Isolation of *Psychrobacter sp. Sarcosine-02u-2 clonal population* From Rasmusen Library Public Use Keyboard

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Introduction

The presence of public use computers on american college campuses is a staple resource. Public computer peripherals such as keyboards have many different users throughout the day. These public keyboards have been shown to have a rich and diverse bacterial microbiome (Blankinship et al, 2013). Keyboards with multiple users have been shown to have a larger chance of "harboring potential pathogens" and computer hardware has been a known vector of disease transmission in hospital settings for many years (Anderson et al, 2008). Public keyboards in non medical settings are not routinely or effectively sanitized. Because of the number of users per public keyboard, hand washing is also important in dictating the presence and diversity of bacteria (Messina et al, 2011).

To investigate the microbial state of public keyboards on the UAF campus I sampled one keyboard available to the public in the Rasmuson Library on the main floor. I expected to find bacteria such as *Staphylococcus aureus, Enterobacteriaceae, Enterococcus faecalis, Bacillus cereus,* or even *Escherichia coli.* These bacterial species have been isolated from multiple-user computer workstations at both educational and medical institutions (Anderson et al, 2008) (Messina et al, 2011). The listed bacterial species are often part of the human microbiota and have the ability to survive on the sampled surface for some time. *Escherichia coli* are part of the normal human microbiome but but certain subrgoups can be pathogenic. The presence of *E. coli*

on shared, public surfaces can be an indicator of poor handwashing amongst the local users (Semenov et al, 2011). The objective of this sample was to isolate a bacterial culture by swabbing and culturing, characterize the sample by metabolic testing and identify a bacteria by genetic sequencing.

Specifically, I hypothesized I would find a bacterium related to human activity that can survive on a variety of surfaces. I expected to find a specific bacteria that is abundant in the human microbiome and is passed from one person to another by poor hand washing such as bacteria present in human feces, human food crumbs, or bacteria specific to human skin oils. This hypothesis is of interest as it highlights a small component of UAF campus public health and solidifies that the way we interact with public computer hardware matters.

Methods

Using a wet, sterile cotton swab I sampled the key switch surfaces of a public keyboard on the main floor in the Rasmuson Library. I streaked the swab sample onto a Tryptic Soy Agar plate (TSA). I repeated the above process onto a Sabouraud's Agar plate (SA). I incubated for five days at low room temperature of 20°C. I observed 8 to 10 distinct bacterial or fungal colonies on the TSA plate and two to four smaller colonies on the SA plate.

I chose to isolate a distinct, uniform, and separate colony from the TSA plate after five days of incubation. Using the Streak Plate Method (Lab 2 Handout) I begun purifying the culture. The first streak plate was on TSA agar and incubated for three days at 37°C resulting in colony overlap. The second plate I chose to use the same agar but incubated at room temperature

(25°C) for about 72 hours in order to have controlled growth and be able to isolate a seperate and uniform colony. I repeated the process used for the second streak plate two more times. The Streak Plate Method was used in an attempt to isolate a pure and uniform bacterial culture. The presumably pure bacterial culture was then kept on a TSA agar slant in a 4°C refrigerator for the remainder of the experiment as a source bacterial culture for fresh agar plate streaking and for later testing.

In order to determine my bacterial sample's cell wall type I performed a Gram-Stain using procedure from (Lab 4 Handout). While observing the outcome of my Gram-Stain under 1000x total magnification I also noted the cell morphology and looked for uniformity in my bacterial sample.

I prepared a Tryptic Soy Broth (TSB) by inoculating a test tube of the broth with a sample of my isolated bacteria that was stored on a TSA slant. I incubated the TSB at room temperature for three days. Using the TSB as a sample source I attempted to perform a DNA extraction using a *PowerSoil® DNA* Isolation *Kit* and lab procedure outlined by (Lab 5 Handout). My attempt was not successful so the DNA extraction procedure was repeated by Alexis Walker, Teaching Assistant at UAF. The second attempt yielded a viable DNA sample that underwent whole genome shotgun sequencing at the UAF DNA Core Lab by Core Lab Coordinator Kyle Dilliplaine using an Illumina MiSeq System®.

I performed bioinformatic analysis of the sequenced genetic data provided by the UAF DNA Core Lab using procedure outlined in (Lab 7 Handout). DNA sequencing data from my sample was uploaded to PATRIC by Alexis Walker. Using the PATRIC platform I requested a Genome Assembly as well as Metagenomic Binning with Annotation and Taxonomic

Assignment using the uploaded DNA sequencing data. I uploaded my bacterial isolates assembled genome to KIJU® for further taxonomic assignment. I analyzed the bioinformatics reports for taxa, genomic features, protein features, specialty genes, and other physiological traits.

I took a sample of my bacterial isolate and streaked a MacConkey (MAC) agar plate as well as an Eosin Methylene Blue (EMB) agar plate using procedure outlined in (Lab 6 Handout). I used an isolate sample that was streaked on (TSA) and incubated at 25°C for four days to inoculate the (EMB) to test for Gram cell wall type as well as test the bacteria's ability to metabolize lactose. I streaked my bacterial isolate on to the (MAC) to test my samples ability to ferment lactose in the presence of bile salts. I incubated both plates at 25°C for three days then refrigerated them for future observation. I looked for color change on the selective and differential agar plates 7 days after inoculation.

I performed a series of physiological tests on my bacterial isolate using procedure from (Lab 8 Handout). I used an isolate sample that was streaked on to an agar plate and incubated at 25°C for about two days to ensure the colony was active. I performed a Fluid Thioglycollate test by incubating a test tube of thioglycollate with recently cultured and active isolate. Incubation was done by a taking a loop of isolate and performing a stab technique (Lab 8 Handout). This test determines my isolates oxygen class. I performed an Oxidase test using an oxidase test strip. A sterile plastic loop was used to administer an active sample of my isolate on to the strip in order to detect the production of cytochrome *c* oxidase. I performed a Catalase test by exposing a sample of active culture to 3% hydrogen peroxide to determine if my bacterial isolate produced the enzyme catalase. Using my bacterial isolate data I determined an API 20NE Test Strip was

best suited for physiological testing of my bacterial sample. Using (API 20NE Test Strip Protocol) and incubating the test strip for two days at 25°C and one day at 37°C. Because no physiological tests were observed I incubated the test strip for an additional three days at 37°C. I tested my bacterial sample for 20 different physiological traits at once. Testing for physiological traits such as nitrate reduction, indole production, glucose fermentation, mannitol assimilation and urease production.

I tested my isolate for antibiotic resistance (procedure outlined in Lab 9 Handout). To inoculate I used isolate streaked onto (TSA) that was incubated for 24 hours at 25°C. I created a bacterial suspension (procedure outlined in Lab 9 Handout) that was spread across two separate Mueller-Hinton (MH) agar plates and placed four different antibiotic disc samples onto individual, labeled quadrants on each (MH) respectively to test for antibiotic resistance. Totaling eight antibiotics being exposed to the isolate. These antibiotic discs were chosen at random. Antibiotics tested included amikacin, cefazolin, cefoperazone, erythromycin, oxacillin, tobramycin, gentamicin, clynomacin. I incubated for two days at 25°C and made observations about growth around the antibiotic samples. Measuring the diameter of no growth zones and comparing it to an antibiotic zone diameter interpretation table (Lab 9 Handout). I then compared this data to my metagenomic binning data.

Results

My initial keyboard sample yielded 8 to 10 visually distinct bacterial or fungal colonies (TSA) and two to four distinct colonies on (SA). Isolation using the Streak Plate Method resulted in a successful isolation of a pure bacterial colony. The isolate colony morphology had circular

form, with a raised elevation and an entire margin. Colony chromogenesis appeared buff with an opaque opacity and each colony was around 3mm in diameter. The isolate cell morphology was cocci and clustered with uniform cell composition.

Gram Stain results were gram-negative with successful positive and negative controls. MacConkey agar streak resulted in a culture growth with a pink color change of the isolate. (EMB) Streak resulted in culture growth with blue black chromogenesis, with no metallic appearance.

Physiological tests on API 20NE test strip (20NE) resulted in negative results for every single trait. Fluid thioglycollate testing classified the isolate as an obligate aerobe. Oxidase testing detected the presence of cytochrome *c* oxidase in the isolate. The catalase test had a positive result for the presence of catalase in the in the isolate.

Genomic analysis classified the isolate as *Psychrobacter sp. Sarcosine-02u-2 clonal population* using 1191 spades contigs. KIJU taxonomic assignment concluded that 97% of the isolate belonged to the Moraxellaceae family and the isolate was predominantly related to (70%) *Psychrobacter sp. SHUES1* with eleven different *Psychrobacter* species each respectively, composing less than 4% of the DNA sample (Figure 1). PATRIC binning yielded one bin and identified 0.8% of the tested sample was composed of contamination (Figure 5). KIJU detected 8 bacterial species that were not a *Psychrobacter* species, each composing 1% of the classification analysis respectively and did not classify 1% of the data used to generate the taxonomic assignment (Figure 1).

PATRIC detected 2759 protein coding regions in the genome assembly (Figure 3). PATRIC detected two virulence factor genes including pilG a twitching mobility gene and katA

a Catalase gene(Figure 4). PATRIC identified two genes associated with antibiotic resistance within the genome. Including gene tetH a Tetracycline resistance gene, coding for a EFS efflux pump (Figure 2). (MH) Agar antibiotic resistance testing identified two antibiotics the isolate is resistant to, oxacillin and clindamycin, with no visible border between the antibiotic disc and colony growth. Taxonomic assignment suggests culture isolation by streak plate method successfully isolated a *Psychrobacter ssp.* Colony, specifically consisting of the species *Psychrobacter sp. Sarcosine-02u-2 clonal population* (Figure 1) (Figure 5).

	PATRIC	RefSeq
Hypothetical proteins	676	0
Proteins with functional assignments	2083	0
Proteins with EC number assignments	0	0
Proteins with GO assignments	670	0
Proteins with Pathway assignments	0	0
Proteins with Subsystem assignments	0	0
Proteins with PATRIC genus-specific family (PLfam) assignments	2558	0
Proteins with PATRIC cross-genus family (PGfam) assignments	2576	0
Proteins with FIGfam assignments	0	0

Figure 4. Table of protein coding sequences identified by PATRIC from isolate DNA. This table is an overview of the abundance of identified protein coding regions present in the isolate sequencing data.

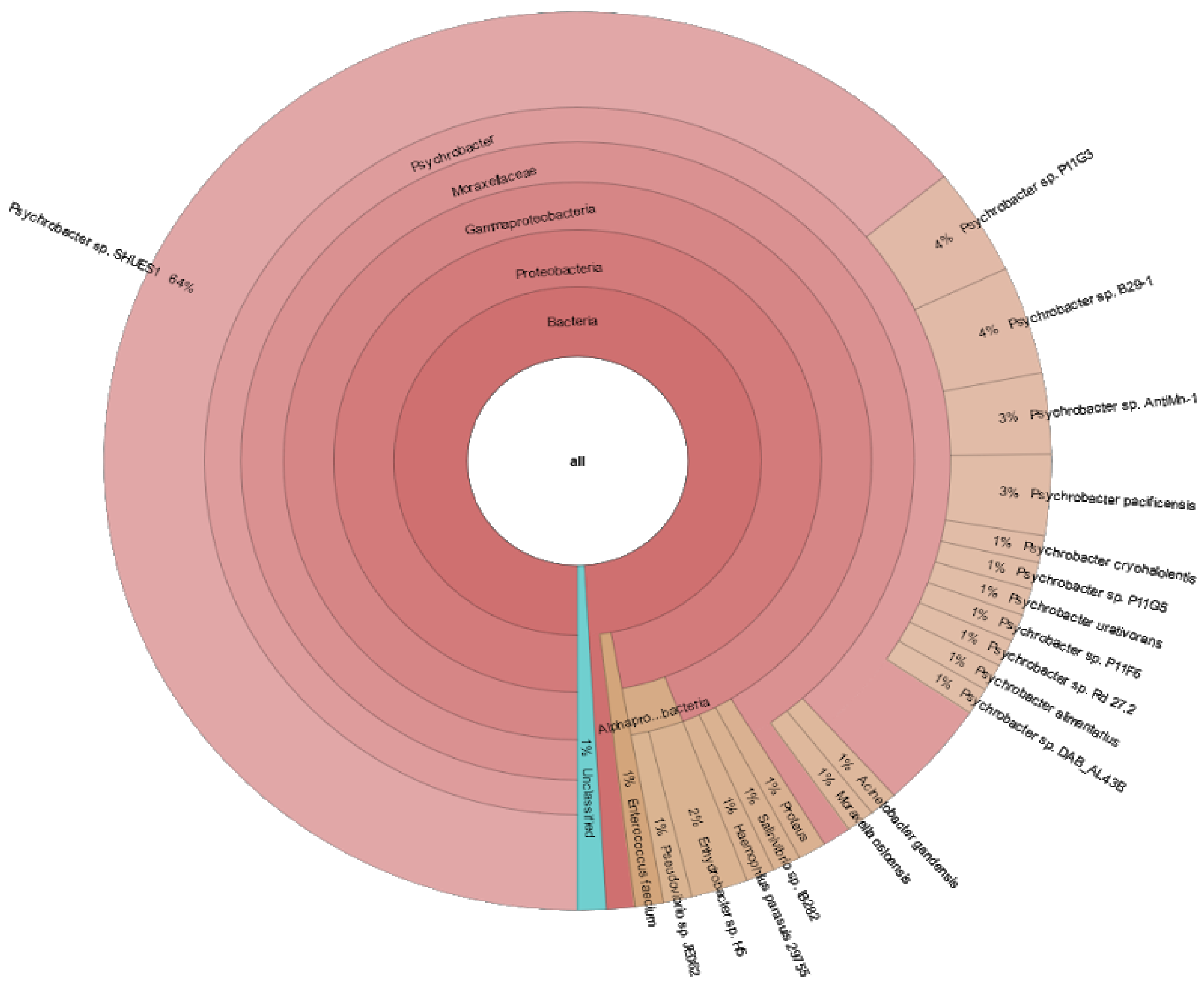

Figure 1. Krona taxonomic classification generated by KIJU. Providing a visualization of taxon abundance. Displays isolate relationship to genus, family and class as well as species with closely related genomes by match proportion.

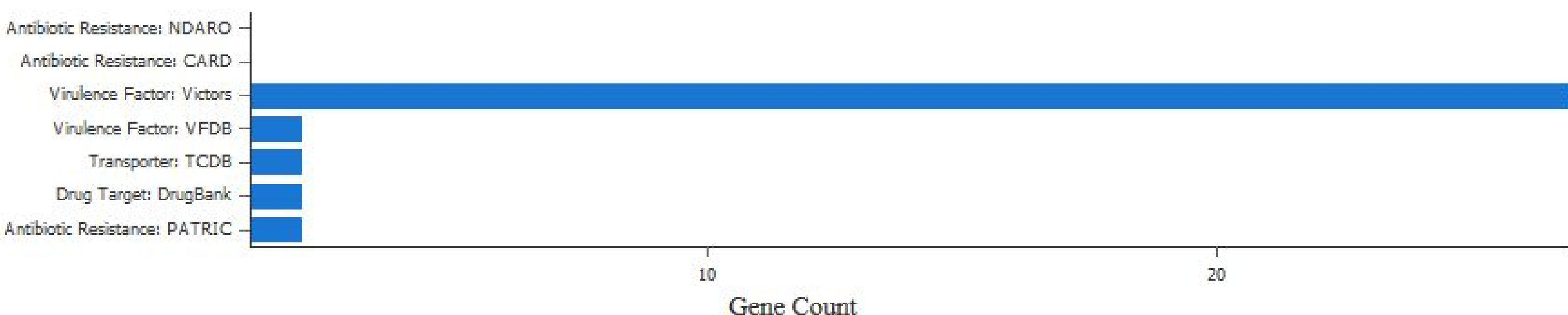

Figure 2. Bar graph generated by PATRIC metagenomic binning displaying abundance and type of specialty gene present in Psychrobacter sp. Sarcosine-02u-2 clonal population.

Sequence Info	
Contigs	88
Genome Length	3086329
GC Content	43.68672
PATRIC CDS	2759

Figure 3. Genome features of Psychrobacter sp. Sarcosine-02u-2 used for PATRIC bioinformatics. Displays the number of protein coding regions identified and classified during binning.

Reference Genome	Coarse consistency (%)	Fine consistency (%)	Completeness (%)	Contamination (%)	Contig count	DNA size (bp)	Contigs N50 (bp)	Mean Coverage	Potentially Problematic Roles	Good PheS
2058324.3	99.4	99.0	100	0.8	88	3086329	83268	22.71	22 roles	Y

Figure 5. Metagenomic binning table generated by PATRIC displaying data behind taxon assignment and specific binnig results. Lists reference genome number and percent match to isolate.

Discussion

Streaking of the initial keyboard sample resulted in a surprisingly high number (4) fungal colonies growing on the initial sample TSA plate (TSA1). I am concluding these were fungal colonies due to their distinct and characteristic culture morphologies. The other six morphologically distinct colonies on (TSA1) seemed like a high amount of diversity for one swabbed keyboard. Multiple-user computer workstations at another university had an average of 20 colonies per square centimeter and identified four different bacteria with unshared genera respectively. All keyboards tested presented both yeast and mold (Anderson G. et al, 2009).

The gram negative stain results were consistent with *Psychrobacter ssp*. cell wall type. Observed colony and cell morphology was also consistent with the taxonomic identification of the isolate (Bozal et al. 2003). Isolate growth on Eosin Methylene Blue agar further supported the gram-negative stain. MacConkey agar is a selective and differential medium that classifies bacteria based on their ability to ferment lactose, tolerate bile salts and grow in the presence of crystal violet; suggesting *Psychrobacter ssp*. could grow in these conditions (Lab 6 Handout).

It is reasonable to suspect physiological tests on API strip came back as false negatives. I discussed the (20NE) test strip choice with Alexis Walker. Considered APIWEB data which suggests the (20NE) strip is a good choice for testing *Psychrobacter ssp.*, and looked at EMB agar results to match my gram-negative isolate with the test strip. This suggests the (20NE) strip and *Psychrobacter ssp*. are a good match for physiological testing. A fresh and active culture should be used to inoculate the API test strip (Supplemental Lab 8 Handout). The culture I used may have been dormant due to the time it spent in refrigeration without another incubation

period before test strip inoculation. Though *Psychrobacter ssp.* can have a cold tolerance that is much lower than refrigeration temperature of 4°C (Bozal et al. 2003).

PATRIC detected a large number of coding regions in the sampled DNA (Figure 4.). PATRIC "Features" search identified genes corresponding to proteins that account for physiological traits *Psychobater ssp.* should test positive for on (20NE). Specifically (20NE) showed negative results for arabinose assimilation, urease, nitrite reductase and indole production. *Psychrobacter* species of human origin have been shown to have these physiological capabilities (Deschaght en al. 2012). PATRIC bioinformatics suggest the reference genome for *Psychrobacter sp. Sarcosine-02u-2 clonal population* contains coding regions for proteins capable of producing these physiological traits within the organism. An example of suspected (20NE) false negative includes mannitol assimilation and glucose fermentation, which is known to not be one of *Psychrobacter ssp*. physiological traits (Deschaght et al. 2012). Additional API testing material was not available to repeat the test.

Fluid thioglycollate test results are in agreement with *Psychrobacter ssp.* being obligate aerobes. Oxidase test and catalase test results further support the genus classification and further identify the isolate physiology (Bakermans et al. 2006) (Deschaght et al. 2012) (Bozal et al. 2003).

Taxonomy assignment of the isolate confidently pointed to the family *Moraxellaceae*. (Figure 1.) and PATRIC metagenomic binning (Figure 5.) suggest a *Psychrobacter ssp*. colony was isolated from a keyboard in the Rasmuson Library. Species identification of the isolate is less clear. The conflicting predominant species identification generated by KIJU and PATRIC is strange. PATRIC taxon analysis data (Figure 5.) presented little impurity, a mean contig

coverage over 20, and only presented 22 problematic roles all of which where either short contigs or already present in a longer strand and did not add depth to the read. Some contigs where too universal or repetitive to classify. The lack of problems with this analysis and a good match to a reference genome would suggest the species classification is accurate.

Kiju allowed 5 mismatches and matched 102 of 103 reads to reference genomes. (Figure 1.) does not list the species PATRIC identified. KIJU uses a different database then PATRIC so it is possible the reference genome one server used was not available on the other. The sample could have also contained several species of *Psychrobacter*. The major species taxonomic assignment by KAIJU was *Psychrobacter* sp. SHUES1 (Figure 1.) This species has genes that suggest it can produce lipase and protease but cannot produce urease. These are a combination of distinct physiologic features (Li et al, 2016). The keyboard isolate shares these traits based on a PATRIC "Features" search of the assembled genome. Further bioinformatics and precision isolation methods would be needed to confidently assign a taxon to species depth.

Psychrobacter ssp. has known tetracycline and streptomycin resistance (Petrova M. et al 2009) along with the oxacillin and clindamycin resistance presented in this case. The resistance genes PATRIC identified might be associated with its resistance to oxacillin and clindamycin as there were 27 listed (Figure 2.). Though I did not find a specific match between oxacillin or clindamycin and a specialty gene responsible for the resistance. Additional classification and genetic testing could identify which genes are responsible for the observed resistance.

Psychrobacter ssp. can live in extremely remote, cold environments (Petrova M. et al 2009) (Bozal et al. 2003). *Psychrobacter ssp.* isolates of human origin are also known (Deschaght et al. 2012). It seems reasonable to identify *Psychrobacter ssp.* in a human setting

located in a subarctic environment. The goal of this experiment was met though the isolate did not indicate a public health threat. Identification of bacteria such as *E. coli* or *E. faecalis* could indicate public keyboards are a potential public health threat (Semenov et al, 2011) (Messina G et al. 2011) (Dogan M et al.2008). Though some *psychrobacter* species can be opportunistic pathogens they are generally not a threat to humans. Public multiple-user computer peripherals should be treated cautiously with respect to public and personal health given the abundance of unclassified distinct colonies present on (TSA1).

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