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The Isolation and Identification of a Microorganism Found on a Cell Phone Case

Introduction

Microorganisms can be found, though not seen, in almost any environment on the planet. Humans inhabit much of the earth, but microbes are able to live anywhere they have a suitable environment. Each microbe can live in a unique environment so some species of microbe can be found almost anywhere on the planet. This means bacteria can even live on us; on our skin. Depending on what microbiome human skin comes in contact with determines the skin’s microbiome. A study was done that shows that the owner of a phone can be identified by the uniqueness of the person’s skin microbiome due to the transfer of microbes between the two surfaces (Lax et al., 2015). This suggests that people’s hands can transfer microbes from anywhere, even surfaces that may be covered in pathogenic microbes. For example, cell phones of health care workers often carry nosocomial bacteria (Mir et al., 2010). The authors recommend that cell phones are cleaned regularly to avoid spreading bacteria further (Mir et al., 2010).

 This project investigates one bacterium of many that grew on a TSA plate inoculated with bacteria from a cell phone; a cell phone that is brought into the bathroom, kitchen and university classes and labs as well as anywhere else this college student goes. Every time the cell phone is used, bacteria transfer onto the surface from hands. Since the cell phone is constantly exposed to air, the bacteria isolated must be aerobic or at least possess the ability to survive in the presence of oxygen.

The objective of this study is to isolate a microbe from the initial growth plate inoculated from a swabbed cell phone. Tests will be run to determine the isolate’s metabolic ability/preferences and the presence of cytochrome c and catalase. In addition, tests for antibiotic resistance will be done. The sequencing of the isolate’s DNA will be most helpful in determining the bacteria’s species. Through the use of physiological and genetic characterization, the isolate’s identity can be determined.

Methods

*Isolation*

To begin isolating a bacterium, I ran a sterile swab soaked in sterile water over the edge of my phone case (Lab 1). I then streaked a tryptic soy agar (TSA) plate by running this swab in a zigzag motion over the surface of the plate. There was growth on the plate within two days of streaking and the first bacteria present was the one I chose to isolate at the end of a total of five days.

 The colony I chose to isolate was a flat, creamy white colony with a dull appearance. A series of three quadrant streaks, over a 13 day time period, were performed using pure culture techniques (Lab 2) to ensure complete isolation of the bacterium. The bacteria were always placed in a 37oC incubator overnight to grow. Each streak appeared to be a pure culture with identical colonies. The bacteria were transferred to sterile tryptic soy broth (TSB) and a TSA slant to preserve the isolate for future use (Lab 4).

*Physiological Trait Evaluation*

 To determine if my isolate was Gram-positive or Gram-negative, I performed a Gram stain according to the protocol in the Lab 4 handout. At the same time as analyzing the Gram stain I also observed the grouping, shape and characteristics of the bacteria under the microscope. A fluid thioglycollate test was performed to determine oxygen class. A catalase test was performed to determine if the bacterium uses catalase to protect itself against toxic oxygenic species. In addition, an oxidase test was used to check for cytochrome c oxidase which indicates the presence of an electron transport chain. I inoculated an API 20E test strip, which contains multiple physiological tests, according to the Lab 6 protocol. After two days of growth in a 37oC incubator, follow up reagents were added and results recorded.

*Antibiotic Susceptibility*

 Physiological tests were done to determine what type of antibiotics, if any, can inhibit the growth of my isolate. This was done using a disk diffusion test which can show whether an organism is susceptible, resistant, or intermediate in its response to a certain antibiotic as described in the Lab 9 handout.

*Genetic Analysis*

 In addition to physiological tests, I obtained DNA sequences from my isolate and analyzed the data to determine the species name. The DNA was extracted by cell lysis (breaking cells open to release DNA), removal of inhibitors and proteins (purification of the DNA) and obtaining a pure solution of DNA (usually in the buffer) using the PowerSoil DNA isolation protocol outlined in Lab 6. My DNA sample, along with the class’s samples, was sent to the Arctic Biology DNA Core Lab to be sequenced.

 To analyze my DNA sequences I used the computational methods through BaseSpace outlined in Lab 7. The data was processed through SPAdes Genome Assembler to assemble the genome of my isolate. I used the Kraken metagenomics program through BaseSpace to view the taxonomic assignment determined by the program by matching the sequences to others in a database. The final step of my analysis was the functional gene annotation which determined the possible functions of my isolates genes.

Results

*Physiological Tests*

The bacteria isolate had a circular, flat colony with a creamy, white dull appearance. When Gram-stained the microbes appeared rather large with evidence of nuclei inside the rod-shaped bacterium. The purple color of the stain indicated it is a Gram-positive organism: the rods were connected in long chains.

The fluid thioglycollate test showed growth through the whole tube but the aerobic zone was thickly coated with growth which suggests a facultative oxygen class. Both the oxidase and catalase tests showed positive results, which means the isolate has the ability to protect itself from oxygen species using catalase and contains cytochrome c oxidase which is involved in the last step of the electron transport chain. The organism tested positive on the API 20E test strip for ADH, TDA and GEL tests (table 1). ADH indicates the decarboxylation of the amino acid arginine by arginine dihydrolase. Positive TDA means presence of tryptophan deaminase. GEL tests for presence of gelatinase.

*Genetic Testing*

According to 81% of classified reads, the isolate is *Bacillus megaterium* (Chart 1). This is a relatively high percentage of classified reads which means the data is likely reliable. The assembled genome consists of are 374 contigs >100bp with the largest contig being 167446 sequence reads, including 5353 coding regions.113 tRNAs are present in the genome, 0 CRISPRS and 0 rRNAs. Two examples of tRNAs in the genome are tRNA-Arg-[57784,57862]36(tct) and tRNA-Ser c [56615,56707]37(tga). Several functional genes include 30s ribosomal protein S1 homolog which is a hypothetical protein. Second, Uncharacterized HTH-type transcriptional regulator YfiR which is a putative HTH-type transcriptional regulator YfiR. Also, probable spore germination protein GerPF, a putative spore germination protein GerPF.

*Antibiotic Resistance*

The final sets of tests were antibiotic susceptibility tests. The isolate was tested for susceptibility to cefoperazone, vancomycin, amikacin, trimethoprim, gentamicin, oxacillin, tobramycin, cefazolin. All diameters determined the bacterium is susceptible to all antibiotics (Table 2).

|  |  |
| --- | --- |
| Physiological Test | Result |
| ONPG: test for β-galactosidase enzyme by hydrolysis of the substrate o-nitrophenyl-b-D-galactopyranoside | Negative |
| ADH: decarboxylation of the amino acid arginine by arginine dihydrolase | Positive |
| LDC: decarboxylations of the amino acid lysine by lysine decarboxylase | Negative |
| ODC: decarboxylations of the amino acid ornithine by ornithine decarboxylase | Negative |
| CIT: utilization of citrate as only carbon source | Negative |
| H2S: production of hydrogen sulfide | Negative |
| URE: test for the enzyme urease | Negative |
| TDA: Tryptophan deaminase | Positive |
| IND: enzyme tryptophanase | Negative |
| VP: acetoin (acetyl methylcarbinol) | Negative |
| GEL: gelatinase | Positive |
| GLU: fermentation of glucose | Negative |
| MAN: fermentation of mannose | Negative |
| INO: fermentation of inositol | Negative |
| SOR: fermentation of sorbitol | Negative |
| RHA: fermentation of rhamnose | Negative |
| SAC: fermentation of sucrose | Negative |
| MEL: fermentation of melibiose | Negative |
| AMY: fermentation of amygdalin | Negative |
| ARA: fermentation of arabinose | Negative |

**Table 1:** The results of the API test strip and oxidase test. Note positive results TDA, ADH, and GEL tests.



**Chart 1:** Krona Classification chart of taxonomic classification from genetic testing analyzed through BaseSpace. Shows that 81.6% of reads were analyzed and those reads classify organism as Bacillus megaterium.

|  |  |  |
| --- | --- | --- |
| Antibiotic | Diameter | Susceptible diameters |
| Cefaperazone | 25 | >21 |
| Vancomycin | 20 | >17 |
| Amikacin | 21 | >17 |
| Trimethoprim | 25 | >16 |
| Gentamicin  | 28 | >15 |
| Oxacillin  | 19 | >20 |
| Tobramycin | 25 | >15 |
| Cefazolin | 28 | >18 |

**Table 2:** Antibiotic resistance/susceptibility diameters after 2 days shows that bacterium is susceptible to all tested antibiotics.

Discussion

 According to genetic testing, the isolate from my phone case is *Bacillus megaterium*. This genetic testing is enough to determine the identification of the bacteria. The literature is also consistent with this identification. The bacterium is easiest to identify because of its large size and production of endospores (Vary, 1994). When I viewed my bacteria under the microscope for the gram-stain, I noticed that the bacteria appeared very large; it was possible to see structures within the microbe. For the purposes of physiological identification, I compared *B. megaterium* to literature on the group of *B. subtilis* in some cases since, until recently, these have been grouped together based on physiological traits (Beesley et al., 2010) (Logan and Berkeley 1983).

Diversity has been found within *B. megaterium* now that genetic testing is more widely used.  It was previously thought that *B. megaterium* was taxonomically close to *B. subtilis,* but studies using 16S rRNA identification has found that there is a cluster of other *Bacillus* species that are more closely related to *B. subtilis* (Vary, 1994).

The literature I found which was specific to *B. megaterium,* indicates the bacterium lives in soil and utilizes aerobic respiration (Vary et al., 2007). It is capable of a wide variety of functions and adaptations. There has been an instance recorded that *B. megaterium* possessed gas vesicle genes, which would be great for a soil dwelling organism since soil is often flooded (Li and Cannon, 1997). This suggests that even though the microbe lives in soil, it still requires oxygen to survive.

There were several tests that I did not perform that would further support this identification. Literature indicates that *B. megaterium* has the ability to form endospores (Vary, 1994). To observe the presence of endospores, I could have prepared a slide of the isolate stained with malachite green (lab 4). In addition, my isolate could have been tested for resistance to penicillin since all members of the *Bacillus* genus are considered resistance except the disease causing member, *B. anthracis* which is the cause of anthrax (Celandroni et al, 2016).

The presence of *B. megaterium* on my phone case is well supported by physiological tests, genetic tests, and environmental preferences of the bacterium. The bacterium can live anywhere there is oxygen and a food source. *B. megaterium* often feeds on food sources we are familiar with such as honey, and fish (Vary, 1994). It is not a concern that the bacterium resides on my phone case since it is commonly known to be non-pathogenic (Celandroni et al, 2016). This bacterium has many useful applications such as the ability to metabolize cyanide (Castric and Strobel, 1969). Metabolizing a dangerous substance that can contaminate drinking water makes it a good candidate for water purification since the microbe itself is safe for humans.

Future research, as suggested by the literature, is needed for the identification of the many strains of *Bacillus megaterium.* Only with the recent advancements in genetic testing, has it been found that this bacterium is more taxonomically diverse than previously expected.

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