

Oral Microbiome Isolation of *Chryseobacterium indologenes*

Introduction:

The human microbiome contains a vast diversity of microbial inhabitants based on location on or in the body. The oral microbiome contains the second most diverse bacterial community in the human body (Verma 2018) and this commensal diversity is beneficial for maintaining homeostatic health of the individual. Commensal diversity is beneficial in preventing any single microbe from over colonizing. Many of the oral microbes have pathogenic attributes but are often opportunistic and affect immunocompromised individuals when there is an increased biofilm (Bowen 2018). Cariogenic bacterial and pathogenic bacteria can thrive in the oral cavity due to the high nutrient sources. These pathogenic microbes are kept in lower concentrations due to the commensal bacteria that thrive in the oral microbiome (Avila 2010).

The purpose of my study was to isolate a bacterium from the oral cavity and view what microbe would be easiest to isolate. The oral cavity is of particular interest to me because of my desire to work in the dental field and the overall connection between the oral microbiome and human health. There are increased health risks associated with improper oral hygiene and oral diseases (He 2015). Over the course of the bacterial isolate project, I hypothesize that I will colonize a common commensal bacterium due to the abundance of this genre of bacterium. The most prevalent oral bacteria are often *Streptococcus spp.* although the sampling location will change what other types of bacteria present (Aas 2005).

Methods:

The following conditions were set for maximum bacterial presence: the evening before inoculation I did not brush my teeth nor ingest any water and then in morning I immediately

swabbed the specific areas. I utilized a tongue depressor to retract my left cheek and a sterile cotton swab was rotated on the distal-buccal surface of teeth numbered 14 and 15 for a duration for thirty seconds. The R2A (Reasoner's 2A agar) plate was inoculated by zig-zag motion of the moistened cotton swab across the plate. The plate was covered and wrapped in Parafilm then placed in a warm dark area for initial growth.

Bacterial isolation commenced 48 hours later by using the aseptic technique and the pure culture technique dictated in Lab 2 to move a single colony from the R2A plate and inoculate a TSA (Tryptic soy agar) plate. It was then placed in a 37°C incubator for 24 hours. This process was repeated 4 times over the following days until the bacterial growth on the TSA plates appeared to be uniformed in shape and color. The TSA plates were periodically refreshed utilizing the lawn technique to preserve bacterial life.

Gram-staining was performed in Lab 4 with the bacterial isolate to determine cell wall structure. Following the gram-stain testing, we utilized the aseptic technique to inoculate a TSB (Tryptic soy broth) tube and an agar slant for future genetic testing. DNA extraction was performed in Lab 5 with the PowerSoil DNA Isolation Kit then submitted to the UAF DNA Core Lab for whole genome analysis via Illumina MiSeq DNA sequencer. The genomic sequencing analysis of bioinformatics was performed via PATRIC and Kronas during Lab 7.

Additional testing was done in Lab 6 to verify cell wall structure by inoculating a MAC (MacConkey Agar) plate and an EMB (Eosin Methylene Blue) plate via aseptic and the zigzag technique to spread the culture on the agar. The MAC plate is used for enteric bacterial growth and to determine fermentation of lactose, while the EMB plate is used for enteric bacterial growth and determining lactose and sucrose fermentation.

In lab 8, physiological testing was performed to test oxygen class, presence of aerobic electron transport chain, catalase presence, and physiological processes. The fluid thioglycolate test utilized a tube of soft agar made from fluid thioglycolate. The aseptic technique was used to inoculate the tube which was then labeled and placed in the 37°C incubator for 48 hours. The catalase test was performed by placing a small amount of 3% H₂O₂ on a clean microscope slide then using a sterile inoculation loop; a small amount of the bacterial isolate was emulsified in the H₂O₂. To perform the oxidase test, an oxidase test strip was placed in a clean petri dish and a small amount of DI water was used to moisten the center of the strip. Using a sterile plastic loop, a small amount of the bacterial colony was smeared onto the moistened oxidase test. To determine roughly 20 different physiological processes, I used a 20 NE API test strip and followed the manufacturers' protocol to inoculate the entire strip. It was then covered, labeled and placed in the 37°C incubator for 48 hours before follow-up testing and interpretation. During Lab 9 we performed antibiotic testing on our isolates in which we placed eight different antibiotic discs on two Mueller-Hinton agar plates that were inoculated.

Results

Physical Properties

The morphology of the bacterial isolate on TSA plates were small round deep yellow-orange colonies with smooth borders and were dull with a slight sheen when rotated and tilted in the light as shown in Figure 1. Older isolations turned a deep dull orange color after sitting in the

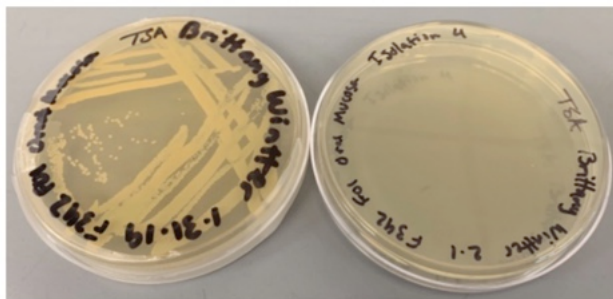


Figure 1: TSA plates during pure culture isolation

refrigerator. The bacterial isolate sparingly grew on the EMB plate and were colorless with no green sheen; showing that the isolate did not ferment lactose. No growth was exhibited on the MAC plate.

The results for the Gram-staining showed that the isolate is a Gram-negative bacterium and appeared as rod-shaped bacilli, roughly 1 μm in size as shown in Figure 2.

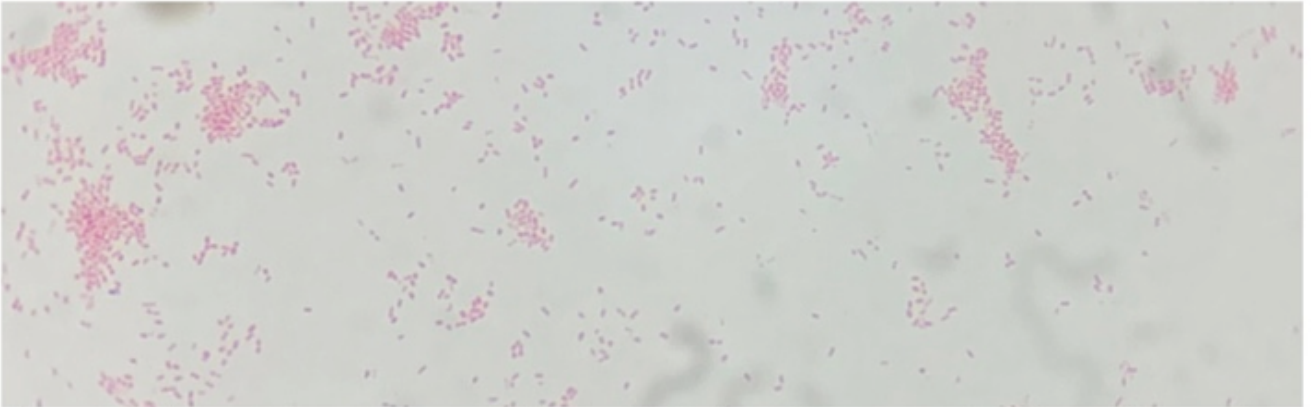


Figure 2: Microscopic appearance of bacterial isolate; 1000x magnification oil-immersion

Microscopes viewing of the isolate indicated that the bacterium formed in clusters. The small size made detailed viewing of the bacterium difficult.

Genetic Properties

The genetic analysis of the isolate on PATRIC showed there was one good bin for *Chryseobacterium indologenes* with 26 contigs, the longest contig is 832583 bp and the shortest contig is 496 bp with the total genome length assembles being 5058585 bp. The Patric analysis indicated that the *C. indologenes* had a high antibiotic resistance of 33. Genes for oxidase, catalase, and aquaporin z were present as well. The specialty gene list present on Patric was roughly 40 with many categorized as hypothetical proteins. The features list in Patric was immense, roughly 4800 results were found, including NADH hydrogenase, Glycosyl transferase, and Alanine transaminase.

Figure 3 shows the Krona Chart with 88% of the genome being that of *Chryseobacterium*.

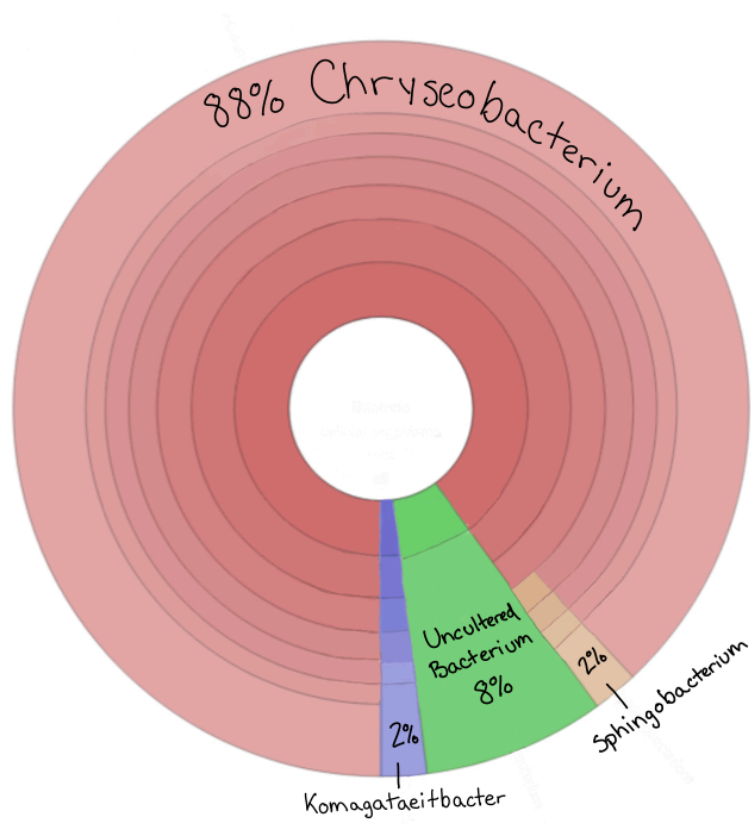


Figure 3: Krona chart showing genus *Chryseobacterium* as major contributor

Increasing the max depth in the Krona chart shows that the *Chryseobacterium* can be separated into further species as shown in Table 1.

Table 1: Krona Percent

<i>Chryseobacterium indologenes</i>	48%
<i>Chryseobacterium</i> sp. UNC8MF colony	22%
Uncultured bacterium	8%
<i>Chryseobacterium</i> sp. CBo1	2%
<i>Sphingobacterium</i> sp.	2%
<i>Komagataeitbacter nataicola</i>	2%

The closer view of the Krona chart shows that there are also three additional bacterium present: *Sphinogobacterium*, *Komagataeitbacter nataicola*, and uncultured bacterium with the three combined equaling 12% of the genomic data.

Physiological Testing:

The physiological testing results indicated that *Chryseobacterium indologenes* has a catalase positive response, an oxidase positive response and that it is an obligate aerobe through the fluid thioglycolate test. The 20 NE API test strip showed all of the tests were negative except for the ESC and GEL. The positive results for the ESC shows that the isolate can use β -glucosidase and the positive GEL shows it can use protease during hydrolysis.

The antibiotic test shown in Table 3 indicates that *Chryseobacterium indologenes* is highly resistant to many antibiotics.

Table 3: Antibiotic Test Results

Antibiotic	Diameter (mm)	Susceptible, Intermediate, Resistant
Ampicillin [Am 10]	Overgrown	Resistant
Cefoperazone [CFP 75]	9 mm	Resistant
Clindamycin [CC 2]	14 mm	Resistant
Erythromycin [E 15]	15 mm	Intermediate
Oxacillin [Ox 1]	Overgrown	Resistant
Penicillin [P 10]	Overgrown	Resistant
Tetracycline [Te 30]	Slight Halo; immeasurable	Resistant
Vancomycin [VA 30]	16 mm	Intermediate

There was only two antibiotics, Erythromycin and Vancomycin, that showed intermediate diameters of inhibition.

Discussion

My original hypothesis of the bacterial isolate from my oral cavity being a common commensal bacterium was not supported with the testing that was conducted. The isolate that was identified as *Chryseobacterium indologenes* is not identified as a common commensal bacterium.

Chryseobacterium sp. is a bacterium that is uncommon in humans but is considered pathogenic.

Cases studies have indicated that there is a lower than normal virulence due to is being opportunistic in immunocompromised individuals (Agarwal 2018). Research has shown that this is a commonly present bacteria in hospital settings and in the environment (McKew 2014). Lin et al. 2010 case study concluded that *Chryseobacterium indologenes* is a non-motile, oxidase positive, gram-negative bacilli which also confirms physical properties that were found during isolation. Case study by Jain et al. 2018 also supported my findings that the bacterium showed resistance to most antibiotics but showed susceptibility to Vancomycin. While my isolate only showed an intermediate diameter of inhibition, it could be that with a stronger dosage of Vancomycin there would be an increased susceptibility.

The closer look into the Krona chart indicated that there is a low percent certainty [44%] that the isolate is actually *Chryseobacterium indologenes*. This low certainty might indicate that the bacterial isolate could share very similar genomic information with *Chryseobacterium indologenes* but does not mean that it is an identical match. The physiological findings do point towards the isolate being *Chryseobacterium indologenes* due to the gram-negative, oxidase and catalase positive, and antibiotic resistance which is a commonality between the Patric results and lab testing. The 20 API NE test did not provide very much information because when searching though the BacDive website I could not find any *Chryseobacterium* in any of the tests. I did a search in each of the tests to ensure I was not missing the information, but it was not present, so I interpreted the two positive results for the 20 API NE based on the Lab 8 protocol sheet. I did encounter difficulty keeping my TSA plates alive after spring break. I had two labs, Lab 8 and Lab 9, that my plates and slants were nonvital, but my TSB broth was able to inoculate a new TSA plate. After inoculating a new TSA plate for each of those experiments I was able to perform the testing but viewing the newest plate after Lab 9 indicated that there was some form of contamination due to three large canary yellow colonies with irregular borders and raised

convex centers. I do not believe that this skewed my results due to the findings of the antibiotic testing being similar to the expected results via Patric.

Chryseobacterium indologenes was previously classified as a member of the genus *Flavobacterium* (Vandamme 1994). There is not much research information about this microbe prior to the reclassification and most of the information available is in clinical settings such as hospitals and very little environmental data is present. In conclusion, my isolate appears to be an unusual pathogenic bacterium that is not normally present in the oral microbiome. This might have appeared in my oral cavity when I used a non-sterile tongue depressor to retract my buccal mucosa to reduce contamination on the sterile cotton-tipped applicator, but I cannot prove this. Further testing would need to be conducted on the same teeth using a sterile tongue depressor and on the non-sterile tongue depressors.

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