BIOL 342 Honors, Tuesday 9-12 **Vanessa Santana**

Isolation and Identification of *Staphylococcus saprophyticus* from a pet cat's mouth

Introduction

The isolation and identification of common microbes provides insight into the health of animals and people. The understanding of what microorganisms may be found in animals is essential to understanding the origin and epidemiology of zoonotic diseases.

The common domestic cat (*Felis catus*) is an excellent example of animal that may harbor pathogens that could potentially be harmful to humans. The saliva of cats may contain strains of *Staphylococcus* bacteria that are resistant to antibiotics (Lilenbaum et al. 1999), which could be dangerous to pet owners or professionals that may come into contact with domestic cats.

In this project I isolated a bacterium from my pet cat's mouth. My objective was to test this microbe in order to characterize and identify it. The tests performed include gram staining, physiological tests such as the oxidase and catalase tests and the use of an API strip. DNA was then extracted from a pure culture of the isolate and sequenced using next-generation sequencing at the University of Alaska Fairbanks DNA core lab. The information gathered in the tests was then compared to the scientific literature for verification of the identity of the microbe.

The bacterium that I isolated, *Staphylococcus saprophyticus*, causes urinary tract infections in humans, in particular women. It is still unclear the extent to which *Staphylococcus saprophyticus* strains from cats may or may not be responsible for human urinary tract infections (UTIs) (Widerström et al. 2007). Given that the transmission of bacteria from the oral cavity of pet cats to humans is very common (Booij-Vrieling et al. 2010), further studies could evaluate the epidemiology of UTIs caused by *Staphylococcus saprophyticus* strains that originate in pet cats. This would allow us to understand any risks that may be associated with interacting with domestic cats.

Methods

Sample collection and isolation: A sterile cotton swab was used to collect a saliva sample from the inside of a pet cat's mouth. The samples were inoculated onto a Tryptic soy agar plate (TSA), which was stored at room temperature (25°C) for 5 days, as described in Lab Handout 1. A white colony was selected from the growth on the TSA plate and then isolated using the pure colony streaking method described in Lab Handout 2. Six quadrant streaks were necessary until I obtained a pure culture. All plates that were inoculated for isolation were incubated for approximately 2-3 days at 37°C.

Gram staining: Gram staining is a differential staining technique that identifies which bacteria are gram positive and gram negative. As described in Lab Handout 4, gram positive bacteria stain purple and gram negative bacteria stain pink/red. This is because gram positive bacteria have a thicker layer of peptidoglycan in their cell walls, which prevents any crystal violet from leaving the cells once they have been stained with it. Gram negative bacteria have this peptidoglycan walls, through which crystal violet dye is removed from the cell when the cells are washed with ethanol.

DNA extraction: I extracted DNA from my pure culture using a Power Soil DNA extraction kit as described in Lab Handout 5. This kit allows us to extract DNA from the culture by lysing the cells and then purifying the DNA sample. I had inoculated a Tryptic soy broth (TSB) test tube with my culture for this extraction but it appeared to have been contaminated during the week prior to the DNA extraction lab. Therefore, I extracted DNA directly from a sample taken from a TSA plate containing a pure culture of my isolate.

DNA sequencing and data analysis: the entire genome of my isolate was sequenced in the University of Alaska Fairbanks Core Lab using next generation sequencing. The results were analyzed using BaseSpace, as described in Lab Handout 7. BaseSpace is a cloud-based computational tool that assembles the genome and then compares it to a database of known sequences. It gives the most probable identity of the species of the strain as well as information about the genome's GC content and details on genes that

the microbe has. Examples of sequences that this program identifies includes antibiotic resistance genes, functional genes and number of tRNAs. I used the BaseSpace applications SPAdes genome assembler to assemble the genome, Kraken metagenomics to identify my isolate and ran PROKKA in metagenome mode to identify functional genes.

Physiological Testing: Several physiological tests were performed on my isolate as described in Lab Handout 6. The physiological tests include:

- Oxidase test: this test identifies if the microbe has cytochrome c oxidase. This test was performed by placing a small amount of my isolate on an oxidase strip card.
- Catalase test: this test identifies if the microbe has the enzyme catalase. It is performed by placing a drop of H_2O_2 on a small sample of the microbe from the pure culture.
- Fluid thioglycollate test: This test identifies the oxygen class of my isolate. A tube of soft agar was inoculated with a sample of my isolate and incubated it at room temperature. After 3 days I checked to see how far down in the medium the microbes had grown by analyzing the turbidity of the soft agar.
- API E test strip: This test strip performs a variety of physiological tests. The test strip was inoculated according to the protocol in Lab Handout 6. It was then incubated at 37 °C for 3 days. This strip tests for phenotypic characteristics that allow further verification of the identity of my isolate.
- API Staph test strip: Similarly to the APIE test strip, this strip performs a variety of physiological tests. However, the results can be used for the identification of species within the genus *Staphylococcus*. I followed the protocol REF 20 500 provided in lab for this test. After the strip was inoculated I incubated it at 37 °C for 3 days.

Testing on differential and selective media: I inoculated 3 plates of selective and differential media with my isolate and incubated them for 5 days at 37°C, according to the protocols described in Lab Handouts 8 and 10. The differential media were:

- Eosin Methylene Blue (EMB) Agar: this medium is selective for gram-negative bacteria and identifies microorganisms that ferment lactose and sucrose.
- MacConkey Agar (MAC): this medium selects for gram-negative bacteria and has a pH indicator that reveals if acidic lactose fermentation is taking place.
- Mannitol Salt Agar: This media contains a pH indicator that changes color when mannitol is fermented, which lowers the pH.

Antibiotic resistance: According to the protocol in the Lab 9 handout, I inoculated 2 Mueller-Hinton agar plates with my isolate. On each plate I placed four different antibiotic disks in each quadrant to test for resistance. After an incubation period of 2 days at 37°C, I measured the diameter around the antibiotic disks that did not have growth and compared these values to a reference table provided in the handout.

Antibiotic resistance to Neosporin: Following the protocol described in the Lab 9 handout, I inoculated one Mueller-Hinton agar plate with my isolate. I then placed three drops of the antibiotic ointment Neosporin (active ingredients Bacitracin, Neomycin, Polymixin B and Pramoxine HCl) and incubated the plate at 37°C for 2 days. I then measured the diameter around the drops that did not contain growth.

Test for hemolysis with blood agar plates: A blood agar plate was inoculated with my isolate. I also added a small sample from my original culture to one side of the plate for comparison. After the plate was incubated at 37°C for 2 days, it was then compared to images of alpha and beta hemolysis available on the Vumicro website, to determine the type of hemolysis that my isolate does to the red blood cells in the agar. This determines if the isolate is pathogenic.

Biofilm formation test: To test if my isolate forms a biofilm, I followed a lab protocol provided by Dr. Mary Beth Leigh. Four tubes containing TSB and 1% of glucose were inoculated: the positive control tube was inoculated with *Bacillus cereus*, the negative control was not inoculated and two tubes were inoculated with my isolate. The tubes were incubated at 37 °C for 14 days. After incubation, the tubes were stained with crystal violet so that any biofilm that may have formed could be observed.

Results

Gram staining: The Gram staining process resulted in the majority of bacteria from my sample appearing purple under the microscope, with a coccus shape (Figure 1). The purple color indicates that my isolate is Gram positive.

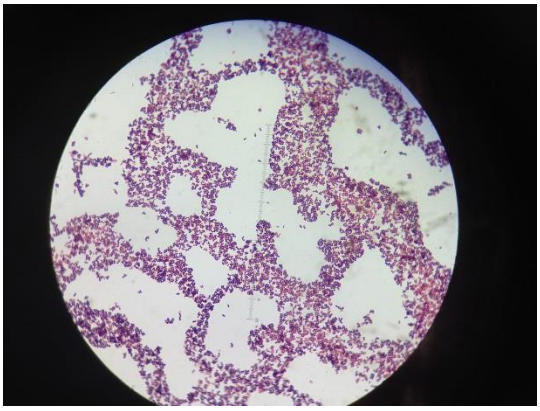

Figure 1: Gram stain of the isolate. Magnification 40X.

DNA sequencing: I obtained 219 contigs from my genome assembly. My longest contig was 83915 base pairs long. My isolate contains 61 tRNAs and was found to have 60 coding regions in its genome.

Regarding the species identification of my isolate, the majority of the reads that I obtained were unclassified. The greatest proportion of reads were identified to be of the species *Staphylococcus saprophyticus*. Of the reads that were classified, the majority belonged to the genus *Staphylococcus*.

Physiological Testing: The oxidade test result was negative indicating that my isolate does not have cytochrome c oxidase. The catalase test result was positive indicating that my isolate has the enzyme catalase. In the fluid thioglycollate test bacteria grew in the soft agar until just below the surface, in the oxic zone, indicating that my isolate is aerobic.

The results of the API E and API Staph test strips can be found in table 1. The API Staph test indicated that my isolate is of the genus *Staphylococcus*.

Table 1: API E and API Staph results

API E			**API Staph**		
Code on strip	**Test**	**Result**	**Code on strip**	**Test**	**Result**
ONPG	Beta-galactosidase	positive	GLU	Acidification (D-glucose)	positive
ADH	Arginine DiHydrolase	negative	FRU	Acidification (D-Fructose)	positive
LDC	Lysine DeCarboxylase	negative	MNE	Acidification (D-Manitose)	positive
ODC	Ornithine DeCarboxylase	negative	MAL	Acidification (D-Maltose)	positive
CIT	CITrate utilization	negative	LAC	Acidification (D-Lactose)	positive
H2S	H2S production	negative	TRE	Acidification (D-Trehalose)	positive
URE	UREase	negative	MAN	Acidification (D-mannitol)	positive
TDA	Tryptophane DeAminase	negative	XLT	Acidification (Xylitol)	negative
IND	INDole production	negative	MEL	Acidification (D-Meibiose)	negative
VP	Acetoin production	positive	NIT	Reduction of nitrates and nitrites	positive
GEL	GELatinase	positive	PAL	Phosphatase Alcaline	*
GLU	fermentation/oxidation - glucose	positive	VP	Production of acetyl-methyl-carbinol	positive
MAN	fermentation/oxidation - mannitol	negative	RAF	Acidification (Rafinose)	negative
INO	fermentation/oxidation - inositol	negative	XYL	Acidification (Xylose)	negative
SOR	fermentation/oxidation - sorbitol	negative	SAC	Acidification (Saccharose)	negative
RHA	fermentation/oxidation - rhamnose	negative	MDG	Acidification (Methyl-D-Glucopyranoside)	negative
SAC	fermentation/oxidation - saccharose	negative	NAG	Acidification (N-Acetyl-Glucosamine)	negative
MEL	fermentation/oxidation - melibiose	negative	ADH	Arginine Dihydrolase	negative
AMY	fermentation/oxidation - amygdalin	negative	URE	Urease	positive
ARA	fermentation/oxidation - arabinose	negative			
OX	cytochrome-oxidase	positive			

*reagent for this test was unavailable

Testing on differential and selective media: My isolate did not grow on the Eosin Methylene Blue (EMB) Agar. This medium is selective for Gram-negative bacteria so this indicates that my strain is not gram negative. My isolate also did not grow on the MacConkey Agar (MAC). MAC is selective for Gram-negative bacteria so this indicates that my strain is not gram negative. The pH indicator did not change, which indicates that fermentation did not take place when I inoculated this plate with my isolate. This is to be expected as my organism did not grow on this medium.

My isolate did grow on the Mannitol Salt Agar. In addition, the pH indicator of the mannitol salt agar changed from pink to yellow, which indicates that the pH dropped due to the fermentation of mannitol. Therefore my isolate ferments mannitol.

Antibiotic resistance: My isolate was resistant to the antibiotic Trimethoprim. It was susceptible to all other antibiotics tested when compared to reference diameter values provided in the Lab 9 handout (Table 2).

Table 2: Antibiotic test results

Antibiotic	Diameter (mm)	Result
Gentamicin	31	Susceptible
Vancomycin	22	Susceptible
Tetracycline*	40	Assumed susceptible
Cefazolin	35	Susceptible
Amikocin	32	Susceptible
Trimethoprim	0	Resistant
Erythromycin*	30	Assumed susceptible
Tobramicin	32	Susceptible

*indicates that reference values for diameter were not available. Diameters were large so the isolate was assumed to be susceptible to the antibiotics

Antibiotic resistance to Neosporin: My isolate appears to be susceptible to Neosporin as there was no growth around the drops of the antibiotic. The diameters were approximately 14 mm. Although no reference values were available for the diameters, my isolate appears to be susceptible to Neosporin (Figure 2).

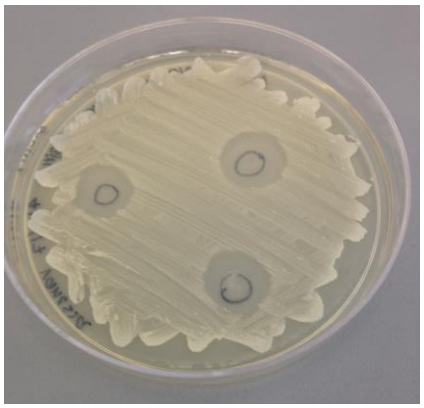

Figure 2: Isolate susceptibility to Neosporin

Test for hemolysis with blood agar plates: The area around where my isolate grew on the blood agar plate appeared green, which when compared to images on the Vumicro website, appear to correspond to alpha hemolysis (figure 3). Alpha hemolysis indicates that my isolate is pathogenic.

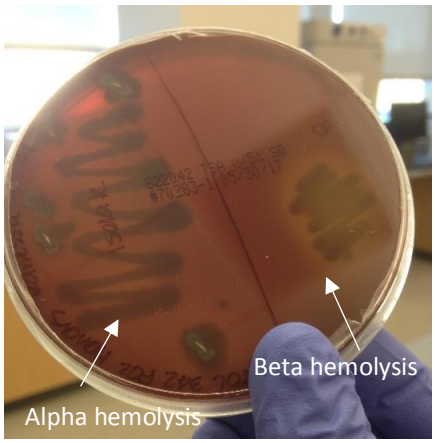

Figure 3: Blood agar plate inoculated with my isolate (left) forming alpha hemolysis. The right side of the blood agar appears to have beta hemolysis.

Biofilm formation test: the biofilm formation test revealed that my isolate forms a biofilm (Figure 4). The crystal violet stain indicated that a biofilm had formed in the tube, similarly to the positive control. The negative control did not form a biofilm.

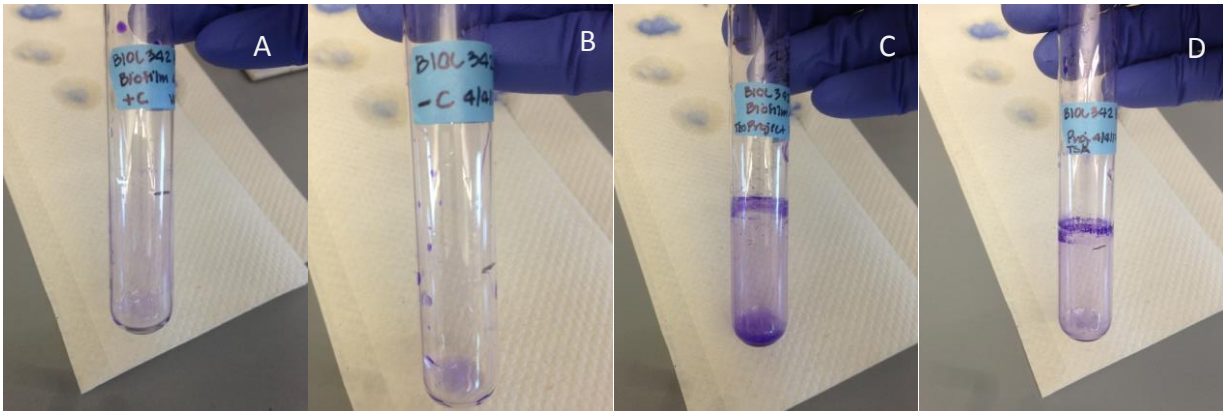

Figure 4: Biofilm formation test. The positive control (A) and both isolate samples (C and D) formed biofilms. The negative control (B) did not.

Discussion

Testing of my isolate revealed that it has several physiological characteristics that are coherent with the environment in which it was found. This environment, a pet cat's mouth, is constantly exposed to oxygen when the animal breaths, eats, plays or does any other activity that involves opening his mouth and exposing it to air. It is also a warm environment that contains a variety of nutrients from the cat's diet, which I make sure contains a variety of different foods. Overall I would expect to find an organism that can ferment several types of sugars, grows well at body temperature (37 °C), that can perform aerobic respiration and that survives in oxic environments. This was indeed confirmed by the several tests performed.

Staphylococcus strains are commonly found in the mucosa of animals, including cats and humans (Raz et al. 2004). Differentiation among *Staphylococcus* species may be challenging as many share colony morphology and other physiological characteristics (Ferreira et al. 2012).

The main feature of *Staphylococcus saprophyticus* that differentiates it from other *Staphylococcus* species is its capability to ferment mannitol (Shittu 2006). My isolate fermented mannitol when cultured on a mannitol salt agar plate. Since my genomic testing revealed similarity with several other *Staphylococcus* species such as *Staphylococcus aureus and Staphylococcus intermedius*, the identification of my isolate's capability to ferment mannitol was important for the final identification of my isolate's species.

Many studies have focused on the pathogenicity and epidemiology of urinary tract infections associated with *Staphylococcus saprophyticus* (Ferreira et. al 2012; Kuroda et al. 2005). Further studies could look at the specific strains that are associated with these infections and investigate their origins. I did not expect to find a species that is most associated with human urinary tract infections in my pet's mouth. This demonstrates that it is very possible that there are more risks that we immediately imagine with handling animals that we live with every day. It would be interesting to sample more cats and other pet species to see how commonly this species is found on them and if they represent an infection risk to humans.

References

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