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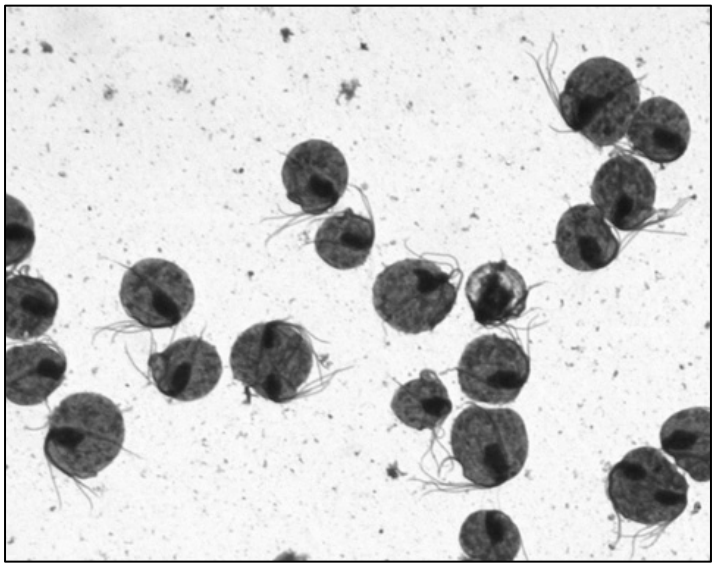

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Cover photo:
A photomicrograph of a prepared slide of *Trichomonas vaginalis* trophozoites magnified 400x. (H. Wilson)

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Microbial Life on the Stadium Turf

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Students in the fall 2007, non-major's microbiology class sampled microorganisms present on the artificial turf of the Sierra College football field. Six nutrient agar plates were exposed to the turf surface in various locations and 16 cultures were established from selected colonies. Cultural characteristics were recorded, Gram stains were made and the wall character and morphological features of each cell type were recorded. DNA was extracted from 15 different-looking pure cultures using a combination of boiling and beating with glass beads. The polymerase chain reaction was used to amplify 16S ribosomal-DNA from each sample, PCR products were subjected to gel electrophoresis and then stained with ethidium bromide, cut from the gels and purified with QIAquick PCR purification kits (Qiagen). Purified DNA samples were taken to the UC DNA sequencing facility in Davis and the resulting electropherograms were analyzed, edited and compared with those available in gene banks through the BLAST algorithm of NCBI. The cultures were determined to include eleven different species within five Gram-positive genera including *Bacillus*, *Paenibacillus*, *Exiguobacterium*, *Streptomyces* and *Microbacterium*. None of the organisms isolated were found to be human pathogens, all were found to be associated with soil, and all could easily have gained access to the field from the air or from the feet of athletes using the field.

INTRODUCTION

Sierra College's Homer "Buzz" Ostrom stadium, first used during the 2006 football season, is equipped with a modern artificial-turf playing surface. Although public access to this playing surface is restricted, and the turf appears clean to the casual observer, the field is used for football games, practices and a variety of Physical Education classes. Given that microorganisms inhabit nearly all types of environments, both natural and artificial, it stands to reason that microorganisms are present on the surface of this field.

The class project, decided upon during the first laboratory meeting of the fall, 2007 Biological Sciences 44 students, was to attempt to identify a number of microorganisms present on the artificial turf in the Sierra College football stadium. To accomplish this the class decided that samples would be taken from various sections of the field. Cultures would be allowed to grow from these, and then class members would select specific examples and develop pure cultures by streaking samples from isolated colonies onto new agar plates. One portion of the semester project would involve maintaining these cultures throughout the semester, while another portion would involve completing the various

procedures necessary to determine the identity of each organism type.

Assuming a variety of different bacteria species would be found on the artificial turf field, another goal of the class project was to determine if or not any of these organisms might pose a threat to the athletes and other individuals using the field. Most bacteria encountered in the environment are not pathogenic to humans and are unlikely to cause infection or disease; therefore, another portion of this investigation would be focused on other significant aspects or interesting features of the organisms encountered. A plausible explanation for how microorganisms could gain access to the field would also be proposed.

MATERIALS AND METHODS

Multiple microbial samples were collected from the artificial turf surface within the football stadium by opening six Petri plates containing nutrient agar and bringing the agar surface of each plate into direct contact with the turf. Sample locations were recorded for all plates as indicated in table 1, and they were returned to the microbiology laboratory for incubation at room temperature.

Location of Field Sample Taken	Source Colonies By Number
50 yard-line within white area of "S"	Colonies #9 and #10
45 yard-line green area at east edge of field	Colonies #7 and 8
5 yard-line white area at east edge of field	Colonies #1, 2, 3 and 14
5 yard-line green area at west edge of field	Colonies #4, 5 and 6
20 yard-line white area at east edge of field	Colonies #11 and 12
20 yard-line green area at west edge of field	Colonies #13, 15 and 16

Table 1 – Location of field sample and colony numbers selected from each plate.

Following one week of incubation, the culture plates were inspected for microbial growth, and variations in colony morphology were noted. Sixteen colonies were selected for sampling based on unique appearance. Each colony was marked with a circle on the plate bottom and each was assigned a number (Table 1). A sample of cells from each marked colony was aseptically transferred to a new nutrient agar plate and each was streaked to produce well-isolated colonies. Each plate was then labeled according to the colony number and field sample location. All plates were incubated at room temperature until growth was evident, and then samples from isolated colonies were transferred to new plates to insure that pure cultures had been established.

The cultural characteristics of the colonies/growth present on nutrient agar were recorded and Gram stains were made for each different isolate. Culture samples approximately 2mm in diameter were transferred into 1.5ml microfuge tubes containing 500µl 10mM Tris buffer (pH 8.5), and 10-15 sterile, 2mm glass beads. Each tube was subjected to 5-10 seconds of vortex mixing to thoroughly suspend the cells in the buffer

solution, and then all tubes were loaded into foam floats and placed in beakers of boiling water for 10 minutes. The tube contents were cooled in an ice bath and then all the tubes were strapped to the platform heads of vortex mixers and subjected to maximum vibration for 10 minutes. This process was designed to break open the bacterial cells, releasing their DNA, and denaturing the enzymes present.

Following chromosome extraction, the Polymerase Chain Reaction (PCR) was used to amplify 16S ribosomal-DNA from each bacterial isolate. A 5µl sample from each boiled and beaten cell suspension was used as template DNA in a reaction mixture containing 25µl *Taq*-polymerase Master Mix (Qiagen), 5µl primer mix, 5µl template DNA and 15µl sterile water. The primer mix contained the bacterial primers 8-forward and 1492-reverse at a concentration of 5mM each. The PCR was completed in a 96-well MyCycler Thermal Cycler (Bio-Rad).

Following amplification, the 16S r-DNA from each isolate was subjected to gel electrophoresis. The total volume from each PCR tube was loaded into the wells of an agarose minigel submerged in TBE buffer, and subjected to 56 Volts of electric current for 30 minutes. Each gel was then stained with ethidium bromide, rinsed in cold water and then placed on a UV transluminator for observation. A clean razor blade was used to cut each fluorescent DNA band from its gel, each sample was weighed, and then each was placed in a clean, sterile 1.5ml centrifuge tube labeled with the culture number. When all samples had been cut and weighed, the DNA from each was purified with a QIAquick gel extraction kit (Qiagen). The purified DNA samples were then transported to the College of Biological Sciences DNA Sequencing Facility at UC Davis.

The sequencing primer used for each sample was the internal bacterial primer 533-forward. Sequencing results were emailed to Sierra College as ab1-files and were accessed by students using iBook computers, MAC OSX and the software program 4Peaks. The nucleotide sequence from each electropherogram was copied into a word file, where it was analyzed and edited. The edited nucleotide sequences were then compared to those available in public databases through the BLAST (Basic Local Alignment Search Tool) algorithm of NCBI (National Center for Biotechnology Information). BLAST results were examined and species showing 99% or greater sequence similarity with the query were recorded.

RESULTS

Some of the 16 cultures isolated from the football field were determined to contain the same types of organisms, and some were eliminated from the set, but 11 different species were ultimately identified. Cultures #1 and #5 formed circular, light cream-colored colonies with undulate margins, raised elevation, smooth-shiny surface texture and opaque optical character. Some colonies were well isolated and ranged in size from 2-4mm in diameter. Both cultures showed a strong tendency to swarm on fresh solid media forming thin layers of effuse growth. In a Gram stain the cells appeared to be purple bacilli with numerous

white, ellipsoidal, centrally located endospores. The sporangia were not swollen. Cell size was somewhat variable but most cells were 0.5-0.8μm in diameter and 2-5μm in length, arranged as single cells. BLAST results indicated that the 16S ribosomal-DNA nucleotide sequences from these cultures showed 99-100% similarity with several strains of *Bacillus pumilus*.

Cultures #2 and #3 formed circular to irregular off-white to tan-colored colonies with entire to undulate margins, raised elevation, rough-shiny surface texture and opaque optical character. These typically ranged in size from 3-4mm in diameter. In a Gram stain the cells appeared to be purple bacilli with numerous white, ellipsoidal, centrally located endospores. The sporangia were not swollen. Cell size was somewhat variable but most cells were 0.7-0.9μm in diameter and 3-4μm in length, arranged as single cells. BLAST results indicated that the 16S ribosomal-DNA nucleotide sequences from these cultures showed 99-100% similarity with several strains of *Bacillus subtilus*.

Culture #4 formed circular to irregular off-white colonies with filamentous margins, raised elevation, rough-shiny surface texture and opaque optical character. These ranged in size from 5-8mm in diameter. In a Gram stain many of the cells appeared pink, but some were purple bacilli. They appeared long and slender, 0.3-0.5μm in diameter and 4-6μm in length, with terminal, spherical endospores and swollen sporangia. They were arranged as single cells. Chromosomal DNA was extracted from this culture, but PCR amplification was not completed due to time constraints. No BLAST results were obtained.

Culture #6 formed circular to irregular, pale creamy-yellow colonies with undulate-spreading margins, raised central elevation with flat borders, smooth-shiny surface texture and semi-translucent optical character. These ranged in size from 4-5mm in diameter. In a Gram stain the cells appeared as large, irregular, purple bacilli ranging in diameter from 0.5-1.5μm and in length from 4-12μm. Some cells contained endospores, but these were also variable in shape. Some were long ellipsoidal structures while others were nearly spherical; their location was central or subterminal. Cell arrangement was also variable, with both single cells and short chains. Some cells were bent and worm-like. BLAST results indicated that the 16S ribosomal-DNA nucleotide sequence from this culture showed 99% similarity with two strains of *Bacillus bataviensis*, one of *Bacillus drentensis* and one of *Bacillus senegalensis*.

Culture #7 formed circular to irregular, light creamy-orange colonies with entire to undulate margins, low-convex elevation, smooth-shiny surface texture and opaque optical character at the center, semi-translucent at the margins. These ranged in size from 2-4mm in diameter. In a Gram stain the cells appeared as oval-shaped purple bacilli ranging in diameter from 0.8-1.0μm and in length from 1.5-2μm. The cells were arranged as single cells and in clusters. BLAST results indicated that the 16S ribosomal-DNA nucleotide sequence from this culture showed 99% similarity with several strains of *Exiguobacterium acetylicum*.

Culture #8 formed circular colonies with filamentous margins, rough to powdery surface texture, convex elevation with a central crater. These were opaque, slate-gray with white on the upper surface and tan with dark gray on the reverse (colors arranged in concentric rings). Their size ranged from 2-5mm in diameter. In a Gram stain the cells formed a tangle of filaments 0.8-1.0μm in diameter with conidiospores arranged in single

chains at some ends. BLAST results indicated that the 16S ribosomal-DNA nucleotide sequence from this culture showed 99% similarity with multiple strains of *Stretomyces ambofaciens*.

Culture #9 formed circular to irregular, off-white to nearly colorless colonies with entire to undulate margins, raised elevation, smooth-shiny surface texture and translucent optical character. These typically ranged in size from 5-9mm in diameter. In a Gram stain the single cells appeared as long, slender purple bacilli ranging in size from 0.3-0.5μm in diameter and 4-7μm in length. BLAST results indicated that the 16S ribosomal-DNA nucleotide sequence from this culture showed 99% similarity with an uncultured clone and 97% similarity with *Bacillus firmus*.

Culture #10 formed pale-colored circular to irregular colonies with pinkish centers and white borders. The margins were undulate to lobate, and the elevation was raised with a convex center, like an egg "sunny-side-up". The optical character was opaque and the size ranged from 8-10mm in diameter. Cells observed in a Gram stain were slender pinkish bacilli arranged as individuals, 0.8-1μm in diameter and 4-7μm in length. Many cells contained ellipsoidal, terminally located endospores. BLAST results indicated that the 16S ribosomal-DNA nucleotide sequence from this culture showed 99% similarity with several strains of *Painebacillus lautus*.

Culture #11 formed circular colonies with undulate margins, raised elevation, wrinkled surface texture, translucent optical character, and tan-colored pigmentation. The largest colonies were 8-10mm in diameter. Cells observed in a Gram stain were purple bacilli, but highly irregular in size and shape. They ranged in diameter from 0.5-1.5μm, many with both slender and fat regions. Though many cells were 2-5μm in length, some were 12 or more. Many cells were curled or worm-like rather than straight, and many contained ellipsoidal endospores located centrally or sub-terminally. Some sporangia appeared swollen, but others not. BLAST results indicated that the 16S ribosomal-DNA nucleotide sequence from this culture showed 100% similarity with *Bacillus niacini*.

Culture #12 formed circular to irregular colonies with spreading, undulate margins, low convex elevation, smooth texture, semi-opaque optical character and creamy-tan pigmentation. These ranged in size from 2-7mm in diameter. Cells observed in a Gram stain were slender purple bacilli with centrally located endospores. Chromosomal DNA was extracted from this culture, but PCR amplification was not completed due to time constraints. No BLAST results were obtained.

Culture #13 formed circular colonies with filamentous margins, convex elevation, rough surface texture, and optical character varying from semi-translucent to opaque. Young colonies appeared gray-tan and mature colonies were white due to the presence of conidiospores. Mature colonies were 4-5mm in diameter. In a Gram stain preparation the cells from this culture formed a tangled mass of filaments 0.8-1.0μm in diameter. No conidiospores were observed, but the colony morphology suggested strongly that conidiospores were present on some filaments. BLAST results indicated that the 16S ribosomal-DNA nucleotide sequence from this culture showed 100% similarity with *Streptomyces flaveolus* and *Streptomyces viridochromogenes*.

Culture #14 formed circular to irregular orange colonies with entire to undulate margins, low convex elevation, smooth-shiny surface texture and translucent optical

character. Their pigmentation was similar to orange marmalade. Most colonies ranged in size from 5-7mm in diameter. In a Gram stain the cells from this culture appeared as tiny, pink rods or coccobacilli, approximately 0.3μm in diameter and 1μm in length. Though most cells were arranged in clusters, they did not appear to be bound to one another in any specific arrangement. BLAST results indicated that the 16S ribosomal-DNA nucleotide sequence from this culture showed 100% similarity with *Microbacterium paraoxydans*. Culture #15 was never fully purified, so was ultimately eliminated from the set.

Culture #16 formed creamy tan-colored, circular to irregular colonies with undulate to lobate margins, raised elevation, smooth surface texture, and opaque optical character. Most were 5-8mm in diameter. In a Gram stain the cells appeared as large, highly irregular purple bacilli. Most cells were 1.5-2.0μm in diameter and 2-5μm in length, but some were 10μm long. Many were curved and worm-like rather than straight. Ellipsoidal endospores were evident within many cells, and appeared to be central in location. Exospores were also prevalent. Some cells were arranged singly and some formed short chains. BLAST results indicated that the 16S ribosomal-DNA nucleotide sequence from this culture showed 99% similarity with *Bacillus megaterium* or *Bacillus flexus*.

DISCUSSION

Although the cells from several of the football field isolates appeared pink or Gram-negative in Gram stain preparations, all of the species identified were Gram-positive. As indicated by the BLAST results, eight were determined to be *Bacillus* species (*Bacillus subtilis*, *B. firmus*, *B. pumilus*, *B. megaterium*, *B. drentensis* and *B. niacini*), two were determined to be *Streptomyces* species (*Streptomyces ambofasciens* and *S. flaveolus* or *viridochromogenes*), one was determined to be *Painebacillus lautus*, one *Exiguobacterium acetylicum*, and one *Microbacterium paraoxydans*. Eleven different species in five different genera were isolated and tentatively identified using 16S r-DNA sequence comparison. None of the species identified are recognized as significant human pathogens.

All the bacteria identified had one feature in common, specifically, all exist in soil. Many of the *Bacillus* species are used in agriculture, which could explain their presence in the soil near the Sierra College location. The *Bacillus* and *Painebacillus* species all produce endospores, dormant structures ideal for survival on the harsh medium of the stadium field. Endospores are resistant to gamma and ultra-violet radiation, dessication, lysozyme, high temperatures, lack of nutrients, and exposure to chemical toxins such as disinfectants. The *Streptomyces* species also produce spores, but these are called conidiospores or conidia. Conidia are reproductive structures formed at the ends of filaments and tend to be present in the air at all times because they are formed in large numbers and are extremely lightweight. Since wind can easily transport both endospores and conidiospores, this alone could explain their arrival at the sampling destination.

Although wind transport provided one possible explanation for the presence of soil bacteria on the stadium's playing surface, it was not the only one. After watching the actions of the Sierra College football team at one of their home games late in the 2007 season, it was

clear how some of these organisms came to the turf. Before the game the players made their way from the locker room to the field, walking two-by-two in a long line, and tracking across various surfaces. They began on asphalt as they exited the locker room and then proceeded onto the natural grass soccer field neighboring the stadium. Since organisms in the genera *Bacillus, Painebacillus, Exiguobacterium, Microbacterium* and S*treptomyces* all inhabit soil and decaying vegetation, they could readily have been picked up on cleats penetrating the surface of the soccer field, and then transferred onto the stadium turf. Members from the opposing team could also transport organisms as they warmed up on the grass field south of the snack bar prior to entering the stadium. Neither team did anything to clean their cleats before taking the field, thus giving the microorganisms a free ride onto the artificial turf.

Once present, the organisms identified might not find survival difficult even though during summer days the turf can get very hot, very fast. As one former football player, Charlie Rapp, described, “It can feel like your feet are melting in your cleats.” Organisms in the genera *Bacillus* and *Painebacillus* can endure the heat by forming endospores, dormant structures highly resistant to damage caused by exposure to high temperatures and high salt concentrations. Organisms in the genus *Microbacterium* are also more resistant to damage caused by high temperatures than are most types of bacteria. Many species within these genera are capable of utilizing unusual organic compounds, as sources of carbon and energy, so might be able to use the rubber-like, artificial "soil" in the stadium as a nutrient source. The filamentous *Strepomyces* species might actually add another sense of realism to the field as they give off an earthy aroma by producing geosmin.

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Isolation of *Acidovorax defluvii* From Goat's Water Trough and Isolation of *Pseudomonas* from Dog's Water Pail.

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We wanted to see if there were similarities between microbes associated with goats and dogs since many people consider goat's dirty animals that will eat anything and dogs are said to be "mans best friend." We took samples of water from both a dog's drinking water pail and a goat's water trough, grew them on TSA, and then isolated one organism from each plate that looked similar to each other. After isolation, we performed various tests and stains to see if we had the same organism. These tests and stains included KOH tests, Gram stains, Endospore stains, capsule stains, and phenol red lactose broth tests. Based on these tests we decided that the organisms were not the same. We could only perform DNA analysis on one organism, so we chose to sequence the goat sample; we sent the products of a polymerase chain reaction and gel electrophoresis to the Division of Biological Sciences Sequencing Facility at the University of California in Davis. We compared our sequence to others on the Basic Local Assignment Search Tool (BLAST) website. We found that the cells we isolated from the goat water were 98% identical to *Acidovorax defluvii*. We then performed oxidase, catalase, and floc tests to confirm the identity. For the dog water sample, we chose to use Bergy's Manual of Systematic Bacteriology to identify our unknown organism. First, we eliminated most of the bacteria that were possibilities. We then decided to try to grow our unknown organism on *Pseudomonas* enrichment medium. Since it did grow on this medium, we performed an oxidase test and a catalase test to confirm the identity. In addition, we grew the dog's isolated cells in *Pseudomonas* broth. After all of these tests, we concluded that we had isolated *Acidovorax defluvii* from the goat water and *Pseudomonas* from the dog water.

INTRODUCTION

What's the difference between an outside dog's drinking water and a goat's trough? We want to find out if goats and dog's have similar microbes since many people consider goats dirty animals that will eat anything while dogs who are said to be "man's best friend." Since the microbes that are on both the dogs and goats faces and in their mouths come into contact with their drinking water, we thought this would be a good way to retrieve an array of their flora. We hypothesize that there will be many different microbes in both water sources, but there should also be at least a couple that are the same. Dogs and goats have close body temperatures and because of this some of the same mesophiles may be a part of their normal flora. However, these animals have a different way of digesting their food. Goats are ruminates; they have a stomach with multiple chambers and they chew their cud (regurgitate their food and chew it). This allows them to digest roughage that many animals

cannot and means they have enzymes and possibly other bacteria that dogs don't. In addition, goats may come into contact with more microorganisms because they eat grass daily. For example, last winter some of our goats became sick and we took them to the veterinarian to find that they had parasitic worms. According to Mindy Hollowell, *Actinomyces, Streptococcus, Granulicatella, Capnocytophaga canimorsus,* and *Porphyromanas salivosa* are all normal flora for a dog (pg. 14-17). On the other hand, normal flora for a goat includes *Entodinium caudatum, Spirochete, Methanosarcina, Entodinum, Ophryoscolex,* and *Diplodinium* (Microbe Zoo). We want to find the similarities in our dog's and goat's water sources. There is one good way to find out ourselves: we will take samples from both the outside dog water and the goat trough, inoculate separate streak plates, and then compare and experiment with colonies that look similar.

MATERIALS AND METHODS

The dog's water sample was obtained from a pail housed outside in the shady part of a porch in western Placer County. The water was supplied by an outside household faucet and provided by Nevada Irrigation District. The goat's water sample was obtained from a trough located outside a home in Greenwood, California. The water was supplied by a well on said property.

A new Ziploc bag was dipped into each source of water. A loopful of each sample of water was streaked (Wilson, 2007, pg. 43-46) onto separate TSA media plates and placed in the incubator for 24 hours at a temperature of 37°C. In order to obtain a pure culture, a similar colony from each plate was re-streaked on separate TSA media plates and placed in the incubator at 37°C for 24 hours. A KOH test was performed (Wilson, 2007, pg. 64) for each sample to determine if the cells were able to be lysed by the 3% Potassium Hydroxide, which is an indicator that the organism is Gram positive or Gram negative. Direct, indirect (Wilson, 2007, pg. 53-55) and Gram stains (Wilson, 2007, pg. 61-63) were performed to determine the cellular morphology. In addition, wet mounts were made to determine motility. To make the wet mount, we put a few loopfuls of water onto a slide, aseptically transferred the cells from the steak plate to the slide with a loop, gently mixed the cells with the water, put a thin piece of plastic over the area that contained the cells, and it was ready to view. Because we had plastic over the slide we couldn't use the microscope's oil immersion objective and had to be very careful not to hit the objective lenses on the plastic. Endospore

(Wilson, 2007, pg. 75-76), capsule (Wilson, 2007, pg. 77-79), and Phenol Red Lactose tests were performed to determine if the organisms produced spores, had capsules, or were capable of fermentation and or produced gas (homofermentative or heterofermentative). The phenol red lactose test was relatively simple to perform: It's a broth medium, so we put water that was inoculated with our cells in a small breaker that was previously sterilized with alcohol and simply poured it in the phenol red medium to fill the tube about halfway. We then incubated it at 37°C for about 24 hours. In addition to confirming cell wall structure, the Gram stains we performed were also used to confirm that the cells had no endospores because our endospore stains weren't as clear as we would have liked.

DNA analysis was performed on the organism that we isolated from a pure culture of the goats water. We extracted chromosomal DNA by boiling the cells in 500 µl of TRIS buffer, with a pH of 8 for 10 minutes. Sasha Warren then added sterile water and bacteria 8-forward (a restriction enzyme) to a microfuge container. After that, the template DNA (chromosomal DNA that we extracted) and the enzyme Taq polymerase (from the bacteria *Thermus aquaticus*) were added. The mixture was placed in ice for 10 minutes and centrifuged. It was then placed in a thermocycler, so a polymerase chain reaction (PCR) would occur. The goal of the PCR was to amplify the 16s ribosomal DNA gene (rDNA). When the PCR was complete, gel electrophoresis was run with 1% agarose gel on the 1500 base pair product. Sasha then brought it to the U.C. Davis Division of Biological Sciences Sequencing Facility. U.C. Davis e-mailed us the sequence and we compared it to thousands of other sequences on Basic Local Assignment Search Tool, a program on the National Center for Biotechnology Information website. We then performed an oxidase test (Wilson, 2007, pg. 176), catalase test (Wilson, 2007, pg. 175-176), and inoculated nutrient broth to see if "flocs" would form to confirm the identity of the goat's cells (Schulze, R. 1999). On all the tests that we preformed, we used a heat sterilized loop to transfer the cells aseptically.

We could perform DNA analysis on only one organism due to time restraints, so we chose to identify the dog's water cells by various tests. To start, we looked through *Bergy's Manual of Systematic Bacteriology* and eliminated most bacteria that pertained to our sample. We already had some experience with *Pseudomonas* and knew it was present in soil, which could have easily gotten into the dog's water, so we decided to streak the dog cells onto *Pseudomonas* enrichment medium. We incubated the *Pseudomonas* plate for about two days at 37°C, but realized it would grow better at room temperature since that's where it was growing in the wild. After that, we performed a catalase test and an oxidase test. Later we aseptically inoculated the dog's cell into *Psedomonas* broth because we were told it was more reliable.

RESULTS

The colonies we chose to isolate and experiment with looked almost identical growing on TSA media. Both had punctiform form, cream color, entire margin, raised elevation, shiny surface texture, opaque optical color, and were about .5 millimeters in diameter. In addition, both had bitter earthy odors (Table 1.0). The direct, indirect, and Gram stains showed that the dog's water cells were about 2 micrometers by 1 micrometer and were short fat rods. The goat's cells were about 3 micrometers by 1.3 micrometers and

were also rods, but longer. However, the cells had the same arrangements: diplobacilli/diplococcobacilli, v-shaped (snapping), and plasiding (Table 1.1). When the wet mounts were performed, the cells from the dog's water would spin for a second or two and then take off in a straight line. While the cells that came from the goat's water moved back and forth just slightly, like they were vibrating (Table 1.2).

Table 1.0

Colony Morphology	**Dog**	**Goat**
Medium used to observe	TSA	TSA
Form	Punctiform	Punctiform
Pigmentation	Cream	Cream
Margin	Entire	Entire
Size in mm	.5mm	.5mm
Elevation	Raised	Raised
Surface Texture	Shiny	Shiny
Optical Character	Opaque	Opaque
Other: Odor	Earthy, slightly bitter	bitter, earthy

Table 1.1

Cellular Morphology	**Dog**	**Goat**
Shape	Coccobacilli	Bacilli
Size	2 μm x 1 μm	3 μm x 1.3 μm
Arrangement	single, diplobacilli, V-shaped, palisading	single, diplobacilli, V-shaped, palisading

Table 1.2

	Dog	**Goat**
Motility	Yes, run and tumble	No, Brownian motion

We completed quite a few diagnostic tests on both organisms. When the KOH tests were performed we had the same results for both organisms; after a few seconds a viscous substance was produced (Table 1.3). Of the areas on the Nigrosin capsule stain that had both a purple background and purple cells, we could not see any rings around the cells (Table

1.3). We also preformed an endospore stain using the Dorner method; from what we could see, all the cells were uniform in color (purple) and had no spots (Table 1.3). The results of the Gram stains were completely pink cells from both the dog and goat samples. The medium of the phenol red lactose broth test that had cells from the dog water remained red with no bubbles in the Durham tube. However, the medium that contained the cells from the goat water turned orange and the top remained red. There were also bubbles in this Durham tube (Table 1.3). The oxidase test paper turned blue/purple when the goat's cells were put on it. (Table 1.3). There was no reaction when hydrogen peroxide was poured over the goat's cells in the catalase test and the bacteria formed chunks in the nutrient broth (Table 1.3).

The dog's cells did, in fact, grow on the *Pseudomonas* streak plate and in the *Pseudomonas* broth. In addition, the oxidative test paper turned blue/purple when the cells were added and the bacteria did not bubble in the catalse test (Table 1.3).

When we received the results from UC Davis, we compared the sequence to others using BLAST (basic local assignment search tool) as discussed previously. The results concluded that the DNA sequence of the organism obtained from the goat's water matched the DNA sequence of *Acidovorax defluvii* with 98% identity. The accession number of organism was Y18616.1 and the ratio of identical nucleotides was 763/770.

Table 1.3

Stain/Test	**Results**		**Conclusions**	
	Dog	**Goat**	**Dog**	**Goat**
KOH	Snot	Snot	KOH+/Gram -	KOH+/Gram -
Gram Stain	Pink	Pink	Gram -	Gram -
Endospore	Uniform in color	Uniform in color	Not capable of producing spores	Not capable of producing spores
Capsule	Cells and background purple/no clear rings around cells	Cells and background purple/no clear rings around cell	does not have capsule	does not have capsule
Phenol Red Lactose Broth with Durham tube	Red; no bubbles	Orange with red top; bubble present	does not ferment lactose nor cause gas	ferments lactose to acid and produces gas
Oxidase	Blue/purple	Blue/purple	Oxidase positive	Oxidase positive
Catalase	bubbling	No bubbles	Catalase positive	Catalase negative

DISCUSSION

Since the colonies we chose to isolate looked identical, we thought that both isolates were the same organism (Table 1.0).

The cell shape and sizes of the cells that we observed from the direct, indirect, and Gram stains lead us to believe that the dog's cells were coccobacilli and the goats were bacilli. At that point we realized that these organisms were not the same. (Table 1.1).

The dog's cells observed on the wet mount that were spinning and then taking off in a strait line we interpreted as bacteria with "run and tumble" motility. In contrast, the movement of the goat's cells led us to the decision that they were only moving from Brownian motion (Table 1.2).

Since the KOH tests both produced a viscous substance (nucleic acid coming out of the cells as they blew up), we concluded that both organisms were KOH positive and thus Gram negative (Table 1.3). Neither type of cell made a capsule because there were no rings around the cells in the Nigrosin capsule stain (Table 1.3). In addition, we concluded that neither organism could produce endospores because there were no spots in the cells when the Dorner method endospore stains and Gram stains were completed (Table 1.3). The phenol red lactose medium staying red with the dog's cells present indicated that the organism is not capable of fermentation with lactose. However, the medium turning orange and producing bubbles in the Durham tube meant that the cell's from the goat's water does indeed ferment lactose to acid and gas (Table 1.3). We interpreted the test paper of the oxidase tests turning blue/purple to be positive for both organisms which means that they have cytochrome C in the electron transport chains (Table 1.3). We knew that the dog's cells were catalase positive because the bacteria bubbled when hydrogen peroxide was added. This meant the Hydrogen peroxide was breaking down to water and oxygen because of the enzyme catalase. On the other hand, we knew that since there was no bubbling with the goat's cells they were catalase negative (Table 1.3). Also, there were chunks that formed in the nutrient broth containing the goat's cells, so we knew the organism produced flocs.

There is limited information about *Acidovorax defluvii* because *defluvii* is a newly discovered species, but the information that we do have is consistent with our tests and stains. This includes the oxidase test, catalase test, flocs, Gram negativity, and cell morphology. We knew the identity of the dog's cells were *Pseudomonas* because the cells grew on the *Pseudomonas* medium and the results of the oxidase and catalse tests were consistent with the genus *Pseudomonas*. Also the Gram stain, cell morphology, and motility were consistent.

In conclusion, we isolated *Acidovorax defluvii* from the goat water and *Pseudomonas* from the dog water. Most species of Pseudomonas are harmless, but "at least one species (*P. mallei*) appears to be a specialized mammalian parasite, while others are occasional animal pathogens" (Palleroni, N., 1984). Since we didn't have the time or resources to identify a species for *Pseudomonas*, we are not sure if this sample of *Pseudomonas* is harmful. From the limited information we have about *Acidovorax defluvii*, it sounds to be harmless to animals and humans; although some species of *Acidovorax* can cause diseases in plants

(Schulze, R., 1999). An interesting fact about *Acidovorax* is that this bacterium is most often found in sludge (Schulze, R., 1999). Overall, we think this experiment shows that any animals that are outside have a risk of coming into contact with potentially harmful agents, from parasitic bacteria to bacteria that are found in sludge.

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Vol. 2, No. 1

A Comparison of Microbes in the Pediatric waiting rooms of both Sutter and Kaiser facilities.

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We obtained organisms from pediatric waiting room toys to be observed and identified. We swabbed toys from both Sutter and Kaiser facilities and did numerous tests on them to decipher what kinds of organisms were living on the toys. We got mixed results being that Kaiser had less bacteria growing than Sutter, but the problem that we encountered was that we did not know whether the organisms growing were harmful or not. The one organism that we did identify was *Methylobacterium extorquens* which is found in the soil and is not harmful. We feel that we just scratched the surface on the amount of research that needs to be done to ensure that these waiting rooms are clean and safe for our kids.

INTRODUCTION

It may seem a strange principle to enunciate as the very first requirement in a hospital that it should do the sick no harm. -Florence Nightingale (1859)

Does taking your child to the doctor for illness risk the possibility of even more infection or sickness due to the cleanliness of the waiting room? That is what we want to find out. Due to the fact that each one of us has children, we wanted to do some research to find out which waiting room had a more sterile environment for our children, Kaiser or Sutter? We all have a vested interest that Kaiser would have better sanitation due to the fact that we all take our children there to be treated. Not to mention, both Jeanette and Dayna work for Kaiser. This experiment will help us to identify any problems that Kaiser and Sutter may have in keeping their waiting rooms and toys clean. We will also be able to share our data with these facilities to ensure that our children are being kept safe in a place where they are supposed to be treated to get better not worse.

We believe that both facilities will have normal children's flora growing on their toys. However, we believe that Kaiser will have less infectious or contagious bacteria due to the fact that their toys (which were recently replaced) are flat surfaced and mounted to the walls. They are also cleaned every night by the sanitation crew. Kaiser sanitation spokesperson stated that they wipe down the wall toys each night with a wet cloth and a heavy cleaning solution diluted with water that requires gloves to be worn. We believe that Sutter will have more infectious or contagious bacteria due to the fact that they have legos that can be held by hand and that can be potentially placed in children's mouths. The receptionist for Sutter commented on the cleaning procedures and stated that she wiped down all the countertops

and handles of chairs in the waiting room, but did not know if or when the legos were cleaned by any cleaning staff.

We also wanted to see if the bacteria were more prevalent in the evening after a long day of sick children playing with them. We hypothesize that both facilities will have a cleaner, more sterile environment in the morning compared to the evening since the waiting rooms are cleaned each night.

MATERIALS AND METHODS

1. Method of organism isolation

We are isolating an organism from a wall-mounted toy for children at Kaiser Permanente and an organism from a lego at Sutter Pediatric Clinic. We used sterile cotton swabs and sterile water to obtain the organisms that we found. We went to both Sutter and Kaiser, found their pediatric waiting rooms and attacked the toys in those rooms.

At Sutter we swabbed a lego both at 8 am and then at 4pm.

At Kaiser we swabbed a toy wall unit at both 8:00 am and 6:00 pm.

After swabbing, we added the organisms to the small test tubes and sterile water to bring back to the lab.

Once back at the lab, we obtained a pure culture of isolated colonies by spread plating the Sutter am and pm test tubes and then the Kaiser am and pm test tubes. We dumped the contents of each tube onto four different nutrient agar plates. We then used a curved sterile plastic stirrer to spread each of the plates with the organisms. We then put all four plates in the incubator at 37 degrees C for 24 hours. Within the span of the project, we

isolated pure cultures several times to determine what each organism was and observe colony morphology.

2. Method to determine Colony and Cellular Morphology

As a group, we decided to take one growing organism from the Sutter pediatric room and one organism from the Kaiser pediatric room and do all of the morphology tests on both of them to determine their characteristics. We performed and indirect stain using nigrosin. We performed the gram stain on the organisms to detect gram positive or gram-negative organisms. We used the Dorner method to look for endospores. Next, we did a capsule stain to look for capsules. We then did a KOH test to see if the organisms were KOH positive or negative. We finished the testing with a wet mount to determine motility. We did not do any physiological tests.

3. Method used to isolate chromosomal DNA

After doing the tests, we decided to DNA sequence the organism from the Sutter facility to find out what it was that was on the legos at Sutter. The goal of this lab was to amplify the 16s ribosomal RNA gene. PCR reaction was run on a 1% agarase gel and the 1500 bp product was purified using the QIA quick gel purification kit (Qiangen). UC Davis purified rDNA was sent to the DBS sequencing facility at UC Davis and sequenced with primer Bact-8-F. The sequence of insolated organisms was compared to those in the database at (NCBI) using (BLAST). The primers used to isolate rDNA were bact8F and 1492R.

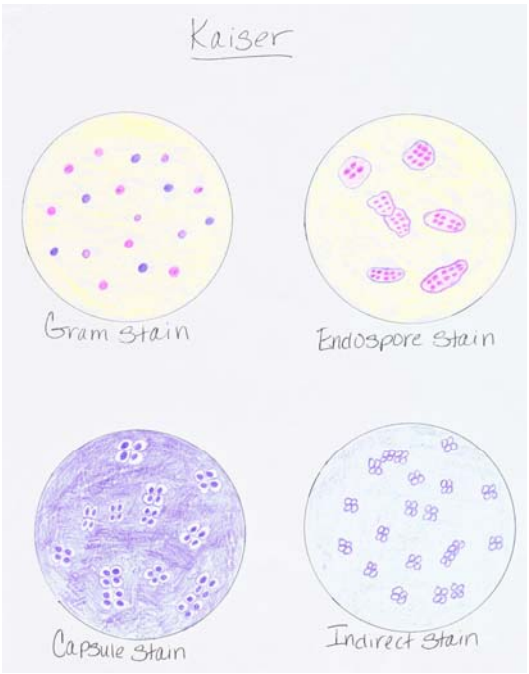
RESULTS

The colony morphology for the **Kaiser organism** on a nutrient agar plate is as follows: circular form, yellow pigmentation, entire margin, convex elevation, dull surface texture, opaque optical character, 1 mm in size and smells like a dirty sweaty locker room. The cellular morphology for the Kaiser organism observed from the indirect slide and wet mount is as follows: cocci shape, tetrads arrangement, 1 length by 1 width. The KOH Test resulted in the organism to not be viscous. The Gram Stain Test showed light pink light purple cells and terminal spores (old organism). The Endospore Stain test produced dark purple cells outside/ pink inside with black dots. The Capsule Stain test produced a white rim around light purple cells and dark purple background.

The colony morphology for the **Sutter organism** on a nutrient agar plate is as follows: punctiform form, pink pigmentation, entire margin, raised elevation, glistening surface texture, translucent optical character, .5mm in size and smells like mushrooms. The cellular morphology observed from the indirect slide and wet mount is as follows: rods shape, clusters arrangement, 4 length by 2 width. The KOH test resulted in the organism to not be viscous. The Gram Stain test produced light pink/ light purple stain with terminal spores (old organism). The Endospore Stain test produced dark purple outside/ pink inside with black dots. The Capsule Stain test showed cells with white rims around light purple cells and a dark purple background.

Our Sutter organism matched the sequence of *Methylobacterium extorquens* with 98% identity. The accession number of organism is AB298401.1 The ratio of identical nucleotides is767/776. The bit Score is 1384 bits (749). The colony morphology for

Methylobacterium extorquens is pink-pigmented bacteria found in the soil and in plants. The cellular morphology for *Methylobacterium extorquens* is gram-negative rods.



DISCUSSION

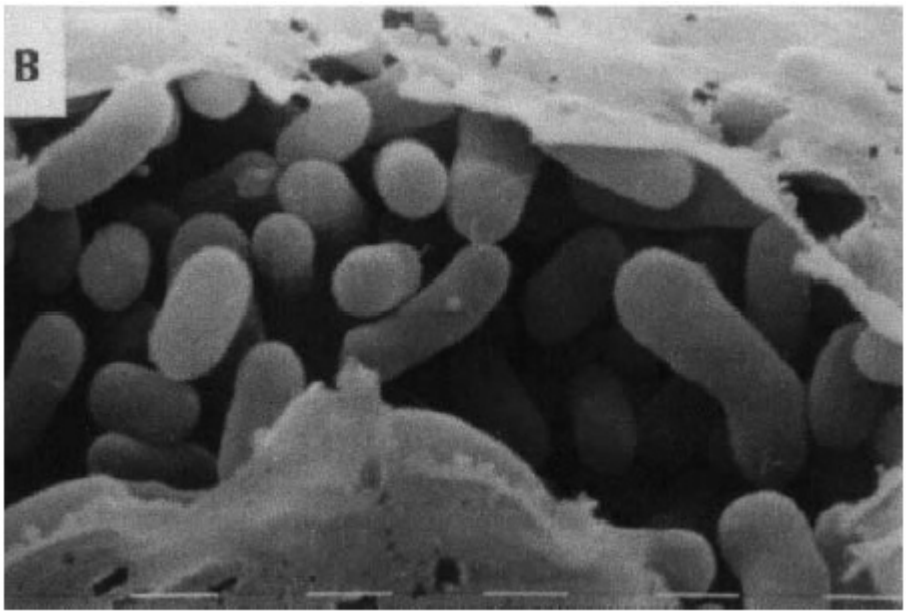
The **Kaiser Organism** tested KOH negative. The organism tested Gram-positive. The organism tested positive for endospores. The organism has a capsule. The organism was not motile.

The **Sutter Organism** tested KOH negative. The organism tested Gram-positive. The organism tested positive for endospores. The organism has capsules. The organism was not motile. The Sutter organism identified post PCR is *Methylobacterium extorquens* an organism found in soil and on leaves and plants.

We believe that our results answered some of our questions and did not answer others. First of all, we wanted to know if Sutter or Kaiser had more bacteria. Sutter did have more bacteria than Kaiser on the plates that we obtained. The problem we encountered is that we did not know all of the kinds of bacteria found whether they were just normal flora or if they were highly pathogenic. We also hypothesized that the morning plates would have fewer bacteria than the evening plates due to children playing with them all day. The results were the exact opposite. The evening plates were covered in bacteria compared to the morning plates.

We came to a roadblock when our organism that came back from UC Davis *Methylobacterium extorquens* was gram negative and all of the research we had done up until that point was a gram-positive stain. This left us wondering where we went wrong. They were both pink pigmented rods, but the main test did not match up. We did another KOH test just to be certain and concluded that although the KOH test came out negative

which makes the organism gram positive, there might not have been enough organism on the slide to produce the desired results. We did not do any other physiological tests due to the fact that we did not know where the mistake occurred and more tests would not help our case. We were also not very successful in finding any information about our organism in the Bergy's manual and resorted to some journal articles and Wikipedia.



Methylobacterium strain (Microbe Wiki 2006)

We found that our data was inconclusive to approach the doctor's offices with any hard evidence that the toys were a hazard to the children and could potentially harm them if played with. Through our journal article research though, we did conclude that our concern about the potential for harm was valid and that it is a hard job to discover a way to make a waiting room kid friendly and welcoming and keep our kids safe from more harm.

ACKNOWLEDGEMENTS

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Vol. 2, No. 1

The Isolation and Identification of an Organism from a One Dollar Bill

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A dollar bill was swabbed and then streaked onto a nutrient agar plate. After several different colonies grew, one was streaked on another nutrient agar plate. Several morphological and physiological tests were preformed to identify the organism. In addition, a polymerase chain reaction was preformed, resulting in a nucleotide sequence. After comparing the nucleotide sequence on BLAST, it was confirmed that the organism isolated was *Micrococcus luteus*, an opportunistic pathogen.

INTRODUCTION

In a society that promotes consumerism, the rate and probability of coming into contact with some form of currency is high. Millions of people come into contact with money on a daily basis, either through the purchasing of products or through the selling of products. Through this daily transaction of buying and selling, the passing of currency from one person to another increases the likelihood of that currency coming into contact with many microorganisms, ultimately spreading to the handler of that currency.

According to Tortora's *Microbiology: an Introduction*, normal flora are permanent inhabitants that normally do not cause disease (Tortora, 2007). Unfortunately, opportunistic pathogens, which are organisms that normally do not cause disease in their normal environment, have the potential to cause disease in different environments (Tortora, 2007).

In two different studies, *Staphylococcus* was found to be the main inhabiting organism. In a study about bacterial flora on money, several different species of *Staphylococcus* was isolated, which are normal flora that have the potential to be pathogenic (Xu, 2005). In a second study on normal flora found on the hand, which could potentially be handling money, *Staphylococcus epidermidis* was found. This particular species has the potential to be pathogenic in people with compromised immune systems (Gunn, 2007).

After reviewing several literatures, the lab group hypothesizes that a dollar bill will contain normal flora or possibly even opportunistic pathogens, most likely a species of *Staphylococcus.* Since each member of the lab group works in a position that main objective is to handle money, such as working as a teller in a bank or as a book keeper for a grocery store, the lab group is interested to see what they are coming into contact with everyday.

MATERIALS AND METHODS

The lab group first took a dollar bill and used a sterile swab, which was dipped into water, to streak the contents on a dollar onto a nutrient agar plate. The plate was then incubated at room temperature for 48 hours. Next, the lab group aseptically streaked one of the isolated colonies that grew onto a different nutrient agar plate by following the streak plate technique (Wilson, 2007, pg. 45-46). The plate was then incubated at room temperature for the next five days.

Following isolation and incubation, the lab group Gram-stained and performed a KOH test on the organism by following the steps in lab syllabus (Wilson, 2007, pg. 62-64). A direct stain was then performed by using the staining agent methylene blue (Wilson, 2007, pg. 54). This was used to determine the organism's cell morphology. Next, the lab group performed a Nigrosin Capsule stain (Wilson, 2007, pg. 77) to verify the cell structure.

In addition to the stains, the lab group extracted some chromosomal DNA in order to determine the genetic sequencing of the organism. This was done by mixing about 2mm of cells in a 1.5 ml tube containing sterile glass beads and 500µl Tris buffer using a 100-1000µl pipette. The tube was then boiled and beaten (Wilson, 2007, pg. 193-194). Next, a Polymerase Chain Reaction (PCR) was setup by using primer bacteria 8-forward (Wilson, 2007, pg. 194). This was done in order to amplify 16S Ribosomal DNA. The instructor, Sasha Warren, performed the PCR reaction. A gel electrophoresis was then run on a 1% agarose gel. The 1500 bp product was purified using the QIA quick gel purification kit (QIAGEN). The purified PCR product was then sent to the Division of Biological Science Sequencing Facility at University of California, Davis where the dideoxy chain termination method of sequencing was performed. This was done to determine the unknown organism's sequence of nucleotides.

After the results were returned, the lab group used the Four Peaks program to analyze the electropherogram results. The sequence was then compared to the database sequences at the National Center for Biotechnology Information (NCBI) and Basic Local Alignment Search Tool (BLAST). Following the identification of the organism, more tests were done to verify the organism's identity.

After finding out the results for our nucleotide sequencing, the lab group preformed a catalase test in order to distinguish between several different types of gram positive cocci (Wilson, 2007, pg 176). Next the lab group preformed a Voges Proskauer test to determine whether the organism was *Staphylococcus* or *Micrococcus* (Wilson, 2007, pg 173-174).

RESULTS

After swabbing the dollar bill and streaking a nutrient agar plate, several different colonies appeared when the plate was incubated at room temperature for 48 hours. The lab group chose a single well isolated colony to be streaked onto another nutrient agar plate. The colony was irregular, had an undulate margin and raised elevation. In addition, it was yellow, shiny, opaque and 8x10mm as shown in Table 1. After performing and viewing a direct stain, it showed that the cells were 1mm in diameter. The cells were round (cocci) and were arranged in clusters (staphylo) and in groups of four (tetrads) as shown in Table 2.

The cells appeared purple during the gram stain. In addition, no slime was observed when the KOH test was performed and the capsule stain showed a purple cell against a purple background as shown in Table 2. The catalase test showed bubbles and the Voges Proskauer resulted with a yellow liquid.

The results of the BLAST indicated that the sequence matched the sequence of *Micrococcus luteus* with 98% identity. The identical nucleotides had a ratio of 763/772 and a bit score of 753. The organism's accession number was DQ855476.1.

form	irregular
margin	undulate
elevation	raised
optical character	opaque
pigmentation	yellow
size (mm)	8x10
surface texture	shiny

Table 1. Colony Morphology

shape	Cocci
size (mm)	1x1
arrangement	Staphylo/ tetrad
KOH	No slime (-)
Gram Stain	Purple (+)
Capsule Stain	Purple cell/ background

Table 2. Cellular Morphology and Stains

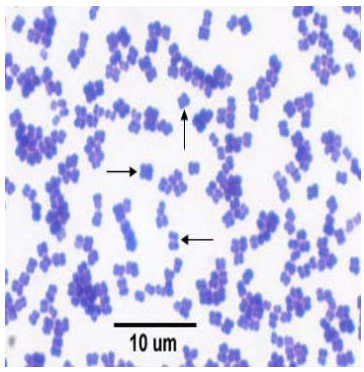
DISCUSSION

The results above indicate that the organism is a Gram-positive organism based on the purple Gram-stain results. The KOH shows that the organism is KOH negative due to the lack of slime. The capsule stain shows that the organism lacks a capsule since there was no ring or halo surrounding the cell, simply purple on purple.

The BLAST results showed that the organism isolated is *Micrococcus luteus.* This organism is found in soil, dust, water, and air. This is normal flora on mammalian skin, which would be easy to transfer from skin to a dollar bill (Kocur, pg. 1015). This can also be an opportunistic pathogen in immuno-suppressed individuals causing septic shock and pneumonia. It is often confused with *Staphylococcus aureus*, which also has groupings of spherical yellow colonies and is gram positive (Kocur, pg. 1015).

The catalase test showed bubbles which indicates that the organism uses catalase. The Voges Proskauer had a yellow (negative) result, which indicates that the organism is not a butanediol fermenter. This agrees with the results from BLAST that the organism is *Micrococcus* not *Staphylococcus* because *Staphylococcus* would have had a red colored (positive) result for the Voges Proskauer test. This also matches the description of the organism in the Bergey's Manual.

Nutrient Agar Plate



Direct Stain

ACKNOWLEDGEMENT

We would like to thank the website Microbelibrary.org for providing the nutrient agar plate picture as shown in the results section.

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Vol. 2, No. 1

Isolation and Identification of *Kocuria rhizophila* From a Table at Applebee's

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When we dine out we expect only to receive what we order. What we don't know is that the table could be home to many types of organisms. In this experiment, we took a sample of the organisms living on a table at an Applebee's restaurant. We grew the sample on nutrient agar, then isolated and identified it as *Kocuria rhizophila*. After isolation, we 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 National Center for Biotechnology Information (NCBI) and Basic Local Alignment Search Tool (BLAST) websites, we found that our isolated organism's nucleotide sequence was 99% identical to that of *Kocuria rhizophila*. To confirm the identity, we observed colony morphology on nutrient agar, performed an Indirect Stain, Gram Stain, Endospore Stain, Acid Fast Stain, Capsule Stain, a KOH test, a wet mount, and a Catalase test. After determining that our isolate was a gram-positive, non-acid-fast, non-endospore forming, non-encapsulated organism that has the same colony morphology as *Kocuria rhizophila*, we concluded this is the organism we isolated from the table at Applebee's.

INTRODUCTION

We have often wondered what all we are being exposed to in restaurants. What kinds of pathogens and bacteria are present on food and commonly-contacted items such as restaurant tables? Some bacteria may be safe or even healthy, but some may be harmful. That harm could make humans ill, and for those humans most at risk, such as the very young, elderly, or those with compromised immune systems it could prove fatal.

Restaurants harbor many potentially harmful organisms. *Salmonella* may infect mayonnaise or salad dressing (Hasegawa, et al., 2003). *Hepatitis A* may be on your dinnerplate (Unicomb, et al., 2007). Food poisoning may be caused by *Clostridium* or *Bacillus* (Tortura, et al., 2007).

We expect to isolate a bacterial organism from the table. Although the table should have been disinfected, it is likely the method of disinfection is not adequate to protect the public's health. Considering media reports of infection of the public dining at restaurants, we expect to find a coliform.

MATERIALS AND METHODS

To collect a sample of the organisms living on the table at Applebee's, we went to the restaurant and swabbed the table. We used a sterile cotton swab, dipped it in sterile water, and swiped it across the table. We then dipped the contaminated swab into a small tube containing 500 micro liters of sterile water to capture any organisms. Next we poured the contaminated water on a plate of nutrient agar and used the spread plate technique to maximize our potential of growing any bacteria we may have captured. We then allowed the organisms to grow on the plate at room temperature (about 21ºC) for 48 hours. Next, we selected a colony to isolate. We isolated this organism onto a sterile plate of nutrient agar and let it grow at room temperature (about 21ºC) for 48 hours.

In order to apply nucleic acid analysis to the identification of our organism we used Polymerase Chain Reaction (PCR). To perform the PCR we obtained a pure culture from our nutrient agar plate. Since our organism is Gram-positive, we added glass beads to our tube with our organism, in order to 'beat it up' and smash the cell wall. The tube was vortexed for 10 minutes following a 10 minute boiling period. The boiled and beaten cell material was then used as our template DNA and we amplified the 16S ribosomal DNA with primers Bacteria 8 Forward and 1492 Reverse. Our PCR product (sample of amplified DNA) was run on a 1% agarose gel, the band cut and DNA purified. The 1500 bp product was purified using the QIA Quick Gel Purification Kit from Quiagen.

Following PCR, we sent the purified DNA to the Division of Biological Sciences DNA Sequencing Facility at the University of California in Davis. The sequencing facility at Davis sequenced our product with Bacteria 8 Forward and 1492 Reverse primers to yield one sequence, which was compared to database sequences at National Center for Biotechnology Information (NCBI) and Basic Local Alignment Search Tool (BLAST). The sequence identification information was then sent to us. After using the National Center for Biotechnology Information (NCBI) and Basic Local Alignment Search Tool (BLAST) websites, we found that our isolated organism's nucleotide sequence was 99% identical to that of *Kocuria rhizophila*. To confirm the identity, we observed colony morphology on nutrient agar, performed an Indirect Stain, Gram Stain, Endospore Stain, Acid Fast Stain, Capsule Stain, a KOH test, a wet mount, and a Catalase test.

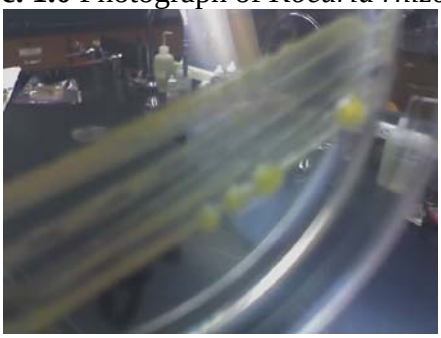
RESULTS

We observed our nutrient agar plate had only one colony type growing. The colony morphology of our isolate is of circular form, entire margin, raised elevation, shiny surface texture, opaque optical character, yellow pigment, 1-3mm, and odiferous (Table 1.0) (Figure 1.0)

Table 1.0

Form	Margin	Elevation	Surface Texture	Optical Character	Pigmentation	Size	Other
Circular	Entire	Raised	Shiny	Opaque	Yellow	1-3mm	Odiferous

Figure. 1.0 Photograph of *Kocuria rhizophila*



The white background and dark purple staphylococci observed in the Gram Stain indicated our organism is Gram-positive (Table 2.0). The Malachite Green Endospore Stain revealed pink staphylococci telling us our organism is not an endospore former (Table 2.0). The Acid Fast Stain revealed blue staphylococci, indicative of non-acid-fast cells (Table 2.0). The Capsule Stain results were a purple-gray background with staphylococci and no capsules (Table 2.0). The KOH test resulted in no viscosity hence the organism is KOH - (Table 2.0). Bubbling occurred during the Catalase test, which tells us our organism uses catalase to break down hydrogen peroxide (Table 2.0). The wet mount indicated no motiliy. After determining that our isolate was a gram-positive, non-acid-fast, non-endospore forming, non-encapsulated organism that has the same colony morphology as *Kocuria rhizophila*, we concluded this is the organism we isolated from the table at Applebee's.

After isolation, we 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 National Center for Biotechnology Information (NCBI) and Basic Local Alignment Search Tool (BLAST) websites, we found that our isolated organism's nucleotide sequence was 99% identical to that of *Kocuria rhizophila*, with 755 out of 761 nucleotides from our PCR having matched *Kocuria rhizophila*, indicating our organism is 99% identical to *Kocuria rhizophila*, Accession # AFO19905 (see reclassification note in discussion below).

Table 2.0

Test	Indirect Stain	Gram Stain	Endospore Stain	Acid-Fast Stain	Capsule Stain	KOH Test	Catalase Test
Result	White cells, dark gray back-ground	White back-ground, dark purple staphyl-ococci	Pink staphyl-ococci	Blue staphyl-ococci	Purple-gray back-ground, white staphylococci 1 mm round	No snot	bubbled
Conclusion		Gram+	Non-endospore former	Non-acid-fast	No capsules	KOH-	Organism has/uses catalase

DISCUSSION

An interesting fact about our organism is that in addition to BLAST matching 6,128,933 sequences out of 22,272,962,094 letters, our organism was recently reclassified from *Micrococcus luteus* to *Kocuria rhizophila* (Tang, Gillevet., 2003).

The identification of our organism is surprising, as we expected to find a coliform which could pose harm to the public. Our organism is "Not pathogenic to plants and animals. Common in soil, dust, water, skin of man and other animals" (Bergey's Manual of Determinative Bacteriology, circa 1974). So while the dining experience included something we didn't order as part of our meal, perhaps not all the hidden 'extras' on our table are harmful, or even going to send us to the hospital. So go ahead, enjoy dining out….but don't lick the table…just in case. ☺

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Vol. 2, No. 1

Got Milk? Isolation and Identification of *Pseudomonas fluorescens* from Lactaid

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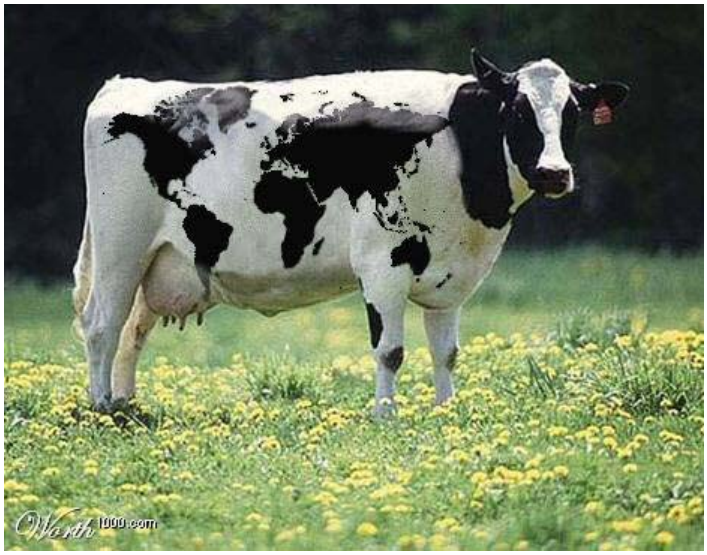
Milk is a staple in most American homes and the American people trust that it is safe to drink before the sale date. It is understood that drinking milk after the sale date is unsafe, but have you ever wondered what is lurking in your milk before the sale date. By isolating microbial growth from various kinds of milk, including; 2% whole milk, organic milk and Lactaid, we were able to answer this question. By performing laboratory tests we were able to isolate and identify the microorganism *Pseudomonas fluorescens* (Tortora, Funke, Case, 2007). *Pseudomonas fluorescens* is known to form a pellicle in liquid broth which it is able to pull nutrients from and is an aerobic respirator, which makes milk the perfect media for it flourish.



INTRODUCTION

GOT MILK? This is a slogan that almost all people in the United States of America have grown accustomed to. People have been told throughout their lifetime the healthy benefits of consuming milk and milk products. It is safe to say that Milk is one of the many substances people use quite often, whether it be consumed as liquid or used in other foods as an ingredient, in fact according to the USDA, in North American alone, 34,589,000 metric tons of milk were consumed in 2006. Today Milk has been recognized as a safe product to drink when consumed before the sale date however this has not always been the case. Before the 1900's diseases such as tuberculosis, diphtheria, polio, salmonella, strep throat, scarlet fever, and typhoid fever were proved to be caused by consuming raw milk. Since

then we have dramatically decreased the risk of these diseases by using pasteurization to reduce the number of viable microorganisms inhabiting milk. Since the 1900's medical and food technology have been on the rise. However, food technology has led to increased risk of chronic diseases such as heart diseases, cancer, and stroke. This has led to an increased awareness on healthy lifestyle and healthy eating. Many people around the country have been going "natural" and eating foods with less additives and chemicals, otherwise known as "organic" foods. Medical technology has been able to develop food products for those unfortunate enough to be Lactose Intolerant by using microbes from yeast and fungi to breakdown lactose before it gets to the digestive tract. How safe is Milk, and what are you really drinking? Most people consume milk on an everyday basis, whether it is organic, regular or Lactaid. For our project we decided to isolate organisms from 2% regular milk, 2% Lactaid, and 2% organic milk to identify which one had the most and least bacteria, and if any have the ability to cause harm. We expect to find microorganisms in all three types of the above mentioned milk, and we hypothesize that Lactaid will have more growth due to the fact that it has already been broken down my microorganisms.

MATERIALS AND METHODS

Our first step was to isolate the organisms from milk. Because we did know the concentration of microorganisms in our milk samples, we decided with the help of our instructor to dilute our samples of milk. Our dilution calculations were as follows: We started with 100 micro liters of milk (Lactaid, regular and organic) and diluted them down into 2 tubes that contained 999 micro liters and 1 tube that contained 900 micro liters. We pipetted 1 micro liter from the original sample into the first tube that contained 999 micro liters, from this tube we then pipetted 1 micro liter into the 2^{nd} tube that contained 999 micro liters, and from this tube we pipetted 100 micro liters into the 3^{rd} tube that contained 900 micro liters. From each tube (1, 2 and 3) we pipetted 100 micro liters onto 3 plates of Nutrient Agar with the total dilutions of 10^{-4}, 10^{-7}, and 10^{-8}. We also plated 100 micro liters of undiluted milk onto nutrient agar. Plates incubated 48 hours at 37 degrees Celsius.

After incubation, plates with growth (undiluted plates) were replica plated on Eosin Methylene Blue for 48 hours at 37 degrees Celsius.

After replica plating, colony from Lactaid plate streaked to Tryptic Soy Agar, and Mueller Hinton, incubated at 37 degrees for 48 hours.

Performed gram stain and KOH test on colonies grown from TSA, isolated chromosomal DNA by boiling it, which caused DNA to blow up. Polymerase Chain Reaction was run to amplify 16S rDNA using primers Bacterial 8-forward and 1492-reverse. PCR reactions run on a 1% agarose gel and the 1500 base pair product was purified using the QIAquick PCR purification Kit. Purified rDNA was sent to the D.B.S. sequencing facility at UC Davis and sequenced with primer Bacterial 8-forward. The sequence from the isolated organism was compared with those in the database at the National Center for Biotechnology Information with the Basic Local Alignment Search Tool.

Performed carbohydrate deep tests using Mannitol, Arabinose, Sucrose, Inositol, Rhamnose and Sorbitol. (Wilson, Harriet and Warren, Sasha, 2007).

RESULTS

Results from Serial Dilution show there was only growth on the plates that were undiluted. No growth on diluted plates. Organic Milk had 105 colonies, CFU/mL is 1.05 X 10^2. Lactaid had 40 colonies, CFU/mL is 4.0 X 10^2. Regular Milk had 3 colonies, CFU/mL is 3.0 X 10^2.

Results from the Eosin Methylene Blue plates were the same for all 3 samples of milk. The organisms grew, agar was red and colonies were clear.

Colony morphology taken from TSA plate with pure culture from undiluted Lactaid. Organism is circular with entire margin, convex, yellow, shiny and opaque, had colonies in size from .5-1 mm and has no smell. Gram stain is red,), KOH test produces no snot.

Sequencing of rDNA from UCD shows that our sequence from the organism isolated from Lactaid matched the sequence of *Pseudomonas fluorescens* with 98% identity. The accession number of the organism is DQ095904.1 and the ratio on identical nucleotides of 760 / 775.

Carbohydrate deep results show yellow tubes in the deeps of Mannitol, Arabinose, Sucrose, Inositol, Sorbitol and not yellow in Rhamnose. (Wilson, Harriet and Warren, Sasha, 2007).

DISCUSSION

Our results showed that the original samples of milk could not have contained very many microorganisms, because of the lack of growth on the diluted plates.

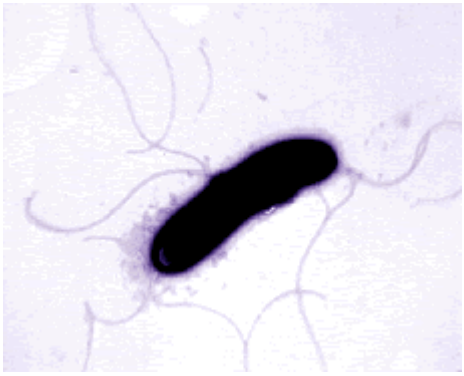
The results of the Eosin Methylene Blue showed that our organism was gram negative and does not ferment lactose.

The results of the TSA plate confirmed our suspicion that Lactaid milk contains microorganisms, even though it has been pre digested. This was shown by the growth of 40 colonies on the plate. Our Gram stain was red which further confirmed that our organism was gram negative. The KOH test produced no snot, which means it is KOH negative.

The results of the carbohydrate deeps were positive for Mannitol, Arabinose, Sucrose, Inositol, and Sorbitol, but not for Rhamnose. (Wilson, Harriet and Warren, Sasha, 2007).

The sample that we sent to UCD to be sequenced was determined to be *Psuedomonas fluorescens*. This bacteria is known to be found in soil and water. It is associated with spoiled eggs, meat, fish and milk. It grows aerobically on carbon enriched media. (Tortora, Funke, Case, 2007).

Our hypothesis was correct in the fact that microorganisms are growing in milk, but was incorrect because the highest concentration of microbial growth was not found in Lactaid. From our results we can assume that *Pseudomonas fluorescens* is a causative agent in the spoilage of Lactaid. It makes sense that *Pseudomonas fluorescens* would be found in Lactaid, because it provides a carbon source and the oxygen aided environment it needs for growth. (Kiendl, Paul, 2006).



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Vol. 2, No. 1

Comparison of the numbers of organisms on different sources of uncooked ground beef.

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Samples of ground beef were obtained from two different sources, and a viable cell count was done to determine the numbers of organisms present in each sample. An organism was isolated from one of the samples and identified using nucleotide sequencing of rDNA. The sequence was compared to those in the database at National Center for Biotechnology Information, and the results indicated a 99% match to *Carnobacterium divergens* and *Carnobacterium maltaromaticum*. The potential pathogenicity of various foodborne organisms was investigated.

INTRODUCTION

Foodborne diseases are a common and costly problem in the United States, and throughout the world. The CDC estimates 76 million people suffer from foodborne illnesses each year in the United States, and of these more than 500 people die. (NAID, 2007).

In addition to the costs in human suffering, foodborne illnesses also exact a staggering financial cost. The National Institute of Allergy and Infectious Diseases reports, "the yearly cost of all foodborne diseases in this country is 5 to 6 billion dollars in direct medical expenses and lost productivity" (NAID, 2007).

There are over 250 different infectious diseases that are spread through our food and water. Some of the most common types are *Campylobacter, Salmonella, Escherichia coli* 0157:H7, *Shigella*, and Hepatitis A. The American College of Gastroenterology identifies raw meat as one of the types of food most likely to carry illnesses (Butler, Martin, 2006).

Many government agencies are concerned with the problems of food safety. In 1994, the USDA instituted a program to detect *E.coli* 0157:H7 in raw ground beef with the goal of reducing the presence of this pathogen (USDA, 2007). The CDC has issued guidelines to help reduce the risk of developing a foodborne disease. These guidelines advise the way meat products are handled can affect the incidence of pathogenic organisms found on meat products.

This study will test the following hypothesis:

The following variables can affect the numbers of organisms found in meat; hand washing practices of the meat handlers, refrigeration and storage variances, work area sanitizing procedures, and the ways different cuts or mixes of meat are processed. Because the numbers of organisms can be affected by so many different variables, the numbers of organisms found on different sources of uncooked ground beef will vary.

MATERIALS AND METHODS

Source of meat samples:

Each of the two samples used consisted of ground beef; 93% lean, 7% fat. The purchase date for both was September 30, 2007. Sample A was purchased at Safeway on Bell Road in Auburn, California. The "sell by" date on the package was October 01, 2007. Sample B was purchased at Bel Air Supermarket on Grass Valley Highway in Auburn, California. The "sell by" date on the package was October 02, 2007.

Determination of microbial numbers:

A viable cell count from each sample was obtained by making a suspension of 1g meat in 1mL distilled water. A serial dilution (Wilson, Warren, 2007, pg. 151-152) was performed on each suspension. The media used was TSA. The plates were incubated at 37^{o}C for 24 hours.

Organism Isolation:

An organism was isolated from a serial dilution plate using the streak plate method (Wilson, Warren, 2007, pg. 47-49). The medium used was TSA. The plate was incubated at 37^{o}C for 48 hours.

DNA Isolation:

Chromosomal DNA was isolated from the organism using the "boil and beat" method (Wilson, Warren, 2007, pg. 193-194). A PCR reaction (ibid pg. 191-194) was run on a 1% agarose gel, and the 1500 bp product was purified using the QIA Quick Gel Purification Kit (Qiagen). The purified rDNA was sent to the DBS sequencing facility at UC Davis and sequenced with primer Bacteria 8-Forward. The sequence of the isolated organism was compared to those in the database at National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST).

Stains and tests:

The organism was subjected to the following stains and tests: KOH test (Wilson, Warren, 2007, pg. 64), Gram stain (ibid pg.61-63), Acid-Fast stain (ibid pg.69-70), Nigrosin capsule stain (ibid pg. 77). The Gram Stain, Acid-Fast stain and Nigrosin capsule stain were viewed with a light microscope using the oil immersion objective with 1000x total magnification. A wet mount was prepared by aseptically transferring a loopful of cells onto a slide with a drop of distilled water. The mixture was covered with a cover slip and viewed with a light microscope at 450X total magnification.

RESULTS

Viable cell count:

The viable cell count for sample A was 2.81 x 10^{9} CFU/mL. The viable cell count of sample B was 4.0 x 10^{7} CFU/mL.

Trial 1:

Sample A (Safeway)	Plate 1: (10^{-9})	Plate 2: (10^{-7})	Plate 3: (10^{-4})
Number of Colonies	5	281	0
Sample B (Bel-Air)			
Number of Colonies	0	4	TNTC

Trial 2:

Sample A (Safeway)	Plate 1: (10^{-3})	Plate 2: (10^{-1})	Plate 3: (undiluted)
Number of Colonies	TNTC	TNTC	TNTC
Sample B (Bel-Air)			
Number of Colonies	TNTC	TNTC	TNTC

Organism Isolation:

The isolated organism viewed on a TSA plate resulted in circular colonies with an entire margin. The pigmentation of the colonies was beige with an opaque optical character. The colonies were raised, with a shiny surface texture and measured between one and three millimeters in size.

DNA sequencing:

The rDNA sequence matched the sequence of *Carnobacterium divergens* with 99% identity. The accession number is AM179875.1. The ratio of identical nucleotides is 767/776. The bit score is 1380 bits.

The rDNA sequence also matched the sequence of *Carnobacterium maltaromaticum* with 99% identity. The accession number is AY543035.1. The ratio of identical nucleotides is 745/786. The bit score is 1236 bits.

Stains and Tests:

A non-viscous solution was obtained with the KOH test. The Gram stain revealed purple cells in the shape of rods and coccobacilli arranged in chains and single cells. The cells measured .5 *u*L width and .5-1 *u*L length. The Acid-Fast Stain resulted in cells stained blue. The cells observed on a wet mount slide did not demonstrate motility. The capsule stain showed the presence of capsules. Endospores were not present.

DISCUSSION

A comparison of the numbers of organisms using the serial dilution method showed Sample A contained a considerably higher CFU/mL in the original culture, 2.8×10^9 CFU/mL, as opposed to Sample B which contained 4.0×10^7 CFU/mL. This result supports my hypothesis that the number of organisms found in various sources of uncooked ground beef will vary.

The serial dilution plates each had from one to four different types of colonies. A colony was randomly selected from one of the plates and isolated. The isolated organism is

KOH negative, Gram positive rods and cocobacilli. The organism is Acid-Fast negative, produces no endospores, and has a capsule.

The rDNA sequence matched with 99% identity both *Carnobacterum divergens* and *Carnobacterium maltaromaticum*. *Carnobacterium divergens* is listed in *Bergey's Manual Of Systematic Bacteriology* with the older name, *Lactobacillus divergens*. No listing was found in *Bergey's Manual* for *Carnobacterium maltaromaticum*.

Carnobacterium are normal flora in meat products. At this time it isn't known "whether differences in the presence of *Carnobacterium* in meat are due to variations in storage conditions or variations in contamination levels at the processing plants" (Leisner, Laursen, Prevost, Drider, Dalgaard, 2007).

Several *Carnobacterial* bacteriocins are known, and, "C. *divergens* and *C. maltaromaticum* have been extensively studied as protective cultures in order to inhibit growth of *Listeria* in fish and meat products" (Leisner et al., 2007).

Although *Carnobacterium* are not known to be pathogenic to humans, there are many other organisms found in ground beef which can pose serious health risks. In order to prevent risk of infection, safe food handling procedures as outlined by the FDA at www.FoodSafety.gov should always be followed when working with raw meat products.

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Vo1. 2, No. 1

Isolation and Identification of *Bacillus subtilis* from Chinese Douchi

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Douchi, also known as the Traditional Chinese fermented black beans, it is the most popular ingredient in Chinese cuisine, and has been used to make black bean sauce for more than 5,000 years. In this experiment, I isolated a sample of douchi and grew them on a nutrient agar plate and identified for isolated colonies. Immediately after the isolation, I used Polymerase Chain Reaction (PCR) and sent the purified DNA sample to the Division of Biological Sciences Sequencing Facility at the University of California in Davis. After obtaining my sequencing results from Davis, I used the Basic Local Alignment Search Tool (BLAST) to compare my unknown sequence to a known sequence on the Blast database. I found that my organism *Bacillus subtilis* was 99% identical to the *Bacillus subtilis* nucleotide sequence. To ensure my results were accurate, I performed a series of both morphological and physiological tests, and confirmed my results with the use of Bergey's Manual of Systematic Bacteriology.

INTRODUCTION

Fermented foods are made by the fermentation of microbes that involves the anaerobic decomposition of organic compound in to acids; the end product may or may not contain microbes (Wilson 135-37). Douchi are halophiles made by fermenting and salting soybeans. The process turns the beans black, soft, and dry. Fermented black beans are strictly used for cooking, never consume by itself. The flavor is salty, spicy, and pungent in smell. Based on the salt tolerance of Douchi I made the hypothesis that my unknown organism might be *Staphylococcus aureus* due halotolerant as well. To test my hypothesis, my family ownes a Chinese Restaurant, so, I had the opportunity to investigate if indead Douchi contain microbes, if so is the organism harmful or cause any side effects to human body.

MATERIALS AND METHODS

In order to collect a sample of the organisms in douchi, I collected some dried douchi from the restaurant and immersed them in sterile water for ten minutes; by doing so I could ensure that all the organisms growing in douchi will be mixed in the solution. Then, I poured the entire contents of the douchi solution (without the black beans) onto an agar plate, then incubated them at 37°C. Next, I chose a colony with a powdery and white colored appearance and re-streaked them again for isolated colonies on to another agar plate.

After I recorded all the organism's colony morphology, I performed various morphological and physiological tests such as Gram Stain, KOH test, Acid- Fast, Endospore Stain, Capule Stain, Flagellar Stain, and Catalase test. From there, I performed a chromosomal extraction from the sample. I transferred a loopful of cells from the agar into a microfuge tube containing buffer solution. Next, I vortexed the mixture until it was evenly blended, and then added glass beads to crush the gram positive cell walls. Afterwards, I placed the microfuge tube with the mixture into the float and allowed it to boil for ten minutes.

After I successfully obtained the template DNA, I used PCR to amplify DNA 16S ribosome using the primer Bacteria 8 Forward and 1492-Revers. Next, 16S ribosomal DNA (rDNA) was isolated using Gel Electrophoresis and the Qiagen Qiaquick PCR purification kit. The purified DNA was then sent to the Division of Biological Sciences Sequencing Facility at UC Davis. I then analyzed the sequence data that was given to me into eletropherograms with 4 Peaks software and compared my nucleotide sequence with those on a database in BLAST.

RESULTS

The colony morphology that I've observed for isolated colonies are of irregular form, serrated margin, flat, dull surface texture, opaque optical character, milky pigment, about 5-8 mm in length, and it smelled really bad, worse than *Serratia*.

Cellular Morphology

Shape:	Size:	Arrangement	Motility
Rods	Length: 3-5μm Width: 1-1.5μm	Streptobaccili	Yes Run & Tumble

Stain & KOH Test Morphology

Gram Stain	KOH Test	Acid-Fast Stain	Endospore Stain	Capsule Stain	Catalase Test
Purple cells	No Viscosity	Red cells	Green cells & central endospores	No white outer membrane	Bubbles observed

For the Gram Stain, I observed purple rods arranged in chains. Next, the KOH test showed me negative results, no viscosity. I saw only red cells in my Acid-Fast Stain, but I did see green terminal endospores for the Endospore Stain. Next, my capsule stain showed no white outer membrane, just the purple cell itself. Following that, my catalase test did form bubbles.

From BLAST, I've discovered the ratio of identical nucleotide of my unknown was 776 out 778, which matched the *Bacillus subtilis* on the database, and 773 out of 776 matched to *Bacillus amyloliquefaciens* (NCBI, 2007)

DISCUSSION

Based on my experimental results, I have learned that my hypothesis was incorrect. I predicted my unknown organism to be *Staphylococcus aureus*, but it violated my Gram Stain results. My Gram Stain showed me that the morphology for my unknown organism was bacilli and the arrangement was in chains; but based on morphological unknown experiments in our earlier exercises 3-6A, "S*taphylococcus aureus* are supposed to be arranged in bunches of cocci. *Staphylococcus aureus* can cause a wide range of illnesses from minor skin infections, such as pimples, impetigo, to life-threatening diseases, such as pneumonia, meningitis, osteomyelitis endocarditis, Toxic shock syndrome, and septicemia" ("*Staphylococcus aureus*"). Contrarily, "*Bacillus subtilis* is not considered a human pathogen; it may contaminate food but rarely causes food poisoning" ("*Bacillus subtilis*").

Based on my morphological tests results I concluded that my organism was gram positive, KOH negative, acid fast negative, and Endospore positive because the green central spores that I observed on the ends of red cells. The tests provided negative results in my Capsule Stain, but the organism did have catalase due to the bubbles that I observed, which enables the organism to convert hydrogen peroxide (H_2O_2) to water and oxygen.

The BLAST showed me there were two organisms that matched my unknown organism's nucleotide: *Bacillus subtilis* and another strain, *Bacillus amyloliquefaciens*. My results showed that my unknown was *Bacillus subtilis* based on the Bergy's Manual, which described *Bacillus subtilis* colonies on agar media as round or irregular; dull surface; become thick and opaque, may be wrinkled and may become cream colored or brown. The spore is central thick but not swollen (Buchanan 531-34). To my surprise every test that bacillus tested positive and negative for was similar for *Bacillus amyloliquefaciens*; Both of the species are found in soil and strong endospore formers. To further my understanding, I did some further research on *B. amyloliquefaciens* and found out that *Bacillus amyloliquefaciens* are species of bacteria that are the source of the BamH1 restriction enzyme. Furthermore, *Bacillus amyloliquefaciens* is known for its ability to degrade proteins (Bauman). In conclusion, I identified my unknown sample organism as *Bacilus subtilis*, although it was very similar to species *B. amyloliquefaciens*, however, their functions are different.

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Isolation and Identification of a Gram-Negative Aquatic Bacterium From Raw Fish Marinated in Citric Acid for Preparation of Ceviche

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This paper discusses the presence of microorganisms on Yellowtail fish that has been marinated in lemon juice. The inspiration for this topic came from the Hispanic dish ceviche, which has become increasingly popular in the U.S. and is consumed by people of all nationalities. The consumption of fish that is prepared without being cooked however brings to surface many concerns regarding bacteria and even parasites. While lemon juice has shown to have an anti-bacterial effect on raw fish, the assumption by many that it cooks it is not true. Rather, the proteins of the fish are denatured by the lemon juice (Hiroyuki, Tetsuro, Masayuki, 2006). This paper compares colony growth of yellow-fin tuna before and after it has been marinated in lemon juice and reveals a significant decrease in colony growth after exposure to the marinade. An attempt to isolate and identify one of the remaining colonies through a series of agar tests leads to the conclusion that the isolated colony is the non-pathogenic species, *Chryseobacterium scopthalmum.*

INTRODUCTION

This project will examine and document potential bacterial growth, which could possibly be found in our sample of raw Yellowtail fish caught in the Gulf of Mexico by Mrs. Valenzuela's husband. The fish was caught in August of 2007, and then frozen in a home freezer until thawed in October 2007 for this experiment. The yellowtail fish will be marinated in lemon juice, and observed for any bacterial growth, which could prove harmful to humans who ingest the fish. Attention will be focused primarily on those microorganisms that will survive the freezing process.

The marinating of raw fish in citric juices and chilies is a key step in the preparation of the dish, "Ceviche". This dish, which has been a staple in many Hispanic countries for generations, has found a renewed interest in this country. With the newfound interest in this dish, it is important that the consumer be made aware of the potential for gastrointestinal upset, and even skin diseases associated with raw fish and seawater contact (1).

New York City is currently conducting an investigation into this popular dish, and the possibility of *Anisakis simplex* contamination. The catalyst for New York City's investigation was an announcement by the Centers for Disease Control in Atlanta in which they reported four cases of infection by the fish tapeworm *Diphyllobothrium latum*, traced to eating raw salmon (2). *Metagonimus yokogawai* and *Heterophyes heterophyes*, both of which are small flukes ingested by humans after eating raw fish have been of particular concern to microbiologists (3). Although many cases of parasites have been found to be a real danger in the consumption of both raw fish and shellfish, this experiment will concentrate on bacterial growth alone.

This project will discuss research done, in which raw fish marinated in citric juices was tested for antibacterial activity against seven strains of *Vibrio* species (4). Testing will be done for *Vibrio vulnicicus* in particular, as this is a halophilic organism that lives in warm seawater (exactly where the fish in question was caught). The bacteria *Vibrio parahaemolyticus* among others, and its tolerance for sub-zero temperatures will be discussed as well, as the fish in question was frozen after being caught.

It is widely believed that in the preparation of Ceviche, the citric acid marinade "cooks" the raw fish without heat, and in so doing, eliminates any bacteria or parasites, which would normally be present in the fish. The consideration is that the citric acid, in fact denatures the protein molecules found in the raw fish through the marinade. The hypothesis for this project is that there will be a significant decrease in microorganisms after the fish has been marinated in the citric acid. This experiment will demonstrate that in denaturing the protein molecules through citric acid marinade potential pathogens are eliminated as well.

MATERIALS AND METHODS

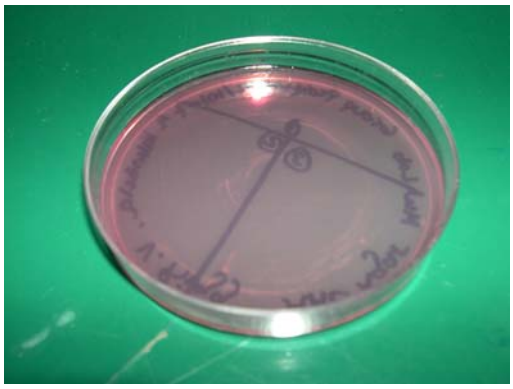
The first step in identifying the growth of microorganisms on the fish was to perform two individual streak plates using nutrient agar. Plate number 1 on the nutrient agar was the raw fish, and plate number 2 was the marinated fish (see tables, plate 1 and 2). A loop was "stabbed" into the two samples of fish, using aseptic technique (Wilson, 2006 p. 43). Following the observation of growth on these plates, colony #2 on plate 2 was selected to perform a pure culture (Wilson, 2006 p. 47). Utilizing the pure culture, a KOH test was then performed (Wilson, 2006 p.64). Retrieval of DNA from the pure culture was performed using the following methods: 1) 10mM Tris buffer. 2): Amplification of 16S ribosomal DNA using the PCR and Taq Master Mix (QIA quick) containing the primers Bacteria 8-Forward Universal 1492-Reverse. 3): Through the use of agarose and TBE buffer gel

electrophoresis of PCR product DNA was performed. 4): The purified DNA was then submitted to the sequencing lab at U.C. Davis (Wilson, 2006 p.191). Upon return of the sequence to Sierra College Biological Sciences department, the Basic Local Alignment Search Tool was used to compare the sequence to many other sequences using through the National Center for Biotechnology website (Wilson, 2006 p. 197). Using the species of organisms that matched most closely with that of the pure culture, five separate tests were performed: a KOH test (Wilson 2006 p. 61), an Esculin Hydrolysis test (Wilson, 2006 p. 174), a Catalase Activity test (Wilson, 2006 p.175) a Triple Sugar Iron Agar test (Wilson, 2006 p. 185), a Urea Hydrolysis test (Wilson 2006 p. 186), and an addition streak plate of the pure culture on MacConkey Agar was done (Wilson 2006 p. 156).

RESULTS

The streak plate of the raw fish had six colonies, (see colony morphology table, plate #1). The streak plate of the marinated fish had three colonies, (see colony morphology table, plate #2. Note that colony 1 in plate #1 is identical to colony 2 in plate #2. Also note that colony 5 in plate #1 is identical to colony 3 in plate #2 (see colony morphology table, plate #'s 1 and 2).

The KOH test results showed the formation of a mucous-like substance. The Esculin Hydrolysis test showed no color change. The Catalase activity test showed no formation of white froth. The Triple Sugar Iron Agar test showed no color change. The additional streak plate on MacConkey agar showed no growth after incubation at 37 degrees Celsius for 48 hours. Finally, after 48 hours of incubation the Urea Hydrolysis test showed no color change.



MORPHOLOGY OF PLATE NUMBER 1 (RAW FISH)

Colony	form	margin	elevation	surface	optical texture	pigmentation character	size
1	Irregular	undulate	umbonate	smooth & shiny	translucent	cream	9mm
2	Circular	entire	raised	shiny	opaque	bright yellow	3mm
3	irregular	lobate	raised	rough & dull	opaque	cream	5mm
4	filamentous	filamentous	flat	rough & dull	opaque	grey	15mm
5	circular	entire	flat	smooth & shiny	opaque	white	1mm
6	irregular	undulate	raised	rough & dull	opaque	cream	5mm

MORPHOLOGY OF PLATE NUMBER 2 (MARINATED FISH)

Colony	form	margin	elevation	surface	optical texture	pigmentation character	size
1	filamentous	filamentous	convex	rough	opaque	cream	5mm
a	irregular	undulate	umbonate	smooth & shiny	translucent	cream	9m
2	circular	entire	flat	smooth & shiny	opaque	white	1mm

DISCUSSION

Many consumers enjoy the superb dish, ceviche, which has raw fish among its many ingredients. This serves as a potential threat to consumers due to the fact that when raw fish is consumed, there is always a potential to find pathogenic organisms as well. It is important to ensure that dishes involving raw fish are prepared properly to prevent illness from these organisms.

The result for the KOH test was the only positive result among the six tests that were performed, implicating that the organism is Gram negative. The Esculin test result showed that the organism is not capable of hydrolyzing esculin and it is not pathogenic. The Catalase test showed that the organism does not break down hydrogen peroxide through catalse enzymes. The TSI test showed that the organism does not ferment glucose, sucrose or lactose and does not produce hydrogen sulfide. The Urea hydrolysis test showed that the organism does not hydrolyze urease. Finally, the absence of growth on the MacConkey agar plate in addition to the Urea hydrolysis test results were consistent with the identification of the aquatic organism *Chyrseobacterium scopthalmum* (5). This organism showed a 98% similarity with the gene bank sample found in the National Center for Biotechnology Information website. Of the three organisms that survived the marinating process, the

organism isolated in this experiment showed no pathogenic characteristics. Successful completion of all tests were performed, however one conflicting result was encountered. Although the organism was identified as Gram negative, it did not grow on MacConkey agar, which selects for the growth of Gram-negative bacteria.

In summary, it has been determined that once raw fish is marinated in lemon juice a significant decrease in bacterial growth can be observed. The initial expectation of this study was to find surviving pathogenic species of *Vibrio* commonly found in aquatic environments (Hiroyuki, 2006). The consumers of these dishes should be pleased to find that the organism identified in this study appears to be non-pathogenic and overall harmless.

ACKNOWLEDGEMENTS

We would like to offer a huge thank you to our husbands, Mr. Randy Rigg and Mr. Mario Valenzuela for "taking care of business" at home while the two of us ignored them and worked on this project. Acknowledgments must also go to Juan Garcia, captain of the boat from which the infamous fish was caught.

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Vol. 2, No. 1

Isolation and identification of unknown bacteria in commercial-grade yogurt

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Staphylococcus pasteuri **was isolated and identified from Mountain High Vanilla Yogurt by various lab tests and was conclusively identified through the use of DNA sequencing. Research indicates that *Staphylococcus pasteuri* is not normally an ingredient used in yogurt production but it has been associated with fermentation of sausages in Italy, and has been found in raw milk and dairy products and most recently in the outer most atmosphere of earth.**

INTRODUCTION

It is important to periodically test food that utilizes live cultures to confirm quality control of these live bacteria. To confirm the content of this product I plan to isolate one of the bacteria within their Vanilla Yogurt, culture it, identify, and confirm that it is indeed one of the bacteria identified as being within this product. I expect to find one of the following species within the sample of Mountain High Vanilla Yogurt: *Lactobacillus bulgaricus, L. acidophilus, L. casei, Streptococcus thermophilus, and Bifidobacterium bifidus.*

MATERIALS AND METHODS

Using aseptic techniques I retained a sample of Mountain High Original Style All Natural Yoghurt: Vanilla. I streaked a sample of yogurt onto a Bromcresal Purple (BCP) Lactose plate. After 48 hrs at 37°C I re-streaked for isolation onto 3 BCP Lactose Agar plates and allowed them to incubate for 48 hrs at 37°C. I also streaked onto a TSA plate and incubated for 48 hrs at 37°C. To determine cell morphology I performed a Gram stain from exercise 6-B (Wilson & Warren, 2007, page. 61-63). To distinguish motility and accurate cell arrangement, I created a wet mount slide using techniques learned in exercise 2A (Wilson & Warren, 2007, page 28). I then performed the KOH test from exercise 6-B supplemental (Wilson & Warren, 2007, page 64) to confirm my Gram stain results. I also performed the Acid-Fast Stain following directions from exercise 6-C (Wilson & Warren, 2007, page 69-70) and a Endospore stain following the Dorner Method from exercise 6-D (Wilson & Warren, 2007, page75-76). I also streaked a Mannitol Salt Agar Plate to determine if my bacteria was salt tolerant and if it could ferment mannitol. I tested its optimum fermentation temperature by using a double concentration phenol red broth, and let it incubate at room temperature for 48 hours, then subsequently incubated at 37°C for another 48 hours. Finally to get conclusive information on my microorganism I prepared it for DNA sequencing by first extracting the chromosomal DNA and then doing a (PCR)

Polymerase Chain Reaction. I isolated the chromosomal gene that codes for the 16S rRNA. I used primers Bact 8F and 1492 R to extracted the chromosomal DNA following the procedures in exercise 16 (Wilson & Warren, 2007, page191-194). The (PCR) Polymerase Chain Reaction product was then run on a 1% agarose gel (Gel Electrophoresis) to isolate my 1500pb PCR product. Sasha Warren then cut the gel, separating my sample and then, melted, spun, and purified it using the QIA quick gel purification kit (Qiagen). The purified rDNA was then sent to the Division of Biological Science (DBS) sequencing facility at UC Davis. When I received the results I analyzed the electropherogram using the 4Peaks program, and then compared the sequence using the National Center Biotechnology Information (NCBI) Basic Alignment Search Tool (BLAST).

RESULTS

The colony morphology of all three separate BCP plate were the same except in regards to size and color. Form: circular, Margin: entire, Elevation: raised, Optical Character: opaque, Surface Texture: shiny, Pigmentation from sample one: yellowish green, Size from sample one: ½ mm; Pigmentation from sample two: green; Size from sample two: 1-2 mm; Pigmentation from sample three: gray blue; Size from sample three: 4 mm. The restreaked product produced identical multicolor results on all three new BCP Lactose agar plates. On TSA, the colonies were all an extremely pale yellow color and did not display any distinct color differences between older colonies and newer colonies. The Gram stain displayed purple bacteria. The bacteria are cocci in shape and measured .5 μm across. The bacteria cluster in the Gram stain. The wet mount displayed non motile cocci that cluster. The KOH test displayed no slimy texture when microorganisms were exposed to 3% KOH. The microorganisms turned blue when subjected to an Acid-Fast stain. Endospore stain, utilizing the Dorner Method, results clear or light pink. In the Bromcresal Purple (BCP) Lactose plate, the first plate streaked with yogurt, displayed a slight yellowing of the agar around the colonies. Subsequent streak plates did not display this agar color change, but the colony formations were yellow. On the Mannitol Salt Agar plate, after 48 hours of incubation at 37°C, the entire plate was yellow. The Phenol Red Broth (double concentration), initial 48 hours at room temperature resulted in no change, it remained red. Subsequent 48 hours at 37°C turned yellow. A small gas bubble about .1 mm, formed inside the Durham tube. The DNA Sequencing matched the sequence of *Staphylococcus pasteuri* with a 99% identity. Accession number of organism: EF127830.1, Ratio of identical nucleotides: 771 / 773, Bit Score: 1415.

DISCUSSION

Believing I had three different bacterial colonies, I restreaked, and analyzed the colony morphologies and concluded that these were in fact all the same organism. I hypothesized that these were a single bacterial strain, and the color differences were an indication of maturity in the colonies, not an indication of distinct bacteria. Furthermore the color distinction was only apparent on BCP Lactose agar; there were no color difference on TSA, as seen in (figure 1).

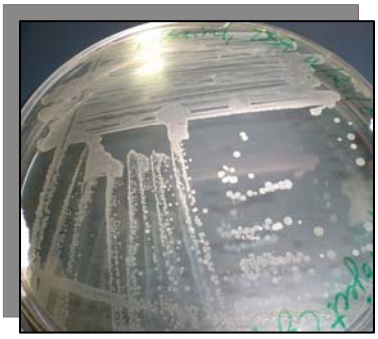

Figure 1.

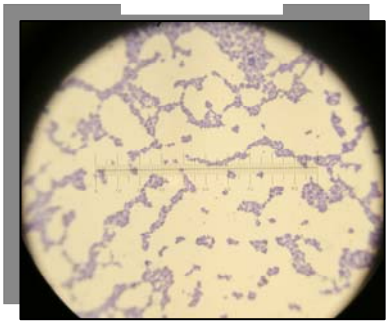

Figure 2.

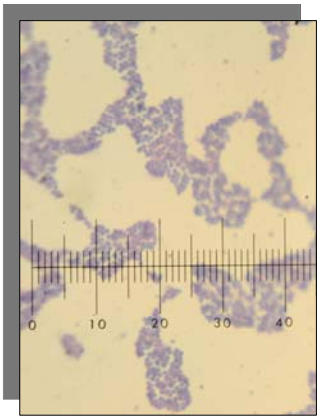

Figure 3.

The Gram stain (figure 2) indicates a G+ Staphylococcus, with thick peptidoglycan walls and techoic acid; therefore it does not have an outer membrane composed of lipopolysaccharides. I performed a KOH test and a wet mount to confirm these results. The wet mount displayed the same cell arrangement as the Gram stain, thus confirming the cell arrangement. The results of the KOH test indicated that when exposed to 3% KOH the integrity of the bacteria's cell wall remained intact, thus confirming the G+ results of the Gram stain, and confirming that this bacteria would not be toxic to humans from its outer membrane.

The acid-fast stain, (figure 3), indicates that even though this bacteria is G+, it does not have a large quantity of mycolic acid in its cell wall. The Endospore stain (Figure 4) indicates that this organism does not produce spores. This indicates that this organism would be susceptible to heat, desiccation, or chemicals. After identifying this organism to a 99% certainty I did some further research and found an article in the *Microbiologist* indicating that this organism can survive in the extreme environment of the outer earth atmosphere. It was found 135,000 feet above earth and was still able to be cultured by researchers from Sheffield University.

The Mannitol Salt Agar results indicated that this organism can utilize mannitol as a potential fuel source. The Phenol Red Broth only changed in color when incubated at 37°C indicates that this organism is more active at 37°C.

At this point in my identifying process I knew that I did not have any of the expected organisms. The final step to determining what I had was to evaluate my microorganisms DNA sequence to that of other known microorganisms. Using BLAST I found that my organism was *Staphylococcus pasteuri*. I further tried to learn about my organism, but was not able to find a current enough Bergey's Mannual in which to compare my test results. I did find an article indicating that *S. warneri* was the most similar species phenotypically, so I compared my test results with that of *S. warneri.* Of the tests given the results matched 100%. Furthermore, I wanted to know the normal environment of *Staphylococcus pasteuri,* so that I could determine if the yogurt was contaminated with a bacterium that would not normally live in that environment. My research indicates that *Staphylococcus pasteuri* has been found in unpasteurized milk and dairy product in Norway, and in Italy in the processing plants used for naturally fermenting

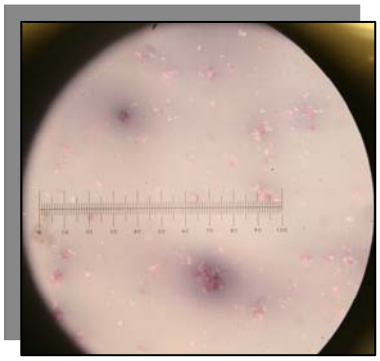

Figure 4.

sausages, and as earlier stated it has even been found in the earth's outer atmosphere at 135,000 feet above sea level.

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Sierra College Foundation, North Valley and Mountain Biotechnology Center at American River College, Sasha Warren.

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Vo1. 2, No. 1

Isolation and Identification of *Staphylococcus hominis* from an unwashed apple.

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In this project, we were interested in finding out what can possibly be found on an unwashed apple. We swabbed an apple, grew all of the bacteria on nutrient agar, picked out an interesting looking colony, and restreaked that colony on nutrient agar. After we had a pure culture, we performed many morphological and physiological tests that would give us the identity of an organism. Also, a DNA sample was taken and sent to the sequencing facility at UC Davis. DNA sequence was compared to the database sequence of BLAST, which identified our unknown organism as *Staphylococcus hominis.*

INTRODUCTION

Apples are eaten all over the world. Most people eat an apple with the skin still on it, which is smart because there are a lot of nutrients in the skin. Or is it that smart to eat it with the skin? Some people may even go as far as not washing the apple and just rubbing the "dirt" off of the peel.

This study was done to see just what kind of bacteria is on the skin of an apple. What we know for sure is that some sort of bacteria will be found. The question is will the bacteria found be a pathogen, an opportunistic pathogen, or just normal flora? Sometimes people are starving, as most college students are, and they will eat anything. The apple used was one that a friend just wiped off with his hands, and was about to eat. Before he sunk his teeth into it, the apple was swabbed for bacteria.

MATERIALS AND METHODS

Sterile cotton swabs were dipped in sterile water and then were swabbed onto the apple. Then the same swab was dipped and swirled around in the tube of sterile water.

Medium-

After swabbing the apple, the small water sample with bacteria was poured out onto Nutrient Agar (NA). It was evenly spread using a sterile glass pipette. Then, the agar plate was incubated at 37°C for 48 hours. Following incubation, the preferred colony was streaked onto another NA plate. This one was incubated for 48 hours at the same temperature, and then left to continue to grow at a room temperature. Once found that it was a *Staphylococcus* genus, it was streaked onto a Blood Agar (BA) plate.

Staining-

An indirect stain was used to observe cell size (Wilson, 2007 p. 53). A standard Gram stain was performed (Wilson, 2007 p. 61). An Endospore, capsule, and acid-fast stain were performed as well (Wilson, 2007 pgs. 69-79)

DNA Extraction/Polymerase Chain Reaction/Gel Electrophoresis-

DNA extraction was performed by boiling a sample in a tris buffer solution. Then the sample was vortexed with glass beads inside the micro tube. When isolated, a Polymerase Chain Reaction (PCR) was performed. This allowed specific segments of DNA to be amplified, specifically the 18s ribosomal RNA. PCR included our Template DNA, Taq polymerase, 2 primers -8-Forward and 1492-Reverse, buffers, and distilled water. When PCR was finished, gel electrophoresis was done. This allows for the separation of DNA fragments based on size. Sample DNA mixed with two dyes was placed into the wells located on one side of the gel, which is composed of agarose, and was subject to an electrical current. After gel electrophoresis, the DNA was cut from the gel and purified using the QiA purification kit and sent to the sequencing facility at the University of California at Davis. After the electropherogram came back from Davis, 4 Peaks software was used to view the data. The data came back as a contiguous sequence, so no editing needed to be done. Following, the Basic Local Alignment Search Tool (BLAST) was used to see which organism was closest to the one found on the apple.

Other tests-

The KOH test was performed to confirm the gram stain. Urease, catalase, and coagulase were other tests used to confirm the organism.

RESULTS

The colonies from the Nutrient Agar plate were:

- Form - circular
- Margin - undulate/lobate
- Elevation - umbonate
- Optical character - opaque
- Size - about 1-5 mm
- Color – cream
- Surface texture – shiny
- Odor - wet dog

The colonies from the Blood agar plate had growth.

The indirect stain (figure 1) showed that the cells were in a staphylococcus arrangement and had a cocci shape. They were an even circle with a diameter of 1.1 µm. A wet-mount stain showed that the organism did not move. The Gram stain (figure 2) came out with the cells stained purple. The KOH test proved to be in agreement with the Gram stain. The KOH stain came out with no snot. An acid-fast stain (figure 3) showed the organism stained blue. The endospore stain (figure 4) was used via the malachite green method. The result was that the cells were stained pink/purple. The capsule stain (figure 5) showed halos around the cells with the background stained purple and the cell stained purple.

The DNA analysis that came back proved that the organism found on the apple was *Staphylococcus hominis*. It showed up with 99% identity. The accession number of the organism is AY030318.1. The ratio of nucleotides was 771/777. The bit score was 1397 bits.

The result of the urease test came out with the agar being hot pink color. The catalase test showed that there were bubbles when the organism was mixed with hydrogen peroxide. The coagulase test came out with the mini tube still in liquid form.

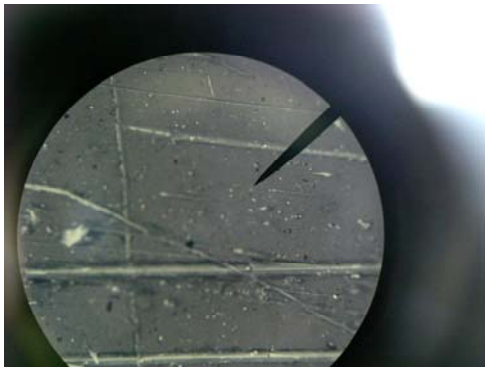

Figure 1

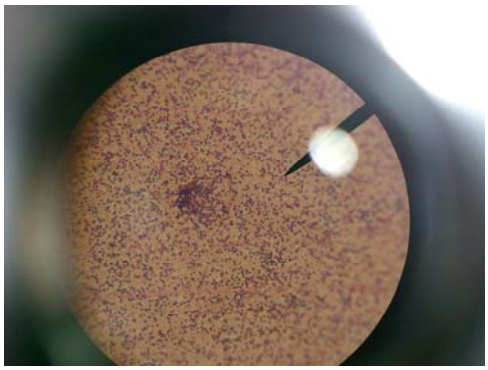

Figure 2

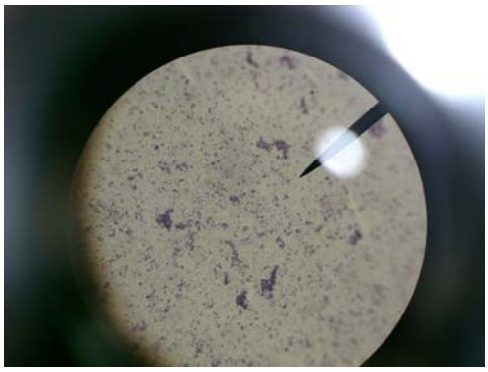

Figure 3

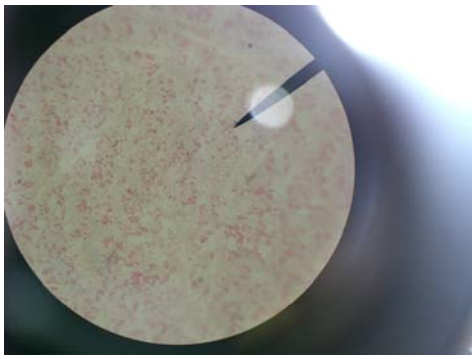

Figure 4

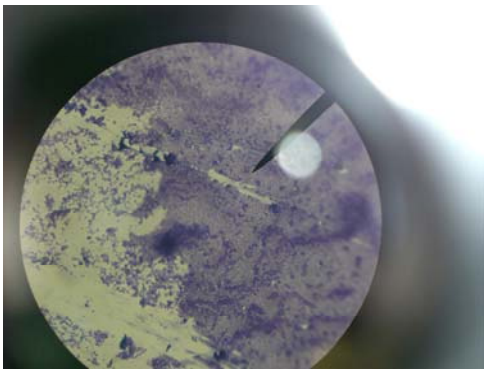

Figure 5

DISCUSSION

After conducting a series of tests, we have come to conclude that our organism is *Staphylococcus hominis*. The catalase test that was performed came out positive, meaning that the organism had catalase. The organism did not have coagulase, because the result of the coagulase test was negative. This means the liquid which was inoculated with the organism did not solidify after the proper incubation time. One other physiological test was performed via the urease test, which came out positive. The organism had urease to break down urea. Organism had the ability to grow on Blood agar plate but showed no hemolysis. The above tests confirmed what the Bergey's Manual said (Cloos, 1025).

The Gram stain provided us with the confirmation that the organism we had was a Gram + cell, and the KOH test helped to confirm the Gram stain. Under the Gram stain the cells were also in a staphylococcus arrangement which also confirmed the catalase test. The indirect stain also confirmed the arrangement of the cells as staphylococcus. In the endospore stain, the cells were pink/purple with no endospores present. The acid-fast stain stained the cells blue indicating that our organism did not have much mycolic acid in its cell wall. In the capsule stain, the cells were stained purple and the background was stained purple as well. We had halos around the cell indicating that the cell had a capsule around it.

When our electropherogram came back, it was the best and most accurate confirmation. It proved that our organism was *Staphylococcus hominis*. The results that were on BLAST matched our organism's DNA to those in the database with 99% identity, matching 771 out of 777 nucleotides.

Again, we must go back to the question asked in the Introduction, "Will the bacteria found be a pathogen, an opportunistic pathogen, or just normal flora?" Bergey's Manual states that *Staphylococcus hominis* is an opportunistic pathogen (Cloos, 1025). This means that it might not be too smart to eat the apple unwashed. We do not know if this bacterium is present on every single apple, but there is no need to risk the chance of getting infected. Please, wash your apples before you eat them.

ACKNOWLEDGEMENTS

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Sasha Warren, thank you for giving us the reference of the Bergey's Manual on the last day!!! ☺

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Vol. 2, No. 1

How your horse may be like a Mastadon from the ice age
Or
An ancient little microbe we found while messing around with horse feces

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We looked through the fecal matter of three horses in hopes of finding some parasites since parasites are very common in horses. Failing that, we hoped to isolate a microbe for examination. We did not find any parasites through a visual scanning, but we did isolate and identify a microbe by the name of *Kurthia gibsonii* through a series of morphological and physiological tests. While researching this microbe, we found out some interesting things about its history.

INTRODUCTION

Knowing that horses are notorious parasite carriers, we decided to try to find, identify and isolate a parasite from random samples of horse feces. Two of the samples of the feces were found at the trailhead of the Western States Trail in Auburn, California while the third sample was from a quarter horse named "Nifty" who had just been brought in state from Texas. Nifty had not been well taken care of and we hoped there would be some parasite (for our sake, not Nifty's). According to the American Veterinary Medical Association, some common parasites that horses carry are strongyles, bots, ascarids and pinworms. (1) (4)

Failing any finding of parasites we hoped to isolate and identify a microorganism that is common to the intestinal tract of a horse such as *Escherichia coli* or *Salmonella typhimurium*. (2) (3)

MATERIALS AND METHODS

The first thing we needed to do was a make a broth of our feces samples and label them “HorseA”, “HorseB” and “Nifty”. This was accomplished by adding a small amount of fecal sample to a Nutrient Broth. We allowed the tubes to incubate at 37degrees C for 48 hours and then moved them to our drawer where they were kept at room temperature (25degrees C) for the remainder of the time. Once we were satisfied with having some happy microbes and/or parasites in the broth samples, we scanned a wet mount of each fecal sample to search for parasites. This was done with the microscopes in the classroom using the 40x objective. Much to our disappointment (though I am sure Nifty was happy) we found no parasites.

Since our horses appeared to be parasite-free, we decided to see what microbe we could isolate from the samples. Using a sterile loop tool, we transferred a loopfull of the broth onto a Nutrient Agar plate. We did this for each broth. We allowed these to incubate at 37 degrees C for 48 hours. These plates resulted in a myriad of colonies. The ones on the plate for HorseA looked to be the most morphologically interesting, so we began the arduous isolation of a solitary colony. This was a very difficult thing as it turned out. After finally getting a plate with what look like a pure sample, we were plagued by a filamentous growth that kept creeping across the plate as the colonies grew. We restreaked the organism a total of 7 times before we gave up. At around the 3rd streaking of the organism, we were required to prepare a sample of the DNA to be sent to Davis for DNA sequencing. This preparation was done by a method called mini prep. To do this, the organism was boiled and then placed on the vortex. This allowed the DNA to be denatured and exposed. The purified RNA was sent to DBS at UC Davis and sequenced with primer Bact-8-f and subjected to DNA sequencing. The PCR was run on a 1% agarose gel and the 1500bp product was purified using QIA quick gel purification kit (Qiagen).

While UC Davis had our DNA sample, we ran a series of morphological tests on our microbe. We performed a Gram stain, a capsule stain, endospore stain, an acid-fast stain and a KOH test.

The results of the DNA sequencing came back from UC Davis. At this point, we used the program called, “Four Peaks” to change the electropherogram into a series of lettered bases. Using this sequence, we applied it to the program called BLAST (Basic Local Alignment Search Tool) that is housed in the website for the NCBI (National Center for Biotechnology Information). This is a program that allows for the comparison of a DNA sequence to the sequences that are housed in NCBI’s DNA library.

At this point we ran a series of physiological tests on the organism to further verify its identity. These tests were a Vogues-Proskauer test to check for butanediol fermentation, a Urease test to check to the capability of urease production and a SIM (Sulfur Indole Motility) test to check for the production of Hydrogen Sulfide, the capability of movement and of breaking down Tryptophan to form indole.

RESULTS

The morphological tests showed our organism to be Gram +, non-encapsulated and non-acid-fast. We were also able to identify our organism as a bacillococci with a possible palisade arrangement. The cells measured 2-3 micrometers long. The colony morphology showed this organism to be entire, lobate, yellow, raised, shiny, opaque with a branching of filamentous areas. The more circular colonies measured 2mm across while the filamentous portion spread out in a lawn.

The results for the physiological tests were a negative for the Vogues-Proskauer, proving this organism does not ferment buntanediol. The SIM showed the organism to be motile, negative for hydrogen sulfide production and negative for indole production. The urease test also had a negative result.

According the Bergey's Manual, all of our tests were performed correctly as our results matched those in the manual. According to the Bergey's manual, the description of genus cellular morphology for *Kurthia gibsonii* is regular, unbranched rods, occurring in chains. They were 0.8 – 1.2 micrometers in diameter and 2-4 micrometers long. Colony morphology was yellow to cream, circular and entire and in a successful isolation, filamentous growth occurs within 2-3 days.

Species morphology from the Bergey's manual (page 1255-1258) was the same as that for genus.

The physiological results in the Bergey's manual showed the organism to be Gram positive, not acid fast, unable to ferment butanediol, unencapsulated, unable to produce urease, unable to produce H2S, unable to produce indole and some strains showing motile.

The results of the search through the BLAST program are as follows:
Identification of the bacteria *Kurthia gibsonii* (strain WAB1921, family: firmicutes) was 99% accurate with 757/778 bases matching and 7 gaps. The accession number is gi/109942127/AM184261.1 .

DISCUSSION

Parasites are very common among horses (4) and it is surprising that we failed to find one. We also had expected to find an enteric that was common to horses. However, we found an organism that seems to be very prevalent in South America as we found numerous articles from that country on *Kurthia gibsonii*. It is also commonly found in livestock feces. There is very little reference to this microbe indicating it is either not very prevalent or that it is harmless enough that it is of little interest to humans. However, we did find one thing very interesting about this little microbe, it was found in the fossilized remains of a Mastadon by a man named Belikova in 1980. This is indeed a microbe with a history.

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Is Your Old Makeup Hazardous?

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Among our choices of suggested topics for this project, we decided to test old makeup for microbial contaminants. We as well as many women we know, hang onto our makeup for way longer than we should. This lead to the question; how long should you hold on to your makeup? We tested a variety of old cosmetics and although we did not find anything extremely hazardous to our knowledge, we still came to the conclusion that it is not a good idea to hang onto old cosmetics.

INTRODUCTION

Have you ever wondered how long you should hold on to your makeup? When does your makeup actually expire. According to The Cosmetics Products Safety Regulations of 1996, only products that won't last longer than 30 months need to show a use-by date, whereas all other products don't need one (3). Our experiment is to see what bacteria can be found in makeup that has been kept for a long period of time. We will be testing mascara, lip gloss and liquid eyeliner. The majority of the bacteria we expect to find are normal flora, however there are also several other infectious bacteria that we could find. Liquid products—foundation, mascaras or moisturizers—are the most likely to harbor bacteria because water is their main ingredient. And bacteria thrive in water (5). One type of bacteria we would expect to find is *Stappylococcus aureus* which known to cause styes. The next possible bacteria would be *Nieserria gonorrhoeae* and is known for causing redness, swelling, and puss the known symptoms of pink eye. In our research we found that 92% of women are putting themselves at risk of exposing themselves to these types of bacteria (1). "Your eyes and lips are openings to your mucous membranes," says Karyn Grossman, M.D., a dermatologist based in Los Angeles, "so never share your lipstick or eyeliner. You could put yourself at risk of a staph infection or viruses like hepatitis, herpes, mononucleosis, or even warts (2)." Bacteria will flourish in moist warm environments. As soon as you open your cosmetics the clock starts ticking.

MATERIALS AND METHODS

The first thing we had to was obtain samples from our four different types of cosmetics. Using a moist cotton swab we took samples from lip gloss, Carmex, liquid eyeliner, and mascara and added them to water creating a mixture. The Carmex and eyeliner were streaked on Brain Heart Agar. The lip gloss and mascara were streaked on Blood Agar. The plates were incubated a 37 degrees Celsius for 24 hours. We also re-streaked our mascara, lip gloss, and eyeliner products on Blood Agar taking samples directly from the

makeup to the plate using a cotton swab. Using aseptic technique we then isolated colonies from the liquid eyeliner sample and the mascara sample in an attempt to obtain a pure culture. The next step was to find out if our bacterium was gram positive or gram negative. We performed a KOH test (potassium hydroxide) which according to the lab syllabus can confirm whether bacteria are gram positive or negative (64). Using aseptic technique we placed a sample of the bacteria on a glass slide and added a loop full of potassium hydroxide to the sample. In addition to the KOH test we also performed a Gram stain. This stain served as a way for us to view our bacterium's colony morphology. We then performed the VP (Voges Proskauer) test. According to the syllabus this test is used to identify organisms that produce acetoin which is a neutral fermentation product (174). For this test we transferred 1 Ml of broth to a tube with a screw top and added 18 drops of Barrett's reagent A and Barrett's reagent B. I closed the tube and shook it vigorously for 1 minute. After letting the tube rest for 10 to 15 minutes I picked it up and shook it again until the color changed. Catalase test: According to the lab syllabus the catalase test is used to determine if bacteria can catalyze the breakdown of hydrogen peroxide (175). We placed a sample of the bacteria on a glass slide and added 3% hydrogen peroxide to the sample. Following this test we performed the Citrate test which according to the lab syllabus is used to determine the ability of an organism to utilize citrate as its sole carbon source (181). I stabbed a sample of the unknown bacteria into a tube and streaked it on an agar slant. Last but not least in order to obtain our DNA sample we performed PCR (193).

RESULTS

When viewing our first agar plates no bacterium was present. So we had to use a different method of obtaining a sample. Our second try was successful. The liquid eyeliner that was streaked on brain heart agar yielded opaque, yellow, dull colonies that were irregular in form, undulate in margin and umbonate in elevation. The colonies measured about 5 millimeters and had a foul odor. Our mascara sample on blood agar yielded very unusual colonies. These colonies produced an opaque red and orange pigment. They were irregular in form and had lobate edges. They looked like the popular children's candy Pop Rocks! Our KOH test results came out negative (no snot). The KOH did not coagulate and therefore confirmed the bacteria were gram positive. Our Gram stain gave our bacteria a purple color in cocci shaped clusters approximately 1 by 1 micrometers in size. The purple color of our bacteria confirmed that our bacterium is gram positive. For our VP test, if the broth culture turns red it is positive for acetoin whereas if the broth culture remains yellow it is negative for acetoin. Our result was VP negative meaning that our bacterium does not produce acetoin. For our catalase test the results yielded lots of bubbles on the slide concluding that our bacterium was positive for catalase enzymes. For our Citrate test it included the pH indicator Bromethymol blue which will turn from green at a neutral pH to blue at a higher pH. Our test yielded a blue result meaning our bacterium is positive for citrate utilization.

BLAST results: My blast sequence matched the sequence of *Micrococcus luteus* with 98% identity.

Accession number of organism: EF187229.1

Ratio of identical nucleotides: 759/768
Bit score: 1371 bits

DISCUSSION

Our Gram stain yielded a purple color meaning our bacterium was gram positive. This means that the surrounding cell wall is a rigid structure made of peptidoglycan. Due to the fact that there is the possibility of error with performing a Gram stain a KOH test is performed in conjunction to confirm whether an isolated bacterium is gram positive of gram negative. Our KOH test did not coagulate confirming that our bacterium was indeed gram positive. Our Catalase test was positive which means, according to our syllabus, our bacterium uses catalase enzymes that are essential for aerobic growth of microorganisms. Our VP test results were negative, concluding that our bacterium does not break down glucose to produce acetoin. The citrate test was positive meaning that citrate was utilized meaning the resulting growth will produce alkaline products. According to the DNA results of our PCR, our unknown bacterium was *Micrococcus luteus*. These bacteria are a common human skin contaminant and are relatively harmless to humans because they maintain a saprotrophytic lifestyle and are distributed by mammalian skin, beach sand, and water. They can also be found in fresh water and soil. *Micrococcus luteus* is considered normal flora, which was what we expected to find as a normal contaminant in our makeup samples.

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Vol. 2, No. 1

The Isolation and Identification of the Opportunistic Pathogen *Pseudomonas putida* from a Private Bathroom Sink

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In this experiment, public and private bathrooms bacteria is examined and compared to provide evidence that private bathrooms contain a greater variety of bacteria. The results of our 2 series of samples were grown on Nutrient Agar and Tryptic Soy Broth, respectively. We isolated an organism and preformed a series of morphological and physiological tests. Then, a Polymerase Chain Reaction (PCR) was performed on our isolated organism using its purified rDNA, and sent to the D.B.S. Sequencing Facility at UC Davis. The organism was identified as *Pseudomonas putida* and later confirmed using the Bergey's Manual. *Pseudomonas putida* is a Gram negative opportunistic pathogen often found in soil and will thrive in moist environments that can be found upon bathroom sinks.

INTRODUCTION

The common statement private bathrooms are cleaner than public bathrooms will be put to the test in this experiment. We will be sampling the transient bacteria found on the exit handles of 4 different bathrooms, 2 private and 2 public. In this experiment we expect to find common bacteria found on human skin and feces, many that can be transmitted via the fecal oral route. A study done by Chuck Gerba and Denise Kennedy of the University of Arizona claim public restrooms are breeding places for disease causing bacteria, particularly those found in the intestinal tract, or fecal bacteria(Riddle, 1997). Bacteria we expect to find are *Staphylococcus* and *E. Coil*, and possibly *Salmonella, Streptococcus,* and *Camphylobacter*. We will examine, analyze, and compare the amount of colonies found in public versus private bathrooms, and what types of bacteria are most prevalent. Our hypothesis is that private bathrooms will contain more bacteria than public bathrooms, due to the possibly of animals in the home and frequency and efficiency of cleaning.

MATERIALS AND METHODS

We streaked our samples collected from the exit handles, using sterile swabs, of 2 private bathrooms and 2 public bathrooms on Nutrient Agar (NA) plates and incubated them at 37 degrees C. Due to results of the handle experiment (see below), samples from bathroom sinks were later swabbed and streaked onto 4 Tryptic Soy Broth (TSB) plates and incubated at 37 degrees C. We recorded all colony morphology found on the various plates of the 2 series and kept our chosen organism alive by re-streaking on Tryptic Soy Broth and Mueller Hinton plates and kept at room temperature. We preformed a KOH test, Gram stain, Oxidase test, Wet-Mount Motility Test, and streaked our organism onto a Pseudomonas

plate containing Sodium Benzoate. We performed a chromosomal DNA extraction of our chosen organism by mixing it with a Vortex mixer and then boiling it for 10 minutes. In the next step we preformed a polymerase chain reaction, PCR, to amplify the 16s ribosomal RNA gene (rDNA) of our sample, using the primers Bact 8-Forward and 1492 Reverse (Warren pg 193,194). PCR reactions were run on a 1% agarose gel and the ~1500 base pair product was purified using QIAquiuk PCR purification kit (Quigen). Purified rDNA was sent to the D.B.S. Sequencing Facility at UC Davis and sequenced with the primer Bact 8-Forward. The sequence from the isolated organism was then compared with those in the database at the National Center for Biotechnology Information (NCBI) with BLAST, Basic Local Alignment Search Tool (Warren 2007).

RESULTS

In our 1st series of samples from exit handles, we initially yielded no growth on any of the 4 NA plates. In our 2nd series, we swabbed bathroom sinks. The 2nd series yielded an extraordinary amount of growth. All 4 TSB plates yielded results too numerous to count. We noted though, that the public bathroom plates appeared to contain similar colony morphology throughout. The private bathroom plates, however, appeared to contain many different colonies morphologies. Two weeks after the 1st series of samples were collected, a second look was taken to reveal colonies that had previously not been accounted for. Both private bathrooms produced colony growth, though of less substance, similar to the morphology of the 2nd series private bathroom plates. Our selected organism was taken from the 2nd series private bathroom plate B, it was 2mm, circular, entire, convex, smooth, slightly translucent, and produced an odor. Our KOH test was positive indicating the organism was likely Gram -. The Gram stain revealed very small, ~1-2 um, pink rods. The organism was a Gram – bacilli. The PCR results revealed that our isolated organism was *Pseudomonas putida* with 1464 base pairs and 99% accuracy matching 772/776 nucleotides. Tests were preformed to compare the PCR results with those of the Bergey's Manual of Systematic Bacteriology for that particular organism (Palleroni pg 165-168). The Oxidase test was positive for Cytochrome C producing a slight silver blue pigment. The wet mount Motility test revealed a positive, very high level of active motility. The organism also lacked a pigment (beige)(Palleroni pg 165-168). The organism was also grown on the medium, *Pseudomonas* agar, to determine that the organism was in fact *Pseudomonas* and could digest Sodium Benzoate as its only carbon source (Warren pg 89).

DISCUSSION

The common theory is that public bathrooms are "dirtier" or contain more bacteria and microorganisms than a private residences bathroom. We sought to give evidence that a private bathroom contained a greater amount of colonies and growth of different bacteria that a public bathroom. Our general hypothesis evolved from the idea that the home environment is a particularly easy place for microorganisms to spread from person to person through cross contamination, especially due to the fact that much activity goes unmonitored like effective cleaning and hand washing versus a public bathroom's maintenance (Britton

2003). We expected to find a numerous amount of enteric bacteria stemming from the fact that they habitate in feces. Enterics can be shed in large numbers from the feces when a member of the household is ill and could be spread be unwashed hands or fomites to places like the handles of the bathroom doors and faucets (Britton 2003). One of the most common bacteria Lynda Britton found and in our experiment, however was *Pseudomonas. Pseudomonas putida* is an organism commonly found in soil that will thrive in moist environments, optimally at room temperature, when isolated in clinical specimens is most commonly found in non-sterile sites and is considered to have pathogenic significance (PHAC 2000). *Pseudomonas* is know to be an opportunistic pathogen, which is an organism that does not in its normal habitat cause disease, but if it is introduced to, for example, broken skin or a mucous membrane it can cause an opportunistic infection (Tortora pg 425). In March of 2001, *Pseudomonas putida* was discovered in 10 infants in the neonatal intensive care unit of Farhat Hached Hospital, Sousse, all developed an infection some Bacteremia despite the fact that all infants umbilicus were cleaned with an antiseptic (Elsevier 2004). This outbreak shows how *Pseudomonas putida* could be an opportunistic pathogen, by using a susceptible portal of entry in an immuno-suppressed infant's umbilical catheter. After our initial series of swabs of bathroom exit handles didn't yield the results we sought, we decided to test a more porous source in our 2nd series, like the bathroom sink as opposed to the metal exit handles. Sinks contain the greatest reservoir of bacteria colonies in restrooms, due to their accumulations of water that will become breeding grounds for organisms (Trubo 2002). A thought to our isolated organism *Pseudomonas putida* rather than coliform bacteria, surely more numerous in the most potent source of bathroom bacteria was that it simply could survive on the carbon source from soaps collected in the sink the sample was swabbed from. This common soil dweller could have very well been carried inside by either a member of the household or an animal and washed into the sink. Another interesting finding in our experiment was colony growth compared between the 2 regions tested, private vs. public. The public bathrooms yielded a numerous amount of growth, however, overall colony morphology was very similar throughout, with a variation between 2 and 3 different colony types. The private bathrooms yielded a comparable amount of growth, but its overall colony morphology produced a copious amount of variation of size, pigment, and elevation. We suspect this would imply a larger variation of bacteria in a private bathroom, versus a public bathroom's yield of 2-3 common bacteria. Though we did not isolate a coliform bacteria as we predicted, we did provide evidence that a private residence contains a greater variety of bacteria growth as opposed to a public bathroom and the residence of the opportunistic pathogen, *Pseudomonas putida.*

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Isolation of *Escherichia coli* from Well and Water Sources

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The contamination of *Escherichia coli (E. coli)* in water sources has been of concern in recent years due to possible illness related to the specific strain of bacteria *E. coli O157:H7*. The purpose of this experiment was to attempt to isolate any strain of *Escherichia coli (E. coli)* from two water sources: well water from Cool, California and water collected from Placer County Irrigation water. After running a variety of tests, our research team found no *E. coli* of any strain in either source, but the team was able to isolate a particular Gram negative organism from Cool, California's well water and send it to UC Davis's Division of Biological Sciences Sequencing Facility. It was found that the organism was *Sphingopyxis alaskensis*, an organism isolated and commonly found in the coastal waters of Alaska.

INTRODUCTION

The purpose of this experiment is to attempt to isolate *Escherichia coli (E. coli)* from irrigation and well water sources including; Cool, CA well water and Placer County irrigation water. This comparative analysis will help determine which water source contains the highest concentrations of *E. coli* contamination. This will also be important in determining which water source may present more of a health risk for human consumption (Clark, 2007).

If *E. coli* is detected in water, it is an indication of fecal pollution. Most likely the strain of *E. coli* is not a harmful pathogen, but the indication that other pathogenic intestinal microorganisms could also be present. Therefore, this experiment will be important in determining which water source could also be contaminated with other pathogenic microorganisms (Ibekwe, Watt, Shouse, Grieve, 2004.; Nordstrom and Vickery, 2007.).

If any *E. coli* is found, we expect to see a higher concentration of *E. coli* in the irrigation and less in the well water source. This is because irrigation sources are theoretically not as carefully regulated as well, leaving many possible contamination sources. (Clark, 2007).

MATERIALS AND METHODS

The first method we used to isolate an organism from our water sources was Phenol Red Lactose Broth (with Durham tube) which tests for bacteria that ferments lactose to produce an acidic product and whether it produces a gas along with the acidic product. We

inoculated the broth with our water samples by pouring our water samples into the Broth mixture just until the volume of liquid in the tube was doubled (around 10ml). We then incubated the tubes for 48 hours.

The second method we used to isolate an organism from our water sources was to streak our water samples onto Nutrient agar and McConkey's agar. Nutrient agar is not selective or differential so most organisms will grow on this type of meida. McConkey's agar (MAC) is both a selective and differential type of media. The selective portion promotes the growth of one type of organism while inhibiting the growth of others. The differential portion allows for different organisms to appear differently based on their physiology. MAC contains an acidic stain (Crystal Violet) which kills Gram positive bacteria but allows Gram negative bacteria to grow, this is what makes the media selective. MAC also contains neutral red (a pH indicator). If the organism ferments lactose and produces an acidic product the media will turn a fuchsia color. If the organism does not ferment lactose, or produces a basic product the media will stay red, this is what makes the media differential. After streaking our water samples on to both types of media we incubated them at 37 degrees Celsius for 48 hours.

We performed a KOH test which tests to see an organism is gram negative or Gram positive. If the organism is gram positive the sample will have a watery texture. If the organism is Gram negative the sample will have a snotty texture. This indicates that the KOH was able to penetrate the cell wall and blow up the DNA. The snotty texture comes from the nucleic acid (DNA) present in the organism.

The next procedure was to perform a Gram stain. The Gram stain is a direct and differential stain. The direct portion indicates that the stain colors the cell (positive cation). The differential portion indicates that different organisms look differently based on their physiology. Gram negative bacteria have a thin cell wall, an outer membrane, and Lipopolysaccharide (LPS) coming off of the membrane. Gram positive bacteria have a thick cell wall, no outer membrane, and teichoic and lipoteichoic acids within the cell wall. We first smeared a sample of cells from our Nutrient agar plate onto a glass slide. Then we let the slide air dry. Next we heat fixed the glass slide by running it over an open flame a few times. This was done in order to kill the organism and fix the cells in place so that they do not move. After that, we were able to start the staining process. Crystal Violet was applied to the slide first and left to absorb for 60 seconds. This stain will color all of the cells violet. Then we rinsed the slide. Next was Gram's Iodine which was applied to the slide and left to absorb for 60 seconds. This stain is used as a mordant and makes the stains stick to the cells more. Then we rinsed the slide again. We then used decolorizer (acetone and alcohol) which decolorizes the Gram negative bacteria. This is done fast so that the gram negative cells become decolorized but not too long as to decolorize some of the Gram positive cells. Then we rinsed the slide again. Saffranin was applied to the slide last and allowed to absorb for 60 seconds. This stain is the counter stain and stains the gram negative bacteria a reddish/pink color so that they can be differentiated from the Gram positive bacteria (purplish/blue). The slide was then left to air dry so we could view the slide with a microscope at 1000X.

For the Gram negative organism, the next procedures were to perform a catalase test, oxidase, bile esculin hydrolysis, citrate, urease, oxidation fermentation (O/F), SIM, and TSI. The first procedure was the catalase test on our organism from Cool, CA well source. We

performed a catalase test in order to detect whether the organism contains catalase. When placed in hydrogen peroxide, an organism that has catalase will hydrolyze hydrogen peroxide into water and oxygen. The oxidase tests for cytchrome c in an organism's electron transport chain. A positive test result is a change in color to a blue/violet color. The bile esculin hydrolysis test shows whether or not the organism is bile salt resistant (halophile) and whether it can hydrolyze esculin to produce a black precipitate. A positive test will have growth and produce a black precipitate indicating it can hydrolyze esculin. The citrate test tests whether or not the organism can use sodium citrate as a carbon source. If an organism can do so, a positive test shows a blue color. Urease tests whether or not the organism has urease. A positive result will show a pink color because it has the enzyme urease to hydrolyze urea into ammonia. An oxidation/ fermentation test tests whether or not an orgranism can ferment glucose and produce an acidic product either with or without a gas. A positive test is yellow tubes (the organism can ferment glucose) with or without bubbles or cracks (indicating gas).

To determine motility of the Gram negative organism a wet mount is helpful. Once the organism is placed on a glass slide along with deionized water, it is placed under the microscope and is easy to determine whether the organism is motile or not. However, it can be somewhat difficult to determine whether the movement is due to Brownian motion or if the organism is actually moving. Motility indicates that the organism can run and tumble.

In this experiment we added 2mm of our unknown organism (cells) from the Cool, CA well water plate into a microphage tube with 500 microliters of TRIS buffer. Next we did a quick vortex to mix up the cells. Since we believe that our sample is gram negative, we did not need to add the glass beads. We boiled the microphage tube with our unknown cells for 10 minutes in a boat float. The boiling served as the tool necessary to obtain the template DNA (blow up the DNA). Again, since we believe that our sample is Gram negative, we did not have to vortex for 10 minutes. This is our template DNA. We then gave our sample to Dr. Sasha Warren to complete the Polymerase Chain Reaction (PCR) process.

To set up the PCR reaction Dr. Sasha Warren took our samples and added 15 microliters of sterile water, 5 microliters of primer mix (Bacteria-8-Forward and 1492-Reverse are the primers), 5 microliters of our template DNA, and 25 microliters of Taq master mix (used instead of DNA polymerase because it is a hyperthermophile) to a new microphage tube. This microphage tube was then put into the thermocycler (cycles temperature). The thermocycler cycles the temperature between; 94 degrees Celsius to denature the DNA (for 1-3 minutes), 55 degrees Celsius to anneal the DNA (for about 1 minute), and 72 degrees Celsius for extension (for 1-2 minutes). This is done in order to allow the DNA replication to take place in vitro. The PCR reaction is a method to perform DNA replication in vitro. It amplifies the 16S ribosomal RNA gene from our sample.

The PCR reaction was then run on a 1% agarose gel and the 1500 base pair (bp) product was purified using the QIAquick Gel Purification Kit (Qiagen). The purified product was sent to the UC Davis Division of Biological Sciences Sequencing Facility to be sequenced.

Since our PCR did not turn out we had to go back and redo the above procedures. After this was done we gave it to Dr. Sasha Warren to be taken to UC Davis D.B.S.S.F and sequenced again.

RESULTS

According to the chart below, Cool, California well water shows viscosity on the KOH test, indicating a positive test. By having a positive KOH test, there is no peptidoglycan outer membrane which allows the KOH to penetrate the cell wall and lyse the membrane to release the nucleic acid from the organism. A positive KOH test also indicates that this organism is gram negative. This was reinforced by the conclusions of the Gram Stain test, showing a pink/red color.

Cool, CA well water:

Colony morphology:

Medium used to observe colonies: Nutrient Agar	
Form: Circular	Pigmentation: Yellow
Margin: Entire	Size in mm: 1-2 mm x 1-2 mm
Elevation: Raised	Surface Texture: Glistening / shiny
Optical Character: Translucent	
Other: No detectable odor	

Cellular Morphology:

Shape: Rods	Arrangement: Bacilli (some streptobacilli)
Size: .2 -.5 micrometers x .5 – 3.0 micrometers	Motility: motile

Stain & KOH Test Morphology

Stain or Test	**Results**	**Conclusion**
KOH	Viscosity	KOH +
Gram Stain	Pink rods	Gram -

BLAST results:

The isolated organism's sequence matched the sequence of *Sphingopyxis alaskensis* with __99__% identity.

Accession number of organism: CP003569
Ratio of identical nucleotides: 1375 / 1375
Bit Score: 1354

Regarding colony morphology, our organism shows circular form, entire margin, raised elevation, glistening or shiny surface texture, translucent optical character, yellow pigment, and measured approximately 1 -2 millimeters in size. The cellular morphology showed pink, rods found commonly in a streptobacilli pattern approximately .2 - .5 x .5 – 3.0 micrometers.

Gram negative tests:

Test	**Results**	**Conclusion**
Catalase	Bubbles	Org. has catalase
Oxidase	Purple	Org. has Cyt C in ETC
Bile Esculin Hydrolysis	Growth & Black	Org. is halophile & can hydrolyze esculin
Citrate	Green	Org. cannot use sodium citrate as carbon source
Urease	Pink	Org. has urease
Oxidation/Fermentation	Green	Org. cannot ferment glucose
Wet Mount	Movement	Org. can do run and tumble

Based on the nucleotide sequence received from the UC Davis Division of Biological Sciences Sequencing Facility we were able to utilize reported sequences and identify our unknown organism through the National Center for Biotechnology Information (NCBI). The program used to compare our contiguous sequence to database sequence is called Basic Local Alignment Search Tool (BLAST). The sequence matched the sequence of *Sphingopyxis alaskensis* with a 99% identity, accession number of CP003569, a ratio of 1375 / 1375 identical nucleotides and Bit Score of 1354.

According to the above tests run on our Gram negative organism, the organism has the enzymes catalase and urease, cytochrome C in its electron transport chain, is a halophile and can hydrolyze esculin. The organism cannot use sodium citrate as a carbon source or ferment glucose.

Placer County Irrigation water

According to the chart below, Placer County Irrigation Water shows no viscosity on the KOH test, indicating a negative test. By having a negative KOH test, there is a peptidoglycan outer membrane which does not allow the KOH to penetrate the cell wall and lyse the membrane to release the nucleic acid from the organism. A negative KOH test also indicates that this organism is Gram positive. This was reinforced by the conclusions of the Gram Stain test, showing a purple color.

Colony morphology:

Medium used to observe colonies: Nutrient Agar	
Form: Circular	Pigmentation: Off-white (dark)
Margin: Undulate	Size in mm: .5 mm – 5 mm
Elevation: Flat - raised	Surface Texture: Glistening/ shiny
Optical Character: Opaque	
Other: No detectable odor	

Cellular Morphology:

Shape: Coccobacilli	Arrangement: Diplococcobacilli
Size: 1- 2 x 3 -4 micrometers	Motility: Non-motile

Stain & KOH Test Morphology

Stain or Test	Results	Conclusion
KOH	No viscosity	KOH -
Gram Stain	Purple coccobacilli	Gram +

Regarding colony morphology, our organism shows circular form, undulate margin, flat to raised elevation, glistening or shiny surface texture, opaque optical character, dark off-white pigment, and measured approximately .5 - 5 millimeters in size. The cellular morphology showed purple coccobacilli found commonly in a diplococcobacilli pattern approximately 1 - 2 x 3- 4 micrometers.

This organism was not sent to UC Davis for sequencing; therefore, Polymerase Chain Reaction (PCR) was not conducted for this sample.

DISCUSSION

After performing the Gram test on the Cool, California well water sample obtained this fall, we found that the organism was pinkish under the microscope indicating that the organism was indeed Gram negative. Along with this test, we also performed a KOH test

(resulting in a KOH positive) which, by showing viscosity, confirmed our initial conclusion of a Gram negative organism.

The Cool, CA well water organism was isolated to UC Davis Division of Biological Sciences Sequencing Facility to be identified. After running the BLAST we were able to come to the probable conclusion that the organism isolated was *Sphingopyxis alaskensis*. This is because 1375 out of 1375 nucleotides of the unknown organism matched with this known organism with a 99% certainty.

Sphingopyxis alaskensis species is one of the most numerically abundant microbes found in oligotrophic marine waters and is an important contributor of biomass. It was very interesting to find *Sphingopyxis alaskensis* in the Cool, CA well water since is stated that it normally grows off of the coast of Alaska. *Sphingopyxis alaskensis* is in the Domain Bacteria, Phylum, Proteobacteria, Class Alphaproteobacteria, Family Sphingomonadales, Genus *Sphingopyxis*, Species *alaskensis.* The picture below is a picture we were able to obtain from the internet of *Sphingopyxis alaskensis* and matches the appearance of our organism viewed in Sierra College's Microbiology lab (Schut, 2004).

After performing the Gram negative tests, a catalase test was conducted. We were able to determine that *Sphingopyxis alaskensis* has catalase (positive catalase test showing bubbles). This result indicates that the organism can breakdown hydrogen peroxide into water and oxygen. After performing the oxidase test it has been determined that the organism has cytochrome c in its electron transport chain (positive oxidase test showing a blue/purple dot on the oxidase test paper). The organism is probably a halophile and can hydrolyze esculin since it was positive for the bile esculin hydrolysis test as well. The citrate test showed that the organism does not have citrate and was confirmed in Bergey's Manual. Nevertheless, it cannot use sodium citrate as a carbon source. Showing a positive urease test result, we conclude that the organism has urease to breakdown urea (with water) into ammonia, a basic product. Also, the organism cannot ferment glucose since the results of the oxidation/fermentation test were negative. To be sure, the oxidation/fermentation test was

run two times, resulting in the same data and solidifying our conclusion that the organism cannot ferment glucose.

The results of the Placer County Irrigation Water indicate that the organism is Gram positive (confirmed by a negative KOH test: no viscosity) and is coccobacilli shaped. Since the organism is Gram positive, we did not perform the Gram negative tests we performed on our other organism. We did not send this isolated organism to the UC Davis Division of Biological Sciences Division; therefore, we do not know the identity of this organism.

Although we were not able to isolate *E. coli* from our water samples, we were able to learn a lot of valuable information about the other types of organisms we isolated. This may not answer our hypothesis about whether the concentration of potentially harmful organisms is more abundant in well versus irrigation; however, the identification of *Sphingopyxis alaskensis* in the Cool, CA well water may prompt others to investigate this organism more thoroughly in water supplies.

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Vo1. 2, No. 1

Isolation and Identification of Gram-Positive *Micrococcus luteus* from an Air Plate Exposed in a Heritage Park Apartment in Roseville.

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Micrococcus luteus* was isolated from an air plate exposed in a refrigerator for one hour. A pure culture was obtained by repeatedly streaking samples from isolated colonies on nutrient agar. Morphological features were determined using Gram Staining and Acid-Fast Staining. Physiological properties were determined using the KOH test, Motility test, MR-VP test, Citrate utilization, Urea hydrolysis and aerobic acid formation on a Glucose slant. Genomic DNA was extracted using a boil and bead-beating method and a portion of 16S r-DNA was amplified using the PCR. After gel electrophoresis and purification of this DNA, it was taken to the sequencing facility at UC Davis. Sequence results were edited and compared to GenBank data using the Basic Local Alignment Search Tool available through NCBI. Following tentative identification, the Bergey's Manual was consulted to determine which additional tests should be performed to verify the results. The culture isolated from refrigerator air was ultimately identified as *Micrococcus luteus.

INTRODUCTION

This project was initiated as an independent investigation assigned by the Sierra College Department of Biological Sciences in Rocklin, CA to all microbiology students. The organisms were initially obtained by exposing a nutrient agar plate made in the laboratory to the air in a refrigerator for one hour. The bacteria isolated were ultimately identified as *Micrococcus luteus*, organisms that live on the epidermis of animals, particularly humans (Bauman, 2004). These organisms can be opportunistic pathogens in immune compromised individuals, but are not usually harmful because they live off the dead particles associated with human skin. In this sense, they are also known as being saprophytic (university of Texas, 1995). *Micrococcus luteus* are Gram-positive cocci that typically appear in irregular clusters in stain preparations. In nature they are found in dust, soil, water and air. *Micrococcus luteus* provide some protection against pathogens because they live symbiotically on the skin surface, not allowing other more pathogenic organisms to colonize and grow there.

MATERIALS AND METHODS

The sample was obtained by exposing a plate of nutrient agar to the air in a refrigerator in an apartment in Roseville, California for one hour. Following exposure, the plate was incubated for two days at room temperature in a lab drawer. Isolation was

accomplished by streaking a sample taken from one isolated colony onto a second nutrient agar plate and incubated at 37 degrees for two days. A Sample taken from one of the colonies growing on this plate was then streaked onto a new nutrient agar plate and was maintained in a room temperature lab drawer. A Gram stain, a KOH test, and a wet mount were prepared and observed. Cell and colony morphology were recorded from the Gram stain and the colonies on nutrient agar. Cultural characteristics of colonies were confirmed with direct observation and stereomicroscopy.

Extraction of genomic DNA and DNA processing was also performed. Genomic DNA was extracted using the boil and bead-beating method (6) and portions of the 16S r-RNA genes were amplified with the polymerase chain reaction. The primers used were bacteria 8-forward and universal 1492-reverse. Following amplification, agarose gel electrophoresis was used to isolate the resulting 1500 base pair segments of ribosomal-DNA. These were stained with ethidium bromide, and then the instructor, Harriet Wilson, cut the target DNA band from the gel and purified the PCR product with a QiAQuick PCR purification kit by Qiagen. The purified ribosomal-DNA was taken to the College of Biological Sciences DNA Sequencing Facility at UC Davis for sequencing with the internal primer 533-forward. The electropherogram and nucleotide sequence obtained was then analyzed and edited using the 4 peaks computer application. The sequence data was then compared to other sequences in the GenBank, EMBL, DDBJ, and PDB databases using the National Center for Biotechnology Information Web page and the Basic Local Alignment Search Tool algorithm available there.

Volume 2 of the 1984 version of the Bergey's Manual of Systematic Bacteriology (the Ordinary Gram-positive bacteria) was consulted to determine which physiological tests could be performed to verify the information gained through the NCBI BLAST search. The following physiological tests were performed with samples taken from the plate culture: O/F, MR-VP, Oxidase, Catalase, Citrate utilization, growth on Mannitol Salt Agar, Urea hydrolysis, and Sulfur Indole Motility tests.

RESULTS

Colony morphology as observed on nutrient agar included yellow pigmented, circular, entire, raised, shiny-wet looking, opaque colonies ranging in size from 1 to 5 mm in diameter. The cells observed in the Gram stain were spherical (cocci), 1-2.5 micrometers in diameter arranged in irregular clusters, they were dark purple. In the Acid Fast Stain the cells appeared as blue spheres arranged in groups of four (tetrads). Cells exposed to 3% KOH produced no snot-like viscosity. Observation of cells in a wet mount yielded no evidence of motility. The cells moved with the liquid and displayed only Brownian motion.

Analysis of the culture's 16S r-DNA nucleotide sequence in comparison with others in the public databases showed 100% sequence similarity with *Micrococcus luteus* strain EHFS1_SO4Ha. Comparison of the sequence obtained with the 533-forward primer to the gene bank sequences using the Basic Local Alignment Search Tool showed 786 out of 786 nucleotides matched pairwise. The gene bank sequence was 1375 bases in length and the bit score was 1447 (Accession # EUO71593).

Data obtained with the physiological tests performed yielded the following: The unsealed tube used in the O/F test contained green agar with a little yellow at the top and no bubbles in the Durham tube; the sealed tube contained only green agar. The Citrate utilization test showed green throughout the medium. Completion of the methyl red test yielded a yellow-colored liquid within the original broth tube, and the broth in the Vogues Proskaur test tube turned a light orangish-yellow. In the Sulfur Indole Motility test tube the medium remained clear yellow with minimal growth only along the inoculation line. The Kovac's reagent added to the tube remained yellow. Light purple streaks were observed on the filter paper when performing the oxidase test, and bubbles appeared when the culture was exposed to 3% hydrogen peroxide. When grown on Mannitol salt agar, the culture formed small colonies, not very numerous and surrounded by little pink zones. The culture grew on the glucose agar slant, but did not change the color of the agar, i.e., it stayed red/pink.

Table 1

TEST	MY TESTS	Bergey's
Oxidase test	+	+
Glucose slant (aerobic acid formation)	-	-
Urea hydrolysis	-	-
Citrate utilization	-	-
Oxidation/fermentation or O/F test	Oxidative	Oxidative
Growth on 7.5% NaCl (Mannitol Salt Agar)	+	+
Methyl-red/Voges Proskauer or MR-VP tests	-/-	-/-
Catalase test (3% hydrogen peroxide)	+	+
Sulfur, Indole, Motility tests (SIM medium)	-	-
Gram Stain	+	+

DISCUSSION

The organisms isolated from the refrigerator air were determined to be Gram-positive with both the Gram stain and the KOH test. The culture proved to be non-fermentative with the O/F test, showing no gas or acid formation under anaerobic conditions. The MR-VP tests indicated the culture was negative for both acid and acetoin formation, which is normal for non-fermentative organisms (3). Cells from these yellow-pigmented colonies on nutrient agar were found to be oxidase-positive and non-motile in both SIM medium and in a wet mount. They cannot utilize citrate as their only carbon source, and cannot hydrolize urea. Their ability to withstand 7.5% NaCl (as indicated in table 1), was not unexpected as the 1986 Bergey's Manual confirmed that many species of *Micrococcus* are somewhat salt tolerant because they can be found living on the mildly salty surface of human skin (4). The culture was negative for the production of aerobic acid on both glucose and mannitol.

Results obtained with nucleotide sequence analysis using the Basic Local Alignment Search Tool of NCBI indicated that a 786 base portion of the culture's 16S r-DNA was 100% similar to that of *Micrococcus luteus* strain EHFS1_SO4Ha. This finding, plus the results obtained with the physiological tests performed suggest strongly that the culture isolated was *Micrococcus luteus*. These organisms are common to the environment and not harmful to humans or other animals under normal circumstances. They can help defend the body by preventing the growth of potentially pathogenic bacteria, and contribute to the formation of body odor by breaking down compounds present in sweat (5). This identification was important in that it shows humans do live amongst and interact with microorganisms they may not be aware of. It also shows that *Micrococcus luteus* can survive suspended in air inside a refrigerator.

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Vo1. 2, No. 1

Isolation of Gram-positive *Bacillus megaterium* From a Dumbbell at a Local Gym

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A bacteria culture isolated from a 15-pound dumbbell at a local gym was determined to be *Bacillus megaterium*. To assist in this identification, colony morphology was documented, a DNA analysis was conducted involving 16S ribosomal DNA nucleotide sequencing, a series of slide stains were prepared and observed and enzymatic testing was completed including a catalase test, MR-VP tests, carbohydrate slant tests, a starch hydrolysis test and a citrate utilization test. The incidence of bacteria on gym equipment is a reality along with the risk of contracting an illness from bacteria located on gym equipment. Through the use of proper sanitization techniques prior to leaving the gym it is possible to avoid some of those risks.

INTRODUCTION

When a person goes to the gym it is in the hopes of becoming healthier and stronger. A reputable gym will typically employ a cleaning company to maintain the cleanliness of the gym and its equipment, but with so many people frequenting the gym, it is naive to assume that the equipment is free of bacteria.

If equipment at the gym does contain a variety of microorganisms then it would suffice to say that a random sample taken from a piece of equipment would indeed prove the existence of bacteria. Keeping this hypothesis in mind, a project was conducted to determine not only if bacteria inhabit the various pieces of equipment, but what exactly can be found.

MATERIALS AND METHODS

To determine if bacteria were present, the handle of a 15-pound dumbbell was rubbed with a sterile cotton swab dampened with sterile de-ionized water. A Petri plate containing nutrient agar was inoculated and left to incubate agar side up at room temperature. Within 48 hours a variety of bacterial colonies had formed. Upon choosing a single, well-isolated colony, another Petri plate containing nutrient agar was streaked to develop a pure culture. Following isolation, different identification procedures were completed including a KOH test, an indirect stain, and an endospore stain. The indirect stain was prepared using the reagent Nigrosin and the endospore stain was prepared using the Malachite Green Stain. Additional tests completed were found in the Microbiology Laboratory Manual (5).

A chromosome extraction was completed using the following procedure: A loopful of cells was aseptically transferred into a sterile tube containing 500µL of 10mM Tris

buffer. Sterile glass beads were added and the solution was briefly vortex mixed. The tube was then placed in a foam float and boiled gently for 10 minutes. Once cooled, the tube was placed into an ice bath to cool and then strapped to a vortex mixer and vibrated for 10 minutes (5).

Following the chromosome extraction, materials were prepared to carry out the polymerase chain reaction. The mixture placed in a sterile tube included 25µL of Taq Polymerase Master Mix (Qiagen), 5.0µL primer mix (Bacteria 8-Forward and 1492-Reverse primers), 5.0µL of the template DNA, and 15µL of sterile, distilled water with a pH of 8.0. The tube was placed into an ice bucket and then a thermal cycler for approximately 3 hours. The PCR product DNA was then run in an agarose gel and made visible with Ethidium Bromide. The DNA was then cut from the gel and purified with a QIAQuick gel extraction kit.

The purified DNA sample was later taken to the College of Biological Sciences DNA Sequencing Facility at UC Davis, California, and the sequence data from three primers was returned electronically. The evaluation of the electropherograms and final editing was completed using Macintosh OSX and 4Peaks software. The entire nucleotide sequence was uploaded into the National Center for Biotechnology Information (NCBI) website to be compared with data in the database through the Basic Local Alignment Search Tool (BLAST) algorithm (1).

Having gained information from the NCBI database and the BLAST algorithm, it was possible to research various features of the bacteria isolated by consulting the Bergey's Manual of Systematic Bacteriology. A number of enzymatic tests were then performed to confirm those results. The physiological tests performed included a catalase test, MR-VP tests, starch hydrolysis test, citrate utilization test, and carbohydrate slant tests (aerobic acid formation) with glucose, arabinose, and mannitol.

RESULTS

The colonies observed on the pure culture plate were circular to irregular, entire to undulate, raised, shiny when young, but dull with age, opaque, light cream-yellow in color and ranging in size from 1-5mm in diameter. Results obtained with the KOH test showed no change in viscosity, confirming that the bacteria had a Gram-positive type wall with thick peptidoglycan. An indirect stain was performed to determine the size and shape of the cells. The cells appeared ellipsoidal and ranged in size from 1.5 to 5µm in length with centrally located endospores and non-swollen sporangia. Their shape was short rods and/or bacilli. Their arrangement was chains and/or single cells.

The results obtained from the DNA analysis and BLAST indicated that the 16S r-DNA from the dumbbell isolate showed a 100% similarity with a GenBank sequence from *Bacillus megaterium*. The sample showed 1310/1310 bases matching pairwise with the strain DPBS17, accession number EU249559 (1).

The results obtained during the series of test are identified below. The morphological features observed on the Nutrient agar plate were compared to those listed in the Bergey's Manual and are shown for comparison (2).

The series of enzymatic tests conducted to confirm the NCBI BLAST results yielded the following. The catalase test produced bubbles when 3% H_2O_2 was added to a sample of the culture which indicated a positive test. The Voges-Proskauer Test came back negative for acetoin as indicated by the presence of a yellow-brown color. The Methyl -Red portion of the MR-VP test produced a yellowish-brown color also indicating it was negative for large quantities of acid. The culture grew on a starch agar plate, but when Gram's iodine was added, the medium turned brown indicating the starch had not been hydrolyzed. The citrate test displayed a negative result as the agar in the slant remained green. Two of the carbohydrate slants, glucose and mannitol contained a yellow-colored medium indicating aerobic acid formation from these two carbohydrates was positive. The test for aerobic acid from arabinose was negative which was indicated by a bright pink agar slant. These results were compared to those found in the Bergey's Manual and are listed below (2).

Table #1: Colony Morphology.

Colony Morphology	**15lb. dumbbell handle**	**Bacillus megaterium as listed in Bergey's Manual.**
Form	Circular to Irregular	Irregular
Margin	Entire to Undulate	Not listed
Elevation	Raised	Raised, wrinkled
Surface Texture	Shiny, sometimes dull	Dull to glossy
Optical Character	Opaque	Not listed
Pigmentation	Light, cream yellow	Yellowish, darkens with age
Size (diameter in mm)	Variable; 1-5mm	Variable
Other: Media used	Nutrient agar	Nutrient agar

Table #2: Enzymatic Testing

Test Conducted	**15 lb. dumbbell handle**	**Bacillus megaterium as listed in Bergey's Manual.**
Catalase	Positive	Positive
MR-VP	Negative	Negative
Starch	Negative	Positive
Citrate	Negative	Positive
Glucose	Positive	Positive
Arabinose	Negative	Variable
Mannitol	Positive	Variable

DISCUSSION

The bacteria existing on the handle of the 15-pound dumbbell were determined to be *Bacillus megarterium.* The taxonomic lineage for this species is: Bacteria; Firmicutes; Bacillales; Bacillaceae, Bacillus (1). *Bacillus megaterium* will grow in minimal medium without any special added growth factors (3) and will typically colonize in less than a 24-hour period on nutrient agar. Because they form endospores, they are very resistant to radiation, heat, and other environmental conditions. The majority are mesophiles, and their

optimum growth temperature lies between 30 and 45 degrees (4). According to Todar's Online Textbook of Bacteriology, cultures of *Bacillus megaterium* are commonly used as a soil innoculant in agriculture and horticulture as well as many Gram-positive studies in laboratory settings (4). *Bacillus* species are used in many medical, pharmaceutical, agricultural, and industrial processes that take advantage of their wide range of physiologic characteristics and their ability to produce a host of enzymes, antibiotics, and other metabolites (4). Certain strains of *Bacillus* are dangerous, such as *Bacillus cereus*; which may cause food poisoning or *Bacillus anthracis*; which is the causative agent in anthrax (4); however, no information was evident to prove that *Bacillus megaterium* poses any real threat which is a good thing because it can be found in a wide variety of places, including on 15-pound dumbbells at the local gym.

When using equipment at the local gym, it's important to remember that reservoirs are prevalent everywhere and fomites provide a viable source for bacteria, therefore careful hygiene is of utmost importance. The bacteria that were identified living on the equipment may have no adverse effect on one person, but to another who may suffer from an immunocompromised immune system it could prove serious. While using the equipment in the gym remember to keep your hands away from your mouth and eyes at all times. Also, before leaving, take the time to go to the restroom and wash your hands thoroughly. If these recommendations are followed, the chance of acquiring anything that could compromise your immune system would be greatly reduced.

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Isolation and Identification of Bacteria Collected from a Dog's Mouth in Rocklin, California

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An unknown type of Gram-negative, rod-shaped, strongly oxidase and catalase-positive bacteria was collected and identified from the mouth of a family dog. 16S ribosomal-DNA sequencing and biochemical testing confirmed the culture's identity. Sequencing results exhibited 99% similarity with a Gene Bank entry; accession number AF395911.1. This isolate had long been known as CDC group M-5, until 1993 at which time the culture was given the name *Neisseria weaveri*. Prior to 1993, studies showed that M-5 was often isolated from dog bite wounds. Subsequent investigations have confirmed *N. weaveri* is found as part of the normal flora in dog mouths and is associated with dog bite wounds.

INTRODUCTION

The purpose of this investigation was to isolate and identify a sample of bacterial flora from a dog appearing to have relatively clean teeth, but somewhat fowl-scented breath. It has long been said that dogs have cleaner mouths than humans, yet they tend to feed on any and everything they come into contact with regardless of how sanitary the items may be. How many times has the family dog been found eating out of the trash or eating the cat's waste? How frequently have they then decided to give their master a big lick across the face? With dog behavior firmly in mind, the questions arose as to what microorganisms might live inside a dog's mouth, and if present, could they be pathogenic, or do they form part of the dog's normal flora living in perfect harmony. This project afforded the investigator the opportunity to isolate one of the many types of organisms present in a healthy dog's mouth, and to relate the findings to pathology.

MATERIALS AND METHODS

The dog used for this identification was a four-year old domesticated Boxer. A specimen was collected using a sterile cotton tip applicator by rubbing the tip of the applicator at the gum line against plaque located on one of the dog's back molars (see Fig. 1). Other than the presence of this brown looking plaque and a somewhat fowl odor, no apparent infection or decay was observed in the dog's mouth.

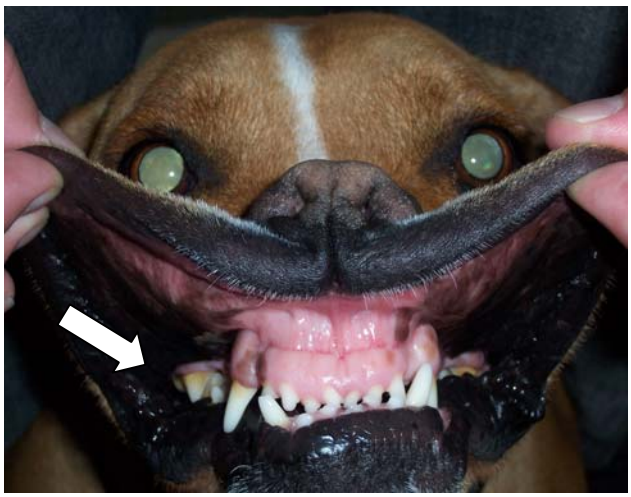

FIG. 1. Arrow shows location of specimen collection.

The sample was immediately streaked onto an agar plate containing enriched medium (Columbia CNA with 5% sheep blood) and incubated at room temperature for 48 hours. Various colonies grew, but only one isolated colony was selected and transferred by means of a sterile wire loop to another blood agar plate. A pure culture with well-isolated colonies was obtained and a Gram stain and potassium hydroxide (3% KOH) test were performed to determine the cell wall type. A sample colony was then used to extract chromosomal DNA. A loopful of cells was added to 500µl of 10mM Tris buffer (pH 8.5) in a sterile 1.5ml microfuge tube and the sample was agitated on a vortex mixer to mix the sample with the buffer solution. The mixture was then boiled for ten minutes to release DNA from the cells and to denature the cellular enzymes present (14).

The Polymerase Chain Reaction (PCR) was then used to amplify a portion of 16S ribosomal-DNA using the primers Bacteria 8-Forward and Universal 1492-Reverse. The reaction mixture contained Taq-polymerase master mix (Qiagen), template DNA from the boiled cell mixture, a primer mix and sterile distilled water (Qiagen). The PCR product DNA was then subjected to gel electrophoresis. The DNA sample was mixed with tracking dye containing xylene cyanol and bromphenol-blue and placed into the wells of an agarose mini-gel. Upon completion, the gel was stained with ethidium bromide solution to make the PCR product DNA visible. The visible DNA band was then cut from the gel and purified with a QIAquick gel extraction kit (Qiagen). The purified product was then sent to the Division of Biological Sciences sequencing facility at UC Davis, and nucleotide sequence data was generated using the primers bacteria 8-forward, internal 533-forward and universal 1492-reverse. Results in the form of electropherograms were returned electronically to the Sierra College Microbiology laboratory for evaluation and edited using Mac OSX and the software program 4Peaks. The sequence data obtained from the subject culture was compared to others available in the pubic databases by accessing the National Center for Biotechnology Information (NCBI) website Basic Local Alignment Search Tool (BLAST) feature (14). After the BLAST results provided a reasonably certain identification for the culture, the 2005 version of Volume 2 of the Bergey's Manual of Systematic Bacteriology

(Betaproteobacteria) was consulted to determine which physiological tests should be used to further confirm the identification of the unknown culture. Live samples from the isolate were then subjected to various enzymatic tests. These included glucose, sucrose and lactose slants to determine if or not the culture could form aerobic acid through carbohydrate utilization, an oxidase test and a catalase activity test. In addition, a wet mount motility test was performed and a Mac Conkey's agar (MAC) plate was inoculated to determine if or not the culture could grow in the presence of crystal violet. A separate blood agar plate was heavily inoculated and used to determine the type of hemolysis reaction the organisms were capable of.

RESULTS

The blood agar plate presented circular colonies 1-2mm in diameter with entire margins and raised elevation after 48 hours of growth. The surface of the colonies appeared shiny, the optical character was semi-opaque, and the pigmentation was a dark-milky creamy color. The second blood agar plate grew colonies that caused the formation of a slight green coloration in areas of heavy growth. A slight fowl odor was detected. The Gram stain indicated pink Gram-negative rods of varying lengths with most being from 1.5 – 2.0μm in length and from .5 – 1μm in width. Most of these bacteria were arranged as single-cells, but some occurred in short chains. The rods had distinctly parallel sides with rounded edges (Fig.2).

When a sample of fresh culture material was rubbed onto filter paper wetted with oxidase test reagent, it turned dark purple within 5-10 seconds. The material on the toothpick used also turned dark purple. When a sample of cells was placed onto a clean glass slide with a drop of 3% hydrogen peroxide, the reaction was characterized by rapid development of a white froth. The glucose, sucrose and lactose slants containing the pH indicator phenol red did not change color, but remained red/pink. The wet mount motility test revealed cells not capable of moving against water currents under the coverslip, and there was no growth on the MAC agar plate after 48 hours of incubation.

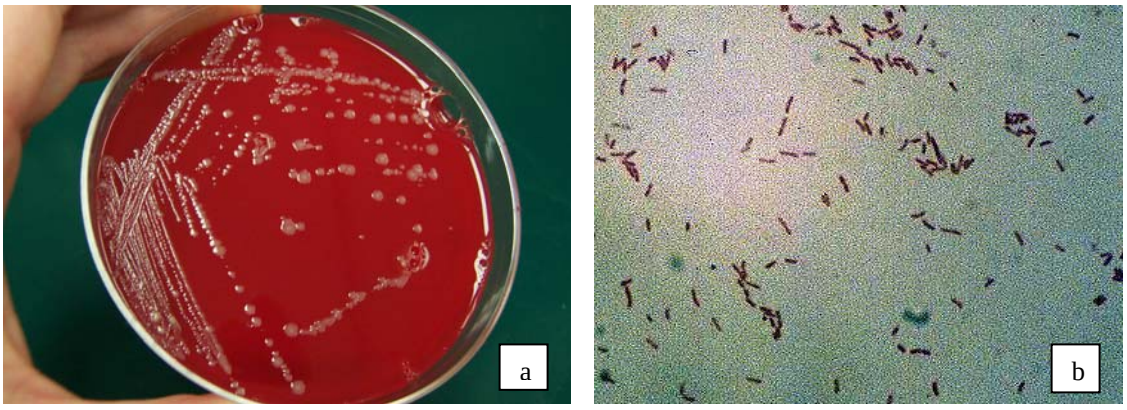

FIG. 2. a) *Neisseria weaveri* colonies on sheep blood agar at 48 hours of growth; b) and representative cell morphology of Gram-negative rods (6).

The electropherogram analysis and GenBank comparison with the NCBI, BLAST was completed on December 7, 2007. The identity of the species showing the greatest similarity at 99% was *Neisseria weaveri* isolate VA6362grgr_2001, with the lineage Bacteria; Proteobacteria; Betaproteobacteria; Neisseriales; Neisseriaceae; Neisseria. The query length was 1439 bases with 1438/1439 bases matching pairwise. The GenBank sequence showing greatest similarity was 1462 bases in length, the bit score was 2652 and the accession number was AF395911. There was also a second GenBank entry, accession number L10738.1 with 99% similarity to the query. This isolate was also identified as *N. weaveri* with a bit score of 2619 with 1426 out of 1429 bases matching pairwise.

DISCUSSION

The culture isolated from the dog mouth used in this investigation contained Gram-negative, rod-shaped, non-motile cells arranged singly and in short chains. The oxidase test was strongly positive confirming the presence of cytochrome c oxidase. The catalase activity test was also strongly positive indicating the presence of catalase enzymes. The culture did not form aerobic acid when grown on glucose, sucrose and lactose agar slants, and it would not grow on MacConkey's agar containing crystal violet. Areas of heavy growth on blood agar indicated weak α-hemolysis of sheep erythrocytes was possible. All of these results are consistent with those indicated in the Bergey's Manual as being characteristics of *Neisseria weaveri*.

Following the identification of *N. weaveri*, further research was completed to determine if or not this bacterium should be present in a dog's mouth. *N. weaveri* was previously identified as CDC group M-5, a type of Gram-negative bacteria associated with dog bite wounds. The Special Bacteriology Reference Laboratory, Menignitis and Special Pathogens Branch of the Centers for Disease Control and Prevention, in Atlanta, GA, had received more the 160 different isolates for identification between 1960 and 1992 and all were categorized as CDC group M-5 (1). In 1993, the 16S r-RNA sequence for strain CDC 8184 was deposited in GenBank under accession number L10738, with the proposed name of *Neisseria weaveri* as a new species. This species was named in honor of Robert E. Weaver, for his contributions to the identification and classification of unusual pathogenic and opportunistic bacteria (1, 8). These bacteria have been isolated as normal flora from dog's mouths (3, 5), and further isolated as gingival flora. A study collecting gingival flora from 50 dogs, identified M-5 (along with many other flora), as being the most frequently isolated bacteria (12). There are several reports of *N. weaveri* being isolated from dog bite wounds dating back to 1962 in which the isolate was referred to as M-5 (1, 8). These bacteria are known to be commensal in the upper respiratory tract of dogs (9). One isolated case identified *N. weaveri* as being the cause of a lower respiratory tract infection in a 60-year old man. (11). Another isolated case identified *N. weaveri* from a wound infection resulting from a tiger bite in a 7-year old girl (4).

Although *Neisseria weaveri* has been isolated from infected bite wounds, its occurrence is generally less common (10) than some other potential pathogens. *Pasteurella multocida* and *Staphylococcus aureus* are the species most frequently isolated from bite wounds (3). In addition, *N. weaveri* is one of only two (the other being *N. elongate* of

human origin) *Neisseria* species that have the rod or bacillary morphology (13). Due to the uniqueness of these rod-shaped *Neisseria,* some laboratories may confuse them with the more pathogenic species of *Pasteurella* or *Moraxella* (6). Typically, dog bite wounds are treated relatively quickly due to concerns about rabies or tetanus and due to the need of repairing the damaged tissue. This coupled with the sensitivity of *N. weaveri* to penicillin may explain why the isolation of these bacteria from bite wounds occurs less commonly (10). *N. weaveri* has been isolated from dogs with periodontal disease. Diseases of the teeth and gums are fairly common in dogs in large part due to the poor care provided by their owners (5). It is logical to conclude that because all dogs lick (and some bite), their normal oral flora could be passed to a susceptible human host and have the potential of becoming opportunistic pathogens.

After using DNA sequencing and BLAST search results, the Bergey's Manual, and a number of physiological tests available in the laboratory, it was concluded with practical certainty that the bacteria isolated from the subject dog's mouth were *Neisseria weaveri*. The odor emitted by colonies growing on blood agar plates was familiar and strongly resembled that noted originally as associated with the dog's breath. Although this feature was very subjective, it was, none-the-less, noted. The tests preformed, as described above, provided results consistent with various published study results. The bacteria isolated from the dog's mouth were non-motile, strongly oxidase and catalase-positive, did not grow on Mac Conkey's agar with crystal violet, and did not form aerobic acid from the carbohydrates glucose, sucrose or lactose. The cell and colony morphology were also consistent with those described in published works (1, 8, 13). Although materials were not available in this laboratory, further biochemical analysis would reveal these bacteria can use nitrite, but not nitrate as a final electron acceptor and show a weak yet positive phenylalanine deaminase reaction (1, 8). Nitrate reduction involves the enzyme nitrate reductase, and phenylalanine deaminase medium tests the ability of organisms to produce the enzyme deaminase (2). These tests would be significant in distinguishing between *N. weaveri* and some other, similar oxidase-positive species (1).

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Vol. 1, No. 2

Isolation and Identification of an Airborne *Staphylococcus lugdunensis* from a Teenager's Room

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A bacterial colony was selected from a variety of colonies present on an agar plate exposed to air in a cluttered teenager's room. Pure culture and Gram stain characteristics were established and DNA was extracted and amplified via the Polymerase Chain Reaction (PCR). The PCR product was purified and submitted to the UC Davis DNA Sequencing Facility for sequencing. The resulting 16S r-RNA gene nucleotide sequence was then compared to the public database available at the National Center for Biotechnology Information (NCBI) using the BLAST algorithm. Comparison results indicated the organisms under investigation were 99% similar or phylogenetically related to *Staphylococcus lugdunensis* (Accession: AB009941). Negative coagulase test results confirmed that the organisms differ from *Staphylococcus aureus*, a type of coagulase-positive staphylococcus, frequently a source of misidentification for *S. lugdunensis*.

INTRODUCTION

Staphylococcus lugdunensis is a type of Gram-positive and coagulase-negative organism usually found on human skin in the inguinal area of the body. It was first described in 1988 as a cause of aggressive endocarditis but is now considered an emerging etiologic agent of many serious human infections such as ostemyolitis, arthritis, septicemia, soft-tissue abscess of the pelvic girdle region, and catheter, prosthetic joint, and urinary tract infections (1,3,4). These organisms have also been identified as etiologic agents of numerous infections associated with biofilm formation, a property that leads to high resistance to antibiotics (2). *Staphylococcus lugdunensis* share many biochemical properties with *Staphylococcus aureus,* but do not produce a free coagulase. They also differ from the majority of other *Staphylococcus* species because they are capable of decarboxylating the amino acid ornithine (3).

MATERIALS AND METHODS

A nutrient agar plate (NA) was exposed to air in a teenager's room for about 24 hours with the lid off, and brought back to the Sierra College Microbiology Lab to incubate at room temperature in a lab drawer for two weeks. In order to limit the scope of the study, only one type of colony, a small circular bright-yellow colony, was selected for the study. A sample from the colony was streaked onto a new NA agar plate and incubated at 37°C. After 48 hours of incubation, the well isolated colonies (faint yellow as pure culture) were

tested with 3% KOH, a Gram and a Nigrosin-indirect stain (5). In addition, a coagulase test using a small plastic tube containing 0.5ml of coagulase plasma was performed after the identification of the organism was achieved through the DNA analysis procedure described below.

Chromosomal DNA was extracted by boiling a mass of the test cells in 500µl of 10mM Tris buffer (pH 8.5) in a test tube containing glass beads for 10 minutes. These cells were then beaten by vortexing the mixture for 10 minutes to ensure cell wall breakage. A PCR mixture was prepared by adding 25µl Taq DNA Polymerase Master Mix (Qiagen), 5.0µl primer mix (Bacterial 8-Forward and Universal 1492-Reverse), 5.0µl of template DNA extract, and 15µl of distilled water (pH 8.0) to a PCR tube and then placing it in ice. The mixture was processed through a thermal cycler for amplification of 16S ribosomal DNA. The total PCR product DNA was then subjected to Gel electrophoresis in an agarose gel with TBE buffer. This was stained with ethidium bromide and then the DNA fragments were cut from the gel and purified using a QIAquick Gel Extraction Kit (Qiagen). The purified DNA was then taken to the ^{uc}DNA Sequencing Facility for sequencing. Sequencing results were obtained in the form of electropherograms, one for each primer used, bacteria 8-forward, 533-forward and 1492-reverse. These were edited using 4-peaks software and converted to a word-file representing an editable base sequence. Suspected base sequence overlaps and discrepancies were edited out and the resulting sequence was compared to the National Center for Biotechnology Information (NCBI) public database of reference organisms using the Basic Local Alignment Search Tool (BLAST).

RESULTS

During the first week after exposure to air the NA plate exhibited little or no growth. Extensive growth of several bacterial and fungal colonies appeared on the plate after the second week of incubation. The colonies were morphologically diverse in color (tan, yellow, orange, and black) and colony growth patterns. The small, bright-yellow colonies, selected and isolated for the study, exhibited a very slow growth pattern and decreased color intensity (from bright-yellow to faint-yellow) after the first isolation pass (Table1, Figure 1). The KOH test result was negative for slimy-viscosity formation. Gram and indirect stains showed dark purple, Gram-positive cocci arranged in clusters (Table2, Figure 2).

Table 1: Colony Morphology

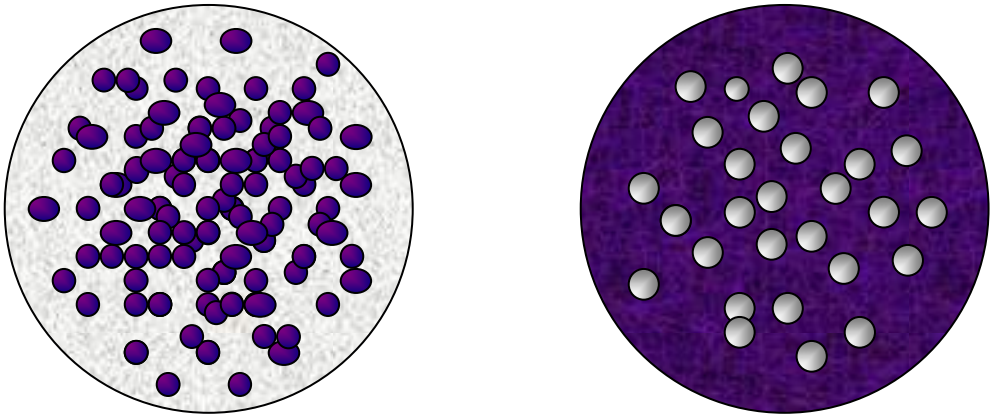
Size	small (1-3mm)	**Surface Texture**	smooth
Form	circular	**Optical Character**	opaque
Margin	entire	**Pigmentation**	yellow
Elevation	convex		

Table 2: Cell Morphology

Gram Stain	Gram-positive
Shape (Gram stain)	cocci
Size (indirect stain)	0.5 - 1.0 µm
Arrangement (indirect stain)	clusters

Figure 1: Streaked NA Plates

Figure 2: Stain Characteristics



Gram Stain **Indirect Stain (Nigrosin)**

Result from the 16S ribosomal-DNA comparison to the NCBI public database showed a strong similarity (99%) between the unknown organisms and *Staphylococcus lugdunensenis* (Accesion: AB009941). Negative coagulase test results further confirmed that the organisms isolated from the room air are most likely not *Staphylococcus aureus*, a coagulase positive type of organism sharing many biochemical properties with *Staphylococcus lugdunensis*.

```
LOCUS       AB009941                1492 bp    DNA     linear   BCT 09-
JAN-1998
DEFINITION  Staphylococcus lugdunensis gene for 16S ribosomal RNA.
ACCESSION   AB009941
VERSION     AB009941.1  GI:2760733
KEYWORDS    16S rRNA; rrn.
SOURCE      Staphylococcus lugdunensis
  ORGANISM  Staphylococcus lugdunensis
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1
  AUTHORS   Takahashi,T., Satoh,I. and Kikuchi,N.
  TITLE     Phylogenetic relationships of Staphylococcus based on 16S
rDNA sequence analyses
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DISCUSSION

This study included only one biochemical test, the coagulase test, because most biochemical tests used for bacterial identification give the same results for both *Staphylococcus lugdunensis* and *Staphylococcus aureus*. The organisms were also not tested for ornithine decarboxylation, though a positive ornithine decarboxylase can identify *Staphylococcus lugdunensis* with a considerable degree of accuracy (3). Materials necessary for an ornithine decarboxylase test were not readily available in the laboratory.

Information provided in the Manual of Clinical Microbiology stated that *Staphylococcus lugdunensis* colonies are usually large (4-7mm) and the organisms grow readily on enriched media such as blood agar and chocolate agar (3). The unknown organisms used for this study were slow-growing and formed colonies that were smaller than expected. This was probably due to the fact that these organisms were grown on NA instead of an enriched medium.

ACKNOWLEDGMENTS

Special thanks to Ms. Harriet Wilson, professor of Microbiology at Sierra College, for her invaluable instruction and for her tireless effort and support during the process of DNA extraction, amplification, and analysis; Mr. Jim Wilson for his computer assistance with the sequencing data; North Valley and Mountain Biotechnology Center and Sierra College foundation for the continuous financial assistance; and personnel of the UC Davis Sequencing Lab for their valuable DNA sequencing services.

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In the Bedroom: The Isolation and Identification of *Staphylococcus xylosus*.

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The bacteria utilized in this investigation were randomly selected for testing from a plate left open to air in a teenage boy's bedroom. A pure culture was established and the morphological features of colonies and cells were documented. A portion of 16S r-DNA from the isolate was amplified with the PCR, subjected to gel electrophoresis, purified in the microbiology laboratory and then sequenced by personnel in the ^{UC}DNA Sequencing Facility at UC Davis. Upon return of the electropherogram data, a BLAST comparison was used to determine that DNA from the air isolate showed greatest sequence similarity with bacteria in the genus *Staphylococcus,* species *xylosus* and *saprophyticus*. Consultation with the Bergey's manual showed these two species to be highly similar, but distinguishable on the basis of aerobic acid formation from arabinose. Following completion of this test, the culture was identified as *Staphylococcus xylosus*.

INTRODUCTION

Though it is not possible for organisms to utilize air for food, bacteria are certainly supported by and carried through the air. It is amazing to leave a plate exposed to what appears to be open air and see what grows on that plate 48-72 hours later. Colonies form in different colors, shapes and sizes, causing observers to wonder what people are exposed to each day without even being aware of it. Bacteria live and reproduce in soil, in water, on plant surfaces and in association with humans and other animals. They are literally all around us, everywhere and all the time.

Any parent of teenage boys knows that they are sometimes not the cleanest or most sanitary of individuals. The 17 year-old brother of the author frequently forgets (or just doesn't bother) to pick up his room, do his laundry, or otherwise maintain cleanliness in his living space. One day, after he and a few of his somewhat unhygienic friends infested his room for a few hours, an open Petri dish was left on his bed to see what kinds of contaminants teenage boys might be carrying. The organisms found on the Petri dish in the days that followed were not exclusively normal flora. However, dirty teenage boys are not the only individuals exposed to and breathing in bacteria on a daily basis. This is common to everyone, everywhere in the world.

Observation of the plate left in a seemingly normal room was mildly disturbing and thought provoking. Is this what people are exposed to day in and day out and is this what

we inhale? What types of organisms are actually present, and why are people not becoming ill from them…because on the plate, they certainly look sickening.

MATERIALS AND METHODS

The initial step used in determining what grows in a teenager's room was to leave a nutrient agar plate open in an area of the room where it could collect the most bacteria. The bed was not made, is in the center of the room and had people sitting on and around it, so it was chosen as a good place to leave the plate. After being open and exposed for approximately 90 minutes, the nutrient agar plate was sealed with tape and returned to the microbiology laboratory two days later. Growth visible on the agar surface was very exciting: all sorts of organisms of different colors were growing. The colony chosen for testing was selected because it was visually appealing: yellow and star-shaped. A sample was collected and streaked onto a new plate of nutrient agar that was maintained at room temperature in a lab drawer. Several days later, a pure culture was observed on the plate.

After a pure culture had been obtained, a Gram stain and a KOH test were completed to determine the cell wall composition of the organisms present. Following this, the very important DNA analysis of the unknown organisms was initiated by extracting the chromosomal DNA with cells boiled in 10mM Tris buffer solution. Glass beads were then used to beat the cells and break open the cell walls. Next, the amplification of the 16S r-DNA was completed using the Polymerase Chain Reaction and Taq Master Mix (Qiagen). The primers used were Bacteria 8-forward and 1492-reverse. Following amplification, Gel Electrophoresis was conducted using agarose and TBE buffer. Purification of the DNA samples was completed with the help of a QIAquick Gel Purification Kit. When the DNA samples were purified successfully, the finished product was taken to the ^{uc}DNA Sequencing Facility, located in Storer Hall at the University of California, Davis. The sequencing primer used at this facility was bacteria internal 533-forward.

After the sequencing data was received by the college it was posted on the microbiology web site and the electropherograms were evaluated and edited using Mac OSX and the 4Peaks program. The final step in the DNA analysis for this project was to compare the sequence data received from UCD to the information available in the public databases; accomplished by accessing NCBI and using the BLAST algorithm. When the BLAST results had been obtained, the Bergey's Manual of Systematic Bacteriology was consulted to determine what additional tests could be conducted to verify the identification. A test to determine aerobic acid formation on arabinose was then completed.

RESULTS

The colonies growing on the pure culture plate measured 1-4mm in diameter and were highly pigmented with a bright orange-yellow color. Their shape was irregular, like little flowers or stars. They were raised with an undulate margin and their surface appeared to have a slightly shiny sheen when seen magnified with the stereomicroscope. A Gram stain showed the cells to be Gram-positive and arranged in clusters; they resembled little grape bunches. Individual cells ranged in size from 0.8 to 1.2mm in diameter.

Results obtained with the NCBI BLAST showed the 16S r-DNA sequence from the air isolates were 99% similar with a nucleotide sequence from *Staphylococcus xylosus*; however, there was also a close match with a nucleotide sequence from *Staphylococcus saprophyticus*. When the Bergey's Manual of Systematic Bacteriology was consulted, it showed that the majority of the characteristics listed for these bacteria were very similar, but that they differed in their ability to form aerobic acid from xylose, mannose and arabinose. When this culture was streaked on a carbohydrate slant containing arabinose, the medium turned from red to yellow in color indicating aerobic acid formation and the culture on top turned an opaque cream color.

DISCUSSION

The two species of *Staphylococcus* showing high 16S r-DNA nucleotide sequence similarity with the air isolate were *S. xylosus* and *S. saprophyticus*, and are very closely related to one another. Both are coagulase negative, facultative anaerobes capable of both respiratory and fermentative metabolism. Like several other species of *Staphylococcus*, they are resistant to antibiotics, such as Novobiocin and Erythromycin, and susceptible to Penecillin, Methicillin and Teicoplanin. Biochemically they are highly reactive, and able to produce aerobic acid from several carbohydrates. According to the Bergey's Manual the only tests showing variation between these two species were aerobic acid formation from xylose, mannose and arabinose. Since arabinose was the only carbohydrate from this set available in the laboratory, the differentiation was dependent on this test. The medium inoculated with the air culture turned yellow, testing positive for aerobic acid formation from arabinose. This canceled out *S. saprophyticus*, as a contender because they are unable to form acid from arabinose.

Staphylococcus xylosus are relatively common Gram-positive staphylococci. They are found most frequently on the skin of humans and animals, in animal foods (like milk, cheese and sausages), and in the environment. This *Staphylococcus* species is one of the main cultures used for meat fermentation. It is mostly non-pathogenic; however, there are a few strains, *S. xylosus* 00-1747 and *S. xylosus* S04009 that cause dermatitis in mice and mammary infections in cows. The reported cases of infection have been vastly more in animals than in humans; however, it is thought to be a causative agent in some cases of pyelonephritis in humans.

As stated, *Staphylococcus xylosus* are primarily non-pathogenic organisms and this is the reason they do not make humans sick. It is important to remember that not all bacteria are disease causing, and that although on the plate they may look disgusting, many of them are harmless. Looks can sometimes be deceiving.

One theory about how the *Staphylococcus xylosus* ended up on the agar plate exposed to air is that they came from one of the humans present. These organisms are often found growing on the skin of humans and animals as part of their normal flora. The bacteria could have been on someone's skin cells, and when these were shed, they floated onto the plate. Another possibility is that the bacteria were associated with food being consumed in the room, such as cheese or sausage. These could also have entered the air as the boys ate

and talked. In either case, when provided with a suitable environment, the bacteria grew and formed interesting colonies.

**The author is pleased to state that since the young man observed the microbes growing on the Petri dish exposed to the microbes from his room, he made considerable effort toward becoming a cleaner individual.

ACKNOWLEDGEMENTS

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Vol. 1, No. 2

Isolation of *Bacillus* from a Fresh Water Fish Tank Filter

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A sample of bacteria collected from a tropical freshwater fish tank from Grass Valley, CA was isolated and a pure culture was established on nutrient agar. A Gram stain and a KOH test were completed to determine cell wall composition. After chromosomal DNA extraction, Polymerase Chain Reaction amplification and gel electrophoresis, a sample of purified 16S r-DNA was taken to U.C. Davis for nucleotide sequencing. When the electronic version of the nucleotide sequence was returned, it was edited and pasted into the Basic Local Alignment Search Tool of NCBI for comparison with data in GenBank. The bacterial culture isolated showed 98% similarity with three different types of organisms: *Bacillus cereus, Bacillus thuringiensis,* and *Bacillus anthracis*. In an attempt to determine which of these organisms was cultured from the fish tank the following tests were run on these organisms: Methyl-Red, Voges-Proskauer, aerobic acid formation from glucose, mannitol and arabinose, starch hydrolysis, citrate utilization, urea hydrolysis, glucose gas production, and a catalase test. The results obtained from these tests did not verify which species was cultured. A more sophisticated test would be needed for positive identification. According to research, many microbiologists believe these three organism types are of the same species, which has potential consequences since these are all endemic in many areas of the world and presumably interact with each other in the environment (2,6).

INTRODUCTION

Curiosity about the types of organisms growing in the filter of a fish tank led to the initiation of an investigation focused on that environment. It was expected that the bacteria present in the filter might be unusual in a household, but residing there because of the moist environment. In addition, since the water leaving the filter aerates the tank and some water splashes into the air, it was suspected that some bacteria living in the filter might also be found floating in droplets in the surrounding air of the room. Little information was available regarding the types of bacteria likely to be found living in a fish tank; however, two types of beneficial bacteria are *Nitrosomonas,* which convert ammonia into nitrites and *Nitrobacter,* which convert nitrites into nitrates. These bacteria keep the ammonia produced by fish waste from becoming toxic to the fish, and without them the fish in the tank can potentially die (3).

MATERIALS AND METHODS

A sample was taken from the fish tank filter under investigation by swiping an area of the filter just barely underwater with a sterile cotton swab. The filter was a Penguin Biowheel 100 mounted on the back of a 10 gallon fish tank containing 2 fish, a Placostamus and a Gourami. The temperature of the tank was 76 degrees Fahrenheit. The cotton swab was rubbed over the agar surface of a nutrient agar plate. A pure culture was established by streaking a sample from an isolated colony on the original agar plate onto a new plate of a nutrient agar.

Following isolation, the cultural characteristics of the isolate were documented and a KOH test was conducted to determined the cell wall composition. A Gram Stain, a Malachite-Green endospore stain, and a nigrosin indirect stain were also prepared in order to determine cell size and shape along with the presence of endospores and their size, shape and location.

After cell and colonial features had been recorded, chromosomal DNA was extracted from the culture using the following procedure. A sterile wire loop was used to aseptically transfer a blob of cells from a single colony into a tube of Tris buffer. Several 2mm glass beads were also added. The mixture was gently vortexed to mix the bacteria with the Tris buffer and then gently heated in boiling water, using a foam float. Following 10 minutes of boiling, the glass beads were used to beat the tube contents for 10 minutes while the tube was strapped on a vortex mixer.

The next step in the identification of the unknown culture involved placing a sample of its chromosomal DNA in a thermal cycler to amplify the 16S r-DNA through a process known as the Polymerase Chain Reaction. Taq Polymerase was added to the chromosomal DNA, along with dNTP's and Oligonucleotide primers called Bacteria 8 forward and 1492 Reverse. The tube was placed in a thermal cycler and the DNA was denatured at 94 degrees Celsius for four minutes. The thermal cycler then completed 35 cycles of denaturing (94 degrees Celsius), annealing the primers for 45 seconds at 55 degrees Celsius and extending (making new DNA) for 2 minutes at 72 degrees Celsius. During the final cycle, the denaturing step was omitted and the extension step was increased to 5 minutes.

Following amplification, the resulting PCR product was mixed with the loading dyes, xylene cyanol and bromphenol blue and placed into an agarose gel for gel electrophoresis. After about 45 minutes gel was stained with Ethidium Bromide and then the DNA was cut and purified using a QIAQuick gel purification kit. The purified DNA sample was taken to the University of California at Davis, College of Biological Sciences DNA sequencing facility. U.C. Davis sent back electropherograms of the nucleotide sequences and these were evaluated and edited. The final sequence was transferred to the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information's website and compared to data available in GenBank.

RESULTS

Colonies grew quite rapidly on the nutrient agar plates inoculated, with off-white glistening, raised colonies, ranging from 2 - 6mm in size after 48-72 hours. The colonies

had an irregular border with lobate edges that were fairly translucent and opaque centers. The odor that emanated from them especially after a couple days of being closed in a plastic bag was a not unpleasant, slightly sour smell.

The Gram stain revealed purple rods in chains. In less then 2 weeks there were so many spores that it was hard to find cells that did not have spores. The rods were 1.5 micrometers in diameter and 4-5 micrometers in length. When 3% KOH was applied to a mass of bacterial cells they did not produce any viscosity. The results obtained with the NCBI BLAST indicated the 16S r-DNA from the fish tank isolate was 98% similar to homologous DNA from three types of organisms: *Bacillus anthracis, Bacillus cereus,* and *Bacillus thuringiensis.* The following tests were conducted to further analyze the characteristics of the culture. (The results for *Bacillus thuringiensis, cereus and anthracis* on the following chart were taken from #4 works cited page).

tests	Unknown bacteria	*Bacillus anthracis*	*Bacillus cereus*	*Bacillus thuringiensis*
Catalase	Positive	Positive	Positive	Positive
Methyl red	Positive			
Voges-Proskauer	Negative	Positive	Positive	Variable
Glucose aerobic acid	Positive	Positive	Positive	Positive
Glucose gas	Negative	Negative	Negative	Negative
Mannitol aerobic acid	Negative	Negative	Negative	Negative
Arabinose aerobic acid	Negative	Negative	Negative	Negative
Urease present	Negative			
Starch hydrolysis	Negative	Positive	Positive	Positive
Citrate utilization	Negative	Variable	Positive	Positive
Endospores	Positive	Positive	Positive	Positive
Cell diameter	1.5 micrometers	>1 micrometer	>1 micrometer	>1 micrometer
Spore shape	Ellipsoidal	Round: negative	Round: negative	Round: negative
Swollen sporangium	Negative	Negative	Negative	negative
Gram stain	Positive	Positive	Positive	Positive

DISCUSSION

The three types of organisms identified as being possible matches with the fish tank isolate have a wide range of uses and/or problems they cause. *Bacillus thuringiesis* are ubiquitous in soil and on plants. They produce a crystal protein that is toxic when eaten by insects that have an alkaline gut pH. For this reason, certain strains have been approved for use as an insecticide since the 1960's (7). Because these bacteria are endemic in our environment, most people have been exposed to them and they are believed to be safe for humans (1). There are many different strains currently being used for specific insecticides. For example, *Bacillus thuringiensis* strain *israelensis* is used to control mosquitos, and strain *aizawai* is used to control wax moth larvae and the Diamondback moth caterpillar. (1,7).

Robert Koch first isolated *Bacillus anthracis* in 1877. These were the first bacteria proven to cause illness. They are the causative agents for anthrax, a disease primarily affecting animals. *Bacillus anthracis* can be found in the soil, however their prevalence varies throughout the world. They can be found in Nebraska, California and Texas as well as other areas in the United States. While humans don't typically acquire anthrax naturally, the bacteria produce endospores, which reside in the ground and in animal remains for many years. Contact with an infected animal's remains, including hair, bones, excrement or flesh can transfer the disease to humans. (7) In the last several years Anthrax has gained notoriety as an agent for use in biological weapons.

The third potential match for the fish tank isolate was *Bacillus cereus.* These organisms are causative agents for food poisoning of 2 different types. The first occurs within 1-5 hours after ingestion and involves vomiting. The second occurs 8 -16 hours after consumption and involves diarrhea and abdominal pain. The food poisoning occurs because the endospores of *Bacillus cereus* are heat resistant and survive cooking and pasteurization and then begin growing when the food is improperly stored. *Bacillus cereus* cannot be avoided because it is ubiquitous in nature. The best prevention for avoiding food poisoning is to **cook** and **store** food properly. (3)

According to an article published in Applied and Environmental Microbiology, the only difference between *Bacillus cereus* and *Bacillus thuringiensis* is the genes coding for insecticidal toxins present on the plasmids of *Bacillus thuringiensis.* Furthermore, "strains isolated from the soil demonstrated a very high diversity in multilocus genotypes indicating that *B. cereus* and *B. thuringiensis* exhibit a low degree of clonality and the exchange of genetic material occurs frequently in their natural environment"(2). Essentially, the differences between *B. cereus, B. thurengiensis* and *B. anthracis* are due merely to genes carried on their plasmids. It is believed by some, that these three types of bacteria are actually the same species. There is concern that because all three bacteria appear to be the same species and that *B. thurningiensis* and *B. cereus* have exchanged genetic material the possibility exists that *B. anthracis* could potentially exchange genetic materials with them also. Since *B. thuringiensis* is used frequently in our environment and waterways, *B.Cereus* is endemic in our soil, and *B. anthracis* carries the threat of being a virulent pathogen, microbiologists are looking for ways to easily and accurately tell them apart. (2)

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Isolation of *Microbacterium testaceum* from Normal Flora – What's On Your Hands?

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On the first day of instruction, students in the 7:50 lab were instructed to form groups of three and to each place one finger on a nutrient agar plate. After several days of incubation, colonies formed, and one of these was streaked onto a new nutrient agar plate to form a pure culture. A series of procedures were conducted to determine the identity of this culture including; morphological observations, Polymerase Chain Reaction, gel electrophoresis, DNA purification and sequencing, electropherogram evaluation and editing through the program 4Peaks. After 16S ribosomal-DNA nucleotide sequence data was collected it was compared to that contained in public databases through the NCBI BLAST program. The culture isolated from the fingerprint showed high sequence similarity with *Microbacterium testaceum.* Because this species was not included in the Bergey's manual, information was obtained from the Internet to determine physiological tests needed to verify its identity. Due to lack of time, only three tests were performed, an oxidase test, a catalase test and a wet mount to determine motility.

INTRODUCTION

Human skin is sterile during fetal development, but during and after the birth process multiple different types of bacteria quickly colonize the infant's skin. Some of these bacteria will remain throughout the life of the individual, but some reside there only temporarily. As humans age, their skin is exposed to many different environments, and their microbial populations change also. Microorganisms established as more-or-less permanent residents are referred to as normal microbiota, or normal flora, while those present only temporarily, for a few hours, days or weeks, are called transient microbiota or transient flora. Transient flora can be various kinds of bacteria obtained through contact with any of the day-to-day objects people interact with.

In order to determine whether the organisms present on the hands of students in the Microbiology class at Sierra College were normal flora or transient flora, fingers of students were pressed onto a nutrient agar plate. Colonies formed within a couple of days, a selected colony was then isolated onto a new nutrient agar plate. This established a pure culture that was used to for all procedures and tests necessary to unveil the identity of the unknown microorganism. After this project, the determination of how important it is to use aseptic technique before eating, touching hands to mouth or any other kind of contact with the mouth will be obvious.

MATERIALS AND METHODS

On the first day of instruction, students in the 7:50 A.M. lab were instructed to form groups of three and to each place one finger on the surface of a nutrient agar plate that had been divided into four quadrants. This was accomplished, and the plate was placed into a lab drawer. After several days of incubation at room temperature, colonies formed, and one of these was streaked onto a new nutrient agar plate to form a pure culture. After well-isolated colonies with consistent morphology were established, their characteristics were recorded and tests were conducted. The morphological features of the colonies present on nutrient agar were determined through direct observation, but to discover the shape, size and structural characteristics of cells, a Gram-stain and indirect stain were prepared. DNA analysis was the next procedure in the identification process. A sample of the unknown culture was placed in 10μm of Tris Buffer and boiled for 10 minutes; it was also beaten with glass beads. A region of the 16S ribosomal-RNA gene was then amplified with the polymerase chain reaction using the primers bacteria 8-forward and universal 1492-reverse. The PCR product DNA was used to perform gel electrophoresis and was then stained with ethidium bromide. The DNA was cut from the gel, purified with a QIAquick gel purification kit and taken to the UC Davis DNA Sequencing Facility. The results from the DNA sequencing were returned electronically and were edited using the 4Peaks program. The DNA sequence data was compared with that in GenBank using the NCBI BLAST.

The results obtained from the BLAST provided a tentative identification of the unknown microorganisms; but the Bergey's Manual did not contain any information about the species. The PubMed feature of NCBI was used to obtain additional details about the microorganisms isolated. Due to time conflicts, not many physiological tests could actually be performed, but an article from the *International Journal of Systematic and Evolutionary Microbiology* provided the results for many tests that could have been conducted in class. The three tests that were actually performed included a motility test, an oxidase test and a catalase Test. The motility test required that a wet mount be prepared by placing a sample of the organisms on a clean glass slide along with a loop full of deionized water. The slide was then observed with a compound microscope using the oil immersion lens. The oxidase test involved rubbing a sample of the unknown organisms onto filter paper saturated with oxidase test reagent. Direct observations were made within a few minutes after doing this to determine the results. The catalase test involved placing a sample of the unknown culture on a clean glass slide and then adding 3% H_2O_2. The results were observed directly and recorded.

RESULTS

The colonies formed on the nutrient agar plate were punctiform to circular, entire, convex, glossy and shiny, semi-translucent with an orange pigment to them. They ranged in size from 1-3mm in diameter. The Gram-stain revealed purple Gram-Positive bacilli organized as single-cells, in pairs and in V-shaped patterns. The indirect stain showed this same pattern, while the background was purple and the cells appeared white or colorless. The size of these cells ranged in size from 1-2μm in length and 1-1.5μm in diameter. The

wet mount showed no sign of motility, while being observed through the microscope. The cells just flowed with the direction of water moving under the coverslip. The oxidase test was negative because the color of the cell blob did not change to a purple color. The catalase test was positive as well because bubbles were formed when 3% hydrogen peroxide was added to the cell sample. Other tests could have been performed on this culture, but time ran out; these are displayed in the table shown.

Tests Performed	**Results**
Gram-Stain	Gram-Positive
Wet Mount	Non-motile
Catalse Test	Positive
Oxidase Test	Negative
Tests that Could be Performed	**Potential Results**
O/F Test	Respiratory
Urease	Negative
TSI	Negative for H2S
MR/VP	Positive/Negative
Glucose	Negative
Sucrose	Positive
Xylose	Positive
Rhamnose	Negative
Inositol	Negative
Sorbitol	Negative
Arabinose	Positive
Fructose	Positive
Mannitol	Positive
Maltose	Positive

Results obtained with the NCBI BLAST showed the 16S ribosomal-DNA nucleotide sequence from the fingertip isolate demonstrated 99% similarity with a GenBank sequence from *Microbacterium testaceum* (DSM 20166). Only 1405 out of 1415 bases matched pairwise with all of the mismatched pairs occurring in the bacteria 8-forward section of the sequence. Nine occurred between base #138 and #178 on the query and one occurred at base #337.

DISCUSSION

Microbacterium testaceum is a fairly recent addition to the genus *Microbacterium*, recorded in 1994 during a revision of the genus. Phylogenetically, this species is closely related to *Microbacterium oleivorans* and several uncultured *Microbacterium* species. The taxonomic lineage for this organism is as follows: **Kingdom:** Bacteria, **Phylum:** Actinobacteria **Class:** Actinobacteria, **Order:** Actinomycetales, **Family:** Microbacteriaceae, **Genus:** Microbacterium, **Species:** *testaceum.* This species was previously recorded under

other genera including *Brevibacterium*, *Aureobacterium* and *Curtobacterium*. Although this particular species of *Microbacterium* is not described as being a pathogen, other *Microbacterium*, *Brevibacterium* and *Aureobacterium* species have been found in association with catheter-related bacteremia, and other clinical samples. Some information recorded about the genus *Micrbacterium* deals with its capability to utilize oil and colonize oil transport pipelines. When not found in these settings, bacteria in the genus *Microbacterium* apparently in live soil. Since this sample was found on the fingertip of a student, it's no wonder aseptic technique is such an important procedure in lab and should be one every person uses after touching everyday objects and before making hand to mouth contact.

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Isolation and Identification of *Bacillus licheniformis* from an Air Plate Collected from a Family Kitchen – *Bacillus licheniformis*… It's What's for Dinner

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A bacterial culture was isolated from an air plate obtained from a family kitchen. The unknown culture was identified through isolation, morphological observation, Polymerase Chain Reaction, gel electrophoresis, and sequencing completed by personnel at the UC Davis DNA Sequencing Facility. The nucleotide sequence electropherogram returned from the sequencing facility was evaluated and edited using 4peaks software. It was then compared to data available through the National Center for Biotechnology Information (NCBI) website using the Basic Local Alignment Search Tool (BLAST) algorithm. The culture was identified as *Bacillus licheniformis*.

INTRODUCTION

Food safety is serious and important; therefore cleanliness in the kitchen helps keep food safe to eat. Food preparation in a clean environment becomes exponentially more important when that food will be eaten by people who are immune-compromised and therefore more susceptible to severe food borne illnesses: pregnant woman, young children, the elderly, and people with diseases such as cancer and AIDS. A bit of extra cleaning, sanitizing, and using surface disinfectants can eliminate these bacterial contaminants from your food preparation area, thereby preventing ailments that may yield sore throats, common colds, gastro-intestinal distress, or even a life-threatening illness.

The goal of this investigation was to expose a nutrient agar plate to open air in a family kitchen for one hour and then assess what organisms would develop. The experiment was conducted to demonstrate whether or not current cleaning practices left the family kitchen free from potentially hazardous microorganisms. If cleaning practices are sufficient, the experiment should not yield harmful bacteria and only indicate those bacteria that are considered normal in the air. If current cleanliness is insufficient, then the microbes yielded from this experiment will prove to be hazardous to the family's health, and current cleaning practices should be re-evaluated.

MATERIALS AND METHODS

Isolation of the subject culture of *Bacillus licheniformis* was accomplished by exposing a nutrient agar plate to the open air in the kitchen of a family home. This plate was placed on the counter, opened and exposed to air in the kitchen for approximately one hour.

The plate was then brought back into the laboratory and placed in the laboratory drawer for incubation at room temperature. After several days had passed, the nutrient agar revealed that several colonies had formed with varied morphology displaying various colors, forms, surface textures, sizes, and optical characteristics. An off-white culture with very interesting colony morphology was selected among the many colorful choices of colonies on the plate. Taking a single colony from the original plate and streaking it onto a new nutrient agar plate accomplished preparation of a pure culture. This process was repeated multiple times and a pure culture was finally established after the third transfer.

A Nigrosin indirect stain was prepared and observed in order to confirm cell shape and size. Cell wall composition was determined by completing a Gram stain. To confirm that the correct results were achieved, a KOH test was also performed. When observation of the Gram stained sample indicated only one type of organism was growing on the plate, chromosomal DNA was extracted from the sample.

DNA analysis was performed using the following methods: DNA was extracted from the sample by vortex mixing and boiling it in 10mM Tris buffer and then beating it with glass beads. Using Taq polymerase and the oligonucleotide primers Bacteria 8-forward and Universal 1492-reverse the Polymerase Chain Reaction amplified the 16S ribosomal-DNA from the culture. After amplification, gel electrophoresis was run to isolate the PCR product DNA, and this was cut out and purified using a QIAQuick PCR purification kit by Qiagen. The purified PCR product DNA was then sent to the DNA Sequencing Facility at UC Davis. The gene sequences sent back were evaluated and edited with the 4peaks software program using the Mac Operating System X. The final sequence was then compared with those available through the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) (1).

RESULTS

The colonies observed on the pure culture plate varied in size from 1-4 mm wide and 2-15 mm in length. The colonies were off-white with an opaque optical character, and were shiny in some places and dull in others. The margins were irregular but appeared lobate when viewed under the stereomicroscope. The colony elevation was raised to convex. These bacteria were observed to be Gram-positive and rod-shaped. They tested KOH negative, indicating they had thick peptidoglycan walls. When viewed with compound microscopy these *Bacillus* were observed to produce endospores that were central in location and ellipsoidal in shape. When viewed in a wet mount, the cells were found to be motile.

When the nucleotide sequence from the 16S ribosomal-DNA taken from the air isolate was compared to data stored in the gene banks through the NCBI BLAST, it was found to be 99% similar to multiple different strains of *Bacillus licheniformis*. These were the only *Bacillus* species the sequence matched with.

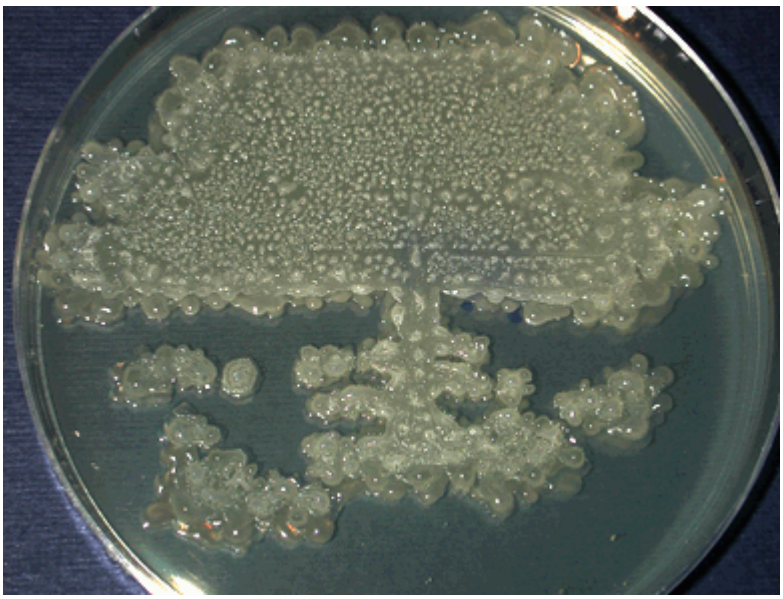

Fig. 1 – A nutrient agar plate showing colonies of *Bacillus licheniformis*.

DISCUSSION

Bacillus licheniformis is a very common species of microorganism found in soil. It contributes to nutrient cycling, displays antifungal activity, and is used in the manufacture of a variety of enzymes. Researchers suggest that these bacteria act against fungi by producing an antibiotic agent, and possibly an anti-fungal enzyme.

Bacillus licheniformis is an interesting type of bacteria because it affects humans in many different ways and has many uses in industry. Human infections involving these bacteria occur only when preceding cases of trauma or immune-suppression allow them to multiply within the body. Generally the possibility of infection by *Bacillus licheniformis* is low and it is not considered a pathogen by most sources. Its signficance in the clinical setting is arguable by some in the scientific community.

Bacillus licheniformis is a common species associated with the spoilage of milk, packaged meats, and some canned goods. Food poisoning can be caused by these organisms and is characterized by diarrhea, with vomiting occurring in half of the cases. Its use is not recommended on plants that may be used for food or feed because it may be associated with food poisoning in humans and reproductive failure in cattle, sheep, and pigs. *Bacillus licheniformis* are important commercial bacteria because they are used to produce enzymes, mainly alpha-amylases and proteases. These enzymes are manufactured in large quantities through fermentation (3). They are then used in many different ways as listed below:

1. They are added to cleaning detergents to improve their effectiveness.
2. They help break down organic stains that are otherwise hard to remove.
3. They are used for de-hairing and batting in the leather industry.

4. The alpha-amylases produced are used for the de-sizing of textiles and starch modification for sizing of paper.
5. *Bacillus licheniformis* is also used to produce the polypeptide antibiotic Bacitracin, which is an additive in topical ointment to prevent minor cuts and abrasions from developing infections, and is also added to eye ointment to treat minor bacterial infections of the eyelids.
6. The soil based organisms, *Bacillus subtilis* and *Bacillus lichenformis*, have been shown to inactivate HIV, SIV and other lipid-enveloped viruses, along with mycoplasmas, fungus and bacteria by producing 'surfactin' - a lipopeptide antibiotic (4).

It is comforting to know that *Bacillus licheniformis* is a species present in household detergents, common in the soil, and not likely to cause severe harm to humans. These facts make it less alarming that these bacteria were discovered in a family kitchen. While *Bacillus lichenformis* are relatively common in the soil environment, they are also important to humans in many different ways. In the future we may find even more uses for their enzymes as well as gain a better understand of how much virulence they actually possess.

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Identification and Isolation of *Acinetobacter radioresistens* from a Contact Lens

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A contact lens was exposed to the surface of a blood agar plate in an attempt to determine if any pathogenic organisms or organisms capable of causing infections were present and would grow. A culture ultimately identified as *Acinetobacter radioresistens* was chosen from the many colonies that grew and was subjected to a variety of tests including staining to determine cell morphology, observation of colony morphology and hemolysis reaction on blood agar. DNA extraction, PCR, gel electrophoresis and comparison of the resulting 16S r-DNA nucleotide sequence with those in public databases were completed. Enzymatic tests including oxidation/fermentation, oxidase, catalase and citrate utilization were also completed in order to identify the culture. The organisms identified were found to be opportunistic pathogens in immune deficient individuals and are recognized as causing nosocomial infections in hospital settings. These bacteria can also exist on the skin of normal, healthy individuals, committing no harm. The findings of this investigation suggest it is highly advisable that people properly clean contact lenses before and after each use and be aware of what they can carry and expose a person's precious eyes to. These findings will hopefully encourage people to think about and take seriously the care they give their contact lenses.

INTRODUCTION

This project was initiated to determine what types of microorganism might be found living on the surface of a contact lens formerly worn in the human eye. A second goal was to determine if any of the microorganisms living on a contact lens surface could be pathogenic, potentially pathogenic, or capable of causing infection or irritation within the human eye.

A young, sixteen-year old woman's contact lenses were the basis for this investigation. The subject individual used Acuvue Advance with Hydra Clear lenses by Johnson and Johnson. These are soft, month-by-month lenses, which means they are disposable, and designed to be discarded after being worn for one month. Observations made many times throughout the month of contact use determined that the lids for the contact's container were frequently left off the cases at night and that various types of contaminants were allowed to enter the contact solution in the container. These included hair, mascara, and dust. After a month of wear, the bacterial content of the poorly cared for contacts was investigated.

MATERIALS AND METHODS

This project was initiated by inoculating a plate of sheep blood agar with a used contact lens. This was accomplished by placing the lens over a fingertip and sliding it over the agar surface several times. After a week of incubation in a lab drawer at room temperature, several different colonies grew on the blood agar. A sample from one isolated colony was chosen and was streaked onto a new blood agar plate using aseptic technique. After two different attempts at obtaining a pure culture, a plate containing well-isolated colonies with consistent morphology was finally obtained. A Gram-stain of this culture was made in order to view the cell arrangement, shape, and to determine the cell wall composition. A potassium hydroxide (3% KOH) test was also performed to confirm the wall composition.

A blob of actively growing cells was mixed with 500µl of 10Mm Tris buffer solution (pH 8.0) in a 1.5ml centrifuge tube and then boiled for ten minutes to break down the cell wall and extract the chromosomal DNA. Then the PCR was used to amplify a portion of the 16S ribosomal-DNA. The reaction mixture included Taq Master Mix (Qiagen), primer mix, the subject's DNA, and water. The bacteria primers used for the PCR were Bacteria 8-Forward and Universal 1492-Reverse. Following the PCR, Gel Electrophoresis was used to separate the PCR product from other materials. An agarose gel was submerged in TBE buffer and then the total volume of PCR product was loaded into the wells. The gel was stained with ethidium bromide and then the subject's DNA was cut out and purified using a kit process: a QIAquick Gel Purification Kit. The purified DNA sample was then taken to UC Davis to the College of Biological Sciences DNA Sequencing Facility in Storer Hall. The sequencing primers used there were 8-Forward, 533-forward and 1492-Reverse. Electropherograms generated with the three primers and indicating the subject's 16S r-DNA nucleotide sequence were then posted on the Microbiology Web Site for Sierra College. These were then evaluated and edited using Mac OSX and 4Peaks. The nucleotide sequence for the unknown subject was then compared with other sequence data information available in public databases through the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) algorithm (5).

After a tentative identification was obtained from the NCBI BLAST results, the Bergey's Manual of Systematic Bacteriology, Volume 2 (2005) was consulted, and then a number of enzymatic tests were performed. These included an oxidation/fermentation or O/F test, a catalase activity test, an oxidase test, a hemolysis reaction on blood agar test, and a citrate utilization test.

RESULTS

The colonies found growing on the blood agar plate were circular to irregular in form with entire margins and a raised to low convex elevation. Their surface texture was shiny and glistening and their optical character was opaque. The colonies appeared milky white and were 1-3mm in diameter. This culture stained pink or Gram-negative in the Gram Stain, but barely showed up (the cells were almost clear even in the third test). The cell arrangement appeared to be single-cells and the shape was both cocci and baccili (the

culture was pleomorphic). When exposed to 3% potassium hydroxide in the KOH test the culture produced a thick viscosity and slime indicating it had thin peptidoglycan walls or had a Gram-negative type wall.

Results obtained with the NCBI BLAST and the public database indicated the subject microorganisms found on the contact lens showed 99% sequence similarity with a 1459 base sequence from the 16S r-DNA genes of *Acinetobacter radioresistens*. The subject sequence matched 1428 out of 1433 bases and the bit score was 2623.

The O/F tubes inoculated with the subject organism remained green under the vaspar seal and green with some yellow coloration at the surface of the unsealed tube. This indicated they had a respiratory metabolism but formed some aerobic acid from glucose catabolism. The culture developed bubbles when exposed to 3% hydrogen peroxide so was catalase-positive, but did not form any purple color when rubbed on filter paper saturated with oxidase test reagent, so was oxidase-negative. There was no change in the color of the blood agar behind the colonies, so they were only capable of gamma-hemolysis. When grown on a citrate agar slant, the culture turned the medium deep blue, so tested positive for citrate utilization.

The Colony Morphology:
Form: circular/ irregular
Edge: entire
Elevation: raised/ convex
Surface texture: shiny/glistening
Optical character: opaque
Pigmented: milky white or non-pigmented
Size: 1-3 mm.
Spread/ isolated colonies

Enzymatic test results:
O/F Test: Respiratory
Catalase Activity: Catalase-positive
Oxidase Test: Oxidase-negative
Hemolysis test: gamma- hemolysis
Citrate Utilization test: Positive

DISCUSSION

The culture isolated from the contact lens was determined to be Gram-negative with the KOH test, but was difficult to see in a Gram-stain. It was catalase-positive, oxidase-negative, respiratory and able to utilize citrate as a sole carbon source. These features and the morphological findings were consistent with the description found in the Bergey's manual for *Acinetobacter radioresistens*.

The cells of *Acinetobacter radioresistens* are short, Gram-negative bacilli, measuring 1.0-1.5 by 1.5-2.5 microns during active growth; they often become more spherical during their stationary phase. They are often difficult to stain, do not form endospores, are non-motile but appear to display “twitching motility”, and grow best between 33-35 degrees Celsius (2). They are strictly aerobic/ respiratory and can use a varied selection of organic materials as carbon sources. “*Acinetobacter* is a genus of opportunistic pathogens in the proteobacteria group, species of which are distributed in widespread, diverse habitats." (1). They are found in soil and in water, and can reside, possibly indigenously, on human skin and in the human respiratory tract where they may or may not be pathogenic (2).

Acinetobacter species are also widely distributed in hospitals, where they pose the danger of transferring resistance to other hospital-inhabiting bacteria. “Up to 27% of hospital sink traps and 20% of hospital floor swabs have yielded isolates of *Acinetobacter*." (1).

Acinetobacter radioresistens can be considered as a cause of opportunistic infection in immune deficient patients (4). “These bacteria can cause nosocomial infections, such as: bacteremia, secondary meningitis, pneumonia, and urinary tract infections in hospitalized patients, especially those dependent on ventilators in Intensive Care Units." (2). These bacteria have been found to contaminate respirators and hospital air when there are colonized patients present, as well as nearby bed blankets and bed curtains. *Acinetobacter radioresistens* is well adapted for survival in the hospital environment and carriage on human skin (3). Current studies are being performed on methods for hospital air purification in order to lower the prevalence of these pathogens among compromised patients (1). Although, *Acinetobacter radioresistens* can cause a lot of trouble for immune compromised individuals, in healthy humans, it is normal to have some amount of *Acinetobacter* on the skin surface. “As many as 25% of healthy adults do in fact harbor these bacteria (1).”

The morphological, physiological and genetic findings of this investigation indicate it is most likely that *Acinetobacter radioresistens* was dwelling on the contact lens tested. This is likely also because *Acinetobacter radioresistens* can reside on human skin and the contact lens was previously handled by an individual’s fingers. Since *Acinetobacter radioresistens* can be opportunistic pathogens in association with immune deficient individuals, it is highly advisable to clean contact lenses well before and after each use, to use fresh conditioning solution and to put the lids on the contact lens cases when storing the lenses overnight to help decrease the potential for contamination with this and or other bacteria.

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