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Effects of Fasting on the Oral Microbiome

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ABSTRACT

Humans are mostly made up of microbes. Multiple studies have estimated that bacterial cells outnumber human cells in the body, with our digestive systems harboring the highest concentrations of bacterial life. The oral microbiome is densely populated and of significant physiological importance. These microbial communities maintain tooth and gum health, aids in pre-digestion of foods, and even plays a defensive role — guarding their host from external threats that may lead to oral and systemic pathologies. Studies have shown that fasting can affect the gut microbiome, but there is currently insufficient research on how fasting intervals can affect the oral microbiome. This study will compare the oral microbiome of subjects with similar dietary and oral hygiene habits (n=3) before and after a 24-hour fasting period. If the abundance and diversity of bacteria in the subjects' mouths change in a consistent and significant manner, then intermittent fasting could play a role in reshaping the oral microbiome and influence human wellbeing.

INTRODUCTION

The microbial profile of the **oral microbiome** is enormous and second only to that of the gut. There are over 700 varying species of bacteria (1) that reside in the human mouth, and types of microorganisms such as fungi and protozoa as well. The mouth is a moist and nutrient rich environment — the perfect home for a microbe. In macroscopic terms, the oral microbiome is responsible for halitosis, cavities, and can even influence taste perception to various foods. (2)

Fasting is a long-standing and integral part of many cultural and religious practices, and a common method of health maintenance and malady treatment in alternative medicine. Evidence of fasting traditions exist throughout Islam, Christianity, Hinduism, and Buddhism. Studies have shown that intermittent fasting can reshape the microbiome of the gut (3), but there has been insufficient research on how brief fasting intervals can affect the oral microbiome in particular.

Given the importance of bacteria to human health and wellbeing, an investigation on the relationship between short term fasting and the human oral microbiome is of significant interest.

In this study, we will observe the changes in quantity and morphologies of bacteria swabbed from the oral microbiome under normal conditions and after 24 hours of fasting. We predict that the overall abundance and diversity of the oral microbiome will decrease during fasted conditions. No food will be introduced over the 24 hour fasting window, reducing the availability of nutrients in the environment, which may limit the growth and survival of some microorganisms. As a result, there may be a decrease in the concentration of bacteria in the oral cavity.

However, it remains a possibility that the overall abundance of bacteria will decrease, but the diversity of the oral microbiome will instead increase due to the shift in the environment favoring different species of bacteria. (4) For example, populations of beneficial *lactobacilli* overtaking sugar consuming *Streptococcus mutants* (5), a consistent culprit for the formation of cavities. This shift in the oral microbiome during fasting could have implications for oral health. While lactobacilli are considered beneficial bacteria that can help maintain oral health, an overgrowth of any one species can also be problematic.

If the **abundance** and **diversity** of bacteria in the participants' mouths change in a consistent and significant manner, then intermittent fasting could play a role in reshaping the oral microbiome and influence human wellbeing.

MATERIALS AND METHODS

Participants (n=3) will swab their mouths before and after a 24-hour treatment of fasting. Participants will not be able to eat, drink, smoke, or chew gum through the fasting period. To ensure participant safety throughout the experiment, water consumption is allowed and encouraged. Oral swabs will be directly plated as a positive control and transferred into solution for dilution, incubation, and calculations.

Pre-swab participant guidelines

1. Fasting: participants will abstain from food, drinks (other than water), gum for 24 hours prior to the swabbing procedure.

2. Non-fasted: participants will follow their normal dietary routine and not make any significant changes to their diet or lifestyle in the 24 hours leading up to the swabbing procedure.

3. Smoking and alcohol: Participants should avoid smoking or consuming alcohol for at least 24 hours prior to the swabbing procedure, as these substances can also affect the composition of the oral microbiome.

4. Oral Hygiene: participants should maintain their current oral hygiene habits as this is a controlled variable which could significantly affect the oral microbiome.

Swabbing & Culturing Procedure

1. Using a sterile swab, rub the inside of both cheeks, the gums, the tongue, and the roof of the mouth. Make sure to cover as much area as possible.

2. Smear the swab in a crisscross motion on the surface of a TSA plate (positive control).

3. Discard the swab in the biohazard container and repeat step 1.

4. Place the swab in a tube of 10 ml of sterile saline and cover tightly.

5. Vortex the tube for 10 seconds to thoroughly homogenize the solution.

6. Discard the swab in the biohazard container.

7. Dilute the sample according to **Fig 1**

8. Inoculate 3 TSA plates of decreasing concentrations by spread plating.

9. Incubate 37°C for 48 hours total.

Calculations and Testing: The number of colony forming units (CFU) on valid plates (30 - 300 CFU) will be quantified. Through log reduction, the given CFU values will be used to back calculate the original sample concentration. (See Table 1B) With this data set, a paired T-test will be conducted to test for significance ($\alpha = 0.05$).

RESULTS

There is significant diversity in the bacterial life that inhabits the oral microbiome. This number exceeds over 700 different species of bacterial organisms. Although there is a large variety of different bacteria, there are dominant species such as streptococcus mutans that attribute to a large portion of bacteria within an individual (6). The results between the bacterial growth of each participant displayed the dissimilarity of an individual's oral microbiome under non-fasted and fasted conditions. The results were heavily skewed — Each participant had a different number of morphological features present under each condition. This observation could be attributed to the diets of participants not being regulated. From a non-fasted to a fasted state; One participant had an increase in different number morphological colonies present,

Figure 1.
Dilution Scheme

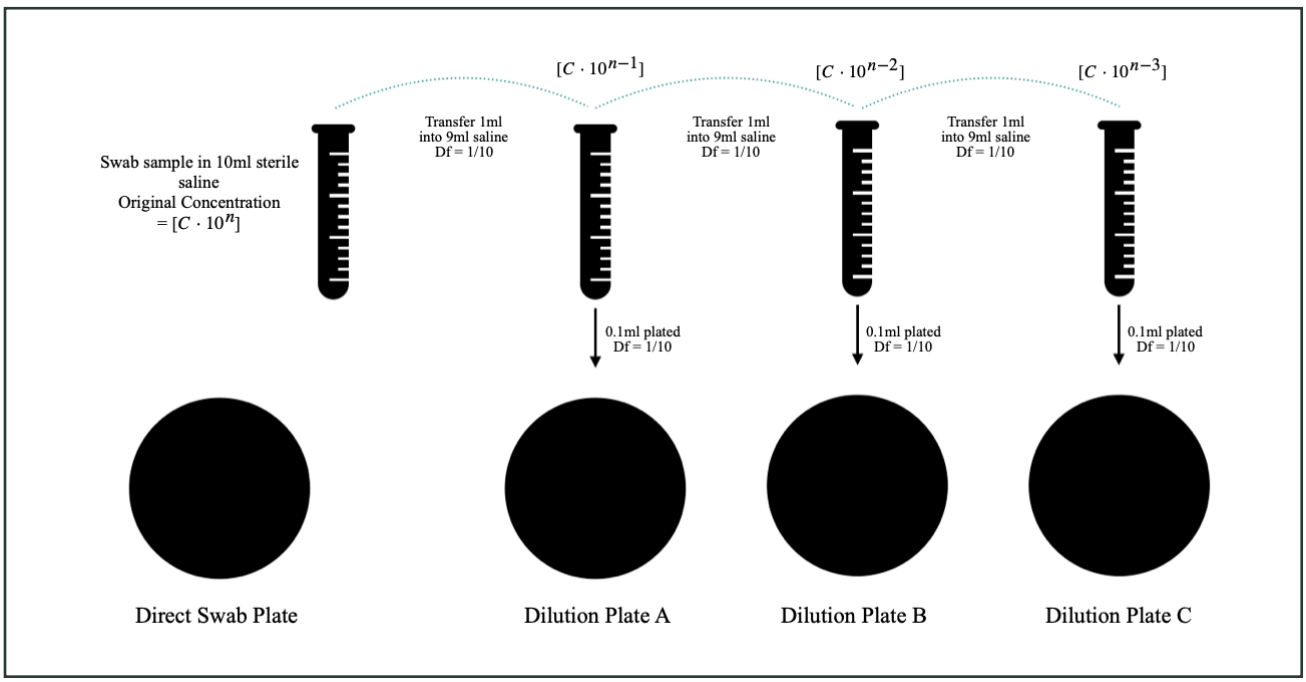
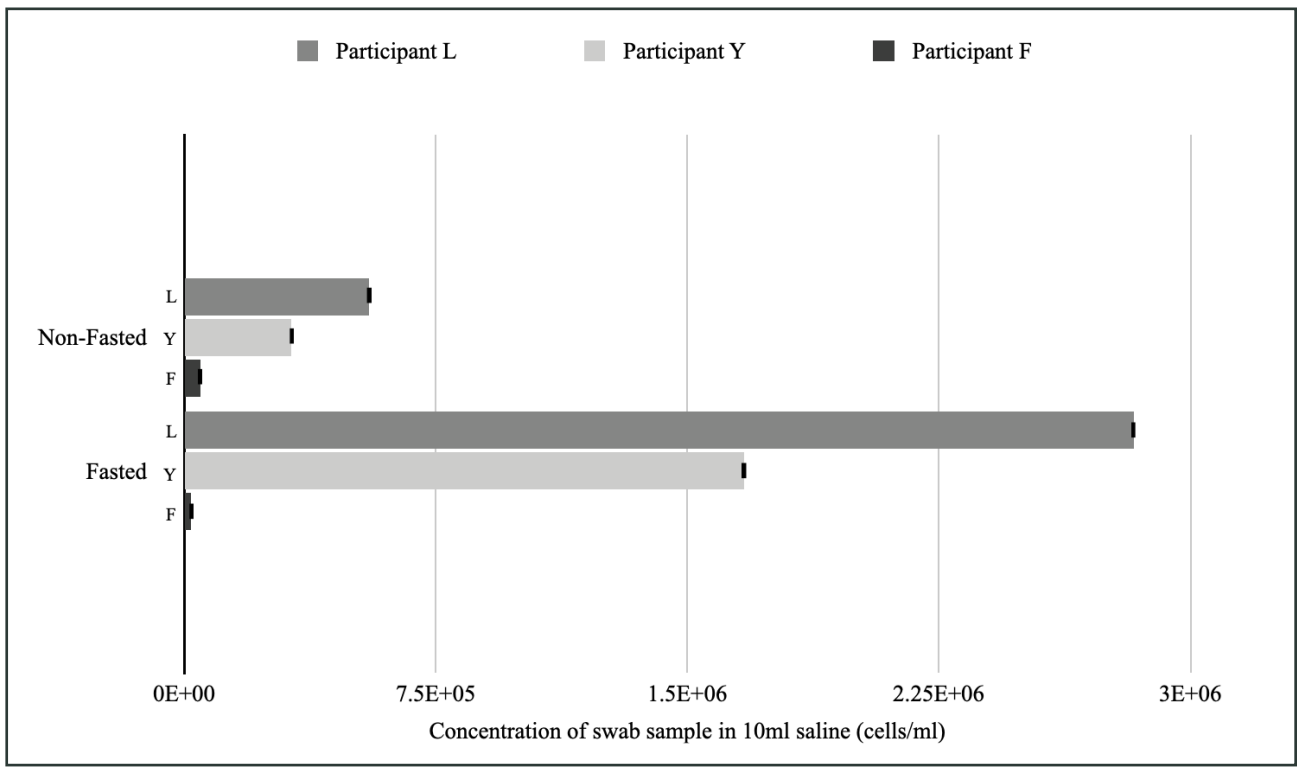


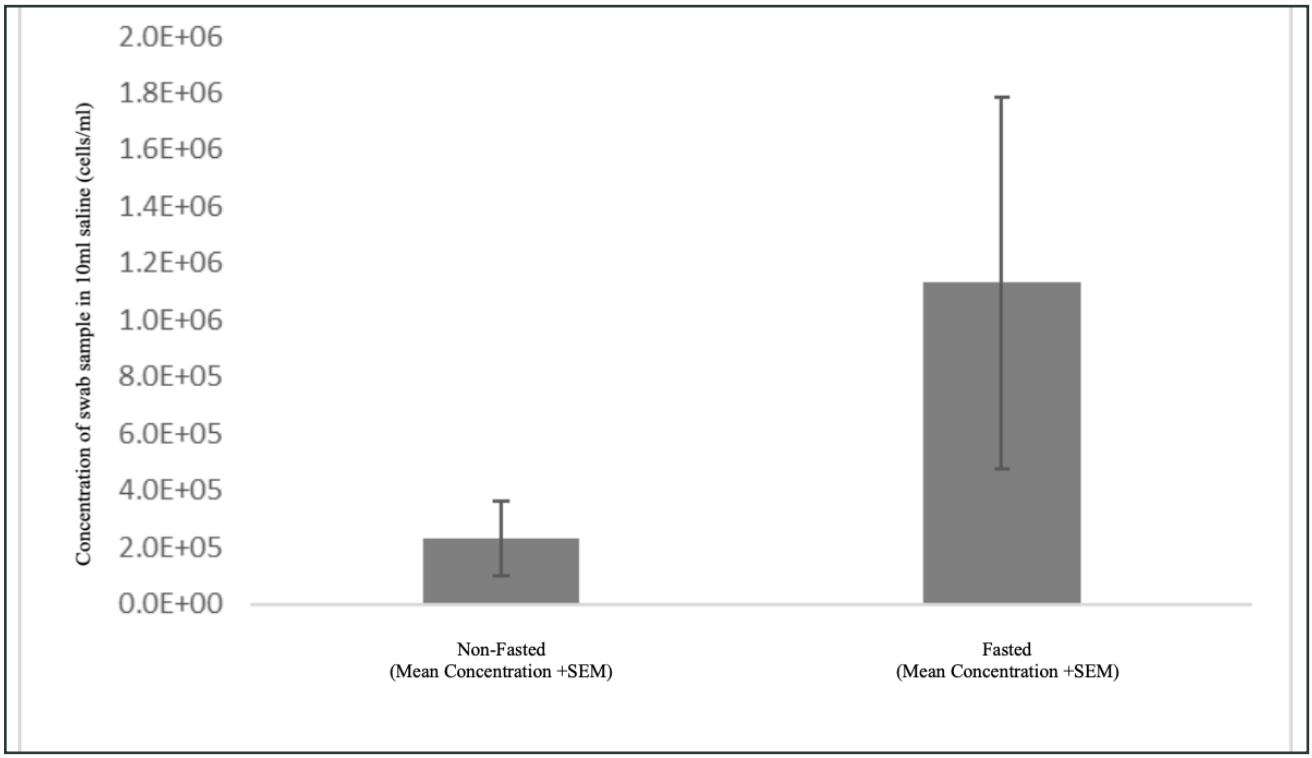
Table 1.
CFU/Morphology counts & calculated concentrations

Participant	Non-Fasted (nf)			Fasted (f)			
	CFU	Concentration	Number of Morphologies	CFU	Concentration	Number of Morphologies	% Change
Participant L -(C),(C)	55	5.5 E+^{05}	2	283	2.83 E+^{06}	3	414.54%
Participant Y -(C),(C)	32	3.2 E+^{05}	5	167	1.67 E+^{06}	3	421.87%
Participant F -(B),(A)	48	4.8 E+^{04}	3	208	2.08 E+^{04}	5	-56.67%

Graph 1.
Bacterial concentration of swab sample in saline of 3 participants before and after fasting treatments. Results of the paired-t test indicated that there is a large, non-significant difference between pre-fast (M = 306000 ,SD = 251292.7) and post-fast states (M = 1506933.3 ,SD = 1411681.3) [t(2) = 1.8, p = .215.]



Graph 2.
A comparison of mean concentration based on fasting conditions. Results of paired t-test indicated that there was no significant relationship between fasting conditions. Before (M=229500.8, standard error= 132502.3), after (M=1130201, standard error= 652521.7) [t(2)=1.791, degrees of freedom= 3, p-value= 0.215]



one a decrease, and another stayed consistent. In Figure 2A-C, there are noticeable differences between the features of each participant's bacteria growths. No significant proposal can be inferred given the skewed distribution, but there is an observed change in the type of bacteria present influenced between the two treatments.

DISCUSSION

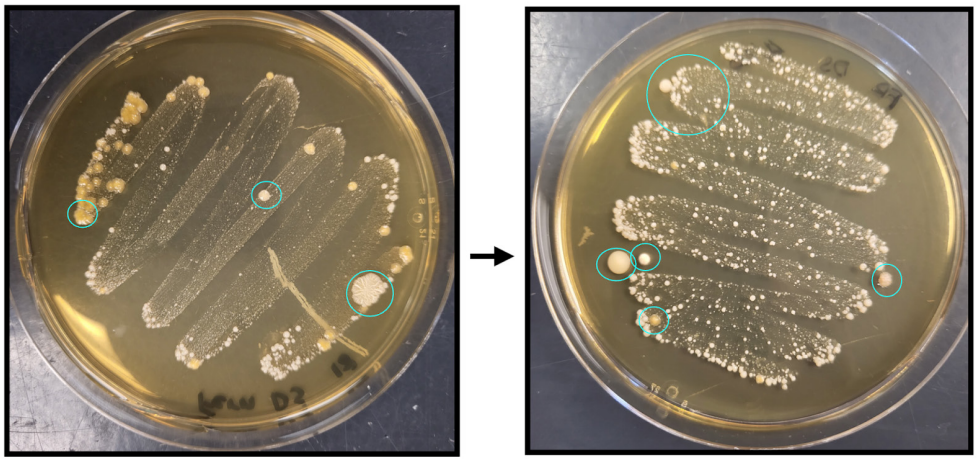
The results from the dilution plate calculations showed a non-significant large difference in the concentration of oral swabs between normal conditions and post-treatment conditions (Paired T-test, $\alpha = 0.05$). While there was a notable increase (+414.54%%, +421.87%) in bacterial concentration for two subjects, a modest decrease (-56.67%) was observed in the third participant. Thus, while there was a large change in bacterial concentration in the mouth, it cannot be stated with certainty that these fluctuations were due to the fasting treatment. It is interesting to note, however, the magnitude of fluctuation between the two periods — it is clear that the population of the oral microbiome is both highly varied and variable in healthy individuals. (7)

Various morphological changes were also observed. For example, in the case of participant Y, the pre-fast oral microbiome was dominated by highly motile rod-shaped bacteria. After fasting, sessile, cocci bacteria were by far the most prevalent.

Overall, there was no unidirectional shift in abundance or level of diversity due to the fasting treatment. This is possibly due to the 'starting state' of the participants' oral microbiome, in terms of their dietary and oral hygiene habits. As an example, if a participant is in the habit of drinking black coffee in the morning, they may be limiting the populations of microbes in their mouth for the next few hours due to the antibiotic effects of caffeine (8). During the fasted swab, the same participant would not have consumed coffee that morning, resulting in a notable increase in oral microbe concentration.

There are also several experimental limitations to consider. Notably, while the swabbing/dilution plating method employed was effective in measuring changes to the microbiome, it is not an effective method to determine the total quantity of bacteria in the human mouth. The swab inoculated saline sample may not be fully indicative of the oral bacterial population. In addition, swabbing is highly limited by human error, and

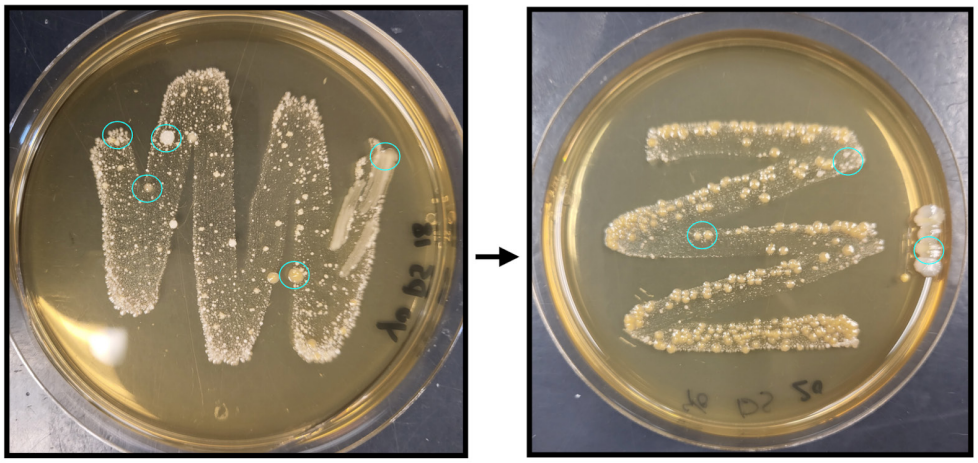
Figure 2A.
Participant F Oral Swab Morphological Changes (Left Plate) non-fasted state;3 morphologies present. Elevations; raised and convex. Forms; Rhizoid and circular shapes throughout. Margins; curled (Rhizoid shaped) and Undulate (small circle). (Right Plate) Fasted state; 5 morphologies present. Forms; irregular and circular. Elevations; Pulvinate(swelled) and convex. Margins; Entire(large circle), Erose (irregular shape; furry looking), and Lobate(different sizes of the same colonies)



Fernie 4/18 - Regular Conditions

Fernie 4/20 - Fasted Conditions

Figure 2B.
Participant Y Oral Swab Morphological Changes (Left Plate) non-fasted state;5 morphologies present. Elevations; Convex and raised. Forms; circular and irregular shapes throughout. Margins; Lobate(different sizes of the same colonies) and Undulate (small circle). (Right Plate) Fasted state; 3 morphologies present. Forms; irregular and circular. Elevations; Convex and Pulvinate(swelled). Margins; Entire(large circle), Undulate (small circle) and Lobate(different sizes of the same colonies)



Yo 4/18 - Regular Conditions

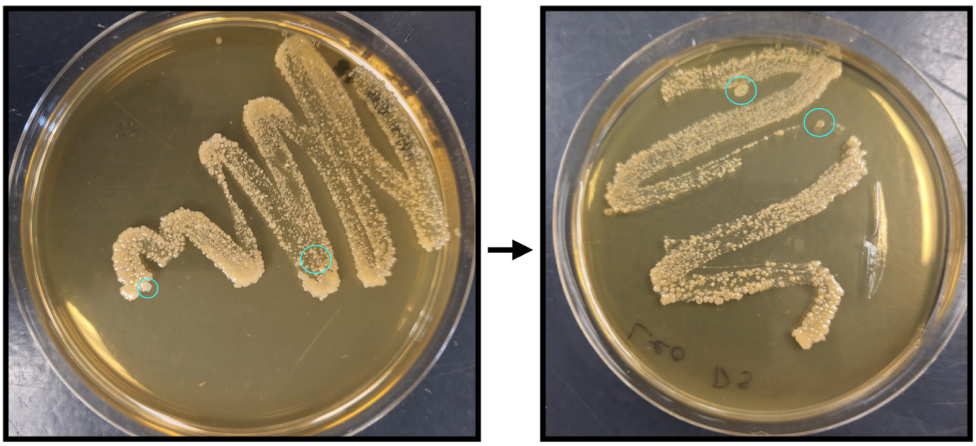
Yo 4/20 - Fasted Conditions

changes in swabbing pressure and technique can easily affect quantitative measures. Furthermore, variations in participants' diets and oral hygiene habits (toothpaste brand, brushing style, and brushing pressure) could have also had a significant effect on the concentration and population diversity of microbes in the mouth.

Significantly, recent studies have re-confirmed the estimation that 50% of the oral flora is unculturable (9). Given this figure, it is highly likely that many populations of oral bacterium were not captured on the TSA plates, further limiting quantitation and morphotyping steps. To rigorously assess the quantity and distribution of microbe phylotypes within oral microbiome, it would be necessary to analyze larger numbers of samples (10) and utilize genotyping tools.

It would be of great interest to reconduct a similar experiment with a significantly larger sample size, higher degree of control over participant oral hygiene and dietary practices, and access to genomic technologies such as qPCR.

Figure 2C.
Participant L Oral Swab Morphological Changes. (Left Plate) Non-fasted state; 2 morphologies present. Forms; circular, elevation is convex, margins are undulate (small circle) and entire (large circle). (Right plate) Fasted state; 2 morphologies present. Forms are circular and irregular, elevations are convex and flat, margins are undulate (upper circle) and entire (lower circle). There was minimal morphological change for this participants.



Leo 4/18 - Regular Conditions

Leo 4/20 - Fasted Conditions

There is still much to learn about the oral microbiome and emerging genomic sequencing tools may aid in unlocking its complexities.

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