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Biol 342 F01

Lab Report

Identification of Unknown Microbe through Physiological and Genetic Characteristic

Introduction:

It will not be an understatement to say that microbes are everywhere. This is due to their great diversity and numbers. Some of the characteristics include being able to produce their own forms of energy through various methods, such as photoautotrophs (uses light energy) and chemolithotrophs (uses inorganic compounds). Another way that allows microbes to be so ubiquitous is that they are capable of "natural dispersal" which allows them to spread and reproduce "even over large distances" [1]. This idea that bacteria are everywhere, and their specific characteristics determine which bacteria are present in a given location is called the Principal of ubiquity.

With this idea in mind, being able to take a sample of multiple microorganisms and isolating them in order to accurately identifying them through their unique characteristics is an important skill. Unfortunately, the number of kinds of bacteria that can be grown in culture is severely limited. There have been improvements in using mimicked natural environment to culture bacteria previously impossible but for the sake of this experiment, the kinds of bacteria that could be grown was limited [3].

For this research the sample was taken from my computer mouse. The microbes living on the computer mouse may get their nutrients possibly from any human hand that regularly comes

in contact with it. Therefore, one can assume that the computer will share most of its bacteria flora with the human skin. When it comes to bacteria on the human body, they can be categorized into two groups, resident and transient. Resident meaning bacteria that originate on the human body and transient meaning bacteria that were moved from another location onto the human body. Some examples of possible bacteria that can be found on the human skin, and therefore a computer mouse, include *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa*, which are all resident bacteria, and *Escherichia coli*, which is a transient bacterium [2]. All these bacteria are capable of infecting human, however, the human immune system is capable of preventing that unless the immune system is somehow compromised by factors such as AIDS or immunosuppressant drugs. This makes them an opportunistic pathogen. It is important to note that although they are capable of infection, it does not make them always harmful. For example, these bacteria may prevent other more harmful bacteria from growing on the skin by competing for resources [3].

A particular unknown microbe was isolated and characterized through physiological and genetic tests. The particular unknown microbe was determined to be a *Staphylococcus epidermidis*.

Methods:

The bacteria were collected from a computer mouse by using a sterile cotton swab. Using the cotton swab, the bacteria were streaked across a TSA plate in a zig-zag pattern. The plates were left in room temperature for a 6 days, however no growth was observed over the week. One colony of bacteria was grown and isolated by using a quadrant streak technique covered during Lab 2. This technique involves using a sterile loop to spread the bacteria over 4 different section

of the TSA plate. By spreading the bacteria over more area, it becomes easier to separate one colony from another. The original TSA plate and the newly streaked plate were incubated in 37°C for a week. At this time, I noticed that the isolated colony looked uniform in shape, color and luster. Afterwards I grew the isolate on a new TSA plate to get fresh samples of the isolate. The bacteria will be grown in new plates or broth accordingly almost every week to provide new samples to test for each individual tests.

A Gram stain test was done to the isolate using techniques covered in Lab 4. A Gram positive and Gram negative bacteria stains different colors, purple for positive and pink for negative, which can then be observed through the microscope to determine whether the particular isolate is a Gram positive or Gram negative bacteria.

More phenotype was done using an API 20E test strip, fluid thioglycollate test, oxidase test and catalase test using techniques covered in Lab 6. A fluid thioglycollate test uses a test tube filled with a soft thioglycollate medium to determine the oxygen class of the bacteria. Fluid Thioglycollate test can tell us what type of metabolism the bacteria have because bacteria with different oxygen class will grow on different area of the tube. The isolate was grown in the thioglycollate medium in 37°C for three days before observing growth. Oxidase test and catalase test will react at the presence of cytochrome c oxidase and catalase enzymes respectively. Both of these tests can determine if the bacterium is capable of cellular respiration. An API 20 E test strip tests 21 different type of physiological traits. The test includes traits such as production of certain enzymes, ability to reduce nitrates, and the ability to metabolize certain type of molecules. After the isolate was inserted into each test, the strip was left to grow in an incubator at 37°C for three days before the results was recorded.

The genotype of the bacteria was also tested. Firstly, the DNA of the bacteria was isolated using the Powersoil DNA isolation technique covered in Lab 5; then by using the extracted DNA, the genome of the bacteria was sequenced by Institute of Arctic Biology DNA Core Lab. The most likely identity of the bacteria was chosen during Lab 7. The isolation of the DNA was done in three general steps, first the cell was lysed to release the DNA, then inhibitor and proteins were removed for a purer DNA sample, and finally the DNA was isolated. After the genome was sequenced, we used Base Space apps to determine the genotype characteristics of the isolate. First app was the SPAdes Genome Assembler, which we used to test number of usable contigs to determine whether or not the isolate was usable or not, and GC content of the isolate DNA. Next, the Kraken metagenomics app was used to narrow down the isolate into the most likely species. Finally, the Prokka genome annotation app was used to find any functional genes that were present in the DNA.

The last test we did to test the isolate was an antibiotic susceptibility test, covered in Lab 9. The isolate was evenly spread on a TSA plate with antibiotic plates on four sections of the plate and grown over a period of three days in a 37°C incubator. The diameter of the no growth zone was measured and compared to known data to determine susceptibility.

Results:

The bacteria isolate grew in a dull and yellow, almost beige, and circular colonies in varying sizes (Figure 1). It also grew best in warm 37-degree incubator. Under a microscope, the bacteria were cocci shaped and around 1 micrometer in diameter. After gram staining, the bacteria stained purple, meaning it is a gram positive bacteria.

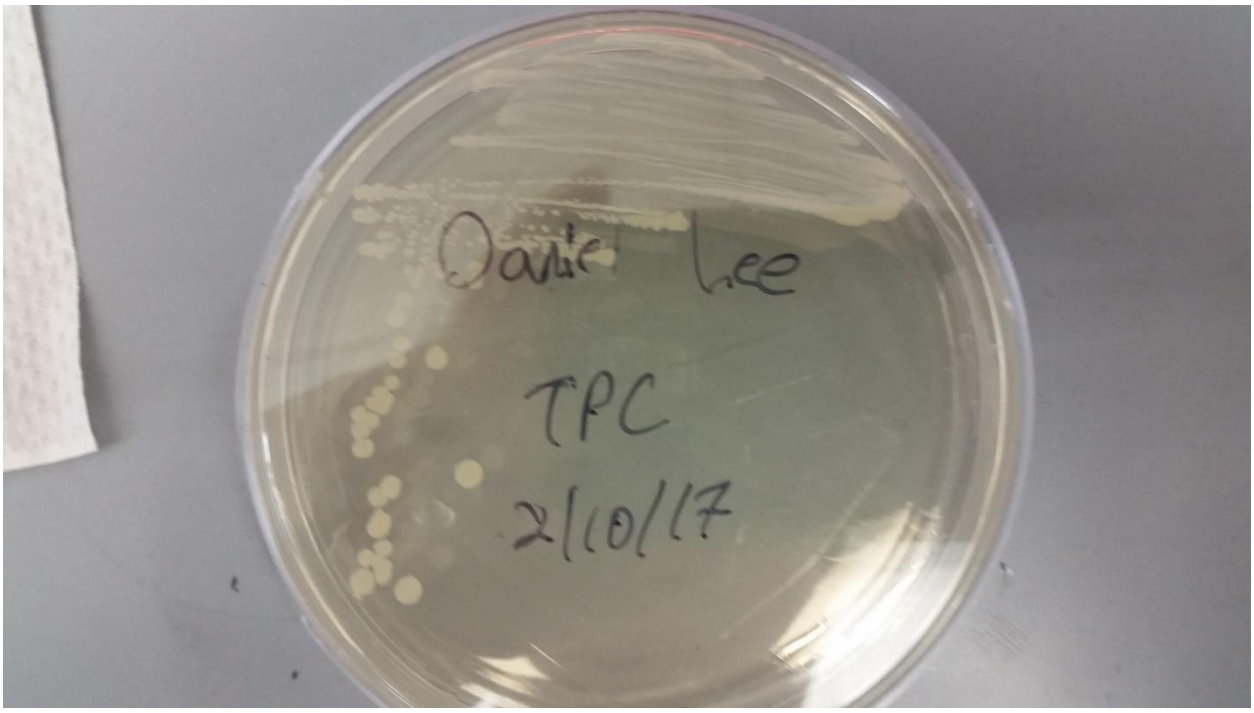

Figure 1: Quadrant streak of bacteria

The bacteria grew throughout the soft fluid thioglycollate medium and therefore it was a facultative anaerobe. The catalase came out as negative, meaning it does not product the enzyme. The API 20E test showed mostly negative results. The only positive results were ADH, URE, and N2 tests (Figure 2). This corresponds to presence of arginine dihydrolase enzyme, urease enzymes, and the ability to reduce nitrogen to N2 gas.

Figure 2: Results of API 20E test

The bacteria showed ability to grow in Eosin Methylene Blue (EMB) agar but not in MacConkey Agar (MAC) (Figure 3)

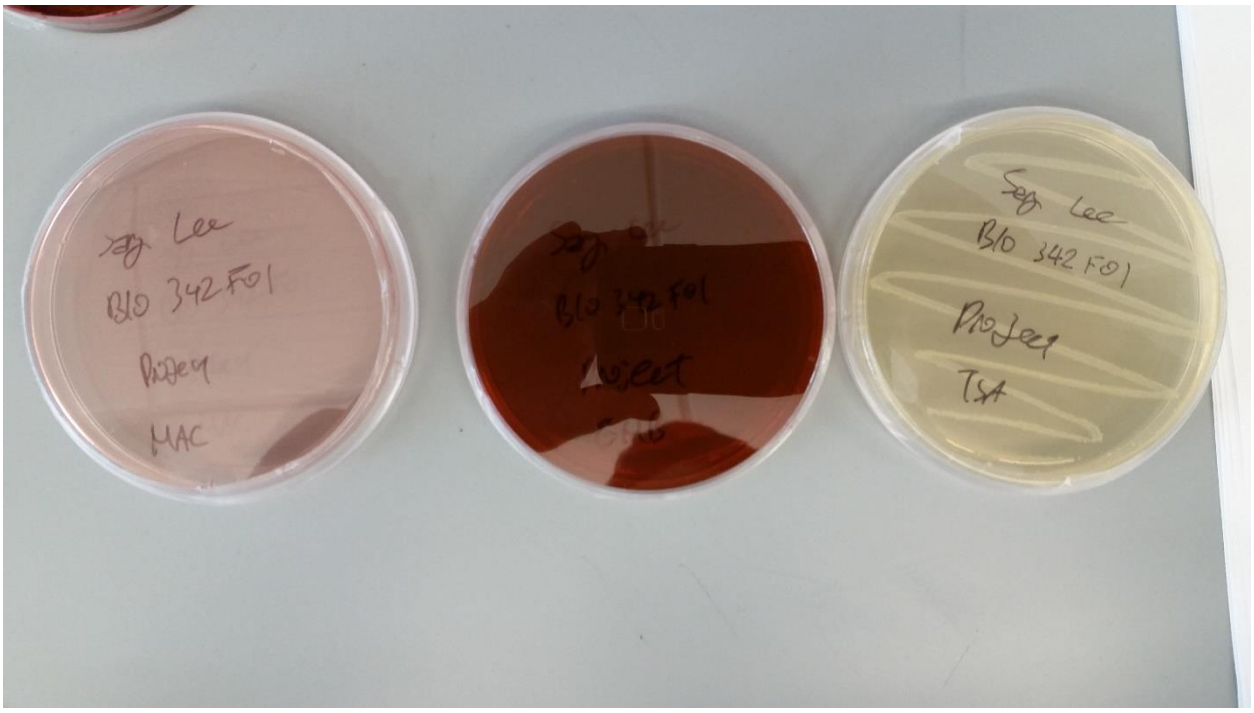

Figure 3: Results after 3 days of growth in MAC, EMB and TSA agar plates respectively

The genotype testing showed 94.70% confidence level of certainty of the bacteria being *staphylococcus epidermidis*.

The antibiotic test showed that the bacteria was susceptible to all antibiotic tested, with no growth zone over 34mm on all antibiotic disks. The antibiotics tested were Amikacin, Cefazolin, Cefoperazone, Gentamicin, Oxacillin, Tobramycin and Vancomycin.

Discussion:

The shape and distribution of the bacteria on the TSA plate under microscope was consistent with the literature. The isolate was coccus in shape and clumped into smaller groups of 10-30 bacterium per group. The size was also similar to literature at around 1 micrometer in diameter. After Gram staining, the bacteria were clearly purple in color. *S.epidermidis* is a Gram-positive bacteria, so this was to be expected.

Since *Staphylococcus Epidermidis* is a gram positive bacteria, it should not have been able to grow on EMB and MAC medium. I thought I saw some growth on these plates, which should not have been possible. This may have been due to me thinking that the streak marks were growth when they were actually just markings left by the loop, or the loop may not have been completely sterile and another bacterium grew on the plates.

The physiological tests were also consistent with the literature, notably *Staphylococcus epidermidis* is a facultative anaerobic and grew throughout the fluid thioglycollate tube [6]. *Staphylococcus epidermidis* is also capable of reducing nitrate, as shown in the API 20E test [6]. In the beginning, there was a bit of concern over the fact that most of the tests on the API 20E test were negative, and I wasn't sure if I had not given the tests enough time. However, this may be due to the fact that API 20E test is mostly for Gram-negative bacteria according to the biomerieux website that sells them [7]. *Staphylococcus epidermidis* is a Nitrogen reducing bacteria, and this correlated with the results [6]. However, I could not find any peer reviewed data supporting whether *Staphylococcus epidermidis* had arginine dihydrolase or urease, this may mean that the results were false positive. *Staphylococcus epidermidis* is capable of fermenting glucose but the API 20E test came out negative, which further demonstrated inconclusiveness of API 20E test [6]. Therefore, I determined that further testing with the API 20E test would be unhelpful.

The bacteria were very susceptible to all antibiotics tested. However, the literature states that the *Staphylococcus epidermidis* is normally resistant to common antibiotics [5]. It was more susceptible to vancomycin, which according to literature, is most effective antibiotic used against *Staphylococcus epidermidis*. The specific strand of *Staphylococcus epidermidis* may be more

susceptible to antibiotics but it seems unlikely. The antibiotic resistance of *Staphylococcus epidermidis* is an important area of study because this bacterium is an opportunistic pathogen. This bacterium causes infections especially in hospitals where immune system of patients can be compromised. *Staphylococcus epidermidis* is also capable of creating biofilm on implants, which combined with the antibiotic resistance, makes them very dangerous [6].

The genotype testing was very useful in specifying the species with 94.70% confidence level for *Staphylococcus epidermidis*, with 239,693 classified reads out of total of 253,456 reads. However, the DNA showed some impurities, maybe from another bacteria DNA due to improper technique. This did not impede the identification of the bacteria but shows room for improvements. Some of the functional genes found in the DNA include genes for glucose uptake, GlcU, cell division protein YtgP and a multidrug resistance protein, EmrK.

In conclusion the numerous tests were mostly consistent with the literature except antibiotics susceptibility test and the EMB/MAC medium test. The tests that we've done are more selective for Gram negative bacteria and for future testing we could use more tests that can be done to distinguish the characteristics of a gram positive bacteria. It made perfect sense that *Staphylococcus epidermidis* would be found on computer mouse, since it is often in contact with the hands of users, from which it could have transferred over.

References:

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