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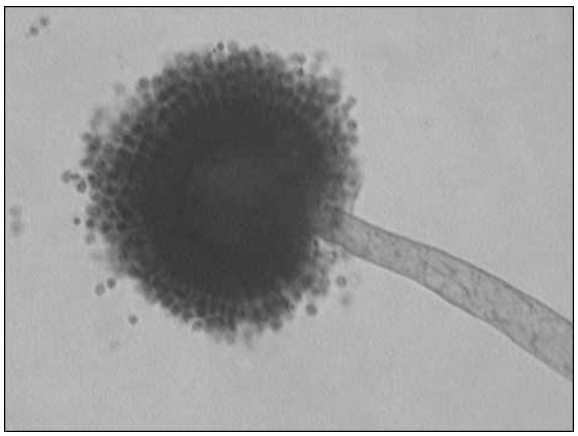

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The editorial staff wishes to thank Elaine Atnip and Jim Wilson for their support and assistance throughout the semester.

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Cover photo:
A photomicrograph of a prepared slide showing the asexual conidiospores of Aspergillus magnified 400x. (H. Wilson)

SIERRA COLLEGE JOURNAL OF MICROBIOLOGY

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Isolation and characterization of a yogurt sample produced from Rocklin, California

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Received 15 December 2006/Accepted 10 December 2006

Homemade yogurt was isolated from a sample made during the microbiology laboratory. Isolate colonies were obtained through the streak plate technique. This isolate was characterized through biochemical tests showing that the genus of bacteria that was isolated is of the genus *Staphylococcus epidermidis.*

INTRODUCTION

Yogurt is produced with a yogurt starter that usually a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in a ratio of 1:1. During the early stages of yogurt formation, the cocci present grow faster than the rods, and are primarily responsible for acid production (lactic acid), while the rods add flavor and aroma. The associative growth of the two organisms results in lactic acid production by either of the two when grown alone, and acetaldehyde (the chief volatile flavor component of yogurt) is produced by *L. bulgaricus* when growing in association with *S. thermophilus* (1).

The first step in yogurt preparation is reducing the water content of either whole or skim milk by at least one-fourth. This is easily accomplished by adding approximately 5% by weight milk solids or condensed milk to a sample of ordinary milk. The concentrated milk is then heated to 82-93°C for 3-5 hours. The finished product is usually cooled prior to consumption, and typically contains around 10^9
organisms per gram.

MATERIALS AND METHODS

A yogurt sample was made on October 3 from 1 quart pasteurized milk, 1/3 cup nonfat dry milk and 1/3 commercial, unflavored yogurt. First I added nonfat dry milk to the quart of fresh milk. Then I heated the milk to 120°F and then let it cool to 110°F. After that I removed ½ cup of the warm milk (110°F) and thoroughly blended it into the commercial unflavored yogurt. I then blended this mixture into the remaining warm milk. I put the milk mixture into a large sterile container and covered it. Next I placed the container in a pan of water maintained at 110°F. I let the milk ripen at that temperature until it was thick and had a tart, acid flavor. I then incubated it and let it chill in the refrigerator (1).

A loopful of the yogurt was transferred onto a BCP plate which was used as a standard media. A streak plate was made from a single colony. Once growth appeared on the streak plate a gram stain was performed in order to determine whether a pure culture existed on the streak

plate. When it was determined that the initial streak did not contain isolate colonies another streak plate was made using a single colony from the original streak plate. Once sufficient colonies formed on this plate another gram stain was performed to see if the streak plate contained a pure culture. Once it was determined that the streak plate contained only one type of bacteria I could move on to run some tests for the unknown of my bacteria.

Biochemical tests for bacterial characterization

The following biochemical tests were performed to characterize the isolated bacterium. A regiment of tests was designed to determine what the isolate was in the yogurt sample. Extraction of chromosomal DNA was done. A polymerase chain reaction (PCR) amplification was performed to allow specific segments of DNA to be amplified by utilizing some characteristic features of DNA. A gel electrophoresis of PCR was performed to allow DNA fragments to be separated on the basis of size and observed with the naked eye. Gel electrophoresis involves loading DNA samples into wells located at one end of a gel composed of agarose or polyacrylamide and subjecting them to an electric current. In our laboratory we mixed or DNA samples with a loading dye containing two types of stain/dye, xylene cyanol and bromophenol-blue. To run this type of test we used a purification kit called QiA quick PCR. Once our DNA was all cleaned up it was sent out to U.C. Davis Sequencing facility. Analysis of the electropherogram was completed to find my unknown. An electropherogram is a data collection software and computer analysis and is used to generate a visual record of the DNA sequence. The electropherograms generated by automated sequencers are four-color chromatograms displaying sequencing results as a series of peaks. A catalase and oxidase test was performed with groups of cells taken directly from the streak plate containing a pure colony. A gram stain was also performed using a group of cells taken directly from a pure streak plate. A MR-VP test was performed using a loopful of cells and inoculating a MR-VP broth. A coagulase test was performed by taking a group of cells and inoculating plasma. Lactose, arabinose, raffinose, sucrose and mannitol test were all performed by taking a group of cells and inoculating the slants. A urea hydrolysis test was performed by inoculating one slant of urea agar with my unknown organism by spreading growth over the entire slant surface. The remaining two tests included streaking a mannitol salt agar plate and tryptic soy agar plate to see if isolated colonies would form.

RESULTS

The results of the lactose, arabinose, raffinose, sucrose, MR-VP and mannitol tests are summarized in table 1. These series of tests test for the ability for fermentation with production of acid and gas (1). The results of the gram stain showed that the cells are in pairs or tetrads. The positive result of the catalase showed the bacteria are probably tolerant of aerobic conditions (1). The negative oxidase test tells us that our organism does not contain cytochrome C. The coagulase test came out negative. Coagulase is an enzyme produced by certain bacteria that catalyzes the coagulation of blood plasma. The tests tell us that our blood plasma remained a liquid. The result of the Vogues Proskauer test was negative which tell us that no acetoin formed. The methyl red test (MR) was positive for lots of acid. The result of the urease test is positive which means the organisms make urease and urea hydrolysis will result in ammonia formation (alkaline) (1). The mannitol salt agar plate grew isolated colonies along with the tryptic soy agar plate.

Table 1:

Biochemical Test	Results
Lactose	Positive (+)
Arabinose	Negative (-)
Raffinose	Negative (-)
Sucrose	Positive (+)
Mannitol	Negative (-)
Urease	Positive (+)
Catalase	Positive (+)
Oxidase	Negative (-)
Coagulase	Negative (-)
Methyl red	Positive (+)
Vogues Proskauer	Negative (-)

To figure out my unknown microorganism I went to NCBI, BLAST and obtained a sequence data and compared it to known sequences using the NCBI Blast. The identify of my species was *Staphylococcus epidermis.* The lineage included: Kingdom: bacteria, Phylum: firmicutes, Class: bacillales, Order: bacillales Family: Staphyloccaceae Genus: *Staphylococcus*. The query length was 807 and the gene bank length was 1498. My % similarity was 99% and number of bases matching pair wise was 796/800 without editing. The bit score was 1524 and accession number was AY741152.1

DISCUSSION

Staphylococcus epidermidis is a member of the bacterial genus *Staphylococcus,* consisting of Gram-positive cocci arranged in clusters. It is a catalase-positve and coagulase-negative and occurs frequently on the skin of humans and animals in mucous membranes. Due to contamination, *S. epidermidis* is probably the most common species found in laboratory tests (2).

Although *S. epidermidis* is usually non-pathogenic, it is important cause of infection in patients whose immune system is compromised, or who have indwelling catheters. Many stains produce a slim (biofilm) that allows them to adhere to the surface of medical prostheses. *S. epidermidis* is often resistant to a wide variety of antibiotics, including penicillin and methicillin.

Colonies of *S. epidermidis* are typically small, white or beige, approximately 1-2 mm in diameter after overnight incubation (2).

ACKNOWLEDGMENTS

Thanks to Sierra College Foundation for funding us money to run a microbiology laboratory. Thank you toThe North Valley & Mountain Biotechnology center at ARC for the lab supplies. Lastly, but not least we thank Harriet Wilson for advice and assistance in media preparation and laboratory.

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Isolation and Identification of Gram-positive Bacterium from Air Sample Collected in Citrus Heights

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This experiment was designed to isolate an airborne organism from an air sample collected in my living room. *Deinococcus* is microbe that can be found in the air. It grows in tetrads of Gram-positive cocci and although their cell walls are similar to Gram-negative bacteria, they stain purple like Gram-positive organisms. Results of DNA sequencing indicated that the organism isolated in this experiment was included in this genus. The information gathered in this experiment was insufficient to determine the exact species of *Deinococcus* isolated; however, the results of microbial tests indicated that it was likely from this genus. Based on results of other research studies and lack of information in the *Bergey's Manual*, it is highly likely that there are many species of *Deinococcus* that have not been identified at this date.

INTRODUCTION

Many species of bacteria are naturally occurring in the air. This experiment was designed to isolate one of these organisms from an air sample from my living room. *Deinococcus grandis*, a deeply branching, Gram-positive bacterium, was found to exist in the air in my living room. Deeply branching bacteria are named for their rRNA sequences and growth characteristics and are similar to the earliest bacteria. *Deinococcus* grows in tetrads of Gram-positive cocci and although their cell walls are similar to Gram-negative bacteria, they stain purple like Gram-positive organisms. *Deinococcus* is an interesting genus in that it can evade radiation damage due to the unique packaging of its DNA, radiation-absorbing pigments, and enzymes that repair damage due to radiation. (Bauman, 323)

MATERIALS AND METHODS

Materials: Nutrient agar, glucose medium with and without Durham tubes, microscope slides, potassium hydroxide (KOH), 3% hydrogen peroxide, Triple Sugar Iron Medium (TSI), laboratory microscope, Gram stain and capsule stain dyes and reagents, PCR ingredients including primers, dNTPs, *Taq* Polymerase, thermocycler, agarose gel, electrophoresis set up.

Methods: A Nutrient agar plate was left open in my living room for one hour. The plate was allowed to incubate at room temperature for two days. I selected a colony from the plate and restreaked on Nutrient agar to obtain a pure culture. Once the culture grew I performed a Gram stain on the organism, KOH test, capsule stain, and made a wet mount. 16SrDNA was isolated from the bacterial genome using the boiled and beat method and was then put through the

polymerase chain reaction with the primers 8 Forward Bacterial, and 1492 Reverse. The PCR product underwent gel electrophoresis and was cut out of the gel and sent to UC Davis for sequencing. The UC Davis facility used the Dideoxy Chain Termination Method to obtain sequences as well as an additional primer 533 Forward. The 3 sequences were viewed with 4 Peaks software. A contiguous sequence was obtained by removing overlapping sequences and combining the three fragments. This contiguous sequence was submitted to the Basic Local Alignment Search Tool on the National Center for Biotechnology Information website and the closest matches were displayed. Based on the results of BLAST search, Bergey's Manual was used to obtain information about the Genus of bacterium isolated and to determine additional physiological tests to be performed. The physiological tests included glucose fermentation, Triple Sugar Iron, catalase test.

RESULTS

Isolation of a pure culture on nutrient agar resulted in colonies with red, circular, lobate, convex, opaque, shiny with thick crusty edges, and a size of 1-4mm. Gram stain showed purple rod-shaped cells measuring approximately 1 micron in a variety of arrangements with the most frequent being pairs. KOH test resulted no change in the liquid. The cells were pink with a white space around them after capsule stain. BLAST analysis indicated a 93% similarity to *Deinococcus grandis* from the lineage Bacteria; *Deinococcus-Thermus; Deinococci; Deinococcales; Deinococaceae; Deinococcus.* The Query length was 499 base pairs and the gene bank length was 707bp. Bit score was 680 and the accession number was AJ585238. Organism produced bubbles when exposed to hydrogen peroxide. When incubated in glucose medium, tube remained red.* However, when inoculated on TSI medium, tube turned yellow.

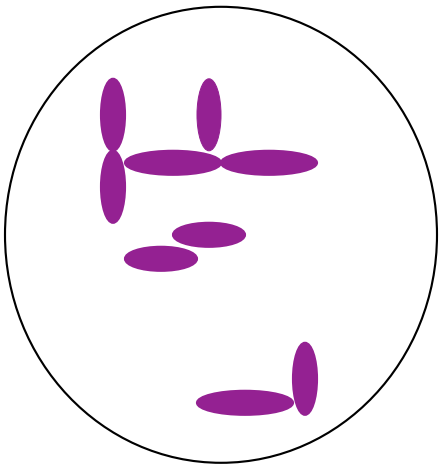

Table 1.1 Cell Morphology after Gram stain.

Test Name	Result
Gram stain	+
KOH test	-
Wet mount	-
Catalase	+
Glucose	- *
TSI	+

Table 1.2 Results of Physiological Test.
*See discussion section for explanation of result.

DISCUSSION

Initial observation of this organism led me to determine that it had rod shaped cells, but after further research it was determined that the cells were actually very large cocci. Because the BLAST search resulted in a 93% match in genetic sequence, it is likely that with a longer gene sequence would give a more accurate result. Also the Bergey's manual contained only information on the genus *Deinococcus* and not the *grandis* species, therefore, I was not able to determine which physiological test would be most beneficial in determining species. Result of the glucose fermentation test showed a negative result/no fermentation. This could have been due to a bad batch of glucose as others have had problems in this lab with glucose fermentation results. To further test this theory, TSI medium was inoculated and turned yellow indicating that the organism was able to ferment at least one of the three sugars.

Another species in the Deinococcus genus, *Deinococcus mumbaiensis* also shares a 94% similarity to *Deinococcus grandis* in 16S rRNA gene sequence. It also has red pigment, is non-motile, and has pleomorphic cells. In contrast, this species is Gram-negative. (Ravindranath). Deinococcus grandis stains Gram-negative with rod shaped cells whereas other species of *Deinococcus* cells stain Gram-positive and are spherical. (Suresh)

The information gathered in this experiment is insufficient to determine the exact species of *Deinococcus* isolated; however, the results of microbial test indicate that it is likely from this genus. Based on results of other research studies and lack of information in the *Bergey's Manual*, it is highly likely that there are many species of *Deinococcus* that have not been identified at this date.

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Isolation of Antimicrobial Components of Northern California Lichens

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In this experiment, two species of lichen, *Parmelia flaventior* and *Evernia prunastri* common to Northern California, were tested for antimicrobial properties (1). First, the lichens were subjected to a disk diffusion test against *Escherichia coli* (Gram negative), *Bacillus cereus* (Gram positive), and *Aspergillis* (mold) for antimicrobial activity. Both species demonstrated activity on the Gram positive plate. Consequently, a procedure designed for extracting organic acids was completed using both species of lichens. The final products obtained in this procedure were tested in different concentrations (5, 10, 25 and 50 percent) against the same bacterium mentioned above. Both final products prevented the growth of the Gram positive species indicating the isolation of the desired compound was achieved. Further tests may be completed to identify the compounds, if time permits

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INTRODUCTION

Historically, a large portion of the world's medicine has been derived from plants and fungi. Penicillin is derived from fungi, and salicylic acid (active ingredient in aspirin) is found in the plant Genus *Salix* (1). Lichens are another important, untapped resource of potential medicinal marvels.

Lichens (pronounced li-ken) are an organism consisting of a symbiotic relationship between fungi and green (Phylum: *Chlorophyta*) or blue-green algae (Phylum: *Cyanobacteria*). This composite organism behaves as an independent plant, with the algae manufacturing sugars via photosynthesis and the fungus metabolizes these sugars and composes most of the plant body. There are three different growth forms of lichens: foliose (leaf-like), fruticose (shrubby or hair-like), and crustose (crust-like) (2). The two species utilized in this experiment are *Parmelia flaventior* (foliose) and *Evernia prunastri* (fruticose). Lichens are unique organisms, which are difficult to study due to their extreme diversity, adaptability and the fact they are derived from organisms in two different kingdoms. Despite this difficulty, many lichens have been shown to demonstrate antibacterial properties, thus promoting an interest in this characteristic feature and advanced studies of the organism in general. In most cases, the component that is antimicrobial is an organic acid produced by the lichen (4).

MATERIALS AND METHODS

Lichen Test

Place a small amount of lichen (about .5 gram) in a sterilized mortar and add a small amount of sterile water (about 2 ml). Using a sterilized pestle, grind up the lichen in the water

until a paste-like texture is obtained. Using the prepared lichen paste, the disk diffusion method is performed using *Escherichia coli* (Gram negative), *Bacillus cereus* (Gram positive), and *Aspergillis* (mold). A control is used to demonstrate what negative results look like (lawn of bacteria on agar plate). Allow the plates to incubate overnight (approximately 12 hours). Observe the results and take note of any zones of inhibition of bacterial growth (Figure 1).

Extraction of Organic Acids

For this procedure, a large quantity of lichen is required (30 grams or more is recommended) (3). In this particular trial, 17.8 grams of *E. prunastri* and 20.1 grams of *P. flaventior* were used. Triturate the lichen to powder using a blender. If blender is not adequate, a mortar and pestle with a small amount of clean, coarse sand works very well. Transfer the powder to a Hirsch funnel and an exhaustive extraction with chloroform is performed under vacuum. The chloroform helps to remove all undesired impurities in the powder. Add approximately 120 mL of chloroform ($CHCl_3$) to the powder in the Hirsch funnel and allow to slowly flow through the powder under vacuum. Repeat this procedure using fresh chloroform until it does not exemplify activity on thin layer chromatography (TLC) paper under ultra violet light. To test for ultra violet activity, take a small amount of the extracted chloroform and spot on TLC paper and observe under ultra violet light (Figure 2). Allow remaining powder to thoroughly dry under vacuum to ensure all chloroform is removed. Discard the chloroform layer. Add approximately 120 mL of acetone (C_3H_6O) to the remaining powder in the Hirsch funnel and vacuum under filtration. The acetone will extract the desired product from the powder. Repeat this procedure until the acetone is not UV active. After completion, the remaining powder can be discarded. Evaporate the acetone in a fume hood. The remaining solid is solubilized with approximately 75 mL of ethyl ether. This solution is treated with approximately 10 mL of a 5% solution of sodium bicarbonate ($NaHCO_3$) in a separatory funnel. Drain off the aqueous layer into an Erlenmeyer flask. Repeat this procedure four times. Add 1M sulfuric acid to the aqueous layer until the solution is acidic. Test the aqueous layer with pH paper to ensure the solution is acidic. The desired acid will precipitate out of solution and allow the solution to cool under ice for approximately 10 minutes. Filter the solution under vacuum and allow the solid to dry overnight (3).

Antibacterial Tests for the Extracted Solid

Take the solid obtained in the preceding procedure and using water make liquid solutions in the following concentrations: 5%, 10%, 25%, and 50%. Perform a disk diffusion test using these solutions and test against the same bacterium used in the first test. Incubate the plates at room temperature for 24 hours. Observe the results and take note of any zones of inhibition of bacterial growth. Procedure is replicated to ensure accurate results.

RESULTS

Figure 1: Example of Agar Plate

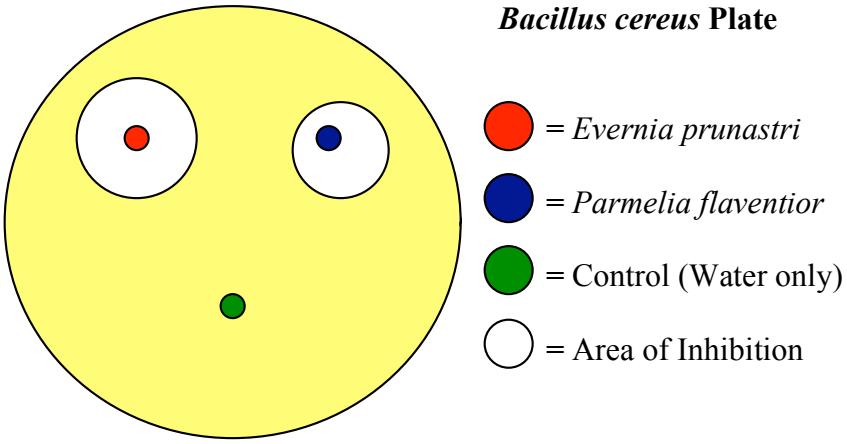


Figure 2: Example of TLC Plate

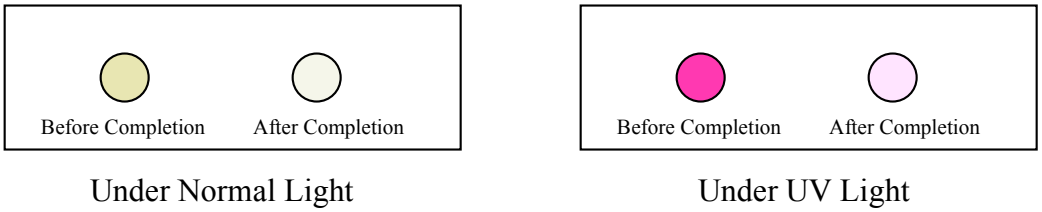


Figure 3: Chart of Lichen Treatment (Before Extraction)

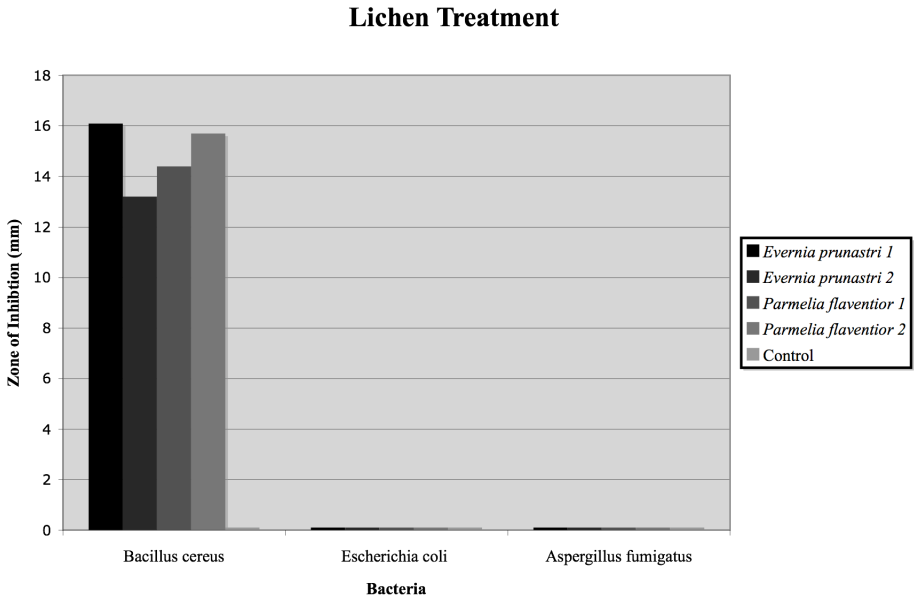


Figure 4: Chart of Lichen Treatment (After Extraction)

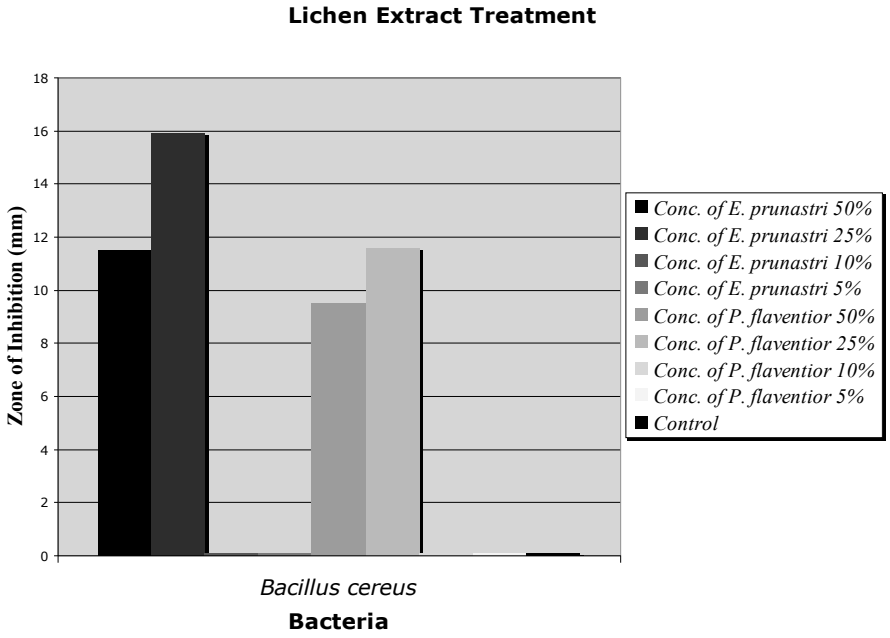


Figure 5: Results of Extraction

Example procedure used 65.5 grams of lichen and produced 9.25 grams of acid (example procedure from (2)).

Evernia prunastri

$$\text{Ex. Yield} = \left(\frac{17.8\text{ g}}{65.5\text{ g}}\right) = 27.18\% \text{ x } 9.25\text{ g} = 2.51\text{ g}$$

Parmelia flaventior

$$\text{Ex. Yield} = \left(\frac{20.0\text{ g}}{65.5\text{ g}}\right) = 30.53\% \text{ x } 9.25\text{ g} = 2.88\text{ g}$$

Evernia prunastri

After extraction total mass = .050 g

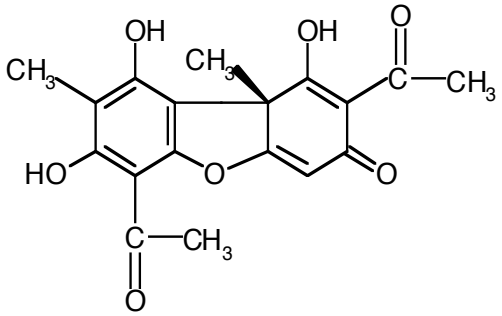
$$\%\text{ Yield} = \left(\frac{.050\text{ g}}{2.51\text{ g}}\right) \text{x } 100\% = 1.9\%$$

Parmelia flaventior

After extraction total mass = .693 g

$$\%\text{ Yield} = \left(\frac{.693\text{ g}}{2.82\text{ g}}\right) \text{x } 100\% = 24.6\%$$

Figure 6: Common Organic Acid Found in Lichens (5):



Usnic Acid

DISCUSSION

The isolation of antimicrobial components from two species of lichens showed significant results. In the first disk diffusion test, using whole species of lichens and water, *Evernia prunastri* (fruticose) demonstrated the greater zone of inhibition (16.1 mm). An explanation for this occurrence could be higher production of the acidic component in the lichen, the acid produced by this lichen may be superior in bacterial inhibition or there may have been greater extraction purity (3). To further confirm these results, the isolated acids from both species of lichen were tested in different concentrations. As expected, the isolated product from the fruticose lichen demonstrated greater bacterial inhibition. Furthermore, in both extracted acids the prevention of bacterial growth was shown to be the greatest when the acid was at 25% concentration. Intuitively, one would believe a higher concentration of acid would be greater in preventing bacterial growth, however the results in this experiment show that a lower concentration is superior. Further testing of the exact composition of the acids would be necessary to help explain these results. Finally, the percent yield was low in this experiment due to inconsistencies in the extraction procedure.

In conclusion, my research demonstrated that two Northern California species of lichen show antimicrobial activity against gram-positive bacteria. This is significant because research on lichens is very limited and these results show further studies will yield great outcomes. Medical researchers are constantly seeking out new sources of medicine and the largely untouched world of lichens may provide investigators with some innovative remedies. Although the products in this experiment demonstrated antimicrobial properties, their actual significance is still unknown. Further tests are required to confirm their specific identities, at which point additional conclusions can be made.

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Identification and Isolation of *Lactococcus lactis* from cultures of buttermilk

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Unknown cultures of *Lactococcus lactis* were identified from organisms in buttermilk through Polymerase Chain Reaction isolation of the 16s ribosomal RNA. Identification programs were used followed by confirmatory tests. The organism was thoroughly observed and researched to determine results and specific characteristics. *L. lactis* is a very important ingredient found in milk and dairy and has been around for centuries.

INTRODUCTION

I was curious to find out what one of the main organisms is in buttermilk. By selecting the organism that look like it grew the most I was able to isolate it. *Lactocuccus lactis* is a common contaminant in milk and dairy, and a microorganism in many kinds of cheese. The source of this organism is in raw milk and milk products, plants and nonsterile frozen and dry foods (Hardie 1909). The culture being tested was found in buttermilk. The species *L. lactis* has two subspecies *lactis* and *cremoris*. Proteins in milk are coagulated by cultures of *Llactis* to form curds ansd whey(Bauman 735). *L. lactis* was formerly identified as *Streptococcus lactis* in the Bergey's Manual. *Lactococcus* is different from other lactic acid bacteria, they have pH, salt, and temperature tolerances for growth (Systematic). *Lactococcus* is a lactic acid bacteria that includes members of the genus *Streptococcus* Group N in the Lancefield scheme of grouping (Systematic). It contains glycerol teichoic acid in its cell wall. Its ability to ferment lactose and digest casein is regulated by two plasmids, Phosphoenolpyruvate dependent phosphotransferase and beta-phosphogalactosidase. In addition to fermentation, *L. lactis* can produce an antibiotic, nisin that inhibits gram positive organisims which may be plasmid mediated (Hardie 1909).

MATERIALS AND METHODS

Buttermilk culture was grown on nutrient agar and incubated in a drawer at room temperature for 48 hours. Organism was isolated and streaked onto nutrient agar, also grown at room temperature. From the growth on this plate colony morphology was detected, then a gram stain was prepared to determine cell morphology and gram results. Using the pure culture, genomic DNA was isolated by using two millimeters of organism and tris buffer in a tube with glass beads. This culture was boiled for ten minutes . After allowing it to cool, the tube contents were vortexed for ten minutes to insure that cell walls were broken, because organism was gram positive. The template DNA is used to set up a Polymerase chain reaction (PCR) mixture. Template DNA is mixed with Taq polymerase, primer mix, and distilled water. The primers used

were bacteria-8 forward, and universal 1492 reverse, to amplify 16s ribosomal DNA during the polymerase chain reaction. The PCR was processed in a thermal cycler: DNA heated to 94' for four minutes; cooled to 55' for 45 seconds to "anneal"; and heated to 72' for 2 minutes for "extension." This process was repeated 35 times, on the final turn extension time was increased to 5 minutes and denaturing step was omitted. After the PCR was amplified the DNA was used to conduct gel electrophoresis to isolate 1500 base pair PCR product. The 1500 bp band was cut out by Dr. Sasha Warren using QiAQUICK PCR Purification Kit by QIAGEN. This purified our product away from primers, enzymes, and genome. The purified 16s ribosomal DNA was sent to the Division of Biological Sciences Sequencing Facility at UC Davis, where it was sequenced with Bacteria 8 forward primer that was sent and converted the sequence to 4 Peaks where I was able to analyze the electropherogram with the program. The sequence from 4 Peaks was copied to National Center for Biotechnology Information (NCBI) where a Nucleotide-nucleotide Basic Local Allignment Search Tool (BLAST) was formatted. BLAST gave information to identify and also general facts for the organism following PCR and gel electrophoresis. I was able to conduct physiological tests to further confirm identity: carbohydrate deeps; glucose, lactose, raffinose, sorbitol, sucrose, and mannitol, also observations of growth on Blood agar, and esculin hydrolysis. Test were also performed to test for presence of endospores, and if the organism was motile.

RESULTS

TEST-Performed	OBSERVED RESULTS	EXPECTED RESULTS
Blood Agar	gamma (no) hemolysis	Weak alpha or gamma Reaction
Esculin Hydrolysis	Negative- no change	Negative
Glucose	Acid Production/ferment	Acid Production
Lactose	Acid Production/ferment	Acid Production
Raffinose	Noacid /no fermentation	No acid
Sorbitol	Acid production/no fermentation	No acid
Mannitol	No acid/no fermentation	Not fermented
Sucrose	No Acid/no fermentation	Not fermented
Motility	No motility	Non-motile
Endospores	No formation	Not formed

Table 1.1 Observation of Test Results for *Lactococcus lactis*. This is a chart comparing the expected results to the results of the Bergy's manual.

NCBI results:*Lactococcus lactis subsp.* Gene for 16s RNA partial sequence. Length-1499, score 1518 bits (766), identities 776/778, 99% accuracy, gaps 1/778, strand plus/plus. Lineage: *Bacteria, Firmicutes, Lactobacillales, Streptococcaceae, Lacticoccus*
The cells were circular, opaque and were about .5-2 micrometers . They were shiny and convex grown on nutrient agar. When grown on Blood Agar the cells had the same features, but grew better.
Observation of a gram stain showed purple cocci in pairs and some in chains, and they were no larger than .5-1.5 micrometers in size.

DISCUSSION

Based on results from multiple test I can confirm that the organism is *Lactococcus lactis*. The organism was able to ferment lactose and glucose as well as produce gas in the presence of glucose and lactose. Multiple carbohydrate deeps were performed to confirm that this organism was no able to ferment or produce acid in their presence. The organism showed the ability to grow on Blood agar, and did not show any hemolysis. Bergy's manual states that cells normally are ovoid cells elongated in a chain, 0.5-1.0 mm in diameter. Mostly in pairs or short chains. The observation of motile forms is rare. Acid fermentation in the presence of glucose and lactose (Hardie 1066). The organism being identified was extremely similar to the characteristics of *L.lactis* in the Bergy's Manual. These basic factors were extremely helpful in final conformation of the choice of this organism. Following a PCR, the clarity of the electropherogram was useful to perform Nucleotide-nucleotide blast. BLAST obtain a 99% accuracy that the unknown organism is *L. lactis*. This showed that 776 of the 778 identities of the organism matched. After getting the polymerase chain reaction information I was able to further confirm the identity of the unknown organism by comparing the results of multiple physiological tests to what was expected in the Bergy's Manual (see table 1.1). By using the NCBI website, to perform BLAST, I was able to find matches to my unknown organisms, perform tests and match my results to those in the Bergy's Manual to confirm its identity.

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Isolation and Identification of a Gram-Negative, Opportunistic Pathogen, From a Toothbrush in Meadow Vista, California; After the Toothbrush was treated with Listerine.

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For this independent project I wanted to find out what microorganisms might inhabit my toothbrush, and even further what ones wouldn't be harmed by Listerine. I grew an organism cultured from my toothbrush (post Listerine treatment) on nutrient agar, isolated a colony and processed it for a DNA template. The template was then run through a polymerase chain reaction and sent to the U.C. Davis Facility for nucleotide sequencing. A contiguous sequence was then compared to database nucleotide sequencing using the Basic Local Alignment Search Tool which identified my unknown organism as *Enterobacter hormaechei*. This organism is a fairly newly identified species (1989) and so was not in the Bergeys Manuel. After following up with research and confirming tests I verified that my organism could be *Enterobacter hormaechei,* a facultative anaerobe, gram-negative bacilli, that ferments lactose. It is an opportunistic pathogen and is from a strain most closely linked to *Enterobacter cloacae,*

INTRODUCTION

In our Microbiology class we cultured "air" plates from our homes and discovered a multitude of microorganisms happily flourishing. This led me to wonder what was living on my toothbrush. I decided to take a sample from my toothbrush after soaking it in Listerine. Several colonies were isolated and I chose to test and identify one – which turned out to be *Enterobacter hormaechei.* After researching Enterobacters and toothbrushes I discovered that my organism is quite native to the toothbrush. Findings from a study done to evaluate bacterial survival rate on toothbrushes after being decontaminated with antimicrobial sprays revealed that 83.3% of the samples were contaminated. Of these 46.7% were infected with Gram-negative bacilli. In my research I ran across a number of studies around this same dilemma, including numerous toothbrush inventions (even one that uses UV light!) aimed at ridding the toothbrush of its inhabitants. *Enteobacter hormaeche* happens to be recently identified (1989) and consists of 23 strains – 22 of which have been isolated from humans.

MATERIALS AND METHODS

First I dipped my toothbrush in Listerine and then obtained a sample from it using a sterile swab which I then inoculated on a nutrient agar plate. After growth was evident I further isolated one colony, from the several found, for further testing and identification. Colony

morphology was observed and a Gram Stain and KOH test were performed. After determining that my unknown was Gram stain = negative I followed the procedure for DNA isolation in order to obtain a DNA template and then innoculated it with an already prepared PCR mixture. This PCR mixture consisted of Taq polymerase, 2 primers – 8 forward and 1492 Universal Reverse as well as buffer and distilled water. The sample was given to my instructor to run through the thermocycler and then purify to be sent to the Division of Biological Sciences Sequencing Facility at U.C. Davis. U.C. Davis used Dideoxy Chain Termination Method to replicate, label and sequence the gene that codes for 16s rRNA. I then received a visual record, an electropherogram, of my unknown DNA sequence. Using the 4 Peaks program I was able to print my approximate 1500 bp sequence. I then deleted overlapping sequences and ended with one contiguous sequence. To identify my unknown organism I used the National Center for Biotechnology Information website and the Basic Local Allignment Search Tool program in order to compare my continguous sequence to database sequences. After obtaining these results I compared my findings to those in the Bergeys Manuel Volume 1 as well as to some internet sites. In order to verify my results I performed the following tests: Methyl Red / Voges-Proskauer, Citrate Utilization Test, Catalase Activity Test, Lactose and Glucose deeps, and Wet – mount motility.

RESULTS

The DNA sequencing results found my unknown organism to be *Enterobacter hormaechei* 1514bp with linear DNA. The database compared it to 4,588,399 sequences and 18,475,705,801 total letters. Query length was 781 and the strain is EN – 562T. The results were 99%compatible.

Wikipedia.org lists this organism as a newly identified strain (1989) most closely linked to *Enterobacter cloacae.* Bergeys Manuel does not list this organism.

Colony morphology showed circular form with entire margins, convex, shiny and translucent approximately 1-2.5 mm., non-pigmented. Following a Gram Stain I observed pink, short rods single and diploid and size was approximately .5 microns wide x 1-2 microns long at 100x. KOH test was snotty. Wet-mount showed definite motility. VP test results – medium turned red, MR medium yellow, citrate tube turned blue, catalase test showed bubbles. Lactose and glucose deeps turned yellow and bubbles were present in glucose tube.

DISCUSSION

My unknown organism is Voges-Proskaur positive, Methyl Red negative, Catalase and citrate positive and very motile. The Gram stain results were negative and KOH test was positive. All of these test results are consistent with *Enterobacter cloacae* the organism in Bergeys Manuel closest to *Enterobacter hormaechei.* Colony morphology is consistent as well. According to Microbe Wiki *Enterobacter hormaechei* is a nosocomial opportunistic pathogen known to cause infections in hospitals. It is a motile, rod-shaped facultative anaerobe that ferments both lactose and glucose with gas production. The Journal of Clinical Microbiology writes that *Enterobacter hormaechei* was proposed as a new species in September, 1989 and it consists of 23 strains most

closely related to *E. cloacae*. *Enterobacter hormaechei* is found on humans, animals, plants and in the soil. My results also seem to clearly indicate that Listerine does not kill this organism!

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INTRODUCTION

What yeast is used to make Sierra Nevada Pale Ale, a popular locally brewed product? Almost all beers use yeast called *Saccharomyces cerevisiae*. However, there are many strains of this yeast. Historically, "*Pale ale* was a term used for beers made from malt dried with coke…. By 1830 onward the expressions *bitter* and *pale ale* were synonymous.." (wikipedia.com). The Sierra Nevada Brewery website says that the Pale Ale is made with "Two-row Pale & Caramel" malts, and has a bitterness units rating of 37 (Sierranevada.com). They also explain it is a "Top-fermenting Ale Yeast".

Wyeast Laboratories sells yeast for home brewers, and in their offerings of strains that make Top-fermenting Ale Yeast they list strain "1056" and say "Commercial examples may include**:** Sierra Nevada Ales…" (Wyeastlab.com). Our experiment was to find out any more specific information about the particular strain.

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MATERIALS AND METHODS

We purchased a bottle of Sierra Nevada Pale Ale in Rocklin, CA at a local grocery store. We extracted a precipitate from the bottom of the bottle and streaked it on a Yeast agar plate to isolate and grow a pure culture. We took additional agar and poured other plates for future use. We observed the colony morphology including smell and performed a direct and indirect stain (using Nigrosin and Congo Red) and a wet mount. We also isolated the genomic DNA for (with a vortex mixer), did a Polymerase Chain Reaction procedure amplifying for the gene for 18s ribosomal RNA using primers, 817 forward and 1536 reverse. A Gel Electrophoresis procedure was performed by the renowned Dr. Sasha to isolate the 719 base pair length of DNA. Once the isolation was complete the DNA was cut out of the section and purified using QiAQuick PCR Purification Kit by Qiagen. This section of DNA was then sent to UC Davis, to the Division of Bioscience Sequencing Facility. We then analyzed the file sent back containing the electropherogram with the Four Peaks program. We then used the base pair sequence from the Four Peaks program to compare with the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information website (ncbi.nlm.nih.gov).

We also did a taste comparison by fermenting the Sierra Nevada Yeast in apple juice, creating apple cider. We performed the same experiment of making cider with store bought active dry yeast (Bakers yeast), allowing the liquids to ferment for 4 weeks. The only ingredients were the apple juice and yeast.

RESULTS

The Colony Morphology was:
Form: Circular, Margin: Entire, Elevation: Convex, Surface texture: Shiny, Optical character: Opaque, Pigment: Cream, Size: 2-3mm by 2-3mm, Smell: like yeast or bread.

The Cell Morphology was:
Size: 9 micrometers
Indirect stain: The cells stood out well and were formed like what is shown in Figure 1.

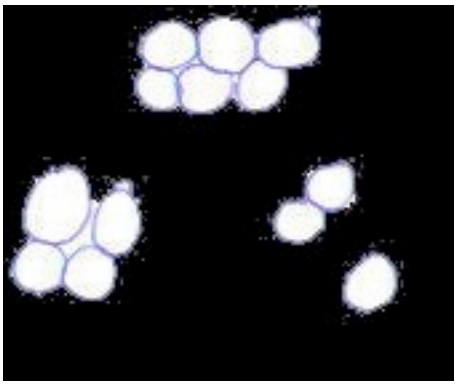

Figure 1: A drawing of the yeast from the indirect stain, viewed at 100x magnification

Direct stain: Despite a slightly smaller appearance the results were the same as for the Indirect stain (Fig.1).
Wet Mount: We could not see any movement and we saw what is shown on Figure 2.

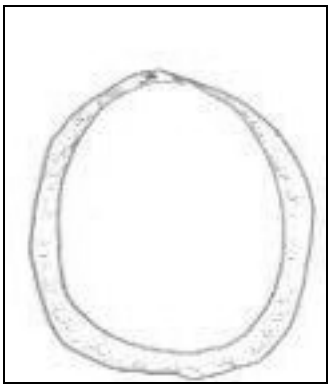

Figure 2: A drawing of the yeast from the wet mount, viewed at 450x magnification

Electropherogram: There were 7 nucleotides in the sequence that were questionable out of the 731bp long sequence.
Results of BLAST: 4,629,701 sequences and 18,602,024,809 total letters were searched in the BLAST data base and there were 4 different Saccharomyces cerevisiae strains that scored 1326 as well as 6 other Saccharomyces.
Cider Taste Test: The Sierra Nevada yeast cider tasted flat and bitter with a waxy after-taste. The store bought Baker's Yeast tasted similar but with less bite and a quickly fading after-taste in comparison to the cider made with Sierra Nevada.

DISCUSSION

We conclude that Sierra Nevada Pale Ale is made from *Saccharomyces cerevisiae*. The BLAST sequence was determined to be 99% accurate at a score of 1326 bits. There are 6 other species of *Saccharomyces* that fit the same accuracy: *bayanus, kudriavzevii, mikatae, cariocanus, paradoxus,* and *pastorianus*. We still conclude, however, that the species in the case of Sierra Nevada Pale Ale must be *cerevisiae* because all our research into brewing shows the use of that particular yeast. It is the yeast that is "capable of tolerating high alcohol

concentrations" (Skinner, 1930). Also, *cervisiae* tends to a "round shape" while *ellipsoidues* is a more "elliptical shape" (Skinner, 1930).

Even at doctorfungus.org we found that "the 'baker's' or 'brewer's' yeast, *Saccharomyces cerevisiae* is used in food industry in production of various food stuffs, wines, and beers."☺ More specifically, that it is "Kingdom: Fungi, Phylum: Ascomycota, Class: Hemiascomycetes, Order: Saccharomycetales, Family: Saccharomycetaceae, Genus: *Saccharomyces*." However, the strain is less clear. We found four strains that were equally probable and had 99% accuracy. They were strain CICC1600, strain ALI 308, strain J2, and strain GK3. We concluded it was not possible to prove if the strain really is "1056" as Wyeast Laboratories theorizes with this research and these researchers.

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Isolation of *Aspergillus niger* from the air.

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The *Aspergillus* species is ubiquitous in nature. It is primary found as a saprotroph living on dead leaves, compost piles, and other decaying vegetation. *A. niger* is a filamentous fungi or mold that produces dark brown or black branching hyphae and spores from biseriate phialides. It is regarded as an opportunistic pathogen that may cause hypersensitivity reactions, otomycosis and pulmonary aspergillosis. Although *A. niger* has this dark side, it is also widely used for the production of citric acid used in soda and production of many enzymes including enzymes used in the food industry.

INTRODUCTION

As a microbiology requirement, we were to investigate and isolate an organism from our environment. A nutrient agar air plate was brought home and placed near an air conditioning vent in my son's bedroom. My son has been coughing chronically for over two years and doctors can not offer any explanations or answers yet thus far. Although DNA testing confirmed my organism, Aspergillus niger is considered one of the most common and easily identifiable species of the genus Aspergillus. It is primarily identified through its morphological features over biochemical and genetic characteristics used to classify bacteria.

Aspergillus niger reproduce asexually, they are highly aerobic and can grow utilizing carbon sources from monosaccharides and polysaccharides. In addition to carbon sources such as glucose, fructose and starch, they are also capable of growing in places where nutrients are scarce such as damp walls thus contributing a mildew problem. A. niger is usually identified by their white to yellow mat which develops black conidia. Texture is downy to powdery. Conidial heads radiate, conidiophores are round, rough walled and brown to black in color. They look like a long handle with a beaded brush on the end.

MATERIALS AND METHODS

Extraction of Chromosomal DNA. A sample was taken from a pure culture plate, transferred into a tube of Tris buffer with glass beads and vortex-mixed to evenly distribute sample in the buffer solution. Next the tube was boiled for ten minutes, cooled in ice and then vortex-mixed for ten minutes.

PCR Amplification. The materials used had a specific order or arrangement, thus Taq DNA Polymerase master mix (Qiagen) was added to a clean sterile tube followed by fungus 8 forward and fungus 1492 reverse primers, the template DNA and sterile distilled water. The tube was placed into a thermal cycler for approximately three hours during which temperatures cycled through 94° C to denature the DNA molecules, 55° C to anneal the primers to the DNA strands and 72° C which allows Taq polymerase to build new DNA strands

Gel Electrophoresis. A process that allows DNA samples to be observed recognized and identified. The fungus DNA samples were mixed with a loading/tracking dye composed of xylene cyanol and bromphenol-blue which allows samples to be visible while loading the wells of an agarose gel and allows for DNA tracking as the dye particles move down the gel. This procedure exposes DNA samples to an electric field that separates DNA samples on the basis of size. A chemical called ethidium bromide is added to the gel after the dye has moved ¾ the way down the gel. When exposed to ultra violet light, ethidium bromide produces an orange fluorescent coloring that allows for visibility of DNA banding patterns which are compared to a known standard to determine the approximate size of the fragments.

QIA Quick PCR Purification Kit. The DNA was cut from the gel and then sent to the Division of Biological Sciences sequencing facility at UC Davis. The results were sent back in the form of an electropherogram.

Electropherogram. The nucleotide sequence was edited and copied to the National Center for Biotechnology Information (NCBI) website which was used to access the Basic Local Alignment Search Tool (BLAST) to identify the unknown fungus.

RESULTS

NCBI BLAST results 99% match for *Aspergillus niger*.
Lineage: Eukaryota; Fungi; Ascomycota; Pezizomycotina; Eurotiomycetes; Eurotiales; Trichocomaceae; Mitosporic Trichocomaceae; Aspergillus
Query length: 731
Gene bank length 1692
Number of bases matching 703/704
Bit score 1392
Accession number AF548064

DISCUSSION

Aspergillus niger is an opportunistic pathogen primarily to those with compromised immune systems. Pulmonary aspergillosis is associated with a chronic cough, chest pain, breathlessness and coughing up blood. The fungus spores are breathed in and create a ball of hyphae within the upper lobes of the lungs. The chances of developing this disease are rare and seen primarily in people with severely compromised immune systems. Aspergillus can cause otomycosis, fungal ear infections which cause pain, and temporary hearing loss. Otomycosis may lead to damage to the ear canal and tympanic membrane if left unchecked. A. Niger may cause hypersensitivity reactions such as asthma which are generally not life threatening.

Aspergillus niger is primarily used to make large quantities of citric acid by way of a fermentation process. Citric acid is used widely in the food and pharmaceutical industries. It serves as a tart flavoring and used to complement fruit flavors in carbonated beverages, jams, candy, sherbets and wine. Citric acid reduces the pH in some canned foods to make heat treatment more effective. In pharmaceuticals, citric acid is the source in effervescent tablets. It is also used as a blood anticoagulant.

A. niger is used to produce many enzymes for a large amount of different biochemical processes. The enzyme pectinase is produced by Aspergillus niger and is capable of hydrolyzing fruit pectin which is typically used in fruit juice and wine production. Catalase is an enzyme used to remove excess hydrogen peroxide, used as a whitener in cheese and milk products. A. niger is also used for biotransformations and waste treatment. There were too many interesting developments from this fungi to mention in one paper, however the positive aspects of A. niger seem to out weigh the rare risks associated with the fungi.

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Isolation and Identification of two Gram-positive *Bacillus* growing on a make-up sponge from Corinne's house.

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In this experiment we used a make-up sponge with a little bit of make-up on it to determine what organisms were growing on it. We performed stains, physiological and morphological tests, and DNA processing to determine the identity of organisms growing on the blood agar plate. After sending our PCR product to the UC Davis sequencing center, we were able to analyze these results and ensure what our organisms actually were.

INTRODUCTION

What is a person really putting on their face when they put their make-up on? Most women think they are covering up the bad but in actuality they may be putting on the bad. Make-up has been proven to be carriers of microorganisms, for example, *Staphylococcus epidermidis* and *Bacillus thuringiensis. S. epidermidis* is a normal microbial inhabitant of human skin and mucosal surfaces. It has the ability to spread to the blood through breaks in the skin. *S. epidermidis* is an opportunistic pathogen, which means when given the possibility can become a pathogen. It is most susceptible to intravenous drug users, newborns, and elderly individuals. It also has the ability to be resistant to phagocytosis and antibiotics. *B. thuringiensis* is a naturally occurring bacterial disease of insects. It is considered safe to humans, but is an active ingredient in some insecticides. It is used to fight against caterpillars, mosquitoes, and beetles. During the isolation process of microorganisms found in our make-up, *S. epidermidis* and *B. thuringiensis* were two that were actively growing.

MATERIALS AND METHODS

We obtained our culture from Fair Oaks, CA in Corinne's house. We used a make-up sponge with make-up blotted on it. We isolated this culture by streaking the face make-up sponge on blood agar plates and incubated it at 37 C. We found two different types of organisms growing on the plate. We analyzed both organisms and grew each one separately to isolate it on blood agar plates. The organisms required different specific physiological tests, however.

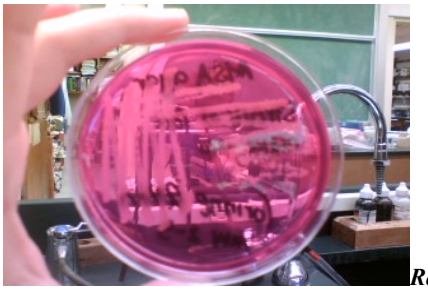
For both the organisms we looked at the colony morphology. We then performed a gram stain to determine what the cell wall consisted of and to determine the bacterial morphology. We also performed an indirect stain to further analyze the cells. After that, we performed a KOH test to understand if the organism is resistant to reagent. We performed an isolation of genomic DNA. We boiled for 10 minutes with beads and shook violently with the vortex. We then amplified 16s ribosomal DNA by P.C.R and primers 8 bacteria forward and 1492 reverse. We then used gel electrophoresis to see if there are 1500 base pairs which will prove rDNA was successful. When then cut 1500 base pairs out of the gel and melted the gel by adding chemicals. We loaded it onto columns to purify PCR product. We then sent it to *UC Davis Division of Biological Sciences Sequencing Facility* for DNA sequencing of the organism. When UC Davis returned our product, we analyzed the electropherogram with the 4 peaks software. We compared our sequence to the database sequencing with the BLAST program of National Center for Biotechnology Information (NCBI).

For the first organism we completed a Vogues Proskauer test. Then we grew the organism on a MSA plate to find out if the organism is capable of growth in a high salt content environment. The last test that was performed was one in which we used mosquito larvae, from pond water at sierra college, and placed them in a test tube filled with distilled water. We then mixed in a good majority of the organism from the isolated blood agar plate. This was used to examine the results of the mosquito larvae when digesting the unknown organism.

For the second organism we performed a gram stain, KOH test, an indirect stain, and a motility test on our organism. The following physiological tests were used to determine the identity of the unknown organism; Catalase Test, Vogues Proskauer, and a Coagulase Test.

RESULTS

In analyzing the colony morphology of the first organism, we observed filamentous form, lobate margin, flat, rough and opaque colonies in tan color. After performing a gram stain we observed purple cells. In the indirect stain, we observed rod shaped cells that were 9 um in size. Following a KOH test, we observed no snot. The PCR product was sent to UC Davis to identify our organism. After this, we performed the test where we used mosquito larvae. We observed that only 1 died after 48 hours and then 2 died 2 days after that. After a total of 9 days, we observed that all the mosquito larvae were dead. After performing this experiment, we observed the results of the Vogues Proskauer test. We found that the indicator remained orange when tested. The last test was growing our organism on MSA plate. We observed that many colonies grew, yet the media stayed pink.



Result of MSA streak

For the second organism we observed the colony morphology which was circular, entire, raised, smooth, opaque, and a whitish-yellow pigment (Wilson, 2006). The following test observations are displayed in the table to follow:

Stains:

Motility	No movement
Indirect Stain	Circular cells about 1.5 micrometers long
Gram Stain	Purple circular cells
KOH	No snot

Physiological Tests:

Catalase Test	Bubbles formed
Vogues Proskauer	Red
Coagulase Test	Liquid Solution

DISCUSSION

In performing all of these experiments, we were able to identify both of the organisms. For the first organism, in the gram stain, we observed purple cells which means that the organism is gram positive. This is due to the fact that the cells keep the crystal violet due to the thicker

wall of peptidoglycan. In the indirect stain, we found rod shaped cells. This shows that the cells are bacilli. The KOH test proved to be negative which meant there was no snot. This showed that the organism was also proven to be gram positive. This is due to the thick peptidoglycan walls of these microorganisms that make them resistant to the KOH. When we performed the DNA tests, we sent it to the lab at UC Davis to sequence it and send it back to us for further analyzing. We used BLAST to show us that closest matches of the DNA amplified with the PCR we sent in to the results that they have in their databases. We had two organisms that were identical 98% matches. One was identified as *Bacillus cereus* and the other was *Bacillus thuringiensis*. Both these organisms are almost identical. "DNA/DNA reassociation studies indicate that this species is genetically closely related to *B.anthracis, B.cereus, and B.mycoides*(Bergey's Manual 1135). When referring to Bergey's Manual, almost all the tests of these two organims came out with the same results. According to Dr. Sasha Warren and Dr. Harriet Wilson from Sierra College, they advised me to perform a test with mosquito larvae and my organism to see if they survive when using it for nutrients. *(shown below)*. After about 9 days, we observed that

mosquito larvae test

all the mosquitos died. Since it took a long time for the mosquitos to die, we performed two tests that had oppossing results for the two organisms. We performed the Vogues Proskauer and observed an orange color in the test tube. This showed that it was negative for fermenting butane diol. In the Bergey's Manual it showed that the *B.cereus* should have been a positive test and turned the reagant red, whereas the *B.thuringensis* was dependent. The last test we performed to ensure that our results were correctly identified, was the growing on MSA plate. We observed many colonies, which showed that this organism can grow in the prescence of high salt tolerated environments. According to Bergey's Manual, *B.cereus* was very dependent and *B. thuringensis* was positive for growing on MSA. Therfore, we concluded that since *B.cereus* was very dependent, it meant that *B.cereus* may not be able to withstand high concentrations of salt. With all these results, we concluded that *B. thuringensis* was the unknown organism found on our blood agar plate.

For the second organism, we concluded that having to grow our organism on Blood Agar shows that the organism is a fastidious (picky) eater (Wilson, 2006).

Our gram stain was purple showing that the organism is gram positive, and contains many layers of peptidoglycan with techoic acid, forming a thick wall. The KOH test confirmed our results of a gram-positive organism because it produced no snot, so the cell wall was not destroyed (Wilson, 2006). The Indirect Stain allowed us to view the shape and size of our organism;

staphylococci about 1.5 micrometers in length and width. Our organism was not motile when viewed on a wet mount slide. Because the Vogues Proskauer Test was red, our organism is a "Butane Diol Fermenter" and produces acetoin (Wilson, 2006). The Catalase Test produced bubbles showing that the organism has catalase, allowing the organism to break down Hydrogen Peroxide into water and oxygen (Wilson, 2006). In the Coagulase Test, the organism remained liquid meaning that our organism is not able to clot blood plasma using coagulase (Wilson, 2006). After comparing our results to the Laboratory Manual's Dichotomous Key, our organism's test results proved that our organism was *Staphylococcus epidermidis*.

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Isolation of bacteria that grows in yogurt.

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I isolated bacteria from yogurt to identify the organisms used in the fermentation of dairy products. I streaked the bacteria onto agar to obtain isolated colonies. I used the cells in those colonies to perform a series of tests and stains to help me in identifying the organism. I was able to isolate the DNA from some of those cells to use for a PCR. Those results I used in gel electrophoresis. These fragments were sent to UC Davis for sequencing. The sequence that I received back from the lab I input into the National Center for Biotechnical Information's Basic Local Alignment Search Tool for identification of my organism. The identification was that of *Lactobacillus Casei*. Based on these results I performed further tests to verify the characteristics of the organism.

INTRODUCTION

I isolated bacteria from yogurt to identify what microorganisms are used in the fermentation of yogurt. The microbe was identified as *Lactobacillus Casei*. These bacteria can be found in raw and fermented dairy products, fresh and fermented plant products and the reproductive and intestinal tracts of humans and other animals. It is a "health-promoting live culture" and it is often used as starter cultures in milk fermentation and for flavor development in some cheese varieties (UC Regents 2004).

MATERIALS AND METHODS

I took a sample of our yogurt from the fermentation lab we participated in. I streaked the culture onto a BCP lactose agar plate and a nutrient agar plate. I did a Gram stain with the cells that grew. I also did an indirect stain with nigrosin to check for cell size, shape and arrangement. I mixed 3% KOH with a cell sample. With methylene blue, I performed an acid-fast stain. And I also did an endospore stain with the malachite green method. I used some of the bacterial growth from the BCP plate by scraping off a colony with a sterile loop, and mixing it with tris buffer in a PCR tube. I boiled the tube for 10 minutes to release the DNA from the cells. A PCR was performed. The PCR results were used for gel electrophoresis. The gel results were sent to the UC Davis sequencing lab for processing. I input the sequence that was returned from UC Davis into the National Center for Biotechnical Information, Nucleotide Basic Local Alignment Search Tool. Based on the microbe identification, I performed a series of tests. Those tests included a Methyl Red and Vogues Proskaur, oxidase and catalase, urea hydrolysis, citrate, Sulfur Indole Motility, Triple Sugar Iron, glucose, lactose, arabinose, sucrose, mannitol, sorbitol, inositol, and raffinose (carbohydrate) deeps.

RESULTS

There was more cell growth on the BCP lactose agar plate than the nutrient agar plate. On both plates the colonies were punctiform, had entire margins, round in shape, convex elevation, smooth surface texture and they were opaque with no pigment. The BCP lactose agar was yellow and purple. Following a Gram stain, I observed purple, rod-shaped cells. I again saw rod-shaped cells that were forming chains in the indirect stain performed with nigrosin. The KOH test result was negative and no "snot" was evident. The cells stained blue with methylene blue in the acid-fast stain. The endospore stain resulted in cells stained reddish-pink with no evidence of green. The UC Davis sequencing resulted in a sequence 675 nucleotides in length. When inputted into the Basic Local Alignment Search Tool (BLAST) it compared my sequence to 4,588,399 sequences in its gene bank and 18,475,705,801 nucleotides. The organism's DNA is 83% similar to the DNA of *Lactobacillus casei*. The Methyl Red test was red and the Vogues Proskaur test did not change the culture's color. The cell sample did not have a reaction on the oxidase test paper, no color developed. I did not observe any bubbles in the catalase test. The urea hydrolysis slant remained orange, it did not turn pink and the citrate slant remained green, it

did not turn blue. The Sulfur Indole Motility test showed no movement in the agar away from the original stab, there was no presence of black in the agar and the agar remained its original color. The Triple Sugar Iron test also did not have any black results and the agar slant and butt were yellow. In the sucrose, arabinose, mannitol, sorbitol, inositol and raffinose deeps the agar remained pink. The glucose and lactose deeps were yellow.

DISCUSSION

While both the BCP lactose agar and the nutrient agar yielded colony growth, the BCP lactose plate had significantly more growth. The colonies on both plates were too numerous to count, but the organism was able to grow. More growth on the BCP lactose plate indicates that the nutrients in the agar were selective for my organism. The yellow agar of the BCP lactose agar indicated that the organism was fermenting one or more of the nutrients and producing acid.

The cells staining purple in the Gram stain indicated that the organism has a thick cell wall and is Gram positive. The KOH test further supported this. With no "snot" resulting, I observed that there was no lysing of cell membranes, also indicating that the organism is Gram positive. The nigrosin stain made the shape and arrangement apparent and I observed streptobacilli. The acid-fast stain was negative; the cells stained blue which means that there is no mycolic acid present in the cell walls. The endospore stain indicated that the organism does not form spores.

The UC Davis sequencing results were not sufficient enough to make a complete positive identification of the organism in the BLAST database. While the program did make an ID, the organism is only 83% similar to the organism identified in the BLAST results. This means that the PCR was not effective in making a sufficient amount of copies of the DNA sequence or that my attempt at releasing the DNA from the cells during boiling failed.

The organism does not produce hydrogen sulphide, which was indicated by the Triple Sugar Iron and Sulphur Indole Motility tests. I observed the SIM agar and the organism is not motile and did not grow flagella when nutrients were no longer present. The organism also did not use tryptophan to produce indole. The yellow agar slant and butt in the TSI test indicates that the organism ferments one or more sugars. After completing the carbohydrate deep tests, I can confirm that the organism ferments 2 sugars, glucose and lactose. The yellow agar in these tests results from acid production. With the Methyl Red and Vogues Proskaur tests I was able to identify that the organism is a mixed acid fermenter and produces lots of acid, but does not produce acetoin as its fermentation product.

The organism does not hydrolyze urea as indicated by the negative test result. The citrate test was also negative which means that it does not use citrate as its primary carbon source. No bubbles in the catalase test means that the organism does not split hydrogen peroxide into oxygen gas and the negative result of the oxidase test indicates the absence of cytochrome C in the organism. These two results means that the organism is an anaerobe and the Bergeys Manual lists this particular species as a facultative anaerobe (pgs 1208-1214).

I knew the general categories of bacteria present in yogurt, but the tests I performed were important for narrowing down the possible identifications of the organism I isolated. I found it interesting that a fellow classmate used the same yogurt culture in class to isolate bacteria from and we each ended up with a different species. Both are present and necessary in the fermentation of dairy products. While our approach in identifying our organisms was very much

the same (ie; tests and results) my classmate observed an organism very different from mine when we looked at one another's Gram stains. What I conclude from this is that I would not have been able to completely identify my organism without the BLAST results and the Gram stain, but the tests did help me to identify and narrow down what category my organism was in and what other tests I needed to do.

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Isolation of bacteria from work pants used in the Burn Unit at UC Davis Medical Center.

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This was an independent project that was done outside of class, in which we conducted tests to identify two unknown microorganisms. We were supplied with the material and computer technology in our microbiology lab to perform these tests. Aseptic technique was used throughout all of our tests. All data was carefully recorded by drawing illustrations and recording all of the information we obtained. Our nucleotide sequence was compared to data recorded in the public database of National Centers for Biotechnology Information (NCBI) and was found to be a 99% match with previously identified strains for both of the microbes tested. Microorganism (1) is a Gram-negative, facultatively anaerobic, Catalase positive, Oxidase negative, Citrate positive bacteria. This microorganism can be pathogenic to humans. For this organism the entire battery of tests didn't match

with the Bergey's manual. Professor Wilson and we believe it is because we didn't select the organism with the highest Bit score from the NCBI. Microorganism (2) is a Gram-positive, Oxidase positive, bacterium capable of fermenting glucose. Microbes (2) do not have much clinical significance.

INTRODUCTION

We thought it would be interesting to know what was growing on my husband's work pants. He works in the Burn Unit at U.C. Davis Medical Center in Sacramento, CA. Due to his direct contact with patients, who have large colonizing wounds, we predicted that there would be a large variety of growth on the agar plate. On September 2, 2006, we placed a Nutrient Agar plate inside a freshly disinfected utility sink. Next, we shook the pants over the plate for ten seconds. The pants were then laid carefully around the open plate for one hour, thereafter sealed tightly with tape and brought back to the microbiology lab at Sierra College.

MATERIALS AND METHODS

Pure Culture. The plate was then placed into our lab drawers and allowed to grow over the weekend. We chose two of the most unique colonies growing on the Nutrient Agar plate. Aseptically, two different organisms were streaked onto separate agar plates and placed into the lab drawers to allow time for colony formation.
Nutrient Agar is a complex medium because its ingredients are in crude form and provide the necessary nutrients for growth. The chemical composition for Nutrient Agar is unknown and it varies slightly from batch to batch. After a number of days, we had observed that we had success in isolating our distinct microorganisms into pure cultures. 9

Polymerase Chain Reaction. On September 29, 2006, we aseptically restreaked a new Nutrient Agar plate to obtain a fresh pure culture so the Polymerase Chain Reaction could be completed during the following class period. A Polymerase Chain Reaction was used in order to amplify the 16s ribosomal DNA of our unknown bacteria. In vitro, the DNA was mixed with Taq polymerase, primers, Tris buffer solution, and water. The primers used to anneal the DNA were bacteria-8 forward and 1492 reverse. The Tris buffer solution contains the dNTP's. The mixture was placed into a thermal cycler. The PCR involves alternately heating and cooling the preparation so the enzymes used to catalyze the building process must be able to remain functional after being subjected to high temperatures. At 94° C the DNA is denatured and the Hydrogen bonds are broken. The thermal cycler then cools to 55° C so the primers can anneal. Finally, the temperature is increased again to 72° C to allow the Taq polymerase to build new complimentary DNA strands from the five prime to three prime ends. This allowed the DNA to replicate approximately one million times through the amplification. 9

Gram Stain. We prepared two Gram stain slides. Crystal Violet was used as the primary stain and was responsible for coloring all present cells purple. Next, the mordant, Grams Iodine was applied. A decolorizing agent was used to remove the previously applied stain. Finally,

Safranin was then used to counter stain any Gram-negative cells by staining the walls a bright pink color. 9

KOH Test. 3% KOH was added to cells to determine the composition of the cell wall. Gram-positive cells tend to be resistant to the KOH because their walls are made of a thick wall of peptidoglycan. If the mixture becomes viscous or "snot-like", it is because the KOH is responsible for the lysis of the thinner walled Gram-negative cells. This process will cause the cell to lysis and the protoplasm to spill out. 9

Indirect Stain. We prepared an Indirect Stain using Nigrosin to contrast and allow for accurate measurements of the cells being viewed. This technique is responsible for the background dark which leaving the bacteria without color. 9

Gel Electrophoresis. On November 16, 2006, we ran our Gel Electrophoresis. This is a technique which allows for DNA fragments to be separated on the basis of size. 9

We filled all six wells with our PCR product and tracking dye with a digital pipette. The tracking dyes were xylene cyanol and bromphenol-blue and were used as a weight in sinking the DNA into the wells through the buffer solution. Thepower was then turned on and the slightly negative DNA slowly traveled towards the positive anode. This process ran until all the DNA was separated from the dyes.

Our gel was then stained with Ethidium bromide so it would be seen on the transilluminator. Thereafter, the gel was cut, cleaned and sent to the U. C. Davis DNA lab for sequencing. 9

Oxidative/Fermentation Test (O/F Test). An Oxidation/Fermentation test to determine whether the organism's metabolism was either respiratory or fermentative was performed. The final electron acceptor determines the end products formed. The end products typically formed by respiratory organisms are carbon dioxide and water.

Two agar deeps containing glucose as the carbon source and Bromothymol blue as the pH indicator were used in this test. Of the two agar deeps, one contained a Durham tube which was sealed with vaspar. The vaspar served two purposed. The first was to keep volatile amines within the tube. The second was to ensure fermentation, given the fact that the organism is capable of the process. 9

Methyl Red-Voges-Proskauer (MR-VP) Test. The Methyl Red (MR) and Voges-Proskauer test was performed on a single sample of culture. The composition of this medium was 0.7% peptone, 0.5% dextrose (glucose) and 0.5% K_2HPO_4 in distilled water.

The phosphate is responsible for acting as a buffer and does not allow changes in the pH indicator unless they are responsible for a large formation of acid. When the glucose in the MR-VP is catabolized in the presence of oxygen, the fermentation products are oxidized to carbon dioxide and water.

In order to obtain results, the Voges-Proskauer test selects for a neutral fermentation product called acetylmethyl carbinol (acetoin). This was performed with the addition of 18 drops of Barritt's reagent. The solution was shaken vigorously for 1 minute and then allowed it to rest for 15 minutes before shaking it again.

The Methyl Red quantitative test was performed next to test for a large production of acid from the fermentation of glucose. In the original tube, several drops of the pH indicator, Methyl Red, were added to determine whether a color change occurred. 9

Citrate Utilization Test. A few organisms possess the capability of taking in citrate as their only carbon source. This acid is an organic compound which is continuously catabolized

within the Krebs cycle. Sodium citrate serves as the carbon source while the pH indicator is Bromothymol blue. 9

Gelatin Hydrolysis. Gelatin is the protein obtained by the hydrolysis of collagen, a component of animal connective tissue. It is the substrate used in the testing for proteolytic enzymes in microbes. The exact amounts of gelatin and water are liquid at room temperature and are solidified in an ice bath (ours were cooled at room temperature). If the organisms have hydrolyzed the gelatin, the medium will remain liquid when cooled. 9

Starch Hydrolysis. The starch hydrolysis test is used to determine whether an organism can hydrolyze starch through enzymatic activity. This test is often used to identify the *Bacillus* and *Pseudomonas* species of bacteria. A starch agar plate is streaked with the organisms and incubated in the lab drawer until isolated colonies are grown. A drop of Grams iodine reagent is placed directly onto an isolated colony. A color change to a dark purple-black indicates starch is not being hydrolyzed. However, if the agar under the colonies is clear, the organisms are hydrolyzing the starch. 9

Carbohydrate deeps. The deeps of carbohydrates were used for testing the organism's ability to ferment a number of individual carbohydrates and subsequently produce both acid and gas. A wide variety of acids and gases may be produced. The pH indicator used was phenol red. The carbohydrate deep used was glucose, which remained pink indicating there was no production of acid from glucose and other sugars in the pentose medium. 9

RESULTS

Organism 1:
Pure Culture
Colony Morphology on Nutrient Agar
Size= 1-5 mm
Form= Irregular
Margin=Undulate
Surface Texture= Shiny and wrinkled
Elevation= Raised with a hole in the middle
Optical Characteristics=opaque
Pigmentation=Golden yellow
Odor=Unpleasant smell

Colony Morphology on Mueller Hinton Agar
Size= 2-4mm
Form= Irregular
Margin=Undulate
Surface Texture= Shiny and wrinkled
Elevation= Raised with a hole in the middle
Optical Characteristics=opaque
Pigmentation=Golden yellow
Odor=Unpleasant smell

Oxidative/Fermentation Test
Facultative anaerobic

Gram Stain
Gram-negative

Indirect Stain
Bacillus shaped rods, single and v-shaped
Rods ranging from .08-1.0µm wide by 2.0-5.0µm in length

KOH Test
Gram-negative

Oxidase Test
Oxidase negative

Citrate Utilization Test
Citrate positive

Gelatin Hydrolysis
Gelatin hydrolysis negative

Starch Hydrolysis
Starch hydrolysis negative

Polymerase Chain Reaction
Professor Wilson reran the PCR on October 13, 2006, because the first test did not contain any DNA.

Gel electrophoresis
The sequence data was returned from the Division of Biological Sciences DNA Sequencing Facility at UC Davis electronically. The electropherogram we received was then edited to obtain a more accurate nucleotide sequence for the unknown microorganism sequenced. A Basic Local Alignment Search Tool (BLAST) was used to compare this nucleotide sequence to others in the system.

Pseudomonas oleovorans
Length=828
Bits Score=1554
Identities=800/803 (99%)

Organism 2:
Pure Culture
Colony Morphology on Mueller Hinton Agar
Size= .3-.8mm
Form= Circular

Margin=Entire
Surface Texture= Smooth and glistening
Elevation= Raised to Convex
Optical Characteristics=opaque
Pigmentation=Contains both white and off-white colonies
Odor=unpleasant smell

Colony Morphology on Nutrient Agar
Size= 1-5mm
Form= Circular
Margin= Entire
Surface Texture= Smooth and glistening
Elevation= Raised to Convex
Optical Characteristics=opaque
Pigmentation=Contains both white and off-while colonies
Odor=unpleasant smell

Gram Stain
Gram-positive bacteria

Indirect Stain
Cocci and short rods
Cocci ranging in .5-1μm, and rods ranging from 1-2μm in width by 2-3 μ in length

KOH Test
Cell mixture remains liquid, thus serving as an indication of a gram-positive organism

Oxidase Test
Oxidase positive; the cells turned slight purple after three to four minutes. Weakly positive

Methyl Red-Voges-Proskauer (MR-VP) Test
Voges-Proskauer – Acetoin negative
Methyl Red Test- acid negative

Citrate Utilization Test
Citrate utilization positive

Gelatin Hydrolysis
Gelatin hydrolysis positive

Carbohydrate deeps
Glucose negative

Polymerase Chain Reaction
The PCR was ran on October 13, 2006, by Professor Wilson

Gel electrophoresis
The sequence data was returned from the Division of Biological Sciences DNA Sequencing Facility at UC Davis electronically. The electropherogram we received was then edited to obtain a more accurate nucleotide sequence for the unknown microorganism sequenced. A Basic Local Alignment Search Tool (BLAST) was used to compare this nucleotide sequence to others in the system.

Brevibacterium iodinum
Length=812
Bits score=1485
Identities=782/785 (99%)

DISCUSSION

With the entire panel of tests completed, the results were compared to the *Bergey's Manual* Volume Two, 2nd ed. According to the book, the organisms should be able to hydrolyze starch, and fluoresce on MHA agar; our specimen (1) did neither. *Pseudomonas oleovorans* should also be Oxidase positive; however, our specimen was not. This may possibly be attributed to the age of the Oxidase reagent in the microbiology lab. It did agree with the results from the book that the microbes were Gram-negative, Gelatin hydrolysis negative, and Catalase positive. 1, 5

Pseudomonas in general, can be described as Gram-negative, non-spore forming, straight or slightly curved rods. Typically they are motile with amphitrichous flagella. However, the *P. oleovorans* in our project did not have flagella and were not motile but demonstrated the Brownian motion. 7

In general, members of the genus can be found in soils, fresh and salt water environments. *Pseudomonas* is able to use a wide variety of organic compounds as its carbon and energy sources. Also, they can be associated with plants and animals as normal flora as well as pathogens. An example is *Pseudomonas aeruginosa,* which can cause infections in wounds, burns and the respiratory system. 7, 9

Though we have identified our specimen as *P. oleovorans*, we have reason to believe these may not be entirely precise for our microbe (1). An organism with a slightly higher bit score may be the correct microorganism. *Pseudomonas psychrotolerans* has the same yellow, leathery, dry, wrinkled colonies that form within 48 hours as described in the literature we used. However, the bit score with the higher number had only a general bacteria name, *Pseudomonas* sp. The distance tree on the NCBI website shows *P. oleovorans* and *P. psychrotolerans* are right next to each other. 6, 8

The taxonomy of *Pseudomonas oleovorans* is: *Bacteria, Proteobacteria; Gammaproteobacteria, Pseudomonadales; Pseudomonadaceae; Pseudomonas*. 3

The results for *Brevibacterium iodinum* were compared to *Bergey's Manuel* Volume Two. The colony morphology was in fact incredibly different than the source indicated. The resulting colonies should produce extra cellular purple crystals on a variety of media when in reality no crystal formation was present for our culture. Cells most frequently appear gram-negative when they are in reality gram-positive. In very young cultures (about 8h) cells

frequently stain half gram-negative, while the other half stains gram-positive. This can, and does cause confusion for many individuals studying such microorganism. 2

After performing the Oxidase test, the results show the organisms were weakly Oxidase-positive. According to the Bergey's Manual, the organisms should be strongly positive. The weakly positive result may be due to the age of the microbiology lab's reagent. The expiration date is 2001. 2

Sources indicated that *Brevibacterium* consists of non-motile, gram-positive rods which require nucleic acid analyses in identification of the organism. These organisms reside on the human skin and are found in dairy products such as cheese. 4

The microorganism *Brevibacterium iodinum* isn't of much clinical significance, but there have been a number of previously reported cases caused by *Brevibacterium* species. One of the case reports found during our research performed on *Brevibacterium* was *Brevibacterium otitidis* endocarditis. This sixty-eight year old female had noted to have malaise, loss of appetite, and fatigue. According to doctors, "Findings of the remainder of her physical examination were remarkable, including the examination of neurological, respiratory, abdominal, and musculoskeletal systems." After six blood samples, cultures yielded organisms that were initially identified as diphtheroids, but after amplification yielded *Brevibacterium otitidis.* 2, 4

Brevibacterium organisms can cause clinically significant disease. In this particular case only four reports were recorded. The empiric selection for antibiotics included those with low Minimal Inhibitory Concentrations (MIC) and a relatively low risk of drug interaction with Warfarin. 4

The taxonomy of *Brevibacterium iodinum* is*: Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Micrococcineae; Brevibacteriaceae; Brevibacterium*. 3

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U.C. Davis College of Biological DNA Sequencing Facility

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Isolation of a Gram Positive bacteria and a Gram Negative bacteria from an air plate in a kitchen.

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Two different organisms were isolated from an air plate in a kitchen. Through research, it was discovered that one of these organisms, *Massilia timonae*, is an infectious agent. After many physiological tests and observations, it was clearly determined that the identities of these two isolated organisms are *Deinococcus radiopugnans*, a Gram-positive bacteria, and *Massilia timonae*, a Gram-negative bacteria.

INTRODUCTION

We are reporting on the morphological and physiological test results of two organisms isolated from an air plate. These organisms were found to be *Deinococcus radiopugnans*, a

Gram positive bacteria, and *Massilia timonae*, a Gram negative bacteria. We felt that it was important to understand the characteristics of these organisms because they were initially growing in a kitchen. Through research it was found that *Deinococcus radiopugnans* can potentially withstand high levels of ultra violet radiation and gamma radiation. This species is also mesophilic and will grow at 37 degrees Celsius. Also, *Deinococcus radiopugnans* is heat resistant. This information tells us that the bacteria can potentially infect a human (Brooks & Murray, 1981). The isolated Gram negative bacteria *Massilia timonae* has been isolated from the blood of an immunocompromised male patient. The bacterium was isolated from the patient's blood with the onset of a high fever. However, due to the patient's immune system deficiency, he could not produce specific antibodies. This caused much difficulty in verifying the involvement of *Massilia timonae* with the patient's condition. The isolation of the bacterium from this patient led scientists to propose it as a new taxon (Birtles, La Scola, Mallet, & Raoult, 1998). In another case, a 36-year-old immunocompetent male had undergone orthopedic surgery. His wound became infected with what was found to be the second strain of *Massilia timonae*, this time infecting a healthy individual (Lindquist et al., 2003).

MATERIALS AND METHODS

Initially, the two bacteria were found on an air plate which had been left on a kitchen counter in Nevada City, California, for one hour. There were several different colonies present on the air plate, but we chose two specific colonies to isolate. After complete isolation on nutrient agar, we noted morphological characteristics of the colonies on the plate and the cells under a microscope. Smears of the two different bacteria were Gram-stained and examined. A 3% KOH test was performed on both species on a glass slide.

Isolation of the genomic DNA for each bacterium was performed. Small samples of each organism were vortex mixed and boiled. The resulting DNA samples were amplified in the polymerase chain reaction (P.C.R.), specifically the 16S rDNA gene. The primers used were Bacteria 8 forward and Universal 1492 reverse. A gel electrophoresis of the amplified DNA was completed. The gene of interest was cut out of the gel with a razor blade. The P.C.R. product was purified with the QiA Quick PCR purification kit by Qiagen. The purified product and the primer Bacteria 8 forward were then sent to the Division of Biological Sciences Sequencing Facility at UC Davis for sequencing. Using the Four Peaks computer program, the resulting sequence was analyzed and compared to database sequences at the National Center for Biotechnology Information (NCBI). The search tool used was the Basic Local Alignment Search Tool (BLAST).

The following physiological tests were performed with both organisms: Oxidase, Citrate, Triple Sugar Iron (TSI), Sulfur Indole Motility (SIM), Esculin Hydrolysis, and Catalase. Also, the two organisms were plated on Mueller Hinton agar to amplify possible pigment production.

RESULTS

After plating the organisms on nutrient agar, both were able to grow within twenty-four hours at room temperature. There appeared to be no specific nutrient or other growth requirements for either bacterium. Observation of colony morphology for the *Deinococcus*

radiopugnans is as follows: form is circular, margin is entire with a convex elevation; surface texture is shiny, and the optical character is opaque; colonies have a bright red pigment, and their size is 1-2 millimeters in diameter; slightly rotten smell after a few days of growth. Colony morphology for the *Massilia timonae* is as follows: circular form with an undulate margin; elevation is raised; surface texture is glistening with a translucent optical character; produces a light yellow pigment; size of 1-2 millimeters in diameter; musty, putrid smell after a few days of growth. 3% KOH test for *Deinococcus radiopugnans* had no snot. KOH test for *Massilia timonae* had snot.

After viewing the Gram stain under a light microscope, the *Deinococcus radiopugnans* stained a magenta to purple color. The cell morphology was small round cells in bunches. Each cell was approximately one micron in diameter at the magnification of 1000X. The *Massilia timonae* stained a pale pink color. The cell morphology was small isolated rods, many were in clusters. Each rod was approximately 0.5 microns in width and 2 microns in length at the magnification of 1000X.

After isolation and sequencing of the 16S rDNA genes were complete, the result of each showed that that the organisms were most likely *Deinococcus radiopugnans* and *Massilia timonae*. The following is the data retrieved from the BLAST search within the NCBI database:

	Deinococcus radiopugnans	*Massilia timonae*
Sequences	4,580,296	4,580,296
Bases compared	18,456,161,821	18,456,161,821
Matching bases	512/536	572/596
Gaps	5/536	5/536
Query length	677	651
Gene bank length	1469	1458
% similarity	95%	96%
Bit score	842	955

Table 1. Data from BLAST and NCBI

Lineage for the *Deinococcus radiopugnans* is *Bacteria; Deinococcus-Thermus; Deinococci; Deinococcales; Deinococcaceae; Deinococcus.* The lineage for *Massilia timonae* is *Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Oxalobacteraceae; Massilia.*

The following are the results for the physiological tests performed:

	Deinococcus radiopugnans	*Massilia timonae*
Oxidase	(+) Blue cells on paper	(+)Blue cells on paper
Citrate	(--) Green tube	(+) Blue tube
TSI	(--) Red-orange tube	(--) Red tube
SIM-sulfur	(--) No black color change	(--) No black color change
SIM-motility	(+) Horizontal spreading of growth	(+) Horizontal spreading of growth
SIM-indole	(--) No color change with Kovac's reagent	(--) No color change with Kovac's reagent
Esculin Hydrolysis	(--) brown tube	(--) brown tube
Catalase	(+) bubbles	(--) no bubbles

Table 2. Results of Various Physiological Tests

DISCUSSION

We determined, through observation and tests, that the organism *Deinococcus radiopugnans* is a Gram-positive coccus. Our observations showed the characteristics of a staphylococcus, but the Bergey's Manual states that it is found in tetrads or dividing pairs. It is strictly aerobic, which is why no fermentation tests were performed. The organism has cytochrome-C in its electron transport chain, which had a redox reaction with the reagent in the oxidase paper. It can not use citrate as a carbon source. *Deinococcus radiopugnans* cannot ferment glucose, sucrose, or lactose upon testing. It is capable of motility, but the Bergey's Manual stated that it is nonmotile, which differs from our test results. It cannot produce hydrogen sulfide in the presence of iron or break down tryptophan to indole. The organism cannot break down esculin, but it can detoxify hydrogen peroxide to form oxygen and water. *Deinococcus radiopugnans* produces a bright red pigment on all plate mediums tested.

Massilia timonae is a Gram-negative bacillus in isolated or possibly palisade forms. It is an aerobic organism, which is again why no fermentation tests were used. This organism has cytochrome-C in its electron transport chain. Also, it can use citrate as a carbon source. It is unable to ferment glucose, sucrose, and lactose, as was predicted. The organism is motile and does not produce hydrogen sulfide when in the presence of iron. Also, *Massilia timonae* cannot break down tryptophan to indole and does not have the enzymes to break down esculin. This organism cannot detoxify hydrogen peroxide to oxygen and water. This must mean that it has a different enzyme to detoxify the toxic oxygen species in order to be aerobic.

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Isolation and Identification of Gram-Positive *Micrococcus luteus* From water in a Beta fish bowl

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I obtained a sample of water from my Beta fish bowl. I isolated and identified one of the organisms growing on a Nutrient Agar plate. For the identification of the organism, I conducted a series of stains and physiological tests that helped confirmed data obtained from DNA sequencing. Both, information of the DNA sequence and my test results showed that the organism of interest was *Micrococcus luteus.*

INTRODUCTION

This is and Independent Investigation project assigned by the Sierra College Department of Biological Sciences in Rocklin, CA to all the Microbiology students. I was curious about the

organisms that were growing in my Beta fish's bowl, so I decided to randomly isolate one of the organisms for its identification. The organism I identified was *Micrococcus luteus.* This organism is found in many types of environments including, soils, dust, fresh water, and on the skin of humans and animals (Baird-Parker, 1929). Fortunately, I don't have to worry about *Micrococcus luteus* living in my Beta fish's bowl, since it is not pathogenic to plants and animals (Baird-Parker. 1929).

MATERIALS AND METHODS

Using a disposable pipette, I obtained a sample of water from the Beta fish bowl in my bedroom. I inoculated a Nutrient Agar plate. I stored it in my room at room temperature. After two weeks, I randomly picked one of the many different organisms growing on the plate to obtain a pure culture. I streaked it on a new Nutrient Agar plate and stored it in the lab at room temperature.

Once I had a pure culture, the first steps I took were the determination of culture morphology and the determination of cell morphology through a Gram stain and an indirect stain. I also did a KOH test to confirm the Gram stain result.

The next step was to isolate the chromosomal gene that codes for 16S rRNA. I extracted the chromosomal DNA using the boil and beat method and amplified it by PCR using Bact 8 Forward and 1492 Reverse primers. The PCR product was then run under Gel Electrophoresis to isolate the 1500pb PCR product of interest, cut out and purified using QIA quick PCR Purification Kit by Qiagen. The 1500bp product was sent to the Division of Biological Science Sequencing Facility at U.C. Davis. The Bact 8 F primer was used for DNA sequencing. I analyzed the electropherogram using 4 Peaks and compared the sequence using the National Center Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLAST). The final tests were glucose fermentation, MR-VP, and motility test.

RESULTS

The organism forms yellow circular, convex, colonies with an entire edge. They are also glistening and opaque and about 1-2 mm in size
Following an indirect stain, the cells were spherical and 1.5 um in diameter arranged in clusters and tetrads.

Gram stain	Purple spherical cells
Indirect stain	1.5 um cells (diameter)
Glucose: fermentation and acid production	No change→yellow
MR	No change→yellow
VP	No change→yellow
Motility	None

Electropherogram analysis and genebank comparison with NCBI, BLAST:
The identity of the species my organism showed greatest similarity (99%) with was *Micrococcus luteus*.

DISCUSSION

The organism isolated from a water sample of a Beta fish bowl is Gram-positive cocci as shown by the Gram stain. These cocci show no evident motility and are easy to see under a light microscope due to their fairly big size. The Gram-positive cocci section of the Bergey's Manual of Determinative Bacteriology, listed four genera of the family *Micrococcaceae*. At this point, only having information about the colony and cell morphology and knowing that the cocci were not motile, I speculated that the organism belonged to either the *Micrococcus* or the *Staphilococcus* genus.

After comparing the DNA sequence for 16S rRNA using NCBI, BLAST, I found that the organism was *Micrococcus luteus.* To confirm this result I ran two physiological tests: a glucose fermentation test and an MR-VP test. My results were negative for both. The organism was unable to ferment glucose, did not produce acid in the MR test and did no form any acetoin in the VP test. The glucose test results were consistent with data in the Bergey's Manual. The Bergey's manual does not give results for the MR-VP test, but it does show that the organism cannot form acid.

There are three species of genus *Micrococcus*: *M. luteus, M. roseus,* and *M. varians.* My organism forms yellow colonies, and of the three species, only *M. luteus* and *M. varians* form yellow colonies. Furthermore, *M. luteus* does not produce acid in a glucose medium, whereas *M. varians* does produce acid. Since my organism does not produce acid, I concluded that this organism is indeed *Micrococcus luteus.*

Acknowledgements

I want to thank my Microbiology teacher, Sasha Warren, for her guidance throughout the semester. I also want to thank instructors Elaine Atnip and Harriet Wilson for helping with some of the lab procedures. Also, thanks to the Sierra College Foundation for providing the facilities and materials for my project and to the North Valley and Mountain Biotechnology Center at A.R.C.

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Isolation and Identification of a Gram-Positive Organism From an Air Plate

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Micrococcus luteus **was isolated from and air plate that was exposed in a garage for one hour. It was isolated by allowing it to grow on a nutrient agar media plate, removing a colony and restreaking it to make a pure culture to test. These colonies that were isolated were tested using Gram staining, acid-fast staining, KOH test, motility and glucose fermentation tests. The genomic DNA was isolated and amplified using a PCR reaction. After isolation of DNA it was sent to the Sequencing facility at UC Davis. When the sequencing was finished it was compared to other DNA sequences using the Basic Local Alignment Search Tool to identify the organism. After being identified, Bergey's Manual was used to identify which test should be performed to ensure that the organism that BLAST had identified as the organism isolated was correct. After performing all of the tests, the organism was identified as *Micrococcus luteus.***

INTRODUCTION

This project was to isolate and organism from the air. The organism that I isolated by exposing a nutrient agar plate to the air in my garage for one hour was *Micrococcus luteus.* *Micrococcus luteus* can be found in many places such as the human skin, water, dust, soil, meat and dairy products. Micrococcus is generally thought of as a harmless bacterium, but there have been rare cases of infections in people with compromised immune systems. Recently, this organism was recognized as an opportunistic pathogen and has been implicated in recurrent bacteremia, septic shock, septic arthritis, endocarditis, meningitis, intracranial suppuration, and cavitating pneumonia in immunosuooressed patients.

MATERIALS AND METHODS

In order to isolate and organism from the environment, a nutrient agar air plate was exposed for one hour in a garage. An isolated colony was removed from the plate using aseptic technique and restreaked on another nutrient agar plate. The organism was allowed to grow at room temperature until isolated colonies were growing. An indirect stain was performed to view the morphology of the organism. A Gram stain was performed to identify if the organism was gram positive or gram negative, and observed under a microscope. A KOH test and an Acid fast test were also done to test the organism. After the initial tests were finished, the DNA was extracted from the organism using Polymerase Chain Reaction to amplify the 16s ribosomal DNA using oligonucloetide primers Bacteria 8 forward and Universal 1492 reverse. A gel electrophoresis was run to isolate the 1500 base pair PCR product. The PCR product was cut out and purified using a purification kit made by Quagan. This product was then sent to the Division of Biological Sciences Sequencing Facility of UC Davis to be sequenced. UC Davis sent an electropherogram that was accessed using the program 4Peaks to be observed. The information that was obtained with 4Peaks was then copied and sent to the National Center for Biotechnology Information and run through their Basic Local Alignment Search Tool (BLAST) to be compared to the other DNA information in their database. After being compared to the database, identification was obtained with a 99% similarity to another organism in their gene bank. After identification, the Bergey's Manual Volume 2 was used to obtain information on the tests that would need to be performed to find out if the identification from BLAST was correct. The Bergey's Manual stated that if the organism was *Micrococcus luteus* it should not ferment glucose, should not have motility and should be MR-VP negative. These three tests were performed on the organism. For testing motility, the organism was mixed with water on a slide and this wet mount was observed under the microscope. For the carbohydrate deeps, raffinose, sucrose, lactose, sorbitol, rhamnose, mannitol, arabinose, and inositol were inoculated with the organism and incubated over night. For the MR-VP test, the organism was inoculated into a deep of MR-VP broth culture to test for fermentation products produced.

RESULTS

The colonies isolated on the nutrient agar plate were opaque and golden-yellow in color, circular, entire, convex, smooth, shiny, and 1.5 to 2.5 mm in width. Following an indirect stain, I observed sphere shaped cells that were 1.0 to 3.0 um in diameter and in bunches of four cells.

Following a Gram stain, I observed purple sphere shaped cells in bunches of four cells. Following and endospore stain, there were endospores present. After performing a KOH test, I observed no change. Following an acid fast stain, I observed blue sphere shaped cells in groups of four cells. When a motility test was performed on the organism, there was no movement observed in the microscope. The next test was the carbohydrate deeps that were inoculated with the organism. The tubes were incubated over night. The color in the tubes did not change, but stayed red in color. The last test, the MR-VP test, the results were no color change for either of the tests. The BLAST results were that the organism had the greatest similarity to *Micrococcus luteus.* The lineage is Bacteria; Acinobacteria; Actinobacteria; actinobacteridae; Actinomycetales; Micrococcineae; Micrococaceae; Micrococcus. The query length is 749. The gene bank length is 999. The % similarity is 99%. The number of bases matching pair wise is 746. The bit score is 1461.

DISCUSSION

The cells that were observed in the Gram stain were gram-positive tetradcocci. They were KOH negative, non-acid fast, endospore positive, did not form capsules, and had no motility. The organism was negative for fermentation of sugars in the carbohydrate deeps and the MR-VP tests performed. According to Bergey's Manual, *Micrococcus luteus* should be MR-VP negative, and should usually not produce acid from carbohydrates. Also the Bergey's Manual states that the colonies are golden-yellow in color, spheres that are 1.0 – 2.0 um in diameter, and non-motile. I did not have any conflicting results that may lead me to conclude that this is another organism. With a 99% match to *Micrococcus luteus* on the Basic Local Alignment Search Tool, I would conclude that this organism is *Micrococcus luteus.*

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Isolation and Identification of a Micrococcus on an Air Plate From an Apartment in Roseville California

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Isolation and identification of an unknown from an air plate was determined by performing Polymerase Chain Reaction, gel electropheresis, and sequencing. Physiological tests were also preformed and morphological observations were made to determine the identity of Micrococcus luteus.

INTRODUCTION

Microorganisms are everywhere, including the air. Outside and inside air both carry dust, dirt, smoke, and numerous bacteria that are associated with humans. One of these bacterium which was isolated from an airplate in an apartment in Roseville California is called Micrococcus luteus. The bacteria Micrococcus luteus is an organism that lives on the epidermis animals, particularly humans (Bauman, 2004). Micrococcus luteus can be an opportunistic

pathogen, but it is not usually harmful because they live off of human's dead particles, also known as being saprophytic (University of Texas, 1995).

MATERIALS AND METHODS

For this isolation, I set a nutrient agar plate at room temperature on the counter near a fan. I left the nutrient agar fully exposed to the circulating air for an hour and then closed the plate. After three days, many different colonies had begun to grow on the nutrient agar. Using aseptic technique, I streaked a second nutrient agar plate with only Micrococcus luteus. After the pure culture had grown for a few days, I took record of bacteria's morphology and I preformed a gram stain to determine if it was gram positive or gram negative and to determine the cell shape and arrangement. Next I preformed a KOH test to confirm the results of the gram stain and a motility test to see if my orgaism was motile. Then I isolated the genomic DNA by obtaining a recent culture of the bacteria and using the boil and beat method with the Vortex Mixer to break up the clump and break the cell wall. Next, I amplified the 16s ribosomal DNA using the Polymerase Chain Reaction and the oligonucleotide primer called Bacteria 8 Forward. Then I used Gel Electrophoresis to isolate approximately 1500 base pairs of the Polymerase Chain Reaction product that represented the ribosomal DNA. Next, my professor, Dr. Sasha Warren, cut the ribosomal DNA out of the gel with a razor blade and purified it using "QiAQuick PCR Purification Kit" by Qiagen. Finally, it was next sent to the Division of Biological Sciences Sequencing Facility at UC Davis who sequenced it with Bacteria 8 Forward primer. Upon receiving the results, I analyzed the electropherogram using the "4 Peaks" software program and compared my sequence using the National Center for Biotechnology Information and utilizing the Basic Local Alignment Search Tool. Based on the results of the DNA, I looked up my microorganism in the Bergey's Manuel and preformed these physiological tests: oxidase, citrate, carbohydrate deep containing glucose, growth of the microorganism at 37 degrees Celsius, and growth on Manitol Salt Agar.

RESULTS

(When analyzing the bacteria's morphology, I noticed yellow pigmented circular colonies, approximately 1-2mm in length, that appeared smooth and convexed. The results of the Gram Stain showed purple cells in the shapes of cocci approximately .9-1.8 micrometers in diameter, arranged in tetrads and clusters. The KOH test resulted in no snot and the cells during the motility test appeared to not be moving on their own. Results of the Polymerase Chain Reaction, the sequencing, and the Basic Local Alignment Search Tool, suggested that my microorganism was Micrococcus luteus. My physiological test results are shown in this table.

Oxidase Test:	+
Citrate Test:	-
Glucose Carbohydrate Deep:	-
Growth at 37 degreed C:	+
Growth on Manitol Salt Agar:	+

DISCUSSION

The observations that I made about my bacteria's morphology means that the bacterium is yellow in color and that the colonies are circular, convex, and smooth and/or shiny. Results from my gram stain mean that the cells are Gram positive cocci arranged in tetrads and clusters and measurements of the individual cells are approximately .9-1.8 micrometers in diameter. The KOH results mean that the organism is KOH negative, which also verifies that the organism is Gram positive. Results of the combined tasks of the Polymerase Chain Reaction, gel electrophoresis, and sequencing all worked to determine the identity of my microorganism which came out to be Micrococcus luteus. This result was verified with the Basic Local Alignment Search Tool which compared my sequence with 4,580,296 other sequences and found a 99% similarity. The results of my physiological tests were found to be accurate based on the information about Micrococcus luteus contained in the Bergey's Manual. The oxidase test proved to be positive meaning that Micrococcus luteus contains cytochrome c in its electron transport chain. Citrate test was negative meaning that it does not use citrate as a carbon source. The glucose carbohydrate deep was also negative meaning that Micrococcus luteus does not turn glucose to acid or it does not ferment glucose. The positive test results of the separate growth tests mean that it can grow at moderate to fairly high temperatures and that it can grow on a medium that has approximately 7.5% salt.

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Harriet Wilson
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Isolation of *Bacillus Thuringiensis* from airplate in Rocklin, California

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Bacillus Thuringiensis **is a naturally occurring insecticide found in the soil. In this project I obtained colonies from an airplate, isolated an unknown organism, and used Standard Microbiology diagnostic tools to determine the genus and species of this organism. After running the tests the organismed turned out to be *Bacillus Thuringiensis.***

INTRODUCTION

Bacillus Thuringiensis, also known as Bt, is a Gram positive bacteria found naturally in the soil. *Bacillus Thuringiensis* is commonly used as a natural insecticide because it produces delta-endotoxin which paralyzes the digestive system of the insect (Cranshaw, 2006). Bt is used world wide for many different types of insects. It is non-toxic to humans, which is what makes it a preferred choice over chemical insecticides. This organism was isolated as part of an

Independent Investigative Project in Sasha Warren's Biological Science 4 class at Sierra College in Fall semester 2006. The goal of the project was to isolate and identify an unknown organism from the environment.

MATERIALS AND METHODS

Organism was isolated from a nutrient agar plate left open in a kitchen for one hour in Rocklin, California. The organism was pure cultured on a nutrient agar plate. I began with an indirect stain with Nigrosin, and then performed a Gram stain in conjunction with a KOH test, and then an endospore stain.

Next, I isolated the genomic DNA by boiling cells and buffer, and vibrating them with glass balls. We used the Polymerase Chain Reaction to amplify 16s ribosomal DNA using primers Bacteria 8 Forward and 1492 Reverse. We then used gel electrophoresis to isolate approximately 1500 base pair of the PCR product, then cut out and purified the product with QIAquick PCR Purification Kit by Qiagen. The product was sent to UC Davis in the Division of Biological Sciences Sequencing Facility and was sequenced using Bacteria 8 Forward. I analyzed the electropherogram using the Four Peaks software and then used the Basic Local Alignment Search Tool on the National Center for Biotechnology's website to compare my genomic sequence to previously documented and archived sequences.

In addition, I performed a Vogues-Proskauer and Citrate Utilization test, struck for isolated colonies on MSA, and inoculated a test tube with water and a mosquito larvae.

RESULTS

My organism has colony morphology that consists of: Irregular form, filamentous margin, flat elevation, rough surface texture, opaque optical character, white color, is three to five millimeters in size, and forms a filamentous effuse layer on the agar after initial growth.

Following an indirect stain with Nigrosin, cells are rod shaped, arranged in pairs, and are approximately 8 micrometers in size. Following a gram stain, cells are dark purple. On a motility test no movement is observed. An endospore stain with Malachite green shows central spores.

PCR and gel electrophoresis worked. When compared to other genomic sequences, my sequence showed a 99% match *Bacillus Cereus* and *Bacillus Thuringiensis*. The Vogues-Proskauer test turned yellow indicating a negative test. Citrate remained green in color, which indicates a negative test. Growth occurred on both the Nutrient Agar and Mannitol Salt Agar plates. Also, the mosquito larvae test was positive because it killed the larvae.

DISCUSSION

From all my results, I conclude that the organism I isolated is *Bacillus Thuringiensis*. At first it was difficult to determine whether the organism was *B. Cereus* or *B. Thuringiensis* because they are both nearly identical in colony morphology, how they look under a microscope, results of biochemical tests, and even how their genomes look. The thing that swayed me in the direction of *B. Thuringiensis* is 3 things. First, the organism tested negative for Vogues-Proskauer. According the the Bergey's Manual, *B. Cereus* always tests positive for VP and B.

Thuringiensis depends on the strain; about 11-89% test positive. Second, *B. Thuringiensis* can withstand high concentrations of salt and *B. Cereus* can't, so when growth on MSA occurred, I leaned more towards Bt. Finally, the giveaway test was the mosquito larvae test. When introduced to insect larvae only *B. Thuringiensis* kills them, so when the mosquito died it was a good indicator that the bacteria was indeed *B. Thuringiensis*. It is important that a bacteria like B. Thuringiensis is found naturally because of its beneficial properties.

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The Isolation and Identification of Cell Phone Bacteria

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After taking a microbiology course, I find myself observing the filth all around but especially the microbe-infested objects I touch. I became interested in finding out what is growing on my husband's cell phone. So I performed some tests. In short, I swabbed his phone ran some tests and discovered exactly what exactly was growing. My result was the bacteria *Micrococcus luteus*. M. luteus is a organism found in many places but most of all on the skin as an organism involved in normal flora. I have determined that it is not necessary to be so overly aware of all of the bacteria surrounding me. The organism I isolated is not typically, pathogenic and very few are.

INTRODUCTION

Microorganisms are everywhere! Some are pathogenic, but many are not. To spend time thinking about the microbe-infested objects that are touched throughout life is enough to drive a

person completely mad! My husband runs a construction company, and honestly I do not believe that he washes his hands at all throughout the day. One object that I know is in his hands all day long is his cellular phone. With all the recent hype about antimicrobial cell phones, I started to become a little curious about what is actually growing on his cell phone. I have not been able to pin point any reports on disease caused by bacteria infested cellular phones, but I suppose that would be a hard case to prove. So my curiosity over came me and I decided to find out exactly what it is that my husband holds in his hands and puts on his face daily.

MATERIALS AND METHODS

A swab was collected from the keypad subjects cell phone. AQ-tip was used to collect the swab and it was streaked onto a nutrient agar plate. The plate was incubated at 20°C (room temperature) for one week. Following incubation, the growth of a variety of bacteria colonies was observed. Some were white, others were creamy white and still others were mustard yellow. The mustard yellow colony was chosen to isolate and identify. The single colony was streaked onto a new nutrient agar plate and incubated once again at 20°C (room temperature) for one week.

Following isolation, a sample of the organism was smeared onto a slide and a gram stain was performed. Other physiological tests were performed, including an MR/VP test, a catalase test, an oxidase test, and a motility test. Subsequent to the gram stain a 100-1000µl micropipette was used to add 500µl of buffer to a 1.5ml centrifuge tube and approximately 2mm of the unknown organism as well as sterile glass beads were added to the tube. The centrifuge tube was placed on the vortex for a moment to mix all of the ingredients. The tube was the placed into a foam floater in a beaker of boiling water and boiled the tube for ten minutes to release the DNA from the cells. The tube was chilled in an ice bath and once cooled, placed on the vortex once again and vortex-mixed for ten minutes.

To perform the Polymerase Chain Reaction amplifying 16s rDNA, a 0.2ml PCR tube was obtained along with the appropriate micropipettes (10-100µl & 0.5-10µl) and the following reagents were added: 25µl of Taq DNA Polymerase, 5.0µl of primer (bacteria 8 forward and universal 1492 reverse), 5.0µl of DNA template, and 15µl of sterile distilled water. The PCR tube was kept on ice the entire time. My instructor, Sasha Warren, was responsible for combining all of these ingredients and placing the 0.2ml PCR tube into the thermocycler where it ran through an amplification process that lasted three hours.

Following the PCR production process, gel electrophoresis was performed. Gel electrophoresis is a technique or a procedure that allows DNA fragments to be separated on the basis of size and observed with the naked eye (Wilson, 2006). 20 mL of agarose gel was prepared and poured onto a glass slide with a plastic comb producing wells in the gel. After solidification, the comb was removed and the gel was placed into the gel electrophoresis box, which sends electrical current through the gel. The electrical current allows the negatively charged bacteria to travel towards the positively charged anode in the box. A buffer solution was poured over the top of the agarose gel. Next, a micropipette was used to load 5µl of the unknown organism mixed with loading dye containing bromophenol-blue and xylene cyanol into one of the wells formed by the comb. Also 5µl each of plasmids, DNA, and DNA ladder, all mixed with the loading dye were pipetted into separate wells. DNA ladder was used for calibration to

determine the base pair sequence of my organism. Once all of the wells were loaded the lid was placed on the box and the voltage was turned up to 45 volts.

Upon completion of gel electrophoresis, the unknown organism was sent to The UC Davis Sequencing Facility to perform the dideoxy chain termination method of sequencing, to determine the sequence of nucleotides in the PCR product. The lab technicians at UC Davis integrated an analog of dNTP: dideoxynucleoside triphosphate (ddNTP), which lacks a 3′ OH group, into the DNA strands during replication. Once the sequencing was complete, the electropherogram was analyzed using the Four Peaks Program. The sequence was compared to other DNA sequences on the Basic Local Alignment Search Tool.

RESULTS

The mustard yellow colony isolated was pinpoint, entire around and the edges, convex, and shiny; it was approximately 1μl in diameter and smelled like dirt. Under the microscope, with a gram stain, the unknown organisms cell morphology appeared as gram-positive round shaped cells arranged in clusters. A negative result was obtained for the MR/VP test performed, as well as the oxidase test, and positive results were obtained for the catalse, and motility tests.

DISCUSSION

Based on the results from the electropherogram obtained through PCR, gel electrophoresis, and dideoxy chain termination, it has been determined that the organism has a base pair sequence that most resembles that of *Micrococcus luteus* isolate SCH0405 16s ribosome RNA gene partial sequence. It was determined that the organism had a 99% similarity match with 769 base pairs matching out of 774 in the query length. *Micrococcus luteus* is found in the family Micrococcacae. Subsequent to performing a gram stain it was determined that the organism is in fact gram-positive cocci arranged in tetrads. According to Bergey's manual, *Micrococcus* is in fact the only genera within the family Micrococcacae that is arrange in tetrads of cocci (*****). The result of the cell morphology from the gram stain corresponds with the identity of the species from the electropherogram.

A MR/VP test was performed and the diagnosis was that the organism was not capable of fermentation. The result coincides with that of the information in the Bergey's manual. According to the Bergey's manual *M. luteus* is a strict aerobe and is only capable of aerobic respiration (*****). The next test performed was the oxidase test and the results were unexpected. It was found that the organism was oxidase negative. *M. luteus* is an oxidase positive organism. I believe the accurate explanation of having conflicting test results would be that the oxidase test disc was in fact old and therefore not producing accurate results. The catalase test was clearly positive, resulting in bubbles upon mixing 3% hydrogen peroxide with the organism. The last performed was the motility test. A positive was obtained. Being new to microbiology and slightly under experienced I may have mistakenly confused motility with Brownian motion, and that is a possible explanation for the conflicting result.

In conclusion, without the electropherogram, I do not believe that the tests I performed would have been enough evidence to determine the identity of the organism. Running four tests and having two of them with conflicting results to that of the Bergey's manual confused things.

Upon further investigation, I have discovered one last test would have helped me to determine the accurate identity of my organism: growth of my organism on inorganic nitrogen agar.

ACKNOWLEDGEMENTS

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Isolation And Identification Of Gram Positive Organisms Found On Yuba City Air Plate

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Airborne, yellow, white and orange bacteria were isolated on an air plate to obtain further information about the species. These organisms were found to be Gram positive Staphylococcus and tetrad cocci. Based on physiological, morphological and DNA techniques the organisms were identified as *Staphylococcus saprophyticus, Rhodococcus terrae and Micrococcus luteus.*

INTRODUCTION

The goal of this investigation was to identify unknown organisms that were grown and isolated using an air plate. In doing this and performing many experiments the organisms have been identified as *Staphylococcus saphrophyticus*, *Micrococcus luteus*, and *Gordonia terrae*.

Micrococcus luteus is a gram positive spherical saprotrophic bacterium that belongs to the micrococcaceae bacterial family. An obligate aerobe, *M. luteus* is found in soil, dust, water,

and air, and as part of the normal flora of the human skin. The bacterium also colonizes in the human mouth, mucosae, oropharynx and upper respiratory tract. *M. luteus* is relatively resistant to reduced water potential and can tolerate drying and high salt tolerant concentrations fairly well, making it a halophile. Although *M. luteus* is non-pathogenic and usually regarded as a contaminant, it should be considered as an emerging nosocomial pathogen in immunocomproised patients. (3)

Staphylococcus saphrophyticus is a gram positive bacterium that belongs to the Staphylococcacea family, and is often implicated in urinary tract infection. *S. saphrophyticus* is rarely found in healthy humans, but is commonly isolated from animals and their carcasses.

Gordonia terrae is a gram positive bacteria belonging to the family Gordinaceae. It is an aerobic chemoorganotrophic with an oxidative type of metabolism. It mostly grows on 30°C media but some require thiamin. It has a rod-coccus growth cycle. It is sensitive to lysozyme. *G. terrae* has been linked to cases of catheter-related bactericidal. Also patients who had primary diagnosis of cancer experienced non neutropenic fever due to *G. terrae*.

MATERIALS AND METHODS

An air sample was collected on nutrient agar and incubated at room temperature for 48 hours and examined for growth. The sample was collected in Yuba City by exposing the plate for one hour in a bedroom which was placed on a dresser, with no air conditioning or heater on. Upon examination of this plate three types of organisms appeared to be growing. The next step was to isolate pure colonies for each organism. Isolated pure cultures were grown, and the following procedures were performed; Gram Stain, Indirect Stain, Motility Test and KOH Test. In order to isolate genomic DNA the following was done to each organism: vortex machine was used for 25 seconds to extract the DNA. In order to amplify 16s ribosomal DNA, Polymerase Chain Reaction was performed using Bacteria 8 Forward and 1492 Reverse primers. Gel electrophoresis was then used to isolate 1500 base pairs of PCR product. The gels containing the 1500 base pairs were cut out and purified with QiAQuick (PCR purification kit by Qiagen). The pieces of gel were sent to the Division of biological Sciences Sequencing Facility at U.C. Davis, which was sequenced using Bacteria 8 Forward primer. Upon returning from U.C. Davis, using the 4 Peaks program, the sequences were then analyzed and compared to online data base sequences using the National Center for Biotechnology Information and the Basic Local Alignment Search Tool. Based on these results, the following physiological tests were performed; Oxidase, Vogues Proskauer, Citrate, Esculin Hydrolysis, Urease, Catalase, Hemolysis, Coagulase, and Carbohydrate Deeps (glucose, sucrose, arabinose, manitol, lactose, inositol, sorbitol, rhaminose).

RESULTS

On nutrient agar colonies of *Mirococcus luteus* appear as gossy, yellow, circles, raised off of the plate, with an irregular edge, that are 2.0 mm in diameter. *Staphylococcus saprophyticus* appear as shiney white circles, slightly raised, with smooth edges, that were 4-5 mm in diameter. *Rhodococcus terrae* appear as orange, circles that were 2mm in diameter and slightly raised.

Table 1.

Tests	**Organism #1 Believed to be *Micrococcus luteus***	**Organism #3 Believed to be *Staphylococcus saprophyticus***	**Organism #2 Believed to be *Rhodococcus terrae***
Oxidase Test	-	-	N/A
Voges Proskauer Test	-	+	N/A
Citrate test	-	N/A	+
Esculin Hydrolysis	-	N/A	N/A
Urease Test	-	-	+
Fermentation of Glucose	-	N/A	N/A
Fermentation of Arabinose	N/A	-	N/A
Fermentation of Sucrose	N/A	+	N/A
Fermentation of Mannitol	N/A	d	+
Fermentation of Lactose	N/A	d	N/A
Fermentation of Inositol	N/A	N/A	-
Fermentation of Sorbitol	N/A	N/A	+
Fermentation of Rhamnose	N/A	N/A	+
Coagulase Test	N/A	-	N/A
Catalalase Test	N/A	N/A	+
Hemolysis	N/A	-	N/A
Motility Test	Non motile	Non motile	Non motile
KOH Test	KOH - no snot	KOH – no snot	KOH- no snot
Gram Stain	Clusters of purple circles that were 1µm micron in length	Clusters or four circles that were 1.7µm in length	Clusters of purple circles that were 1µm in length
Indirect Stain	Black background white cell	Black background white cell	Black background white cell
Capsule Stain	-	N/A	N/A

*N/A – non applicable
*d – inconclusive

DISCUSSION

According to Bergey Manual (4) Gram positive cocci that form clusters of tetrads can be found in the Mirococcacea family. The species that form these tetrads is *Mirococcus luteus*. This species exhibit glossy yellow pigmentation, and appear convex, with udulate egde. *M. luteus* is oxidase positive, although the results for the unknown organism were conflicting due to inadequate methods of testing in the classroom. The Voges Proskauer test for the isolated organism was found to be negative for fermentation. The result of the citrate test was negative, as was the urease test result. In the stab glucose agar, the organism showed no sign of fermentation. A capsule stain was performed, to confirm the organism had no capsule. KOH test was negative, reaffirming that the organism is Gram positive. A motility test was done, confirming a non motile organism. The results from the esculin hydrolysis test were negative. Based on our results from Table 1 and in comparison to the Bergey Maual , we believe that this isolated organism #1 from the air plate is *Micrococcus luteus.* (4)

Gordonia terrae was not in the Bergey's Manual but its old name was *Rhodococcus terrae*. (6) According to the manual it would test positive for the following tests: urease, citrate, fermentation of maltose, sorbitol, inositol, mannitol but unable to ferment inositol. I conducted the same experiment and my results agreed with the burges manual. Catalase test the when added H2O2 the organism showed bubbles meaning it has the enzyme to catalyze the break down of H2O2. Urease test - We tested if the organism hydrolyzes urea and the medium changed to hot pink meaning more alkaline such as ammonium was present. The organism does hydrolyze urea. CHO deeps -the organism fermented the following carbohydrates into acid: sorbitol, rhaminose, and mannitol. The medium didn't change to yellow for inositol it changed into pink meaning negative for inositol and it doesn't ferment it. According to the Bergey Manual (2) it also tests positive for the fermentation of maltose but we did not have the supply for that experiment. The organism showed no motility when observed on a wet mount. Citrate test - We tested if the organism was able to utilize citrate as a sole source of carbon and the medium changed blue meaning the residual media was alkaline and it does use citrate as a carbon source. According to the Bergey's Manaul, the second organism was found to be *Rhodococcus terrae*.

We referred to the Bergey's Manual once more and conducted many of the same tests were done in order to confirm the third organism. The oxidase test result was negative, along with the results for the urease test, hemolysis test, and the coagulase test.

A positive result for the Voges Proskauer test implicates that this organism is a butaindiol fermentor. A motility test confirmed the organism to be non motile. KOH test was negative, indicating that the organism is Gram positive. Inoculating carbohydrate deeps resulted in the following; the organism does not ferment arabinose, it does ferment sucrose, and the results for mannitol and lactose were inconclusive. According to these results, the Bergey's Manual states that the organism is *Staphylococcus saprophytucus* (5).

AKNOWLEDGEMENTS

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Isolation and Identification of *Bacillus drentensis* from the Environment

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A Gram-positive or Gram-variable, facultatively anaerobic, motile organism with tapered rods occurring singly and in pairs was isolated from the air in Roseville, Califronia. The isolated colony was subject to various staining techniques, physiological tests, Polymerase Chain Reaction, gel electrophoresis and gene bank comparison. The organism was subsequently identified as Bacillus drentensis.

INTRODUCTION

The isolation of *Bacillus drentensis* from the environment started simply to identify the unknown. There are billions of microbes which we encounter in our everyday lives but to know exactly what is living around us and how they affect us has been a curiosity of many since before

the days of Pasteur. I began this project by isolating a bacterial culture from the environment to which I am most familiar, my bedroom in Roseville, California. This organism was identified as *Bacillus drentensis* after being subject to various staining techniques, physiological tests, Polymerase Chain Reaction, gel electrophoresis and gene bank comparison.

MATERIALS AND METHODS

On October 23, 2006, a Petri dish containing nutrient agar was opened in a bedroom in Roseville, California for one hour then stored in a drawer at Sierra College for a week to allow for growth. Using a week old, pure bacterial culture, colony morphology was observed along with various staining methods and physiological tests to identify cellular morphology and physiology. These included a Gram stain, KOH test, wet mount testing for motility, and a Malachite Green stain. On October 30, 2006, chromosomal DNA was extracted from a week old bacterial sample boiling for ten minutes. Because a Gram stain indicated a Gram-negative organism, sterile glass beads were not added. The isolated genomic DNA went through a Polymerase Chain Reaction (PCR) which amplified 16s ribosomal DNA using Bacteria 8 forward and Universal 1492 reverse primers. Gel electrophoresis of the PCR product was completed on November 1, 2006. Approximately 1500 base pair PCR product was cut out then purified using QIA quick PCR. Purification kit by Qiagen was used. Next, the mixture was given to Sasha Warren to be sent to the Division of Biological Sciences Sequencing Facility at University of California, Davis to be analyzed. Here, Bacteria 8 forward primer was used. On November 20, 2006, electropherogram analysis using 4 Peaks and gene bank comparison using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information website was completed. Based upon the results, more tests were done for confirmation of the organism including Lysine decarboxylation, Citrate utilization, Sulfur Indole Motility to test for hydrogen sulfide and indole production, Urea hydrolysis, and carbohydrate deeps including glucose, lactose, arabinose, mannitol, rhamnose, and sorbitol.

RESULTS

The shiny, off white colonies isolated are approximately 1-2mm wide, slightly irregular, entire and convex with an opaque optical character. Under microscopic observation, following a Gram-stain, pink, rod-shaped cells occurring singly and in pairs were viewed. A wet mount proved to be positive for motility and the Malachite Green stain showed spherical endospores located centrally within the cells. Electropherogram analysis and gene bank comparison indicated the unknown organism to be Bacillus *drentensis*, an organism with a lineage as follows: *Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.* Its Query length of 822, Gene bank length of 1492, a 98% similarity, 809/822 number of matching bases pair wise, a 1495 Bit Score and Accession number DQ275176. Based on the results of the confirmatory tests, reactions for lysine decarboxylation, citrate utilization, hydrogen sulfide production, indole production and urease are negative. Acid without gas is produced from glucose and lactose carbohydrate deeps. Acid is not produced from arabinose, mannitol, rhamnose and sorbitol carbohydrate deeps.

DISCUSSION

Bacillus drentensis is a Gram-positive or Gram-variable, facultatively anaerobic, motile organism with tapered rods occurring singly and in pairs (Heyrman, 2004). I found this organism to be Gram-negative after completing the Gram stain. This could have been because it is said to be a Gram-variable organism or because of over decoloization. Because my then unknown organism was thought to be a Gram-negative, during the isolation of chromosomal DNA process, the second step (using glass beads then beating) was not done. This organism, identified with BLAST to be *Bacillus drentensis*, had a 98% similarity. This organism was not found in the Bergey's Manual but I was able to compare my results to an article on the internet from Wikibooks. Following the confirmatory tests, all of which matched my unknown organism to *Bacillus drentensis,* I concluded that the unknown organism I isolated from the air in Roseville was *Bacillus drentensis*, the species *drentensis* being of Drente, a province in The Netherlands (Heyrman, 2004).

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The Strength of a Common Household Cleaner on Selected Gram-Positive Bacteria

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Clorox Anywhere Spray is a new household product I have been using to clean the counters in my kitchen. I laid out an open nutrient agar plate on a counter I often use to cook and clean my food. I performed carbohydrate slants and sugar tests. I also Gram stained, capsule stained, and did a Kirby Bauer test on the cleaner. I laid the bacteria out in the light to check for pigment and incubated it at two different temperatures. I attempted to grow it on MacConkey's and brain heart infusion agar.

INTRODUCTION

For my independent project I decided to test out one of the latest household cleaners on bacteria I discovered growing on my kitchen counter. Clorox Anywhere spray is a new cleanser

I have used on my counter tops that claims it has the same killing power of bleach without the obnoxious fumes. It has a large impact on my family as the counter from which I chose to sample bacteria is the one on which I prepare most of my family's meals. It turns out that the organism I had taken from my kitchen counter was actually a Gram + organism by the name of *Brevibacterium antarcticum* otherwise known as *Exiguobacterium aestuarii.* It is an opportunistic pathogen so it is essential to my family's well being that I get a more efficient cleaner.

MATERIALS AND METHODS

Nutrient agar to collect an air sample from the environment I cleanse with the cleaner. I laid out an open nutrient agar plate on the counter for approximately one hour. The air conditioner was on in my home and I had just cleaned the counter with the Clorox Anywhere Spray that morning. The home was approximately 75 degrees Fahrenheit and it was eight o'clock pm. I then picked one of several bacterial samples that grew to identify. I went on to perform a gram stain, capsule stain, and KOH test. I also inoculated the bacteria in SIM to check for sulfur, indole, and motility. Genomic DNA was isolated by boiling a sample for ten minutes and then using the vortex mixer to shake the sample for ten additional minutes. 16s rDNA was amplified using Polymerase Chain Reaction and adding primers Bacteria 8 forward and Universal 1492 reverse. Gel electrophoreses was performed to isolate approximately 1500bp of PCR product that represented the 16s rDNA. Mrs. Sasha Warren then cut the band out of the gel using a kit supplied by QIAQuick Qiagen that purified the PCR product. This removed the genomic DNA and primers. The cut-out rDNA was then sent to the Division of Biological Sciences Sequencing Facility at UC Davis. It was sequenced with Bacteria 8 forward primer. An electrogram was sent back. I analyzed it with the 4Peaks program on the Apple computer provided. It was then compared to the DNA sequences in the National Center for Biotechnology Information (NCBI) and put into the Basic Local Alignment Search Tool (BLAST) their website provides. After the results were obtained I performed the following tests: lysine decarboxilayse, glucose gas, arabanose, sucrose, esculin, catalase, oxidase, and Vogues-Proskauer. I also streaked the sample on EMB and MacConkey's agar and placed in the incubator at 37 degrees Celsius for three days. This was done to verify if it was a Gram + or – organism and backup results performed before the DNA was identified. I then streaked the organism again on MacConkey's agar and nutrient agar and grew them in my drawer between 20-30 degrees Celsius. This was performed to check for growth under different temperatures. I then streaked a nutrient agar plate and left it out to grow in a lighted environment. This was to check for pigment change if exposed to light. I inoculated the sample on nutrient and bloods heart infusion agar when I performed a Kirby Bower test on the Clorox Anywhere Spray.

RESULTS

The colony morphology included a sample that was opaque, cream color, circular, entire, and umbonate. The largest colony measured 4mm. The gram stain yielded purple cocci shaped bacteria 2.27 microns in length yielding the results of a Gram + organism. The KOH test yielded no consistency confirming the Gram + test and the sample had a white ring around the bacteria

when the capsule test was performed confirming that a capsule was present. When I initially checked the DNA sequence it came back with a Gram – organism by the name of *Vibrio alginolyticus*. After closer evaluation it was determined that the incorrect sequence had been put into National Center for Biotechnology Information (NCBI). That would explain the reason for the Gram – organism and at that point I had already inoculated all of the media for the following tests: Lysine decarboxilayse, glucose, arabanose, sucrose, Vogues Proskauer, and esculin. It turns out the organism I had taken from my kitchen counter was actually a Gram + organism by the name of *Brevibacterium antarcticum* otherwise known as *Exiguobacterium aestuarii* with a 99% similarity. The number of matching bases was 1489. The SIM test was yellow and cloudy. The catalase test yielded bubbles when introduced to hydrogen peroxide therefore producing a positive result. The oxidase test yielded no color and yielded negative results. The organism did not grow at the incubated temperature of 37 degrees Celsius yet grew on my nutrient agar plate in my drawer. It did not grow on the MacConkey agar. The pigment of the bacteria did change slightly to a yellowish cream instead of a more basic creamy color. The bacteria inoculated on the nutrient and brain heart infusion The Kirby Bauer test showed no zones of inhibition.

DISCUSSION

The tests I performed confirm the DNA analysis sent back from UC Davis. I am now confident of the results obtained due to the trial and error I witnessed during the procedure. The Bergey's Manual confirms that *Brevibacterium antarcticum* is Catalase positive, oxidase negative and, under certain circumstances, the shape of the bacteria will change under the microscope as the sample gets older. It also stated the organism will change pigment if exposed to light. It is an obligate anaerobe and, as a result, all of the carbohydrate tests I performed yielded a negative result. The oxidase test yielded no color, revealing no cytochrome C was present. I also observed capsules and this is typical of this organism under the circumstance that the stain was perform three to four days after the bacteria had been inoculated on the media. The SIM test was yellow and cloudy, meaning it does not make sulfur, yellow meaning it does not ferment indole and cloudy means that it is modile. It did not grow on the MacConkey agar confirming it is not a Gram – organism. The Voges-Proskauer test yielded a positive result, meaning it forms acetoin. The lack of Zones of inhibition for the Kirby Bauer test reveals that the cleaner does not kill the sampled bacteria. This is probably why the bacteria were present in the first place. Also, this organism originates from cheeses and fish, (Bergey Manual, 1312). I sliced cheese on a cutting board on that part of the counter and I have a fish tank on my adjacent kitchen counter. Some puzzling results were that it does not typically grow on nutrient agar which is where the original sample was taken from. It is also a rare bacterium that was originally isolated in Lake Fryxell, Antarctica (Schuman, 1171). It has also been isolated from a tidal flat of Daepo Beach (Yellow Sea) near Mokpo City, Korea, (Lee, 885). There were many articles I found written on the topic but most were written in German and very hard to understand. The Bergey's Manual lists this as an opportunistic pathogen and, therefore, is a bit alarming to me. Evidently I will have to use hard bleach to clean my counters in the future!

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I would like to acknowledge the following organizations; Sierra College Foundation for the computers used to analyze the DNA that was sent back from UC Davis. Also, we would like to thank North Valley and Mountain Technology Center and American River College for donating some of the equipment used in these experiments.

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The Isolation and Identification of a strictly aerobic, Gram-positive Micrococcus from Rocklin California Air.

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I isolated and identified a species of Micrococcus luteus from the environment. I used Polymerase Chain reaction process with electrophoresis and electopherogram analysis. This was to enter a sample strand of rDNA into the Basic Local Alignment Search Tool to compare and identify the sequence to other sequences in a series of databases. To back this up I used a variety of morphological and physiological tests and compared them to the bergey's manual of systematic bacteriology to confirm results. I found that *Micrococcus luteus* was successfully isolated and identified from the original air sample.

INTRODUCTION

Microbes are an undeniable part of us as humans. They are beneficial as our natural symbiotic flora; they are also harmful in certain cases as parasitic pathogenic organisms. I thought it would be interesting and possibly beneficial to know what is floating around an ordinary Rocklin apartment. My sample being from the air in the kitchen would possibly deem beneficial if the organism happened to be pathogenic. *Micrococcus luteus* was isolated and is normal flora of the human skin, is found in water, dust, soil, dairy products, and beer. *M. luteus* and its common relationship with animals and the environment is rarely found to be infectious and is only considered a opportunistic pathogen to severely immune compromised individuals. Being part of the human skins normal flora it does interact with the organ. *M. luteus* provides some symbiosis not allowing possible pathogenic organisms to colonize on the skin.

MATERIALS AND METHODS

The sample was obtained by exposing a plate of nutrient agar to the air in a kitchen in Rocklin California for one hour. It was then incubated for two days at room temperature. Isolation was done by streaking one colony on to nutrient agar and incubated at 37 degrees for two days. I then did a gram stain, KOH test, wet mount, and observed and recorded colony morphology. I also did the following physiological tests: O/F, MR-VP, Oxidize, Citrate utilization, Mannitol salt agar, and Sulfur Indole Motility,

Isolation of genomic DNA and DNA processing was performed. First 16s Ribosomal DNA was amplified using the Polymerase Chain Reaction process with the primers bacteria 8 forward and 1492 reverse. Then a gel electrophoresis was done to isolate a 1500 base pair segment to represent the ribosomal DNA. Then instructor Sasha Warren cut the band out and purified the PCR product with the QiAQuick PCR purification kit by Qiagen. Then the purified ribosomal DNA with Bacteria 8 forward primer was sent to the Division of Biological Sciences Sequencing Facility as UC Davis for sequencing. They provided an electropherogram of the sequence. I then analyzed and edited the nucleotide sequence using the 4 Peaks computer application. Then the sequence was compared to other sequences in GenBank, EMBL, DDBJ, PDB databases. They were compared to the databases using the National Center for Biotechnology Information web page and entered into the Basic Local Alignment Search Tool program.

RESULTS

Observing the colony morphology on nutrient agar I saw yellow pigmented, circular, entire, raised, shiny, and opaque colonies. Observing a Gram Stain I saw circular cells of 1-3.5 micrometers in diameter arranged in irregular clusters, they were dark purple. KOH produced no snot or viscosity. Looking at a wet mount I saw no motility of the cells. The O/F test had green with a little yellow at the top and no bubbles in the non-sealed tube; in the sealed it was green. The Citrate utilization test showed green throughout the medium. Observing the methyl red test the tube remained yellow, and the vogues proskaur test the tube turned a light orangish yellow. In the Sulfur Indole Motility test the tube was yellow and had only growth along the inoculation line, and after adding kovacs reagent the reagent and tube remained yellow. I observed a light

purple streak when performing the oxidase test. On mannitol salt agar colonies did appear but not very numerous and were very small with a little pink around them. After analyzing the organism's nucleotide sequence using the Basic Local Alignment Search Tool it found that 765 out of the 766 nucleotides analyzed matched.

Test	My Tests	Bergey's
Citrate	-	-
O/F	Oxidative	Oxidative
7.5% NaCl	+	+
MR/VP	-/-	(N/A)
Catalayse	+	+
SIM(motility)	-	-
Gram Stain	+	+

-Table 1

DISCUSSION

I found the organism to be Gram-Positive after doing a gram stain and checking with a KOH test. It proved to not be fermentative after the O/F test showed no gas or color change. I checked this with the MR-VP test and found both results to be negative and this is normal in non-fermentative organisms (Wieser, 2002). Then observing yellow pigmented colonies on nutrient agar, small (1-3.5micrometers) coccus, oxidative positive, non-motile in SIM and wet mount, can not use citrate as only carbon source, and ability to withstand salt (table 1). I compared them to The Bergeys manual and found them to be similar (Bergy's, 1986). I also found and is confirmed by the MSA plate that many species of micrococcus are somewhat salt tolerable because of them living on the mildly salty skin of the human body (Shana, 2006).

The Basic Local Alignment Search Tool showed a 765/766 nucleotide match giving a 99% probability that the organism was indeed *Micrococcus luteus*. I feel that even though I identified a common organism to the environment and is not harmful under normal circumstances. My identification was important and shows that we do interact and live amongst organisms that we may not be aware of. I know that the next time I smell myself or someone's body odor I will know that it was caused by *Micrococcus luteus* breaking down the compounds in sweat (Smith, 1999).

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Isolation and Identification of Two Different Organisms from an Air Plate in Auburn California

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Two different organisms were isolated from an air plate grown for one hour in the bathroom of an Auburn California home. The DNA from the two organisms were sequenced then compared to a database to determine the identification of the organisms. Morphological tests as well as multiple physiological tests were done on the two organisms to confirm the results of the sequencing. The two organisms were found to be *Staphylococcus warneri* and *Micrococcus luteus*.

INTRODUCTION

We began with a nutrient agar plate that was exposed to air for one hour in the bathroom of an Auburn California home. We did this to determine what organisms were present in this environment. We expected to find normal flora of adults, and would have been concerned to find pathogenic organisms. We isolated two unique colonies from this plate to identify them.

We found the colonies to be *Staphylococcus warneri* and *Micrococcus luteus*. Both organisms are found on the skin of humans as normal flora. *Staphylococcus warneri* is a gram positive cocci that is usually found in irregular clusters. It is normally harmless and can be found in the air and soil. (Wikipedia, 2006). *Micrococcus luteus* is also a gram positive cocci that appears in irregular clusters. It is found in dust, soil, water and air. It can colonize in the mouth, mucosae, oropharynx and upper respiratory tract in humans. Both organisms can occasionally cause disease in immunocompromised patients. (Wikipedia, 2006).

MATERIALS AND METHODS

We exposed the nutrient agar plate to the air for one hour. It was placed on the counter next to the sink in the bathroom below a closed window. The door to the bathroom remained open during the full hour of exposure. No person entered the room during that time period. The plate was incubated at room temperature for 48 hours in the drawer of the microbiology lab room. After that time two colonies were chosen for isolation. We used aseptic technique throughout all procedures. Each colony was individually streaked onto a new nutrient agar plate to isolate them to determine colony morphology. To establish cell morphology we performed a gram stain, followed by a KOH test to confirm our gram stain results.

When we had pure colonies we isolated our genomic DNA by adding our cells to buffer and mixing beads. We then vortexed the mixture and boiled it for ten minutes. We then vortexed it for ten more minutes. This gave us our ribosomal DNA (rDNA) template. We then performed Gel Electrophoresis to isolate approximately 1500 base pairs, which represents our 16s rDNA. The 1500 base pair band was cut from the gel then purified and amplified using the Q.A. Quick Polymerase Chain Reaction Purification Kit by Qiagen. The product of our Polymerase Chain Reaction (PCR), plus the primer bacteria 8 forward was sent to the Division of Biological Sciences Sequencing Facility at U.C. Davis. They obtained a nucleotide sequence for each of our organisms. We then analyzed the sequence using the program Four Peaks and compared the results using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST). The BLAST program compared our sequences to database sequences to identify our organisms.

To confirm the identity of our organisms we did multiple physiological tests. On *Staphylococcus warneri* we performed the following tests: Methyl Red- Vogues Praskour (MR-VP), Motility, Catalase, Mannitol Salt Agar (MSA), Sucrose, Urease and Coagulase. For *Micrococcus luteus* we did the MR-VP, test, Motility test, Catalase test, Glucose test, and the Triple Sugar Iron test.

RESULTS

Using the Basic Local Alignment Search Tool we obtained results for our two organisms. The first organism received a score of 1394 bits (703), and 345 of 751 base pairs were identified to be *Staphylococcus warneri*, giving it 99% accuracy. It had a length of 1470, an expect of 0.0,

gaps of 6 out of 751 (0%), and the strand was plus/plus. The second organism received a score of 1001 bits (505) and 700 of 751 base pairs were identified to be *Micrococcus luteus*, giving it 93% accuracy. It had a length of 1477, an expect of 0.0, gaps of 13 out of 751 (1%), and the strand was plus/plus.

The two organisms had a series of morphological and physiological tests done. The first organism was gram stained and revealed purple cocci in irregular clusters. The KOH test results showed no snot. The colonies were circular, entire, raised, shiny, opaque, and gray, smelled of wet dog and measured 2mm. The Catalase test displayed bubbles. The MR-VP test both turned red. On Mannitol Salt Agar the colonies grew and turned the medium yellow. The Coagulase results were liquid. The wet mount slide showed only Brownian motion.

For the second organism the gram stain showed purple cocci in irregular clusters. The KOH test had no snot. The colonies were circular, entire, umbonate, glistening, opaque, and beige, smelled of wet dog and measured 4mm. The Catalase test had bubbles. The MR-VP test was yellow. The Glucose test remained red. The Triple Sugar Iron (TSI) slant and butt remained red. Only Brownian motion was observed on the wet mount slide.

DISCUSSION

Based on the data from the Basic Local Alignment Search Tool (BLAST) we found the first organism to be *Staphylococcus warneri*. The purple gram stain proved that our organism is gram positive. The no snot KOH test confirmed gram positive. The gram positive irregular clusters matched the expected results for *Staphylococcus warneri*. (Wikipedia, 2006). The wet mount Motility test showed Brownian motion but no organism motility. The MR-VP results were both red, confirming that the organism is both a mixed acid and butanediol fermentor. A liquid Coagulase test told us that the organism does not contain coagulase. The hot pink Urease test showed that the organism produces a basic end-product. The yellow Sucrose test shows that it ferments sucrose to acid. We grew the organism on MSA which turned yellow around the growth, proving that the organism ferments mannitol and that it is salt tolerant. The Catalase test results had bubbles telling us that it contains catalase and therefore converts hydrogen peroxide to water and oxygen gas. According to the Bergey's Manual of Systemic Bacteriology First edition, volume 2 (1986), these tests and the colony morphology characteristics were expected results for *Staphylococcus warneri*.

The results form BLAST concluded our second organism to be *Micrococcus luteus*. The gram stain showed purple irregular clusters that proved the organism to be gram positive. There was no snot produced by the KOH test also proving the organism to be gram positive. This organism had negative results for the Motility test, with only Brownian motion observed. The negative yellow results of the MR-VP test showed that the organism is not a mixed acid or butanediol fermentor. We did a TSI test which showed no growth in the butt and showed red in the butt and slant. This told us that the organism is a strict aerobe. The pink glucose test showed that this organism does not ferment glucose. Bubbles were seen on the Catalase test which means that the organism contains catalase. The morphological and physiological test results matched the expected results found in the Bergey's Manual of Determinative Bacteriology, Eight edition (1974). The test results confirmed that this organism is *Micrococcus luteus*. (Wieser, M. et al, 2002).

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Isolation and Identification of *Kaistobacter koreensi* from a dog.

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During our Microbiology introduction, the class was informed that an independent project would be due at the end of the semester. Independent projects were at our discretion and the methods used can be obtained in the lab syllabus or taught in lab. I used our air plate project as the source of my independent project. Two types of microbes grew on my air plate and I chose the one with a pink-pigment. I used scientific sources as well as running test within the lab to determine the name and characteristics of my bacteria. After all was said and done, I have the name of my bacteria (*Kaistobacter koreensis)* but no further information.

INTRODUCTION

I thought about many different objects that I would like to test for this project. One that came to mind was the paw of my dog. However, she lives indoors and do I really want to know what she is carrying around. Second, I thought it would be interesting to test the water from out hot tub. However, hot tub water is treated with chlorine and I may not get much growth on agar. Third, I thought about testing what may be growing on my counter tops. I consider myself to be a pretty good housekeeper so I decided I really didn't want to know. Last, the class had a small project to do at home that involved nutrient agar that each student made on their own. Once the agar was set, it was to be taken home and exposed to air anywhere within the home. It would then be returned to the classroom for lab. The dustiest part of my home is in the living/dining room area. Most of the human/canine traffic is in and out of this room. I exposed my agar to the air for 1 hour, then sealed my petri dish and returned it to my class drawer in lab. The following week we checked are plates and I was not surprised to find some growth on the nutrient agar plate. I had a pink - pigmented colony and one that was opaque beige. After some thought, I decided to pursue the pink –pigmented colony on the agar as my independent project. What is floating in the air around my home??

MATERIALS AND METHODS AND RESULTS

Once it was decided that I would be testing for the unknown pink –pigmented colony growing on nutrient agar, I needed to isolate the colony. I restreaked a nutrient agar dish and returned it to my lab drawer for later use. Once a pure culture was formed, a sample of my unknown bacteria was removed and processed per the lab syllabus for DNA. The sample of DNA was then sent to the UC Davis- College of Biological Science DNA Sequencing Facility. This procedure took a few weeks to complete.

In the meantime, I removed samples from the unknown bacteria in order to make some slides for viewing. I made an indirect slide and a Gram–stain slide. From the Gram-Stain, I determined my unknown to be Gram-negative bacteria with bacilli and no endospores.

The results from UC-Davis were returned and I then followed procedure to run the extracted DNA through the gel electrophoresis for DNA fingerprinting. Several weeks' later DNA nucleotide sequences were placed in an electropherogram. The electropherogram was copied onto a word document and the website for NCBI (National Center for Biotechnology Information) was accessed to BLAST (Basic Local Alignment Search Tool) nucleotide sequence and find the identity of my unknown bacteria. The identity of the unknown organism growing on my plate turned out to be ***Kaistobacter koreensis.*** The information from the NCBI BLAST site indicates that it is 16s ribosomal RNA gene partial sequence. The lineage is: *Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Shingomonadaceae; Kaistobacter.* The query length is 815, gene bank length is 1420, similarity is 99%, number of base match pairs is 793/796, the bit score was 1555 and the accession number is AY769084. However, there is no further information regarding this organism.

I decided to run a few tests to give me more information. I started with the **Catalase** test and received a positive result. I later took a sample from my ***Kaitobacter koreensis*** and proceeded with an **oxidase** test. My bacteria gave me a positive result. The last test I performed on my bacteria was the **Oxidation/Fermentation** test to determine the type of metabolism. The bacteria showed no fermentative ability. Its metabolism is oxidative. At this point of the experiment, I was no closer to determining what my bacteria was or where it originated.

I was a bit frustrated about the bacteria and Professor Wilson suggested checking the Stanford University website for more information. The website accessed was http://highwire.stanford.edu. This website also did not have any information regarding ***Kaistobacter koreensis***. However, it did pull up other bacteria in the species ***koreensis.*** The term or species ***koreensis*** pertains to Korea where the organism was originally isolated. Most of the genuses in this species are Gram-negative, non-spore forming, rod-shaped bacilli, colonies are circular, opaque, and convex, Catalase positive, Oxidase positive and O/F negative with a YELLOW-PIGMENT. The ***Kaistobacter koreensis*** was close other than the color of the pigment.
I searched several different website to try and find more information but, I was unsuccessful.

In conclusion, I have to assume this bacteria floating in my home must come from one of the following suspects: the air plate was placed on my dining room table that sits under an air conditioning vent and near a couple of plants, I have two very active boys that are constantly running through this room and last, I own a dog that is constantly shedding in this room. I am somewhat reassured that this is a bacteria I do not need to worry about since most of the organisms I looked up that were similar in name and characteristics to my organism were not harmful.

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