

Nikolas Pitsikoulis¹, Roddic Chen², and Dr. Marianne Williams³

¹Queens High School for the Sciences, ²Benjamin N. Cardozo High School, ³Queensborough Community College

Abstract

In this study, we aim to document the various species of transient (foreign) and residential (remain rooted within your skin) bacteria found within your hands and determine their effects on human health. To achieve this goal, we took advantage of residential bacteria's ability to remain embedded in the skin even after an extensive hand washing, allowing us to collect 20 samples, 10 of which were collected before a rigorous hand washing after a full day, and ten taken immediately after a thorough wash. DNA barcoding and Biolog were performed on the samples to analyze their properties, allowing us to determine whether our samples contained harmless, opportunistic, or pathogenic bacteria.

Introduction

Transient and residential bacteria are the primary forms of bacteria that occupy an individual's hands. Residential bacteria are often ingrained within the skin and remain there even after a thorough hand washing, while transient bacteria are picked up during day-to-day activities. Transient Bacteria may be removed by a thorough hand washing (NIH.gov, 1970). We aimed to analyze and determine the nature of various residential and transient bacteria using Biolog and barcoding techniques in this study. Our initial hypothesis, prior to any experiments, predicted that residential samples will contain bacteria that isn't harmful to human health, while transient samples were to contain bacteria that could lead to injury and illness if ingested.

Materials and Methods:

1. Samples and Collection:

20 samples are to be collected in total, with 10 samples being collected from each participant, respectively. We performed 12 consecutive hours of touching common surfaces and not washing our hands. Portions of each of the samples from our hands were transferred onto a soy agar plate before being incubated at room temperature for 24 hours and held in storage until further steps began roughly 3 days later.

2. DNA Extraction and subsequent analysis:

The Chelex Isolation Protocol was used to isolate and amplify the desired DNA of our samples for DNA Barcoding to be employed. In this process, bacterial colonies were allowed to incubate on a soy agar plate before being removed and added into Eppendorf Tubes containing 10% Chelex solution. The sample was then grinded before being heated and submerged in water for 10 minutes. Finally, after a 30-second centrifuge, the resulting supernatant was transferred to a clean tube before beginning the PCR. After this process, the samples were ready to undergo the full polymerase chain reaction (PCR), allowing us to amplify our data for observation. Gel electrophoresis was conducted to determine the viability of samples, which allowed us to determine that samples 1, 2, 3, 4, 5, 7, 14, 15, 16, 19, and 20 were all viable samples that could be sent to the lab for further analysis done through DNA barcoding. These samples were labeled in integers from 1-11 in numerical order before being sent for further analysis. In addition to the analysis done through Barcoding, Biolog was also used. Biolog requires the incubation of samples in a similar manner to Barcoding. Unlike barcoding, however, PCR isn't utilized. Instead, the samples are transferred to a clean inoculation tube before being transferred into a reservoir and eventually lab, after a brief transferring process.

Barcoding

10% Chelex Solution (100 µL), two sterile plastic pestles, pipet tips, tissue samples (bacterial colonies), Microcentrifuge tubes (1.5mL), aluminum foil, and a mug of boiling water.

Biolog

Inoculation tubes, agar-streaked plates, inoculating fluid, and reservoirs.

Sample #:	Barcoding results:	Nature of the specimen:
1	<i>Bacillus Pumilus</i>	Opportunistic
2	<i>Bacillus Circulans</i>	Opportunistic
3	<i>Bacillus Cereus</i>	Pathogenic
4	<i>Bacillus Pumilus</i>	Opportunistic
5	<i>Bacillus</i>	Unclear
6	<i>Paenibacillus anaericanus</i>	Opportunistic
7	<i>Kocuria rhizophila</i>	Opportunistic
8	<i>Bacillus subtilis</i>	Beneficial
9	<i>Bacillus</i>	Unclear
10	<i>Pantoea stewarti</i>	Opportunistic/Pathogenic
11	<i>Bacillus endophyticus</i>	Opportunistic

*samples 1, 3, 5, 7, 9 and 10 represent transient bacteria while the remaining samples represent residential bacteria.

Identified Species:	Nature of Bacteria:
<i>Clavibacter michiganensis</i>	Mostly Harmless
<i>Pantoea dispensa</i>	Opportunistic
<i>Rummeliibacillus pynus</i>	Mostly Harmless

Results: The combination of Barcoding and Biolog techniques produced a plethora of data ripe for analysis. Out of the two methods, however, our barcoding data proved to provide more information. Data from the barcoding data established that sample 1 was identified as *Bacillus Pumilus*, a species of bacteria commonly associated with agriculture and may have been found on our samples due to contact with plants and soil. *Bacillus Pumilus* is also an opportunistic bacterium, meaning that it could lead to infection if exposed to individuals that are immunocompromised. Samples 2 and 4 were identified as *Bacillus Circulans*, another species of bacteria commonly associated with soil. Like the previous sample, it's also opportunistic, and can lead to sepsis if presented the opportunity to. Samples 3 was identified as *Bacillus Cereus*, a well documented pathogen that can induce severe food poisoning on consumption. Samples 5 and 9 were identified as members of the genus of *Bacillus*, but no further classifications could be made, making it quite difficult to determine the nature of these samples. Sample 6 was identified as *Paenibacillus anaericanus*. Like previous samples, *Paenibacillus anaericanus* is commonly associated with soils, and rarely causes infection. Sample 7 was identified as *Kocuria rhizophila* and is associated with soils, rarely causing infection. Sample 8 was identified as *Bacillus subtilis*, a bacteria associated with soils and the GI tract. It's often acknowledged for its contributions to a healthy GI tract and is important for proper digestion. Sample 10 was identified as *Pantoea stewarti*, a plant pathogen well known for being closely related with *Escherichia coli*. *Pantoea stewarti* has been known to rarely lead to infections if presented the opportunity. Finally, we identified sample 11 as *Bacillus endophyticus*, a soil dwelling bacteria associated with plant pathogens. Our Biolog data identified 3 species of bacteria within our samples, *Clavibacter michiganensis*, *Pantoea dispensa*, and *Rummeliibacillus Pynus*. *Clavibacter michiganensis* is mostly known for its damaging effects on plant life. *Pantoea dispensa*, is another plant pathogen, which can rarely infect humans if given the opportunity. *Rummeliibacillus Pynus* is a spore forming bacteria that is mostly harmless to humans.

Discussion

The findings of this study provided some evidence for our initial hypothesis but remained inconclusive for the most part. This conclusion can be derived from the fact that most, if not all, samples tended to be opportunistic bacteria, with the only notable exceptions being *Bacillus Cereus* and *Pantoea stewarti*, being the only inherently pathogenic organisms found in this study. While these specimens were classified as being in the transient group, such a limited data pool doesn't provide us with enough information to make an entirely informed conclusion. One reason this data may be inconclusive could be related to the lack of data that could be extracted from samples 6, 8, 9, 10, 11, 12, 13, 17, and 18. In addition, samples 5 and 9 could only be identified as members of the genus *Bacillus*, a classification too broad to interpret properly. Combining this with the relatively small set of data extracted from the Biolog procedure creates a scenario where less data is available to analyze. Redoing the barcoding procedure could fix these issues in future alliterations of this experiment.

References:

Gen III test panel. Biolog. (2023, August 2). <https://www.biolog.com/products/microbial-identification-microplates/gen-iii-test-panel/>

"Disc Diffusion (Kirby-Bauer) Antimicrobial Susceptibility Testing." *YouTube*, YouTube, 27 July 2020. www.youtube.com/watch?v=M-szoknT00.

"polymerase chain reaction (PCR)" *Biology animation library - csh dna learning center*. Cold Spring Harbor Laboratory DNA Learning Center. (n.d.). <https://dnalc.cshl.edu/resources/animations/pcr.html>

DNA learning center barcoding 101. (n.d.). <https://dnabarcoding101.org/lab/protocol-2.html#alternate>

"Search NCBI Databases - NLM." *National Center for Biotechnology Information*, U.S. National Library of Medicine, www.ncbi.nlm.nih.gov/search/. Accessed 17 Apr. 2024.