Identification and Characterization of *Staphylococcus hominis*

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Introduction

Many microorganisms play a significant role in our daily lives. With over 2 billion species, microbes make up about 60% of the biomass of earth [1]. While some microbes may be harmful to humans, there are many more that are safe, helpful, and only mildly annoying. Human skin hosts many highly specific microbial communities. To better understand the harmless microbes that we encounter during our daily lives, I took samples of several locations, isolated a colony of bacteria, and carried out multiple tests to determine what bacteria I had isolated.

Staphylococcus hominis (S. hominis) is one of the mildly annoying microorganisms. It can be found on many surfaces including human skin and is responsible for body odor. Along with other coagulase-negative staphylococci, *S. hominis* is a natural part of the flora of the human skin and mucous membranes and are capable of producing very large colonies [2], [3]. *S. hominis* breaks down naturally occurring molecules in sweat into thioalcohols which are the cause of body odor [4].

The sample of *S. hominis* that I isolate came from a makeup sponge, often used for applying makeup to a person's face. The initial bacteria most likely came from my body, since the sponge touches my face and the skin on my hands every day. Makeup sponges and brushes almost always carry an array of bacteria including *Pseudomonas aeruginosa,* strains of *Staphylococcus* (including *Staphylococcus aureus)*, as well as yeasts and fungus [5]. I carried out several tests, including both physiological and genetic, to identify the bacteria and some of its characteristics.

Methods

Sampling and Isolating

To obtain a sample of microorganisms for isolation, I followed the procedure in Lab Handout 1. I first selected a location to take samples from and then used a swab to obtain the sample. To grow a sample on Tryptic Soy Agar (TSA) plate, I wet a sterile swab with sterile water and rubbed it on my makeup sponge. A makeup sponge is used to apply liquid or cream makeup to a person's face. It comes into contact with the microbes on my skin daily. After swabbing the sponge, I swabbed the TSA plate in a zigzag motion so that the entire plate was covered. I left the plates out at room temperature for several days. During the first four days, I saw no growth. On the fifth day, I placed the plate directly under a lamp to provide a little extra heat. The plate started showing growth by the end of the fifth day. On the end of the seventh day, there was significant growth on the TSA plate (Figure 1).

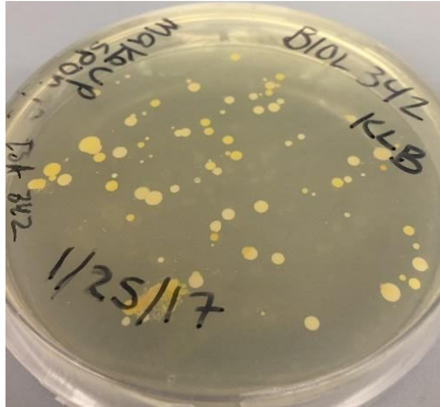

Figure 1. TSA plate growth after seven days.

To isolate one bacterium, I followed the procedure in Lab Handout 2. This included using a sterile inoculating loop to pick up one colony from the original TSA plate and make a streak late (Figure 2) on a new TSA plate. I chose a colony that was light yellow and slightly shiny. It was not as shiny as some of the other colonies on the plate, but was not at all as dull as some of the others. I left the new streak plate in the 37ºC incubator for two days, then used one of the isolated colonies to make a new streak plate, which I left in the incubator for three days.

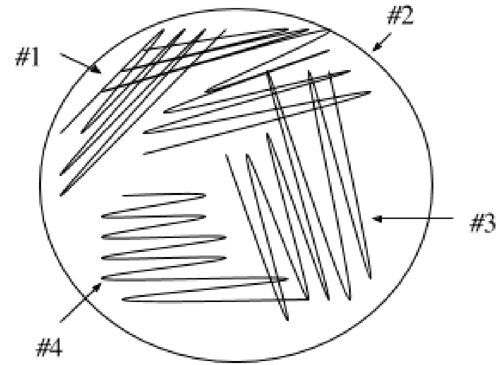

Figure 2. Quadrant streak method

Identifying and Characterizing

To begin narrowing down the possibilities of my bacteria's identity, I performed a Gram stain. I followed standard Gram staining protocol, as outlined in Lab Handout 4. I started by making three circles on a microscope slide using a wax pencil. Using a sterile metal inoculating loop, I transferred one loopful of *E. Coli* broth culture into the first circle as a Gram-negative control. I then transferred one loopful of *Bacillus cereus* broth culture into the third circle as a Gram-positive control. I performed various physiological tests to narrow identification possibilities down even further. These physiological tests included a fluid thioglycollate test, an oxidase test, catalase test, and an API 20E test strip which contained multiple metabolic tests.

DNA extraction from the bacteria was done according to the procedure in Lab Handout 5. This included several steps that led to a product of pure DNA to be used for sequencing. I followed the steps as written, which included cell lysis, removal of inhibitors and proteins, and obtaining a pure solution of DNA in a buffer. After I completed these steps, I was left with a tube containing a small amount of pure DNA in a buffer. This DNA was sent to the UAF DNA Core Lab for sequencing. When DNA sequencing was complete, I ran the sequence through several programs on the BaseSpace website, including Kraken Metagenomics and GENIUS Metagenomics databases. GENIUS Metagenomics was able to give me the most thorough and accurate results.

To determine susceptibility to antibiotics, I followed the procedure in Lab Handout 9. I tested 10 antibiotics using the Kirby-Bauer method. Two antibiotic tests were replicated because the antibiotic disk fell too close together when I was initially placing them on the agar.

Results

Attempts at sampling and isolating a bacterial sample were successful. I isolated a colony and continued to grow it on TSA plates successfully. Colonies were small, yellow, and slightly shiny, but not completely reflective. They were perfectly circular, grew individually of one another, and rose less than 1 mm in height. . Cells were nonmotile Gram-positive cocci and grew clustered together. Identifying and characterizing the isolate began with determining that is was Gram-positive. The results of the physiological tests and the API 20E test strip (Figure 3) revealed that the isolate is a strict aerobe, produces the enzyme catalase, and has cytochrome *c* oxidase. Other positives included production of citrate and production of glutinase. These results were not similar to results from other strains of *Staphylococcus* [6].

Test	Results
Oxidase	+
Catalase	+
Fluid thioglycollate	aerobe
ONPG	-
ADH	-
LDC	-
ODC	-
CIT	+
H2S	-
URE	-
TDA	+
IND	-
VP	+
GEL	+
GLU	+
MAN	-
INO	-
SOR	-
RHA	-
SAC	-
MEL	-
AMY	-
ARA	-

Figure 3. API 20E test strip results

GENIUS Metagenomics database analysis concluded that the DNA sample was 89% *Staphylococcus hominis*. The other 11% contained *Pseudonocardia spinosispora* and *Micrococcus luteus* which probably came from contamination during the DNA extraction process.

Figure 4. Zones of diameter around antibiotic disks

Antibiotic resistance testing concluded that this isolate of *S. hominis* was very susceptible to all antibiotics tested. This was evident by the diameter of the zone around the antibiotic disk in which there was no bacterial growth, which can be seen in Figure 3. All zones of diameter were large enough to indicate strong susceptibility.

Discussion

The isolate of *S. hominis* grew as expected according to literature [7]. I found the best growing time in the 37ºC incubator to be 3 days. Any longer than that and colonies in the fourth quadrant of a streak plate would start to grow together. It did not grow well at room temperature and initially took five days to show any growth at all when incubated at room temperature. Growth did not begin until the agar plate was placed directly under a lamp where the temperature was

slightly greater than room temperature. The isolate was Gram-positive, as was expected based on current literature and other *Staphylococci* [7].

In relation to the literature, the API 20E test strip results were not consistent with results of most other strains of *Staphylococcus*. [8]. This could be because the API 20E test strip is meant for use with Gram-negative bacteria, according to the BioMérieux website [9]. There are other tests on the market for Gram-positive bacteria and ones specifically for Staphylococci.

DNA extraction was done by me alongside another student's isolate, which increased the risk of contamination. This needed to be done because the student was ill that day and the DNA extraction needed to be completed at the same time as everyone else's. There was, in fact, some contamination in my DNA isolation which was seen by the GENIUS Metagenomics program. The contaminant was also found by metagenomic databases in the other students isolate, although they were unable to identify their isolate completely. It is likely that the contamination came from the lab or my skin and not from the other students isolate.

While there are some S*taphylococci* that are resistant to specific antibiotics, a number which has risen over the last 20 years [10], my isolate was susceptible to all antibiotics tested. This makes sense because it is a relatively harmless bacteria that is part of normal human flora. It also probably initially came from my body and I have not taken antibiotics or used antibiotic substances (such as antibiotic creams) in a very long time. The bacteria living on my body have not had a need to develop a resistance to any antibiotics.

Overall, identification and characterization went as expected. There were no surprises or abnormalities outside of the API 20E test strip. All of the information I obtained from physiological, morphological, and genomic tests fit into established standards for *Staphylococci spp.*

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