BIOL 342 F02

Lab Report

Courtney Hill

An Exploration of Bacteria Found on a Personal Keyboard

Introduction

The use of technology has greatly improved medical and teaching practices and continues to be an effective resource for those purposes. However, issues continue to arise when cellphones, computers, and laptops in medical or laboratory settings act as harbors for not only benign microbes but also pathogenic ones. Contamination and spreading of these pathogens is propagated as cell phones and laptops are continually touched and exposed to various people and locations. Specifically, public computers are notorious as reservoirs for pathogenic bacteria (Anderson & Palombo, 2009). Therefore technology used by healthcare workers can become a disease gateway from hospitals to the public and vice versa. (Ustun & Cihangiroglu, 2012). Efforts to address these issues start with attempts to characterize these microbes and understand effective measures to prevent their spread.

In addition to the quest for identification of the microbes prevalent on surfaces of technology, it is apparent that methods to prohibit the growth of dangerous microbes must be harnessed (Gashaw et al., 2014). Antibiotic resistance has become common for organisms found on these surfaces and will become a larger problem if not acknowledged (Blankinship, et al.,

2013). For this reason, research efforts continue to probe and examine what kind of antibiotic resistance occurs on these surfaces and how it can be combatted.

To propel these understandings forward, this project isolated a microbe from a sample collected from a personal laptop keyboard. Genetic and physiological tests were conducted on the selected bacteria to confirm DNA sequencing results with testable characteristics. Identification was further studied to look at the viability of the bacteria against antibiotic agents. This paper presents an evaluation of one type of bacteria present on the surface of laptop keyboards and how the traits indicative of that bacteria can prove success on other technological surfaces as well an explorative look at the antibiotic resistance of the bacteria.

Methods

Collection and Isolation

To collect the sample I ran a swab dampened with sterile water over a personal laptop keyboard. I then streaked the swab over a plate of tryptic soy agar (TSA). The sample grew for a week at 20 ℃ in dark conditions. From there, a single colony was selected and streaked out three times to ensure a pure culture. The selected colony was yellow and circular with a convex elevation. Each instance the cultures grew on TSA plates and were incubated at 37℃.

Morphological and Physiological Tests

Once pure, the first test that was performed on the isolated bacterium was a Gram stain for determination of Gram-positive or Gram-negative (Lab 4 Handout). Physiological tests were conducted;detailed procedures and techniques for which can be found in Lab 6 Handout. These

tests were ran with a fresh culture that had been incubated at 37℃. Included in these procedures were:

1. The oxidase test to determine if the bacteria produced cytochrome c oxidase, an indicator of an electron transport chain
2. A test for the catalase enzyme, to detect if the bacteria has the ability to withstand oxidative damage
3. The fluid thioglycollate test to identify the oxygen class of the bacteria
4. An API 20E test strip was also inoculated to test for many characteristics of the bacteria including presence of various enzymes, decarboxylation of certain amino acids, fermentative capabilities of different sugars, as well as other tests. The breadth of tests in this strip can be found at the end of this report. After inoculation the test strip was incubated at 37℃ for 2 days before interpretation.

Genomic Testing

DNA was extracted from the isolate using the PowerSoil DNA extraction kit from MoBio (Lab 5 Handout). Centrifugation was conducted at 10,000 x g throughout and the final extraction was held in the 4C until sequencing. Sequencing was carried out using the Illumina MiSeq DNA sequencer. Analysis included genome assembly, taxonomic assignment, and functional gene annotation using BaseSpace(Illumina) (Lab 7 Handout).

Additional Tests

The ability of the isolate to grow on selective agars was also tested. The bacteria was streaked on both Eosin methylene blue agar and MacConkey agar, which both select against

Gram positive bacteria . Antibiotic susceptibility of the isolate was also explored (Lab 9 Handout). Antibiotics that were tested include Clindamycin, Gentamicin, Tetracycline, Piperacillin, Cefazolin, Erythromycin, Oxacillin, and Trimethoprim. Plates were allowed to grow for 2 days at 37℃ before measurements were recorded.

Results

Morphological Observations

Colonies were round and convex with even margins and pale yellow hue. A streak plate showing the colony morphology of the isolate can be seen in Figure 1. Under the microscope, individual cells were spherical and arranged in tightly clustered chains. Physiological Tests

The series of physiological tests conducted are reported in the Table 1. The majority of the tests on the API test strip were negative. The Gram Stain concluded that the isolate is Gram +; The isolate was not able to grow in the selective agars MacConkey or Eosin methylene blue.The fluid thioglycollate revealed that the bacterium was able to grow marginally throughout the media but was most successful in an oxic environment, meaning that the isolate is likely an obligate anaerobe. The isolate was oxidase - and catalase + suggesting the isolate is an enteric species and has protective mechanisms against toxic forms of oxygen.

Genomic Data

Genome sequencing was successful and provided strong confidence of identification to the species level. Assembly status applied by BaseSpace is recorded in Table 2: Assembly Report. There were 716 contigs recorded and 2138 coding regions were found. Taxonomic confidence levels are summarized in Table 3: Statistical Classification. Figure 2 shows the

assignment for each level of taxa. With 93% confidence to the species level the isolate was identified as *Staphylococcus aureus.*

Antibiotic Resistance Tests

Results from testing the isolate against a variety of antibiotics showed that the strain was susceptible to all. Table 4 summarizes the antibiotics tested and the diameters of the zones of inhibition around each. The isolate was not resistant against any drugs tested and was susceptible by large margins to the majority.

Discussion

Characterization of the bacteria isolated from the keyboard was verified using both genetic and physiological tests. DNA sequencing indicated the sample was *Staphylococcus aureus.* This taxonomic identification was corroborated with the variety of morphological and physiological tests conducted. *S. aureus* is a Gram-positive, oxidase negative, facultative anaerobe. These characteristics are consistent with my results. Additionally, the ability of my isolate to grow out on and ferment mannitol agar is indicative of *Staphylococcus aureus*. A color change of the mannitol agar from red to yellow indicated fermentation and a resulting drop in pH, another character suggestive of *S. aureus*.

S. aureus is a common bacterium that resides in the human nose and on skin. It is an opportunistic pathogenic and associated with skin infections. The bacteria can become more dangerous when entering the bloodstream. Recently, it has become an immediate health care problem as strains of methicillin resistant *Staphylococcus aureus* (MRSA) found in hospitals attack the immunocompromised. With little treatments options the disease is dangerous and

hospitals and nursing homes are at high risk (Peters, 2017). Schools have also become a concern for the pathogen as sports provide contact routes for skin infection to spread (Mahoney, 2015). Efforts to halt antibiotic resistance and find new targets for developing drugs are the at the pinnacle for this bacteria future study of this bacterium.

Tests for resistance against various antibiotics suggested the strain of *S. aureus* that I isolated is susceptible to a variety of antibiotics. While methicillin was not explicitly tested for, oxacillin, another penicillinase-stable penicillin was tested. The isolate was highly susceptible suggesting this strain of *S. aureus* is not a concern with respect to treatment options. Additionally, the mecA gene, which has been correlated to antibiotic resistance in strains of *S. aureus* (Beck, et al, 1986), was not identified in the genome assembly. This shows that the strain of *S. aureus* I collected has not developed methods of antibiotic resistance.

It is common that this species of bacteria will be found on keyboards. The majority of bacteria found on technology is from human contamination. Since *S. aureus* is a microbe normally found on humans the transfer of the bacteria can be easily facilitated from hands or sneezes. Survival and propagation of bacteria is dependent on nutrient availability. Food particles on a keyboard could be a leading factor for microbial success as well improper cleaning methods. Differences on microbial communities on keyboards differ in personal and health settings. Studies have exposed that public keyboards have pathogenic strains of MRSA (Kassem, 2007). It is essential that the public is aware of this threat and is informed on ways to prevent the spread of the bacteria. Healthcare settings should be wary in cleaning methods of technology as to not produce more antibiotic resistant bacteria.

Future research should include testing of different keyboards, potentially ones that are used by the broader public, as to gain a better understanding of what microbes can and do live on these surfaces. Private keyboards are as not as much of a risk considering that they are in a relatively controlled environment as they are accessed by less people. In addition to researching public keyboards, it may be more useful to focus on those utilized in public and healthcare settings, as these are environments where microbes such as MRSA are more prevalent More broadly, steps should be taken to sequence and identify broad samples of S. aureus to locate the causes of pathogenicity and how they can be targeted.

References

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Appendix

Extent of Tests in API 20E (http://microbeonline.com/)

ONPG: test for β-galactosidase enzyme by hydrolysis of the substrate o-nitrophenyl-b-D-galactopyranoside
ADH: decarboxylation of the amino acid arginine by arginine dihydrolase
LDC: decarboxylations of the amino acid lysine by lysine decarboxylase
ODC: decarboxylations of the amino acid ornithine by ornithine decarboxylase
CIT: utilization of citrate as only carbon source
H2S: production of hydrogen sulfide
URE: test for the enzyme urease
TDA (Tryptophan deaminase): detection of the enzyme tryptophan deaminase: Reagent to put- Ferric Chloride.
IND: Indole Test-production of indole from tryptophan by the enzyme tryptophanase . Reagent- Indole is detected by addition of Kovac's reagent.
VP: the Voges-Proskauer test for the detection of acetoin (acetyl methylcarbinol) produced by fermentation of glucose by bacteria utilizing the butylene glycol pathway
GEL: test for the production of the enzyme gelatinase which liquefies gelatin
GLU: fermentation of glucose (hexose sugar)
MAN: fermentation of mannose (hexose sugar)
INO: fermentation of inositol (cyclic polyalcohol)
SOR: fermentation of sorbitol (alcohol sugar)
RHA: fermentation of rhamnose (methyl pentose sugar)
SAC: fermentation of sucrose (disaccharide)
MEL: fermentation of melibiose (disaccharide)
AMY: fermentation of amygdalin (glycoside)
ARA: fermentation of arabinose (pentose sugar)

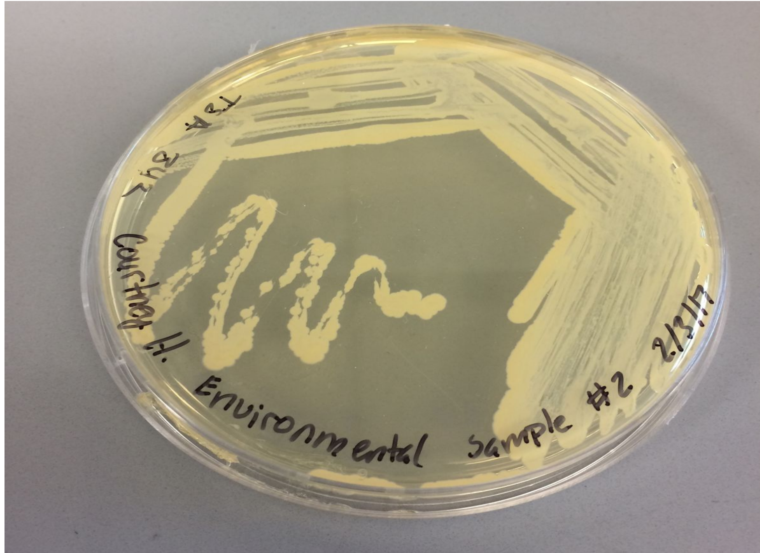

Figure 1. This streak plate is showing the colony morphology of the isolate, colonies are noted as yellow, round and have convex elevations

Physiological Tests			
Test	Result	**Test**	Result
Catalase	+		
Oxidase	-		
Gram	+		
Oxygen Class	facultative anaerobe		
API 20E			
ONPG	+	GLU	-
ADH	+	MAN	-
LDC	-	INO	-
ODC	-	SOR	-
CIT	+	RHA	-
H2S	-	SAC	-
URE	+	MEL	-
TDA	-	AMY	-
IND	-	ARA	-
VP	-	NO2	-

Table 1. Summarization of the various physiological tests conducted on the isolate. + signs indicate a positive result.

Assembly Report

Assembly	contigs	scaffolds
# contigs (>= 0 bp)	1068	1065
# contigs (>= 1000 bp)	716	713
Total length (>= 0 bp)	2511710	2511798
Total length (>= 1000 bp)	2284164	2284252
# contigs	980	977
Largest contig	36037	36037
Total length	2475183	2475271
GC (%)	33.36	33.36

Table 2. Various data collected from the assembly of genome sequence. (Retrieved from Illumina: BaseSpace)

CLASSIFICATION STATISTICS

Taxonomic Level	Reads Classified to Taxonomic Level	% Reads Classified to Taxonomic Level
Domain*	113,555	95.04%
Phylum	113,393	94.91%
Class	113,350	94.87%
Order	113,375	94.89%
Family	113,360	94.88%
Genus	113,099	94.66%
Species	111,793	93.57%

Table 3. Summary of confidence levels associated with each level of taxa for the genomic sequencing of the isolate (Retrieved from Illumina: BaseSpace)

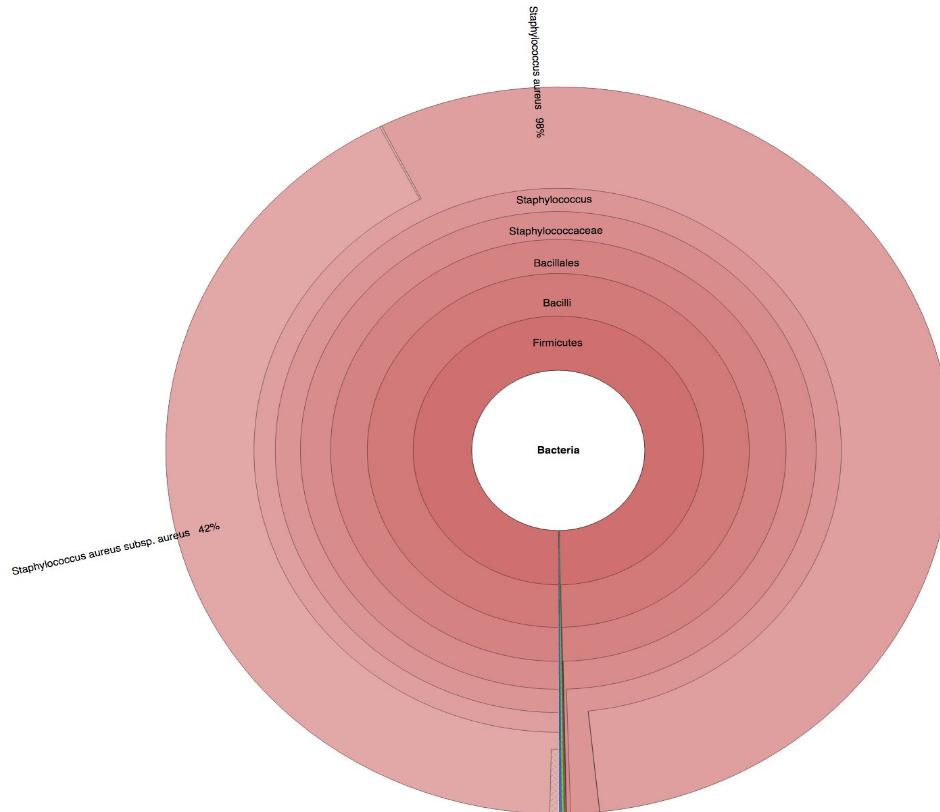

Figure 2. Representation of class assigned for each level of taxonomy. Each confidence level can be correlated with table above. (Retrieved from Illumina: BaseSpace)

Antibiotic Susceptibility Test					
Antibiotic	Inhibition Zone (mm)	Result	Antibiotic	Inhibition Zone (mm)	Result
Clindamycin	25	susceptible	Cefazolin	34	susceptible
Gentamicin	20	susceptible	Erythromycin	24	susceptible
Tetracycline	30	susceptible	Oxacillin	19	susceptible
Piperacillin	19	intermediate	Trimethoprim	15	intermediate

Table 4. Results of testing isolate against various antibiotics. Inhibition zone is measured in mm and result was conferred with literature. (Becton Dickinson, abridged)