yielded a $10^7$ dilution of the tested solution. Five microliter aliquots of the $4^{th}$, $5^{th}$ and $6^{th}$ wells from all conditions were spread over the entire surface of agar plates that were pre-labeled with matching numbers. All plates were incubated at 37ºC for 24-48 hours.

After the optimal bacterial growth period was reached, the number of colonies per plate was determined. To account for dilution, the following formula was used to calculate the original number of bacterial colony forming units per ml of solution:

$$\frac{\#CFU}{\text{volume plated}} \times \text{dilution factor} = \text{CFU/ml}$$

Figures 1 – 3 illustrate the plate counting method.

**Agar-Well Diffusion Method:**

The antibacterial activity of the tested solutions was also studied by the agar-well diffusion method with bacteria cultured on their proper agar. 26,33 Four hundred microliter suspension of the bacteria was plated on the agar in petri dishes (10 cm in diameter). Four 10 mm in diameter wells per plate were made using a trephine and numbered according to the tested solution (100µl) to be added in them. The plates were then placed at 4ºC for 2 hours to allow pre-diffusion of the tested solutions before they were placed in the
incubator at 37ºC for 24-48 hours. In addition, another set of plates was placed straight in the incubator without the pre-diffusion phase; but no notable differences in bacterial growth inhibition was noticed. The diameter of inhibitory zones around the wells was then measured to determine the antimicrobial activity of the tested solutions. The chlorhexidine mouthwash, a 5µl, 10µl of 100% ethanol in 1ml broth, and 5µl of 95% ethanol in 1ml broth were used as controls. Figure 4-6 illustrate the inhibition zone experiments for different tested solutions and microorganisms.

**Statistical Analyses**

Statistical analyses were performed using SPSS software version 21. One-way analysis of variance (ANOVA) was used to compare the inhibitory effect of the different compounds on the selected microorganisms with 95% confidence intervals. Pairwise post hoc tests were performed to determine which compounds were statistically significantly different from others. All p-values < 0.05 were considered statistically significant.
Results

**Serial Dilution and ELISA Plate Data Analysis:**

Using the serial dilution method, the means of colony forming units per volume corresponding to each tested solution against all bacterial strains are listed in Table 3.

*Streptococcus mutans:* The mean [standard deviation (SD)] number of *S. mutans* colonies treated with monocapric acid (46,666 [23,094.01]) was the least and thus had the highest antimicrobial activity compared to both concentrations of coconut oil 100µg/ml (146,666.7 [41,633.32]) and 200µg/ml (146,666.7 [83,266.64]) and monolauric acid (106,666.6 [23,094.01]). Treatment with Coconut oil with concentration of 100µg/ml resulted in the highest mean number of colonies compared to all other tested solutions. With reference to the number of colonies for *S. mutans* counted in the baseline broth plates, the treatment with both concentrations of coconut oil showed a statistically significant difference with p-values less than 0.05. Table 4 lists the p-values comparing the pairs of solutions. In addition, Figure 1 shows the experiment plates for *S. mutans* and Figure 5 shows a graphical representation of mean values for this bacterial strain with standard deviations.

*Lactobacillus fermentum:* comparing the means for the *Lactobacillus fermentum* colonies after mixing with the tested solutions, we found that treating with monolauric acid resulted in the fewest colonies formation (133,333.3 [30,550.50]). Both concentrations (100µg/ml and 200µg/ml) of coconut oil had the same mean (140,000.0), but different standard deviations [20,000.0] and [34,641.02], respectively. The mean [SD] of colony count for the treatment with monocapric acid was the highest (120,000.0
There were statistically significant differences among the groups (p < 0.05). With reference to the number of colonies for *Lactobacillus fermentum* counted in the baseline broth plates, the treatment with both concentrations of coconut oil showed a statistically significant difference with p-values less than 0.05. Table 4 lists the p-values of all tested solutions, whereas Figure 2 shows the experiment plates of *Lactobacillus fermentum* and Figure 6 shows a graphical representation of mean values with standard deviations.

**Streptococcus mitis**: the mean colony number detected after treatment with monocapric acid was the least compared to the other tested solutions (333.3 [416.33]). On the other hands, treatment with 100µg/ml coconut oil resulted in the highest number of colonies growth (1,866.7 [642.91]). According to the one-way ANOVA results, the differences were statistically significant between the groups (p-value = 0.005). With reference to the number of colonies for *S. mitis* counted in the baseline broth plates, the treatment with both concentrations of coconut oil showed a statistically significant difference with p-values below 0.05. Table 4 lists the p-values of all compounds. Figure 3 shows the experiment plates for *S. mitis* and Figure 7 shows a graphical representation of mean values with standard deviations.

To detect the difference between the effectiveness of different tested solutions as anti-microbial agents, one-way ANOVA was used to compare the means. The results were statistically significant with p-values of <0.001 for *S. mutans*, <0.001 for *Lactobacillus fermentum* and 0.005 for *S. mitis*. 
Chlorhexidine had the highest antimicrobial effect with mean [SD] of (0.0 [0.00]) for S. mutans, (13,333.3 [23,094.01]) for Lactobacillus fermentum and (0.0 [0.00]) for S. mitis. These results were statistically significant (p < 0.05). Tables 4 and 6 list the means and p-values.

**Inhibition Zone Data Analysis:**

Using the agar-well diffusion method, the means of inhibition zone measurements around each tested solution against all bacterial strains are listed in Table 5. The inhibition zone was determined by measuring the diameter of the clear zone in the agar after incubation at 37 ºC for 24-48 hours. Figure 4 depicts the inhibition zones of different test solutions as it was applied to the selected microorganisms and Figure 8 shows a graphical representation of mean values with standard deviations.

**Streptococcus mutans:** monolauric acid and monocapric acid showed the second highest inhibition zones with mean [SD] of (20.0 [11.45]) and (19.2 [3.70]) mm, respectively, compared to both concentrations of coconut oil. Coconut oil had a very small inhibition zones of (12.0 [1.41]) and (11.0 [0.82]) mm for 100µg/ml concentration and 200µg/ml concentration, respectively. Coconut oil showed 42% and 39% inhibition for 100µg/ml and 200µg/ml concentrations, whereas monolauric acid showed 68% and monocapric acid showed 71% inhibition, respectively. These results were statistically significant between the groups (p < 0.05). Table 6 lists the p-values of all the tested solutions.

**Lactobacillus fermentum:** after the chlorhexidine, the second largest mean [SD] inhibition zone was found with the monolauric acid tested on Lactobacillus fermentum strain with the width of (16.0 [3.74]) mm. All other tested solutions demonstrated slight
inhibition of bacterial growth around the wells and all were statistically significant between the groups with p-values listed on Table 6. Coconut oil showed 50% and 45% inhibition for 100µg/ml and 200µg/ml concentrations, whereas monolauric acid showed 67% and monocapric acid showed 57% inhibition, respectively.

**Streptococcus mitis:** monolauric acid showed the second highest mean [SD] inhibition zone measured compared to all other tested solutions (22.2 [4.15]). The mean inhibition zone around the wells that contained monocapric acid or 100µg/ml coconut oil were about the same width of (14.0 [6.36]) and (14.3 [4.27]) mm, respectively. The 200µg/ml coconut oil had almost no inhibition against the *S. mitis* strain and showed a width of (11.3 [1.89]) mm clear zone. Yet there was statistically significant differences among the groups (p <0.001) and the effect of monocapric acid and coconut oil was statistically significant as seen in the p-values listed on Table 6. Coconut oil showed 50% and 39% inhibition for 100µg/ml and 200µg/ml concentrations, whereas monolauric acid showed 77% and monocapric acid showed 49% inhibition, respectively.

Comparing the means of the effect of the agents on the microorganisms growth using one-way ANOVA, the results were statistically significant with p-value = 0.003 for *S. mutans*, p-value < 0.001 for *Lactobacillus fermentum* and p-value < 0.001 for *S. mitis*. Chlorhexidine treatment resulted in the highest inhibition zone of bacterial growth with mean [SD] of (28.3 [5.77]) for *S. mutans*, (24.0 [1.00]) for *Lactobacillus fermentum* and (28.7 [5.13]) for *S. mitis*. These results were statistically significant (p = 0.041, <0.001, and <0.001). Tables 7 and 9 list the means and p-values. For comparison purposes, the percent inhibition for chlorhexidine was set to 100% and the rest of compounds were compared to it.
As expected, among all the tested solutions, chlorhexidine mouthrinse had the best inhibitory effect against all bacterial strains tested using both serial dilution and plate counting method and agar-well diffusion method.

**Discussion**

The results of this *in vitro* study indicated that 200µg/ml of coconut oil, and monocapric and monolauric acid solutions showed variable antimicrobial activity against three types of strains of oral microorganisms associated with oral infections: *S. mutans*; *Lactobacillus fermentum*; and *S. mitis*. In addition, the effect of chlorhexidine on the selected microorganisms was similar to earlier studies.\(^{11-13}\) Specifically, *S. mutans* and *S. mitis* were completely eliminated by this solution and a reduction in the colony forming units of *Lactobacillus fermentum* was seen. The inhibitory effect of coconut oil on the growth of *S. mutans*, *Lactobacillus fermentum* and *S. mitis* strains was slight compared to the other solutions both using the serial dilution and the agar well diffusion methods (Table 3 and Table 5).

Using the serial dilution and plate counting method, monocapric acid displayed the most antimicrobial activity against *S. mutans* and *S. mitis* compared to the coconut oil and monolauric acid. On the other hand, monolauric acid exhibited better activity against *lactobacillus fermentum* strain than coconut oil and monocapric acid.

Using the agar well diffusion method, monolauric acid exhibited significantly more bactericidal activity against *Lactobacillus fermentum* and *S. mitis* strains than coconut oil and monocapric acid, while monocapric acid showed more antimicrobial activity against
S. mutans (Table 3 and Table 4). Both concentrations of coconut oil (100 and 200 µg/ml) were the least bactericidal compounds against S. mutans and Lactobacillus fermentum, except S. mitis with relatively larger inhibition zone. Monocapric acid was clearly more active against S. mutans than Lactobacillus fermentum and S. mitis strains. Additionally, S. mutans was the most sensitive microorganism to both monocapric and monolauric acids.

Coconut oil contains 92% saturated fatty acids; approximately 50% of which is lauric acid and 5% is capric acid.\textsuperscript{25} The study showed that the antimicrobial activity of coconut oil was consistent with, but slightly less than its main ingredients. The reason may be possibly due to the solubility of coconut oil in ethanol and the coexistence of its specific fatty acids in comparison to a concentrated single fatty acid. In addition, the esterification of lauric acid and capric acid to glycerol derivatives resulting in monolaurin and monocaprin, respectively, increases their antimicrobial effects.\textsuperscript{26} The results from our study conform to similar studies in the literature. While Taheri et al.\textsuperscript{32} showed that coconut flour did not have any antimicrobial activity irrespective of its concentration, other studies showed appreciable antimicrobial activities of other forms of coconut extracts and ingredient such as oil,\textsuperscript{27,29,30} water extract,\textsuperscript{28} monolauric acid,\textsuperscript{31,34} and monocapric acid.\textsuperscript{24,34} Most of these studies demonstrated the antimicrobial efficacy of coconut oil against S. mutans, Lactobacillus fermentum, and S. mitis, among other oral microorganisms.\textsuperscript{34,35}

In our study, in order to use the coconut oil and the medium-chain fatty acids (monolauric acid and monocapric acid) in the culturing media, they needed to be dissolved in ethanol. This solubility requirement could be a major concern if very high concentrations were
needed to achieve specific antimicrobial activity levels. However, the coconut oil and the fatty acids functionally inhibited the selected microorganisms at the µg/ml levels. Additionally, serial dilutions of the coconut oil and fatty acids from stock solutions were required, thus, the ethanol solvent was also diluted several folds in each specific media. Furthermore, the diluted ethanol from stock solutions was used as a negative control in all experiments with no antibacterial activity. Although the negative control showed no significant antibacterial activity, there is a possibility that the inhibitory activity of the fatty acids could be enhanced in the presence of ethanol, which could not be disregarded in the study. This does not invalidate the importance of the observations of the effect of the coconut oil and monolauric and monocapric acids solutions on the bacteria that were selected for this study.

Although the exact antimicrobial mechanism of coconut oil is still unclear, some studies have hypothesized that medium chain fatty acids (monolauric and monocapric) had the capacity to alter bacterial cell walls, penetrate and disrupt cell membranes, inhibit enzymes involved in energy production and nutrient transfer, leading to the death of the bacteria.²⁷

Coconut oil and its medium chain fatty acids are generally recognized as safe substances that have a long history of use in food products.²³ Consequently, these compounds may have the potential to be promising, non-chemically based antimicrobial ingredients for mouthrinses, thus, deserving further study to determine whether they may be used as alternatives to chemically-based ingredients.
During the study, the commercially available forms of coconut oil imposed a limitation on the experiment due to the difficulty to solubilize it in the culture media. This could be addressed by using different coconut extracts or designing a specific medium with enhanced solubility conditions. Furthermore, to extend our study, more oral microorganisms such as *Actinomyces*, *Candida albicans* and *S. sanguinis* should be tested. Also, it is recommended to carry similar studies examining whether the addition of salivary enzymes to coconut oil solution would enhance its antimicrobial activity against oral microorganisms.

**Conclusion**

We conclude that coconut oil has the potential to be antimicrobial agent to reduce cariogenic microorganisms in the oral cavity. Future studies should be specifically designed to examine the potential biological role of coconut oil and its fatty acids in the characteristics of oral biofilm formation and dynamics.
References


Appendix A: Tables

Table 1: Selected microorganisms with their characteristics

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Classification</th>
<th>Rule</th>
<th>Environment</th>
<th>Settings</th>
<th>Cultivation Medium</th>
<th>Cultivation Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. mitis</em></td>
<td>Normal</td>
<td>Flora</td>
<td>Aerobic</td>
<td>37°C</td>
<td>Tryptic Soy Broth &amp; Agar, and defibrinated Sheep Blood</td>
<td>24 hrs</td>
</tr>
<tr>
<td><em>S. mutans</em></td>
<td>Disease</td>
<td>Caries</td>
<td>Aerobic</td>
<td>37°C</td>
<td>BHI Agar/Broth</td>
<td>48-72 hrs</td>
</tr>
<tr>
<td><em>Lactobacillus fermentum</em></td>
<td>Disease</td>
<td>Caries</td>
<td>Aerobic</td>
<td>37°C</td>
<td>Lactobacilli MRS Agar/Broth</td>
<td>24-48 hrs</td>
</tr>
</tbody>
</table>

* The above settings, cultivation medium and duration for each microorganism are obtained from the supplier ATCC [http://www.atcc.org](http://www.atcc.org)

Table 2: Optical Density measurements for various dilution ratios for the tested microorganisms

<table>
<thead>
<tr>
<th>Dilution</th>
<th><em>S. mutans</em></th>
<th><em>Lactobacillus fermentum</em></th>
<th><em>S. mitis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary stock</td>
<td>1.164</td>
<td>2.273</td>
<td>2.115</td>
</tr>
<tr>
<td>Dilution (1:5)</td>
<td>0.220*</td>
<td>0.667</td>
<td>0.635</td>
</tr>
<tr>
<td>Dilution (1:25)</td>
<td>0.039</td>
<td>0.118*</td>
<td>0.117*</td>
</tr>
<tr>
<td>Dilution (1:125)</td>
<td>0.007</td>
<td>0.023</td>
<td>0.020</td>
</tr>
<tr>
<td>Dilution (1:625)</td>
<td>0.004</td>
<td>0.005</td>
<td>0.002</td>
</tr>
</tbody>
</table>

The asterisk (*) corresponds to the optical density measurements of the selected dilution ratio for test microorganisms
Table 3: Colony count of bacterial strains tested following application of monocapric acid, monolauric acid, 100µg/ml and 200µg/ml coconut oil

<table>
<thead>
<tr>
<th></th>
<th>S. mutans</th>
<th>Lactobacillus fermentum</th>
<th>S. mitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (CFU/ml)</td>
<td>SD</td>
<td>Mean (CFU/ml)</td>
</tr>
<tr>
<td>Coconut oil 100µg/ml</td>
<td>146666.7</td>
<td>41633.32</td>
<td>140000.0</td>
</tr>
<tr>
<td>Coconut oil 200µg/ml</td>
<td>146666.7</td>
<td>11547.01</td>
<td>140000.0</td>
</tr>
<tr>
<td>Monocapric Acid</td>
<td>46666.7</td>
<td>23094.01</td>
<td>120000.0</td>
</tr>
<tr>
<td>Monolauric Acid</td>
<td>106666.7</td>
<td>23094.01</td>
<td>133333.3</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>0.0</td>
<td>0.00</td>
<td>13333.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>146666.7</td>
<td>41633.32</td>
<td>193333.3</td>
</tr>
<tr>
<td>Broth</td>
<td>466666.7</td>
<td>115470.05</td>
<td>306666.7</td>
</tr>
<tr>
<td>ANOVA p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
Table 4: The inhibitory effect of the different tested compounds on *S. mutans*, *Lactobacillus fermentum* and *S. mitis* bacterial strains using the serial dilution and plate counting method.

<table>
<thead>
<tr>
<th></th>
<th><em>S. mutans</em></th>
<th></th>
<th><em>Lactobacillus fermentum</em></th>
<th></th>
<th><em>S. mitis</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean diff.</td>
<td>p-value</td>
<td>Mean diff.</td>
<td>p-value</td>
<td>Mean diff.</td>
<td>p-value</td>
</tr>
<tr>
<td>Coconut oil 100 µg/ml vs. Ethanol</td>
<td>0.00</td>
<td>1.000</td>
<td>53333.33</td>
<td>0.926</td>
<td>400.00</td>
<td>0.996</td>
</tr>
<tr>
<td>Coconut oil 200 µg/ml vs. Ethanol</td>
<td>0.00</td>
<td>1.000</td>
<td>53333.33</td>
<td>0.926</td>
<td>133.33</td>
<td>1.000</td>
</tr>
<tr>
<td>Monocapric Acid vs. Ethanol</td>
<td>100000.00</td>
<td>0.479</td>
<td>73333.33</td>
<td>0.748</td>
<td>1133.33</td>
<td>0.618</td>
</tr>
<tr>
<td>Monolauric Acid vs. Ethanol</td>
<td>40000.00</td>
<td>0.985</td>
<td>60000.00</td>
<td>0.879</td>
<td>933.33</td>
<td>0.791</td>
</tr>
<tr>
<td>Chlorhexidine vs. Ethanol</td>
<td>146666.67</td>
<td>0.120</td>
<td>180000.00</td>
<td>0.027*</td>
<td>1466.67</td>
<td>0.336</td>
</tr>
<tr>
<td>Coconut oil 100 µg/ml vs. Chlorhexidine</td>
<td>146666.67</td>
<td>0.120</td>
<td>126666.67</td>
<td>0.194</td>
<td>1866.67</td>
<td>0.127</td>
</tr>
<tr>
<td>Coconut oil 200 µg/ml vs. Chlorhexidine</td>
<td>146666.67</td>
<td>0.120</td>
<td>126666.67</td>
<td>0.194</td>
<td>1600.00</td>
<td>0.248</td>
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<td>Monocapric Acid vs. Chlorhexidine</td>
<td>46666.67</td>
<td>0.967</td>
<td>106666.67</td>
<td>0.360</td>
<td>333.33</td>
<td>0.999</td>
</tr>
<tr>
<td>Monolauric Acid vs.Chlorhexidine</td>
<td>106666.67</td>
<td>0.406</td>
<td>120000.00</td>
<td>0.241</td>
<td>533.33</td>
<td>0.982</td>
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<tr>
<td>Ethanol vs. Chlorhexidine</td>
<td>146666.67</td>
<td>0.120</td>
<td>180000.00</td>
<td>0.027*</td>
<td>1466.67</td>
<td>0.336</td>
</tr>
<tr>
<td>Coconut oil 100 µg/ml vs. baseline Broth</td>
<td>320000.00</td>
<td>0.000*</td>
<td>166666.67</td>
<td>0.045*</td>
<td>466.67</td>
<td>0.991</td>
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<tr>
<td>Coconut oil 200 µg/ml vs. baseline Broth</td>
<td>320000.00</td>
<td>0.000*</td>
<td>166666.67</td>
<td>0.045*</td>
<td>733.33</td>
<td>0.918</td>
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<tr>
<td>Monocapric Acid vs. baseline Broth</td>
<td>420000.00</td>
<td>0.000*</td>
<td>186666.67</td>
<td>0.021*</td>
<td>2000.00</td>
<td>0.089</td>
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<tr>
<td>Monolauric Acid vs. baseline Broth</td>
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<td>173333.33</td>
<td>0.035*</td>
<td>1800.00</td>
<td>0.151</td>
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<tr>
<td>Chlorhexidine vs. baseline Broth</td>
<td>466666.67</td>
<td>0.000*</td>
<td>293333.33</td>
<td>0.000*</td>
<td>2333.33</td>
<td>0.035*</td>
</tr>
<tr>
<td>Ethanol vs. baseline Broth</td>
<td>320000.00</td>
<td>0.000*</td>
<td>113333.33</td>
<td>0.296</td>
<td>866.67</td>
<td>0.840</td>
</tr>
</tbody>
</table>

* The asterisk (*) corresponds to statistically significant (p < 0.05) results
Table 5: The means of inhibition zones around tested compounds against *S. mutans*, *Lactobacillus fermentum* and *S. mitis* bacterial strains.

<table>
<thead>
<tr>
<th></th>
<th><em>S. mutans</em></th>
<th></th>
<th><em>Lactobacillus fermentum</em></th>
<th></th>
<th><em>S. mitis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (mm)</td>
<td>SD</td>
<td>Inhibition %</td>
<td>Mean (mm)</td>
<td>SD</td>
</tr>
<tr>
<td>Coconut oil 100 µg/ml</td>
<td>12.0</td>
<td>1.41</td>
<td>42%</td>
<td>12.0</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.3</td>
<td>4.27</td>
</tr>
<tr>
<td>Coconut oil 200 µg/ml</td>
<td>11.0</td>
<td>0.82</td>
<td>39%</td>
<td>10.9</td>
<td>1.18</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.3</td>
<td>1.89</td>
</tr>
<tr>
<td>Monocapric Acid</td>
<td>20.0</td>
<td>11.45</td>
<td>71%</td>
<td>13.6</td>
<td>3.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.0</td>
<td>6.36</td>
</tr>
<tr>
<td>Monolauric Acid</td>
<td>19.2</td>
<td>3.70</td>
<td>68%</td>
<td>16.0</td>
<td>3.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22.2</td>
<td>4.15</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>28.3</td>
<td>5.77</td>
<td>100%</td>
<td>24.0</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>28.7</td>
<td>5.13</td>
</tr>
<tr>
<td>Ethanol</td>
<td>11.0</td>
<td>0.71</td>
<td>39%</td>
<td>10.0</td>
<td>0.00</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>0.00</td>
</tr>
<tr>
<td>ANOVA p-value</td>
<td>0.003</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 6: The inhibitory effect of the different tested compounds on *S. mutans*, *Lactobacillus fermentum* and *S. mitis* bacterial strains using the Agar-well diffusion method.

<table>
<thead>
<tr>
<th></th>
<th><em>S. mutans</em></th>
<th></th>
<th><em>Lactobacillus fermentum</em></th>
<th></th>
<th><em>S. mitis</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean diff.</td>
<td>p-value</td>
<td>Mean diff.</td>
<td>p-value</td>
<td>Mean diff.</td>
<td>p-value</td>
</tr>
<tr>
<td>Coconut oil 100 µg/ml vs. Chlorhexidine</td>
<td>16.33</td>
<td>0.055</td>
<td>12.00</td>
<td>0.000</td>
<td>14.42</td>
<td>0.014*</td>
</tr>
<tr>
<td>Coconut oil 200 µg/ml vs. Chlorhexidine</td>
<td>17.33</td>
<td>0.037</td>
<td>13.13</td>
<td>0.000</td>
<td>17.42</td>
<td>0.002*</td>
</tr>
<tr>
<td>Coconut oil 100 µg/ml vs. Ethanol</td>
<td>0.75</td>
<td>1.000</td>
<td>2.00</td>
<td>0.920</td>
<td>4.25</td>
<td>0.847</td>
</tr>
<tr>
<td>Coconut oil 200 µg/ml vs. Ethanol</td>
<td>0.25</td>
<td>1.000</td>
<td>0.88</td>
<td>0.998</td>
<td>1.25</td>
<td>0.999</td>
</tr>
<tr>
<td>Monocapric Acid vs. Ethanol</td>
<td>8.75</td>
<td>0.451</td>
<td>3.60</td>
<td>0.444</td>
<td>4.00</td>
<td>0.851</td>
</tr>
<tr>
<td>Monolauric Acid vs. Ethanol</td>
<td>7.95</td>
<td>0.553</td>
<td>6.00</td>
<td>0.048*</td>
<td>12.20</td>
<td>0.018*</td>
</tr>
<tr>
<td>Chlorhexidine vs. Ethanol</td>
<td>17.08</td>
<td>0.041*</td>
<td>14.00</td>
<td>0.000*</td>
<td>18.67</td>
<td>0.001*</td>
</tr>
<tr>
<td>Monocapric Acid vs. Chlorhexidine</td>
<td>8.33</td>
<td>0.592</td>
<td>10.40</td>
<td>0.001*</td>
<td>14.67</td>
<td>0.008*</td>
</tr>
<tr>
<td>Monolauric Acid vs. Chlorhexidine</td>
<td>9.13</td>
<td>0.496</td>
<td>8.00</td>
<td>0.010*</td>
<td>6.47</td>
<td>0.529</td>
</tr>
<tr>
<td>Ethanol vs. Chlorhexidine</td>
<td>17.08</td>
<td>0.041*</td>
<td>14.00</td>
<td>0.000*</td>
<td>18.67</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

* The asterisk (*) corresponds to statistically significant (p < 0.05) results
Appendix B: Figures

Figure 1: Agar plates for *S. mutans* with different tested solutions.

A) chlorhexidine, B) 100 µg/ml coconut oil, C) 200 µg/ml coconut oil, D) monolauric acid, E) monocapric acid, and F) ethanol control
Figure 2: Agar plates for *Lactobacillus fermentum* with different tested solutions.

A) chlorhexidine, B) 100 µg/ml coconut oil, C) 200 µg/ml coconut oil, D) monolauric acid, E) monocapric acid, and F) ethanol control
Figure 3: Agar plates for *S. mitis* with different tested solutions.

A) chlorhexidine, B) 100 µg/ml coconut oil, C) 200 µg/ml coconut oil, D) monolauric acid, E) monocapric acid, and F) ethanol control
Figure 4: Inhibition Zone plates for *S. Mutans* with different tested solutions. Monocapric (1), monolauric acid (2), 100 µg/ml coconut oil (3), 200 µg/ml coconut oil (4), and 2) control compounds: Chlorhexidine (ch), ethanol (5, 10, 95 % concentration)

Figure 5: Inhibition Zone plates for *Lactobacillus fermentum* with different tested solutions. Monocapric (1), monolauric acid (2), 100 µg/ml coconut oil (3), 200 µg/ml coconut oil (4), and 2) control compounds: Chlorhexidine (ch), ethanol (5, 10, 95 % concentration)

Figure 6: Inhibition Zone plates for *S. Mitis* with different tested solutions. Monocapric (1), monolauric acid (2), 100 µg/ml coconut oil (3), 200 µg/ml coconut oil (4), and 2) control compounds: Chlorhexidine (ch), ethanol (5, 10, 95 % concentration)
Figure 7: The antimicrobial effect of the tested solutions on *S. mutans* using the serial dilution method
Figure 8: The antimicrobial effect of the tested solutions on *Lactobacillus fermentum* using the serial dilution method
Figure 9: The antimicrobial effect of the tested solutions on *S. mitis* using the serial dilution method
Figure 10: The mean inhibition zones of the tested solutions on oral microorganisms using the agar-well diffusion method
Appendix C: Media Preparation Instruction

**ATCC Medium: 44 Brain Heart Infusion Agar/Broth**

**Agar Medium**

- Brain Heart Infusion Agar (BD 211065) . .52 g
- DI Water ... 1000 ml

Autoclave at 121°C.

**Broth Medium**

- Brain Heart Infusion Broth (BD 237500) .. .37.0g
- DI Water .1000 ml

Autoclave at 121°C.

*This product is commercially available from BD.

To make medium from scratch, follow formulation below:

**Brain Heart Infusion Composition**

- Calf Brains, infusion from . .200 g
- Beef Hearts, infusion from . .250 g
- Proteose Peptone ... 10.0 g
- Dextrose .. .2.0 g
- NaCl ... 5.0 g
- Na₂HPO₄ ... .2.5 g
- DI Water .1000 ml

Final pH 7.4 +/- 0.2

*Omit agar for broth medium.

**Final Product Description**

- Medium should be tan in color.
- Recommended storage temperature: 2-8°C.
**ATCC Medium: 260 Tryptic Soy Agar/Broth with 5% Sheep Blood (defibrinated)**

**Agar Medium**
Tryptic Soy Agar (BD 236950)………………40 g  
Sheep Blood (defibrinated)…………………50 ml  
DI Water……………………………………950 ml

Autoclave at 121°C. Cool sterilized medium to ~47°C. Aseptically add 50 ml of room temperature defibrinated sheep blood. Gently mix and dispense as required.

**Broth Medium**
Tryptic Soy Broth (BD 211825)……………30.0 g  
Sheep Blood (defibrinated)…………………50 ml  
DI Water…………………………………..950 ml

Follow same procedure as agar medium above.

If commercialized product is not available, Tryptic Soy Agar can be made from scratch, by following the formulation below:

**Tryptic Soy Agar Composition**
Tryptone…………………………15.0 g  
Soytone…………………………5.0 g  
NaCl…………………………….5.0 g  
Agar…………………………….15.0 g  
DI Water…………………………950 ml

pH to 7.3 +/- 0.2. (Tryptone-Pancreatic Digest of Casein; Soytone-Papaic Digest of Soybean Meal). To make Tryptic Soy Broth from scratch, follow Tryptic Soy Agar formulation but omit agar.
ATCC Medium: 416 Lactobacilli MRS Agar/Broth

**Agar Medium**
Lactobacilli MRS Agar (BD 288210)..........................70 g
DI Water.................................................................1000 ml

Boil to dissolve agar. Autoclave at 121°C.

**Broth Medium**
Lactobacilli MRS Broth (BD 288130)..................55.0 g
DI Water.................................................................1000 ml

Autoclave at 121°C.

*This product is commercially available from BD.

To make medium from scratch, follow formulation below:

**Lactobacilli MRS Broth Composition**
Proteose Peptone #3.................................10.0 g
Beef Extract.......................................................10.0 g
Yeast Extract......................................................5.0 g
Dextrose..........................................................20.0 g
Sorbitan Monooleate.................................1.0 g
Ammonium Citrate........................................2.0 g
Sodium Acetate........................................5.0 g
MnSO₄ x H₂O..................................................0.05 g
Na₂HPO₄.........................................................2.0 g
DI Water..........................................................1000 ml

Final pH 6.5 +/- 0.2
Appendix D: Culture Preparation Instruction

STREPTOCOCCUS MUTANS
(ATCC® 25175™)

Please read this FIRST

Storage Temp.
Frozen: -80°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Propagation Section

Biosafety Level 1

Description

Designation: NCTC 10449 [IFO 13955]
Deposited Name: Streptococcus mutans Clarke
Product Description: Type strain

Propagation

Medium
ATCC® Medium 44: Brain Heart Infusion Agar Broth
ATCC® Medium 260: Tryptic soy agar broth with defibrinated sheep blood

Growth Conditions
Temperature: 37°C
Atmosphere: Aerobic

Propagation Procedure
1. Open vial according to enclosed instructions.
2. Using a single tube of #44 broth (5 to 6 mL), withdraw approximately 0.5 to 1.0 mL with a Pasteur or 1.0 mL pipette. Rehydrate the entire pellet.
3. Aseptically transfer this aliquot back into the broth tube. Mix well.
4. Use several drops of the suspension to inoculate a #260 agar slant and/or plate.
5. Incubate the tubes and plate at 37°C for 24 to 48 hours.

Notes

Colonies in #260 agar are punctiform, white, dry, flat, and entire.
Additional information on this culture is available on the ATCC web site at www.atcc.org.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.
**ATCC**

Product Sheet

*Lactobacillus fermentum*  
(ATCC® 11976™)

Please read this FIRST

Storage Temp.  
Frozen: -80°C or colder  
Freeze-Dried: 2°C to 8°C  
Live Culture: See Propagation Section

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**Biosafety Level**  
1

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**Intended Use**

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

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**Citation of Strain**

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Lactobacillus fermentum*  (ATCC® 11976™)

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**Notes**

On #416 media two colony types were observed. Colony type 1 was circular, entire, convex, and white. Colony type 2 was circular, entire, flat, dull, and tan.

Additional information on this culture is available on the ATCC® web site at www.atcc.org.

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**References**

References and other information relating to this product are available online at www.atcc.org.

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**Biosafety Level: 1**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org

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Please read this FIRST

Biosafety Level

2

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Streptococcus parasanguinis (ATCC® 903™)

Description

Designation: [SK 132]

Deposited Name: Streptococcus milior Schottmuller. Formerly Streptococcus milius.

Antigenic Properties: Type II

Propagation

Medium

ATCC® Medium 260: Trypticase soy agar/broth with defibrinated sheep blood

Growth Conditions

Temperature: 37.0°C

Propagation Procedure

1. Open vial according to enclosed instructions.
2. Using a single tube of #44 broth (5 to 6 ml), withdraw approximately 0.5 to 1.0 ml with a Pasteur or 1.0 ml pipette. Rehydrate the entire pellet.
3. Aseptically transfer this aliquot back into the broth tube. Mix well.
4. Use several drops of the suspension to inoculate a #260 agar slant and/or plate.
5. Incubate the tubes and plate at 37°C for 24 hours.

Notes

On #260 plates, colonies are entire, glistening, circular, smooth, small, and exhibit alpha-hemolysis.

This strain is cited as producing an aminopeptidase (1).

Additional information on this culture is available on the ATCC® web site at www.atcc.org.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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Additional information on this culture is available on the ATCC web site at www.atcc.org

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