The Effect of Marijuana on The Oral Microbiome

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THESIS

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SUMMARY

The changes in the oral microbiome of marijuana smokers versus non-smokers were investigated. Smokers were strictly marijuana and not tobacco users, and non-smokers utilized neither product. 11 test subjects and 16 control subjects participated in the study.

Bacterial swabs were taken and analyzed from two oral locations: lateral border of the tongue and the oropharynx. Bacterial DNA was extracted utilizing the Quick-DNA™ Fungal/Bacterial Miniprep Kit. Relative abundances of bacterial species across test and control samples were determined via a two-stage Polymerase Chain Reaction (PCR) or "Targeted Amplicon Sequencing" (TAS) approach, which targeted the 16S ribosomal RNA gene.

Statistical differences ($p<0.05$) were noted in the relative abundances of bacterial taxa at the genus and subfamily level at both sites, lateral border of the tongue and oropharynx. However, when accounting for multiple testing utilizing the Benajmini Hochberg test, the false discovery rate (FDR) was ≤0.01 in bacterial taxa from the lateral border of the tongue only.

The study showed that four taxa had differentially relative abundances at the lateral border of the tongue of marijuana users compared to non-users. Three organisms were identified at the genera level *Rothia*, *Variovorax*, *Fusobacterium* and one at the family level, *Bradyrhizobiaceae* (FDR ≤0.1). Of these, *Rothia* (FDR<0.065*)*, *Variovorax*

(FDR<0.065), and *Bradyrhizobiaceae* (FDR<0.073) were found to have higher relative abundances in the marijuana user group, while *Fusobacterium* (FDR<0.065), was increased in the control group.

I. INTRODUCTION

Marijuana is the most commonly used recreational drug in the United States. In 2015, it was reported that 22.2 million U.S. individuals ≥12 years old had used marijuana in the past month. With a shift toward legalization of the drug, including 29 states, the District of Columbia, Guam and Puerto Rico, questions arise regarding the health implications of marijuana utilization (Center for Behavioral Health Statistics and Quality 2016) . Short term marijuana use has been associated with impaired motor coordination, altered judgement, psychosis and paranoia. Long term effects, especially early in adolescence, have demonstrated cognitive impairment and altered brain development (Kalant 2004). Additional effects on systemic health and disease continue to be evaluated. For example, marijuana smoke is said to have carcinogenic capabilities as it contains aromatic hydrocarbons, nitrosamines, and benzopyrene (Cho et al. 2005); however, the evidence of its association with lung cancer is largely inconclusive (Ribeiro and Ind 2016). In some histological studies, bronchial biopsies showed precancerous changes. However, several studies on the matter do not account for confounding factors such as tobacco smoking (Ribeiro and Ind 2016). Nonetheless, the overall risk of cancer associated with marijuana smoking is lower than that with tobacco smoking (Volkow et al. 2014). Furthermore, the cardiac effects of marijuana smoking include acute tachycardia and vasodilation, increasing oxygen demand. In relatively healthy

individuals, this may be regulated, but in those with underlying cardiomyopathies or comorbidities, cardiac ischemia may result (Cho et al. 2005).

Intraorally, frequent recreational cannabis (FRC) users, including marijuana and hashish, were associated with higher odds of severe periodontitis, including deeper probing depths and more clinical attachment loss. When excluding former and current tobacco users, inferior periodontal status was still twice as likely in FRC users versus non-users (Shariff et al. 2017). The term "cannabis stomatitis" has been used to describe the oral epithelial changes that occur with inhalation and chewing of cannabis. These changes include leukoedema of the buccal mucosa and hyperkeratosis, and with chronic use could include chronic gingival inflammation and leukoplakia (Cho et al. 2005). Finally, cannabinoids (i.e. tetrahydrocannabinol and cannabidol) are anti-inflammatory in nature due to their capacity to stimulate apoptosis, prevent cell proliferation, and diminish cytokine production (Volkow et al. 2014).

The association between microorganisms, health and disease, is being studied extensively, and advances in research and data analysis have brought about stronger evidence of such a relationship. The human microbiome is defined as microbial communities, including bacteria, viruses, and fungi, that inhabit the human body. The Human Microbiome Project (HMP) works to provide microbial community data as a reference for further metagenomics analysis (Human Microbiome Project Consortium 2012a). Using this database, organisms can be identified from the different

microhabitats, working toward eventually determining their role in health and disease. Studies through the HMP have identified a range of microorganisms that can likely be located in healthy individuals. Focusing on one type of microbe, bacteria, in for example the oral cavity, the following genera are the most common: *Streptococcus*, *Veillonella*, *Granulicatella*, *Gamella*, *Actinomyces*, *Corynebacterium*, *Rothia*, *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Capnocytophaga*, *Nisseria*, *Haemophilis*, *Treponema*, *Lactobacterium*, *Eikenella*, *Leptotrichia*, *Peptostreptococcus*, *Staphylococcus*, *Eubacteria*, and *Propionibacterium* (Zarco et al. 2012). Changes in microbe diversity at a body site could be indicative of disease. (Lloyd-Price et al. 2016). Changes in the microbiological environment can present a shift that allows microbes once considered favorable, or at worst innocuous, to become contributors to disease. These changes can include the relationship between the host and the microorganism, relative abundance increases, and procurement of virulence traits (Zarco et al. 2012). Usually the commensal microbiome has certain functions to maintain host homeostasis and health. When a shift in microbiome occurs, also known as dysbiosis, certain functions can be altered and a disease state can present (Lloyd-Price et al. 2016). Changes in the microbiome can lead to disease, or perhaps a disease is creating an environment for different bacteria to flourish that cause a shift in the host's ability to maintain homeostasis.

The gut is one of the better studied areas of the microbiome, including it's role in disease. Low diversity in the gut microbiome has been linked to obesity and

inflammatory bowel disease (Human Microbiome Project Consortium 2012b). A review of gut bacteria showed that mice with ulcerative colitis had less diverse colonic bacterial communities and that this decreased diversity is an indicator of acute inflammation. Furthermore, in individuals with active and inactive Crohn's Disease, their fecal microflora contained significantly greater enterobacteria versus healthy subjects (Zhang et al. 2015). The gut microbiome has been shown to play a role in overall systemic disease. Dysbiosis in the gut microbiome is associated with the presence or development of disease states such as metabolic syndromes, diabetes, alcoholic liver diseases, nonalcoholic fatty liver disease, and nonalcoholic steatohepatitis (Betrapally et al. 2016). The relationship between the gut microbiome and these diseases can be attributed to several processes. For example, the intestinal mucosal barrier (IB) ensures that intestinal contents such as microorganisms and pro-inflammatory molecules do not translocate to the rest of the body. The IB integrity is upheld by adequate intestinal microbiome (IM) homeostasis, and when there is gut dysbiosis, the IB is compromised. This can lead to increased permeability and subsequent bacterial translocation, as well as small intestinal bacteria overgrowth (SIBO), which have been shown to be present in liver conditions such as nonalcoholic fatty liver disease (Abdou et al. 2016).

The oral microbiome is capable of impacting disease locally in the oral cavity. Oral microbiome shifts are long known to be linked to dental caries, and play a role in

periodontal disease initiation and progression. Furthermore, researchers have noted taxonomic differences in samples collected from intraoral sites, saliva, and lesions in patients with oral squamous cell carcinoma (OSCC). When compared to healthy controls, OSCC lesions showed increased bacterial diversity and increased relative abundances of certain taxa at the phylum (*Spirochaetes, Fusobacteria, and Bacteroidetes)* and genus (*Fusobacterium, Treponema, Dialister, Catonella, Filifactor, Peptococcus, Parvimonas, Peptostreptococcus, Campylobacter, and Pseudomonas*) levels (Al-hebshi et al. 2017; Zhao et al. 2017). When evaluating salivary samples, the highest abundant genera in OSCC patients were *Veillonella, Neisseria, Streptococcus, and Prevotella* (Yang et al. 2018). The corroboration of this data in regards to specific taxa is still underway; however, it is apparent that the oral microbiome does appear to be distinct in the presence of disease, namely OSCC, though a causative role for bacteria in OSCC incidence or progression is unclear.

Disease states in different areas of the body are associated with the oral microbiome and oral diseases. For example, a 10 year follow up of a large population-based cohort associated periodontitis with cancer mortality, namely pancreatic cancer (Heikkila et al. 2018). Investigating this relationship further, studies have found that certain oral pathogenic microbes are associated with and/or represent markers of distant disease sites. Salivary samples of patients with precancerous lesions of gastric

cancer (PLGC) were less diverse than healthy controls, and possessed increased levels of *T. denticola* and *A. actinomycetemcomitans* (Sun et al. 2017). Furthermore, individuals with relatively higher abundances of oral *P. gingivalis and A. actinomycetemcomitans* were associated with an increased risk of pancreatic cancer (Fan et al. 2018). And finally, *P. gingivalis* and *T. denticola* have been associated with tumorigenesis of esophageal cancer (Gao et al. 2016; Narikiyo et al. 2004).

It is evident that the presence and/or dysbiosis of microorganisms play a role in disease states locally or at distant areas of the body. This begs the question, what factors are capable of influencing these changes? It is known that environment or lifestyle can alter the human microbiome, which in turn can play a role in disease development (Turnbaugh et al. 2007). The implications of diet, alcohol, and stress on the gut microbiome have been investigated. Diet has proved to be a driving force of microbiome changes in the gut. In an animal study, gut bacteria differed in mice fed with a Western-diet versus a low-fat-chow-diet. Mice fed a Western-diet, that of high fat and sucrose content, had increased relative abundance of *Bacteroidetes* and *Proteobacteria*, with a decrease in *Firmicutes* and *Lactobacilli*, specifically members of *the L. gasseri* species (Tachon et al. 2014). In evaluating short-term diets in human studies, consumption of an animal-based diet showed an increased abundance of *Alistipes, Bilophila, and Bacteroides,* known bile-tolerant organisms. This group also

showed a decreased abundance of *Firmicutes* (David et al. 2014). Reports have shown a cause and effect relationship between diet, microbiome, and disease. A plant-based diet was shown to increase *Bifidofacterium, Lactobacillus*, while decreasing *Bacteroides and Clostridum perfingens*. These changes increased short chain fatty acids (SCFA), which are anti-inflammatory and important for maintenance of the mucosal barrier, hence decreasing inflammation. An animal-based diet was shown to do the opposite, decreasing SCFA and increasing trimethylamine N-oxide (TMAO), a pro-atherogenic compound, therefore leading to cardiovascular disease and inflammatory bowel disease (Singh et al. 2017). Mice that were chronically fed ethanol resulted in increased abundance of gram negative *Proteobacteria* and gram positive *Actinobacteria*, and a decreased *Bacteriodetes* and *Firmicutes* (Lara Bull-Otterson et al. 2013). Stress on the host has shown to result in reduced number of *Lactobacilli* while increasing *E.coli* and *Pseudomonas* in the gut microbiome (Lutgendorff et al. 2008).

Tobacco smoking, already well-known for its implications on health can effect oral bacteria. Smoking increases the risk for heart disease, stroke, diabetes, tooth loss, pre-term births, and male infertility. Additionally, smoking can cause cancer in almost any part of the body, and increases the risk of dying due to cancer. Overall, smoking affects the body causing decreased immune function and increased inflammation (CDC Office on Smoking and Health 2017). The effect of smoking on the gut microbiome was

evaluated in subjects considered healthy versus those with Crohn's Disease. Smokers with Crohn's Disease possessed higher amounts of bacterial group *Bacteroides-Prevotella* versus non-smokers with Crohn's Disease. Additionally, healthy subjects who were smokers also exhibited increased *Bacteroides-Prevotella* compared to healthy non-smokers (Benjamin et al. 2012).The oral and nasopharyngeal microbiomes are also subject to changes due to external factors, such as smoking. The subgingival microbiome of periodontally healthy smokers was distinct from non-smokers, with increased pathogens such as *Fusobacterium nucleatum*, *F. naviforme*, *Filifactor alocis*, *Dialister microaerophilus*, *Acinetobacter species,* and *Pseudomonas,* with decreased health-compatible commensal organisms such as *Streptococcus sanguinis*, *Neisseria subflava* and *Hemophilus parainfluenzae* (Mason et al. 2015). Data obtained from mouthwash stimulated saliva samples revealed the oral microbiome of current smokers is distinct from that of former and never smokers. Phylum level increases of *Firmicutes* and *Actinobacteria* were observed, with decreases in *Proteobacteria.* Genera *Lactobacillus*, *Streptococcus* (*Bacilli*), *Veillonella*, and *Bifidobacterium* (*Actinobacteria*) were enriched, while *Neisseria*, *Porphyromonas* and *Capnocytophaga* were diminished. Species level analysis revealed increased anaerobic *Bifidobacterium longum, Atopobium spp., Actinomyces spp.,* and *Rothia mucilaginosa,* and depleted *Neisseria subflava* and *Corynebacterium* (Wu et al. 2016). In evaluating the mucosa surrounding healthy and diseased dental implants, differences were again

noted in the microbiomes of smokers versus non-smokers. The microbiome of smokers comprised elevated abundances of *Treponema*, *Prevotella*, *Propionibacterium*, *Pseudomonas*, *Lactobacillus*, *Propionibacterium* and *Rothia*. Of note, the latter three groups were seen exclusively in the smokers (Tsigarida et al. 2015). Finally, swab cultures of the nasopharynx of smokers revealed changes including a decrease of aerobic and anaerobic bacteria with pathogen-interfering capabilities, in addition to more frequent isolations of *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*. Interestingly, these organisms were previously shown to be more abundant in individuals prone to sinusitis and otitis media (Brook and Gober 2005).

As previously explained, microbiome alterations are observed in certain disease states, as well as with exogenous factors, such as tobacco use, implying an association between these entities. At this point, the overall evidence surrounding the effects of marijuana on systemic disease is suggestive but inconclusive. Marijuana's role as an exogenous factor for disease has also been investigated to an extent, but better understanding of this drug is needed. As discussed, the microbiome and its subsequent changes represent a promising component to the host health-disease axis and its contributors. The continued research on the microbiome has provided understanding of core microorganisms that live in symbiosis and provide functional roles for host homeostasis. Seeing as several factors can alter the microbiome and contribute to or induce disease, the question arises, can marijuana do the same?

Therefore, the aim of this study was to evaluate if marijuana use via inhalation is associated with differences in the oral microbiome compared to non-marijuana use. It was hypothesized that differences in relative abundances of microbiota will be seen in the mouths of marijuana users versus non-users.

II. MATERIALS AND METHODS

A. Subject Selection

Individuals who reported currently using marijuana were recruited from the University of Illinois at Chicago College of Dentistry. Subjects were asked to participate in the approved study (IRB/ACC protocol: 2012-1030), provided with an information sheet, and written consent was obtained (Form 1).

The following inclusion criteria were applied:

1. Systemically healthy
2. Daily or almost daily marijuana use in the past month (defined as using marijuana on 20 or more days in the past month) (Azofeifa et al. 2016)
3. Never smoker (tobacco)

The following exclusion criteria were applied:

1. Current medication use
2. Overt oral diseases or conditions
3. Current or former tobacco smokers

An attempt was made to recruit age and sex-matched controls. Subjects were asked to participate in the approved study, provided with an information sheet, and written consent was obtained (Form 1).

The following inclusion criteria were applied:

1. Systemically healthy, no medical conditions.

2. Never-user (tobacco nor marijuana)

Subject data including age, sex, frequency of use, was obtain. Subjects were assigned an identifying number.

Figure 1. Patient consent form and HIPAA waiver

9.1

UNIVERSITY OF ILLINOIS AT CHICAGO
Research Information and Consent for Participation in Biomedical Research

UIC Oral Cancer Community Clinic Outreach

You are being asked to participate in a research study. Researchers are required to provide a consent form such as this one to tell you about the research, to explain that taking part is voluntary, to describe the risks and benefits of participation, and to help you make an informed decision. You should feel free to ask the researchers any questions you may have.

PRINCIPAL INVESTIGATOR NAMES AND TITLES
Guy Adami, Associate Professor, Department of Oral Medicine and Diagnostic Sciences. College of Dentistry,
ADDRESS AND CONTACT INFORMATION
-801 S Paulina (MC 838), Chicago, IL 60612; Phone, 5-4311, Email: gadami@uic.edu

EMERGENCY CONTACT NAME AND INFORMATION
Name: Guy Adami
Contact Information: Phone: 6-6251 E-mail: **gadami@uic.edu**

SPONSORS
This study is funded by the Department of Oral Medicine & Diagnostic Sciences at the College of Dentistry at the University of Illinois at Chicago.

WHY AM I BEING ASKED

You are being asked to be a subject in a research study about oral cancer. In this study, we would like to find new ways to improve the early detection of changes in the mouth that may lead to cancer.

You have been asked to participate in the research because we and/or your referring physician or dentist have found a lesion in your mouth that may be cancerous or pre-cancerous.

Your participation in this research is voluntary. Your decision whether or not to participate will not affect your current or future dealings with the University of Illinois at Chicago. ***If you decide to participate, you are free to withdraw at any time without affecting that relationship.***

Approximately 180 subjects will be involved in this study at UIC.

WHAT IS THE PURPOSE OF THIS RESEARCH?

Oral cancer is believed to result mainly from exposure to damaging chemicals as found for example in cigarettes. Not everyone exposed to these damaging chemicals get cancer. Certain people are more likely than others to get oral cancer. This research is being done to better understand why certain people are more likely to get oral cancer than others. We believe what we inherit from our parents, the presence of certain bacteria, and a poor immune protection contribute to a higher risk of getting oral cancer.

Your genes may make you more or less likely to get cancer. Certain bacteria are suspected to be linked to cancer formation by producing cancer causing chemicals. Your immune system may not be as functional against the cancer as it should be. These three possibilities will be tested.

WHAT PROCEDURES ARE INVOLVED?

Tissue will be removed from you to obtain a diagnosis of disease, part of your treatment, or testing to determine the nature of the disease and how best to treat you. After the tests are done, a small amount of tissue is usually leftover. After the doctors have ensured your specimen is no longer needed for your care, the extra material is usually discarded or destroyed, or you may choose to donate the tissue for medical research.

Our research consists of the use of a soft brush to collect cells and bacteria from the surface of your mouth. Additionally you will be asked to fill out a questionnaire about your health. This questionnaire will ask you about your age; ancestry; length of time with sexual partner; your general and oral health; use of tobacco, marijuana, and betel nut containing products; use of alcohol products; and diet. We will also ask you to donate a teaspoon or so of blood for the analysis of your immune cells.

The visit will take approximately 45 minutes.

During your standard visit for care you will be asked to participate in a research study.

This research is being performed at the College of Dentistry, in either the Oral Medicine or Oral Maxillofacial Surgery clinics

The study procedures are:

A short questionnaire to gather information as stated above. This will permit us to understand better the reason you have a possible change in your mouth.

For research purposes, an image will be taken of the biopsy area using a special device that uses light in a way that is similar to how an ultrasound device uses sound waves to produce an image.

Several samples will be taken. These samples provide us with microorganisms and cells. This provides us the opportunity to identify these microorganisms. In addition, we obtain cells and these cells provide us hereditary material to observe the activity of various genes (material passed from your parents to you that determines your make-up) that may put you at risk for oral cancer. We will also determine based on your genes your race.

A soft bristle brush is passed on the surface of the lining of the throat, tongue, or other mouth site. This soft brush will capture bacteria and yeast sitting on the surface. This brush will also capture cells forming the lining of the throat, tongue or other mouth surfaces. After obtaining bacteria or yeast on the bristles of the brush we place the brush into a tube and shake the brush a few times. The tube will now contain

these microorganisms. Another sample using the brush to capture cells that form the lining are placed in another tube with a different solution. The purposes for these harvests are given above.

You should experience no pain, bleeding or discomfort from this capture.

We want to obtain information about your immune system. To obtain the cells that can be studied and provide this information we will ask you to provide some blood.

To obtain blood from your arm we will use a sterile standard blood test kit. This will require a quick wipe with an alcohol cotton cloth square and then a blood test. The blood is collected using a tube with a colored top (purple or lavender). This tube contains chemicals to stop the blood from clotting and permits us to obtain the white blood cells.

GENETIC TESTING

In one study, we will obtain cells from tongue and throat for genetic information. This information will tell us about the ability of your cells to produce cancer causing chemicals. This information is also important because we will compare presence of these genes (material passed from your parents to you that determines your make-up) to other genes that identify your race. This knowledge will help us to improve our understanding of genes and association to cancers of the throat and mouth and the role genes may play in health and disease.

-I agree to allow genetic testing to be performed on my BLOOD OR TISSUE sample for the current present research study. **Initials**: _____

-I agree to allow my BLOOD OR TISSUE to be kept by Dr. Guy Adami and his colleagues at the College of Dentistry, for use for future genetic research to learn more about how to prevent, detect, or treat cancers of the throat and mouth. **Initials**: _____

-I agree to allow my BLOOD OR TISSUE to be kept by Dr. Adami and his colleagues at the College of Dentistry, for use by other researchers for future genetic research to learn more about how to prevent, detect, or treat other health problems. **Initials**: _____

-I agree to allow the researchers to contact me about future genetic research. **Initials**: _____

TISSUE BANKING

We will bank samples of microbes, oral cells and pieces of suspected oral cancer tissue. Blood that you have provided will also be frozen.

Some tissue samples are large enough for freezing. Tissues coded but de- identified that you have provided tissues are collected for future investigation. These tissues could include normal, pre-cancer and cancer tissues. Tissues will be placed into a freezer located in the College of Dentistry. This freezer is a locked room that requires a scanned ID to enter.

WHAT ARE THE POTENTIAL RISKS AND DISCOMFORTS?

-The likely risks and discomforts expected in this study are minimal. Use of an oral brush can cause a break in the lining of the throat or mouth and produce small bleeding. If this occurs we will stop immediately and control the bleeding with pressure with sterile cotton gauze.

You may experience a faint feeling, some swelling from the blood draw, for this reason we ask you elevate your arm after drawing blood. To prevent infection we will apply a bandaid which you will keep in place the rest of the day. You may notice a black and blue area from bruising at the site of drawing of the blood but this should gradually decline in color and disappear within a week. We ask you to phone Dr. Guy Adami (312-966-6251) if you experience any pain or swelling in the next few hours.

You may experience some stress or discomfort while filling up the medical questionnaire. Be assured that Dr. Schwartz and his colleagues will make the outmost effort to keep your information private.

Imaging of your tissue is only for research purposes, and is not expected to not cause you any harm, or directly benefit you.

There is a risk that someone could get access to the genetic information we have stored about you. Genetic testing can create information about a person and their family's personal health risks and can cause or increase anxiety, and /or interface with your ability to get insurance or a job, and can even lead to discrimination. Patterns of genetic variation also can be used by law enforcement agencies to identify a person or his/her blood relatives. There are laws against this kind of misuse, but they may not give full protection. There may be other unforeseen privacy risks. We believe the chance these things will happen is very small, but we cannot make guarantees. Your privacy and the confidentiality of your data are very important to use and we will make every effort to protect them.

There is a new Federal law called the Genetic Information Nondiscrimination Act (GINA). In general, this law makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. However, it does not protect you against discrimination by companies that sell life insurance, disability insurance, or long-term care insurance. GINA also does not protect you against discrimination if you have already been diagnosed with the genetic disease being tested.

WILL I BE TOLD ABOUT NEW INFORMATION THAT MAY AFFECT MY DECISION TO PARTICIPATE?
During the course of the study, you will not be informed of any new findings (either good or bad), but you will informed of changes in the risks or benefits resulting from participation in the research or new alternatives to participations, that might cause you to change your mind about continuing in the study. If new information is provided to you, your consent to continue participation in this study may be re-obtained.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

You will be compensated a sum of $20 for participating in this study. It is hoped that the knowledge gained from this research may benefit others. We hope to produce images of tissues changes through a scan that will show disease or normal tissue. We also hope to identify a new relationship between genes, microorganism, immune activity and risk for cancer of the mouth.

WHAT OTHER OPTIONS ARE THERE?

You do not have to be in this study to be assessed or treated for oral cancer.

This research does not involve treatment for oral cancer. If a diagnosis of cancer is made your doctors will help you to plan for further treatment outside of this research.

WHAT ABOUT MY PRIVACY AND CONFIDENTIALITY?

The only people who will know you are a research subject are members of the research team, and if appropriate, your clinicians and nurses. No information about you, or information provided by you during the research, will be disclosed to others without your written permission, except if necessary to protect your rights or welfare (for example, if you are injured and need emergency care, or when the UIC Office for the Protection of Research Subjects monitors the research or consent process) or if required by law.

Study information which identifies you and the consent form signed by you will be placed into a password coded file and not copied except for examining the research by the UIC Office for the Protection of Research Subjects the UIC Institutional Review Board or State of Illinois Auditors, as required by law.

A possible risk of the research is that your participation in the research or information about you and your health might become known to individuals outside the research.

Tissues collected from you for research purposes will also be coded and stripped of identifiers to protect your confidentiality.

Each individual investigator will provide a code for each subject and sample. Each code is kept in a locked file drawer. The records and codes for tissues samples are placed into a password protected encrypted file on a secure server which will be monitored by the Principal Investigator. When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity.

WHAT ARE THE COSTS FOR PARTICIPATING IN THIS RESEARCH?

There are no costs to you for participating in this research, as stated above on rare occasions a cancer is detected. This requires immediate care and treatment; but treatment costs will be discussed with you outside of your participation in this research.

WILL I BE REIMBURSED FOR ANY OF MY EXPENSES OR PAID FOR MY PARTICIPATION IN THIS STUDY?
You will not be offered payment for participating in this study.

WILL MY CELLS, TISSUES, BLOOD, OR OTHER BIOLOGICAL MATERIALS BE USED TO DEVELOP COMMERCIAL PRODUCTS?
If a commercial product is developed from the tissue or blood samples collected as part of this research project, the commercial product will be owned by the University of Illinois at Chicago and the investigators. You will not profit financially from such a product.

CAN I WITHDRAW OR BE REMOVED FROM THE STUDY?

If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without affecting your future care at UIC.

You have the right to leave a study at any time without penalty. For your safety, however, you should consider the investigator's advice about how to leave the study. If you leave the study before the final planned study visit, the investigator may ask you to complete the final steps.

The researchers and sponsor also have the right to stop your participation in this study without your consent if they believe it is in your best interest, or if you do not follow the study procedures or if new information is identified.

WHO SHOULD I CONTACT IF I HAVE QUESTIONS?

Contact the researchers below if you have any questions about this study or your part in it, or if you have questions, concerns or complaints about the research.
Guy Adami, 312-966-6251, gadami@uic.edu

WHAT ARE MY RIGHTS AS A RESEARCH SUBJECT?

If you feel you have not been treated according to the descriptions in this form, or if you have any questions about your rights as a research subject, including questions, concerns, complaints, or to offer input, you may call the Office for the Protection of Research Subjects (OPRS) at 312-996-1711 or 1-866-789-6215 (toll-free) or e-mail OPRS at uicirb@uic.edu.

WHAT IF I AM A UIC EMPLOYEE?

Your participation in this research is in no way a part of your university duties, and your refusal to participate will not in anyway effect your employment with the university, or the benefits, privileges, or opportunities associated with your employment at UIC. You will not be offered or receive any special consideration if you participate in this research.

REMEMBER:

Your participation in this research is voluntary and anonymous. Your decision whether or not to participate will not affect your current or future relations with the University. If you decide to participate, you are free to withdraw at any time without affecting that relationship.

_____ _____

Signature Date

_____

Printed Name

_____ _____

Signature of Person Obtaining Consent Date (must be same as subject's)

_____

Printed Name of Person Obtaining Consent

University of Illinois at Chicago
Authorization to Use and Disclose (Release) Health Information For a Research Study

"UIC Oral Cancer Community Clinic Outreach"

State and Federal laws, including the Health Insurance Portability and Accountability Act (HIPAA), require researchers to protect your health information. This form describes how researchers, with your authorization (permission), may use and release (share) your protected health information in this research study. **Please read this form carefully.**

You have been asked to take part in a research study. The study has already been described to you in a separate consent form. By signing this form you are permitting [Dr. Schwartz and members of his research team] to create, get, use, store, and share protected health information that identifies you for the purposes of this research study.

Description of protected health information that may be used and released (disclosed or shared)

The health information includes all information created and/or collected during the research as described in the 'Consent for Participation in Research' entitled "*UIC Oral Cancer Community Clinic Outreach"*. Protected health information may include results of tests, procedures or surveys that are part of the research. Health information in your medical record may be used and released if it is needed for the research; for example, past medical conditions or medications or information related to illness or hospitalizations that occur during your participation in the research.

The health information includes: [name, address telephone number, medical record number, date of birth, demographic information (e.g., race, gender), the results of physical exams, blood tests (e.g., human papilloma virus (HPV) and human immunodeficiency virus (HIV) status), other tests (e.g., biopsy results), x-rays, as well as your medical history, use of tobacco, marijuana, betel nut, and alcohol products, and number of significant partners].

Research use of your protected health information:

During the conduct of the research, the researchers may use or share your health information: With each other, with other researchers involved with the study,and with law enforcement or other agencies, when required by law.

Protection of your health information

The researchers **Cancer Center of UIC** agree to protect your health information and will only share this information as described in this Authorization and the research consent form.

B. Sample Collection

Bacterial samples were collected utilizing a sterile swab from four intraoral sites (buccal mucosa, lateral border of the tongue, oropharynx, and keratinized gingiva). Samples were placed into sterile tubes containing TrisEDTA (TE) buffer solution (10mmol Tris-HCl and 1mmol EDTA) with a pH of 8.0.

All samples were immediately placed in a -80°C freezer until DNA extraction could be completed. Bacterial sample tubes included the subject's identifying number and date of sample collection. Additionally, a corresponding letter for each oral site was also labeled on the tube (A: buccal mucosa; B: lateral border of the tongue; C: oropharynx; D: keratinized gingiva).

C. Bacterial DNA Extraction

DNA extraction was completed using the Quick-DNA™ Fungal/Bacterial Miniprep Kit according to the manufacturer's instructions (Zymo Research, Inc.). Briefly, bacterial samples are lysed utilizing BashingBeads™. Then, centrifugation with "Zymo-Spin™" technology allows genomic DNA isolation of DNA on a silica filters, which is washed and then eluted as pure DNA.

D. DNA Amplification and Characterization

Relative abundances of bacterial species across test and control samples were determined via a two-stage Polymerase Chain Reaction (PCR) or "Targeted Amplicon Sequencing" (TAS) approach. In the first stage of PCR, the primers 27F and 534R, with additional common sequences CS1 and CS2, were utilized to polymerize and amplify the V1-V3 variable regions of bacterial 16S ribosomal RNA gene (rRNA). In 10 µl reaction volumes using the KAPA HiFi HotStart PCR Kit, the PCR was carried out as follows: 5 min initial denaturation at 95°C, then 28 cycles of: 95°C for 30", 50°C for 30", 72°C for 60 Bio-rad iCycler." After synthesis, a 2 µl aliqout containing 20% glycerol was loaded on to a 2% Agarose Tris Acetate gel for electrophoresis. This was followed by ethidium bromide staining for DNA visualization to allow quality control of the PCR product.

PCR amplicons were then sent to the DNA Services Facility at the University of Illinois at Chicago for the second stage PCR procedure, where primers are added to label DNA from each sample with a distinct DNA barcode. Sequencing was performed at the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign (UIUC). Briefly, new primers were utilized that hybridized to the CS linker sequences, and containing linker sequences, Illumina adapters, and a sample-specific barcode. PCR was again carried out for approximately 8 cycles under the conditions mentioned above after removal of nonspecific DNAs, DNA sequencing was

carried out on an Illumina MiSeq sequencer using a standard V3 chemistry with pair-end, 300 base reads. Fluidigm sequencing primers, targeting the CS1 and CS2 linker regions, were used to initiate sequencing. Demultiplexing of reads was performed on instrument.

E. Bioinformatics Analysis

Binning of similar 16s rRNA sequences into operational taxonomic units (OTU) was completed utilizing raw paired-end FASTQ files using QIIME2.0 as described previously (Caporaso et al. 2010). In this case, reverse (V3) reads were utilized and sequences were trimmed if the average quality was lower than 20. Taxonomy assignment was completed by BLAST against the Human Oral Microbiome Database (HOMD), with 98% match identity. (Dewhirst et al. 2010). Each OTU with 2 or fewer sequence reads for a sample was omitted and OTUs present in fewer than 10% of samples were omitted A biological observation matrix (BIOM) was generated at taxonomic levels from phylum to genera.

F. Statistical Analysis

BIOMs were used for analysis of similarity (ANOSIM) tests to determine if microbial communities were significantly different between groups, and to identify taxa which were significantly differentially abundant between the test and control groups. Differences in abundance between the groups were tested using DEseq2. Briefly, DESeq2 models the raw counts, normalizing the data while taking into account

differences in sequencing depth. It then fits the data to a negative binomial model and performs the Wald or Likelihood Ratio Test to estimate the significance of differences. False discovery rate–corrected *P* values were estimated using the method of Benjamini Hochberg for all taxa comparisons via DeSeq2 software. Significance was set at $p<0.05$, or FDR < 0.10, as applicable.

III. RESULTS

A. Study Subjects

27 total subjects participated in this study, 11 marijuana users and 16 control subjects. The marijuana group (test) consisted of two females and nine males, ages 18-49 years old (mean: 28 years, median: 25 years) (**Table I**).

Table I. Subject data for marijuana users (MJ).

MARIJUANA USERS (MJ) SUBJECTS		
Age	**Gender**	**Frequency of Use**
49	F	Daily
42	M	Daily
29	M	4 days/week
29	M	4+ days/week
26	M	4 days/week
25	M	Daily
25	M	Daily
25	M	Daily
23	M	Daily
18	M	Daily
18	F	Daily

The non-users (control group) were age and sex matched, and consisted of two females and 14 males, ages 18-49 years old (mean: 26.06 years, median: 24.5 years) (**Table II**).

Table II. Subject data for non- users (control).

CONTROL SUBJECTS	
Age	**Gender**
49	F
35	M
32	M
27	M
27	M
27	M
26	M
25	M
24	M
22	M
22	M
22	M
21	M
20	M
20	F
18	M

A Mann Whitney test for unpaired data was performed to evaluate significant difference between the two groups in terms of mean age. The result was not statistically significant at $p<0.05$ (p-value = 0.585).

B. Alpha and Beta Diversity of Microbiome Composition

Samples from the lateral border of the tongue (site B) and the oropharynx (site C) were processed and sequenced as described in the materials and methods section. For the lateral tongue samples, the average DNA sequence read count was 37,109 in each sample, with a range of 32,013 to 40,508. For the oral pharyngeal samples, the average read count was 37,722, with a range of 8106 to 42,783.

Beta diversity, the amount of variation in taxonomic composition amongst the groups, was evaluated using non-metric multidimensional scaling (NMDS). This can be quantified using Analysis of Similarities (ANOSIM), which determines the level of differences between two groups. The beta diversity of the samples from the lateral border of the tongue between the marijuana (MJ) and control groups showed no significant difference, with relatively similar populations ($R=0.49$ at $P< 0.189$). The beta diversity of the samples from the oropharynx between the marijuana and control groups showed a small difference, with borderline statistical significance ($R=0.10$ at $P<0.055$). The lateral border of the tongue was represented by 69 different operational taxonomic units on the genus level and higher (OTU). **Figure 2** represents the relative abundance of the top 20 OTUs in this site, where the five most abundant genera were *Streptococcus* (32%), *Haemophilus*(11%), *Rothia* (10%), *Gemella* (8%), and *Veillonella* (6%). The relative

abundance of the top 20 OTUs are further shown amongst the individual groups, marijuana and control (**Figure 3**).

Figure 2. Site B: Taxonomic profiles at the genus level, relative abundance (all samples). The twenty most abundant genera/subfamilies are shown.

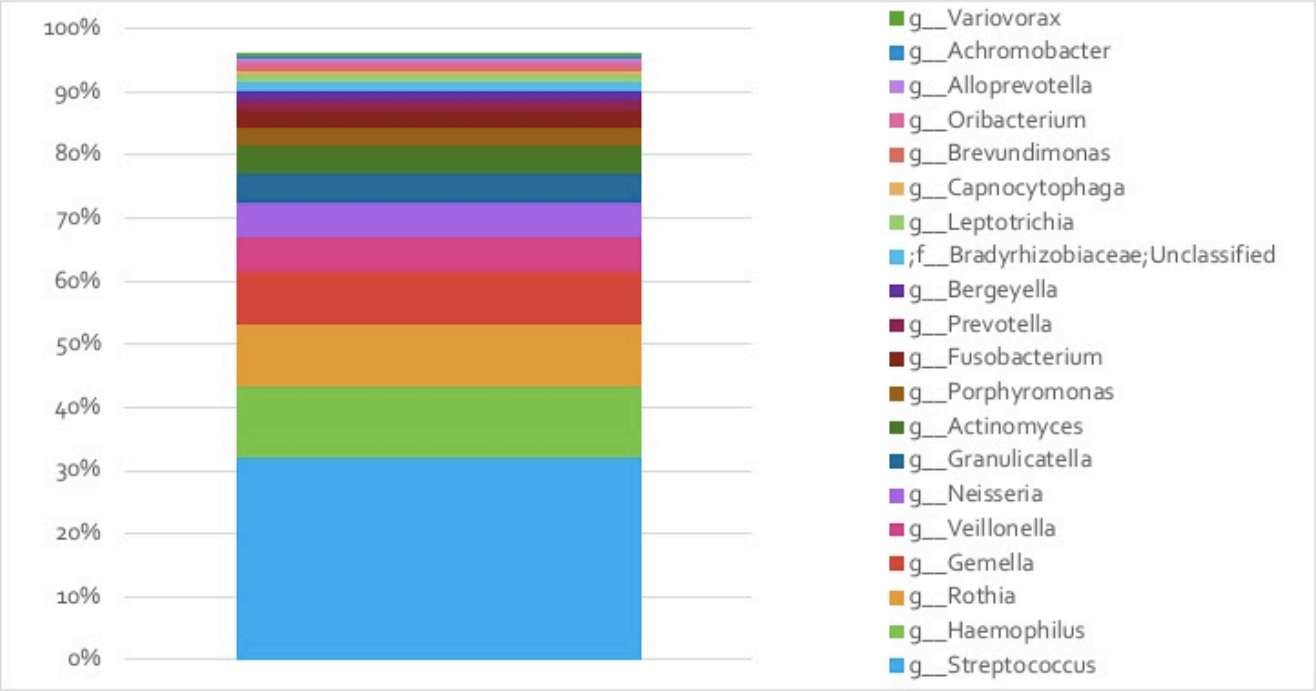


Figure 3. Site B: taxonomic profiles at the genus level, relative abundance (Marijuana vs. Control). Twenty most abundant genera and subfamilies.

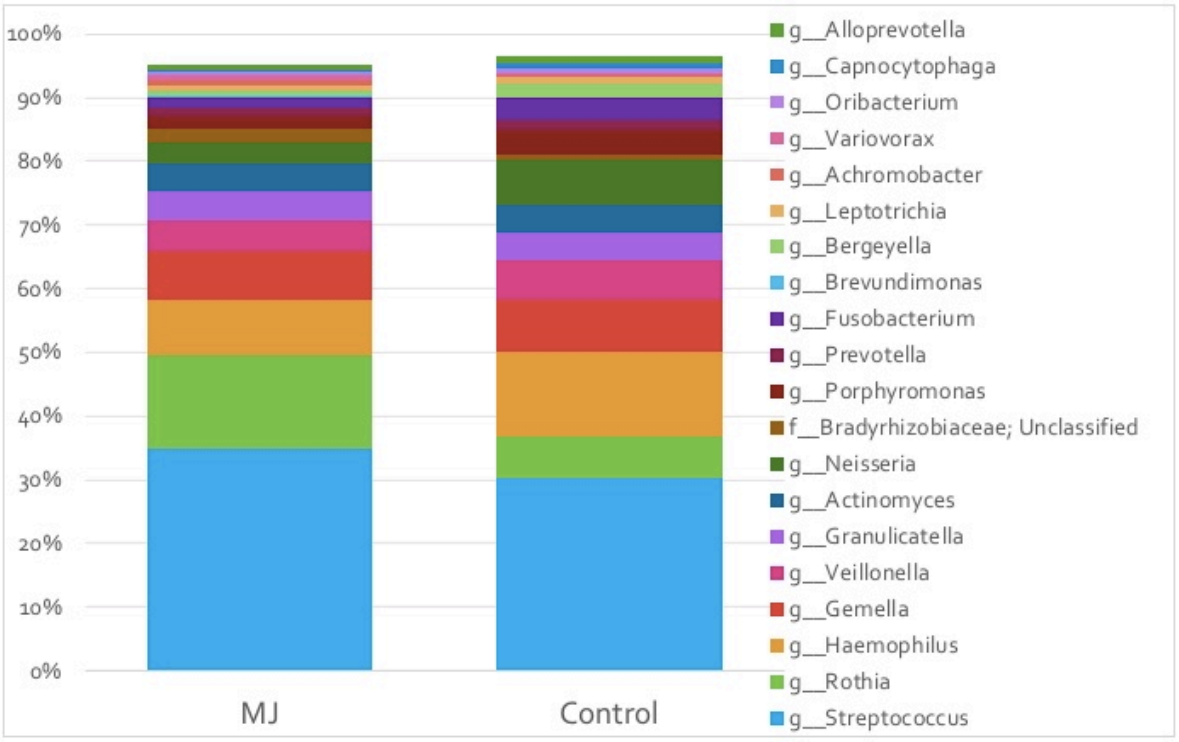


Figure 4 shows a side by side comparison of the relative abundances of each of the top 20 OTUs amongst the marijuana and control groups. False discovery rate (FDR) was adjusted for multiple testing via the Benjamini Hochberg procedure, and four OTU's were identified as having significant differences in their relative abundance in the marijuana group compared to the control group (**Figure 5**).

Figure 4. Site B: taxonomic profiles, relative abundance side-by-side comparison (Marijuana/Control) for the 20 most abundant taxa on the genus/subfamily level.

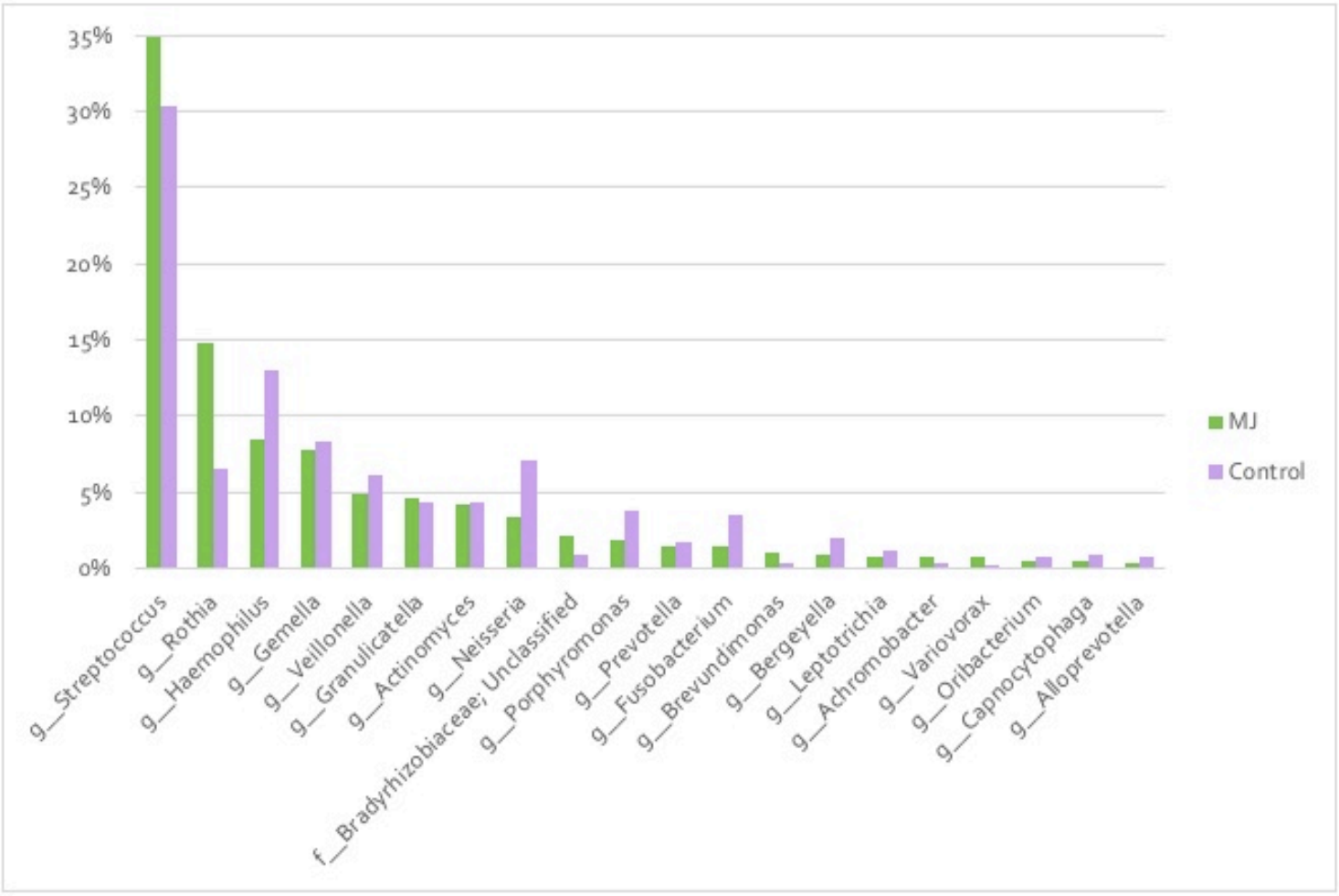
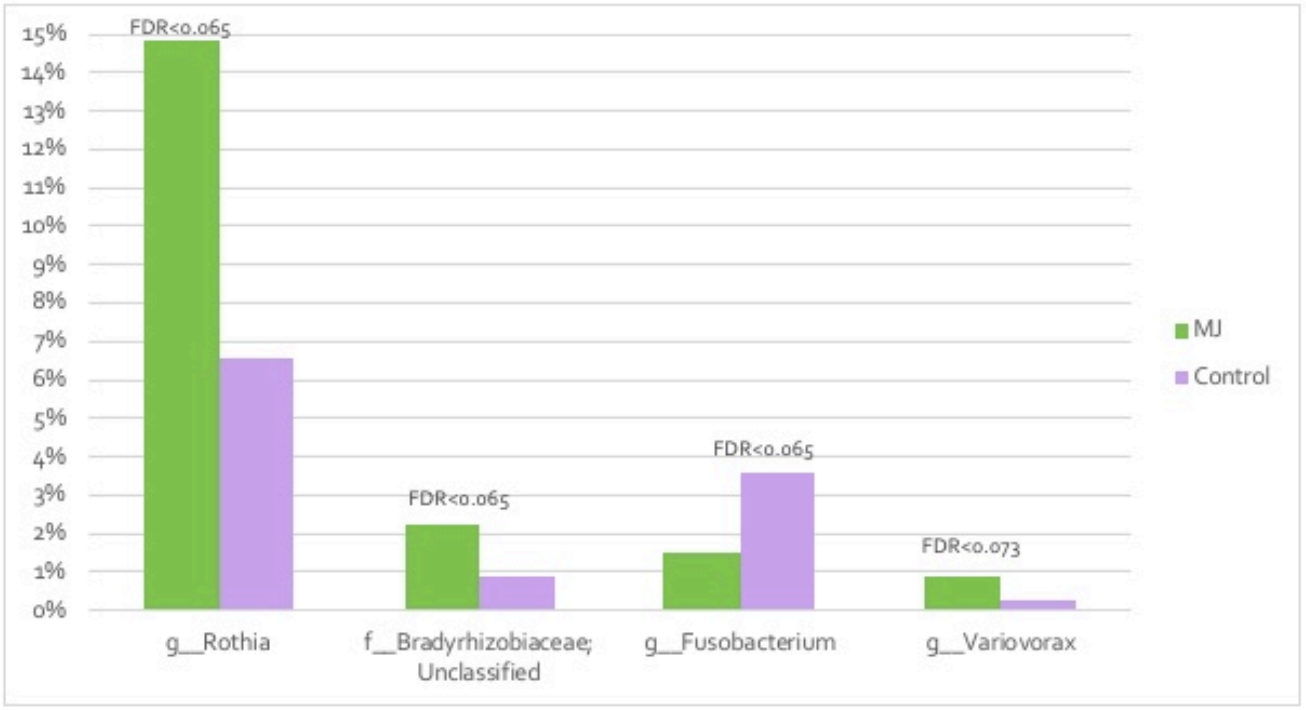


Figure 5. Site B: abundance levels for 4 genera/subfamilies differentially abundant in the two groups based on FDR < 0.10.



Three organisms were identified at the genera level *Rothia*, *Variovorax*, *Fusobacterium* and one at the family level, *Bradyrhizobiaceae* (FDR ≤0.1). Of these, *Rothia* (FDR<0.065), *Variovorax* (FDR<0.065), and *Bradyrhizobiaceae* (FDR<0.073) were found to have higher relative abundances in the marijuana user group, while *Fusobacterium* (FDR<0.065), was increased in the control group.

The oropharynx (site C) amongst both groups was represented by 77 different operational taxonomic units (OTU) at the level of genus and higher. **Figure 6** represents the relative abundance of the top 20 OTUs in this site, where the five most abundant genera were *Streptococcus* (38%), *Actinomyces* (10%), *Prevotella* (8%), *Veillonella* (5%), and *Gemella* (5%).The relative abundance of the top 20 OTUs are further shown amongst the individual groups, marijuana and control (**Figure 7**).

Figure 6. Site C: taxonomic profiles at the genus level, relative abundance all oropharyngeal samples with the top 20 shown.

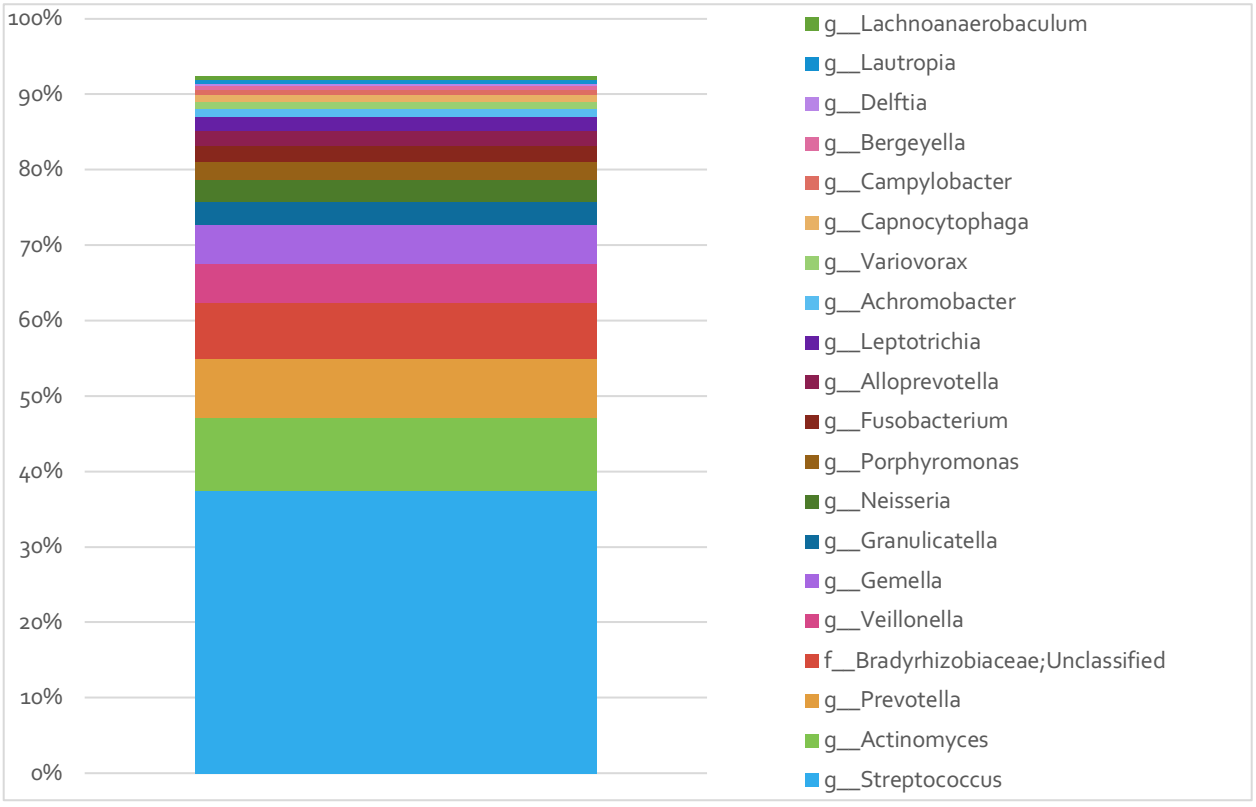


Figure 7. Site C: taxonomic profiles, relative abundance top 20 genera and subfamilies (Marijuana/Control)

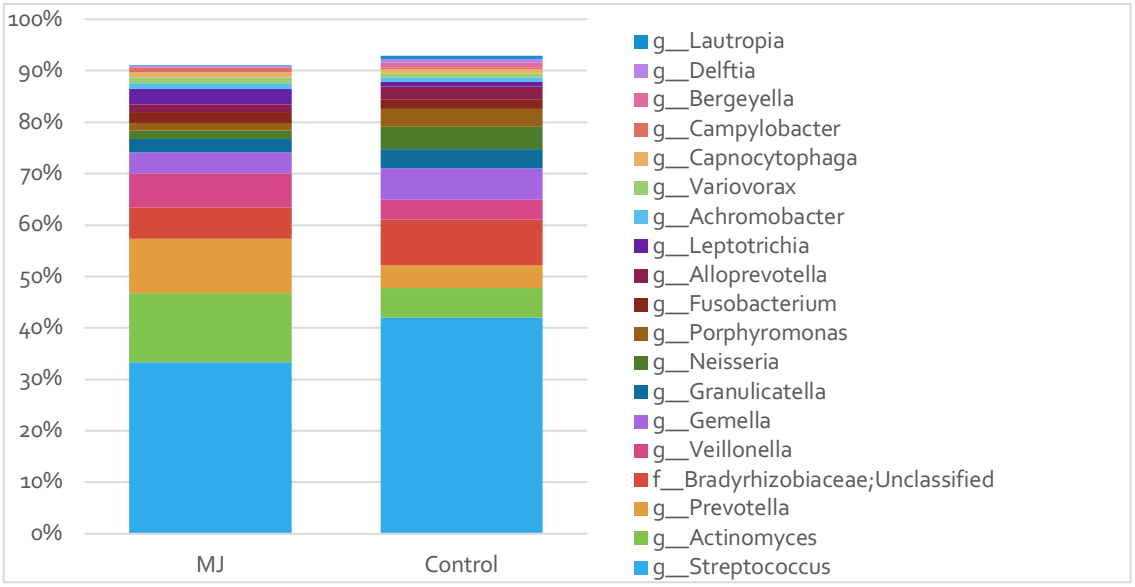


Figure 8 shows a side by side comparison of the relative abundances of each of the top 20 OTUs amongst the marijuana and control groups; however, after taking into account statistical effects of multiple testing by determination of FDR, no significant differences were noted in the relative abundances of organisms between the marijuana users and control groups at this site (**Figure 9**).

Figure 8. Site C: taxonomic profiles, relative abundance side-by-side comparison- top 20 genera subfamilies (Marijuana/Control)

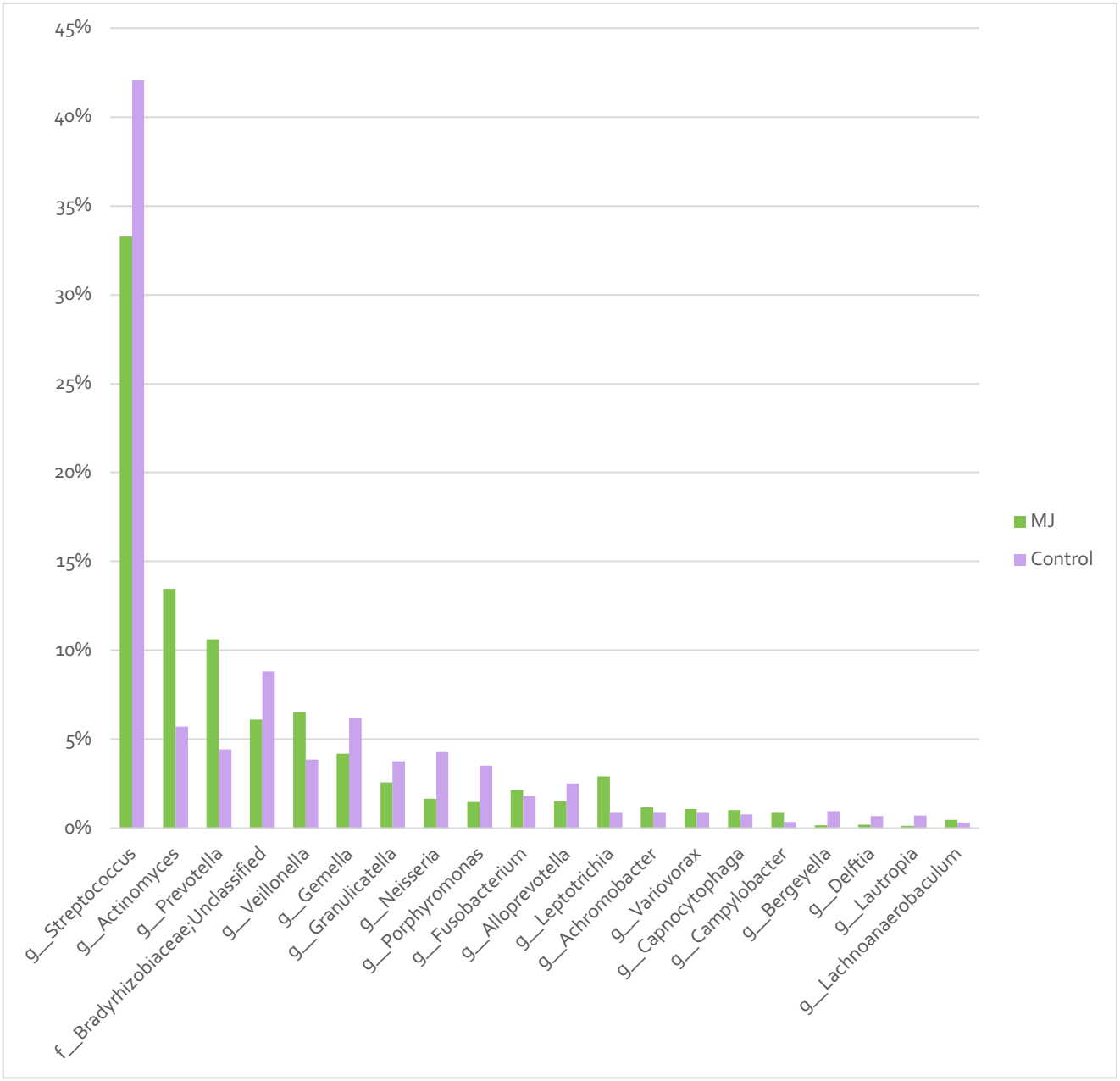
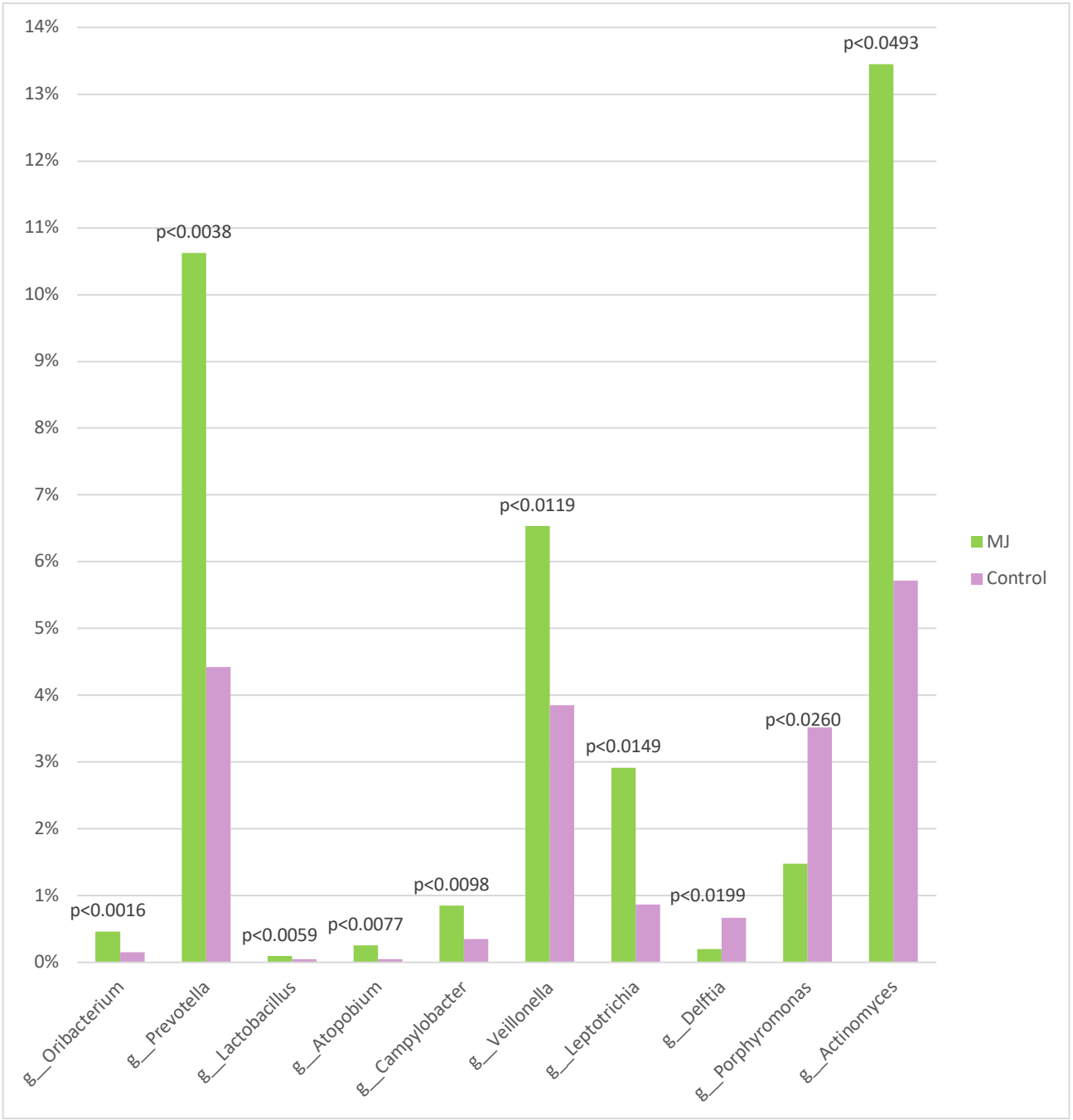


Figure 9. Site C: Shown are abundance levels for 10 genera differentially abundant in the two groups based on P<0.05



Linear discriminative analysis (LDA) effect size (LEfSe) was utilized to reveal which taxa were driving divergence between the two groups at site B (**Figure 10**) (Segata et

al. 2011). The green labeled genera (BM) represent those more abundant in the marijuana group, and the red (BC) represent the genera that are more abundant in the control group. In order to evaluate the evolutionary closeness of these bacteria, a cladogram is depicted (**Figure 11**). This takes into account the similarities that should exist amongst bacteria of the same family. Ideally, the driving taxa should be similar and related in their phylogenic taxonomy.

Figure 10. The LEfSe reveals differentially abundant taxa found on the tongue in marijuana users versus controls.

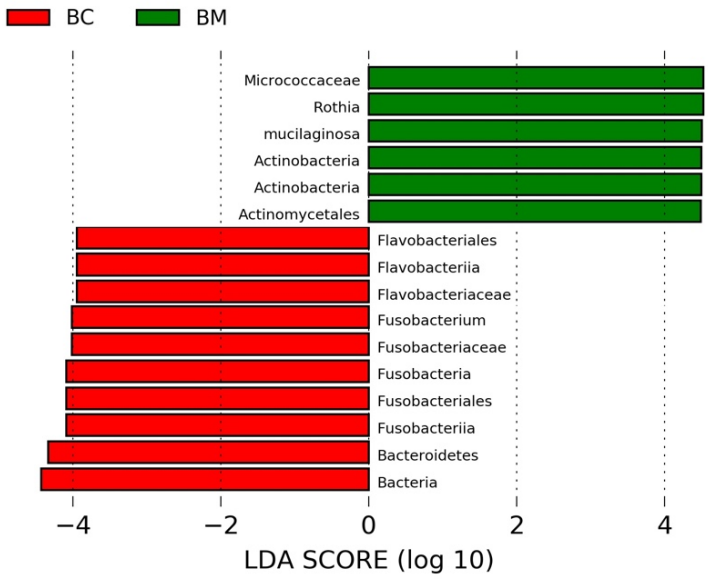
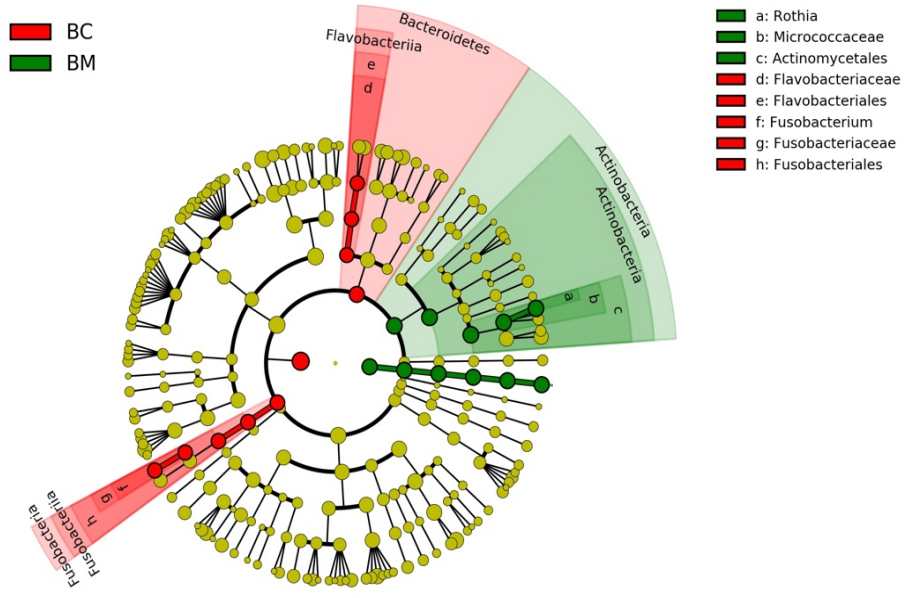


Figure 11. Cladogram. Highlights phylogenetic relationship of taxa that differ in abundance between the two groups



IV. DISCUSSION

Several resources report the diseases that are tentatively associated with marijuana use, mainly on the basis of epidemiological studies. As discussed earlier, associations have been made between marijuana and impaired cognitive function, tachycardia and the potential for cardiac injury, lung inflammation, chronic bronchitis, and periodontal disease (Volkow et al. 2014; Shariff et al. 2017; Cho et al. 2005). Marijuana, or cannabis, refers to the dried leaves, flowers, stems, and seeds from the hemp plant *Cannabis sativa*. The main psychoactive chemical in marijuana is Δ-9-tetrahydrocannabinol (THC). THC closely resembles the chemical in the brain known as anandamide, allowing THC to alter normal brain communication. The plant also contains over five hundred other chemicals, including over one hundred compounds that are chemically related to THC, called cannabinoids (Arteaga 2017; NIDA 2018). While studies have taken place over the years in regard to the effect of cannabinoids, much less is known about how exposure to the many other chemicals found in the marijuana plant may affect health status. Epidemiological studies mainly focus on an association and do not reveal a causative role for marijuana in disease.

The results of this study suggest that daily or almost daily inhalation of marijuana in the past month correlates with differentially abundant taxa of the oral microbiome, in samples taken from the lateral border of the tongue. Four OTU's were identified as having significant differences in their relative abundance between the marijuana users

and non-users (FDR ≤0.1). These included the genera *Rothia*, *Variovorax*, *Fusobacterium* and the family *Bradyrhizobiaceae*. The *Fusobacterium* genera showed decreased relative abundance in the marijuana group, where the others were increased. According to the HOMD, at this time *Variovorax* has a single species in the oral microbiome, and therefore our genera likely corresponds to that species, *Variovorax Paradoxus*. *V. Paradoxus* is a nutritionally diverse organism, commonly isolated from soil. Strains of this organism are capable of degrading several compounds, including explosives and pesticides. It is also an organism that promotes plant growth and increases its resistance to disease and heavy metals. Furthermore, it has the ability to interfere with the communication of other bacteria and can closely interact with other biota, like plants, in numerous ecosystems. Finally, *V. Paradoxus* was recently identified in the human oral cavity as a member of methylotrophic community, which is an organism that can utilize one-carbon compounds, such as methane, as their carbon source. (Han et al. 2011) .

Overall, these bacterial changes noted in the mouths of marijuana users may cause disease, change how marijuana affects the body, or perhaps do nothing in regard to health.

Our study begins to look at changes in the body that may act as indicators of health risks. The term dysbiosis is used to refer to negative changes in human microbiome from the typical microbiome in those without disease. We have seen diet induce gut

dysbiosis, changing the predominant organisms of the gut microbiome, and in turn altering gene expression, which can promote health or disease (Singh et al. 2017) . Furthermore, smoking has been clearly identified as a risk factor for cancers of the head and neck and periodontal disease (CDC Office on Smoking and Health 2017), while also causing changes at the level of the microbiome, which may contribute to the development and/or exacerbation of disease (Wu et al. 2016). In the oral cavity, salivary oral microbiome changes were linked to oral squamous cell carcinoma and pancreatic cancer (Zhao et al. 2017; Fan et al. 2018).

Could the oral microbiome changes seen in marijuana users be linked to any of the disease conditions associated with marijuana usage? There has been research that found a 2.6 times more likely association of primary squamous cell carcinoma of the head and neck in marijuana users, once adjusting for cigarette smoking, alcohol use, and other risk factors (Z. F. Zhang et al. 1999). However, this finding has not been consistent amongst all studies (Arteaga 2017). Nonetheless, the process by which marijuana could act as a carcinogen or direct inducer of disease is still to be investigated. Largely, it is difficult to say what the exact mechanism of the relationship is, as the observations linking the presence of certain bacteria in the gut or oral cavity and distal disease are still being formed as well. With a few notable exceptions, such as the direct role of *H. Pylori* in gastric cancer, it unclear how these bacteria would cause disease (Y. Zhang et al. 2015). They could do so by perturbing the immune system, inducing inflammation,

changing metabolism of nutrients in the gastrointestinal tract and mouth, or by directly making pathogenic molecules. These studies that aim to pinpoint marijuana effects will demand analysis of additional changes in the oral cavity that occur with the change in bacteria.

While this study serves as a pilot, suggesting that exposure to airborne marijuana daily is sufficient to change oral bacteria, of course a prospective study is needed to verify a cause and effect relationship. This may be done with medical marijuana users who are newly introduced to usage of the drug. In addition, we need to study more subjects in order to increase the statistical power of the study so that we can establish on the species level which bacteria have been altered in abundance with marijuana usage. By identifying marijuana associated bacteria on the species level, we will better be able to determine what changes may occur. In this study, we chose two sites to analyze, oral pharynx and the lateral border of the tongue, based on their propensity for oral disease and oral pharyngeal cancer (The American Cancer Society). The other sites in which samples were obtained, such as the keratinized gingiva, were not analyzed at this time due to their intra-subject variability to bacterial identification thought due to variable hygiene between subjects.. A study with patients that are instructed regarding uniform oral hygiene might be necessary to evaluate the effect of marijuana on the oral periodontium.

Therefore, similar to the relationship observed between diet, smoking, microbiome changes, and disease states, the same model may prove beneficial in understanding the role marijuana plays in this continuum. The data in this study infers that microbiome changes can be seen with marijuana use. Further research should include a larger sample size to validate and expand findings, determining taxa at the species level to better understand their role in the potential relationship between marijuana and disease.

CITED LITERATURE

Abdou, Reham M., Lixin Zhu, Robert D. Baker, and Susan S. Baker. 2016. "The Gut Microbiota and Nonalcoholic Fatty Liver Disease." *Digestive Diseases and Sciences* 61 (5) (/05/01): 1268-1281.

Al-hebshi, Nezar Noor, Akram Thabet Nasher, Mohamed Yousef Maryoud, Husham E. Homeida, Tsute Chen, Ali Mohamed Idris, and Newell Johnson. 2017. "Inflammatory Bacteriome Featuring Fusobacterium Nucleatum and Pseudomonas Aeruginosa Identified in Association with Oral Squamous Cell Carcinoma." *Scientific Reports* 7 (1) (May): 1834. doi:10.1038/s41598-017-02079-3.

Arteaga, Sarita. 2017. "Marijuana use and Oral Health | Continuing Education Course."

Azofeifa, Alejandro, Margaret E. Mattson, Gillian Schauer, Tim McAfee, Althea Grant, and Rob Lyerla. 2016. "National Estimates of Marijuana use and Related Indicators - National Survey on Drug use and Health, United States, 2002-2014." *Morbidity and Mortality Weekly Report. Surveillance Summaries (Washington, D.C. : 2002)* 65 (11): 1-28. doi:10.15585/mmwr.ss6511a1.

Benjamin, Jane L., Charlotte R. H. Hedin, Andreas Koutsoumpas, Siew C. Ng, Neil E. McCarthy, Natalie J. Prescott, Pedro Pessoa-Lopes, et al. 2012. "Smokers with Active Crohn's Disease have a Clinically Relevant Dysbiosis of the Gastrointestinal Microbiota." *Inflammatory Bowel Diseases* 18 (6) (/06/01): 1092-1100. doi:10.1002/ibd.21864.

Betrapally, Naga S., MS, Patrick M. Gillevet PhD, and Bajaj, Jasmohan S., MD, MS. 2016. "Gut Microbiome and Liver Disease." *Translational Research* 179: 49-59. doi:10.1016/j.trsl.2016.07.005.

Brook, Itzhak and Alan E. Gober. 2005. "Recovery of Potential Pathogens and Interfering Bacteria in the Nasopharynx of Smokers and Nonsmokers." *Chest* 127 (6) (Jun): 2072-2075. doi:10.1378/chest.127.6.2072.

CDC Office on Smoking and Health. "Health Effects of Cigarette Smoking.", last modified -08-28T01:43:42Z, accessed Mar 21, 2018,

Center for Behavioral Health Statistics and Quality. 2015. *Behavioral Health Trends in the United States: Results from the 2014 National Survey on Drug use and Health (HHS Publication no. SMA 15-4927, NSDUH Series H-50)*.

Cho, CM, R. Hirsch, and S. Johnstone. 2005. "General and Oral Health Implications of Cannabis Use." *Australian Dental Journal* 50 (2) (Jun): 70-74. doi:10.1111/j.1834-7819.2005.tb00343.x.

David, Lawrence A., Corinne F. Maurice, Rachel N. Carmody, David B. Gootenberg, Julie E. Button, Benjamin E. Wolfe, Alisha V. Ling, et al. 2014. "Diet Rapidly and Reproducibly Alters the Human Gut Microbiome." *Nature* 505 (7484) (Jan 23,): 559-563. doi:10.1038/nature12820.

Fan, Xiaozhou, Alexander V. Alekseyenko, Jing Wu, Brandilyn A. Peters, Eric J. Jacobs, Susan M. Gapstur, Mark P. Purdue, et al. 2018. "Human Oral Microbiome and Prospective Risk for Pancreatic Cancer: A Population-Based Nested Case-Control Study." *Gut* 67 (1) (Jan 1,): 120. doi:10.1136/gutjnl-2016-312580.

Gao, Shegan, Shuoguo Li, Zhikun Ma, Shuo Liang, Tanyou Shan, Mengxi Zhang, Xiaojuan Zhu, et al. 2016. "Presence of Porphyromonas Gingivalis in Esophagus and its Association with the Clinicopathological Characteristics and Survival in Patients with Esophageal Cancer." *Infectious Agents and Cancer* 11: 3. doi:10.1186/s13027-016-0049-x.

Han, Jong-In, Hong-Kyu Choi, Seung-Won Lee, Paul M. Orwin, Jina Kim, Sarah L. Laroe, Tae-Gyu Kim, et al. 2011. "Complete Genome Sequence of the Metabolically Versatile Plant Growth-Promoting Endophyte Variovorax Paradoxus S110." *Journal of Bacteriology* 193 (5) (Mar): 1183-1190. doi:10.1128/JB.00925-10.

Heikkila, P., A. But, T. Sorsa, and J. Haukka. 2018. "Periodontitis and Cancer Mortality: Register-Based Cohort Study of 68,273 Adults in 10-Year Follow-Up
." *International Journal of Cancer*.

Human Microbiome Project Consortium. 2012a. "A Framework for Human Microbiome Research." *Nature* 486 (7402) (Jun 14,): 215-221. doi:10.1038/nature11209.

Human Microbiome Project Consortium. 2012b. "Structure, Function and Diversity of the Healthy Human Microbiome." *Nature* 486 (7402) (Jun 14,): 207-214. doi:10.1038/nature11234.

Kalant, Harold. 2004. *Adverse Effects of Cannabis on Health: An Update of the Literature since 1996*. Vol. 28. England: Elsevier Inc. doi:10.1016/j.pnpbp.2004.05.027.

Lara Bull-Otterson, Wenke Feng, Irina Kirpich, Yuhua Wang, Xiang Qin, Yanlong Liu, Leila Gobejishvili, et al. 2013. "Metagenomic Analyses of Alcohol Induced Pathogenic Alterations in the Intestinal Microbiome and the Effect of Lactobacillus Rhamnosus GG Treatment." *PLoS One* 8 (1) (Jan 1,): e53028. doi:10.1371/journal.pone.0053028.

Lloyd-Price, Jason, Galeb Abu-Ali, and Curtis Huttenhower. 2016. "The Healthy Human Microbiome." *Genome Medicine* 8 (1): 51. doi:10.1186/s13073-016-0307-y.

Lutgendorff, Femke, Louis MA Akkermans, and Johan D. Soderholm. 2008. "The Role of Microbiota and Probiotics in Stress-Induced Gastrointestinal Damage." *Current Molecular Medicine* 8 (4) (/05/31): 282-298.

Mason, Matthew R., Philip M. Preshaw, Haikady N. Nagaraja, Shareef M. Dabdoub, Anis Rahman, and Purnima S. Kumar. 2015. "The Subgingival Microbiome of Clinically Healthy Current and Never Smokers." *The ISME Journal* 9 (1) (Jan): 268-272. doi:10.1038/ismej.2014.114.

Narikiyo, Michihiro, Chikako Tanabe, Yukishige Yamada, Hiroyasu Igaki, Yuji Tachimori, Hoichi Kato, Manabu Muto, et al. 2004. "Frequent and Preferential Infection of Treponema Denticola, Streptococcus Mitis, and Streptococcus Anginosus in Esophageal Cancers." *Cancer Science* 95 (7) (Jul): 569-574.

NIDA. 2018. *Marijuana*: National Institute on Drug Abuse.

Ribeiro, Luis IG and Philip W. Ind. 2016. "Effect of Cannabis Smoking on Lung Function and Respiratory Symptoms: A Structured Literature Review." *Npj Primary Care Respiratory Medicine* 26 (1) (Dec): 16071. doi:10.1038/npjpcrm.2016.71.

Segata, Nicola, Jacques Izard, Levi Waldron, Dirk Gevers, Larisa Miropolsky, Wendy S. Garrett, and Curtis Huttenhower. 2011. "Metagenomic Biomarker Discovery and Explanation." *Genome Biology* 12 (6) (Jun 24,): R60. doi:10.1186/gb-2011-12-6-r60.

Shariff, Jaffer A., Kavita P. Ahluwalia, and Panos N. Papapanou. 2017. "Relationship between Frequent Recreational Cannabis (Marijuana and Hashish) use and Periodontitis in Adults in the United States: National Health and Nutrition Examination Survey 2011 to 2012." *Journal of Periodontology* 88 (3) (Mar): 273-280. doi:10.1902/jop.2016.160370.

Singh, Rasnik K., Hsin-Wen Chang, Di Yan, Kristina M. Lee, Derya Ucmak, Kirsten Wong, Michael Abrouk, et al. 2017. "Influence of Diet on the Gut Microbiome and Implications for Human Health." *Journal of Translational Medicine* 15 (1) (Apr 08,): 73. doi:10.1186/s12967-017-1175-y.

Sun, Jinghua, Min Zhou, Christian R. Salazar, Rosemary Hays, Sukhleen Bedi, Yu Chen, and Yihong Li. 2017. "Chronic Periodontal Disease, Periodontal Pathogen Colonization, and Increased Risk of Precancerous Gastric Lesions." *Journal of Periodontology* 88 (11) (Nov): 1124-1134. doi:10.1902/jop.2017.160829.

Tachon, Sybille, Bokyung Lee, and Maria L. Marco. 2014. "Diet Alters Probiotic Lactobacillus Persistence and Function in the Intestine." *Environmental Microbiology* 16 (9) (Sep): 2915-2926. doi:10.1111/1462-2920.12297.

The American Cancer Society. "Key Statistics for Oral Cavity and Oropharyngeal Cancers."

Tsigarida, A. A., S. M. Dabdoub, H. N. Nagaraja, and P. S. Kumar. 2015. "The Influence of Smoking on the Peri-Implant Microbiome." *Journal of Dental Research* 94 (9) (Sep): 1202-1217. doi:10.1177/0022034515590581.

Turnbaugh, Ley, Harmady, Fraser-Liggett, Knight, and Gordon. 2007. "The Human Microbiome Project: Exploring the Microbial Part of." *Nature* 449 (7164) (October 18,): 804-810.

Volkow, Nora D., Ruben D. Baler, Wilson M. Compton, and Susan R. B. Weiss. 2014. "Adverse Health Effects of Marijuana Use." *The New England Journal of Medicine* 370 (23) (Jun 5,): 2219.

Wu, Jing, Brandilyn A. Peters, Christine Dominianni, Yilong Zhang, Zhiheng Pei, Liying Yang, Yingfei Ma, et al. 2016. "Cigarette Smoking and the Oral Microbiome in a Large Study of American Adults." *The ISME Journal* 10 (10) (Oct): 2435-2446. doi:10.1038/ismej.2016.37.

Yang, Shun-Fa, Hsien-Da Huang, Wen-Lang Fan, Yuh-Jyh Jong, Mu-Kuan Chen, Chien-Ning Huang, Chun-Yi Chuang, Yu-Lun Kuo, Wen-Hung Chung, and Shih-Chi Su. 2018. *Compositional and Functional Variations of Oral Microbiota Associated with the Mutational Changes in Oral Cancer*. Vol. 77. doi://doi-org.proxy.cc.uic.edu/10.1016/j.oraloncology.2017.12.005..

Zarco, MF, TJ Vess, and GS Ginsburg. 2012. "The Oral Microbiome in Health and Disease and the Potential Impact on Personalized Dental Medicine." *Oral Diseases* 18 (2) (Mar): 109-120. doi:10.1111/j.1601-0825.2011.01851.x.

Zhang, Yu-Jie, Sha Li, Ren-You Gan, Tong Zhou, Dong-Ping Xu, and Hua-Bin Li. 2015. "Impacts of Gut Bacteria on Human Health and Diseases." *International Journal of Molecular Sciences* 16 (4): 7493-7519. doi:10.3390/ijms16047493.

Zhang, Z. F., H. Morgenstern, M. R. Spitz, D. P. Tashkin, G. P. Yu, J. R. Marshall, T. C. Hsu, and S. P. Schantz. 1999. "Marijuana use and Increased Risk of Squamous Cell Carcinoma of the Head and Neck." *Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology* 8 (12) (Dec): 1071-1078.

Zhao, Hongsen, Min Chu, Zhengwei Huang, Xi Yang, Shujun Ran, Bin Hu, Chenping Zhang, and Jingping Liang. 2017. "Variations in Oral Microbiota Associated with Oral Cancer." *Scientific Reports (Nature Publisher Group)* 7 (Sep 1,): 1. doi:10.1038/s41598-017-11779-9.

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