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Bio 342
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Identification of Acinetobacter baumannii

Introduction

The microbial world of a college campus is enormous and unpredictable. With students from all over the world, an assorted variety of microbes can appear from any sample. Since these microbes can use energy from many different sources, they are able to grow in locations that are not ideal for just any organism. However, even with such a large diversity, only a small number of these microbes can actually be cultured. The results from this study will only be a small percentage of what actually exists on the sample (Stewart 2012). For this study, a dorm room door knob was swabbed to identify a species that is able to grow here and be transferred by human skin.

Door knobs are touched by people every day and can be full of bacteria (Tiller et *al.* 2001). Because of this, institutions such as hospitals use materials such as copper alloys to limit bacterial growth (Mikolay et *al.* 2010). Since many prokaryotic species have similar characteristics, a series of genotypic and physiological tests are conducted to properly identify a species. The species identified in this study, Acinetobacter baumannii, is commonly found in a hospital setting. Outside of the hospital, little is known about where this bacterium makes itself at home.

Acinetobacter species are known for being found everywhere including soil samples and on surface water (Peleg et *al.* 2008). A. baumannii is different than the rest of the genus and the optimal habitat for it is not yet defined, although it does not appear to be an environmental organism. However, this species is able to colonize on human skin (Howard et *al.* 2012) and infect immunocompromised individuals. Over the past decade, our understanding of this bacteria has greatly increased due to its rise in antibiotic resistance. There have been strains of A. baumannii reported to be resistant to all known antibiotics that have emerged in just the last 15 years (Peleg et *al.* 2008). By furthering our understanding of this species, hospital stays for immunocompromised patients can become safer.

Methods

I took a sterile swab and moistened it with distilled water. The swab was then rubbed on the doorknob of a UAF dorm room. Once the swab was sufficiently rubbed on the entire sample, it was streaked on a Tryptic Soy Agar (TSA) plate in a zig zag pattern. The plate was sealed with

parafilm, and was kept at room temperature until several colonies had formed. Once the colonies had grown, one of them was chosen to make a pure culture. With a sterilized inoculating loop, a quadrant streak was performed to separate any contamination between colonies. Following the instructions of Lab 2, the plate was incubated for two days at 37˚C then re-streaked. This process was repeated at least three times for all samples until a pure culture was achieved. Once the culture was pure, zig-zag streaks were used to create fresh samples grown in an incubator at 37 degrees Celsius.

A series of physiological tests were used to assist identification of the isolate. To start, the isolate was Gram stained to identify if it was positive or negative (Lab 4 handout). The isolate was used in a fluid thiogycollate test to determine the oxygen class, an oxidase test to determine if the isolate had cytochrome C oxidase, a catalase test to determine if the isolate contains the enzyme catalase, and lastly, the isolate was applied to an API 20 E test strip (Lab 6 handout). This test strip can test for 21 different physiological processes in a period of 48 hours. Review the protocol of Lab 6 for the specific instructions relating to each of the physiological tests. Isolate growth was also attempted on multiple mediums. We used Eosin Methylene Blue (EMB) Agar and MacConkey Agar (MAC) plates to differentiate fermentative species (Lab 8 handout). Lastly, the antibiotic susceptibility of the isolate was tested with six different antibiotics (Lab 9 handout).

Once the physiological tests are complete, I sequenced my isolate's genome for further identification. Using the PowerSoil® DNA Isolation Kit, I extracted the DNA from my isolate to be sequenced and analyzed with bioinformatics (Lab 5 handout, Lab 7 handout). The DNA was sequenced using "next-generation sequencing" with the Illumina MiSeq at UAF's DNA Core Lab. When the sequenced results were returned to me, I used BaseSpace Sequence Hub to analyze my isolate's genomic sequence. By using the apps SPAdes Genome Assembler, Kraken metagenomics, and Prokka Genome Annotation on the BaseSpace website, I was able to identify not only what my isolate was, but also the functional genes that were identified in sequencing (Lab 7 handout).

Results

When grown on a TSA plate, the colonies are round and have a light-yellow color. They are slightly raised from the surface of the agar. The gram stain of the isolate was shown to be Gram-negative, indicated by a red/pink color when they are counter-stained with safranin. While

examining the gram stain under a microscope, it was observed that the bacteria is coccobacillus shaped and 1x1.5 μm in size. The isolate also formed chains or mats of cells (fig.1).

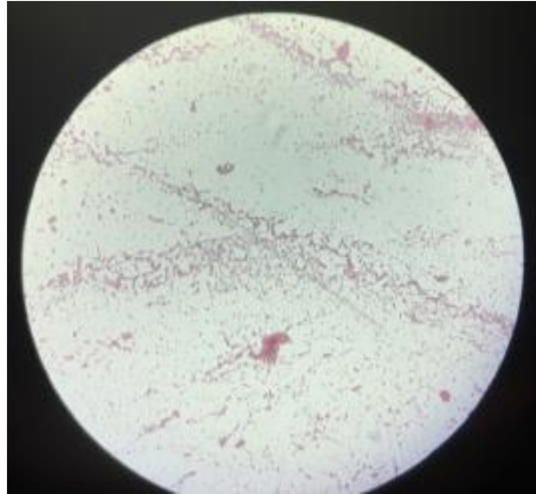

Fig. 1 shows a picture taken at 40X of the isolate after a Gram stain

The fluid thiogycollate test performed in Lab 6 determined the isolate was a strict aerobe since the isolate grew at the surface and just below (fig. 2).

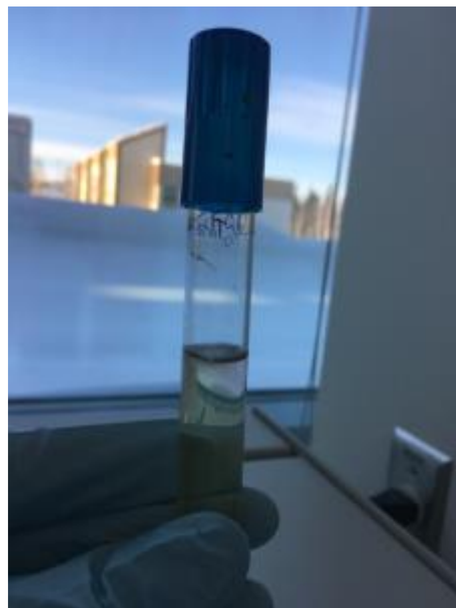

Fig. 2 shows the result of the fluid thioglycollate test

The catalase test showed that the isolate contained the enzyme catalase. When the isolate was placed in 3% H_2O_2, O_2 bubbles formed. The oxidase test was negative with no color change occurring when the isolate was exposed to the test strip. The API strip (fig. 3) had mixed results. Table 1 shows the results of the test strip.

Fig. 3 shows the API test result.

Table 1

Test	Result	Additional Information
ONPG	-	
ADH	N/A	
LDC	-	
ODC	-	
CIT	+	
H2S	-	
URE	N/A	
TDA	-	
IND	-	
VP	-	
GEL	-	
GLU	+	NO_2 test -, N_2 test +
MAN	-	
INO	-	
SOR	-	
RHA	-	
SAC	-	
MEL	+	
AMY	-	
ARA	+	

Table 1 shows the results of the API 20E test strip.

From the sequenced isolate, 43% of the sequenced DNA came back as unclassified. From the remaining 57%, 99.97% of the reads stated the isolate belonged to the domain Bacteria, 99.88% of the reads stated the isolate belonged to the phylum Proteobacteria, 99.82% of the reads stated the isolate belonged to the class Gammaproteobacteria, 99.58% of the reads stated the isolate belonged to the order Pseudomonadales, 99.61% of the reads stated the isolate belonged to the family Moraxellaceae, 99.63% of the reads stated the isolate belonged to the

genus Acinetobacter, and 84.85% of the reads stated the isolate belonged to the species Acinetobacter baumannii. Other possible species include Acinetobacter oleivorans (7.44%), Acinetobacter calcoaceticus (7.19%), and Alteromonas macleodii. A visual representation of this data can be seen in figures 4 and 5.

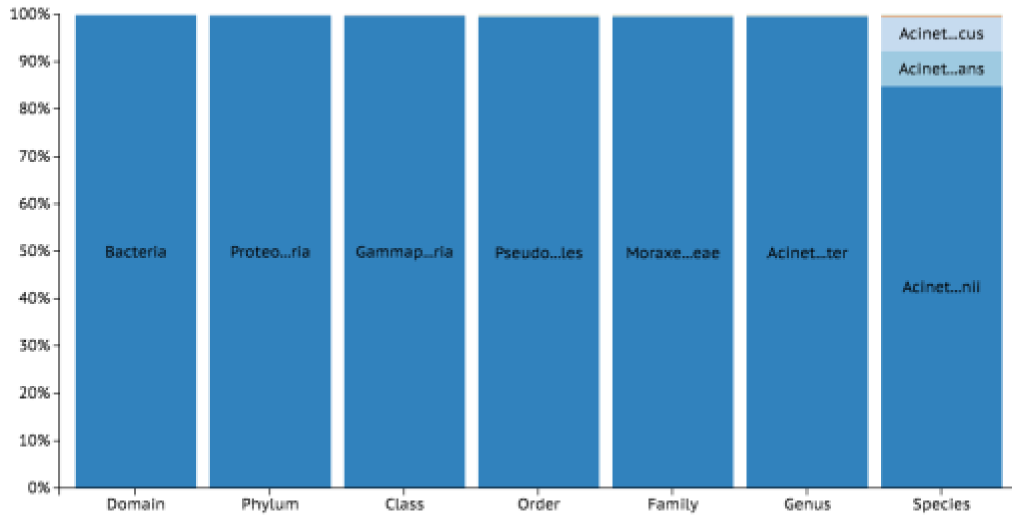

Fig. 4 shows a visual representation of the percent of reads classified to each level.

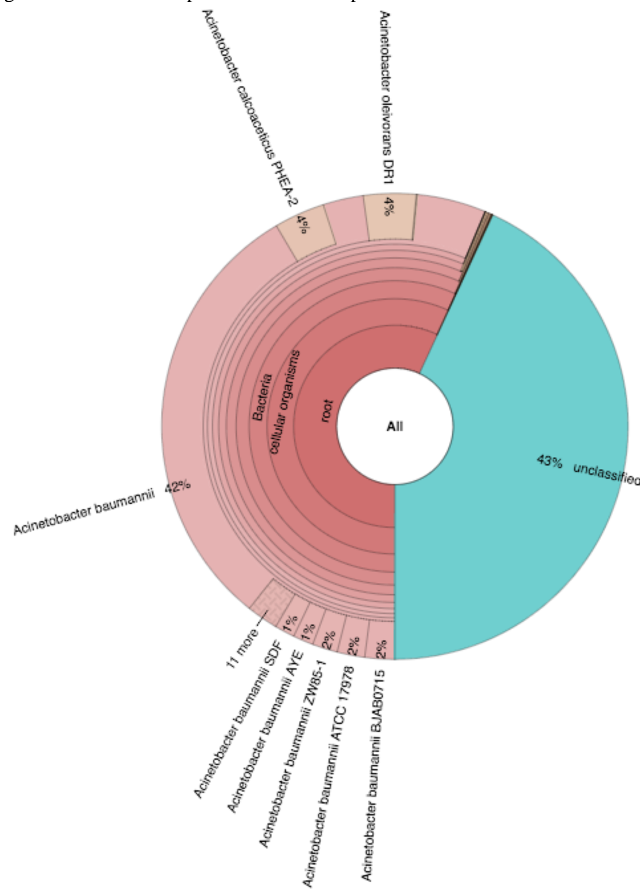

Fig.5 shows a Krona diagram of the analyzed reads.

The isolate showed resistance to both Cefazolin and Vancomycin with zone diameters less than 14 millimeters for both antibiotics, and partial resistance to Trimethoprim with a zone diameter between 11 and 15 millimeters. The isolate was susceptible to the other three antibiotics, Tobramycin, Gentamicin, and Amikacin with zone diameters larger than 15, 15, and 17 millimeters respectively (figure 6).

Fig. 6 shows the results of the antibiotic susceptibility tests.

Discussion

Along with identification methods found in literature, physiological tests and genomic sequencing identified the isolate as Acinetobacter baumannii (Karageorgopoulos and Falagas 2008). While this evidence is convincing, we must still take into consideration that only 53% of the genomic data was identified. This taken into account with the certainty of the genus gives a 48.36% certainty of the species and 56.8% certainty of the genus. Even with the amount of uncertainty, the evidence provided from literature and physiological tests strongly suggests that this isolate is Acinetobacter baumannii.

The physiological tests are all in line with that we would expect to see in Acinetobacter baumanii. As shown in our labs and literature, Acinetobacter baumannii is Gram-negative, an obligate aerobe, catalase positive, and oxidase negative. The identified isolate was also coccobacillus shaped, as to be expected. The species grows at a range of 20-42˚C, but grow

optimally at 33-35˚C. While the isolate shows some degree of antibiotic resistance, it was not as much as literature had suggested. Through these tests, we can confirm that the isolated microbe at least belongs to the genus Acinetobacter.

While the combination of physiological tests and genomic sequencing is convincing, the 43% of unidentified sequences still provides doubt to the identification of the isolate. Horizontal gene transfer is very common through bacterial species. While species share a core genome, there is still extreme amounts of variation in other regions. This could be the reasoning behind parts of the genome being unidentified. Through one study in Argentina public hospitals, it shows that Acinetobacter baumannii is capable of horizontal gene transfer, and four different variations of a membrane protein were found within the species (Mussi et *al.* 2011). By repeating the DNA extraction and resequencing the genome, clearer results could be achieved due to any lab error or contamination.

Acinetobacter baumannii has a reputation for being difficult to treat and an opportunistic pathogen (Howard et *al.* 2012). This means that it takes advantage of those who are already immunocompromised. It is responsible for different infections such as pneumonia, meningitis, urinary tract infections, and more. Due to its opportunistic behavior, it is easily seen why it thrives in hospitals (Howard et *al.* 2012), and as we saw in this study, college dorms. While Acinetobacter baumannii is known for being multi-drug resistant (Howard et *al.* 2012, Perez et *al.* 2007, Eliopoulos et *al.* 2008), the isolate in this study showed complete resistance to only two of the tested antibiotics, Vancomycin and Cefazolin, and partial resistance to Trimethoprim. This is half of the tested antibiotics which is still significant, but literature suggested the bacteria would be much more of a problem.

Even with the inconclusive data we received with this study, the physiological tests still show the isolated microbe belongs to the genus Acinetobacter. Through further testing and repeating tests with inconclusive results, the species of the microbe will be confirmed. The physiological tests conclude that the isolate belongs to this genus, but the genomic data has a large amount of uncertainty when it comes to species. By analyzing the core genome of Acinetobacter baumannii, the isolate species will be confirmed.

Works Cited

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