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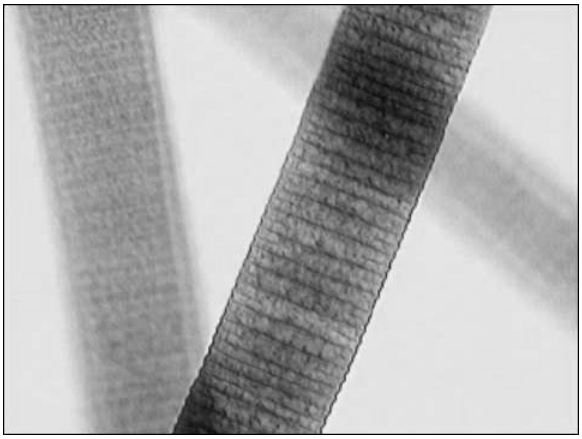

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Cover photo:
A photomicrograph of a prepared slide of *Oscillatoria* magnified 400x. (H. Wilson)

SIERRA COLLEGE JOURNAL OF MICROBIOLOGY

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Isolation and Identification of *Bacillus pumilus* From My Labrador Retriever

MINDY HOLLOWELL

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Received 10 May 2007/Accepted 10 May 2007

The undercarriages of dogs are known to be the home to many types of organisms. In this experiment, I took a sample of the organisms living on my Labrador Retriever's undercarriage, grew the sample on nutrient agar, and then chose to isolate and identify one of the organisms. After isolation, I used Polymerase Chain Reaction (PCR) and sent the purified DNA to the Division of Biological Sciences Sequencing Facility at the University of California in Davis. After using the Basic Local Alignment Search Tool (BLAST) website, I found that my isolated organism's nucleotide sequence was 98% identical to that of *Bacillus pumilus*. To confirm the identity, I observed colony morphology on nutrient agar, performed an Indirect Stain, Gram Stain, a KOH test, an Endospore Stain, and Methyl Red-Voges Proskauer (MRVP) test. After determining that my isolate was a gram-positive, endospore forming, acetoin producing bacteria that has the same colony morphology as *Bacillus pumilus*, I concluded that this is the bacteria I isolated from my dog.

INTRODUCTION

I have an eight year old, black Labrador Retriever named Pepsi who has had skin infections since she was eighteen months old. She has skin that constantly itches, gives off an unpleasant odor, feels like leather, and she sheds all of her fur year-round (more than the average dog). She has lost all the hair around her eyes, on her legs, and undercarriage. Pepsi's veterinarian has completed skin scrapes, flea searches, and full blood analysis. The only abnormality he found was hypothyroidism, which now has been treated for two years. For her skin infection, we have tried allergy shots, corticosteroids, antibiotics, and antifungals; nothing has worked. For this reason, I have decided to use Pepsi as my subject for this project. I will swab her skin on her undercarriage and see what microbial growths appear.

The flora of dogs contains microorganisms from their environment. Since my dog comes into contact regularly with soil, water, and her own feces, I expect to find microbes from these sources on her undercarriage. Many species in the *Bacillus* and *Azotobacter* family are found in soil, as well as *Pseudomonas fluorescens*, and *Clostridium perfringens* (Selected Descriptions of Bacteria). *Escherichia coli*, *Giardia*, hookworm, parovovirus, and *Toxocara canis* (roundworm eggs) are commonly found in the feces of dogs. *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Corynebacteria*, *Mycobacteria*, and *Pseudomonas aeruginosa* are prevalent in the normal flora of dogs (Selected Descriptions of Bacteria).

Many dogs that have skin problems are tested for mange. There are two main types of mange: Sarcopic mange and Demodectic mange. Sarcopic mange, also called Canine Scabies, is caused by *Sarcoptes scabiei canis* (Jeromin, 2002). It is a mite that usually causes problems in older dogs, especially those with hypothyroidism (Jeromin, 2002). This is due to the host's increased susceptibility due to his or her weakened immune system (Jeromin, 2002). Dogs with this type of mites often have crusty ear edges and itchy skin (Jeromin, 2002). In addition, dogs typically start itching and chewing at the infection causing a secondary skin infection called Pyoderma Gangrenousm (Vitale, 2004). The culprit of this skin disease is *Staphylococcus intermedius* (Ihrke, 2002).

On the other hand, Demodectic mange is caused by the overpopulation of *Demodex canis* (Blogg, 1983). This is a mite that most dogs have, but does not cause a problem; however, dogs with too many of these mites may suffer from inflammation, hair loss, and red, scaly skin (Vitale, 2004).

I expect to isolate a bacterium, fungus, type of mite, and/or eggs from a parasite from my dog's undercarriage. Based on her symptoms, I expect to find *Sarcoptes scabiei canis* during this experiment.

MATERIALS AND METHODS

In order to collect a sample of the organisms living on my Labrador Retriever's undercarriage, I used a sterile cotton swab, dipped it in sterile water, and gently swiped it across my dog's undercarriage. Then, I streaked a plate of nutrient agar with the sample-containing swab. I allowed the organisms to grow on the plate at room temperature (about 21ºC) for 48 to 120 hours. Next, I chose a colony with a smooth, yellow colored appearance. I isolated this organism onto a sterile plate of nutrient agar and let it grown at room temperature (about 21ºC) for 48 hours.

After isolating the organism, I performed a gram stain (Wilson, 2006, pg. 44-45) and KOH test (Wilson, 2006, pg. 46). From there, I performed a chromosomal extraction for a gram positive bacteria. I transferred 2 mm of cells from the agar into a microfuge tube containing buffer. Next, I vortexed it until it was evenly suspended, and then added glass beads. I placed the microfuge tube with the cells and buffer into a float and let it boil for ten minutes. Finally, I vortexted it for ten more minutes.

After obtaining the template DNA, I used PCR to amplify rDNA using the primers Bacteria-8-Forward and 1492-Reverse (Wilson, 2006, pg. 151-152). Next, rDNA was isolated using electrophoresis and the Qiagen Qiaquick PCR purification kit (Wilson, 2006, 156-158). The purified DNA was then sent to the Division of Biological Sciences Sequencing Facility at the University of California in Davis. Finally, I used the 4 Peaks computer program to obtain my nucleotide sequence in order to use the Basic Local Alignment Search Tool (BLAST) to see which known bacteria my sequence matches the closest with (National Center for Biotechnology Information).

To verify the identity of my organism, I performed an Endospore stain (Wilson, 2006, pg. 52), an indirect stain (Wilson, 2006, pg. 39-40), a Methyl Red-Voges Proskauer (MRVP) test

(Wilson, 2006, pg. 135-136) and a Beta-Hemolysis test in which I aseptically transferred some of my culture using a loop onto a plate of blood agar and then incubated it at 37ºC for 48 hours. In addition, I streaked a plate of nutrient agar with *B. subtilis* to see how its colony morphology differed from *B. pumilus*.

RESULTS

On nutrient agar, the colony morphology of my isolate is an umbonate elevation, irregular form, undulate margin, dull surface texture, opaque optical character, a beige to yellow pigment, about 0.5 to 6 mm in length, with an odor similar to that of smelly socks.

Table 1.0

Gram Stain	KOH Test	Indirect Stain	Endospore Stain	MR-VP	Beta-Hemolysis
Purple rods with white centers	No viscosity	Single-cell rods	Pink rods with smaller turquoise rods in the center	MR: Yellow VP: Red	Yellow, transparent agar

After performing a Gram stain, I observed purple rods with white centers (Table 1.0). Following the KOH test, I observed no viscosity (Table 1.0). After an indirect stain, I saw single-cell rods (Table 1.0). Following the Malachite Green Endospore Stain, I observed pink rods with small turquoise rods in the center (Figure 1.0). After I performed a Methyl Red-Voges Proskauer test, the MR-VP broth was turned after I added Barritt's Reagent A and B, but there was no color change (stayed yellow) when I added Methyl Red (a pH indicator) to a different test tube containing MR-VP broth (Table 1.0). After completing a Beta-hemolytic test, the blood agar turned yellow and transparent where there were colonies, but remained a dark red color where colonies were absent (Table 1.0).

According to BLAST, 569 out of 574 nucleotides from my PCR were the same as *Bacillus pumilus*. This search tool tells me that the organism I isolated from my dog is 98% identical to *Bacillus pumilus*. (National Center for Biotechnology Information).

DISCUSSION

The purple result I observed from my Gram Stain leads me to believe my organism is gram positive (Table 2.0). The white spots I observed in the middle of the purple rods tells me that my organism contains endospores (Table 2.0). The KOH test verified that my organism is gram positive since no viscosity was observed (Table 2.0). The indirect stain tells me that my isolated organism's cell arrangement is single-cells and its shape is bacillus (Table 2.0). The

Endospore Stain tells me that the organism has ellipsoidal and centrally located endospores (Table 2.0). The Methyl Red-Vogues Proskaur test tells me that acetoin is present and therefore my organism is a butanediol fermentator (Table 2.0). Finally, the Beta-hemolysis test tells me that it is Beta-hemolytic and therefore can break down the red blood cells in the media (Table 2.0).

Because BLAST determined that the nucleotide sequence from my isolated organism is 98% identical to that of *Bacillus pumilus*, I conclude that the microorganism I isolated from my dog's undercarriage is in fact *Bacillus pumilus* (National Center for Biotechology Information).

In addition to the BLAST website, I believe my isolated organism truly is *Bacillus pumilus* because of the information I read in the *Bergey's Manual of Determinative Bacteriology*. This book says that my organism is difficult to differentiate from *B. subtilis*, but I concluded that *B. subtilis* is not my isolate because *B. pumilus* is more smooth and yellow than *B. subtilis* on nutrient agar (Gibson, 1872). When I streaked *B. subtilis* onto one plate of nutrient agar, and *B. pumilus* onto another plate of nutrient agar, *B. pumilus* definitely was more yellow.

Unfortunately, my isolate is not a pathogen and therefore probably does not cause any of my dog's skin infections.

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Isolation of *Bacillus subtilis* from Fermented Food Miso.

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We streaked a miso-paste sample onto nutrient agar plates and incubated one at 37˚C, and one at room temperature. We isolated one of the colonies by restreaking it. We performed various physiological and morphological tests on our unknown organism. We extracted purified DNA from our unknown, sent it to UC Davis, and then with the electropherogram they provided us we matched our unknown to a known organism using a database. These techniques led us to conlude that our organism was *Bacillus subtilis*, and we confirmed this with the use of the Bergey's Manual.

INTRODUCTION

Diseases are caused by a variety of microbes; however not all microbes cause disease, some can be beneficial to our health. For example, fungi are widely used in our daily food production. Some fungi produce antibiotics and cholesterol reducing agents, which are very beneficial to our health. In this experiment we will isolate an organism from miso, fermented soybean paste, which is prepared using a two part fermentation process of soybeans, rise and barley. Miso is a salty condiment that is used in Japanese cooking such as miso soup.

In 1904, microbiologists isolated a pure culture of miso mold, an organism belonging to the single species *Aspergillus orzae* (Shurtleff, 1976). Therefore, we will expect to isolate *Aspergillus orzae* through our miso experiment and we will investigate its characteristics.

MATERIALS AND METHODS

We streaked a sample of Miso onto two Nutrient Agar plates: one was incubated at room temperature; the other at 37˚C. We recorded morphology of all growing colonies, and kept our chosen organism alive by regularly re-streaking it from our room temperature plate. We performed a KOH test, Gram stain, acid-fast stain, endospore stain, and a Vogues Proskaur test. We then performed a chromosomal DNA extraction by boiling a sample and then mixing it with sterile glass beads in a vortex mixer. With this, we used a polymerase chain reaction, or PCR, to amplify DNA 16S ribosome using the primers Bacteria 8 Forward and 1492 Reverse. Next, the DNA was isolated using gel electrophoresis and the Qiagen Qiaquick PCR Purification Kit. Purified DNA was next sent to the Division of Biological Sciences Sequencing Facility at UC Davis. We analyzed the electropherograms with 4Peaks software. We compared our new sequence with those on a database using Basic Local Alignment Search Tool.

RESULTS

We have isolated a pure culture from miso, and we inoculated this pure culture into two different conditions on nutrient agar; room temperature and incubated in 37°C to observe the growth. The outer portion of the room temperature organism has a shiny surface texture and is cream yellow. Its center portion is orange with a dull surface texture. The entire colony is opaque. The colony measured 23mm×16mm and the center part was 8mm×8mm. The colony had an unpleasant smell, like garbage. The organism incubated in 37°C formed a filamentous flat shape with the pigmentation of cream yellow with opaque in optical and its surface texture was dull and a little wrinkled. The colony size was 23mm×15mm. The colony had an unpleasant smell like garbage which is the same smell we observed from room temperature colony.

We have decided to use the organism which grew under room temperature because miso is usually stored in room temperature or refrigerated after opening the package. The organism's cellular morphology showed single bacilli with the size of 3.6μm and no motility. KOH test did not show any snot. Gram stain showed purple palisading rods, 3.6μm, with endospores. Acid fast stain also showed the same size purple palisading rods with pink endospores in the center. Spore is circular and not swollen. Endospore stain showed pink palisading rods, same size, with green spores in the center. Capsule stain did not show any capsule. Vogues Proskaur test show red and Methyl Red test yellow. 16S Ribosomal DNA from the organism was 99% identical to that of *Bacillus subtilis* with score of 1172 bits and 0% gap.

DISCUSSION

Miso is known to be a healthy food. For instance, clinical studies have shown miso to be beneficial in combating cancer. Estrogen-sensitive cancers, such as breast, ovarian and prostate, have been found to be especially responsive to protective Genistein, an isoflavone in miso (Keuneke, 1996). We expected to isolate *Aspergillus orzae* from our miso because *Aspergillus orzae* is an important strain, which yields koji, a starter mold which has a good fermentation "strength," and it produces miso that is characterized by its excellent flavor and aroma. Microbiologists isolated a pure culture of this starter mold in 1904, belonging to the single species *Aspergillus orzae* (Shurtleff, 1976). However, we isolated *Bacillus subtilis*. The Bergey's Manual describes *Bacillus subtilis* colonies on agar media as round or irregular; surface dull; become thick and opaque, may be wrinkled and may become cream colored or brown. The spore is central or paracentral and sporangia not definitely swollen (Buchanan, 1975). These are the same characteristics as we found on our miso experiment. Although we have isolated *Bacillus subtilis*, this does not mean *Aspergillus orzae* was not present; there were multiple colonies growing on our initial plate.

Bacillus subtilis is known to contaminate the starter mold in miso production, and it makes an irregular "Koji," which causes an odd flavor and a darker color than desired in miso, although *Bacillus subtilis* is usually present in miso at a constant concentration. The food industry is trying to find high quality miso without the growth of *Bacillus subtilis* (Onda, 2002). Nevertheless, I made miso soup with the miso paste we took our sample from, and it tasted good.

I did not notice any irregularity in the smell, color, or taste from the miso which turned out to contain *Bacillus subtilis*.

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Staphylococcus pasteuri Isolated from Sacramento County Tap Water

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Received 10 May 2007/Accepted 10 May 2007

A Sample of Sacramento County's tap water was tested from my apartments kitchen sink. Growth was successful using tryptic soy broth. Two types of growth were present when streaked onto nutrient agar. Isolation and analysis of one of the two colonies proved the growth to be a gram-positive species of *Staphylococcus*. To obtain this information and test its safety, I performed a series of tests including Gram stain, KOH and Coagulase. I then completed a PCR reaction and gel electrophoreses. The purified DNA was sequenced and matched with 98% accuracy to *Staphylococcus pasteuri.* This gram positive form of *Staphylococcus* should not be harmful, and unlike gram negative staphylococci, will not cause food poisoning.

INTRODUCTION

There are a number of threats to drinking water: improperly disposed chemicals; animal waste; human waste; pesticides; wastes injected deep underground; and naturally-occurring substances, all of which can contaminate drinking water. ww.epa.gov)

The tap water in Sacramento, CA is filtered through Sacramento Suburban Water District. SSWD filters the water to remove any harmful contaminates that may be in the water before entering a tap. It is then treated for many potential pathogens by high filtration systems and addition of chemicals such as chlorine and ammonia.

Although the Safe Drinking Water Act (SDWA) requires regulation of concentrations of coliform bacteria, and other contaminates it is possible for error to occur.(Coffell, Steve, 1989) I believe there is possibility for Coliform bacteria such as *Escherichia coli* or other organisms such as *Giardia lamblia* to enter the water. The City of Sacramento is one of 65,000 water suppliers across the country wrestling with treating water polluted by sprawl, sewage, factory farms, and industry (http://www.ewg.org).

To determine successful treatment of biological water contaminates and safety from coliform bacteria, I collected a sample of water from the kitchen sink of my Sacramento apartment for testing.

MATERIALS AND METHODS

Phenol Red pH indicator was inoculated with the tap water to determine the possibility of a lactose fermenter. Then, Tryptic Soy Broth (TSB) as an initial growth medium. Using an Inoculation loop and aseptic technique I plated the TSB using the steak plate method to isolate

colonies on nutrient agar. The plate was incubated at room temperature for 24 hours. A Gram-stain was used to differentiate cells based on cell wall and a KOH to prove the results. (Bauman, Robert, 2006) Chromosomal DNA extraction was completed by boiling with glass beads. (Wilson, 2006, pg.151-152). PCR reaction amplified the ribosomal DNA using primers, Bacteria 8 forward and 1492 reverse. (Wilson, 2006, pg.151-152). The DNA was then isolation using gel electrophoresis. (Wilson, 2006, pg.179).The purified DNA was then sent to the Division of Biological Science Sequencing Facility at U.C. Davis for sequencing. Analyzed sequence using 4 Peaks was compared to database on BLAST. (www.ncbi.nlm.nih.gov) An indirect stain determined cell morphology and a coagulase test to determine presence of enzyme coagulase and possibility of food poisoning.

RESULTS

Phenol Red had no change and stayed the original color of red and the Tryptic Soy Broth contained a slight amount of cloudy, white sediment. The growth on the nutrient agar appeared two have two types of colonies; both round with smooth edges, a slight shine, opaque, and white in color. There was a difference between the two in that one was slightly elevated while the other was elevated with an indent in the center. I chose to isolate a colony with the indention in the center and preformed a Gram-stain. The Gram stain showed purple, circular clusters of cells approximately 1-3ul in size. The KOH was not viscous. Cells appeared to be circular clusters in the indirect stain. Coagulase test did not solidify. Blast matched my sequence against its database of 5,140,938 sequences. My sequence matched 742 out of 755 with only 4 gaps giving it 98% accuracy to *Staphylococcus pasteuri.*

DISCUSSION

The conclusions from this lab prove that the isolated growth is safe to drink. After the identification of *Staphylococcus,* it was possible that the water could be hazardous to consume, but the tests that followed proved the waters safety. The first sign to this positive outcome was by the gram stain and KOH test which both identified the organism to be gram-positive. Then, the coagulase test proved that it did not have the enzyme coagulase. Coagulase is an enzyme that will clot blood and can cause food poisoning. (King, Jonathan 1985) The attempt for growth in phenol red was unsuccessful and therefore the organism is not a lactose fermenter and is not a coliform.

When the DNA was analyzed and compared to the database from BLAST it identified the organism to be *Staphylococcus pasteuri*. (www.ncbi.nlm.nih.gov) Little information is available on this specific organism but from the information provided about Staphylococcus and the factors that cause water born illness, it can be said that in February 2007 Sacramento's tap water has successful treatment and regulation of the Staphylococcus contaminate and safety from coliform bacteria..

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A comparison of drinking water as represented in bacteria present in canine mouths

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Received 10 May 2007/Accepted 10 May 2007

We are attempting to show that canine mouths reflect how "dirty" their source water is, one dog drinking from a private domestic well source and the second from a public treated water source. We isolated two microorganism species, one from each dog, and through a series of experiments determined what species they were. Using DNA sequencing, we determined that *Bacillus cereus* and *Gordonia terrae* were present. This was confirmed with additional testing. As these organisms appear in soil, we cannot conclude that water is the source of these opportunistic pathogens.

INTRODUCTION

In an attempt to ascertain whether drinking water sources affect endemic microorganism populations in a subject organism, we have taken samples from the mouths of two dogs (*Canis lupus familiaris)*; one solely drinking water from a treated water source and one from a private domestic well source. Public treated water is often promoted as "safer" than domestic wells, as wells are deemed "untreated" due to the lack of regular testing (as public water sources are required to by State law) and the wide range of caretaking by individual homeowners. If pathogens and/or opportunistic pathogens are identified, this could potentially be linked back to the water source. This would obviously have an effect on human health (National Ground Water Association, 2006). We hypothesize that well water is intrinsically "dirtier" than public treated water. We will attempt to identify two microbe colonies, one from each subject dog, to identify differences and potential dangers in this drinking water.

MATERIALS AND METHODS

The two subject animals were "Scrappy", a 10 pound Rat Terrier dog, and "Mattie", a 28 pound Australian Cattle Dog. To acquire pure samples from the dog's mouths, we used sterile swabs, took samples from the gum line, and inoculated a nutrient agar plate for each individual dog. These samples were retrieved on a Sunday, and the next day, once in the laboratory, we incubated these nutrient agar plates at 37 degrees Celsius for 48 hours. We also sent a water sample off to be analyzed by Cranmer Analytical Laboratory in Grass Valley, CA (Cranmer Analytical Laboratory, 2007). Two tests were performed, one to analyze content of water (including heavy metals) and the other to verify whether coliforms were present in the well water. The

depth of this well was 200 feet (Dave's Water Works, 2007). Back at the laboratory, we transferred some cells from the most populace colonies via sterile transfer loops onto sterile nutrient agar plates. We streaked these new plates to isolate the cultures to get isolated colonies. Once isolated colonies were present, we observed morphology for each organism. From here, we performed a Gram stain as well as a KOH test on each organism (Wilson, 2006). At this time we performed chromosomal extraction to identify each organism. We took a sample from each organism, placed them in a tube which we boiled for 10 minutes and then vortexed, with beads, for 10 more minutes. DNA was gel purified using the Qiagen QIAquick PCR Purification Kit. This was then sent to the Division of Biological Sciences Sequencing Facility at the University of California at Davis. We then analyzed the electropherogram results from UC Davis using the 4Peaks Software. Once a base pair sequence was established, we proceeded to the National Center for Biotechnology Information (NCBI) website. At this website, we used Basic Local Alignment Search Tool (BLAST) to ascertain if we had a match with known organisms DNA sequence (National Center for Biotechnology Institute, 2007). Once a list of organisms were provided, we consulted the "Bergey's Manual of Systematic Bacteriology" to look for ways to confirm which organisms we had. We then completed an oxidase test on the "Mattie" sample organism, and we performed a catalayse and Sulfur Indole Motility (SIM) test on the "Scrappy" sample organism.

RESULTS

The results of the water samples from Cranmer Analytical Laboratory stated that coliforms were not present and that chlorine was not present, which can mask the presence of coliforms (National Ground Water Association, 2006). All other results of the water samples indicated nothing abnormal in the well water sample (see Figure 1 & 2). We performed a Gram stain on both samples, the "Mattie" sample contained a purple, rod-shaped cells approximately 3-4 microns in length (1000x magnification). The "Mattie" sample was KOH test negative. Morphology of the "Mattie" sample was as follows: Circular, undulate, raised, dull or rough, opaque, no pigmentation, and had an average size of 5-8 mm. Also, the "Mattie" sample had a foul odor associated with the sample. The Gram stain on the "Scrappy" sample indicated pink (or red), kidney-bean shaped cells that were approximately 6-8 microns in length (1000x magnification). The "Scrappy" sample was KOH test negative. Morphology of the "Scrappy" sample was as follows: Circular, undulate, raised, glistening, translucent, orange color, no odor, and average size of 4-7 mm. DNA sequencing results using BLAST indicated that the "Mattie" sample was 763 base pairs long and had a 99% match with various *Bacillus* species (including *B. anthracis, B. cereus, B. subtilis,* and *B. thuringiensis*). The Accession number we believed was the most likely candidate was EF025924.1 (*Bacillus cereus*). The "Scrappy" BLAST results showed 1412 base pairs and a 99% match to Accession number DQ180334.1, a strain of *Gordonia terrae*. To further identify our samples, we carried out an oxidase test on the "Mattie" sample, which was positive. We carried out two tests on the "Scrappy" sample which indicated that the organism produced a positive result on a catalayse test and the SIM test indicated a motile, indole negative, organism which did not make hydrogen sulfide.

FIGURE 1

Cranmer
Analytical
Laboratory

Bacterial
Report

1188 East Main Street, Grass Valley, CA 95945-5710 (530) 273-7284, FAX (530) 273-9507 E.L.A.P. Certification No. 1936

Montero, Levi
PO Box 545
Penn Valley CA 95946

Job Number: 1071046
Date Reported: 03/12/07
Date / Time Received: 03/09/07 16:38

Site Description: Kitchen sink
Collected By: Pat Montero
Source Type: Well
Chlorine Residual: None detected

Sample Number: 1071046-1
Date Collected: 03/09/07 16:20
Set Up Time: 03/09/07 16:45
Elapsed Time: 0.4 Hours
Analysis: QT Low Range (24 hr)
Media: MMO-MUG 24hr

	Result	Method	Units
Total Coliform	Absent	SM(18) 9223	MPN per 100mL
E. coli	Absent	SM(18) 9223	MPN per 100mL

No coliform bacteria were detected. The water source may be considered safe from bacterial contamination at this time. This is the only result that passes public health and lending institution guidelines.

Site Description: Outside
Collected By: Pat Montero
Source Type: Well
Chlorine Residual: None detected

Sample Number: 1071046-2
Date Collected: 03/09/07 16:20
Set Up Time: 03/09/07 16:45
Elapsed Time: 0.4 Hours
Analysis: QT Low Range (24 hr)
Media: MMO-MUG 24hr

	Result	Method	Units
Total Coliform	Absent	SM(18) 9223	MPN per 100mL
E. coli	Absent	SM(18) 9223	MPN per 100mL

No coliform bacteria were detected. The water source may be considered safe from bacterial contamination at this time. This is the only result that passes public health and lending institution guidelines.

Page 1 of 1

Ralph G. McKnight

FIGURE 2

Mar 13 07 07:34a p.1

PAT MONTERO
3/9/07
200' DEPTH 7/10± GPM

DAVE'S WATER WORKS
10841 Rough N Ready Hwy
Grass Valley, CA 95945
(530) 273-3220
CSL #766110

H20 LIMITS

Mineral	Rec. Limits	Maximum Limit	Definition	Possible effects if higher than recommended limits
Alkalinity	<5 GPG	30 GPG	Capacity to neutralize acids. Present in Carbonate & bi-carbonate forms commonly	Not in itself dangerous. Possible gastro-intestinal problems if excessive levels.
Chloride	250 MG/L	500 MG/L	Due to salt intrusion(Sea Water),fertilizer,sewage,bleach or chlorine. Present in some rock formations.	Taste if in high levels. Corrosive to metallic plumbing and fixtures. Can leave residue.
Color	<5.0 ICU	15 ICU	Due to water contents such as iron, tannin & organics	Aesthetic considerations, some indication of other possibilities.
Fluoride	<1.5 MG/L	<1.5 MG/L	Used in insecticides and present in some natural ground waters and industrial processes	Protects tooth enamel at 1.2 mg/l. Extremely toxic at very elevated levels.
Hardness	<1.0 GPG	10 GPG	Indicates presence of calcium and magnesium ions in the water.	High soap consumption. High scale build-up and energy use.
Iron Total	<0.3 MG/L	0.3 MG/L	Includes dissolved, suspended organically bound/cholated forms.	Aesthetic, bad taste, staining orange, toxic at extreme levels.(Occasionally)
pH	7.0 units	6.5-8.5	Measure of acids or alkaline nature of a solution.	Taste and corrosion are the major associated problems.
Manganese	<0.05 MG/L	<0.05 MG/L	Includes suspended dissolved and organically bound or complexed forms.	Taste. Black staining and possible damage to fixtures.
Tannin Lignin	<0.2 MG/L	<0.4 MG/L	Decomposition of plants and ligneous materials.	Cholates iron, manganese and other metals. Can cause black or brown staining and odors.
Bulphate	<50 MG/L	500 MG/L	Naturally occurring in most ground water formations, especially associated with water hardness and nitrates.	Bitter tastes, laxative effect, gray water appearance are most noticeable problems. Can affect nitrate removal process.
Turbidity	<5 NIU	5 NIU	Weakly ionized or weakly polar suspended matter.	Tastes, stains or cloudy appearances are most common aesthetic problems. Likely to with chlorination and U.V.
Nitrate	<10 MG/L	<50 MG/L	Naturally occur in some ground water formations. Indicator of industrial or sewage waste or fertilizers.	Can cause fatalities in young children, even in moderate to low levels. Highly cumulative in body. Competitive with hemoglobin and blood for oxygen.
Hydrogen Bulphide Gas	<0.02 MG/L	<0.05 MG/L	Present in many ground water sources from decomposition.	Extreme odors, staining and corrosiveness are most common.
Total Dissolved Solids	<250 MG/L	<500 MG/L	Indicator of dissolved solids content in water.	Depending on type of dissolved solids,tastes, odors, stains, and corrosion may result.
Hemme Iron	<0.3 MG/L	<0.3 MG/L	Usually indicates the presence of tannin or other organics in water with iron.	Staining as with iron, not subject to softener or normal iron filter removal techniques.
Baron	<1.0 MG/L	<1.5 MG/L	Present in some natural ground waters and some industrial processes.	Not toxic to humans. Can be very toxic to most plants and vegetation.
Chlorine	?	?	Disinfectant for drinking water. Sanitizing swimming pools. Treatment of municipal waste water.	May cause cancer to the bladder or colon. May weaken the ammune system.

WELL
PH 6.8 IRON 0 MANG. 0.01 CHLORIDES 80 HD. 14 ALK 11 TDS 240

PH 6.8 IRON 0 MANG. 0.015 CHLORIDES 100 HD. 14 ALK. 13 TDS 255
USE

DAVE'S WATER WORKS
10841 Rough N Ready Hwy
Grass Valley, CA 95945
(530) 273-3220
CSL #766110

DISCUSSION

The results from Cranmer Analytical Laboratories indicated that the well water was in no way intrinsically "dirtier" than the public treated water. As stated earlier, "Mattie" was drinking public treated water, and "Scrappy" was drinking water from a private domestic well source. If the presence of coliforms were present, and found in the "Scrappy" sample, then one could make the accusation that the well water is "dirtier". While it was interesting to find *Gordonia terrae* in the "Scrappy" sample, that in itself does not indicate a dirty water source. The "Mattie" sample, which came from a canine that was drinking only public treated water, carried the Gram-positive, rod-shaped *Bacillus cereus*. *Bacillus cereus* is the only one of the four *Bacillus* species indicated on the BLAST database that was oxidase positive, a result which we observed in our laboratory tests (Claus, et al, 1986). *B. cereus* is endemic and is found in soil or can be a foodborne organism. It is an opportunistic pathogen (Wikipedia, 2007). The "Scrappy" sample, which came from a canine that was restricted to well water, contained *Gordonia terrae*. *G. terrae* is a vibrio shaped cell that is Gram positive, but is slightly acid fast. This is why we noticed a pink/red color on the Gram stain, but no KOH reaction. The catalase test was positive, as would be expected from *G. terrae* (Tsukamura, 1974). The SIM test indicated a species or strain that was motile, although according to the "Bergey's Manual", *G. terrae* was normally immotile. *G. terrae*, normally isolated from soil (Arenskotter, et al, 2004), perhaps indicates that the canine "Scrappy" is either drinking water from a pond or eating soil (picogeophagia). *G. terrae* is also an opportunistic pathogen (Arenskotter, et al, 2004).

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Vol. 1, No. 2

Isolation and Identification of a mesophilic, Gram-positive Cocci from a commercial airplane.

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Our goal for this project was to isolate and identify a bacterium that is found on a commercial airplane. Taking a sterile sample, we identified the morphological and physiological characteristics of our unknown organism and extracted chromosomal DNA to be compared to a database of known organisms. A common organism in the food industry, *Lactococcus lactis* subspecies *cremoris* was found on the headrest of the airplane. This species which is normally nonpathogenic, this subspecies has recently been found to be an opportunistic pathogen for certain individuals.

INTRODUCTION

Every day thousands of people travel by airplane to various destinations. For many, it is an inexpensive alternative to driving, but for others it's the only option. If you think about all the different people traveling worldwide on these planes, who knows what variety of microbial contact they have made. Travelers that are sick are not only miserable but they are exposing every other passenger to the risk of infection from whatever microbe they are carrying. We are attempting to identify just one of the Gram positive bacterium harvested from a short flight to determine its potential effects on unsuspecting passengers.

Before we start, we must discuss some of the resident microbiota living on or in humans. For example, bacteria are mostly strict anaerobes, though some facultative anaerobes are also resident. These bacteria are important in our lower digestive tract and some genera contain: *Escherichia, Clostridium, Lactobacillus, Candida* (fungus), *and Entamoeba* just to name a few (Bauman, 2007). If we were to find *E. coli* as one of our bacteria, we could conclude that there was some sort of fecal contamination since it is only found in our digestive tract. While there are microbes that live inside our bodies, many live on our outer, dead layers of the skin. These microbes must be able to withstand the lower pH, and *Staphylococcus, Micrococcus, Candida, and Malassezia* do just that (Bauman, 2007). Once our Gram positive bacterium has been isolated and identified, assuming it is not part of our normal flora, we may be able to take defensive actions next time we choose to fly. This can only be done by determining what the bacterium is, its metabolism and its physical structure so we can choose the most effective antimicrobial treatment.

MATERIALS AND METHODS

To obtain the unknown organism, a sterile swab sample was taken from the headrest of a commercial airplane and streaked onto a nutrient agar plate. The plate was left at room temperature (approximately 37° C) for two days until colonies began to form. Once growth occurred, it was necessary to complete a Gram stain (Wilson, 2006, pg. 43-45) to determine the type of cell we were looking to identify. From that Gram stain, we then knew which procedure was necessary for chromosomal extraction and performed that with the unknown pure culture.

Using a sterile loop, 2mm of cells were removed from the nutrient agar and mixed together with Tris buffer in a microcentrifuge tube. Glass beads were added to the tube and the combination was mixed by hand for a few seconds. After this, the tube was placed into boiling water for 10 minutes. After boiling, the tube was secured onto the vortex for 10 minutes. With the chromosomal extraction completed, a polymerase chain reaction (PCR) was set up using Bact-8 Forward and 1492-Reverse as specific primers to amplify the rDNA of our unknown organism. The DNA was gel purified using the Qiagen Qiaquick PCR purification kit. The kit requires 15 ml sterile water, 5 ml primer mix, 5 ml DNA, and 25 ml of Taq master mix (Taq polymerase and dNTP's) to be mixed together. The PCR mixture was sent to the Division of Biological Sciences Sequencing Facility at UC Davis. The facility used the dideoxy chain termination method to determine the DNA sequence, and returned the sequence along with an electropherogram obtained using the Four Peaks software. Once the nucleotide sequence was established, we sent the information through the database from the National Center for Biotechnology Information (NCBI) using a Basic Local Alignment Search Tool (BLAST) on the internet. The results from this yielded a match to the unknown organism, allowing for identification.

RESULTS

The Gram stain showed our cells were cocci, arranged in short chains. We performed the indirect stain revealing the size of the cells which was 0.5-1um. The morphology of the organism's colonies were; the cells had a circular form, the margin was entire, it had an umbonate elevation, the surface texture was granulated and rough, the optical character was translucent, there was no pigment, and the general size of each colony ranged from 1-4mm. We preformed many morphological tests and the results are as follows. The cells were purple when performing the Gram stain, the capsule stain was entirely purple with no white lining, and in the endospore stain the entire cell remained pink. When inoculating the organism into carbohydrate deeps, the ribose turned yellow, and the raffinose, rhamnose, sorbitol, and dextrin remained red. We looked at the organism on a wet mount and observed only Brownian motion. The results from our DNA comparison from the BLAST website were as follows: The accession number was CP000425-1. The score was 1332 bits. It was a 93% match, which means that the identity was 747/798. There were only 7/798 gaps which amounted to 0%.

DISCUSSION

The website program BLAST revealed that our organism was Lactococcus lactis with a subspecies of cremoris. This organism is a Gram-positive organism that doesn't contain capsules, and also doesn't form endospores. The organism is able to ferment select sugars including glucose and ribose and lactose. It is unable to ferment raffinose, rhamnose, sorbitol. When it ferments, it produced large amounts of lactic acid. It is also non-motile. This organism is critical for manufacturing dairy products like buttermilk, yogurt, and cheese. It is generally seen as non-pathogenic, however the subspecies cremoris may be an opportunistic pathogen in some compromised individuals. We obtained the organism from the headrest in a plane, which makes it odd that we found this type of organism. Lactococcus lactis is not part of our natural flora; instead it is usually found in dairy products. We speculated on where the organism could have come from and thought that it was probably from the meal trays that are distributed on planes. How it was transferred to the headrest, was most likely from direct contact with the Lactococcus on an individual's hand which was then transferred up to the headrest area. This organism isn't rare in the environment or in close quarters with humans, so having it in an area like an airplane isn't too surprising.

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Vol. 1, No. 2

Isolation of *S. cerevisiae* From Sasha's Husband's Wine

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In the world wine community knowing what makes a wine good is just as important as knowing what makes a wine bad. In an attempt to shine a little light on which agents make a wine bad, β-factors, microorganisms from a sample of bad wine were cultured. The rDNA was isolated and sequenced using PCR methods and diDNA Chain Termination Sequencing and determined to be the yeast, *Saccharomyces cerevisiae* with 99.9% similarity. The results were inconclusive as to what or why there were β-factors in the wine, but *S. cerevisiae* almost certainly fermented the grape sugar into alcohol.

INTRODUCTION

Alcoholic fermentation was one of the first implementations of microbes in production, and has been used for millennia since. Though the mechanisms were not known for a better portion of that time how alcohol was produced, even after the discovery that fermentation was due to microbes rather than higher powers, alcoholic beverages played an important role in history, ranging from a religious symbol to a highly regarded commodity.

Today the microbe that is attributed with alcoholic fermentation is *Saccharomyces cerevisiae*, also known as yeast. It works by the general formula:

$$\text{Sugar} + \text{Yeast} = \text{Ethanol} + CO_2$$

The yeast are able to metabolize sugars for energy and excrete ethyl alcohol and carbon dioxide as a waste product; if the sugars and yeast are in solution, an alcoholic beverage is the result.

However, when a "bad" tasting wine or beer results, one has to wonder what went different about it in the fermentation process. There are three hypotheses to explain the reason for this. The first is there are other microbes in the solution that are also metabolizing ingredients into other unwanted products. There are known aerobic bacteria, *Acetobacter* (Baldy, 79-80) that metabolize ethanol into acetic acid, or vinegar. The second hypothesis is that the yeast themselves have undergone some change in their metabolism, due to mutation, that results in different products. The third is that the ingredients used may not be conducive to making a "good" beverage. From a wine sample, we want to determine if there are bacteria contained within that may be causing the "bad" flavor.

MATERIALS AND METHODS

A sample of wine made by the husband of acclaimed microbiologist, Sasha Warren, was obtained. To determine the best medium for the organisms in the wine, samples were plated to nutrient and potato agars using sterile technique. The morphology of a colony was documented for later consideration.

Upon isolation of a colony, a sample was collected for analysis. First, a negative stain (Nigrosin and Congo Red) was used to prepare the cells for viewing. Once the cells were observed, a sample was re-plated to a nutrient agar plate to keep the sample viable.

Lastly, the organism was prepared for genetic analysis using the Polymerase Chain Reaction (PCR) method (Wilson, pp 149-152). The DNA was extracted by boiling a sample of growth and beating it with beads. Fungus primers 817FWD and 1536REV were used to tag strands of rDNA for PCR sequencing. The DNA was gel purified by using the Qiagen Qiaquick PCR purification kit. Then the samples were sent to UC Davis's Division of Biological Sciences Sequencing Facility. Here, the nucleotide sequences were determined by running a gel electophoresis and analyzing an electropherogram. Four peaks were analyzed by comparing the results to the database using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website (Wilson, 159-167).

RESULTS

Only one type of colony morphology grew on the plates, and more colonies grew on nutrient agar than on potato agar, so this was the culture used for subsequent growth. Colony morphology was determined as follows: circular shapes, entire margin, convex elevation, shiny in appearance, optically opaque, pigmented cream, and 3mm in diameter. Under the microscope, the organism was determined to be 8μm in diameter and made of circular cells that were arranged in small clumps (1-10 cells per clump). (Bauman, 178)

The PCR data matched *Saccharomyces cereviciae* strain FJUYS5 with 99.9% similarity.

DISCUSSION

It was determined that the organism obtained was *Saccharomyces cereviciae*. It was impractical to stain these cells since they were suspected and determined to be eukaryotic. Normally Gram stains and KOH tests, along with metabolic tests, are used to identify prokaryotes.

As no other colonies grew on the plates it seems unlikely that there was an organism other than *S. cerevisiae* unless of course conditions became so unsuitable for the other organism that the cultures died. This scenario is, while improbable, possible. Therefore, the "bad" element, now being termed the β-factor, of the wine was probably not a result of bacteria metabolism. Still, a more in depth study of this should be performed.

As for our second hypothesis of there being a yeast mutant present, this is also not likely to have been the case. The rDNA, highly conserved DNA among organisms, matched *S. cerevisiae* with a 99.9% similarity showing a statistically significant resemblance.

The third hypothesis that the ingredients used may have not produced a "good" flavor, now being termed the γ-factor, was not explored in any depth. Further examination of what went into the wine is still necessary.

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Isolation and Identification of Canine Oral Bacteria from a Bichon Frise

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Claims of a dog's mouth being cleaner than that of a human mouth have been in existence for many years. Without any enzymes within the saliva of a canine mouth, many organisms have a favorable environment in which to grow. The objective of this report was to determine the flora found within a canine mouth. One subject canine, Dylan, was tested. After taking swab cultures of the canine mouth and plating them onto Nutrient Agar, two organisms were randomly chosen. Most frequently found organisms in a canine mouth include: *Actinomyces, Streptococcus, Granulicatella, Capnocytophaga canimorsus,* (Buckley, Elliott, Spratt, Wilson, 2005) *Porphyromanas salivosa, Prophyromonas gulae, and Porphyromonas denticanis* (Canine Oral Health, 2006). Polymerase Chain Reaction technique to amplify rDNA was the first step. Once results were determined from PCR, then physiological tests were completed to confirm results using Bergeys Manual of Determinative Bacteriology as a guide. Results conclude that the two organisms isolated are *Chryseobacterium indologenes* and *Microbacterium oxydans*, neither, of which is part of the normal flora found in a canine mouth, but rather are transient flora.

INTRODUCTION

Claims that a dog's mouth is cleaner than a human mouth have been around for many years. True or not, what kind of organisms do dogs share with humans each time they lap the faces of their owners with their tongues in what has been affectionately termed "doggie kisses"? With no enzymes in the saliva of a dog's mouth (Correa, 2001), organisms find a warm, wet and favorable environment in which to grow. The objective of this report is to determine the flora found within a canine mouth. Dylan, a Bichon Frise dog of 6 years, was the test subject. His mouth was swabbed and plated on Nutrient Agar. After three days of incubation, two organisms were randomly chosen for isolation and tested to determine the type of organisms and whether or not they are that of normal flora or transient flora. The most common organisms typically immunized against from dog bites are that of *Pasteurella multocida* and *Staphylococcus aureus* and at times, *Rabies Virus* (Presutti, 2001). Of the various types of normal flora found in a dogs mouth, we expect one or more of the following: *Actinomyces, Streptococcus, Granulicatella, Capnocytophaga canimorsus,* (Buckley, Elliott, Spratt, Wilson, 2005)
Porphyromanas salivosa, Prophyromonas gulae, or Porphyromonas denticanis (Canine Oral Health, 2006).

MATERIALS AND METHODS

A swab culture was taken from the mouth of a dog, plated on Nutrient Agar and stored at room temperature. After three days of incubation at room temperature, two organisms labeled Dylan1 and Dylan2 were randomly chosen and isolated onto Nutrient Agar plates. A Gram stain (Wilson, 2006, pg.44-45) was performed to distinguish the type of cell wall of each. When completed, we performed a KOH test (Wilson, 2006, pg. 46) to confirm our Gram stain results. Once we determined the type of cell walls our organisms had, we began preparations for Polymerase Chain Reaction to ascertain genetic and molecular information. DNA was boiled and beaten for Chromosomal Extractions. Polymerase Chain Reaction, which included Bacterial 8-Forward and 1492-Reverse primers to amplify rDNA. DNA was gel purified using the Qiagen Qiaquick PCR Purification kit. PCR was sent to Division of Biological Sciences – Sequencing Facility at University of California, Davis. Electropherogram data was analyzed using 4-Peaks Data Software. Compared data sequences were determined using BLAST – Basic Local Alignment and Search Tool on the National Center for Biotechnology Information (NCBI) website. Once results from PCR were concluded, we researched the organisms in Bergey's Manual to determine which physiological tests were necessary to confirm the organism's identity. A Malachite Green Stain (Wilson, 2006, pg. 52) was performed to determine if organisms made endospores. A Nigrosin Capsule stain (Wilson, 2006, pg. 53) was performed to determine if the organisms had capsules surrounding them. A Catalase test (Wilson, 2006, pg 138) was completed to determine whether the organisms broke down hydrogen peroxide. And finally, a separate wet mount of each organism was prepared by mixing the organisms with a droplet of water on a clean slide, covering it with a slip cover and observing it under a microscope to determine motility.

RESULTS

Colony morphology indicates that Dylan1 is yellow color, with optical character transparent and form being circular, raised with undulate edges. The Gram stain results indicate that Dylan1 has pink clusters of coccobacilli shaped bacteria. Snot was observed with the KOH test. No spores were observed. No capsule observed. Catalase test resulted in a small amount of bubbles. Motility test indicated brownian motion. PCR results indicate Dylan1 to be *Chryseobacterium indologenes*, Accession number AYO50493.1. Results were 93% sequencing 372 out of 375 nucleotides.

Colony morphology indicates Dylan2 is beige color with optical character being opaque and glistening and form as filamentous with serrated edges. Gram stain indicates purple rods in a line and cocci in a line. No snot was observed with KOH test. No endospores observed. No capsule observed. Catalase test resulted in small amount of bubbles. Brownian motion was observed. PCR results indicate Dylan2 to be *Microbacterium oxydans*, Accession number AB193267.1. Results were 98% sequencing 774 out of 788 nucleotides.

DISCUSSION

According to PCR results, Dylan1 is *Chryseobacterium indologenes* (NCBI, 2007), a Gram-negative coccobacilli bacteria When we attempted to research *Chryseobacterium* in Bergey's Manual, we could not find this Genus. Upon further research, we discovered that *Chryseobacterium* had been reclassified from *Flavobacterium (*Athanasiou, Chalkiopoulou, Christakis, Legakis, Perlorentzou, 2005). The species, *indologenes*, is a more recent variety and was not specifically mentioned in Bergey's Manual, so general characteristics of *Flavobacterium* were researched. According to Bergey's Manual, Flavobacterium is a yellow-pigmented, Gram-negative coccobacilli bacterium that is Catalase-positive, with no endospores, no capsule and no motility (Bergeys, 1974). Each of these tests was performed on Dylan1. Dylan1 is also yellow-pigmented, Gram-negative, Endospore-negative, Capsule-negative and Catalase-postive. All of our Dylan1 tests confirm that Dylan1 is indeed *Flavobacterium*, more recently known as *Chryseobacterium indologenes*. This particular organism is typically found in soil and plants (Weeks, 1923). Being found in a dog's mouth it would be regarded as a transient organism and not necessarily a part of the normal flora of a dog's mouth. However, this organism has been known to be found in Hospital environments and are known to cause severe infections in humans (Athanasiou, et. al, 2005). Appropriate attention should be given in the case of any dog bite.

According to PCR results, Dylan2 is *Microbacterium oxydans* (NCBI, 2007) a Gram-positive Streptobacilli and Streptococci-shaped bacteria. When we researched *Microbacterium* in Bergey's manual, the species *oxydans* could not be specifically located so general characteristics of *Microbacterium* were researched. According to Bergey's Manual, this organism has a beige-opaque color, is Gram-positive and has diptheroid rods with rounded ends. It also produces no endospores, has no capsule, is KOH-negative and Catalase-positive. (Bergey's, 1974) Tests on Dylan2 resulted in a Gram-positive, KOH-negative, endospore-negative, capsule-negative, non-motile organism. This is confirmation that Dylan2 is that of *Microbacterium oxydans*. Of interest to note, *Microbacterium oxydans* was reclassified from *Brevibacterium oxydans* (Burghardt, Rainey, Schumann, Stackebrandt, Weiss, N. 1999). This organism is typically found in dairy products and dairy utensils (Bergey's, 1974) and would be regarded as transient. It is not a considered part of the normal flora within a dog's mouth.

In summary, the two individual organisms isolated from Dylan, labeled Dylan1 and Dylan2, were not that of normal flora typically found within a dog's mouth, but that of transient flora.

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Isolation of *Rhodotorula mucilaginosa* from Air Exposure

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An unknown microorganism was isolated from an air exposure and identified through Neucleic Acid Sequencing to be *Rhodotorula mucilaginosa*. A series of tests were run to confirm this finding-Fermentation (lactose, sucrose), a halophile test for salt tolerance, and an asexual reproduction test. These tests were consistent with recorded scientific data, showing negative lactose and sucrose fermentation, positive for salt-tolerance, and negative for asexual reproduction through the process of spore-shooting.

INTRODUCTION

A petri dish was exposed, open to the environment of an office space for the period of one hour. One microorganism was selected from the medium and isolated. This organism was then identified through scientific testing, followed by a variety of tests to support or disprove this finding. The tests supported the finding and *Rhodotorula mucilaginosa* was identified as the microorganism.

Rhodotorula mucilaginosa is an opportunistic yeast-like fungus that is salt-tolerant. It is commonly found in the environment (air, water, soil, shower curtains and bathtub grout) and as a commensal microorganism in skin, mucous membranes, nails, cheese and milk products. Taxonomy for the species is: Fungi, Basidiomycota, Urediniomycetes, Sporidiales, Sporidiobolaceae, Rhodotorula, mucilaginosa.

MATERIALS AND METHODS

Stains, Media, Growth Conditions

A Nutrient Agar plate was initially exposed for the period of one hour to the environment, then left to grow for a period of three days. One colony was chosen from the plate and isolated into a pure culture on Nutrient Agar. Colony morphology was noted. Indirect Stain was conducted with Nigrosin and a Gram Stain was conducted. A sample was taken by aseptic means to run a PCR (1536 Reverse) and extract DNA for Nucleic Acid Sequencing. Gel Electrophoresis was run using Qiaquick Gel purification kit (Qiagen Company). Purified DNA was sent to the College of Biological Sciences at UC Davis, to a DNA sequencing facility. The sequence was returned via an Electropherogram and analyzed with the 4 Peaks program, then referenced with the NCIB database through a BLAST program. The organism identified was then tested for inaccuracies through an O/F Test and Carbohydrate Deep Tests for Lactose and

Sucrose fermentation. The halophile test was conducted on MSA and incubated for two days, then removed to room temperature for 3 days, with Nutrient Agar as an incubator control. An asexual reproduction (spore-shooting) test was run, by sealing two Nutrient Agar bottoms to each other-one inoculated, one sterile- with tape and left at room temperature, inoculated side as the ceiling for two weeks.

RESULTS

Colony morphology yielded orangey-pink, circular, entire, flat, smooth, opaque colonies, .25- 2mm diameter. Indirect Stain showed cocci-shaped cells, measuring .5μm to 2 μm in length, .25 μm in width in formations multiple formations- singles, pairs, tetrads, clusters Gram Stain showed Gram-positive cells O/F Test showed no fermentation through gas or pH indicator. The Electropherogram was not edited before being run through the BLAST. Nucleic Acid Sequencing identified the microorganism as *Rhodotorula mucilaginosa* at 99% matching. Carbohydrate Deeps showed negative fermentation for both lactose and sucrose. The Halophile Test on incubated MSA did not yield colony growth. The MSA plate was then kept at room temperature and colony growth was positive. The Nutrient Agar control also yielded no growth while in the incubator. Asexual reproduction via spore-shooting was negative for *Rhodotorula mucilaginosa*, but positive for an invading fungus that contaminated the sample.

DISCUSSION

The species, *Rhodotorula mucilaginosa*, is commonly found in the environment. Although this was an internal setting, it is not considered odd to have isolated it from a room vented by outside air. Colony morphology was consistent with reported observations, and over time, the colonies appeared to become filamentous. Data on the microorganism lists that it is not fermentative to lactose or sucrose, which our testing supported. The Halophile Test showed that although *Rhodotorula m.* is salt-tolerant, it does not show growth in warm temperatures. This is consistent with it being found in external environments, where temperatures remain lower. The spore-shooting test proved that although some species of *Rhodotorula* shoot their spores, this species does not and the success of the invading fungus proved the test to be valid.

As mentioned previously, this is an opportunistic fungus. Research on the species as shown that it is becoming a concern for medical mycology, and has been found in urinary tract infections, Cystic Fibrosis patients, and is a threat to immunocompromised individuals.

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Staphylococcus on Your Stethoscope?

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Nosocomial infection is an increasingly serious problem. A doctor's stethoscope was swabbed to investigate pathogens that linger after frequent disinfection. Multiple colonies were isolated from swabs, and the dominant colony was then subjected to more specific tests. *Staphylococcus epidermidis* was identified as the dominant organism. This presence of this common bacteria, which should have been destroyed by routine sterilization, suggests that present methods of sterilization may be inadequate in controlling this, and other more dangerous bacteria.

INTRODUCTION

Diseases contracted in the hospital setting, nosocomial infections, are typically among the most difficult to treat. The subset of these infections that is caused by bacteria are commonly resistant to multiple antibiotics, as bacteria present in the hospital have been passively selected to survive among the myriad of antibiotics constantly employed in hospitals.

The present study is performed to determine what microorganisms might be present on a doctor's stethoscope used in both inpatient and outpatient environments. The stethoscope was sterilized frequently, usually between each examination, with isopropyl alcohol wipes. Although the bulk of these examinations were performed in a busy outpatient oncology clinic, the stethoscope was also used about every other day for examinations in the hospital. When not in use, the stethoscope was stored in the doctor's laboratory coat.

MATERIALS AND METHODS

Medium

Sterile cotton swabs soaked in sterile culture were used to obtain organisms from the surfaces commonly in contact with patients: the diaphragm and attachment ring of the stethoscope. These organisms then transferred onto a nutrient agar plate and incubated for 72 hours. Following incubation, the selected colony for identification was transferred to a new nutrient agar plate and isolated. The organism was then transferred to a Tryptic Soy Agar (TSA) plate and incubated for 48 hours. Once the *Staphylococcus* genus was suspected from initial plating, a Mannitol Salt Agar (MSA) plate was also streaked. The TSA seemed to be the organisms preferred medium and was therefore utilized for the duration of the project.

Staining

Standard Gram staining was performed. A Nigrosin indirect stain was used to determine cell size.

DNA Extraction/Polymerase Chain Reaction/Gel Electrophoresis

DNA extraction was performed by boiling the sample in a tris buffer solution and then vortexing the organism with glass beads. Once isolated, a Polymerase Chain Reaction (PCR) was used to amplify the DNA. PCR was performed by using 35 cycles of a thermal cycler preceded by a 4-minute denaturation at 94 °C. Each cycle consisted of 45 seconds at 55 °C , 2 minutes at 72 °C, and 30 seconds at 94° C. Following this a gel electrophoresis was completed. The gel was placed over a transilluminator and DNA was cut from the gel using a Quiauick gel purification kit from Qiagen and sent for sequencing by the **Division of Biological Sciences sequencing facility at the University of California at Davis**. After the elecropherogram was returned, 4 Peaks software (©mekentosj.com) was used to view and edit data.

Biochemical Tests

KOH biochemical testing was used to confirm the results of Gram staining. Tests to provide alternative confirmatory data included Methyl Red-Vogues Proskauer (MRVP), Catalase, Oxidase, Urea, and Carbohydrate Slants: Mannitol, Lactose, Arabinose, Glucose, Raffinose.

RESULTS

The colony morphology from initial TSA plating showed circular colonies with a complete edge, flat elevation, with a smooth and shiny surface texture. The colonies were white and opaque, 1 to 2 mm in size. Nigrosin Indirect Staining showed organisms seen at 1000 times magnification. The bacteria were cocci arranged in clusters, 1 micron in diameter. An MSA plate showed small, red colonies, indicating non-pathogenic *Staphylococcus.* The cocci Gram stained positive, and this was confirmed by the KOH test.

Subsequent DNA analysis showed *Staphylococcus epidermidis* or *Staphylococcus capitis* with 98% accuracy. Confirmatory tests showed no acid production from MVRP, Catalase test showed breakdown of Hydrogen Peroxide, Oxidase showed the presence of cytochrome C, and the Urea test showed no ability to hydrolyze Urea. The carbohydrate tests showed no use of Lactose, Arabinose, Glucose, and Raffinose, with marginal use of Mannitol.

When these results were cross referenced with standardized test outcomes (Baird-Parker, p.488-89), it was concluded that *Staphylococcus epidermidis* was the dominant organism collected from the stethoscope. DNA analysis raised the possibility of *Staphylococcus capitus* as the dominant organism, but results or the mannitol tests ruled out this possibility.

DISCUSSION

The possibility of nosocomial infection is considered one of the most formidable risks associated with hospital admission. Indeed, apart from more stringent guidelines from government and private insurers, concern regarding hospital acquired infection is probably the

single most significant contributor to the decreased length of hospital stay when compared to length of stay of twenty or thirty years ago.

With the relative stasis of pharmaceutical companies in the area of development of more novel antibiotic treatments, more and more species of bacteria have developed the ability to resist the common, and even uncommon, weapons in the health care system's armamentarium against bacterial pathogens. With this, the focus has broadened from treatment to include more active means of prevention and education. Almost any modern hospital ward has multiple signs encouraging hand washing and universal precautions. By the doorpost in each room is a canister of bacteriocidal foam. It is interesting to note that these "new techniques" were practiced, or at least known about, before antibiotics were ever invented (Lister, Lancet).

Still, in today's modern health care environment, the physician is ever more pressed for time, and is passively encouraged to cut procedural corners. Physicians cannot bill for washing their hands, or sanitizing exam equipment, but they are penalized for lackluster documentation and incomplete examinations. Often roaming from unit to unit, they may never be in one place long enough to take heed of hospital policy in which other hospital staff is immersed, actively taught, and tested. These inconsistencies put the hurried physician at risk of becoming the weak link in the preventative chain. To compound the problem, the nomadic pattern of the physician within the hospital puts her in a position to spread pathogens more effectively than most other healthcare workers.

Staphylococcus epidermidis is a species that commonly lives on human or animal skin and in mucous membranes. The organism is typically non-pathogenic for the immunocompetent patient. The results may indicate that the stethoscope cultured was in a relatively "clean" condition, as it was non-pathogenic to most patients. Alternatively, the argument may be made that this bacteria is easy to eradicate and should not have been present in any significant amount on an instrument which comes into contact with numerous patients in a single day. The presence of this ubiquitous bacteria on the physician's stethoscope does not necessarily suggest that our current system of infectious disease control works well. For example, if Methacillin Resistant *Staphlococcus Aureus* (MRSA) were out of control on a hospital unit, this might be the local ubiquitous bacteria that was cultured off of the stethoscope.

Objectively, *Staphlococcus epidermidis* is present in such large amounts that it is possible that the colonies isolated grew as a contaminant. They may have present in the physician's coat after the stethoscope was disinfected. To rule out these uncertainties, this experiment would need to be repeated on a larger scale, and in more controlled conditions. Certainly this sampling is not conclusive, but it does point to the need for constant vigilance. In a war where our weapons are becoming less effective, it becomes more important to keep the enemy from ever taking to the battlefield.

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Identification of Oral Bacteria

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Bacteria present in human mouth are causing tooth decay and bad breath. An experiment is done to determine one type of bacteria that are present in human mouth. By using several methods in identification of bacteria, such as isolating pure colonies, KOH test, catalase test, urease test, citrate test, esculin hydrolysis, carbohydrate deeps, and DNA sequencing, an investigator found an organism known as Rothia dentocariosa. Furthermore, some researchers showed that these bacteria can cause diseases other than normal infection in humans' mouth such as endocarditis and infected arteriovenous fistula.

INTRODUCTION

There are many types of bacteria that live in the human mouth. As normal flora help humans to block pathogens, humans are responsible to preserve the normal flora. The first action that humans can do is to determine and acknowledge which bacteria are normal flora and pathogens. The bacteria that are found normally around living creatures are known as normal flora (bacteria used to be classified as kingdom *Plantae*). Other bacteria are potentially pathogens, and can cause disease if the bacteria cells keep growing and reproducing inside the body overweigh the normal level of the presence of these bacteria. This experiment has a purpose to identify one type of bacteria that is a normal flora or potentially pathogens bacteria living in human's mouth. Bacteria samples are collected from an investigator's mouth. Most people experience tooth decay caused by bacteria that consume some leftovers from sweets remaining on their teeth. Some of the substrate that bacteria can metabolize came from human's mouth itself including teeth and saliva (Selwitz et al. 2007). Considering normal flora have important roles in the body, the identification of bacteria living in the human mouth is important. The bacteria that are expected to appear in this experiment are in the genera *Streptococcus*, *Veillonella*, *Rothia*, *Actinomyces*, and *Prevotella* (Aas et al. 2005, Selwitz et al. 2007 and Diaz et al. 2006).

MATERIALS AND METHODS

The tests were using aseptic technique, such as sterilizing wire loop and the mouth of the test tubes. The bacteria in the test tubes are placed in the incubator for 5 days. The sample of cultural bacteria were collected by scrubbing inside the mouth using

a sterile cotton swab and then streaking it to a Brain Heart agar plate. Considering the bacteria in the human's mouth grow at body temperature, the culture was grown in the incubator set on 37ºC. The staining applied in this independent project was a gram stain and an indirect stain using nigrosin. The KOH test is used to determine gram quality of bacteria by using a loop of 3% of potassium hydroxide solution on glass slide then transferring visible amount of bacteria to the solution on the glass slide. The experiment for acids and gas production was done by stabbing carbohydrate deeps. Carbohydrate deeps used were arabinose, glucose, inositol, lactose, mannitol, raffinose, rhamnose, sorbitol, and sucrose. Catalase test was done by putting a visible culture into a glass slide containing a drop of H_2O_2. Then, using a urea agar and citrate agar and streaking the slant, the bacteria are tested for urease and citrate utilization test. For the esculin hydrolysis test, an investigator simply streaked the slant. To test for lysine decarboxylation, two tubes were used: one was a lysine control that does not contain lysine, and the other tube contained lysine. Both tubes were closed with vaspar. To run this test, a visible amount of bacteria colony was placed in the bottom of the tube. Voges Proskauer test was applied using 18 drops of Barrits A and B in small closed tube with 0.1 ml of broth culture. The researcher shook the tube vigorously, and waited for 10-15 minutes to see the change in the solution. Then, Methyl Red test was applied using five to eight drops of pH indicator Methyl red. There were 3 part for sulfur indole motility tests, including hydrogen sulfide (H_2S) production, tryptophan catabolism and a motility test. This was done by stabbing a SIM medium straight to the bottom tube. Triple Sugar Iron test was applied by simply streaking and stabbing slant. After streaking and stabbing slants DNA extraction was done. To extract DNA, a loop of bacteria was transferred to a small closed tube and then vortex mixed the tube until the bacteria dissolve with the 500 µL of tris buffer. In this experiment the bacteria used were gram positive bacteria, so the bacteria are vortex mixed with glass beads. Then it was boiled for 10 minutes and then vortex mixed again. In Polymerase Chain Reaction the primer used in this method is 16S ribosomal RNA. After the DNA extraction, gel electrophoresis was run by loading DNA sample with dye to the gel. The DNA gel was cut physically by Professor Harriett Wilson to be purified by Qiaquick Gel Purification Kit (Qiagen). This purified DNA gel was sent to the College of Biological Science at UC Davis considering they have more DNA sequencing facilities. The data results were returned by electronic mail on the internet. At this time, an investigator viewed the data from an electropherogram by opening software called 4-peaks and copies the sequences to Microsoft Word so the data are able to be edited. By combining and editing 8-forward, 533-forward, and 1492-forward sequences, the bacteria were identified through the Basic Local Alignment Search Tool (BLAST). The investigator simply copied and pasted the sequences that already being combined and edited.

RESULTS

An isolation culture was collected, and the colony morphology on agar plate showed the agreement to the statements in the Bergey's Manual. The colony size was ranging from 0.25 µm to 1 µm in diameter, white, circular, rough, entire, umbonate and curled. In gram stain, the culture sizes were in the range of 1-3 mm. The gram stain showed that the bacteria were gram positive bacteria, also confirmed by a potassium hydroxide test, which showed no viscosity was

formed. Cell morphology on the gram positive showed staphylococcus (clusters cocci) in size range from 0.25 µm to 1 µm. The indirect stain showed inconsistent shape and size including staphylococcus that looks like asexual condiospores in fungi (for example, *Penicillium sp.* And *Aspergillus sp.*) and filamentous cells (Figure 1).

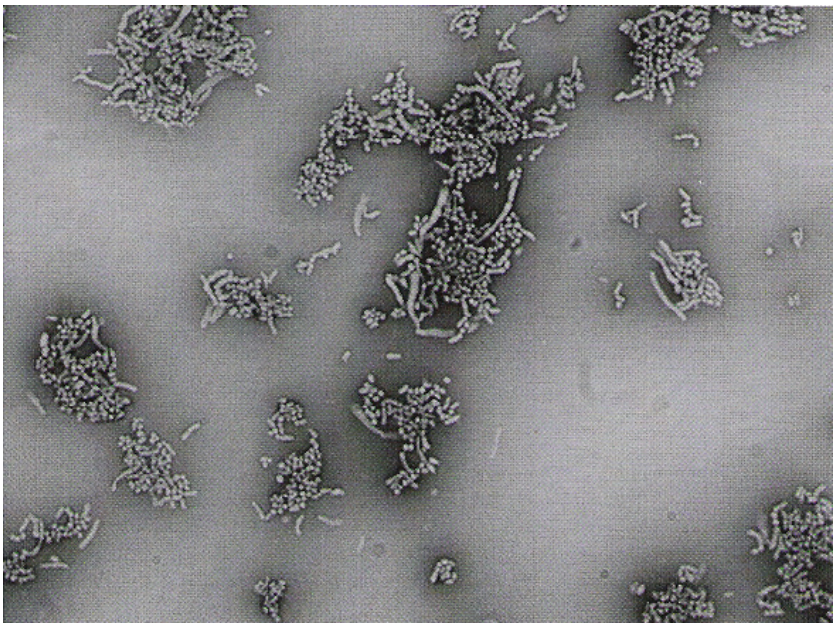

Figure 1. Indirect stain of *Rothia dentocariosa* after 4-5 days grown on brain heart agar.

Acid and gas production by interacting with certain carbohydrates showed that the bacteria fermented only glucose and sucrose, as showed by the change in color on the carbohydrate deeps from red to orange. Other carbohydrates, such as lastose, inositol, raffinose, rhamnose, arabinose, sorbitol, and mannitol, showed negative results because they did not show any reaction on the carbohydrate deeps. According to this experiment, the only enzyme was produced by this bacteria was catalase enzyme because it showed a bubbling reaction when the catalase test was applied. Another test for enzyme presence was the urease test, and it showed no change in agar slant (it stayed orange), which meant a negative result for the production of urease enzyme. In citrate utilization, a green slant tube showed no reaction (it stayed green), which means these bacteria were not able to take in and use citrate or citric acid. The bacteria did not show the ability to break down/hydrolyze on the esculin hydrolysis test. The ability for the bacteria to hydrolyze is shown by the change color from tan brownish of the agar to black. To test for the presence of lysine, a lysine decarboxylation test was applied. This test showed yellow color in both control lysine and lysine tubes. This color indicated these bacteria can ferment only the carbohydrate presence, glucose, but do not contain lysine. The Voges Proskauer test showed a red wine color after vigorously shaking the closed tube, waiting for 10 minutes and continuing to shake the tube again. Red wine color showed a positive result for the formation of acetoin. Then a methyl red test was run, and it showed no change in color (it stayed yellow). The sulfur indole motility tested

for 3 parts and no positive test showed on this experiment. The test for testing the ability to produce hydrogen sulfide gas was negative; the medium that was yellow remained the same. On the indole test, the solution showed yellow after a visible amount of Kovac's reagent was dropped and let sit for several minutes. The last test for motility showed a straight and clear line, which means the bacteria did not move, it just stayed all along the line. The triple sugar iron medium showed no change in color. This lack of change meant that these bacteria did not ferment any of the carbohydrates present, including glucose, sucrose and lactose, and did not produce hydrogen sulfide gas. Then to be more precise, DNA extraction was done to be able to run PCR. The results in the BLAST showed the bacteria are *Rothia Dentocariosa*. Source of the organisms are: *Bateria*, *Actinobacteria*, *Actinobacteridae*, *Actinomycetales*, *Miccrococcinea*, *Micrococcaceae*, and *Rothia*.

DISCUSSION

In 1968, researchers June M. Brown, and Lucille K. Georg discovered organisms known as *Rothia dentocariosa* were different from *Actinomyces* and *Nocardia*. The experiment showed that *Rothia* are not pathogens compared to *Actinomyces* and *Nocardia* even if *Rothia* can cause infection in human mouth. The difference between these genera is hard to identify due to the similarity of cells morphology and physiology (Brown and Lucille et al. 1968). Finally, the experiment revealed the characteristics of *R. dentocariosa* that differentiate this species to *Actinomyces* and *Nocardia*. Other characters such as the preference of aerobic condition, nitrite reduction, and coccoid cells in broth culture differentiate *R. dentocariosa* from *Actinomyces* and *Nocardia*.

In 1993, further experiment was done by Stuart J. Ruben showed that *R. dentocariosa* are potentially pathogen (Ruben 1993). According to Ruben's experiments the infections that *R. dentocariosa* can cause are: "periodontal inflammatory disease, and infected pilonidal cyst, a periappendiceal abscess, five cases of endocarditis and an infected arteriovenous fistula" (Ruben 1993). However, another group of researchers operated a later experiment in 2004 to determine some species in the genera *Rothia*, *Actynomyces*, and *Nocardia* that are included in carcoal-black-pigmented organisms (Daneshvar 2004). During the discussion section of this experiment, the researchers proved that the serious diseases such as endocarditis caused by *R. dentocariosa* are rare due to insufficiency methods in the experiment (Daneshvar 2004).

Another characteristic that was discussed was the ability of *Rothia* to hydrolyze. According to this experiment, esculin hydrolysis showed a negative result. On the other hand, Bergey's Mannual and the experiment of Brown and Georg showed a positive result. Bergey's manual indicated that *R. dentocariosa* have 26 strains that are divided into three groups (Butler 1986). Other experiment showed that most of *Rothia* species are closely related, such as *Rothia mucilaginosa*, *Rothia amarae* and *Rothia* strain CCUG 25688; Y13025 (Aas et al. 2005 and NCBI). This experiment could use a different strain from the experiment in the Bergey's Mannual and Brown and Georg's.

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Isolation, Examination, and Identification of Bacteria from Air Sample Collected in the Kitchen

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Prior to the investigation of my unknown bacteria, I was interested in the field of microbiology and what I could learn from it. When assigned to conduct a research on a microorganism from location of choice, I was intrigued about what I could find in my kitchen. Series of biochemical tests, DNA extraction, staining procedures, and interpretations of an electropherogram led me to the identification of my bacteria being, *Stenotrophomonas maltophilia.* With further research, I learned its negative effects on biological systems, but also its positive outcomes it provides for the human race.

INTRODUCTION

For most people, the world of microorganisms is a fascinating subject. My very own interest with the world of microorganisms was one of my motives for beginning my investigation of bacteria. Upon receipt of this assignment, I was really curious about what sorts of organisms took up residence in my home. Was my home really as safe as it should be? The hard part was, deciding what part of my home I wanted to study. Finally I decided on the kitchen on the basis of this is the part of my home that people spend most of their time in. Not only is it a setting for people, but a place where food was prepared, where dishes were cleaned, where study groups were formed, and where my dog consumed his very own meals. Although I expected to find something harmless or a species that was part of our everyday normal flora, I did hope to find something that had never yet been identified.

MATERIALS AND METHODS

After exposing my nutrient agar plate for one hour on the kitchen counter, I returned by agar plate to school and let it sit in my lab desk drawer for approximately 48 hours to allow for growth. Once I had an assortment of colonies forming on my plate, I chose the colony of most interest to me and transferred it onto another sterile nutrient agar plate using aseptic technique. As soon as I allowed for those colonies to grow, my main goal was for isolation. I inspected my plate to make sure that it was free of any other inhabitants besides the colony of my choice and even took confirmation from my instructor. The moment I was positive of complete isolation of my organism, I began to perform some staining techniques to study the morphology of my unknown organism. The two stains that I performed included a Gram stain and an indirect stain.

Following my stain procedures, I extracted the DNA of my organism in order to identify what my organism was. The first step to this was taking a small sample of my colony and putting it into a Tris buffer solution that was placed into a micro centrifuge tube using aseptic technique. The tube was then vortex mixed for a matter of seconds to distribute the bacteria evenly into the buffer solution. Then, the tube was boiled for 10 minutes for denaturation and the isolation of my DNA to occur. From this point on, my professor took control over the rest of the procedure. After the DNA extraction, my professor set up a PCR tube which was used for the continued identification of my organism. My professor took another micro centrifuge tube and created a mix that consisted of Taq DNA Polymerase Master Mix, two primers (Bacteria 8 Forward and Universal 1492 Reverse), a sample of my DNA which served as a template, and distilled water. She then ran the PCR cycle with this PCR solution with the use of a thermal cycler. Finally, she cut the DNA with a razor blade and used the Qiaquick Gel Purification Kit from a company called Qiagen to purify the DNA. The DNA was put in tubes with a filter in it and rinsed it so that the DNA caught on the filter. She then sent the sample to the UC Davis College of Biological Sciences to complete DNA sequencing and the data was eventually sent to Harriet Wilson, my professor, via e-mail as an electropherogram which requires the 4-Peaks program to view.

Lastly, I performed a series of biochemical tests with the rest of my colonies to test for its physiology. The tests I performed include: KOH test, Oxidation/Fermentation test, an Oxidase test, and a Gelatin test.

RESULTS

In regards to the morphology of my colonies, they appeared a pale, yellow color that was smooth, shiny, and entire in shape. They ranged from 0.5 mm to 2 mm in size. My Gram stain proved them to be Gram-negative, staining pink and being bacilli in shape from 0.5-.08 micrometers in width and 1.0-1.5 micrometers in length. The sizes of my bacteria showed to be similar in my indirect stain.

The biochemical tests I performed can be summarized in Table 1.1 below.

BIOCHEMICAL TEST	**RESULT**
KOH test	Negative (-)
Oxidation/Fermentation test	Negative (-)/ Positive (+)
Oxidase test	Negative (-)
Gelatin test	Positive (+)

TABLE 1.1

The KOH test that I performed was just a confirmation to my Gram stain on whether or not my bacteria were Gram-negative or Gram-positive. The test was negative because as soon as I put a drop of KOH onto a sample of my bacteria, the viscosity increased due to the bacteria being Gram-negative. With the Oxidation/Fermentation test, the tube turned from yellow to green meaning they were positive for fermenting lactose, but were

negative for oxidation because of the lack of gas bubbles or gas formation in the agar. The Oxidase test was a quick test that tested for the presence of cytochrome in my bacteria. Since there was no color change on the filter paper that I smeared the bacteria on, my test was negative. Lastly, in the gelatin hydrolysis test, this tested for whether or not bacteria had the ability to hydrolyze collagen. The gelatin in my test tube stayed liquid with my organisms in it, meaning I had a positive test for the hydrolysis of gelatin by my bacteria.

Then, my professor discussed with me the results of the PCR that was returned to her and showed me the electropherogram of my bacterial DNA. The electropherogram displayed as a row of colorful, clean peaks of the DNA sequence. I didn't perform any editing considering that most of the electropherogram looked ordered and contained only a couple of misread errors in the sequence. After opening a file that consisted of the DNA sequence, we used a program that cut the sequence with primers Bacteria 8 Forward and flipped it with Universal 1492 Reverse. We then copied and pasted this newly modified sequence onto a BLAST program on the NCBI website. After interpreting the results, the closest match in the database to my unknown organism was identified as *Stenotrophomonas maltophilia.*

As soon as I established the identification of my bacteria, I looked it up in the Bergey's Manual of Systemic Bacteria, Second Edition, Volume Two. All the tests I had performed prior to the identification of my bacteria rendered accurate in comparison to what the Bergey's Manual had.

DISCUSSION

In this research of an unknown organism, I have been enlightened by many aspects in the field of microbiology. As far as *Stenotrophomonas maltophilia* is concerned, it has been known to be found in certain human conditions such as cystic fibrosis, bacteremia, urinary tract infection, and pneumonia and has caused second degree infections. Although, according to a journal article in the High Wire Press at Stanford University, research has found that in some cases of patients, they were not able to find a direct link between an infection caused by *Stenotrophomonas maltophilia* being a cause of death (Denton & Kerr, 1998). Studies also show that although strains of *S. maltophilia* were associated with toxicity, it was far less toxic compared to that of the problems associated with *Escherichia coli.*

What I question is how an organism that can cause such infections to a human be residing in a domestic setting such as my kitchen. In further research of my bacteria, I have also discovered, stated in a journal from High Wire Press at Stanford University, that *S. maltophilia* can be found in frozen fish, milk, and even poultry eggs, items that can be seen everyday at home, in the kitchen, where I initiated my research (Denton & Kerr, 1998). Fortunately, aside from the negative implications that *S. maltophilia* has brought about, it has been used for the inhibition of fungal growth, a positive side to *S. maltophilia*.

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Isolation and Identification of Normal Flora Organism Found on Hand

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A nutrient agar plate was inoculated with fingertips that had been thoroughly washed and air-dried. A variety of colonies grew on the plate and one in particular was selected. Once the organism was isolated several biochemical tested were performed on the organism and a nucleotide sequence of the organism was compared to those on BLAST. The results of the tests and the nucleotide sequence indicated that the organism selected was *Staphylococcus epidermidis*.

INTRODUCTION

This organism was selected based on the "Effectiveness of Hand Washing" exercise. There was a variety of organisms growing in the quadrant of the plate inoculated with thoroughly washed, air-dried hands. The purpose of this project was to isolate and identify the organism and confirm the results based on a variety of biochemical test.

The organism selected was *Staphylococcus epidermidis*. *Staphylococcus epidermidis* is a spherical shaped Gram-positive bacteria that is frequently found on the skin surface and mucous membranes of humans and animals. It is considered to be normal flora. It is the most prevalent and persistent species of *Staphylococcus* found on human skin. It is typically a non-pathogenic organism, but it has been found to cause infection in people with compromised immune systems. *Staphylococcus epidermidis* has been found to be a common cause of hospital-acquired infections. It can also cause infection is people who have indwelling catheters because the organism is capable of producing a biofilm that allows them to adhere to medical equipment. Because *Staphylococcus epidermidis* is capable of forming biofilms it is highly resistant to many antibiotics including penicillins and cephalosporins. It can also be resistant to antibodies and phagocytosis. *Staphylococcus epidermidis* can also serve as a reservoir for antibiotic resistant genes. Due to the resistant and the virulence of this organism, researchers are pressured to find alternative means of fighting *Staphylococcus*.

MATERIALS AND METHOD

A nutrient agar plate was inoculated with the fingertips of a hand that had been thoroughly washed and air dried. The plate was incubated at room temperature. A variety of organism developed. One well developed colony was selected to isolate and was aseptically streaked on Nutrient agar using the streak plate technique. The organism was Gram-stained, and a KOH test was performed to confirm results. An indirect stain was performed using nigrosin as

the staining agent to determine organism's cell morphology. Chromosomal extraction took place. A sample of the cells was boiled and beaten using glass beads. 16S Ribosomal DNA was amplified using the Polymerase Chain Reaction (PCR) technique. The PCR was run according to the lab manual using primer bacteria 8-forward and universal 1492-reverse. A gel electrophoresis was performed. DNA was purified using QIA quick gel purification kit (QIAGEN). The purified DNA was taken to University of California, Davis College of Biological Sciences DNA sequencing facility. The electropherograms were edited and combined using 4 Peaks. The National Center for Biotechnology Center (NCBI) website and Basic Local Allignment Search Tool (BLAST) were used to compare the nucleic sequence with gene banks.

Once the organism was identified using BLAST a variety of biochemical tests were run to confirm these results. The biochemical tests run included: Urea hydrolysis, Methyl- Red Voges- Proskaur (MR-VP), Catalase test, Coagulase test, Oxidase test, Hemolysis reaction on blood agar, and carbohydrate slants (arabinose, lactose, mannitol, raffinose, sucrose) (Because Staphylococcus epidermidis is an aerobic organism carbohydrate deeps were melted to be slants and the slants were inoculated).

RESULTS

The colonies formed on the nutrient agar plate indicated a colony morphology of colonies that were opaque, pale yellowish white in color and less than 2 millimeters in diameter.

The Gram-stain stained purple indicating that the organism is Gram-positive. The results of the indirect stain indicated that the cell morphology is cocci shaped cells arranged in clusters. The cell size is approximately 1 micrometer.

The results of the BLAST indicated that the organism had a 99% nucleotide sequence match to Staphylococcus epidermidis.

The results of the biochemical test are as follows: The agar of the urea hydrolysis test was yellowish/ peach indicating negative (-) results and that the organism is unable to make urease enzymes. The MR portion of the MR-VP test was yellowish orange indicating negative (-) results and that the organism does not form large amounts of acid. The VP portion of the MR-VP was brown indicating negative (-) results and that the organism is unable to form acetoin. The catalase test caused bubbling indicating positive (+) catalase results. The coagulase test remained liquid indicating negative (-) results and that the organism does not contain a coagulase enzyme. The oxidase test had no color change indicating negative (-) results and that organism does not form cytochrome C. The Hemolysis test had no change in the appearance of the agar surrounding the colonies indicating a gamma-hemolysis reaction. The arabinose, lactose, mannitol, and raffinose carbohydrate slants had no color change, all agar remained pink indicating negative (-) results and that organism is unable to ferment these carbohydrates. The sucrose carbohydrate tube turned yellow indicating positive results and that the organism is capable of fermenting sucrose.

DISCUSSION

All of the test results matched the results of those found in Bergey's Manual except the urea hydrolysis results, The Bergey's Manual indicated that the results of the urea hydrolysis test should be positive (+) rather than the negative (-) results obtained in the lab. Although the results of this test did not match, the organism should still be identified as Staphylococcus epidermidis, because all other biochemical test results matched as well as the BLAST search. The difference in urea hydrolysis results could be due to an error in procedure.

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