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BIOL F342

**Physiological Characterization and Genetic Identification of *Streptococcus parasanguinis***

**Introduction:**

Microbial growth accounts for the majority of organismal diversity on Earth. Their immense diversity encompasses the high level variability for all varieties of microorganisms. They are noted for adapting to almost every environment present due to their diverse metabolic properties, making them a vital source of knowledge for various life strategies.

Based on the high complexity and diversity of microorganisms, the human mouth was further investigated for the presence of microbial growth. The human mouth microbiome is by far one of the most diverse in the human body, compromised of viruses, fungi, protozoa, archaea and bacteria (Olson, 2015). This significant amount of diversity is a result of the moist and warm environment present in the human mouth, which creates a variety of surfaces that allow colonization of bacterial species (Gibbons et al., 1975). The different habitats of the human oral cavity that promote growth include; the teeth, tongue, cheeks, the hard and soft palates, and the tonsils (Dewhirst et. al., 2010). Due to this high level of diversity, a substantial amount of the oral bacterial biome has yet to be cultured and identified (Olson, 2015).

The objective of this study was to isolate and develop a pure bacterial culture taken from the human mouth. This project was focused on identifying and analyzing bacterial colonization and determining whether the isolated bacterial strain is commonly found in this particular environment. Various physiological tests, along with genetic testing, were performed in order to identify the isolated bacterium. The results of DNA sequence and bioinformatics analysis revealed the presence of *Streptococcus parasanguinis,* which plays a distinct role in dental biofilm formation (Liang et al., 2011). This formation of dental biofilm aids in the regulation and maintenance of microbial ecology, which is crucial for sustaining a healthful state of the oral cavity (Liang et al., 2011).

**Methods:**

To conduct this experiment, I first began with the execution of bacterial inoculation from a human mouth. In order to collect the bacteria, a sterile swab was placed along the lining of the oral cavity and wiped along the inner cheek. The swab was then transferred to a tryptic soy agar, or TSA, plate using a simple zig-zag streaking pattern. The process was then repeated, instead using a Sabouraud’s Agar, or SA, plate. Each agar plate was then sealed using parafilm and stored in a dimly lit environment at room temperature during the observation period. Observations regarding colonization were taken each day following the initial inoculation. Initial bacterial colonization was recorded approximately 72 hours following inoculation.

One week following the inoculation, a single bacterial colony from the initial TSA plate was re-streaked using a quadrant streak method onto a fresh TSA plate in preparation for the development of a pure culture. The streak plate was then placed in a 37 degrees C incubator in order to maximize growth rate. This plate was then observed 48 hours following streaking, when notes were taking regarding the appearance of the colonies. This streaking process was repeating twice more before a pure culture was obtained. Observations were conducted following each streak.

After a pure bacterial culture was obtained, various testing methods were implemented in order to determine the identification of the isolate. A common differential staining method, Gram staining, was conducted in order to classify the culture into one of two main groups: Gram positive and Gram negative. The Gram staining procedure was also utilized in order to characterize the isolate based on its cell morphology (Leigh, 2017). Following the gram staining, a fluid thioglycollate test was conducted to determine to oxygen class of the isolate. The oxidase test was performed in order to determine the presence of cytochrome c oxidase as stated in lab handout 6. Following the oxidase test, a catalase test was performed to determine the presence of the enzyme catalase, which is utilized to catalyze the release of oxygen from hydrogen peroxide. Following these initial tests, a series of various physiological tests were conducted using API 20E test strips. The individual strip contained 21 different tests in a miniaturized format.

In part with these phenotypic tests, genotypic testing was utilized in order to aid in the identification and characterization of the culture. DNA was extracted from the isolate for the purpose of genome sequencing using the PowerSoil DNA extraction kit (Leigh, 2017). Once complete, the extracted DNA was sent to the UAF Core Lab for genome sequencing using Illumina MiSeq technology. Following sequencing, a bioinformatic analysis was conducted using BaseSpace. Apps within this online software including, SPAdes Genome Assembler, Kraken Metagenomics, and Prokka Genome Annotation, were each utilized in order to obtain taxonomic identification of the unknown isolate and the presence of functional genes within the isolate’s genome (Leigh, 2017).

Following genome sequencing, a disk diffusion test was performed on the isolate. The objective of this test was to determine resistance or susceptibility to various antibiotics (Leigh, 2017). To perform this test, a TSB tube was inoculated with the isolate and allowed to grow. The inoculated broth was transferred to a fresh TSA plate. Immediately following the broth to agar transfer, filtered paper infused with antibiotics was placed on the plate. The plate was then incubated until growth was observed. The presence and size of the zone of inhibition was monitored and measured to provide insight on the isolate’s susceptibility or resistance to the tested antibiotics (Leigh, 2017). A total of 8 antibiotics were tested, including Ticarcillin, Tetracycline, Tobramycin, Amikacin, Cefazolin, Cefoperazone, Trimethoprim, and Erythromycin. Guidelines and protocol for this procedure were illustrated in lab handout 9.

**Results:**

Following analysis of the gram staining procedure, it was concluded that the isolate was gram positive. This staining procedure also revealed the cell morphology of the isolate to be relatively small cocci arranged in chains, roughly 1 micrometer in size. When the isolate was plated, colonies were off-white and lightly yellow in coloration. The fluid thioglycollate test unveiled sufficient growth throughout the inoculated TSB tube, indicating that the isolate can perform under both aerobic and anaerobic conditions. Both the catalase and oxidase tests revealed negative results, indicating the lack of both the catalase and oxidase enzyme in the isolate. Table 1 reflects the results of the 21 API tests performed.

Sequencing provided a general breakdown of the genome assembly for the isolate, including values such as the number of contigs and the guanine cytosine content. Genome sequencing also provided taxonomic information regarding the isolate. The genome for the isolate was matched with a database in an attempt to rule out its taxonomic identity. The results of the taxonomic analysis assigned the species name of *Streptococcus parasanguinis,* with a 90.79% confidence level.

The results of the antibiotic susceptibility were inconclusive. The culture was unable to be revived prior to the beginning of this experiment. Instead of using a fresh plate of the isolate, a suspension was used to perform the antibiotic susceptibility testing. Unfortunately, no growth was observed on either of the inoculated plates due to potential death of the isolate. The results of this testing were unable to be utilized for the characterization and identification of the isolate.

**Table 1. Summary of physiological and phenotypic test results**

|  |  |
| --- | --- |
| Test/Observations | Results |
| Gram stain | Positive |
| Cell morphology | Small circular, cocci |
| Fluid thioglycollate | Growth distributed throughout tube (Facultative Aerobe) |
| Oxidase Test | Negative |
| Catalase Test | Negative |
| API 20 E Test | ONPG: negative  ADH: **positive**  LDC: negative  ODC: negative  CIT: **positive**  H2S: negative  URE: negative  TDA: negative IND: **positive** VP: **positive** GEL: **positive** GLU: negative MAN: negative INO: negative SOR: negative RHA: negative SAC: negative MEL: negative AMY: negative ARA: negative OX: negative NO2: negative |
| Antibiotic Susceptibility | Inconclusive |

**Discussion:**

The results of both the physiological and genotypic tests, along with the use of supplemental research, are consistent with the presence of *Streptococcus parasanguinis*. This particular species is a gram-positive, non-motile bacterium of the streptococcus genus, grouped in long, coccal chains. *S. parasanguinis*is noted for being a facultative aerobe, exhibiting growth in both oxic and anoxic environment, as confirmed by the fluid thioglycollate testing. Little information from the API physiological testing was utilized for the characterization of the isolate due to variability in results when compared to previous literature. However, various tests yielded consist results with the presence of *S. parasanguinis,* including the ADH, URE, and MAN tests.

Although most Streptococcal species are considered commensals, a disruption in microbial and host balance often results in the development of disease (Jenkinson et. al., 1997). This disease state is caused by environmental influences including diet or a disruption in the host’s immune responses. This particular streptococcus species is a predominant colonizer on the surface of human teeth. Pili and fimbrae allow this bacterium to easily adhere to surfaces in the human oral cavity (Peng et. al., 2008). This bacterial strain plays a vital role in the formation of biofilms, or dental plague, by aiding in the colonization of other oral bacterial strains (Peng et. al., 2008).

In conclusion, both the physiological tests and genome sequencing support the presence of *Streptococcus parasanguinis*. Due to its high rate of colonization on teeth, it is not shocking that this was isolated from the human mouth. However, little research has been conducted on this particular species of *Streptococcus.* Due to the prevalence of this particular genus, it is often hard to differentiate between different strains. Also, there have only been 280 bacterial species successfully isolated and cultured from the oral cavity (Dewhirst et. al., 2010). It has been estimated that less than half of the bacterial species present in the mouth can be cultivated using microbiological methods, while there are roughly 500 to 700 species residing in this environment.

A broadened understanding of the microbial ecology of the oral cavity is essential for understanding various immunological and physiological functions including food digestion and nutrition, energy generation, and the development and regulation of the immune (Kilian et. al., 2016). Disruptions to the composition of the microbiome can result in harmful consequences to human health. With that being said, there is still a great deal of knowledge that needs to be learned about streptococcal species and other oral microbes so that we are better able to better regulate and control diseases associated with this microbial habitat.

**Literature Cited**

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