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Biology F342

Unknown Bacterium Identification Paper

Intro:

While one may not always recognize it, humans interact with microbes in all aspects of their lives. In fact, one would be hard pressed to find a surface throughout their day that would not be teeming with microbial life. One such surface that most of us interact with on a daily basis is a shower floor. In general, showers are damp, dark places that come in frequent contact with human skin that may carry microbes from outside sources. In addition to bacteria, showers are notorious for hosting different types of fungal life. While this study is concerned specifically with bacteria, a study into the different types of fungus present on a given shower floor would be interesting. A specific shower floor that may have a more diverse and abundant bacterial community than normal would be one that was shared amongst 44 students living in a college dorm. Because of this prediction, I chose to sample the floor of the second floor mens shower in Bartlett Hall on the University of Alaska Fairbanks campus.

Many studies have explored the health and safety risks of using a communal shower and the precautionary steps one should take to protect themselves from unnecessary exposure to harmful bacteria. Pathogenic bacteria such as *Staphylococcus aureus* and *Legionella pneumophila* have been found in great numbers in communal showers in places such as hospitals and prisons. (Wadowsky et al. 1982) To find a high prevalence of these bacteria in a place like a college dormitory would pose a serious health risk to the student health population. Dorms represent a extremely densely populated living area where the spread of disease would be

extremely easy. By sampling from the Bartlett Hall shower, and isolating a pure culture, I hope to identify a specific strain of bacteria found in a dorm shower. Depending on the strain identified, the information gained from my experiment could help one better access the health risks associated with communal showers.

Methods:

The original sample was taken by swabbing the dorm shower floor with a sterile disposable swab and streaking it onto a tryptic soy agar (TSA) plate. Next the plate was sealed with parafilm to protect it from contamination and placed in a dark room temperature environment to grow for a week. After a week, many different types of bacterial colonies were present on the medium. One was chosen and used to restreak a new TSA plate. The second plate was then moved to a 37℃ incubator to stimulate faster growth. Over the course of another week a specific colony was chose and restreaked 3 times until a pure culture was formed. From there the pure culture was moved from the agar plate in a liquid medium of tryptic soy broth (TSB).

After week of growth in the liquid culture, the bacteria was used for DNA extraction. The DNA extraction was accomplished by using the PowerSoil DNA extraction kit and the kit's accompanying procedure. Once the DNA had successfully been extracted the 16s mRNA gene was sequenced using the University of Alaska Fairbanks miSeq DNA sequencer. The sequences were then analyzed using the online DNA analyzing software PATRIC and Kaiju.

In addition to genetic analysis of the bacterial sample, a series of physiological tests were performed. The isolate was subjected to a Gram stain in order gain more information about the structure of its cell wall and membrane. Determining if a bacteria is Gram negative or positive is

also one of the most useful classifying tools. Oxidase and catalase tests were also performed to test for presence of the cytochrome *c* oxidase and catalase enzymes and gain information on the metabolic pathways used by the bacteria. The oxidase test was done using an Oxidase Test Strip, while the presence of catalase was determined by exposing the isolate to hydrogen peroxide. Finally the API test strip Coryne was used to perform various physiological tests at once. API strips are used to determine physiological traits such as glucose metabolism, the presence of urea, and lactose metabolism. The Coryne strip specifically is meant for bacteria that are gram positive, rod shaped, and test positive for catalase. Finally, the isolate was grown on both Eosin Methylene Blue (EMB) and MacConkey (MAC) agars. EMB agar selects for gram negative bacteria, while differentiating lactose and sucrose fermenters. MAC agar also selects for gram negatives, while differentiating between lactose and peptone fermenters.

Results

Physiological Tests

Once the microorganism was isolated it grew best within the 37℃ incubator on a TSA plate. The isolate grew in circular colonies across the entire plate. Colonies were observed to be off white in color with little shine or height above the agar.

Test	Result	Test	Result
Gram	+	Penicillin Resistance	Susceptible
Oxidase	-	Gentamicin Resistance	Susceptible
Catalase	+	Oxacillin Resistance	Susceptible

Thio	Facultative aerobe	Vancomycin Resistance	Resistant
Piperacillin Resistance	Susceptible	Amikacin Resistance	Susceptible
Tobramycin Resistance	Resistant	Clindamycin Resistance	Susceptible

Table 1. Physiological Test Overview

Table one shows the results of various physiological tests performed on my isolate, as well as the results of eight antibiotic resistance tests.

Test	**Result**	**Test**	**Result**	**Test**	**Result**
NIT	+	βGLU	-	RIB	-
PYZ	inconclusive	βNAG	-	XYL	-
PYRA	-	ESC	-	MAN	-
PAL	+	URE	+	MAL	-
βGUR	-	GEl	-	LAC	-
βGAL	+	0	-	SAC	-
αGLU	-	GLU	-	GLYG	-

Table 2. Coryne API Test Strip results

Table 2 shows the results from a Coryne API Test Strip. Some tests within the strip resulted in an inconclusive outcome.

Data from the EMB and MAC selective agars was discarded due to contamination of the plates by a foreign colony.

Genetic Tests

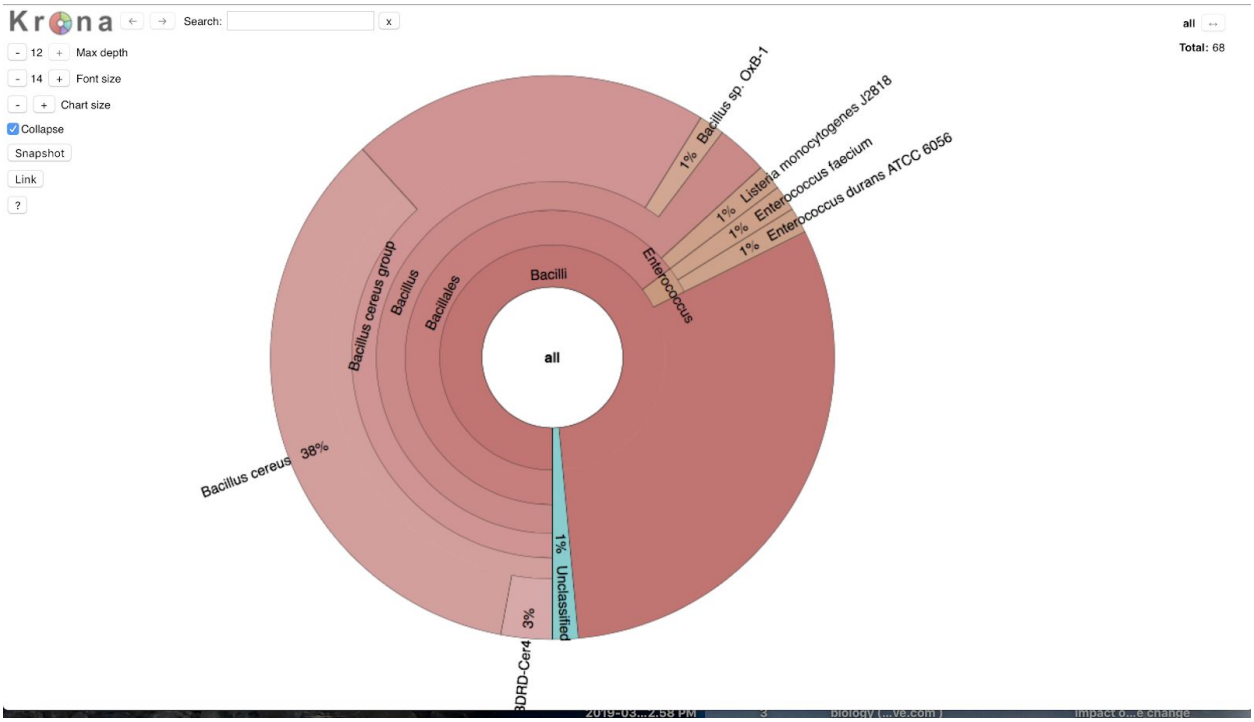

Figure 1. Krona genetic analysis summary

Krona summarized the isolates genetic data into a taxonomical pie chart. The chart shows the percent makeup of each taxonomic group present in the DNA that was sequenced. The largest individual species present was Bacillus cereus at 38% while the most prevalent family was Bacillus cereus group at 59%.

Discussion

After analyzing all of the experimental results from both the physiological and genetic tests I concluded that in all likelihood my unknown isolate was in fact Bacillus cereus. Bacillus cereus is a Gram positive, spore forming, rod shaped bacteria. It is also a facultative aerobe and is capable of producing Catalase (Bottone, 2010). While an endospore stain was not performed,

all other results are substantiated by the physiological tests that were performed. The API test strip test results did not match an online database of API strips that included Bacillus cereus. However, the database tested Bacillus cereus on the 20E test strip, while I used the Coryne API strip. By using the Coryne strip my results may have been significantly skewed.

Bacillus cereus refers to the species that I was able to isolate and identify as well as a larger family of seven different microbes. The closest relative to the species Bacillus cereus is Bacillus anthracis. Bacillus cereus is most often found naturally in soils or freshwater (Granum 2005). From these sources it has been known to infect humans through contaminated food or water. Based on this knowledge, the habitat that my isolate was taken from is not surprising. A shower floor could potentially collect bacteria from many other places people have walked, including various soils. In addition, a shower has a constant supply of fresh water. Within humans, Bacillus cereus is most often known to infect the gastrointestinal tract and cause severe diarrhea (Granum 2005). Bacillus cereus infections are commonly contracted through food poisoning, as its spores are extremely hard to remove and do not die during common sterilization methods such as pasteurization (Fratamico et al. 2010).

While it is not likely that someone will be eating food found on the shower floor, the risk of infection by Bacillus cereus is still high. Someone may have an open wound that comes in contact with the floor, or unknowingly transfer the bacteria from a surface to their mouth by some other mean. Shower shoes should be recommended to anyone using a public shower in order to reduce contact with an infected surface. One may also consider showering directly after the showers receive their daily cleaning. Future studies may include the characterization of all

microbes grown from the original streak, or perhaps the comparison of shower microbiomes across gender.

Works Cited

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